Legacy and Emerging Environmental Organic Pollutants in Human Placenta and Blood

BY

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This dissertation is dedicated to the memory of my loving father. –SD

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CONTRIBUTION OF AUTHORS

Chapter 3 represents a published manuscript (Dassanayake et al., 2009) for which I was the primary author and major driver of the research. Dr. Hwa Wei assisted me with the statistical data analysis. Rachel Chen helped me with sample preparation and literature search. My research mentor, Dr. An Li contributed to the writing of the manuscript. Chapters 4 and 5 represent a series of my own unpublished experiments directed at successfully quantifying environmental organic pollutants in human placenta and blood and understanding the distribution of these chemicals within the feto-maternal unit. I anticipate that this line of research will be continued in the laboratory after I leave and that this work will ultimately be published as part of a co-authored manuscript.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
anti-DP	Dechlorane Plus (anti)
ATBPE	2,4,6-tribromophenyl Allyl Ether
BB153	2,2’,4,4’,5,5’-hexabromodiphenyl
BCF	Bioconcentration Factor in Fish
BDE	Bromodiphenyl Ether
BFR	Brominated Flame-Retardants
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethan
CB	Chloro Biphenyl
CDC	Centers for Disease Control
COC	Chain of Custody
CRM	Certified Reference Material
DBDPE	Decabromodiphenylethane
DCM	Dichloromethane (Methylene Chloride)
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene (1,1-bis-(4-chlorophenyl)-2,2-dichloroethene)
DDT	Dichlorodiphenyltrichloroethane (1,1-bis-(4-chlorophenyl)-2,2,2-trichloroethane)
Dec 602	Dechlorane 602
Dec 603	Dechlorane 603
Dec 604	Dechlorane 604 Component a
dw	Dry Weight
ECD	Electron Capture Device
EHTBB	2-ethylhexyl, 2,3,4,5-tetrabromobenzoate
EI	Electron Impact Ionization
ENCI	Electron Capture Negative Ionization

LIST OF ABBREVIATIONS (continued)

EPA	Environmental Protection Agency
FBDE69	4’-fluoro-2,3’,4,6-tetrabromodiphenyl Ether
FBDE208	4’-fluoro-2,2’,3,3’,4,5,5’,6,6’-nonabromodiphenyl Ether
GC	Gas Chromatography
GM	Geometric Mean
HBB	Hexabromobenzene
HBCD	Hexabromocyclododecane
HCB	Hexacholorobenzene
HCCP	Hexachlorocyclo-pentadiene
HCDBCO	Hexachlorocyclopentadienyl-dibromocyclooctane
HCH	Hexachlorocyclohexane
HSD	Honest Significant Difference (Tukey)
IARC	International Agency for the Research on Cancer
IS	Internal Standard
IUPAC	International Union of Pure and Applied Chemistry
K_{OA}	Octanol-Air Partition Coefficient
K_{OW}	Octanol-Water Partition Coefficient
LOD	Limit of Detection
LOQ	Limit of Quantification
lw	Lipid (Adjusted) Weight
m/z	Mass-to-Charge Ratio
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
MS/MS	Tandem Mass Spectrometer
MSPD	Matrix Solid Phase Dispersion

LIST OF ABBREVIATIONS (continued)

MTBE	Methyl Tertbutyl Ether
N_2	Nitrogen Gas
NCS	National Children's Study
NHANES	National Health and Nutrition Examination Survey
NICHHD	National Institute of Child Health and Human Development
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institute of Health
NIST	National Institute of Standards and Technology
OCP	Organochlorine Pesticides
PBB	1,2,3,4,5-pentabromo-benzene
PBB101	2,2'4,5,5'-pentabromobiphenyl
PBBA	Pentabromobenzyl Acrylate
PBBB	Pentabromobenzyl Bromide
PBCCH	Pentabromochlorocyclohexane
PBDE	Polybrominated Diphenyl Ether
PBEB	Pentabromoethylbenzene
PBT	Pentabromotoluene
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Dibenzo-p-dioxins
PCDF	Polychlorinated Dibenzofurans
POP	Persistent Organic Pollutants
PTV	Programable Temperature Vaporization
QA/QC	Quality Assurance and Quality Control
QQQMS	Triple Quadrupole Mass Spectrometer

LIST OF ABBREVIATIONS (continued)

RPD	Relative Percent Deviation
RSD	Relative Standard Deviation
SRM	Standard Reference Material
syn-DP	Dechlorane Plus (syn)
TBB	1,3,5-tribromobenzene
TBC	1,3,6,8-tetrabromocarbazole
TBCO	1,2,5,6-tetrabromocyclooctane
TBECH	1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane
TboCT	Tetrabromo-o-chlorotoluene
TBPH	Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate
TBpX	2,3,5,6-tetrabromo-p-xylene,
TCDD	Tetrachlorodibenzo-p-dioxin
UIC	University of Illinois at Chicago
wwt	Wet Weight
XFR	Halogenated Flame-Retardants

SUMMARY

A reliable anlytical method was developed and validated to quantify persistent organic pollutants (POPs), including organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polybrominated biphenyl ethers (PBDEs) in human placenta. This method was used to analyze 43 POPs (10 PBDEs including BDE-209, 32 PCBs, and DDE) compounds in 374 placental tissue specimens taken from 210 placentas collected during 2011–2012 from seven states, for the Main Study section of the formative research project "Placenta Project" of the National Children's Study (NCS) of the United States. Of all the samples analyzed, data from 191 placentas with complete location information were selected for statistical data analysis. Lipid content of the samples was not measured due to limitation of the sample amount. The terms "Total PBDE" and "Total PCB" below refer to the sums of the concentrations of all targeted congeners in the respective chemical groups. The data were non-normally distributed. The median Total PBDE, Total PCB, and DDE concentrations were 199 (range 54–867), 679 (range 217–2370), and 76 (range 182–4160) pg/g wet weight (wwt), respectively. For PBDE congener dominance in concentration decreased in the rank order BDE-209 > BDE-47 > BDE-153 > BDE-99. Deca (BDE-209) was detected in all samples and represented 38% of Total PBDE concentration whereas BDE-47 was at 24%. Among all measured PCBs, Chloro biphenyl 101 (CB-101) has the highest concentration followed by CBs 118, 70, 52, 87, 153, and 138. Together CB-101 and CB-118 count for 23% of the Total PCB concentration. The homolog profile for the abundance decreased as penta > tetra > hexa > hepta. Placentas collected from different locations across the United States were assessed for regional differences. Samples collected from Duplin County, North Carolina had the highest median of 290 (range 89–867)

pg/g wwt, whereas Orange County, California had the highest 75 and 90 percentiles of Total PBDE concentration. Orange County, California had the highest Total PCB concentration of 720 (range 308–1780) pg/g wwt, and a DDE concentration of 294 (range 927–4,160) pg/g wwt. A significant site-specific variation was observed for DDE concentrations. Site variation in PBDE concentration was not statistically significant in general; however, a significant difference was found for PCB concentrations between placentas collected in Cache County, Utah, and Salt Lake County, Utah ($p<.05$). Clinical information collected from the study participants had limited use, with several variables of high importance (e.g., race, ethnicity, and body mass index) having high proportions of missing data.

In a parallel Chicago study, 24 sets of matched feto-maternal samples (24 maternal blood, 24 placenta, and 24 cord blood), collected in Chicago, Illinois during 2010–2011, were analyzed for 10 PBDEs, 32 PCBs, 31 OCPs, and 31 non-PBDE halogenated flame-retardants (XFRs). Analytes such as PBDEs, PCBs, DDE, DDT, and hexachlorobenzene (HCB) were detected in all samples. Detection and quantification of most of the OCPs and XFRs were problematic due to low instrument responses, high background, and/or coelution with PBDEs. The detection rates for some analytes were particularly low in blood samples, which might have been caused by the strong acid treatment in the laboratory procedure.

In the Chicago study, the lipid content of the samples was determined gravimetrically. On wwt, maternal blood had higher concentration of Total PBDE (median 134 pg/g wwt) than placenta (median 83 pg/g wwt) and cord blood (median 72 pg/g wwt). Most abundant congeners were BDEs -47 and -209 and the BDE-47: BDE-209 ratio was 1:1 in maternal blood, 1.7:1 in

placenta, and 1:2 in cord blood. Cord blood had the highest lipid-adjusted PBDE concentration (43.5 ng/g lipid), which was significantly different for the placental concentration (10.5 ng/g lipid). Maternal blood concentration (35.8 ng/g lipid) was lower compared to cord blood but the difference was not statistically significant ($p>0.05$). In each matrix PBDEs were highly correlated. Strong correlations also existed between the concentrations in maternal blood and cord blood. DecaBDE in cord blood was an exception because it was significantly correlated only with placental BDE-209 but not with BDE-209 in maternal blood or with any other congeners. Analysis of congener profile in each matrix suggested that placenta selectively retains tetra, penta, and hexaPBDEs. The results also hint on the occurrence of debromination or other metabolic activity on BDE-209 in placenta. Cord blood is apparently accumulating BDE-209, with 46% of its total PBDE being BDE-209.

Wet weight-based median Total PCB concentrations for maternal blood, placenta, and cord blood were 241 (range 65–1190), 274 (range 110–783), and 226 (range 36–515) pg/g wwt, respectively. On lipid normalized basis, the medians were 74 (range 21–208), 112 (range 15–523), and 32 (8–208) ng/g lipid for maternal blood, cord blood, and placentas, respectively. The concentrations of cord and maternal bloods were not statistically significantly different ($p>.05$), and were correlated. Placental levels of Total PCB were significantly lower than those of both cord and maternal blood ($p<.05$); and the correlations between placenta and maternal blood, and between placenta and cord blood, were mostly negative and weak. Similar congener profile was observed in all matrices with CBs 153, 101, 118, 138, 52, 28, 8, 70, and 87 having higher abundances than other congeners. The variation among the three matrices in homolog

contribution to the Total PCB was found to be trivial. PentaPCBs (33%) followed by tetraPCBs (28%) were dominant contributors to the Total PCB.

Among the target OCPs, HCB, p,p'-DDE, and p,p'-DDT were detected in all samples. Wet weight-based median p,p'-DDE concentration was significantly higher in maternal blood. Lipid-adjusted median maternal, placental, and cord blood p,p'-DDE concentrations were 35, 8.9, and 34 ng/g lipid, respectively. The concentrations of p,p'-DDT were much lower than those of p,p'-DDE, with the medians in maternal, placental, and cord blood medians at 6.0, 0.3, and 7.6 ng/g lipid. Median maternal, placental, and cord blood HCB concentrations were 3.2, 0.9, and 4.0 ng/g lipid. All three compounds in each matrix were highly correlated. Detection rates for the target emerging flame-retardants and other pesticides were low, particularly in cord blood samples. This may be a reflection of both the limited sample amount and the inadequacy of the analytical method, which warrants additional optimizations with regard to the extraction, cleanup, and instrumental analyses when these compounds are to be quantitatively analyzed.

To characterize the equilibrium of pollutants between compartments, two ratios of concentrations of pollutants have been used historically. The C/M ratio was used to quantify the ration between concentrations in maternal blood to cord blood while P/M ratio was used to describe the pollutant concentrations in maternal blood to placenta. Higher ratios may be due to rates of transfer between compartments, as well as rates of biotransformation, excretion, and distribution within compartments. Pollutants analyzed in this study displayed varying C/M and P/M ratios. On average, C/M and lipid-based concentration ratios were higher comparative to P/M and weight-based ratios. For both PCBs and PBDEs, the C/M and P/M ratios decreased with

the number of halogen substitution. DecaBDE showed a shift from this trend by having the higher C/M and P/M ratios, questioning the roles played by molecular size and the lipid solubility in crossing biological membranes and/or in their biotransformation, excretion, and distribution.

Overall, the results of this study demonstrated the passing of potentially harmful chemicals into the fetal blood circulation. Presence of POPs in cord blood corroborates the prenatal exposure of human to POPs. Potential for POPs accumulation in cord blood and metabolic transformation in placenta may explain the concentration ratios. Significant correlations existed between matrices indicating the possibility of using placenta as a noninvasive surrogate to predict both maternal and prenatal exposures simultaneously. More studies are essential to validate the findings of this study.

1. INTRODUCTION

1.1. Background

Persistent organic pollutants are of great concern due to their resistance to degradation and their ability to accumulate in human and animal tissues. These include legacy pollutant chemicals such as PCBs and OCPs as well as emerging pollutants like PBDEs.

Legacy environmental pollutants are still ubiquitous around the world even after being banned for decades in most countries. Due to their hydrophobicity, POPs accumulate in lipids of biota and have the potential to biomagnify in the food web (McLeod et al., 2014; Molde et al., 2013; Frederiksen et al., 2009). They have been found in various matrices of the environment including wildlife and human. While the legacy POPs are declining, PBDEs rapidly increased in humans from the 1970s to the mid-2000s in the United States (Schecter et al., 2005), with a doubling time of only 3–5 years in human blood (Hites, 2004). All these POPs have been found in maternal blood, cord blood, and breast milk of humans, indicating that prenatal exposure to these chemicals is occurring (Al-Saleh et al., 2012; Curley, 1969; Dassanayake et al., 2009; Frederiksen et al., 2009; Rappolt and Hale, 1968; Schecter et al., 2003; Shen et al., 2007).

Exposure to various environmental pollutants starting at very early life stages is of particular concern because PCBs, DDE, and PBDEs are suspected endocrine disruptors (Valvi et al., 2011; Lee et al., 2010; ATSDR, 2002; Darnerud, 2008; Norris and Carr, 2006). In-utero and prenatal exposure to POPs have been associated with detrimental effects on the development of the fetus and related to lower birth weight, gestational age, and neuromuscular maturity of the fetus, and intelligence development of children at early ages (Straif et al., 2005; Pathak et al., 2010; Ren et al., 2011; Patayova et al., 2013). Maternal POP exposures have been shown to be

associated with childhood obesity. These exposures may find their way to offsprings through placenta or milk (LaMerill and Birnbaum, 2011) and certain pollutants (e.g DDE) are termed as "developmental obesogens" (Birnbaum, 2013). However, little is known about in-utero exposures and the associated health effects of many emerging POPs. For example, the health outcomes of neonatal exposure to PBDEs are less understood than those related to the legacy POPs.

Placenta has been used as a matrix for analysis in several studies to determine the levels of pesticides, flame-retardants, therapeutic and illegal drugs, and metals (Nanes et al., 2014; Viscaino et al., 2014; Leino et al., 2012; Needham et al., 2010; Dassanayake et al., 2009; Iyengar and Rapp, 2001; Myren et al., 2007; Shen et al., 2007). The placenta offers a unique view of prenatal exposure that may differ from that given by cord blood analysis. Compared with blood, placental tissue offers a measure of longer term exposure to bioaccumulative chemicals during fetal development (Iyengar and Rapp, 2001; Myllynen et al., 2005; Myren et al., 2007). Other benefits of using placentas for prenatal exposure studies include the noninvasive sample collection and a yield of a large amount of tissue, which enables multiple laboratory analyses. This is of particular importance for comprehensive investigations where the collected placentas are subject to multiple examinations for various research purposes. In recent years, placenta analysis has found increasing uses as a noninvasive technique for chemical exposure assessment (Esteban et al., 2009). However, the eveluating for POPs in placenta and placental transfer of POPs are comparatively limited.

Despite the fact that analytical techniques have improved substantially over the last 10 years in terms of accuracy, precision, and sensitivity, analysis of POPs at trace levels is still challenging owing to the ubiquity of POP in the environment. Levels of POPs in laboratory

environment can interfere with the analytical results by introducing false positive results and overestimation. Therefore, the use of a validated analytical method that has been optimized to extract POPs sufficiently from the substrate is important to increase the successful detection and accurate measurement results.

1.2 Aims and Objectives of the Study

The overall objective of this research is to assess the prenatal exposure of humans to persistent organic pollutants including PBDEs, PCBs, and selected OCPs and alternative flame-retardant chemicals by accurately measuring these pollutants in the human placenta tissue, maternal blood, and umbilical cord blood. To achieve the objective, following specific tasks were performed.

- developed and optimized a laboratory procedure to extract PBDEs from human placenta tissue, and modified and validated the method for the analyses of other legacy and emerging pollutants;
- quantitatively measured the concentrations of PBDEs, PCBs and DDE in the placenta tissue samples collected in the NCS Formative Research "Placenta Project";
- statistically analyzed the measured chemical data to assess the concentrations in placental tissue and examined the variations in concentration and congener profile, with regard to collection site and time;
- developed a protocol to collect maternal blood, umbilical cord blood, and placenta samples and health and social demographic information from the pregnant women admitted to the University of Illinois at Chicago (UIC) Medical Center;
- collected samples from the UIC Medical Center and analyzed PBDEs, PCBs, and other POPs in the samples;

- analyzed chemical data to characterize matrix variation with regard to abundance, congener patterns, and distribution patterns;
- Determined P/M and C/M concentration ratios for each target chemical to begin to understand the dynamic processes that may determine, for a given materal blood centration, the in utero exposure to the analyzed POPs.

1.3 Organization of this Dissertation

Following this introductory chapter, the results of a comprehensive literature review are summarized in chapter 2. It provides information on the physicochemical characteristics of targeted POPs, their environmental behavior, the up-to-date findings on human exposure, as well as the adverse effects of POPs on human health, human placenta, placenta analysis, and transplacental transfer.

Laboratory method development is the focus of chapter 3, which describes the efforts leading to the establishment of the analytical capacity for reliable detection and accurate determination of the targeted chemicals in order to meet the objectives of this research. A cost effective, fast, and reliable laboratory procedure was developed to extract PBDEs from human placenta tissue. This method was validated using standard reference materials (SRMs) with certified PBDE concentrations. The procedure was evaluated for the extraction efficiency of chlorinated compounds such as PCBs, DDE, and other targeted chemical analytes.

The major work of this dissertation research consists of two parallel studies, presented in chapters 4 and 5 respectively. As part of the NCS Placenta Consortium, environmental pollutant concentrations of 43 organic pollutants including 10 PBDEs, 32 PCBs, and DDE in more than 300 NCS placenta samples received from seven US geographical locations were determined. The

data were examined to identify the statistical distribution, congener, and homolog compositions, and the variation in chemical concentration among collection sites. The results are presented in chapter 4.

Transplacental transfer of POPs is explored in chapter 5. Twenty-five complete sets of maternal blood-placenta-cord blood (each set coming from a pregnant individual) were collected from the UIC Medical Center. Placenta and blood matrices were analyzed for PBDEs, PCBs, and DDT and its metabolites DDE and DDD. Organochlorine pesticides and novel/alternative halogenated (chlorinated and brominated) flame-retardant (XFRs) compounds were initially screened in all the three matrices to assess the feasibility of detection and quantitation. The data obtained were examined to understand cross-placental transfer of individual chemicals from mother to child.

The concluding chapter is dedicated to summarizing the study findings and delineating the significance of this research with regards to public health policy and practices as well as advances in science.

2. LITERATURE REVIEW

2.1 Persistent Organic Pollutants

Organohalogen Compounds (OHCs) are a group of compounds that are extremely important in the industrial world. They have been extensively used as organic solvents, pesticides, plasticizers, flame-retardants, industrial coolants, ink and dyes, and in many other applications. Slow reactivity, high resistance to transformation, high thermal stability, and low water solubility are some of the industrially favored characteristics of OHCs (Gribble, 2004; Mariussen & Fonnum, 2006). The same characteristics make them persistent and long-lasting in the environment as residues released during or after the initial production or application. Thus most OHCs fall under POP category. Persistent organic pollutants are a subset of persistent, bioaccumulative, and toxic chemicals that are capable of long-range atmospheric transport and deposition, and adversely affecting the environment and human health at locations near and far from their sources (UN-ECE, 1998a). The carbon-halogen bonds in OHCs are highly stable and therefore these compounds are less likely to be reactive. As a result, POPs are highly resistant to degradation processes. They have long half-lives in soil, sediment, and air; over time, concentrations build up as the natural elimination is often slow. The bioaccumulation potential of organic chemicals is often associated with the octanol-water partition coefficient (K_{OW}) and octanol-air partition coefficient (K_{OA}). Those POPs with high K_{OW} (>5,000) and K_{OA} values tend to concentrate in fatty matrices. The presence of halogen atoms prevents the enzymatic degradation of POPs. Lipophilicity and resistance to metabolism ensures steady uptake and storage of POPs, thus resulting in biomagnification in food webs. Bioaccumulation eventually results from the balance between uptake—via dietary ingestion and respiration—and loss—via

growth dilution, respiration, metabolism, and fecal elimination (Campfens & Mackay, 1997; Bartrons et al., 2012). Some POPs are semi-volatiles with vapor pressure between 10^{-4} and 10^{-11} atm at 25°C, and thus have the tendency to enter the gas phase under ambient temperatures. In the atmosphere, POPs partition between the atmospheric gas and aerosol phases. In the absence of breakdown reactions, POPs travel long distances through atmosphere before being redeposited on soil, vegetation, and water. The recurrence of this phenomenon is called the "grasshopper" behavior of POPs and it is responsible for the accumulation of POPs in remote places like the arctic region where they have never been produced (Jones and Voogt, 1999; Bartrons et al., 2012).

The downside of these remarkable industrial chemicals as hazardous pollutants first came to light after the publication of Racheal Carson's *Silent Spring* in 1962. Through years of efforts to control the emission and discharges of POPs into the environment, the Stockholm Convention on POPs entered into force in 2004. As a result, the productions and uses of 12 POPs known as the "dirty dozen" were banned or restricted. By 2013, more than 10 additional compounds (or classes of compounds) have been added by the convention (Stockholm Convention, 2014). These compounds, their original use, and their main adverse health effects are summarized in Table I.

TABLE I

CHEMICALS BANNED (OR WITH RESTRICED USE) BY STOCKHOLM CONVENTION

Chemical	Year banned or restricted	Intended Use
Aldrin	2004	Pesticide
Dieldrin	2004	Pesticide
Chlordane	2004	Pesticide
DDT	2004	Pesticide
Endrin	2004	Pesticide
Heptachlor	2004	Pesticide
Mirex	2004	Pesticide; fire retardant
Toxaphene	2004	Pesticide
PCBs	2004	Insulating fluid; paint additive; lubricant
Hexachlorobenzene (HCB)	2004	Fungicide; additive to fireworks, ammunition, and synthetic rubber
Dibenzodioxins Dibenzofurans	2004	Byproduct of chlorine bleaching by paper mill; combustion; herbicide production
Chlordecone	2009	Pesticide
Lindane (gamma hexachlorocyclohexane)	2009	Insecticide; use to treat lice and scabies in humans
Hexabromobiphenyl	2009	Flame-retardant
Commercial pentaBDE Commercial octaBDE	2009	Flame-retardant
Perfluorooctane sulfonic acid (PFOS) and its salts Perfluorooctane sulfonyl fluoride (PFOS-F)	2009	Nonstick coating; protective coating on carpet and clothing
Alpha and beta hexachlorocyclohexane (HCH)	2009	Insecticide
Pentachlorobenzene	2009	Pesticide byproduct
Endosulfan	2011	Pesticide
Hexabromocyclododecane	2013	Flame-retardant

Modified from Haffner & Schecter, 2014

2.2 Classes of Persistent Organic Pollutants:

2.2.1 Polybrominated Diphenyl Ethers

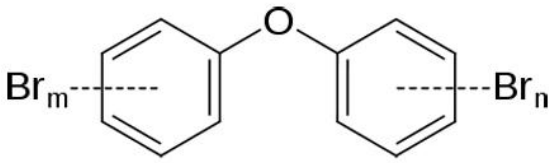


Figure1. Generalized structure of PBDEs (m + n = 1 – 10).

Polybrominated diphenyl ethers are a group of brominated flame-retardants (BFRs). The BFRs represent a major industrial chemical category whose demand has increased dramatically over the past decades. These chemicals are often used in furniture foam; plastics in electronics and computers; wire insulation; drapery and upholstery coatings, TV cabinets, and household appliances; as well as seats and parts in airplanes and vehicles. Flame retardants are helpful in slowing down the ignition and the growth of the flames and therefore are beneficial in mitigating fire damage (USEPA, 2008).

Based on molecular structure, there are ten homologs of PBDEs (mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and decaBDE) depending on the number of bromine atoms attached. The PBDE congeners are named and numbered using the same International Union of Pure and Applied Chemistry (IUPAC) system used for PCBs. Even though theoretically there are 209 BDE congeners, commercially produced PBDE mixtures contain a limited number of congeners, probably because many of them lack stability and tend to debrominate. The PBDEs

were produced in three commercial mixtures: pentaBDE (trade names: DE-60F, DE-61, DE-62, and DE-71); octaBDE (DE-79); and decaBDE (DE-83R, Saytex 102E). The pentaBDE mixtures were primarily comprised of tetraBDEs (especially BDE-47 and BDE-99). The octaBDE mainly was mainly a mixture of heptaBDEs (44%) and octaBDEs (31%–35%) but also contained hexaBDEs (10%–12%) and nonaBDEs (10%–11%). The decaBDE was composed almost entirely of the fully brominated congener, BDE-209 (97%–99%) (USEPA, 2006).

The PBDEs were major industrial products with a total worldwide production of approximately 67,400 metric tons/year (Birnbaum and Staskal, 2004). Of the worldwide production of pentaBDE, 98% was used in North America in 1999; for octaBDE and decaBDE it was 36% and 44% respectively (Birnbaum and Staskal, 2004; EPA, 2006; Hale et al., 2002). By the end of 2004, pentaBDE and octaBDE commercial mixtures were withdrawn from the Asian, European, and US markets, but the decaBDE continued to be manufactured and used through 2013 (Morland et al., 2005; Covaci et al., 2011).

Low water solubility and high affinity to lipids make PBDEs bioaccumulative in the environment. In biological systems, lower brominated congeners (with bromines <7) are more bioaccumulative and persistent than the higher brominated ones owing to the differences in absorption and elimination. Large molecules like BDE-209 are believed to be poorly absorbed and readily eliminated and therefore less likely to accumulate (de Wit, 2002). The PBDEs have been detected in such pristine environments as the arctic region. This suggests that despite their low volatility, PBDEs are capable of long-range transport (Birnbaum and Staskal, 2004). Some important chemical and physical parameters that determine the properties for selected environmentally significant PBDE compounds are given in Table II.

TABLE II

PHYSICOCHEMICAL PROPERTIES OF SELECTED PBDES

Congener	# Br atoms	Molecular Weight	Melting Point (°C)	S_w (mg/L)	Log K_{OA}	log K_{ow}	Vapor pressure(Pa)
28	3	406.9	64	$7x10^{-1}$	9.46	5.53	$2.19x10^{-3}$
47	4	485.8	84	$1.5x10^{-2}$	10.53	6.11	$1.86x10^{-4}$
85	5	564.7	123	$7.86x10^{-5}$	11.66	6.71	$5.11x10^{-5}$
99	5	564.7	92	$9.4x10^{-3}$	11.32	6.61	$1.76x10^{-5}$
100	5	564.7	100	$4x10^{-2}$	11.18	6.51	$2.86x10^{-5}$
153	6	643.6	162	$8.7x10^{-4}$	11.86	7.13	$2.09x10^{-6}$
154	6	643.6	132	$8.7x10^{-4}$	11.93	NA	$3.80x10^{-6}$
183	7	722.5	172	$1.5x10^{-3}$	11.96	7.14	$4.68x10^{-7}$
209	10	959.2	~300	$1.3x10^{-8}$	NA	9.97	$5.42x10^{-11}$

S_w = Water solubility, K_{ow} = n-Octanol-water partition coefficient, K_{OA} =n-Octanol-air partition coefficient, NA = Not Available. (Chen et al., 2003; Tittlemier et al., 2002; Wania and Dugani, 2003)

2.2.2 **Polychlorinated Biphenyles**

Polychlorinated biphenyls are chlorinated hydrocarbons with a general structure as shown in Figure 2. They consist of two connected phenyl rings with chlorine atoms attached to the rings. There are 10 homologs and 209 congeners of PCBs, ranging from one to 10 chlorine atoms at various substitution positions on the two phenyl rings of the molecule.

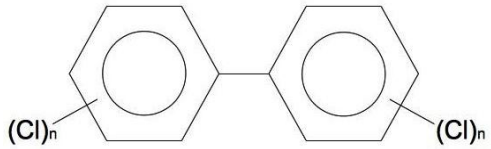


Figure 2. Generalized structure of PCBs (n=1 – 10).

The PCBs are chemically stable compounds that do not react readily with other compounds. They are resistant to degradation, non-water-soluble, and fire resistant. It is because of these attributes that PCBs were remarkable compounds for industrial uses. The chemical properties of the various isomers of PCBs are listed in Table III. The K_{OW} is high for PCBs (log K_{OW} ranging from 4.7–8.3), signifying a strong affinity to lipids. The bioconcentration factor (BCF) in fish is also very high, allowing PCBs to bioaccumulate in fatty tissues with subsequent biomagnification up the food chain.

TABLE III

PHYSICOCHEMICAL PROPERTIES OF THE PCB HOMOLOGS

Homolog Group	MW	# of Isomers	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (Pa)	Water Solubil-ity (g/m^3)	Half-Life* (air, hr)	Log K_{ow}	BCF (13olybro)
Monochlorobiphenyl	188.7	3	25–77.9	285	1.1	4	NA	4.7	2500
Dichlorobiphenyl	223.1	12	24.4–149	312	0.24	1.6	170	5.1	6300
Trichlorobiphenyl	257.6	24	28.87	337	0.054	0.65	550	5.5	$1.6x10^4$
Tetrachlorobiphenyl	292.0	42	47–180	360	0.012	0.26	1700	5.9	$4.0x10^4$
Pentachlorobiphenyl	326.4	46	76.5–124	381	$2.6x10^{-3}$	0.099	1700	6.3	$1.0x10^5$
Hexachlorobiphenyl	360.9	42	77–150	400	$5.8x10^{-4}$	0.038	5500	6.7	$2.5x10^5$
Heptachlorobiphenyl	395.3	24	122.4–149	417	$1.3x10^{-4}$	0.014	5500	7.1	$6.3x10^5$
Ochtachlorobiphenyl	429.8	12	159–162	432	$2.8x10^{-5}$	$5.5x10^{-3}$	17000	7.5	$1.6x10^6$
Nonachlorobiphenyl	464.2	3	182.8–206	445	$6.3x10^{-6}$	$2.0x10^{-3}$	17000	7.9	$4.0x10^6$
Decachlorobiphenyl	498.7	1	305.9	456	$1.4x10^{-6}$	$7.6x10^{-4}$	NA	8.3	$1.0x10^7$

Mackay et al., 2006, Robertson and Hansen, 2001.

Synthesis of PCBs was first published in 1881 (Schmidt et al., 1881) but the production for industrial applications did not begin until 1929 (Cairns et al., 1981; Risebrough et al., 1970). Monsanto was the primary PCB manufacturer in the United States and is estimated to have produced around 60% of total worldwide PCBs (ATSDR, 2010). Aroclor was the trade name used by Monsanto to market different PCB mixtures. The General Electric Company later joined the production and marketed PCB under the name "Pyranol" (Risebrough et al., 1970; ATDSR, 2010; Robertson and Hansen, 2001). Owing to its excessive inertness and heat capacity, PCB was used in numerous industrial applications as coolants, insulators, and lubricants and in products such as electrical transformers and capacitors, in paints and pigments, as plasticizers, fluorescent lighting fixtures, cable insulation, adhesives, floor finishes, copy paper, caulking, hydraulic oils, and microscope oil (Kutz et al., 1991, ATSDR, 2000).

The degree and position of substitution has a major influence on physicochemical properties, such as lipophilicity, volatility, water solubility, biodegradability, as well as toxicity (de Wit, 2002). Twelve of the 209 PCB congeners share certain toxicological properties with the PCDD/F and have been regarded as dioxin-like PCBs (e.g., non-ortho substituted: CB-77, -81, -126, -169, mono-ortho substituted: CB-105, -114, -118, -123, -156, -157, -167, -189). These PCBs have been assigned the toxic equivalents (TEQs) based on 2,3,7,8-TCDD, but they are at least an order of magnitude less toxic than 2,3,7,8-TCDD (Van Oostdam et al., 2004). Dioxin-like PCBs are of high toxicity but their presence in the environment is at trace levels (Voogt et al., 1990; Baars et al., 2004).

2.2.3 **Pesticides**

Organochlorine pesticides are chlorinated hydrocarbons used extensively from the 1940s through the 1960s in agriculture and mosquito control. Included in the list that is commonly referred as the dirty dozen or "black list" are Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor,

HCB, Mirex, and Toxaphene. Uses of many of these pesticides have been cancelled or restricted because of their environmental persistence and potential adverse effects on wildlife and human health (Table I) by the Stockholm Convention as well the US Environmental Protection Agency (EPA).

Aldrin is readily converted to dieldrin in the environment and in plants that take up the chemical. Both persist in the environment and bioaccumulate in biota (Jorgenson, 2001; USGS, 2007). Chlordane and heptachlor are structurally related OCPs. They have been used in the United States until the mid-1980s to control termites, fire ants, and other insects in soil, crops, and buildings (ATSDR, 2002). Technical-grade chlordane had contained 7% *trans*-nonachlor. *Cis*-chlordane and *trans*-chlordane are two predominant isomers in technical chlordane, mixed with many other side products. The most commonly detected and abundant organochlorine pesticide in various environmental and human samples is DDT. More than 70% of DDT formulation is comprised with p,p'-DDT (4,4'-(2,2,2-trichloroethane-1,1-diyl)bis(chlorobenzene). Its major biodegradation products and metabolites p,p'-DDE (1,1-bis-(4-chlorophenyl)-2,2-dichloroethene) and p,p'-DDD (1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl)ethyl]benzene) are also of high persistency and considered as POPs. Hexachlorocyclohexanes (HCHs) are also deemed as persistent toxic substances and are generally accepted as POPs. Technical HCH is constituted by 67%–70% of α-HCH isomer, 13% of γ-HCH isomer, 6% of δ-HCH isomer, 5%–6% of β-HCH isomer and traces of ε-HCH, λ-HCH, and ν-HCH isomers (Fabre et al., 2005). It has insecticide properties because of the γ-HCH isomer, which is commonly referred to as Lindane. This isomer has the highest acute mammalian and insecticidal toxicity, and is used extensively for agricultural and public health purposes in many developing countries. Hexachlorobenzene was used from the 1930s to the 1970s in the United States primarily as a fungicide and in seed treatment. It has been detected in soil, air,

water, and sediment (Barber et al., 2005). It is a persistent chemical and bioaccumulates in both aquatic and terrestrial food chains (ATSDR, 2002).The US EPA cancelled its use in 1984.

2.3 Environmental Release and Occurrence

Persitent organic pollutants have become major global pollutants that are ubiquitously present in the environment. Studies have found these chemicals in almost all environmental matrices such as soil, sediment, sewage sludge, water, indoor and outdoor air, fish and other wildlife, as well as in humans (Hites, 2004; Hale et al., 2003; Darnerud et al., 2001; Stapleton et al., 2005; Song et al., 2004).

First introduced in the 1970s, PBDEs were first detected in the environment in 1979 and in biota in the 1980s (EPA, 2006). Flame-retardants are added to products either as an additive or a reactant. Reactive flame-retardants form covalent bonds and become a part of the polymer matrix. Additive flame-retardants, such as PBDEs, are mixed with the polymer and are not strongly bound to the polymer. Consequently they are more readily released from the product with time (Sjödin et al., 2003), and could also be released to the environment from PBDE manufacturing and processing plants (USEPA, 2008). In addition, recycling, land filling, and incineration of PBDE-containing products can release these chemicals into the environment. While in the environment, higher BDE congeners (e.g., BDE-209) can undergo reductive debromination, thereby resulting in increased concentrations of low brominated congeners in the environment (Stapleton et al., 2004b; Shenker et al., 2008; Kim et al., 2014). The congener BDE47 tends to be found more frequently than other congeners in measurements from humans, fish, and other biota, followed by BDEs -99, -100, -153, and -154. In measurements of house dust, sediments, and indoor air, BDE-209 seems to be dominant (EPA, 2006).

Massive use of PCBs resulted in ubiquitous environmental contamination that was not identified until 1966. Due to concerns over the toxic potential of PCBs, manufacturing was voluntarily suspended in 1977 in the United States. The US EPA began regulating the disposal of PCBs in 1978 and banned their production in 1979 (EPA, 1979). However, PCBs are still released into the environment from different sources such as poorly maintained waste sites that contain PCBs, illegal dumping of PCB wastes, or by the burning of some wastes in municipal and industrial incinerators. A recent study of building sealants in Canada found measurable levels of PCBs in 14% of the buildings tested (Robson et al., 2010). An inventory of all PCBs in Toronto identified 437 metric tons of PCBs, mostly in closed sources such as insulating fluids and oils. This is most likely an underestimate due to the unknown amounts of PCBs in waste facilities and other minor products (Robson et al., 2010). Models estimate up to 880 kg of PCBs could be emitted from the city of Toronto alone each year from known sources (Diamond et al., 2010). The environmental release of pesticides mainly occurs through applications of pesticides. They can also be released during manufacturing and from the landfills contaminated with pesticides (CDC, 2009). As of 2008, quantities of DDT were still being produced in India, China, and the Democratic People's Republic of Korea. In 2007, India alone produced more than six thousand metric tons of the pesticide for export and for local use to control diseases and mosquitoes (van den Berg, 2008). Multiple studies have shown that the levels of several POPs found in the environment, human fat, maternal and cord bloods, and breast milk have decreased after they were phased out (Daucet et al., 2009). With the effect of usage restrictions the serum organochlorine levels in the US population and other developed countries have decreased (Hagmar et al., 2006; Kutz et al., 1991). According to the Food and Drug Administration, HCB levels in food are decreasing. In serum subsamples of the National Health and Nutrition Survey (NHANES) 2001–2002 and 2003–2004, Aldrin levels were below the

limit of detection. Since the 1970s, mean serum levels of DDT and DDE in the US population declined by about fivefold to tenfold (CDC, 2009). But unlike PCB and OCP, a steady increase has been reported for the levels of PBDEs found in the environment as well as in human tissues between 1980 and early 2000 (Turyk et al., 2010; Akutsu et al., 2003, de Wit et al., 2006; Li et al., 2006; Lorber, 2008; Pereg et al., 2003; Schecter et al., 2005). A meta-analysis in 2004 by Hites showed a 100-fold increase in the PBDE concentrations in people during the last 30 years. It also showed that PBDEs have an exponential increase with a doubling time of about five years in humans. Comparison of PBDE concentrations in humans from different countries revealed that the PBDE levels in the US population is approximately 20 times higher than that of European populations (Hites, 2004). In Europe the samples collected during 1997-1998 period showed the highest concentrations of PBDEs. After that the levels were decreased or leveled off. The reason for this observation may be the imposed restrictions and bans on the manufacture, distribution and use of PBDE in the European Union which happened rather earlier compared to that of North America (Darnerud et al., 2002; Law et al., 2006, 2008; Doucet et al., 2009). Likewise, in the United States, especially after the removal of pentaPBDE from the market in 2004, a decreasing levels of PBDE in the environment have been observed by the studies conducted after 2010 (Zota et al., 2013; Stapleton et al., 2012; Dodson et al., 2012; Sjödin et al., 2013).

2.4 Human Exposure and Toxicity

The general population may be exposed to POPs through diet, primarily dairy products, fish, and meat. Infant and fetal exposures occur through breast milk and contaminated maternal blood flow via the placenta. Many studies have reported that POPs such as PCB, DDT, and PBDE as well as their metabolites (DDE, OH-PCH, and OH-PBDE) cross the placenta and reach the fetus (Gómara et al., 2007, 2011).

Human exposure to Aldrin occurs through the diet, inhalation (in places with Aldrin application history), and dermal application. After absorption, Aldrin is rapidly metabolized to dieldrin and rarely detected. Dieldrin accumulates in fatty tissues, and its metabolites are excreted in bile and feces (ATSDR, 2002). It is also excreted in breast milk and can cross the placenta. The elimination half-life of dieldrin is approximately one year (IPCS, 1989; Jorgenson, 2001). Hexachlorobenzine is well-absorbed after oral administration, distributes widely throughout the body, and accumulates in fatty tissues where it persists for years. It is slowly metabolized, and elimination occurs by renal and fecal routes and breast milk. Upon metabolizing, HCB produces pentachlorophenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol, causing secondary exposure (CDC, 2009). Hexachlorobenzine has been classified as possibly carcinogenic to humans by IARC and as "reasonably anticipated to be a human carcinogen" by National Toxicology Program (NTP). Human health effects of chronic low environmental exposures to Aldrin and dieldrin are unknown but acute exposure effects are caused by obstruction of inhibitory neurotransmitters in the central nervous system (Narahashi et al., 1992). Prenatally exposed rodents to dieldrin showed changes in neurotransmitter levels (Sanchez-Ramos et al., 1998) and behavioral changes (Carlson and Rosellini, 1987). Findings are not conclusive of the studies on the estrogenic effect of dieldrin and the association of dieldrin exposure and the onset of Parkinson's disease (Soto et al., 1995; Tully et al., 2000; Corrigan et al., 2000; Kanthasamy et al., 2005; Li et al., 2005). Only a small proportion of DDT is metabolized and excreted; and both DDT and DDE can cross the placenta, resulting in fetal exposure and are excreted in breast milk resulting in infant exposure (Rogan et al., 1986). In laboratory animals, both DDT and DDE are considered as inducers of cytochrome P450 isozymes. Estrogenic and anti-androgenic effects of DDT, o,p'-DDD and p,p'-DDE have been demonstrated (Gray et al., 2006). Animal studies reported reduced fertility, premature delivery, reproductive

organ abnormalities, and altered behavior after neonatal exposure (Gray et al., 2006). High maternal DDE levels may be a risk factor for preterm delivery (Longnecker et al., 2001).

In general, POPs are metabolized by enzymes like cytochrome p-450. PCBs, like many other POPs, are metabolized resulting more polar hydroxylated PCBs (OH-PCBs). These metabolites are removed from the body via feces or urine (Gómara et al., 2011). According to some studies, PCB metabolites also have the capacity to bind to proteins in the plasma (Sandau et al., 2000). In addition to that hydroxylated PCBs have the ability to interact with hormone receptors and interfere with hormonally induced responses such as thyroxin transportation (Mortensen et al., 2007; Braathen et al., 2009). Therefore in establishing the overall toxicity of POPs both the parent compounds as well as their metabolites are needed to be taken into consideration (Gómara et al., 2011, Gutleb et al.2010).

Since PBDEs are present in many critical exposure media, including various foods, indoor air, and house dust, it is assumed that the general population is exposed to these chemicals via inhalation, food ingestion, dust ingestion, and dermal absorption when in direct contact with the products containing PBDEs (Jones-Otazo et al., 2005; Bocio et al., 2003; Bradman et al., 2007; Julie et al., 2007). The PBDEs are primarily indoor air pollutants and higher levels have been found inside homes than in the outside environment (Schecter et al., 2005). This may be due to the migration of PBDEs from products, including furniture and carpet foam and plastics in televisions and computers. Occupational exposure to PBDEs has also been studied. The study populations mostly included computer and electronic maintenance personnel (Sjödin et al., 1999; Jakobsson et al., 2002). Elevated levels of PBDE have often been detected in the occupationally exposed group compared to the controls.

Rodent data indicate that the tetraBDEs and pentaBDEs are well-absorbed and highly distributed to fatty tissues. They are slowly metabolized and poorly eliminated (Hakk and Letcher, 2003; Staskal et al., 2005). In contrast, decaBDE is poorly absorbed with more than 90% of the dose excreted within two days (Mörck et al., 2003). Available data suggest that the acute toxicity of PBDEs is low with no severe toxicities observed in subacute and sub-chronic toxicity studies. Studies conducted in rodents have demonstrated higher hepatotoxic potential of commercial mixtures of octaBDE and pentaBDE compared to decaBDE (Zhou et al., 2002). Developmental neurotoxic effects have been found in rodent studies where exposure to individual PBDE congeners (e.g., BDEs 47, 99, 153, and 209) have been tested (Eriksson et al., 2001; Branchi et al., 2002; Birnbaum and Staskal, 2004; Viberg et al., 2003; Alm et al., 2006). Also PBDEs have been observed to have an effect on thyroid function (primarily observed as T4 hypothyroidism). Exposure to commercial pentaBDE reduced the serum levels of the thyroid hormone T4 (Stoker et al., 2005; Zhou et al., 2002).

Hydroxylated BDEs have been shown to inhibit estrogen sulfotransferase, leading to an apparent estrogenic effect (Kester et al., 2002). Male and female rats exposed to the commercial pentaBDE mixture DE-71 exhibited delayed pubertal development (Stoker et al., 2005). Daily sperm production and spermatid counts were significantly decreased in the rat offspring of dams exposed to BDE-99 in single doses of 60 and 300 μg/kg (Kuriyama et al., 2005).

In carcinogenicity studies with decaBDE at high doses, increased incidences of hepatocellular and thyroid adenomas and carcinomas were observed in mice and increased incidences of hepatocellular adenomas and acinar cell adenomas of the pancreas were observed in rats. Based on two-year cancer bioassay results, the EPA has classified decaBDE as a possible human carcinogen (EPA, 2006).

2.5 Prenatal and Neonatal Exposure

In addition to the toxicity of a xenobiotic, there are multiple factors which determine the magnitude and the impact of the exposure to that agent. Not only the frequency, duration, and the pathways of these exposures, but also the life stage at which this exposure occurs are of fundamental importance. Researchers believe that the most susceptible time period for environmental exposures is in utero through two years of age, especially for some neurobehavioral outcomes (Needham et al., 2005). Conditions associated with premature births and birth defects are among the leading causes of infant death. In 2001, a considerable proportion of children in the United States were born with some type of developmental disorder or chronic health problem. Although the exact causes for these events are unknown, it is reasonable to speculate some linkage to environmental contaminants (Needham et al., 2005). Fetuses as well as infants are considered as sensitive subpopulations because at these stages rapidly developing brain and other organ systems are susceptible to effects of chemical exposures (Howdeshell, 2002). During their development humans may be exposed to toxicants via maternal circulation as fetuses, via breast milk as infants, and by involuntary ingestion as children and adults. Therefore fetal and neonatal exposure can be characterized by measuring the toxicant levels in maternal circulation, placental and cord blood, and breast milk.

The levels of POPs in placenta and cord blood are directly related to the prenatal exposure (Julie et al., 2007). Majority of the prenatal exposure studies have used cord blood measurements. Only a very limited number of these studies have looked into the placental levels. One of the major issues debated in these studies is the cross-placental transfer. For example some have found comparable levels of PBDEs in maternal and fetal circulation favoring complete transfer through placenta (Mazdai et al., 2003; Gómara et al., 2007), while others have found no or low levels of

PBDEs in fetal circulation, suggesting blocking or partial blocking of PBDE transport through the placental barrier (Guvenius et al., 2003; Hirai et al., 2004; Takasuga et al., 2006). Placental resistance to higher brominated congeners has also been discussed in some studies (Guvenius et al., 2003; Schecter et al., 2007; Gómara et al., 2007). Different BDE congeners may cross the placenta at different rates depending on the degree of bromination, lipid solubility, capacity for bioaccumulation, and the lipid level of the placenta (Gómara et al., 2007). The exposure to POPs in utero has been linked to adverse effects on fetuses, including intrauterine growth retardation, neurocognitive deficits, and hormonal and intellectual dysfunctions (Rogan et al., 1986; Jacobson et al., 1990; Jacobson and Jacobson, 1996; Berkowitz et al., 2004; Eskenazi et al., 2009). Exposure to PCBs during early fetal development can cause brain injury at doses much lower than those affecting adult brain functions and an inverse relation between maternal PCB concentrations and cognitive performance in Dutch children at 42 months and 7.5 years of age (Sioen, 2013). Early life exposure to DDT and DDE has been associated with lowered birth weight and shortened gestation, poorer neurodevelopment in children, and breast cancer in adults (Cohn et al., 2007; Al-Saleh et al., 2012; Kezios et al., 2013; Eskenazi et al., 2009). Prenatal exposure DDE is associated with allergy susceptibility biomarkers and increased incidence of asthma in children (Brooks et al., 2007). Eskanazi et al. (2013) found significant associations between both maternal prenatal and childhood PBDE exposures resulting in deficits in attention, fine motor coordination, and cognition in early school-age children.

Disruptions in maternal and fetal thyroid homeostasis can result in neurologic impairment, including developmental delays and decreased IQ in children of mothers with small reductions in thyroid hormone T4 (Pop et al., 2003). Chevrier et al. (2008) observed a negative association between the sum of PCB congeners and free thyroxine as well as between HCB and either free

thyroxine or total thyroxine concentrations in pregnant women. With the exposure to commercial pentaBDE, reduced serum levels of the thyroxine have been observed (Stoker et al., 2005; Zhou et al., 2002). Recent studies have focused on possible obesogenic effects of endocrine-disrupting compounds such as POPs. Experimental evidence suggests that DDT and DDE have the ability to promote some aspects of adipose dysfunction (Warner et al., 2013). Early-life exposure to these chemicals might alter development of adipose tissue structure or it may affect the systems involved in weight homeostasis that can result in obesity as the child ages (Grun and Blumberg, 2009). Positive associations have been reported between prenatal DDE and DDT exposure and overweight status for children with follow-up periods ranging from 14 months to 6.5 years (Mendez et al., 2011; Valvi et al., 2012; Verhulst et al., 2009).

2.6 Alternate (Novel/Emerging) Flame-Retardants

Before 2004, PBDEs were the major brominated flame-retardants used commercially. But the restriction on the use of PBDEs has led to the synthesis of new chemicals that have the potential to be used as flame-retardants (Covaci et al., 2010; Stapleton et al., 2009; Eskanazi et al., 2013). These chemicals are referred to as “alternate,” “new,” “emerging,” “current-use” or “non-PBDEs” or “novel” flame-retardants (Covaci et al., 2010). A large portion of the flame-retardants are brominated ones. About 75 different formulations containing bromine are commercially produced as flame-retardants (Alaee, 2003).

Ethylene bis(tetrabromophthalimide) (EBTBP), decabromodiphenyl ethane (DBDPE), hexachlorocyclopentadienyldibromo-cyclooctane (HCDBCO), 1,2-bis(2,4,6-tribromophenoxyethane (BTBPE), tetrabromobisphenol A-bis(2,3-dibromopropylether) (TBBPA-DBPE), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB or EHTBB) and bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (TBPH / BEHTBP) are examples for some alternative flame retardants

(Covaci et al., 2011). The chemical structures of some BFRs are given in Figure 3. Chemical information regarding production volume, physicochemical properties, persistence and current level in the environment, human exposure, and toxicity and health concerns are largely unknown. Additional research is needed to determine the potential child health consequences of these alternate chemical flame-retardants (Eskanazi et al., 2013). Some studies have reported on the atmospheric transport and the environmental release and existence of a number of alternative flame retardants (Shi et al., 2009; Harju et al., 2009; de Wit et al., 2010). A summary of physicochemical properties is available in Covaci et al. (2011). Similarity to PBDEs is noticeable with regard to volatility, solubility, and lipophilicity and therefore the potential of becoming POPs.

All alternative BFRs are additive-type flame retardants used in various consumer products and are leached into the environments with the aging of these products. Dust generated during car dissembling and metal recycling has been found to be high with BFRs such as BTBPE, DBDPE, and TBBPA-DBPE (Nyholm et al., 2013; Harrad and Abdallah, 2011). Some alternate BFRs like BTBPE and TBBPA-DBPE which are with the potential to bioaccumulate and to biomagnify have been reported to be present in aquatic biota and birds' egg samples (Law et al., 2006; Tomy et al., 2007; Gauthier et al., 2009; Howard and Muir, 2010; Wu et al., 2011; Nyholm et al., 2013).

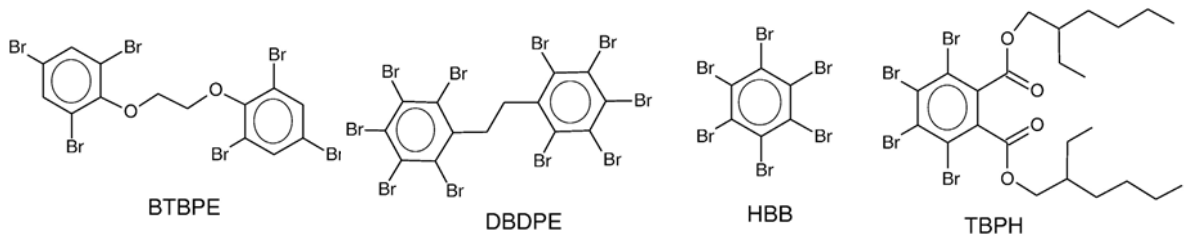


Figure 3. Chemical structures of some "novel" brominated flame-retardants.

2.7 Human Placenta

2.7.1 Morphology and Function

The placenta is an organ that provides the embryo and fetus with nutrients and serves as a mean of elimination of excretory products. The placenta grows exponentially during gestation, from an average of 6 grams at third week of pregnancy to 470 grams at term. The usual term placenta is about 22 cm in diameter and 2.0 to 2.5 cm thick. The maternal surface of the placenta is dark maroon in color while the fetal surface is shiny and gray. Maternal surface is divided into lobules or cotyledons (Yetter, 1998). The placenta is composed of a chorionic shell, off which project tiny hair-like projections (villi), finger-like outgrowths of placental epithelium (trophoblast) and blood vessels with their supportive connective tissue stroma.

The extensions called villi are formed in the placenta and are extended into the uterine wall (figure 4). Maternal blood circulates through the spaces between villi. Fetal blood vessels are formed within the villi. The two circulation systems are separated from each other and normally there is no mixing of fetal and maternal blood within the placenta (Myren et al., 2007).

The functions of the placenta in all species is to be an anchor for attachment, control the maternal environment through release of hormones—e.g., human chorionic gonadotropin, human placental lactogen, progesterone, estradiol—and be a conduit for selective transport of nutrients (oxygen, glucose, amino acids) and waste products (lactic acid, carbon dioxide, bilirubin) between mother and fetus. Such transport is facilitated by the close approximation of maternal and fetal blood supplies within the placenta—e.g., immunoglobulins and vitamin B_{12} (Schneider and Miller, 2010). In addition to transporting some molecules unaltered between fetal and maternal blood, it also consumes a large fraction of certain types of molecules like glucose and oxygen and produces large amounts of lactic acid. Additionally, a number of molecules that cross the placenta are metabolized during passage. Some molecules (e.g., heparin) are not transported at all and the placenta behaves as a barrier for these molecules.

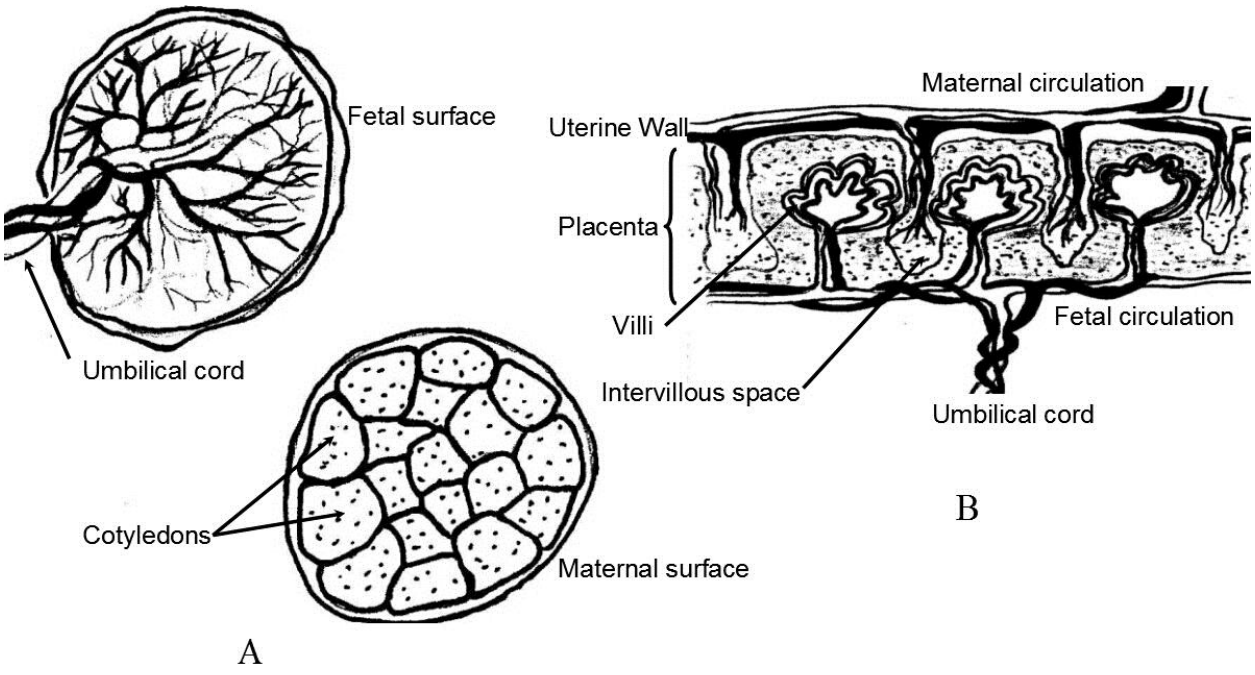


Figure 4. Schematic diagram of human placenta.

A- The maternal and fetal surfaces and the placement of placenta within the uterus
B- Fetoplacental and maternal circulation in placenta

2.7.2 **Transplacental Transfer of Environmental Chemicals**

Pregnant women are exposed to a wide variety of exogenous chemicals. These exposures are commonly due to maternal medication; lifestyle factors such as smoking, drug abuse, and alcohol consumption; or occupational and environmental sources. Concentrations of foreign chemicals in placental tissue have been used to evaluate exposure caused by occupational and environmental sources or maternal smoking (Myllynen et al., 2005; Zadorozhnaja, 2000). During pregnancy, human placenta is an important endocrine organ that synthesizes both steroid and protein hormones as well as carrier proteins for nutrients such as Transcobalamin II for vitamin B-12 (D'Gregorio, 1998). Many environmental pollutants (e.g., pesticides, PCBs, and dioxins) are endocrine disruptors, have the potential to interfere with the hormonal balance in placenta. Some environmental toxicants such as cadmium have been shown to bioaccumulate in placenta (Falcon et

al., 2002) while some are readily cross-placenta and enters the fetal circulation. The thalidomide catastrophe in the 1960s made it evident that chemical compounds can cross the placenta and give rise to serious developmental toxicity. Diethylstilbestrol is a human carcinogen that is transported to the fetus through the placenta and selectively bound to fetal plasma proteins (Miller et al., 1982; Henry, 1986). Recently, it has been shown that environmental pollution may cause mutations in transplacentally exposed fetuses in humans (Myllynen et al., 2005). Chemicals cross the placenta mainly by simple passive diffusion. Other possible mechanisms of placental transport are facilitated diffusion, active transport, pinocytosis, and filtration. The important properties determining the placental transfer by passive diffusion are molecular weight, dissociation (P$k_{a)}$, lipid solubility, and protein binding. Many chemicals serve as ligands for transporter proteins, which facilitate the chemicals crossing through biological membranes (Myllynen et al., 2005; Schneider, 2010). The placental transfer rate varies from chemical to chemical and the effectiveness of the placental barrier is not similar for every compound. For instance, placenta is a weaker barrier for lead than for cadmium because cadmium is bound to sulfhydryl groups (metallothionein) (Breen et al., 1994). The organic form of mercury readily passes through the placenta (Iyengar and Rapp, 2001). Cross-placental transfer of lipophilic organic pollutants like PCBs from mother to fetus have been well-documented (Wang et al., 2006; Chen et al., 2001; Grabic et al., 2006; Jacobson, 1984).

3. MATERIALS AND METHODS

3.1 Laboratory Method Development for Chemical Analysis

3.1.1 Background

Matrix solid phase dispersion (MSPD) was developed in the late 1980s for the extraction of solid and semisolid samples. The principle of this method is to disrupt and disperse the sample in a solid phase sorbent that is present in excess quantity. The basic procedure, which comprises three major steps, is extensively illustrated by Barker (Barker, 2000). The first step involves sample grinding in the presence of excess amounts of sorbent. Then the ground mixture of sample and sorbent is loaded onto a chromatographic column, followed by elution with a suitable solvent. The quality of the MSPD performance depends on multiple factors, particularly the sorbent type and extraction solvent. A careful selection of a combination of factors specific for the analyte and the sample matrix is critical. The advantages of MSPD include its simplicity, efficacy, low cost, and the possibility of simultaneous extraction and cleanup. It has been successfully used to extract trace-level organic pollutants, including PBDEs, from different environmental and biological matrices (Martinez et al., 2005; Gómara et al., 2006, 2007; Kristenson et al., 2006)

An experiment was designed to optimize MSPD method to extract PBDEs from human placenta. The factors considered for the optimization included testing different sorbents, sample conditions (wet, dry), grinding methods (wet grinding and dry grinding), elution solvents, and single and repeated extraction procedures. These factors were tested separately and the best performers in each test were combined to make up the optimized method. The efficiency of the optimized MSPD method for extracting 43 PBDEs was compared to two other widely used extraction methods—i.e., liquid extraction and Soxhlet extraction—by extracting placenta samples obtained from a local

hospital. Validation of the optimized MSPD method was conducted by analyzing matrix spike samples and a fish tissue standard reference material (SRM) with certified PBDE concentrations.

3.1.2 Materials and Chemicals

A standard mixture of 39 PBDEs (BDEs 1–3, 7, 8, 10–13, 15, 17, 25, 28, 30, 32, 33, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 116, 118, 119, 126, 138, 153–155, 166, 181, 183, 190), PCB-204 and decabromobiphenyl (BB-209) were purchased from AccuStandard (New Haven, Conntecticut). Individual PBDE standards (BDEs 28, 47, 66, 85, 99, 100, 153, 154, 183, 196, 206, 207, 209) and ^{13}C-labeled BDE-118 (BDE-118L) were purchased from Cambridge Isotope Laboratories (Andover, Massechussettes). Analytical grade hexane, dichloromethane (DCM) and acetone were purchased from Fisher Scientific. Methyl tert-butyl ether (MTBE) with 99% purity (Acros Organics) was also purchased from Fisher. Bio-beads S-X3 (200–400 mesh) were purchased from Bio-Rad Laboratory (Richmond, California). Bondesil C18 (40 μm) was purchased from Varian Inc. (Palo Alto, California). Anhydrous sodium sulfate, silica gel (100–200 mesh, Davisil Grade 644) and Florisil (60–100 mesh) were purchased from Fisher Scientific. Before use, the sorbents, silica gel, and sodium sulfate were cleaned by washing with acetone, DCM, and hexane and dried overnight in an oven at 150°C. A standard reference material (Lake Michigan fish tissue SRM 1947) was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland). It was stored in a -80°C freezer upon receiving.

3.1.3 Placenta Collection and Pretreatment

Full-term human placenta samples (N=5) were collected from five pregnant women who were admitted to the UIC Medical Center for child delivery. They were asked to sign a written consent form approved by the university's Institutional Review Board before donating their placentas. Collected placentas were immediately stored in a freezer at -20°C. Before extraction,

excess blood from the placenta was wiped and the umbilical cord and other connective tissues were removed. Then, the placenta was cut into small pieces and homogenized in a commercial blender and the homogenate was kept in the freezer. Part of the homogenate was dried using a freeze-dryer (Freezone 4.6L, Labconco, Kansas City, Missouri). Freeze-dried samples were stored in tightly closed amber glass bottles and kept in a desiccator.

3.1.4 **Optimization of Extraction Conditions**

The MSPD method generally has the following steps. Prepare the sample, grinding the sample with a suitable sorbent, packing the sample-sorbent mixture on to a column, and extract the sample using an appropriate solvent. For the optimization, the MSPD extractions were conducted by applying different conditions at each step. Each step was tested sequentially one after the other. To investigate which form of sample is best, fresh sample extraction was compared against freeze-dried sample extraction. Florisil, C18, and silica gel were tested to isolate the best sorbent. Two grinding techniques were tested for blending of the sample with the sorbent: (1) Wet grinding with Sample + Sorbent + 20 mL acetone / hexane (3:1) mixture, and (2) Dry grinding with Sample + Sorbent only. Four types of solvent and solvent mixtures were compared for extraction efficiency: hexane 100%, hexane acetone mixture in 8:2 ratio, hexane DCM mixture in 8:2 ratio, and hexane, acetone, and DCM mixture in 4.5 :4.5:1 ratio. At the end of testing these factors, the effect of reflux was tested by repeating the extraction from one to four times using 100 mL of solvent (e.g., x1= one time extraction using 100 mL, x2 = extraction repeated twice using the same 100ml, x3 = extraction repeated 3 times using the same 100ml)

One of the major steps in the development of the MSPD method was to select the sorbent for sample dispersion. In this study, three sorbents including silica gel, C18, and Florisil were compared, all at a sample-to-sorbent ratio of 1:2 (dry weight). In the literature, ratios varying from

1:1 to 1:4 have been used (Kristenson et al., 2006). For dry samples, the 1:2 ratio was found to be sufficient for proper dispersion of samples, and no improvement in extraction efficiency was observed upon increasing the sorbent amount higher than twice the sample amount (Valsamaki et al., 2006). In this work, the highest PBDE extraction efficiency was observed for C18; while the extraction efficiency using Florisil and silica gel was 73% and 67%, respectively, of that using C18 (Figure 5).

Surrogate recovery for both C18 and Florisil were similar (>92%), but it was lower (60%) for silica gel. However, coextracted lipid was also higher for C18, thus mandating an additional gel permeation chromatography (GPC) procedure for lipid removal. Method blanks revealed that the C18 sorbent has the highest level of contamination, as seen from the elevated baseline and unidentified peaks appearing in the gas chromatography/mass spectrometer (GC/MS) total ion chromatogram, even after intensive washing with solvent prior to use. In addition, C18 is the most costly among the three sorbents.

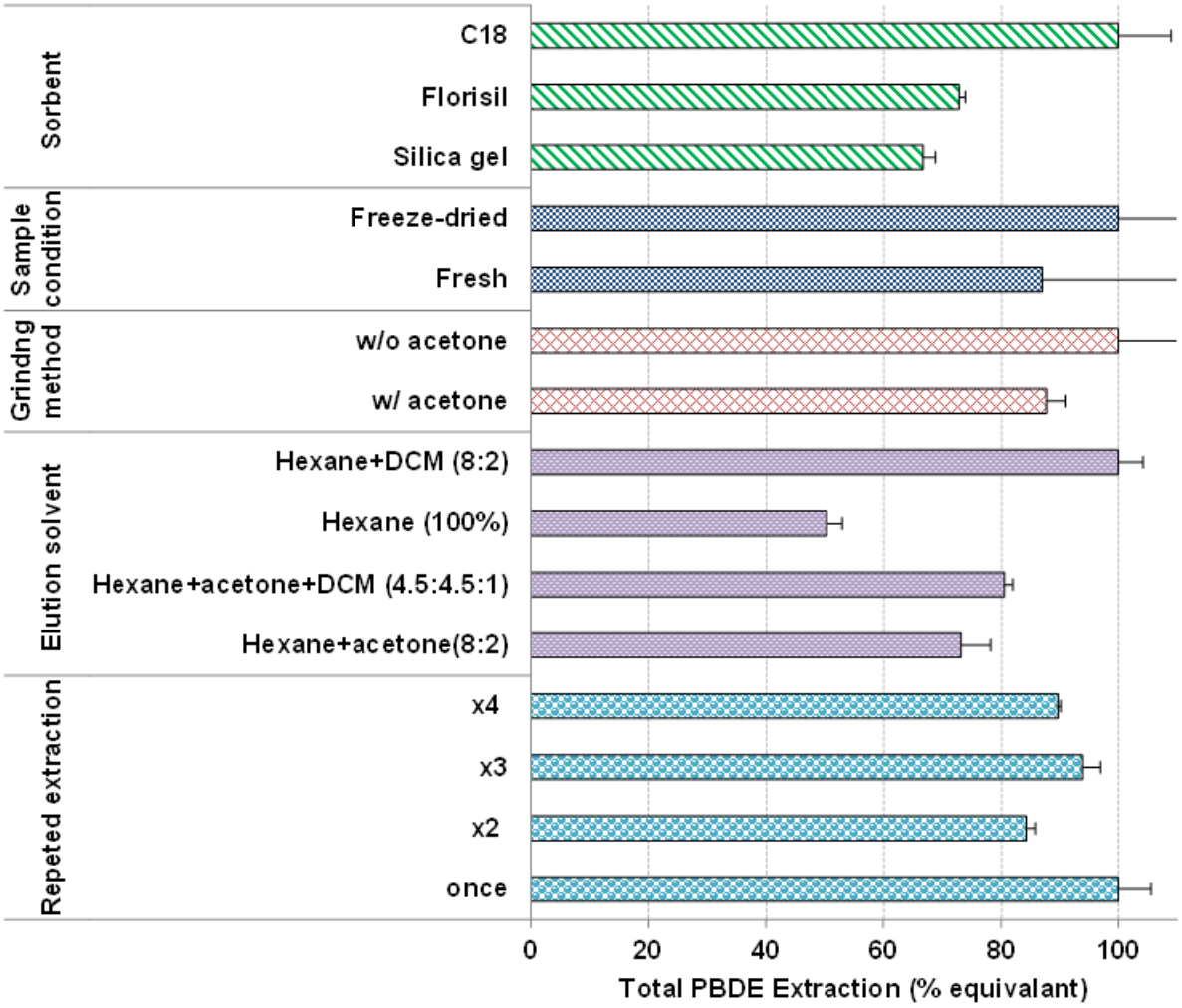


Figure 5. Relative extraction efficiency of total PBDEs under different conditions. The error bar represents one standard deviation of the duplicated analyses.

Based on the results and these considerations, Florisil was chosen as the dispersion sorbent. Compared with C18, Florisil may provide similar or even better abrasive shearing forces in the disruption of the tissue architecture, due to its larger particle size and sharp edges. As magnesium silica gel, Florisil particles may have a more polar surface than the C18-derivatized silica (Barker, 2000). Florisil has also been successfully used to extract environmental pollutants such as PCBs and

pesticides with more than 70% recovery (Li et al., 2003; Morzycka, 2002; Gomez-Ariza et al., 2002; Albero et al., 2001).

The effect of the sample's moisture condition (wet versus dry) on the extraction efficiency of the MSPD method was tested. Two aliquots of 20 g wwt from a homogenized placenta sample were taken and one was extracted as a wet sample and the other was freeze-dried before the extraction as a dry sample. Both were extracted using Florisil as the sorbent in 1:2, sample-to-sorbent ratio. Surrogate recovery was similar for both samples, and the loss of any congener due to freeze-drying was not detected. Lipid extraction appeared to be higher for the dry sample (0.08 g lipids/100 mL solvent) compared to the wet one (0.05 g lipids / 100 mL solvent). Upon comparing the total PBDE concentrations in the extracts of the two samples, the freeze-dried sample was slightly higher than that of the fresh one. But the analytical results were not significantly different from each other (Figure 5). Additionally, freeze-dried samples were convenient in handling, and consumed less amount of sorbent. Also with freeze-drying, the ability to treat a larger quantity of a sample if needed is another great advantage.

Dry grinding of the sample sorbent mixture was tested against the solvent-assisted "wet" grinding. In wet grinding, 20–40 mL of acetone:hexane (3:1) mixture was added to the freeze-dried sample prior to grinding in the glass mortar. In dry grinding, the freeze-dried sample was ground with the sorbent without adding solvent. As shown in the Figure 6, the effect of the grinding method appears to be insignificant on extracted level of PBDEs. However, in contrast to PBDE extraction, the lipid extraction using wet grinding was fivefold higher than using dry grinding (0.125 versus 0.025 g lipids per 100 mL solvent). This suggests that more efficient lipid extraction does not necessarily produce higher PBDE levels in the extract. The polar solvent acetone can denature the protein structure and thereby promote the extraction of polar lipids (phospholipids and cholesterol).

However, highly hydrophobic compounds such as PBDEs may be more associated with nonpolar lipid (triglycerides) in placenta and other biological matrices.

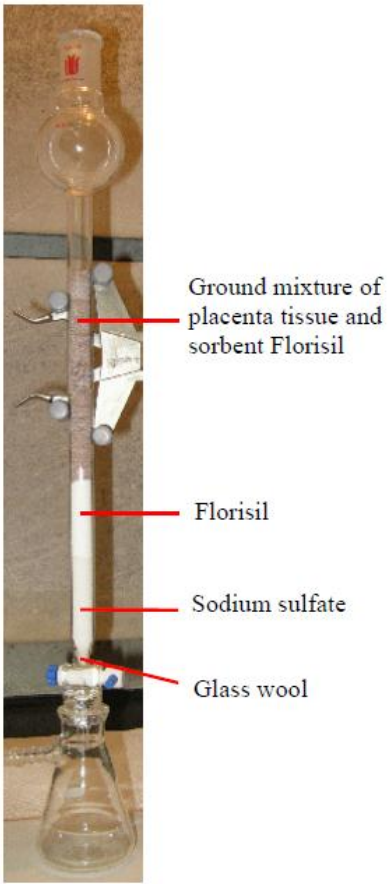


Figure 6. Packed MSPD column.

Selection of a suitable elution solvent is critical in MSPD extraction (Barker, 2000). Hexane and three solvent mixtures were tested, and the resulted total PBDE concentrations are compared in Figure 5. It is obvious that solvent mixtures work better than hexane alone. Even though nonpolar organic solvents like hexane are better solvents for hydrophobic compounds like PBDEs, they lack the ability to penetrate deep into the tissue. Hexane is commonly used in combination with a polar solvent to enhance the extraction efficiencies for biotic samples (de Boer et al., 2001; Saito et al., 2004). In this work, the binary solvent mixtures of hexane and DCM (8:2) gave the highest extraction efficiency for PBDEs. An additional advantage of using this solvent mixture was that it extracted fewer lipids than the other two mixtures.

Elution of the MSPD extraction column is typically conducted once. With an intention to increase the analyte extraction efficiency without increasing the total solvent consumption, the collected eluate was reintroduced to the top of the column after the initial 100 mL elution solvent mixture had passed through the MSPD column. The elution was then repeated for a total of two, three, or four cycles. As seen in Figure 6, a significant difference was not found; in fact, single

elution resulted in higher PBDE concentration than repeated ones. Readsorption of the analyte to the packing material of the column could have occurred during the repeated elution.

Based on these results, following conditions were selected for the optimized MSPD method: sample freeze-drying, Florisil as the sorbent, 1:2 sample-to-sorbent ratio, dry grinding, and 100 mL solvent mixture of hexane DCM (8:2) in a single extraction and elution cycle (Figure 5).

3.1.5 **Comparison of Extraction Methods**

The extraction efficiency of the optimized MSPD method was compared to two other well-established methods, the Soxhlet extraction and liquid-liquid extraction, by analyzing PBDEs in five placenta samples and SRM in duplicate. Approximately 4 g of freeze-dried sample was weighed into a solvent-washed aluminum pan. To the same pan, Florisil was added in a 1:2 sample-to-sorbent mass ratio and transferred into a glass mortar. After adding a known amount of surrogate (BDE118L), the sample-sorbent mixture was thoroughly ground using a glass pestle for approximately five minutes to become a fine powder. A glass column (13 mm id and 30 cm length) was packed from bottom to top with glass wool, prewashed anhydrous sodium sulfate (10 g), Florisil (4 g), and topped with the prepared sample-sorbent mixture. The packed column (Figure 6) was gently tapped to remove the trapped air. The column was eluted with 100 mL of hexane DCM (8:2) solvent mixture. The elution was controlled at 1–2 drops/second using the Teflon clog and accomplished under gravitational flow. When the flow ceased, a gentle vacuum was applied to collect the remainder of the solution in the column.

The liquid-liquid extraction method developed by Jensen and coworkers (Jensen et al., 1983) was used with slight modifications. Briefly, a 50 mL glass centrifuge tube was filled with 4 g of the dried sample and mixed with 15 mL of acetone:hexane (7:2) mixture for 1 min using a vortex mixer. Ten mL of hexane: MTBE (9:1, v/v) were added and mixed for another 1 min, before the

phase separation by centrifugation (Beckman Avanti 30 Centrifuge, Beckman Instruments, Fullerton, California) at 3,000 rpm for 30 minutes. The organic layer was pipetted out into another tube. The extraction was repeated by adding another 10 mL of hexane:MTBE (9:1) mixture. After repeated extraction, the combined organic extract was filtered through a hexane-washed Whatman No. 1 filter paper. The filtrate was then washed with 5 mL of 0.1 M H3PO4 in 1% potassium chloride (KCl) solution by gentle shaking.

In Soxhlet extraction, 4 g of freeze-dried samples were transferred to a Whatman glass microfiber thimble. Extraction flasks (250 mL) were filled with 150 mL of a solvent mixture (acetone, hexane, and DCM in 45:45:10 ratio). The refluxing was adjusted at four cycles per hour, and the extraction continued for 22 hours.

The sample cleanup procedure was similar for all extraction methods. The extract was first transferred into a Kuderna-Danish concentrator for solvent evaporation. Further volume reduction (down to 2 mL) was achieved by a gentle N_2 gas blow. Sample cleanup was accomplished by GPC followed by silica gel chromatography. A glass GPC column (25 mm id × 400 mm long) was manually prepared by packing with S-X3 bio beads. The sample was eluted with 140 mL of hexane and DCM (1:1) mixture. A multilayer silica gel column was prepared using (from bottom to top) 1 g of neutral silica, 1 g of basic silica, 4 g of acidic silica, 1 g of neutral silica, and 5 g of anhydrous sodium sulfate. The preparation of the silica gel column followed the procedures described in EPA Method 1614. Fifty milliliters of hexane were used as the elution solvent. The final volume of the sample was reduced to 1 mL by Kuderna-Danish evaporation and N_2 blow before the instrumental analysis.

Instrumental analyses of PBDEs were performed on an Agilent Model 6890 GC with a Model 5973 MS detector. An Rtx1614 capillary column (15 m × 0.25 mm i.d., 0.1 μm film thickness) was used with helium as the carrier gas. Internal standard CB204 was added to each sample before a GC injection to normalize the peak areas in the quantification of targeted PBDEs. Each sample was introduced into GC/MS through a programmable temperature evaporation (PTV) injection port. The operational parameters of the PTV inlet were optimized previously and have been described in detail elsewhere (Wei et al., 2010; Norlock et al., 2002). In each run, 120 mL was injected using solvent vent mode. The initial oven temperature was 90°C, which lasted for 3 min, and then increased to 140°C at 10°C/min and further to 300°C at 5°C /min. The final temperature was kept for 15 min until the run was completed. Selected ion monitoring (SIM) was used in electron capture negative chemical ionization (ECNI) MS. The mass-to-charge ratio (m/z) values for the monitored ions of individual BDEs and BB-209 were 79 and 81 while they were 428, 430 and 432 for CB-204 and 484 and 486 for BDE-209.

A total of 43 PBDE congeners were analyzed. Identification was based on the retention time matching with the PBDE standards. For quantification, a six-point calibration curve for mono-to-hepta-BDEs was prepared using the 39-PBDE mixture standard, with concentrations extended from 0.024 to 15 ng/mL. A five-point calibration curve with a concentration range from 1 to 38.4 ng/mL was used for the heavy congeners (BDEs 196, 206, 207, and 209). Good linearity was obtained for all the standard curves (r^2=.99 to 1.00). The standard curves were used to derive the response factors for individual congeners against internal standards. Internal standard for mono-to-hepta-congeners was CB-204 while BB-209 was used for heavy congeners.

The total extracted lipid was determined gravimetrically using an aliquot of 1 mL of the eluted extract solution, in order to determine the lipid removal efficiency by the MSPD and to

examine whether the PBDE extraction depends on the extraction of lipid. Efficient lipid removal was desired in order to maximize the advantage of using MSPD procedure in combining the extraction and cleanup steps.

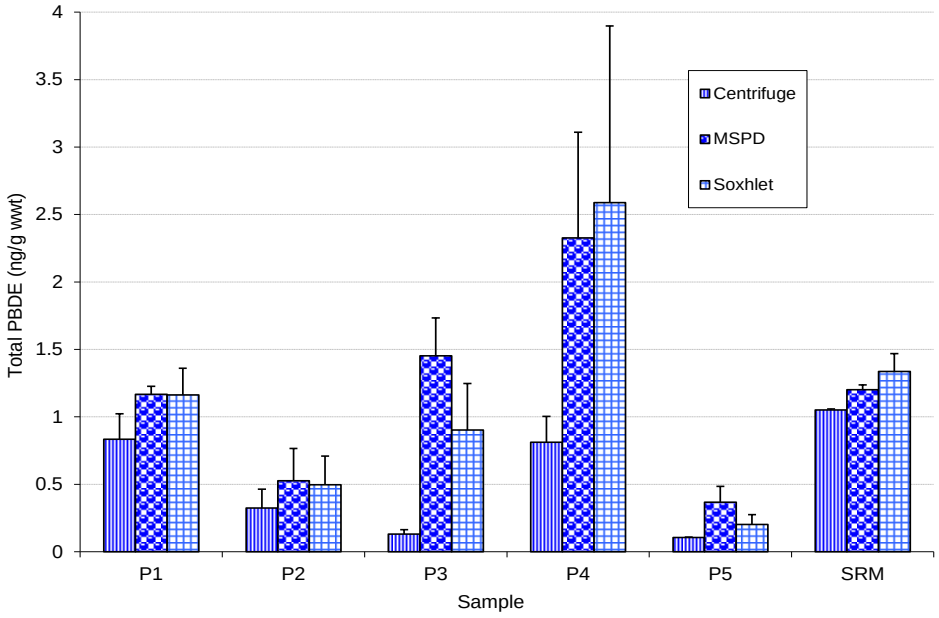
For quality control, procedural blanks were analyzed with each batch of extraction. The PBDE levels found in the blanks were deducted from those of the samples in that batch. A known amount of surrogate BDE-118L was added into all the samples before extraction. The recovery of the surrogate was used to indicate the analytical accuracy and to correct the concentration values for each sample. All analyses were performed in duplicates.

The Student's t-test and two nonparametric statistical methods—paired sign test and signed rank test—were used to compare the experimental results between the extraction methods. The analysis was performed using SAS version 9.0, with the input experimental results expressed in ng/g dry weight (dw) for individual congeners and total PBDEs.

3.1.6 **Results of the Comparison Study**

Figure 7 shows the Total PBDE extraction by the three methods compared in this study. The optimized MSPD method performed similarly to the conventional, robust Soxhlet method. The paired t-test indicated a nonsignificant difference between the two methods, with a p-value that ranged from 0.13 to 0.78 for individual congeners and $p=.5$ for total PBDEs. On the other hand, the efficiency of liquid extraction was significantly lower than that of MSPD ($p<.05$ for seven out of 10 congeners as well as the total PBDEs). The same conclusion was derived from the results of the paired sign test and signed rank test, which compare medians of two repeated group measures.

The advantages of MSPD over Soxhlet extraction are multifold. First, the extraction time was shortened from 22 to 24 hours with Soxhlet to 30–45 min with MSPD. Secondly, solvent consumption was reduced by 50 mL per sample. In addition, the optimized method extracted few lipids, compared to approximately 5% and 4% lipid (dw) extracted using the Soxhlet and liquid methods, respectively. Therefore, lipid removal using the GPC cleanup procedure was necessary for Soxhlet-extracted samples, but not required when the MSPD procedure was used. Nonetheless, it is recommended that further cleanup is conducted in order to maintain the lifetime of the capillary GC column, even though the MSPD extract is of GC-injectable quality (Kristenson et al., 2006; Barker, 2007). In this study, the multilayer silica gel column was used following the elution and concentration steps. This was necessary when large volume GC injection is involved.



Standard reference material (SRM) concentrations are 10 times diluted. The error bars represent one standard deviation of duplicated analyses. P = placenta.

Figure. 7 Total PBDE concentration from 5 individual placenta samples and fish tissue. SRM extracted using MSPD, liquid and Soxhlet extraction methods.

Lipid Extraction: It was found that a higher content of extracted lipids was not accompanied by significantly higher PBDE levels. This observation is in line with previous reports that lipid-normalized PCB concentration is negatively correlated with the content of the extracted polar lipid in tissues (Saito et al., 2004; Ewald et al., 1998). In the literature, chemical concentrations are often presented on the basis of lipid mass. However, the inconsistency in lipid measurement makes it difficult to compare among published data. As suggested by the US EPA, (USEPA method 1614) the PBDE concentrations should be reported on the basis of wet tissue mass, rather than on the basis of the lipid content.

Owing to simultaneous lipid removal, the MSPD method presented here is incapable of simultaneous lipid determination. The optimized method extracted negligible amount of lipids. Two major factors acted in the decrease of extracted lipid, including the use of an elution solvent mixture of hexane and DCM (8:2) to minimize the extraction of polar lipids, and the layer of Florisil in the MSPD column that captured the extracted lipids. The dimension of the MSPD column was also found to be important; longer columns with smaller diameters are more efficient in lipid removal. When MSPD is used, lipid content may be determined by a separate laboratory procedure, such as the one developed by Bligh and Dyer (Bligh and Dyer, 1959).

3.1.7 **Validation of the Optimized Method**

The reliability of the optimized MSPD procedure was examined with matrix spike samples. Three aliquots of the same placenta sample (Table IV, Sp1, Sp2, and Sp3) were spiked with individual standard solutions of BDEs 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209, and kept overnight in a refrigerator to allow proper penetration and mixing. These samples were extracted along with the original, unspiked samples. The reliability of the procedure was further validated by analyzing SRM 1947 (Table V). The fish tissue powder (1.00 g) was spiked with the surrogate,

mixed with 5 g of sodium sulfate in a pre-cleaned aluminum pan, and kept covered in the refrigerator for 12 hours. Extraction of the matrix spike and the SRM samples was accomplished using the optimized MSPD method, and the cleanup and GC/MS analysis were conducted as described above.

TABLE IV

PBDE RECOVERY FROM THE SPIKED PLACENTA SAMPLES

Congener	Spike conc.	Sp1	Sp2	Sp3	Un-spiked	Mean Recovery (%)	RSD (%)
BDE-28+-33	1.32	1.22	1.16	1.22	0.0025	91 ± 0.03	2.71
BDE-47	1.52	1.55	1.47	1.57	0.0775	95 ± 0.06	3.62
BDE-66	1.43	1.52	1.31	1.42	0.0000	99 ± 0.1	7.35
BDE-100	1.60	1.57	1.43	1.52	0.0150	94 ± 0.07	4.71
BDE-99	1.55	1.74	1.47	1.57	0.0325	100 ± 0.14	8.58
BDE-118L	1.39	1.72	1.36	1.51	0.0000	110 ± 0.18	11.59
BDE-85	1.44	1.79	1.47	1.71	0.0150	114 ± 0.16	9.93
BDE-154	1.57	1.72	1.43	1.55	0.0075	99 ± 0.15	9.37
BDE-153	1.54	1.78	1.51	1.62	0.0350	104 ± 0.14	8.31
BDE-183	1.58	1.87	1.69	1.81	0.0100	113 ± 0.09	5.10
BDE-209	1.53	1.98	2.06	1.84	0.4775	97 ± 0.11	5.64

Sp1, Sp2, Sp3: Spiked replicates, Unspiked: original sample before spike, RSD: relative standard deviation, Concentrations are in ng/g dry weight

Determination of the method detection limit (MDL) followed the standard procedure (USEPA SW846). Briefly, the instrumental detection limit was determined as the concentration

capable of producing a GC/MS response three times greater than the noise (S:N=3:1). Then, a homogenized freeze-dried placenta sample, which had been pre-extracted, was divided into seven replicate samples, and each was spiked with PBDE standards at a level approximately equal to five times of the instrumental detection limit. After the analyses, the MDL was calculated from the results by:

$$MDL = SD \times t(n\text{-}1, \alpha = 0.01)$$

where SD is standard deviation of the seven replicate analyses, and $t(n\text{-}1, \alpha = 0.01)$ is the student's t-value for 99% confidence level with n-1 degrees of freedom; it is 3.143 for n=7.

The analytical accuracy is best evaluated by analyzing certified reference material or SRM. However, methods are most often validated by using only standard spiked samples due to the limited availability of suitable reference material (Garzia-Lopez et al., 2008). In this study, the reliability of the optimized MSPD procedure was examined by analyzing both spiked placenta samples and NIST SRM 1947 with certified concentrations for BDEs 47, 66, 99, 100, 153, and 154, and a reference concentration for BDE-28. As presented in Table IV, excellent recoveries of 91%–114% were observed with a relative standard deviation (RSD) of 2.7% to 11.6% for the matrix spike samples. For SRM 1947 (Table V), the results were within 89% to 115% of the certified values for all congeners except BDE-28, for which 49% of the reference value was achieved. The definite reason for the low recovery for BDE-28 was unknown but attributable to the fact that the initial value is only a reference value, which has more uncertainty compared to a certified value. The SRM was also analyzed using the Soxhlet and the liquid extraction methods, and the comparison among the three methods is similar to those using placenta samples.

The detection limits of the MSPD procedure developed in this work are presented in Table VI. The MDLs for 39 out of the 43 spiked congeners ranged from 0.34 to 10.7 ng/kg of wet sample. The BDEs 1–3, and 15 were not observed at the level of the spike.

TABLE V

ANALYTICAL RESULTS OF SRM 1947 ANALYSIS USING OPTIMIZED MSPD EXTRACTION

PBDE	**(A)**	**(B)**	**Mean ± sd**	**RSD (%)**	**Certified concentration (µg/kg ww)**	**Recovery (%)**
BDE-28	1.26	0.94	1.10 ± 0.23	20.8	2.26 ± 0.46*****	49
BDE-47	70.17	67.38	68.77 ± 1.98	2.9	73.30 ± 2.9	94
BDE-66	1.84	1.46	1.65 ± 0.27	16.4	1.85 ± 0.13	89
BDE-100	14.82	15.50	15.16 ± 0.48	3.2	17.10 ± 0.6	89
BDE-99	23.15	20.91	22.03 ± 1.58	7.2	19.20 ± 0.8	115
BDE-154	6.49	6.66	6.57 ± 0.12	1.8	6.88 ± 0.52	96
BDE-153	4.16	4.27	4.22 ± 0.07	1.7	3.83 ± 0.04	110

A, B: sample duplicates

*: A reference concentration (certified concentration is not available)

TABLE VI

METHOD DETECTION LIMITS OF THE OPTIMIZED MSPD METHOD

PBDE	Concentration ng/mL							SD	MDL ng/kg (dw)	MDL ng/kg (ww)
	R1	R2	R3	R4	R5	R6	R7			
10	0.001	0.001	0.002	0.001	0.002	0.002	0.002	0.0006	1.91	0.38
7	0.003	0.002	0.006	0.005	0.004	0.005	0.007	0.0016	5.18	1.04
8+11	0.001	0.004	0.006	0.003	0.004	0.004	0.006	0.0017	5.49	1.10
12+13	0.001	0.001	0.002	0.001	0.001	0.002	0.002	0.0005	1.69	0.34
30	0.002	0.003	0.006	0.004	0.004	0.004	0.005	0.0016	4.91	0.98
32	0.003	0.003	0.005	0.003	0.003	0.006	0.003	0.0012	3.81	0.76
17	0.001	0.004	0.004	0.002	0.003	0.002	0.004	0.0011	3.32	0.66
25	0.000	0.002	0.002	0.002	0.002	0.004	0.004	0.0012	3.63	0.73
28+33	0.003	0.003	0.002	0.003	0.003	0.005	0.004	0.0010	3.11	0.62
35	0.001	0.002	0.003	0.002	0.002	0.003	0.002	0.0006	1.90	0.38
37	0.001	0.003	0.004	0.002	0.003	0.004	0.003	0.0010	3.17	0.63
75	0.005	0.004	0.004	0.003	0.007	0.009	0.005	0.0020	6.37	1.27
49	0.003	0.003	0.003	0.003	0.004	0.005	0.004	0.0007	2.25	0.45
71	0.002	0.005	0.008	0.004	0.004	0.006	0.005	0.0019	5.91	1.18
47	0.013	0.023	0.032	0.023	0.023	0.026	0.018	0.0061	19.11	3.82
66	0.006	0.007	0.009	0.006	0.010	0.010	0.008	0.0016	4.91	0.98
77	0.003	0.004	0.006	0.005	0.005	0.005	0.005	0.0009	2.96	0.59
100	0.009	0.013	0.019	0.016	0.014	0.013	0.015	0.0032	10.01	2.00
119	0.004	0.005	0.012	0.006	0.005	0.006	0.006	0.0027	8.60	1.72
99	0.030	0.032	0.040	0.043	0.039	0.023	0.026	0.0075	23.46	4.69
116	0.004	0.007	0.007	0.009	0.007	0.010	0.008	0.0018	5.76	1.15
118	0.003	0.006	0.004	0.007	0.005	0.009	0.007	0.0022	7.01	1.40
85	0.004	0.007	0.013	0.005	0.008	0.008	0.007	0.0029	9.06	1.81
155+126	0.002	0.004	0.005	0.002	0.004	0.006	0.005	0.0013	4.19	0.84
154	0.006	0.007	0.014	0.010	0.010	0.013	0.011	0.0028	8.82	1.76
153	0.008	0.012	0.013	0.013	0.014	0.015	0.015	0.0025	7.93	1.59
138+166	0.004	0.005	0.009	0.006	0.004	0.006	0.006	0.0015	4.81	0.96
183	0.007	0.012	0.008	0.014	0.014	0.010	0.011	0.0026	8.28	1.66
181	0.015	0.011	0.021	0.019	0.018	0.012	0.018	0.0037	11.55	2.31
190	0.022	0.025	0.017	0.029	0.029	0.014	0.015	0.0064	20.01	4.00
196	0.076	0.126	0.134	0.133	0.138	0.150	0.152	0.0105	32.87	6.57
207	0.042	0.053	0.088	0.059	0.043	0.066	0.057	0.0036	11.46	2.29
206	0.074	0.117	0.080	0.140	0.142	0.100	0.116	0.0080	25.05	5.01
209	0.148	0.109	0.209	0.193	0.180	0.126	0.187	0.0170	53.48	10.70

Sample weight = 1 g dry wt = 5 g wet wt
Final volume (sample) = 1 mL, Injection volume = 120 μL
R: replicates
SD: standard deviation

3.1.8 **Preliminary Sample Analysis**

Table VII summarizes the results of PBDE analyses in the five human placenta samples. The total PBDEs ranged from 385 to 2,370 ng/kg (wwt) among the five placenta samples. Among the 44 PBDE congeners quantitatively analyzed, 22 were detected in all five placenta samples analyzed in this study. The dominant congeners were BDE-47 with a concentration range of 97–947 ng/kg (wwt) and BDE-99 with a range 166–994 ng/kg (wwt). The average concentration ratio of BDE-47 to BDE-99 was 5:4 and together these two congeners accounted for more than 75% of the total PBDE burden in human placenta. The BDEs 85, 100, 153, 154, and 183 were also detected and quantified in all the samples, and they contributed approximately 15% to the total PBDEs. The concentration of BDE-209 in the five placenta samples ranged from <MDL to 29 ng/kg (wwt). Although they did not contribute heavily to the total PBDE concentration, some infrequently reported PBDE congeners, including BDEs 15, 17, 49, 71, 119, 155, 126, 196, 206, and 207, were also present in the placentas. A peak which highly likely represents BDE-208 was also seen in all the samples analyzed. In addition, a number of unidentified brominated compounds were detected, based on the presence and the abundance ratio of the m/z 79 and m/z 81.

TABLE VII

CONCENTRATION OF PBDES IN 5 PLACENTAS (NG/KG,WWT)

Compound	Placenta-1	Placenta-2	Placenta-3	Placenta-4	Placenta-5
BDE-1	nd	nd	nd	nd	nd
BDE-2	nd	nd	nd	nd	nd
BDE-3	nd	nd	nd	nd	nd
BDE-10	nd	nd	nd	nd	nd
BDE-7	nd	nd	nd	nd	nd
BDE-(8+11)	nd	nd	nd	nd	nd
BDE-12+13	nd	nd	nd	nd	nd
BDE-15	1.5	29.5	2	1.5	2.5
BDE-30	nd	nd	nd	nd	nd
BDE-32	nd	nd	nd	nd	nd
BDE-17*	1	<MDL	2	1	<MDL
BDE-25*	1	1	1	<MDL	<MDL
BDE-(28+33)	5.3	2.3	4.8	9.8	3
BDE-35	nd	nd	nd	nd	nd
BDE-37	<MDL	2	<MDL	nd	nd
BDE-75	nd	nd	nd	0.5	nd
BDE-49*	11	5	31.5	3.5	1.5
BDE-71*	5	2.5	15.5	1.5	1
BDE-47	652.8	194	535.5	946.5	97
BDE-66	6.3	1.3	6	10.5	<MDL
BDE-77	nd	nd	nd	<MDL	nd
BDE-100	57.8	48	118.5	192.8	47
BDE-119	4	46	13	39	6.5
BDE-99	290	207.3	636.5	993.8	165.5
BDE-116	#	#	#	#	#
BDE-118	nd	nd	nd	nd	nd
BDE-85	29	10.5	32.8	48	5
BDE-(155+126)	18.5	1	6	3.5	<MDL
BDE-154	34.8	13.3	36.3	55.3	12.5
BDE-153	74.5	23.3	58.5	37.3	18.3
BDE-(138+166)	4.5	4	nd	nd	2.5
BDE-183	8	6.5	6.3	9.3	6.8
BDE-181	nd	nd	nd	nd	nd
BDE-190	nd	2.5	nd	nd	nd
BDE-196	<MDL	<MDL	<MDL	<MDL	<MDL
BDE-208	<MDL	<MDL	<MDL	<MDL	<MDL
BDE-207	<MDL	2.5	6	2.5	3.5
BDE-206	<MDL	<MDL	8	<MDL	<MDL
BDE-209	<MDL	24	28.5	13.5	12
Σ_{44}PBDE	1205	626.5	1548.7	2369.8	384.6

nd: non detect, <MDL: below method detection limit, *: incompletely resolved at the base, #: peak covered by BDE-99 peak

In this work, the MSPD method was optimized to extract PBDEs from human placenta. Florisil is an efficient dispersion sorbent, and 100 mL of hexane/DCM (8:2) solvent mixture is adequate to extract and elute the PBDEs from the MSPD column combining extraction and lipid removal. The MSPD method drastically reduces the extraction time and solvent consumption, in comparison with the traditional Soxhlet method. Moreover, the cleaner extract produced by the MSPD reduces the need for subsequent cleanup efforts. Overall, the optimized method is an efficient procedure to extract PBDEs from human placenta tissue. This work was published and the content was adapted with permission from "Optimization of the Matrix Solid Phase Dispersion Extraction procedure for the analysis of Polybrominated Diphenyl Ethers in Human Placenta. Copyright © 2009, American Chemical Society" (see App. C)

3.2 Method Validation for other Target Analytes

The appropriateness of the optimized MSPD method to extract PCBs and DDE from placenta tissue was tested by extracting blank spikes samples and matrix (placenta) spike samples. For the blank spikes, 2 g of Florisil (sorbent) was spiked with a known amount (2 ng) of individual PCBs and DDE. These samples were extracted and the recovery was calculated. Matrix spikes were done by using placenta samples spiked with known amount (2 ng) of individual PCBs and DDE. After adding standards, the samples were kept overnight to equilibrate. Along with the spikes, an unspiked sample from the same placenta was analyzed to find out the initial level of analytes. This amount was subtracted from the spikes before calculating the recovery.

As shown in Table VIII, the duplicate analysis indicated good precision and accuracy in analyzing PCBs and DDE using the method developed for PBDEs and described above. The recoveries of the spiked PCBs and DDE ranged from 76% to 106% (±1%–4%) for the blanks and from 75% to 104% (±1%–6%) for matrix spikes.

TABLE VIII

PCB AND DDE RECOVERY FROM SPIKED BLANKS AND SPIKED PLACENTA SAMPLES

Blank Spiked (n=2) % recovery					Matrix Spiked (n=2) % recovery			
Analyte, PCB	A	B	Ave Rec	sd ±	A	B	Ave Rec	sd ±
8	76	77	76	1	74	78	76	3
28	82	87	84	3	80	81	80	1
52	91	87	89	3	84	79	82	4
49	90	87	88	2	82	80	81	1
44	91	89	90	1	82	80	81	2
37	95	94	94	1	88	87	88	1
74	95	93	94	1	88	84	86	2
70	96	94	95	1	87	84	85	2
66	96	94	95	1	88	84	86	2
101	98	96	97	2	88	85	87	2
60	95	92	93	2	86	81	83	3
99	96	93	95	2	86	82	84	3
87	100	95	98	4	88	83	85	4
82	100	96	98	3	87	81	84	4
77	107	105	106	2	94	90	92	3
118	105	101	103	3	90	85	87	4
114	103	98	101	3	89	84	86	4
153	100	95	97	3	86	79	83	5
179	95	91	93	3	83	76	79	5
105	105	99	102	4	90	85	87	3
138	104	100	102	3	87	81	84	4
158	102	98	100	3	86	80	83	4
187	100	95	98	3	84	78	81	5
166	100	96	98	3	87	80	84	4
183	96	92	94	3	74	69	72	4
126	108	102	105	4	108	101	105	4
128	105	100	102	4	89	82	86	5
156	107	102	105	4	90	84	87	4
180	102	97	99	3	83	77	80	4
170	101	96	99	3	84	78	81	5
169	101	95	98	4	91	85	88	4
189	101	96	99	3	90	83	86	5
DDE	na	na	na	na	90	81	85	6
52L	na	na	na	na	81	79	80	1

A, B: duplicates, na: not available, Ave Rec: average recovery for the duplicates, sd: standard deviation

3.3 Extraction of Blood

For blood samples, the extraction was conducted by the method described by Hovander et al. (2000) with some modification. The frozen samples were brought to room temperature before weighing out 3 g of whole blood into 50 mL screw cap glass centrifuge tubes. Recovery standards (CB-52L 1 ng, Fluorinated BDE-69 1 ng, and Fluorinated BDE-208 2 ng) were added and samples were votexed about 15 seconds and left undisturbed for 1 hour for thorough stabilization of the surrogates with the matrix. Then 1 Ml of 6M hydrochloric acid (HCl) and 4 mL of 2-propanol were added to the blood matrix and vortexed again to denature the matrix. Into the mixture 5mL of 1:1 hexane:MTBE was added and mixed by shaking the tube. Samples were centrifuged at 3,000 rpm for 20 minutes (Beckman Avanti 30 Centrifuge, Beckman Instruments, Fullerton, California). The organic layer was pipetted into another centrifuge tube containing 4 mL of 1% KCl solution. The extraction was repeated twice using 3mL of the solvent mixture and the organic layers were added into the same KCl solution. After gentle shaking to partition the coextracted non-lipids, the mixture was centrifuged again at 6,000 rpm for 20 min. The organic layer was collected into a pre-weighed glass tube. All the solvents were evaporated and the lipid was determined gravimetrically. The sample was reconstituted with hexane and the volume was brought to 2 Ml by blowing a gentle stream of nitrogen.

3.4 Placenta Lipid Determination

Concentrations of lipophilic contaminants in biological samples are frequently corrected for variation in tissue lipid content (Inouye et al., 2006; Smedes, 1999). Although many methods have been developed for the determination of tissue lipid level, selection of the appropriate method had always been debatable as each of these methods are different from each other and tend to produce different results. The US EPA method 1614 suggests to report PBDE levels on tissue

weight basis rather than lipid weight basis because of the associated ambiguity in the gravimetric lipid determination (Method 1614, USEPA). Nonetheless it is important to have a lipid measurement associated with contaminant data in order to compare between different matrices and different studies (Covaci et al., 2007). The major limitation of the optimized MSPD method for placenta (Dassanayake et al., 2009) is the inability to perform simultaneous lipid determination. Therefore to determine the placenta lipid content for this study we used the method developed by Folch et al. (1956) for total lipids, which has been accepted by many as a standard procedure for lipid determination (Smedes, 1999).

One gram of the dried placenta sample was ground into a fine powder and transferred into a glass test tube. Eight mL of chloroform was added to the test tube, followed by 4 mL of methanol and vortexed for three minutes. The mixture was vacuum-filtered and the vortexing repeated using another 8 mL of chloroform and 4 mL methanol for the residue left on the filter. After filtering again, both fractions of the filtrate were transferred into a 50 mL centrifuge tube. Then 6 mL of 1% KCl solution was added to the centrifuge tube and centrifuged at 3,000 rpm for 30 minutes for the phase separation. The entire lower phase (containing the chloroform and lipids) was removed using a pipette into a graduated measuring cylinder and the volume was recorded. Five mL aliquot was removed into a pre-weighed aluminum pan. The pan was covered and air-dried overnight. The dried pan was weighed and the percent of lipid was calculated by the formula below.

$$\text{Lipid } \% = \frac{(W2-W1)g*Tml*100}{5ml*Wg*6.4}$$

where,

W = weight of the sample (g)

W1 = weight of the empty container where the aliquot is placed (g)

W2 = container + lipid weight (g)

T = total volume of the extract (mL)

A conversion factor of 6.4 was used to convert dry weight to wet weight (freeze-dryer data showed 1 g of dry placenta equals to 6.4 of wet placenta on average, n=50).

Lipids from 51 placenta samples were extracted as above. The average lipid content (wwt) was found to be 0.98% ±0.3%. Placenta lipid content varied over a range from 0.34% to 1.9%.

3.5 Development of Instrumental Method

Gas chromatography coupled to mass spectrometry (GC-MS) has been the most widely used technique for the determination of organic compounds that are sufficiently volatile to evaporate under enhanced temperatures. A complex mixture of a liquid sample is injected into the inlet of a GC, which separates the individual compounds via a capillary column. The MS detects and identifies the compounds by ionization and selectively capturing the ions based on their m/z ratios. For the analytes involved in this study, both electron impact ionization (EI) (Ramu et al., 2007; Hernandez et al., 2005; Smeds, et al., 2001; Li, et al., 2005), and ECNI (Jaraczewska et al., 2006; Saito, K et al., 2004; Raina and Hall, 2008) have been applied. Among a number of major types of mass analyzers, quadrupole filter, which consists of four parallel rods and applied oscillating electrical fields, is the most widely used for targeted, quantitative analyses because of its robust reliability.

The application of tandem mass spectrometry in environmental sample analysis has improved the quality of the analysis by diminishing the undesirable effects caused by sample matrices. Analyzers such as triple quadrupole or ion trap are widely acknowledged in trace -level pollutant analysis due to their ability to remove the background interferences as well as to increase both the

selectivity and sensitivity. These methods have shown excellent results in the determination of pesticides as well as several other organic pollutants in environmental and biological samples (Pitarch et al., 2007; Hernandez et al., 2005; Medina et al., 2009; Frenich et al., 2007).

3.5.1 Polybrominated Diphenyl Ethers and Other Halogenated Flame-Retardents

In this work, separation of PBDE congeners was achieved by injecting large volumes of sample using PTV inlet in solvent vent mode. An RTX-1614 column (15 m x 0.25 mm ID x 0.1 um thickness) was used with helium as the carrier gas at a constant flow rate of 1.5 mL/min. A total of 60 mL was injected in three consecutive injections of 20 mL each with 10 seconds intervals in between. The temperature program for the PTV injection port was from 40°C (holding for 1.5 min) to 300°C at 600°C/min and held for 1min. The vent flow was 100 mL/min. The purge flow was 100 mL/min at a run time of 2.75 min. The initial oven temperature was 80°C, which lasted for 2 min, and then increased to 300°C at 10°C/min. The final temperature was kept for 10 min until the run was completed.

The use of the MS/MS method with ECNI may not be advantageous over single MS for PBDEs amd other BFRs, as only the ion clusters from the fragments $[Br]^-$ and/or $[HBr_2]^-$ were observed in the full scan spectra (Medina et al., 2008). The only feasible transitions corresponded to the fragmentations of precursor ions to give bromine atoms, resulting in low sensitivity and poor selectivity. The MS/MS with EI ionization was found difficult for trace-level analytes because the sensitivity with EI detection is about 20 times lower than ECNI (Eljarrat et al., 2002) and a large drop in sensitivity was observed for congeners with more than six bromine atoms (Stapleton, 2006). It follows that GC-ECNI/MS is the analytical technique currently employed when trace levels of PBDEs have to be determined (Mascolo et al. 2010).

A GC-ECNI/ MS method was optimized in SIM mode using at least two m/z ions, normally the most abundant, selected from the full-scan spectrum. For PBDEs, m/z 79 $(Br)^-$ was selected as the quantitation (Q) ion and m/z 81 and m/z 160 as the confirmation (q) ions. Though the specificity is affected, for PBDEs it is beneficial to use m/z 79 as the quantifier ion while using the m/z 81 as the qualifier ion. As the isotopic ratio for these two ions is close to 1, this increases the sensitivity at trace levels of analytes which in turn aides in the identification of the compounds (Medina et al., 2009). DecaBDE (BDE-209) was quantified using m/z 486 and 488 for $(C_6Br_5O)^-$. Fluorinated BDEs 69 and 208 were used as analytical surrogates for PBDEs but the advantage of fluorine addition could not be used because of the dominant effect of Br- in the spectra. Two internal standards were used for the PBDE analysis: ^{13}C labeled CB205 (for Tri-Hepta BDE) and decabromobiphenyl (BB-209). The monitoring and quantitation ions selected for the compounds analyzed with GC/MS are given in Table IX.

TABLE IX

CHEMICALS ANALYZED IN THIS STUDY AND THE IONS SELECTED FOR THE GC/MS/SIM METHOD

Analyte	Quant and qual ions (Q, q) m/z
tri-heptaBDEs	79, 81, 160
BDE-209	488.8, 486.8, 79, 81
CB-205L (IS for tri-heptaBDE)	441.8, 430.8, 405.8
BB-209 (IS for BDE-209)	79, 81
F-BDE-69, F-BDE-208 (surrogates)	488.8, 486.8, 79, 81

Abr. Name	Full Name	Quant ion Q	Qual ion q	Qual ion 2
TBPH	bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate	463	461	79
EHTBB	2-ethylhexyl, 2,3,4,5-tetrabromobenzoate	79	81	
TBC	1,3,6,8-tetrabromocarbazole	79	81	482
BB-153	2,2',4,4',5,5'-hexabromodiphenyl	79	81	
PBB-101	2,2'4,5,5'-pentabromobiphenyl	79	81	
HBB	hexabromobenzene	79	81	
TBB	1,3,5-tribromobenzene	79	81	
PBB	1,2,3,4,5-pentabromo-benzene	79	81	
PBT	pentabromotoluene	79	81	
TBCO	1,2,5,6-tetrabromocyclooctane	79	81	
PBEB	pentabromoethylbenzene	79	81	
TboCT	tetrabromo-o-chlorotoluene	79	81	
PBBB	pentabromobenzyl bromide	79	81	
PBCCH	pentabromochlorocyclohexane	79	81	
TBECH	1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane	79	81	
HCDBCO	hexachlorocyclopentadienyl-dibromocyclooctane	79	81	
DBDPE	decabromodiphenylethane	79	81	
TBpX	2,3,5,6-tetrabromo-p-xylene, 98%	79	81	
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane	79	81	
syn-DP	dechlorane plus (syn)	654	652	37
anti-DP	dechlorane plus (anti)	654	652	37
mirex	mirex	368	37	
Dec 602	dechlorane 602	340.8	613	37
Dec 603	dechlorane 603	637	635	37
Dec 604	dechlorane 604 component a	79	81	
PBBA	pentabromobenzyl acrylate	79	81	
ATBPE	2,4,6-tribromophenyl allyl ether, 98%	79	81	
HBCDs	α-hexabromocyclododecane	79	81	

3.5.2 **Polychlorinated Biphenyl and Organochlorine Pesticides**

Using an Agilent 7890A GC coupled with an Agilent 7000 triple quadrupole mass spectrometer (QQQMS), analysis of PCBs and OCPs was performed. The GC was equipped with a 30 m × 0.25 mm × 0.1 μm thickness Rxi-XLB column (Restek Corp., Bellefonte, Philadelphia). Multimode inlet injection and oven parameters were determined using hexanic standards to obtain better separation and peak shapes. The best method was set to inject 60 Ul. The initial inlet temperature was held for 1 min at 60°C then ramped at a rate of 600°C/min to 300°C. The vent flow was 100 mL/min and the purge flow was 100 mL/min. The column flow was set at 1.1 mL/min using helium as the carrier gas. The oven conditions were as follows: initial temperature of 45^0C held for 2 min, increased to 150°C at 10°C/min, increased to 200°C at 2°C/min and held for 5 min, followed by an increase to 300°C at 10°C/min and held for 1.5 min. The interface temperature was set at 300°C. For detection, the EI source temperature was set at 230°C, and the ionization voltage was -70 Ev. The temperature of the MS-1 analyzer was set at 150°C, and that of the MS-2 at 150°C. The collision cell gases were helium at 2.25 mL/min and nitrogen at 1.5 mL/min.

Multiple-reaction monitoring (MRM) was used for data acquisition. At least two MS/MS transitions were acquired for each compound in MRM mode in order to get a reliable confirmation of the analyte identity. The dwell time and collision energy parameters were optimized in order to obtain a good chromatographic peak maintaining satisfactory sensitivity for each compound. Tables X and XI show the precursor and product ions selected for the Q and q transitions monitored and the optimized collision energies for PCBs and OCPs analyzed in this study. As the internal standard and the surrogate standard for PCB analysis, ^{13}C labeled CB47 (CB47L) and 52 (CB52L) were used respectively with a Q transition of m/z 303.9 $\rightarrow$ 234 and q transitions m/z 303.9 $\rightarrow$ 232.

TABLE X

PCB PRECURSOR IONS, PRODUCT IONS, AND COLLISION ENERGIES FOR MRM TRANSITIONS

Analyte	Precursor ion	Product ion (Q, q)	Collision energy(Q,q)
DiPCB	221.9	187.1, 152.0	10, 32
TriPCB	255.9	186.0, 221.0	28, 10
TetraPCB	291.9	220.0, 222.0	32
PentaPCB	325.8	256.0, 254.0	31
HexaPCB	359.8	289.9, 287.9	31
HeptaPCB	393.8	323.9, 321.9	31
DDE	246.0, 317.9	176.0, 246.0	30, 24
CB-47L	303.9	234.0, 232.0	31
CB-52L	303.9	234.0, 232.0	31

TABLE XI

OCP PRECURSOR IONS, PRODUCT IONS (QUANT AND QUAL), AND COLLISION ENERGIES (CE) FOR MRM TRANSITIONS

ABR NAME	FULL NAME	PRECURSOR	QUANT ION	CE	QUAL ION	CE
HCCP	hexachlorocyclo-pentadiene	236.8	143	29	167	41
HCB	hexachlorobenzene	283.7	214	37	249	23
	etridiazole	210.8	183	15	211	5
	chloroneb	205.8	191	13	163	21
α-HCH	alpha-hexachlorocyclohexane	218.8	183	7	181	7
	atrazine	214.9	200	7	173	5
β-HCH	beta-hexachlorocyclohexane	218.8	183	7	181	7
	simazine	200.9	173	5	186	5
γ-HCH	gamma-hexachlorocyclohexane	218.8	183	7	181	7
	chlorothalonil	265.7	231	19	229	19
δ-HCH	delta-hexachlorocyclohexane	218.8	183	7	181	7
	alachlor	237	160	9	188	7
	heptachlor	336.8	266	19	302	11
	aldrin	328.7	293	7	257	13
	dacthal	331.7	301	13	223	41
	heptachlor epoxide	389.7	353	5	263	29
	gama-chlordane	409.7	375	7	373	7
	alpha-chlordane	409.7	375	7	373	7
	endosulfan 1	322.7	267	2	195	19
	trans-nonachlor	408.6	300	25	263	27
	dieldrin	379.7	277	11	345	3
p,p'-DDE	p,p'-dichlorodiphenyldichloroethylene	317.7	246	27	283	13
	endrin	344.8	281	9	317	3
	chlorobenzilate	250.8	139	17	223	5
	endosulfan 11	322.7	267	2	195	19
p,p'-DDD	p,p'- dichlorodiphenyldichloroethane	319.8	237	5	235	3
	endrin aldehyde	344.7	281	7	245	17
	endosulfan sulfate	386.8	289	5	219	31
p,p'-DDT	p,p'-dichlorodiphenyltrichloroethane	234.9	165	27	199	19
	methoxychlor	274	239	11	259	11
	cis-permethrin	182.9	168	15	165	13
	trans-permethrin	182.9	168	15	165	13

3.6 Quality Control

General glassware was thoroughly washed with soap and water using brushes, and then rinsed with distilled water. After drying in the air, open ends were wrapped aluminum foil and the glassware was heated in a furnace at 500 °C for at least 12 hours total. Thermally liable glassware was cleaned with soap and distilled water, then air-dried and ultrasonicated with acetone, DCM, and hexane for 5 minutes for each solvent. After cleaning, all the glassware was protected from dust by keeping them in cleaned drawers or wrapping them with aluminum foil. Samples were stored in amber glass containers and clear glassware containing the samples was covered with aluminum foil to protect light-sensitive compounds.

Parallel to every batch of 10–15 samples a procedural blank was analyzed. The procedural blank was prepared with 2 g of Florisil. Average blank level for each congener was determined. If the average blank level for any congener was >30% of the congener level in the sample, the average blank level was subtracted from the sample level. All the samples and blanks were spiked with known amounts of surrogate standards to calculate the analyte recovery. Carbon labelled (^{13}C) CB-52 was used as the surrogate for PCBs and fluorinated BDEs 69 and 208 (FBDE-69, FBDE-208) were used for lighter (3–7) and heavy (8–10) PBDEs respectively.

Duplicate analysis was performed whenever possible to assess the accuracy and the precision of the analysis. Analytical accuracy was also verified by analyzing SRM with certified levels of analytes of interest.

Internal standard (IS) method was used for quantitation with ^{13}C labeled CB-47 and CB-205 and BB-209 as IS. Calibration curves for analytes consisted of 5–7 points with R^2>.99.

The retention time match to the analytical standards, signal-to-noise ratio (s: n) greater than 3, and isotopic ratios were used to accurately identify the target analytes. The quantifier-to qualifier ratio (Q: q) was set to be within ±20% of the expected value. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated for each compound. Values below LOD were substituted with numbers equal to LOD/√2 for data analysis purposes.

3.7 Statistical Data Analysis

All statistical analyses were performed using SAS Enterprise Guide 5.1 (SAS Institute Inc., Cary, North Carolina). Descriptive statistics of exposures with means, medians, 90th and 95th percentiles, and ranges were reported for each matrix. Frequency and univariate procedures were used to analyze the distribution of data, including proportion of samples below the LOD. Normality of the data was assessed by examination of histograms and Kolmogorov-Smirnov, Anderson-Darling, and Cramér-von Mises tests. Bartlett's test or Levene's test was used to determine the equal variance between matrices. Welch's variance-weighted analysis of variance (ANOVA) was used when inequality of variance was observed. Non-normal variables were natural-log transformed before performing parametric data analysis. Prior to transformation, analytes below the LOD were imputed as the LOD/√2. Spearman and Pearson Correlation coefficients and linear regression were used to assess the associations between different analyte groups and clinical variables. Multiple comparisons of means were performed using one-way ANOVA and Tuckey's studentized range test (HSD). Level of statistical significance was set to 0.05.

4. NATIONAL CHILDREN'S STUDY—PLACENTA PROJECT

4.1 Background

Under the Children's Health Act of 2000 (Congress, 2000), the US Congress directed the National Institute of Child Health and Human Development (NICHHD) to conduct the NCS. The goal of NCS is to follow up a prospective cohort of 100,000 US-born children from conception though 21 years of age to examine the influence of children's environment on their health, growth, and development. Children's chemical, physical, biological, and psychosocial environments will be assessed repeatedly in children's homes, schools, and communities throughout the study to better understand the environmental risk factors as causes for disease in children (Landrigan et al., 2006). As one of the most holistic research approach towards studying children health, NCS is expected to form the basis of child health guidance, intervention, and policy (NCS, 2014).

The NCS is being implemented in several phases. At present the 3 main components are the NCS Vanguard Study, NCS Main Study, and NCS Substudies. The Vanguard Study is a pilot study that has been designed to assess the possibility, suitability, and cost of study recruitment, logistics, and operations that are to be used in the Main Study. The NCS Placenta Consortium was established to carry out Project LOI2-BIO-18 (P2-18), which is one of the several formative research projects conducted under the Vanguard phase of the NCS. The purpose of the placenta project is to devise and optimize methods of collection, storage, and transportation of the placenta in order to maintain the overall quality of the samples to ensure uncompromised results from various genomic, environmental, and morphological analyses where placenta can be successfully used (NCS, 2014).

The Placenta project P2-18 consisted of three studies: Pilot, Main, and Morphological studies. The Pilot Study used 43 placentas to establish the methods of collection, preservation, and transport that would ensure the integrity of the tissue specimens; meanwhile various laboratory methods were developed for stem cell isolation; RNA/DNA/miRNA isolation, expression, and epigenetic evaluations; and environmental analyses for known agents of concern. With the procedures established in the Pilot Study, the P2-18 Main study collected placental specimens donated by mothers registered in NCS at Vanguard sites in the United States, and conducted the above-mentioned laboratory studies to further test the capacity and warrant the reliability of the procedures, in preparation for the NCS Main Study. The P2-18 Morphology Study assessed the ability to perform state-of-the-art evaluations on placental tissue shipped from multiple sites throughout the United States for vascular patterning using 3-D imaging, histopathology for infections, and other pathologies using digital examination of the stained slides with association to clinical chart review (Final formative research report: LOI 2-BIO-18, 2013). Twenty institutions were involved in the collection, processing, and/or analyses of the placenta specimens. Placental Processing Center (PPC) at the University of Rochester served as the coordinating center for the project.

As part of the P2-18 Main Study, chemicals of concerns, including organic environmental pollutants such as PBDEs, PCBs, DDT, metabolite DDE, and bisphenol A, as well as many toxic metals, were analyzed in the collected placenta tissue samples. The work on PBDEs, PCBs, and DDE is described in this chapter.

4.2 Placenta Tissue Collection

Seven NCS Vanguard centers contributed from November 2011 through August 2012 in the collection of 210 placentas for the Main Study. Sampling locations are listed in Table XII.

TABLE XII

MAIN STUDY SAMPLE COLLECTION LOCATION, INSTITUTION ASSOCIATED WITH COLLECTION, AND THE ABBREVIATIONS USED IN THE TEXT

Institution	Collection site	Site abbreviation
The Children's Hospital of Philadelphia	Montgomery County, PA	PA_MC
Mount Sinai Ichan school of Medicine	Queens County, NY	NY_QVC
University of California	Orange County, CA	CA_OC
University of North Carolina	Duplin County, NC	NC_DC
University of Utah	Salt Lake City, UT	UT_SLC
University of Utah	Cache County, UT	UT_CACHE
South Dakota State University	Brookings County, SD	SD_BC

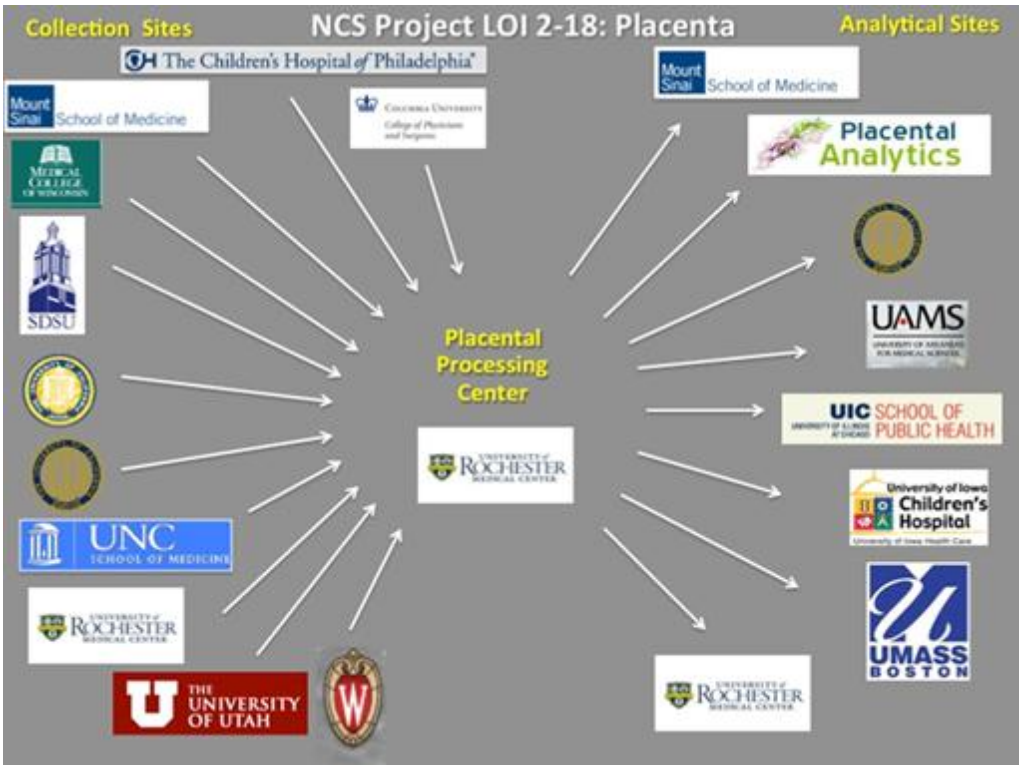


Figure 8. Flow of specimens from the collection sites in the field to PPC and on to analytical sites.

During pregnancy, each study participant signed the general consent form indicating her choice to be a part of the NCS, and the Biological and Environmental Sample Collection consent indicating she agreed to biospecimen collection. In addition, an authorization to obtain information from medical records form was also made by the patient. All personal health identifiers were obtained and retained at the respective Vanguard centers. A unique Placenta Assignment Number was assigned by the Vanguard centers to each placenta. This number was matched with the personal health identifiers information and retained and tracked only at the collecting Vanguard center. A Clinical Abstraction Form was developed for collecting all information required about the mother,

baby, placenta, and pregnancy. Unique analytical numbers were assigned by PPC to each specimen to be analyzed at the different analytical sites.

Each placenta was sampled twice. The first sample was taken as soon as the placenta was available for the sampling. Samples collected thus at the hospitals were transferred on dry ice to the PPC at Rochester, New York. The rest of the bulk placenta was chilled and sent to PPC where the second sample from the placenta was taken. All specimens were collected from grossly normal areas of the villous parenchyma, with exclusion of the decidua basalis and chorionic plate. Each collection was restricted to a placental region. Collections were added to acid-washed 50-mL tubes using plastic (nonmetallic) disposable forceps. Depending on the analyses, samples were sent to their destination under different preservation conditions. For the analyses of PBDEs, PCBs, and DDE, the samples were re-coded at PPC and express-delivered on dry ice to the Environmental Organic Chemistry Laboratory at the UIC School of Public Health. The UIC analysts were blinded to all sample information except the sample wwt and the analytical code given to the sample by PPC. Upon receiving a sample at UIC, the analytical ID of the sample, weight, receiving date and time, sample condition, and storage location were recorded in a Chain of Custody form. Specimens were stored at -20°C until further processing. The eight-step order of sampling plan for the Main Study is given in the Figure 9. Environmental samples were collected per the step-6 of the plan.

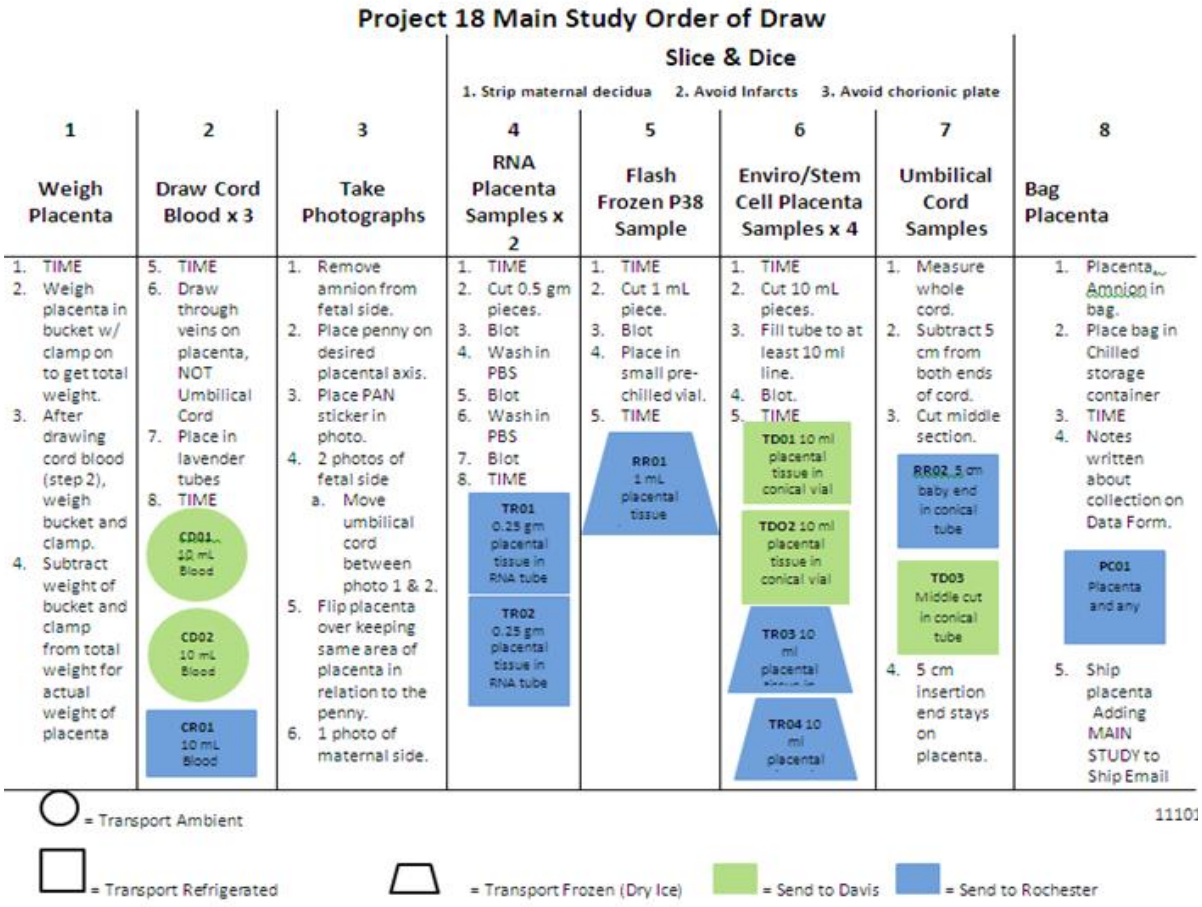


Figure 9. NCS Main Study sampling design for different analyses.

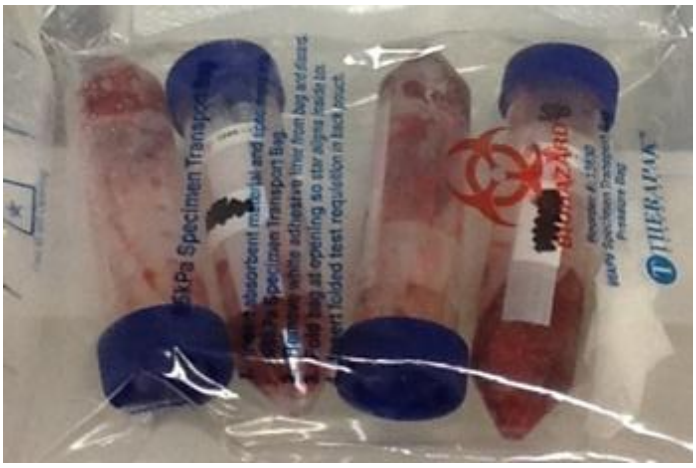


Figure 10. A set of 4 samples in conical plastic tubes, received at UIC.

4.3 Laboratory Analysis

4.3.1 Chemicals

At UIC, the scope of work included the laboratory analysis of 43 individual organic compounds in human placenta samples received from the NCS PPC. The 43 target compounds are given in Table XIII and XIV.

TABLE XIII

LIST OF PBDES ANALYZED IN THE MAIN STUDY

Congener	IUPAC Name
BDE-28	2,4,4’-tribromodiphenyl ether
BDE-47	2,2’,4,4’-tetrabromodiphenyl ether
BDE-66	2,3’,4,4’-tetrabromodiphenyl ether
BDE-85	2,2’,3,4,4’-pentabromodiphenyl ether
BDE-99	2,2’,4,4’,5-pentabromodiphenyl ether
BDE-100	2,2’,4,4’,6-pentabromodiphenyl ether
BDE-153	2,2’,4,4’,5,5’-hexabromodiphenyl ether
BDE-154	2,2’,4,4’,5,6’-hexabromodiphenyl ether
BDE-183	2,2’,3,4,4’,5’,6-heptabromodiphenyl ether
BDE-209	2,2’,3,3’4,4’,5,5’,6,6’-decabromodiphenyl ether

TABLE XIV

LIST OF PCBS ANALYZED IN THE MAIN STUDY

Compound	Scientific Name
PCB-8	2,4'-Dichlorobiphenyl
PCB-28	2,4,4'-Trichlorobiphenyl
PCB-37	3,4,4'-Trichlorobiphenyl
PCB-44	2,2',3,5'-Tetrachlorobiphenyl
PCB-49	2,2',4,5'-Tetrachlorobiphenyl
PCB-52	2,2',5,5'-Tetrachlorobiphenyl
PCB-60	2,3',4',5-Tetrachlorobiphenyl
PCB-66	2,3',4,4'-Tetrachlorobiphenyl
PCB-70	2,3,4,4'-Tetrachlorobiphenyl
PCB-74	2,4,4',5-Tetrachlorobiphenyl
PCB-77	3,3',4,4'-Tetrachlorobiphenyl
PCB-82	2,2',3,3',4-Pentachlorobiphenyl
PCB-87	2,2',3,4,5'-Pentachlorobiphenyl
PCB-99	2,2',4,4',5-Pentachlorobiphenyl
PCB-101	2,2',4,5,5'-Pentachlorobiphenyl
PCB-105	2,3',4,4',5-Pentachlorobiphenyl
PCB-114	2,3,3',4,4'-Pentachlorobiphenyl
PCB-118	2,3,4,4',5-Pentachlorobiphenyl
PCB-126	3,3',4,4',5-Pentachlorobiphenyl
PCB-128	2,2',3,3',4,4'-Hexachlorobiphenyl
PCB-138	2,2',3,4,4',5'-Hexachlorobiphenyl
PCB-153	2,2',4,4',5,5'-Hexachlorobiphenyl
PCB-156	2,3,3',4,4',5-Hexachlorobiphenyl
PCB-158	2,3,3',4,4',6-Hexachlorobiphenyl
PCB-166	2,3,4,4',5,6-Hexachlorobiphenyl
PCB-169	3,3',4,4',5,5'-Hexachlorobiphenyl
PCB-170	2,2',3,3',4,4',5-Heptachlorobiphenyl
PCB-179	2,2',3,3',5,6,6'-Heptachlorobiphenyl
PCB-180	2,2',3,4',5,5',6-Heptachlorobiphenyl
PCB-183	2,2',3,4,4',5',6-Heptachlorobiphenyl
PCB-187	2,2',3,4,4',5,5'-Heptachlorobiphenyl
PCB-189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
p,p'-DDE	4,4'-dichlorodiphenyldichloroethylene

The solvents n-hexane (Optima grade, >99.9%), dichloromethane and acetone (GC grade) were purchased from Fisher Scientific (Hanover Park, Illinois). Other chemicals including sodium hydroxide (1 N) and concentrated sulfuric acid. Florisil (60–100 mesh), silica gel (100–200 mesh, Davisil Grade 644), and anhydrous sodium sulfate were also purchased from Fisher Scientific. Silica gel, florisil and sodium sulfate were activated by heating in an oven at 150°C overnight before use.

Individual PBDE standards (BDEs 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209) were purchased from Cambridge Isotope Laboratories (Andover, Massachusettes). A mixture of 32 PCB congener (CBs 8, 28, 37, 44, 49, 52, 60, 66, 70, 74, 77, 82, 87, 99, 101, 105, 114, 118, 126, 128, 138, 153, 156, 158, 166, 169, 170, 179, 180, 183, 187, and 189), which was formulated for food and human tissue analysis, was purchased from AccuStandard (New Haven, Connecticut). Also purchased from AccuStandard were DDE, 2,2',3,4,4',5,6,6'-octachlorobiphneyl (PCB-204), 4'-fluoro-2,3',4,6-tetrabromodiphenyl ether (FBDE-69), 4'-fluoro-2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether (FBDE-208), decabromobiphenyl 209 (BB-209), ^{13}C labeled 2,2',5,5'-tetrachlorobiphenyl (CB-52L), ^{13} C labeled 2,2',4,4'-tetrachlorobiphenyl (CB-47L), and ^{13}C labeled 2,3,3',4,4',5,5',6-octachloriobiphenyl (CB-205L).

4.3.2 **Sample Pretreatment**

The laboratory procedure used in this work was initially developed for analyzing PBDEs in placenta tissue by Dassanayake et al. (2009), and later adopted and optimized by Nanes et al. (2014) for PBDEs, PCBs, and DDE. Prior to analysis, the samples were defrosted, and the wwt was measured. The samples were then transferred into pre-cleaned freeze-dried flasks and chopped into small pieces using a pair of surgical scissors. The samples were refrozen overnight at -20°C. The samples were lyophilized and stored in airtight desiccators until further preparation.

The extraction of the specimens and cleanup of the extract were conducted by the optimized MSPD method (Dassanayake et al., 2009). Briefly, the freeze-dried samples were weighed and the dry weight was recorded before transferring into pre-cleaned glass mortars. Then 1 ng of carbon-13 labeled (CB-52L), 1 ng FBDE-69, and 2 ng of FBDE-208 were added onto the tissue as surrogate (recovery) standards. Pre-cleaned Florisil was added in a 2:1 sorbent-to-dry-tissue ratio and the mixture was pulverized using a glass pestle for 5 to 7 min.

Matrix solid phase dispersion extraction columns (305 mm long x 13.4 mm i.d.) were prepared by adding from bottom to top: glass wool, 10 g anhydrous sodium sulfate, and 4 g activated Florisil. The column was rinsed with 20 mL of 4:1 hexane-to-DCM mixture and vacuum dried. The tissue-sorbent mixture was then added to the column and the sample was eluted with 120 mL of 4:1 hexane-to-DCM mixture. Extract was collected at a flow rate of 1–2 drops per second under gravitational flow. After collection each sample was concentrated by evaporating the excess solvent first in a rotary evaporator and second by a gentle stream of N_2 to approximately 2 mL.

Cleanup of the extract was performed using multilayer silica gel column chromatography. The hexane-filled glass columns (350 mm long x 11 mm i.d.) were packed from bottom to top with glass wool, 1 g anhydrous sodium sulfate, 1 g activated silica, 1 g basic silica, 1 g activated silica, 4 g acidic silica, 1 g activated silica, and 5 g anhydrous sodium sulfate. After packing, the excess hexane was drained up to the level of sodium sulfate and the column was once washed with an additional 20 mL of hexane. The sample was loaded on to the column and the extract was eluted with 50 mL of hexane. The final extract was concentrated and the volume was made to 1 mL using a micro-volumetric flask. The sample was then transferred to 2 mL amber-glass storage vials and kept in the refrigerator until instrumental analysis.

4.3.3 Instrumental Analysis

Levels of PCBs and DDE were analyzed using the Agilent 7890A GC with a 30 m × 0.25 mm × 0.1 μm thickness Rxi-XLB column (Restek Corp., Bellefonte, Philadelphia) coupled with the Agilent QQQMS. Before the injection, the IS (CB-47L) was added. For each run, a total of 60 μL was injected into the GC via a multimode inlet that was operated in solvent vent mode. The initial inlet temperature was held for 0.75 min at 60°C then ramped at a rate of 600°C/min to 300°C. The vent flow was 100 mL/min and the purge flow was 100 mL/min. The column flow was set at 1.1 mL/min using helium as the carrier gas. The oven conditions were as follows: initial temperature of 45°C held for 2 min, increased to 150°C at 10°C/min, increased to 200°C at 2°C/min followed by an increase to 300°C at 10°C/min and held for 2.5 min. The interface temperature was set at 300°C. For detection, the EI source temperature was set at 230°C, and the ionization voltage was -70 V. The temperature of both MS-1 and MS-2 analyzers was set at 150°C. The collision cell gases were helium at 2.25 mL/min and nitrogen at 1.5 mL/min. Multiple reaction monitoring was used for data acquisition.

For PBDE analysis, an Agilent Model 6890 GC was used with a 15 m × 0.25 mm × 0.10 μm thickness Restek Rtx-1614 capillary column. Before the injection, IS CB-205L and BB-209 were added. The temperature of the PTV inlet was held at 40°C for 1 min then increased at a rate of 600°C/min to 300°C and held for 5 min. The vent flow was 100 mL/min and the purge flow was 100 mL/min. A total of 60 μL of each sample was injected. The carrier gas helium was set at 1.5 mL/min constant flow. The oven conditions were: 80°C for 3 min, increased to 300°C at 10°C/min and held for 10 min. Electron capture negative ionization MS in selected ion monitoring mode was

used for PBDE detection. Methane was used as the reagent gas. The MS ion source, quadrupole, and interface were set at 200°C, 150°C, and 300°C, respectively.

Quantification was performed by the IS method using Agilent MassHunter and ChemStation software. The response factors for individual analytes were obtained using standard calibration curves with 6–7 points. All the PBDE data acquired by the ChemStation software on GC/MS 6890/5973 were converted using method translation to the software MassHunter for the quantitation.

4.3.4. **Quality Control**

One procedural blank was analyzed with each batch of 10–15 samples. The levels of analytes in the blanks were <5% of for majority of the samples (Appendix B, Table XLVIII). The LOD for every compound was calculated using the RSD method with seven replicates. The LODs the detection rates are presented in the Table XLIX and Table L of Appendix B.

Three surrogate standards were added to each sample prior to extraction, including CB-52L, FBDE-69, and FDBE-208, to monitor the analytical accuracy of PCBs/DDE, tri- to heptaBDEs, and decaBDE, respectively. The surrogate recoveries are given in Table LI of Appendix B. As shown in Figure 30 (Appendix B), interpersonal difference existed for the recoveries of FBDE-208. Therefore, the reported concentrations of BDE-209 were corrected based on FBDE-208 recoveries. Standard Reference Material SRM 1947 (Lake Michigan fish tissue) was purchased from NIST. It was analyzed in triplicate, and the results are summarized and compared with the certified values (Appendix B, Table LIII).

4.4 Results and Discussion

4.4.1 Samples

All 384 samples received from PPC were extracted and analyzed. Analytical results for a few samples were unrecoverable due to experimental and sample handling errors. Thus final results are available for 376 samples for PCBs/DDE and 374 for PBDEs.

According to the study protocol, each placenta was to be sampled twice; the first sample (T1) was taken at the hospital, and the second sample (T2) was taken at the PPC.Thus there were 177 placentas which were sampled repeatedly. The effect of collection time on concentration of analytes in placenta was evaluated by examining the concentrations of Total PCBs (Σ_{32}PCB), Total PBDEs (Σ_{10}PBDE), DDE, and BDE-209 in T1s and T2s. Paired sample t-test at α=.05 was used to compare the means of T1 and T2 data sets. None of the analytes showed significant difference between the means for T1 and T2 ($p>.05$). Further data analyses were performed on sample set T1 as it is considered the most representative of the two. Only when data for sample T1 were missing, were the T2 data taken into consideration. Thus a total of 191 placentas were available for further data analysis.

All the analyte concentrations were initially expressed in pg/g wwt. As the optimized MSPD extraction method limits the coextraction of lipids, simultaneous lipid determinations were impossible in this study. Limited sample volume made it impractical to perform another extraction for lipid determination. Lipid normalization is important to compare results across different studies. Though the recommended practice by EPA is to present chemical concentration in fresh weight basis and lipid levels separately, most studies have lipid normalized concentrations only. In the absence of tissue lipid content it is acceptable to use an appropriate proxy lipid value (Rogan et al.,1986; DeKoning and Karmaus, 2000). Placenta lipid content varies from 1% to 1.5% in most

published studies. In an experiment where 51 placenta samples collected in Chicago, Illinois were analyzed for lipid content we found the average placenta lipid content to be 1% to ± 0.3% (chapter 5), which is well within the range of literature values. Based on this finding, 1% of lipid content can be suggested as a reasonable substitute to convert placental wwt-based chemical concentrations into lipid-based concentration for the purpose of interstudy and intercompartmental comparisons.

4.4.2 Quality Asurance and Quality Control

Limits of detection (based on 10g sample) for tri-heptaBDEs ranged from 0.06 to 0.84 pg/g wwt; LOD for decaBDE was 1.95 pg/g wwt. Concentrations below LOD were replaced by the value equal to LOD/√2 before natural log transformation. Median surrogate standards recoveries for FBDE-69 and FBDE-208 were 92% and 99% respectively. Even though FBDE-208 median recovery was excellent, there were some values beyond mean + 3x standard deviations. The BDE-209 results were corrected based on the recoveries of surrogate FBDE-208. A significant background contamination was not observed in the blank analysis though BDEs 47, 99, and 209 were often found in the blanks. Background levels of PCBs were negligible.

4.4.3 Polybrominated Diphenyl Ethers

In all placenta samples BDEs 28, 47, 99, 100, 153, and 209 were detected. The least frequently detected was BDE-66 which was present in only 60% of the samples. The measured concentrations of PBDEs were not normally distributed, and the data were natural log transformed prior to further data analysis. Total PBDE concentration (Σ_{10}PBDE) was calculated by summing up the concentrations of the 10 individual congeners. Table XV summarizes the congener specific concentrations and Σ_{10}BDE in the placenta specimens analyzed in this work. The congener distribution is presented in Figure 12. In the RTX 1614 column BDEs 28 and 33 coeluted and therefore are reported together. The analysis of BDE-154 may be interfered by the presence of

2,2’,4,4’,5,5’-hexabromodiphenyl (BB-153), because these two compounds could not be completely resolved by the GC/MS method used in this study.

Median total PBDE of the 10 congeners was 190 pg/g wwt and for tri-heptaBDEs it was 91 ng/g wwt. These concentration levels were lower than the corresponding data of 330 ng/g wwt and 291 ng/g wwt obtained in the P2-18 pilot study that was completed before the Main Study (Nanes et al., 2014). The time gap between the sampling of the two studies was approximately one year. Though it seems unrealistic to have such a decrease in concentration within one year, Zota et al. (2013) have reported a similar observation. According to their work, total PBDE levels in serum of pregnant women from San Francisco had decreased by 60% from 2008–2009 to 2011–2012. This might be a reflection of the effectiveness of the restrictions imposed on the usage of PBDEs, especially the phaseout of penta and octa commercial mixtures in 2004.

Comparison on lipid normalized (1% lipid content) total PBDE level (19 ng/g lw) revealed that Main Study placental PBDE levels are about an order of magnitude higher than the levels reported in Europe (0.64–1.7 ng/g lw), China (1.02 ng/g lw) and Japan (0.64 ng/g lw) (Main et al., 2007; Strandman et al., 2000; Gómara et al., 2007a, 2007b; Leung et al., 2010; Takasuga et al., 2006) and close to the occupational exposure of 19.5 ng/g lw reported for e-waste workers in China (Leung et al., 2010). This is in line with the PBDE measurements in other matrices in the United States, which are often more than a few times higher than the reported values from other countries (Mazdai et al.,2003; Harrad et al., 2010; Lober, 2008; Schecter et al., 2006; Lenters et al., 2013).

TABLE XV

INDIVIDUAL CONGENER CONCENTRATIONS OF 10 PBDES IN HUMAN PLACENTA ANALYZED IN P-18 MAIN STUDY

Congener	Average	Min	10%ile	25%ile	Median	75%ile	90%ile	Max	% of Total*
BDE-28+-33	2.2	0.2	0.8	1.3	1.9	2.8	4.1	7.9	1
BDE-47	54.8	5.4	14.4	22.9	41.8	63.6	100.3	361.4	23.9
BDE-66	0.6	0.2	0.2	0.2	0.4	0.9	1.4	3.0	0.3
BDE-100	15.9	1.0	2.8	4.8	10.4	21.1	32.7	172.6	6.9
BDE-99	31.7	0.8	3.7	6.3	12.7	56.0	84.9	161.2	13.8
BDE-85	2.7	0.4	0.4	0.8	2.2	3.6	5.2	18.8	1.2
BDE-154	5.2	0.4	0.8	2.0	3.4	6.6	11.8	72.0	2.3
BDE-153	27.8	2.7	6.9	10.2	16.4	29.8	54.7	251.3	12.1
BDE-183	1.8	0.3	0.5	1.0	1.5	2.5	3.2	6.5	0.8
BDE-209	86.2	10.3	28.8	40.1	58.0	111.8	179.9	612.5	37.6
Σ_{10}BDEs	228.9	54.4	87.6	119.2	189.9	305.7	433.3	867.3	

*: Calculated as a percentage of average Σ_{10}BDEs, N=191, Concentration units are pg/g wwt

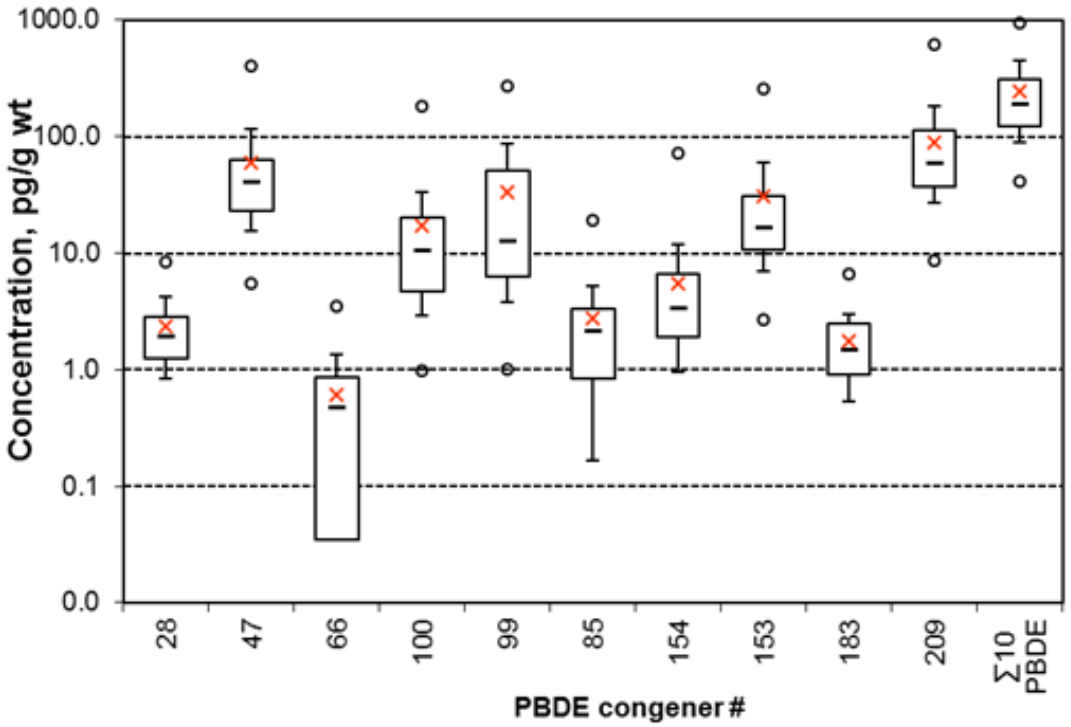


Figure 11. Box and whisker plot of concentration of 10 PBDE analyzed in the P-18 Main Study. (Mean (x), Median, interquartile range, minimum and maximum.)

DecaBDE (BDE-209) was observed as the most abundant among all congeners with a 38% of the total PBDE concentration (Figure 11). This finding was different from several previous studies from the United States where BDE-47 was reported as the dominating congener (Nanes et al., 2014; Dassanayake et al., 2009; Mazdai et al., 2003; Sjödin et al., 2001). Rawn et al. (2011) reported similar results for Canadian placenta where BDE-209 contributed more than 50% to the total. DecaBDE was found to be the major congener in placenta samples analyzed in Spain and Denmark, China and Japan (Frederiksen et al., 2009; Gómara et al., 2007; Zhao et al., 2013; Takasuga et al., 2006), reflecting the pattern of PBDE consumption in Europe and Asia where decaBDE was more prevalent in contrast to North America where the penta product was heavily used. After the penta and octa products were removed from the North American market in 2004,

deca remained as one of the major flame-retardants used for commercial products till 2013. The extended use of deca, in which BDE-209 was the main component, in the absence of penta, might explain the variation in relative abundance of PBDE congeners in placenta.

The Pearson correlations (performed on natural logarithm transformed data) among congeners and with Σ_{10}BDE were examined and the results are shown in Table XVI. All the PBDE congeners were significantly correlated with the Σ_{10}BDE. DecaBDE was the least related to other congeners. It showed negative correlations with BDEs 28, 153, and 183 although they are not statistically significant ($p>.05$). The highest association with Σ_{10}BDE was shown by BDE-100 followed by BDEs 47, 85, and 99 (0.90, 0.89, 0.88, and 0.82 respectively).

TABLE XVI

PEARSON CORRELATIONS MATRIX OF PBDES MEASURED IN MAIN STUDY PLACENTA

	BDE28+33	BDE47	BDE66	BDE100	BDE99	BDE85	BDE154	BDE153	BDE183	BDE209
BDE-47	0.69***									
BDE-66	0.47***	0.67***								
BDE-100	0.44***	0.89***	0.60***							
BDE-99	0.21**	0.73***	0.63***	0.83***						
BDE-85	0.49***	0.75***	0.68***	0.75***	0.73***					
BDE-154	0.20**	0.41***	0.41***	0.48***	0.50***	0.56***				
BDE-153	0.26**	0.44***	0.24**	0.59***	0.34***	0.39***	0.30***			
BDE-183	0.37***	0.30***	0.35***	0.27**	0.11	0.50***	0.20**	0.20**		
BDE-209	-0.06	0.15*	0.19*	0.13	0.20*	0.05	0.22*	-0.03	-0.09	
Σ_{10}PBDE	0.56***	0.89***	0.77***	0.90***	0.82***	0.88***	0.66***	0.56***	0.44***	0.25**

*: p<.05, **: p<.01, ***: p<.001; Correlation analysis was performed on natural log transformed PBDE concentrations.

Main study placentas were collected from seven counties in six states in the United States. Total PBDE concentration for each location is presented in Table XVII. Summary statistics of the Total PBDE concentration distribution for the seven collection sites are shown in Figure 12. Among the collection sites, the highest median PBDE concentration (290 pg/g wwt) was detected for Duplin County, North Carolina, whereas the lowest was seen for Montgomery County, Philadelphia (130 pg/g wwt). The variation in Total PBDE (Σ_{10}PBDE) or Σ_{3-7}PBDE concentration was not significantly different among sites (ANOVA, p=.27, and 0.15). Upon comparing PBDEs on homolog levels, statistically significant differences were found for site-specific tri, hexa, hepta, and deca homolog concentrations (ANOVA, $p<.05$). Penta- or tetraPBDEs do not differ significantly among sites. The results of the multiple means comparison are given in Table XVIII with the location pair and the corresponding BDE homolog that is significantly different for the tested pair.

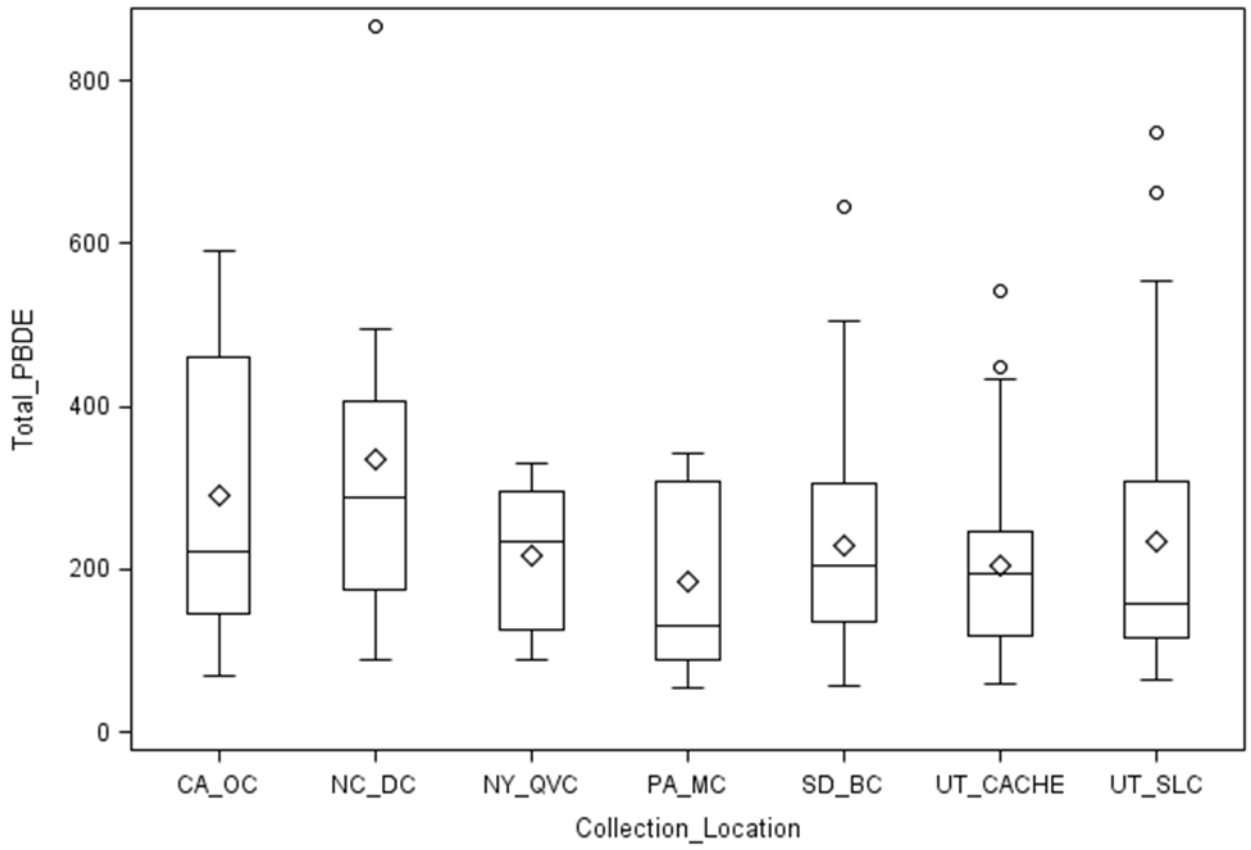


Figure 12. Summary statistics box plot of Total PBDE (in pg/g wwt) variation by collection site. (Mean (diamond), Median, interquartile range, minimum and maximum.)

TABLE XVII

BASIC STATISTICS OF TOTAL PBDE (Σ_{10}BDE) LEVELS IN PLACENTA COLLECTED FROM DIFFERENT LOCATION

Collection Location	N	Mean	Min	Max	5th Percentile	Lower Quartile	Median	Upper Quartile	95th Percentile
CA_OC	17	290.5	69.8	592.0	69.8	145.1	221.6	460.0	592.0
NC_DC	10	335.2	89.4	867.3	89.4	175.0	289.5	407.6	867.3
NY_QVC	8	216.6	88.2	331.2	88.2	126.0	234.4	296.5	331.2
PA_MC	23	185.8	54.4	342.6	67.8	88.2	130.0	307.6	340.9
SD_BC	53	229.4	56.4	645.0	74.5	136.2	205.6	305.7	481.7
UT_CACHE	51	204.5	59.5	541.6	84.1	117.6	194.7	246.1	433.3
UT_SLC	31	235.4	65.5	737.4	70.9	115.6	157.9	308.9	663.6

TABLE XVIII

PBDE HOMOLOG/S AND THE CORRESPONDING SAMPLING SITES WITH SIGNIFICANT CONCENTRATION DIFFERENCES

Sampling location pair tested	PBDE homolog with significant concentration variation (p<.05)*
CA_OC / SD_BC	TriBDE
CA_OC /NY_QVC	TriBDE
UT_CACHE / SD_BC	TriBDE, DecaBDE
UT_CACHE/ NY_QVC	TriBDE
UT_CACHE/ UT_SLC	HeptaBDE
NC_DC / NY_QVC	HexaBDE

* Result of the post hoc multiple means comparison (Tukey HSD) analysis between sampling location and different homolog concentration.

TABLE XIX

CONCENTRATIONS OF PBDES IN PLACENTA TISSUE *

Population location	Year	Number of placentas	Number of BDE congeners	Median $\sum_{3-7}$BDEs (ng/g lw)	Median BDE-209 (ng/g lw)	Lipid (%)	Median $\sum_{3-7}$BDEs (pg/g ww)*	Median BDE-209 (pg/g ww)*	Congener concentration ranking	References
USA	2011–2012	191	10	NA	NA	NA	98.8	58.0	209>47>153>99	This Study
USA	2011	42	10	NA	NA	NA	291	29.4	47>153≈99>209	Nanes et al., 2014
USA	2009–2010	24	10	6.6	3.2	0.98	65.4	26.7	47>209>153>99	This Study, Chicago
China	2009–2011	31	9	3	2.64	1.38	40.99	29.5	209>197>153>47	Zhao et al., 2013
USA	2007–2008	5	42	NA	NA	NA	1205	18.8	47≈99>153	Dassanayake et al., 2009
Denmark	2007	50	12	1.22	1.14	1.21	14.8	13.8	209>47≈153	Frederiksen et al., 2009
China	2005–2007	130	39	0.54	NA	NA	5.4	NA	47>153≈99	Ma et al., 2012
China	2005	5	36	19.5	NA	NA	195	NA	47>153>99	Leung et al., 2010 (e-waste site)
China	2005	5	36	1.02	NA	NA	10.2	NA	47>153>99	Leung et al., 2010 (reference)
Spain	2003–2004	30	15	0.65	1.0	0.7	4.6	7.0	209>47>153	Gómara et al., 2007
Denmark	1997–2001	129	14	1.31	NA	1.09	14.3	NA	153≈47	Main et al., 2007
Finland	1997–2001	56	14	1.18	NA	1.21	14.3	NA	47>153	Main et al., 2007
Japan	NA	10	25	0.25	0.32	3.6	9.0	11.5	209>47>153	Takasuga et al., 2006
China	NA	6	7	2.73	NA	NA	NA	NA	47	Zhang et al., 2008

* lw = lipid weight-based, wwt = wet weight-based. Conversion: wwt (pg/g) = lw (ng/g) × 1000 × Lipid% / 100, where Lipid% is the median or average lipid content reported in each cited literature paper. In the cases where the Lipid% is not available, value of 1% is used. The table was extended from Nanes et al., 2014.

Table XIX summarizes the concentrations of PBDEs in placenta tissue reported worldwide to date. The same data were used to generate Figure 13. The general declining trend of PBDE levels in placenta in the United States (green in Figure 13) from 2007 to 2012 is apparent from these results. Still the concentrations in the United States are much higher (about 10 times) when compared to other parts of the world. While total PBDE is on the decrease, the steady increase is seen for BDE-209 in US placentas. Placenta studies especially for PBDEs are still scarce. Even with the studies available, direct comparison is difficult due to the variability in study protocol. For example, in Table XIX, the number of congeners analyzed, sample size, data interpretation (normalization based on lipid content), are highly variable from study to study.

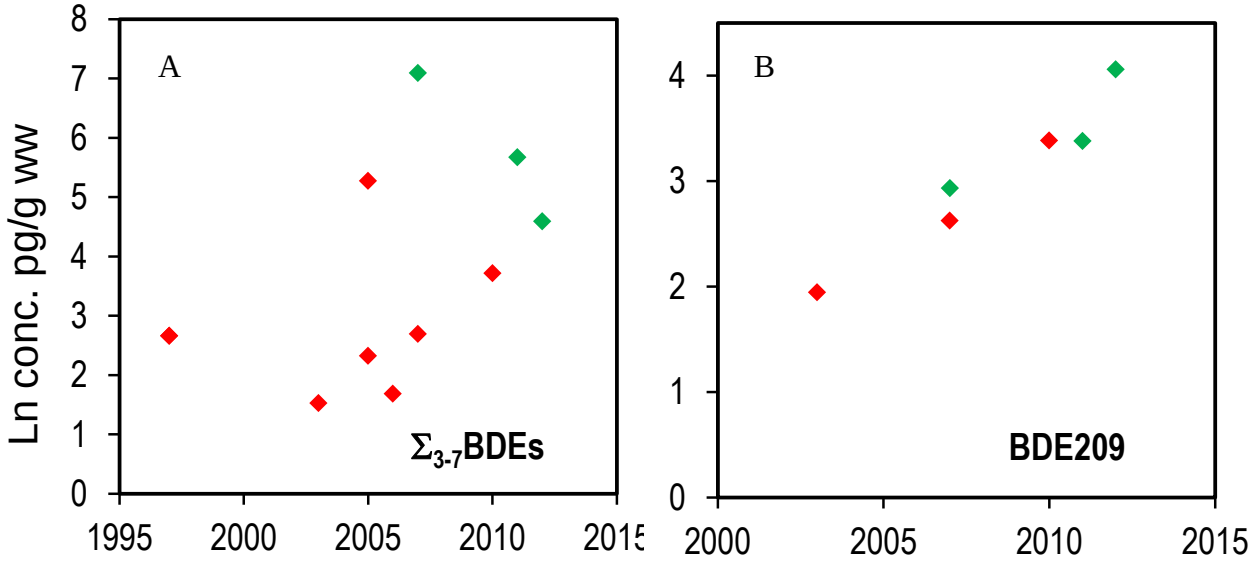


Figure 13. Continental and temporal variation of PBDE concentration in placenta. Panel A: Tri-hepta Total BDEs. Panel B: DecaBDE. Green diamonds: USA, Red diamonds: Asia and Europe, horizontal axis represents the year of sample collection.

4.4.4 **Polychlorinated Biphenyls**

Detection limits for PCB ranged from 0.45 to 0.81 pg/g wwt. Most congeners had a 100% detection rate. The least-frequently detected congeners were CBs 166, 169, 126, and 128. Nondetects were replaced by a value equal to LOD/√2 before the ln-transformation of data to achieve normal distribution in order to perform parametric statistical analysis. Identification of CBs 37 and 77 was problematic due to suspected coelution of an unknown compound. Their peaks on GC/QQQMS chromatogram often failed to meet the qualifier ion criteria. Table XX and Figure 14 show the PCB concentration distribution in 191 placenta samples collected in the Project 18 Main Study. Median Total PCB concentration was 679 with a range of 217–1289 pg/g wwt. Among the 32 congeners measured, CB-101 and CB-118 showed the highest individual concentrations. They collectively contributed 23% to Σ_{32}PCB. Among the more abundant congeners were CBs 70, 52, 87, 153, and 138. Figure 16 presents the homolog contribution to the total PCB concentration. Penta and tetra homologs contributed to the Total PCB the most with 41% and 30% respectively. The PCB congener and homolog pattern in the Main Study showed a deviation from that of the pilot study. In the pilot study the major contributors to the total were CB-52 and the tetra homolog group. In contrast to that, in this study, the penta group and CBs 101 and 118 are dominant. The PCB congener profile reported in published literature varies considerably but often had CBs 101, 118, 153, and 138 as major congeners in the congener profile of placenta (Bergonzi et al., 2009; Gómara et al., 2012; Reichrtová et al., 1999). Two of these studies reported CB-52 as high in abundance.

TABLE XX

SUMMARY STATISTICS OF 32 PCBS AND TOTAL PCB (Σ_{32}PCB) CONCENTRATION IN MAIN STUDY PLACENTA (N=191)

Congener	AM	GM	Min	Max	10th %ile	25th %ile	Median	75th %ile	90th %ile
CB-8	18.4	16.4	3	67.8	7.9	10.2	15.5	24	33
CB-28	25.9	22.2	6.1	74.3	11.7	14.4	21.9	34.2	45.3
CB-52	46	40.4	13.7	142	22.3	28.1	39.9	59.9	75.8
CB-49	18.3	16.4	5.5	54.4	8.7	10.7	16	23.7	30.6
CB-44	34	30.0	9.9	100	16.3	20.6	29.3	43.9	57.3
CB-37	13.3	11.0	2.9	43.9	4.9	6.8	10.7	19.1	24.6
CB-74	24.6	22.2	6.9	82.4	11.7	14.3	21.8	32.4	40.5
CB-70	62.9	54.6	16.4	184.2	27.3	35.6	55.4	84.5	111.5
CB-66	30	27.1	8.1	88.1	13.2	17	25.9	39.7	53.8
CB-60	7.8	6.7	1.7	24.8	3.1	4.3	6.6	10.5	14.4
CB-101	93.1	81.5	24.9	283.4	40.1	55.7	84.9	115.6	152
CB-99	39.7	33.1	11.2	124.3	18	23.7	35.8	51.1	65.2
CB-87	51.6	44.7	12.8	189.1	20.4	29.6	46.5	64.2	91.1
CB-82	10.6	9.0	2.5	41.3	4.2	5.7	8.8	13.3	20.1
CB-77	2.6	2.0	0.5	10.6	0.8	1.2	2	3.5	5.6
CB-118	79.9	66.7	20.6	327.8	33.7	45.3	67.9	99.3	142.8
CB-114	2.3	1.8	0.5	18.2	0.9	1.2	1.9	2.9	3.9
CB-153	51	44.7	11.9	214.2	22.9	32.4	46.3	60.1	84.8
CB-179	2	1.6	0.4	9.3	0.8	1.2	1.7	2.6	3.6
CB-105	28.9	24.5	7	163.3	11.2	15.5	23.3	36.9	52.2
CB-138	40.1	36.6	9.5	172.3	17.9	23.9	36	48.4	67.7
CB-158	3.9	3.3	1	17.8	1.6	2.1	3.2	4.8	7
CB-187	9.2	7.4	1.7	74.8	3.7	5.7	7.7	10.9	15.1
CB-166	0.5	3.0	0.5	1.3	0.5	0.5	0.5	0.5	0.5
CB-183	3.6	4.1	0.9	22.3	1.6	2.2	3.1	4.3	5.6
CB-156	7	5.0	0.6	72	1.8	3.2	5.1	7.9	14.4
CB-180	23.5	18.2	2.5	184.3	6.8	11.1	18	27.2	42.9
CB-170	10.9	7.4	0.8	102.6	2.8	4.7	7.7	11.9	20.5
Total_PCB	749.4	665.1	216.6	2371	341	453.1	678.5	968	1289.2

AM: Arithmetic mean, GM: geometric mean

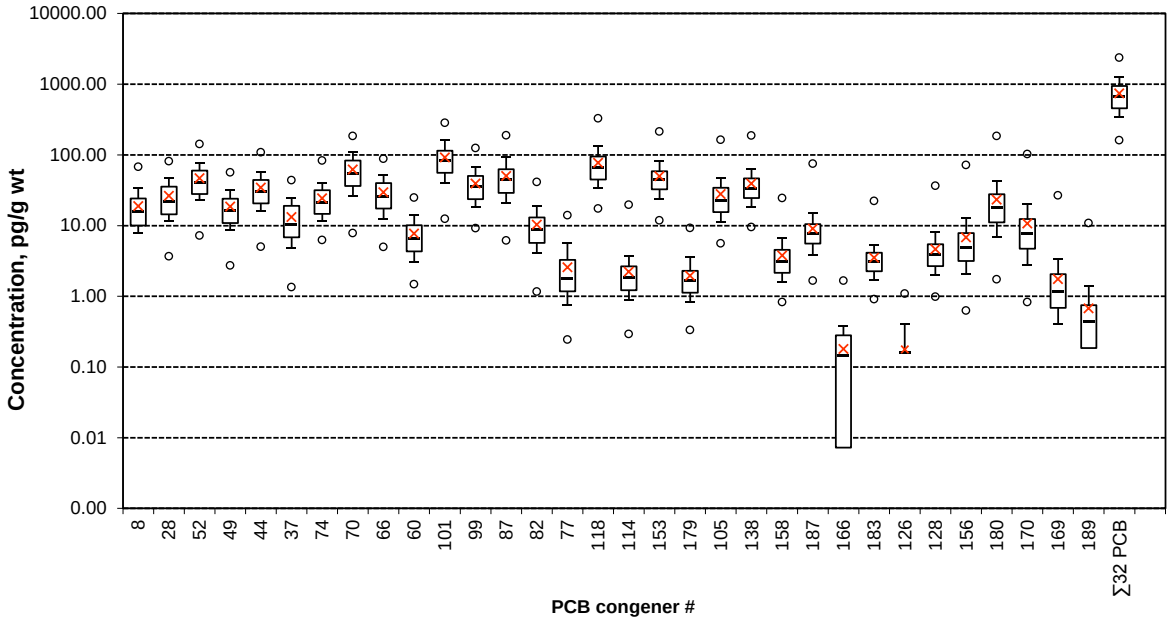


Figure 14. PCB congener distribution in placenta samples collected in the P-18 Main Study.

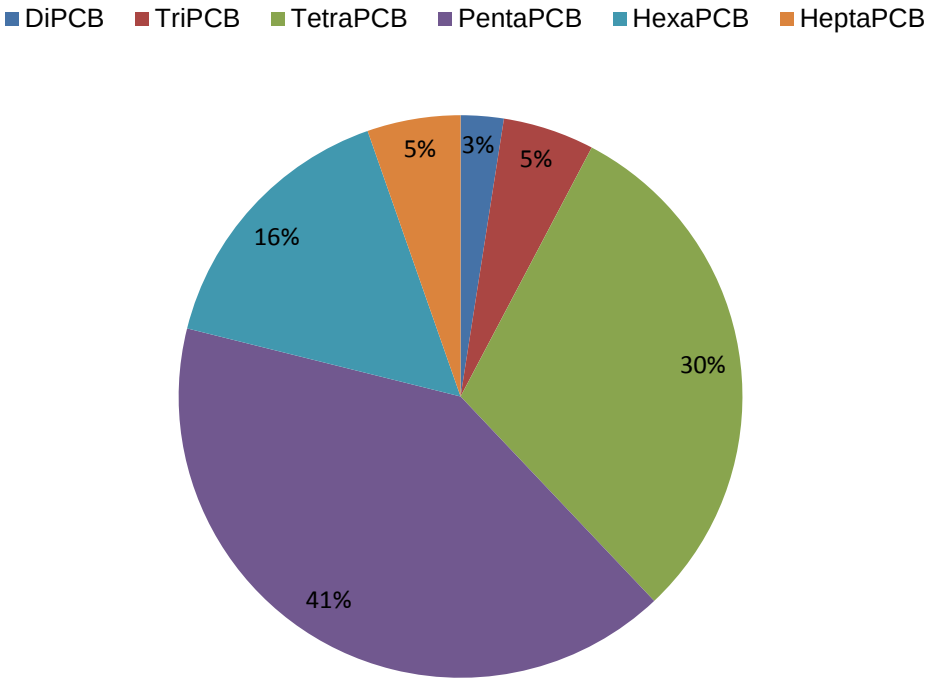


Figure 15. PCB homolog contribution (as a % Total PCB) to the Total PCB in placenta collected in the Main Study.

Collection site-related PCB concentrations are given in Table XXI and their distribution is shown in figure 16. Total PCB (Σ_{32}PCB, Table XXI), Total indicator PCB (Σ_7 of CBs 28, 52, 101, 118, 153, 138, and 180) and Total Dioxin-like PCB (Σ_8 of CBs 77, 105, 114, 118, 126, 156, 169, and 189) levels were compared using the mean concentration (GM) for each collection site. The ANOVA result showed marginal significance for Total PCB (p=.052). Both Total indicator and Total Dioxin-like PCB were found to be significantly different for collection sites (p=.036, 0.038 respectively). Pairwise comparison revealed that Cache County, Utah was significantly higher than Salt Lake County, Utah on all three measures. It would be interesting to study these two counties located within the same state especially with regard to socioeconomics to find a reasonable cause for this difference. Birth weight and gestational age did not show a difference between the two counties (p=.59, 0.82). Salt Lake County study population is predominantly non-Hispanic white. Unfortunately, further comparisons were not possible because information other than birth weight and gestational age was entirely missing for Cache County.

TABLE XXI
SUMMARY STATISTICS OF TOTAL PCB CONCENTRATION IN PLACENTA SAMPLES FROM DIFFERENT COLLECTION SITES

Location	N	GM	Min	Max	10th%ile	25th%ile	Median	75%ile	90thile
CA_OC	17	723.6	308.3	1784.0	333.6	673.5	719.8	857.7	1324.2
NC_DC	10	580.0	341.0	1315.6	349.6	366.0	547.8	799.2	1196.0
NY_QVC	8	773.8	499.7	1345.6	499.7	576.4	720.1	1053.2	1345.6
PA_MC	23	737.3	296.4	1951.9	407.7	453.1	702.5	1076.4	1358.0
SD_BC	51	638.1	248.7	1398.9	344.9	486.3	637.7	885.2	1131.4
UT_CAC	51	733.5	234.5	2371.0	387.2	472.8	698.3	1041.7	1483.0
UT_SLC	31	523.1	216.6	1190.6	269.2	366.5	478.7	748.7	1095.4

Total PCB: sum of 32 PCBs, GM: geometric mean, concentrations are given in pg/g wwt, N: number of placenta from each location

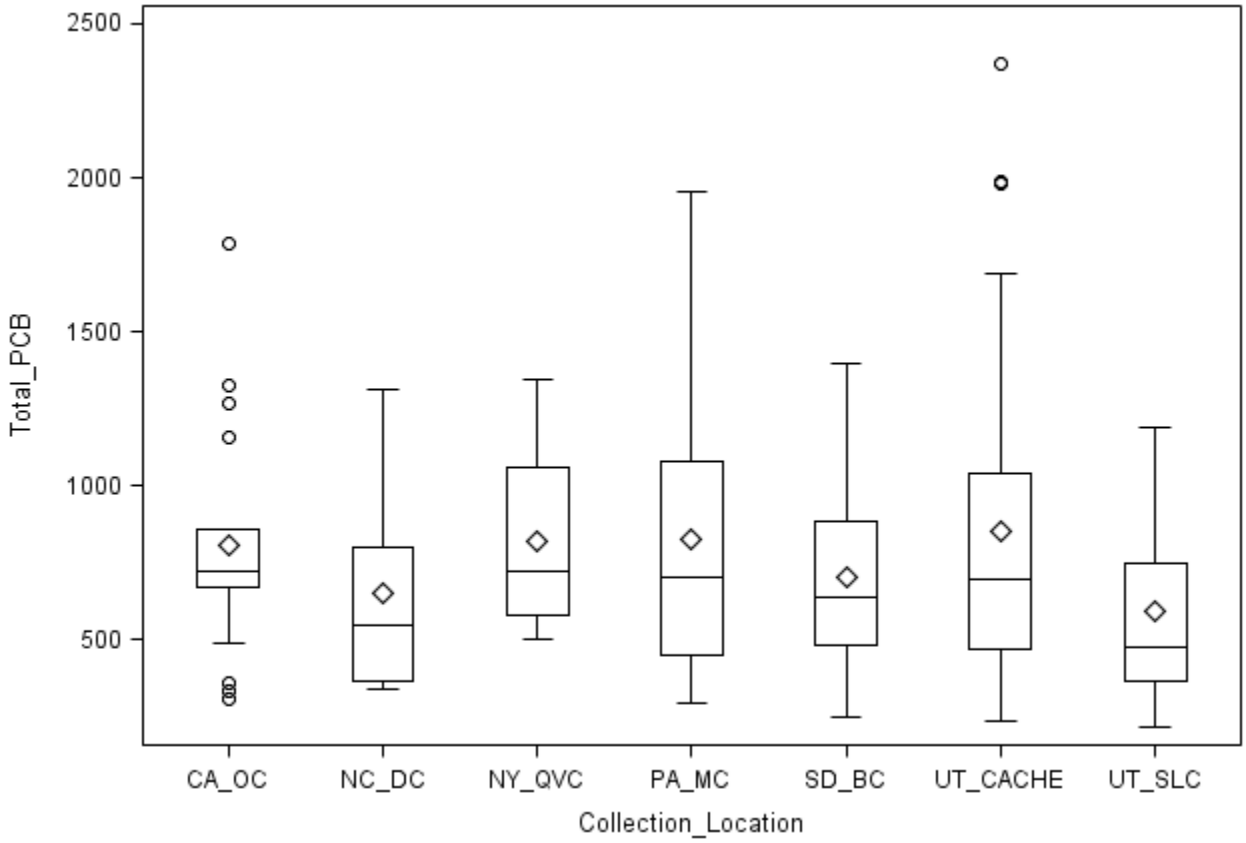


Figure 16. Summary statistics box plot showing the regional variation of Σ_{32}PCB.
(Mean (diamond), Median, interquartile range, minimum, and maximum.)

TABLE XXII

CONCENTRATIONS OF PCBS IN PLACENTA TISSUE *

Country	Year	Number of placentas	Number of PCB Congeners[#]	Median ∑PCBs (ng/g lw)	Reported Lipid (%)	Median ∑PCBs (pg/g ww)	References
USA	2011–2012	191	32	NA	NA	678.5	This Study
USA	2011	42	10	NA	NA	405	Nanes et al., 2014
USA	2009–2010	24	32	27.4	0.98	273.7	This study, Chicago
Brescia, Italy	2006	70	30	92.5	0.97	*897*	Bergonzi et al., 2009
China	2005–2007	130	27	0.89	NA	*8.9*	Ma et al., 2012
Madrid, Spain	2003–2004	17	15	*272*	0.84	2,292	Gómara et al., 2012
Spain	2000–2008	50	12 (DL)	5.46	0.4–1.1	*41*	Fernandez et al., 2012
Central Taiwan	2000–2001	50	12 (DL)	4.33 [a]	NA	*43.6* [a]	Wang et al., 2004
Central Taiwan	2000–2001	50	3 (Ind)	21.3 [a]	NA	*213* [a]	Wang et al., 2004
Taiwan	2000–2001	119	12 (DL)	4.59	0.74	*34.0*	Chao et al., 2007
Taiwan	2000–2001	119	6 (Ind)	22.6	0.74	*167*	Chao et al., 2007
Japan	before 2005	13	12	2.28 [b]	NA	*22.8*	Suzuki et al., 2005
Québec, Canada	before 2002	20	14	173 [a,c]	NA	*1,730*	Pereg et al., 2002
Slovakia (industrial)	before 1999	57	7	NA	NA	NA	Reichrtova et al., 1999
Slovakia (rural)	before 1999	63	7	NA	NA	NA	Reichrtova et al., 1999
Canada	1998–2006	60	7		1.31		Doucet, et al., 2009
(not reported)	before 1996	25	NA	950	NA	*9,500*	DeKoning and Karmaus, 2000
New York, USA	1995–1996	5	3 (DL)	0.018	NA	*0.18*	Schecter et al., 1998
Germany	before 1994	46	NA	248-373	1–1.5	*3,100–4,662*	DeKoning and Karmaus, 2000
United States	before 1986	790	NA	*<9,600*	1–1.5	<12,000	Rogan et al., 1986
Israel	before 1977	19	NA	5027[a]	NA	*50,270* [a]	Polishuk et al., 1977

* lw = lipid weight-based, wwt = wet weight-based. *Italic values* are converted from the reported values using: wwt (pg/g) = lw (ng/g) × 1000 × Lipid% / 100, where Lipid% is the median or average lipid content reported in each cited literature paper. In the cases where the Lipid% is not available, value of 1% is used.

#. DL = dioxin-like PCBs (none-ortho and mono-ortho substituted). Ind = indicator PCBs (all or some of PCBs 28, 52, 101, 118, 138, 153, 180).

a. Average (mean) or geometric mean values, because the median values are not available.

b. Concentrations were originally reported in TEQ values for individual congeners. The total concentration was estimated using ΣPCB = Σ(TEQ/TEF).

c. Estimated from graphics.

This table is an extension from Nanes et al., 2014

Table XXII summarizes the concentrations of PCBs in placenta tissue reported worldwide since 1977. Comparison across these studies is challenging because of the considerable variation in the protocol as well as in the reporting. In general there is an apparent decrease in the concentration from before 2000 to the present. Total PCB concentration in this study is higher than the concentration reported by Nanes et al. (2014) for three US locations. This statement holds true for California, which has been a sampling site in both studies (719 pg/g wwt in this study versus 412 pg/g wwt in Nanes et al., 2014). The reason for this increase is unclear but may be an effect specific to the county where the samples came from.

4.4.5 **Dichlorodiphenyldichloroethylene**

The DDE concentration measured in 191 placenta samples in the Main Study showed an extremely skewed distribution. The concentration spanned from 76 to 4,157 pg/g wwt (Table XXIII). Geometric mean of DDE concentration for all samples was 200 (95% CI, 181–221) pg/g wwt. Normal distribution was not achievable in spite of log transformation of data. The extreme skewness was largely caused by five samples (Figures 17 and 18). The removal of these data assured a near log-normal distribution. Levene's test of equal variance revealed the heterogeneity of variance among sampling locations. Therefore the means were tested by Welch's variance-weighted Anova test. The results showed marginal significance with p=.054. Multiple means comparison (Tukey HSD test) did not indicate any location with significantly different DDE concentration. A nonparametric ANOVA test (Kruskal-Wallis) was also performed using all the 191 measurements including the five extreme values. This test indicated a significant (p=.008) difference in DDE concentrations among sites.

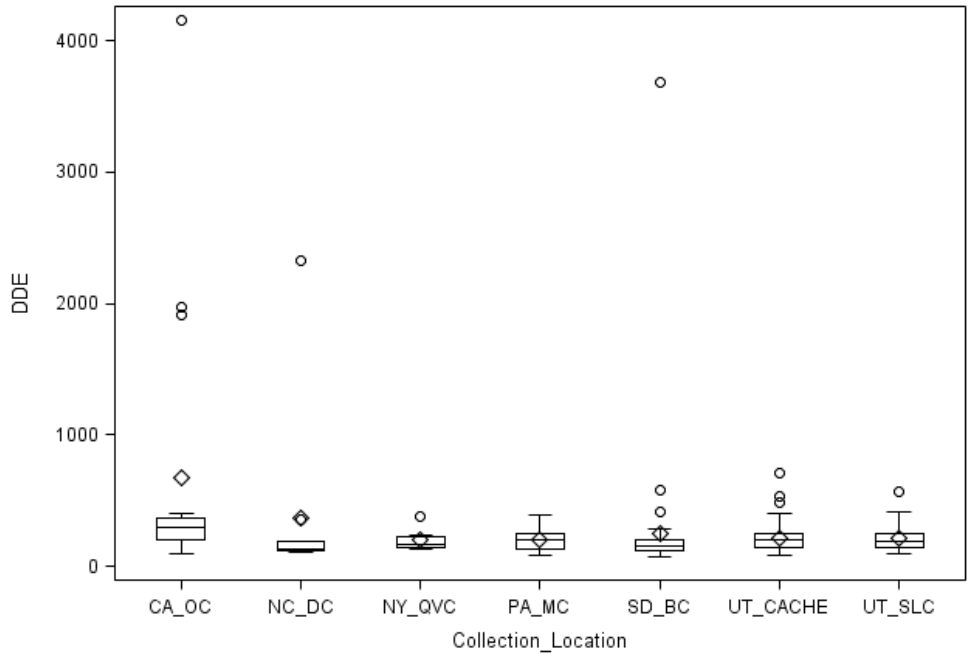


Figure 17. Distribution of p,p'DDE concentration (pg/g wwt) in placenta collected from different sites (N=191).

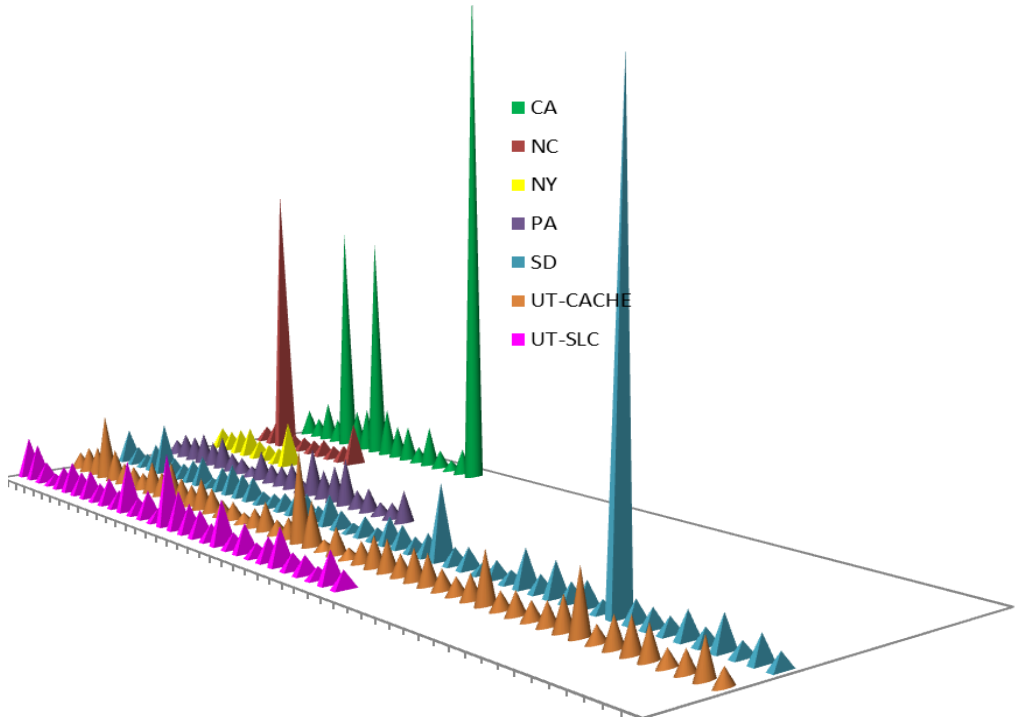


Figure 18. The p,p'DDE concentration of all placenta samples(191) analyzed in the Main Study.

TABLE XXIII

SUMMARY STATISTICS OF p,p'-DDE CONCENTRATION IN PLACENTA COLLECTED FROM DIFFERENT SITES

Collection Site	N	AM	GM	Min	Max	5%ile	25%ile	Median	75%ile	95%ile
CA_OC	17	679.7	365.0	91.7	4157	91.7	200.4	293.7	362.3	4157
NC_DC	10	373.1	200.3	111.8	2326	111.8	115	130.1	186.8	2325.7
NY_QVC	8	197	181.3	131.5	383	131.5	144.4	166.1	219.9	383.2
PA_MC	23	202.7	181.3	87.1	393	96.7	133.2	200.2	250.1	385.2
SD_BC	51	246	181.3	76.2	3679	85.7	125.4	157.2	206.8	418.2
UT_CACHE	51	219	200.3	88.5	709	89.7	145.4	196.7	253.8	483.1
UT_SLC	31	215.4	200.3	97.1	573	98.5	139.3	192.5	254.8	409.5
All sites	191	271.8	200.3	76.2	4157	96.9	137.3	181.6	249.4	450.6

Am: arithmetic mean, GM: geometric mean, concentrations are in pg/g wwt

In spite of the ban more than 40 years ago on the use or manufacture of DDT, its metabolite DDE is still the highest concentration in placenta as a single compound in comparison to PCBs and PBDEs. California, when compared to other sites, had the highest DDE concentration as well as the widest range of exposure to DDE. Three individuals from California had DDE concentrations of more than 2,000 pg/g wwt. This disparity might have been caused by the race/ethnicity differences in the subsamples. California has a larger proportion of Hispanics and immigrants and higher DDE levels have been reported for Hispanics (Perla et al., 2014). This may be particularly true for recent immigrants from Latin America, where regulatory control for DDT is less. The proportion of Hispanics in the California participants is unfortunately unknown in this study and therefore further testing of this theory is not possible. In the P-18 Pilot study also we found higher DDE levels in California samples compared to samples collected from New York and Wisconsin (Nanes et al., 2014). The DDE levels in placenta were found to be significantly correlated with both Total PCB

and PBDE (Spearman r, 0.26, 015; p<.001, p<.05 respectively). Correlations between PCB and DDE are commonly reported in the literature, but such is uncommon for DDE and PBDE. Generally such a correlation is an indication of a similar exposure source. Diet is often discussed as the major source of DDE and PCB, while for PBDE both indoor dust and diet are considered as exposure sources (Zota et al., 2013). As the house dust levels have been decreasing especially after the 2004 pentaBDE ban (Dodson et al., 2012; Stapleton et al., 2012), the contribution of dietary exposure might have become significant for PBDEs. Thus it is possible for DDE and PBDE to have diet as an overlapping exposure source.

In Table XXIV, DDE levels in placenta that have been reported in the published literature are presented. A clear decline in US DDE levels is seen from 1968 to 2012 (Figure. 19). Pilot study and Chicago samples (2009–2010) revealed similar levels of exposure, while an increase from that is seen for Main Study samples. Multiple studies have shown that pollutants found in the environment including DDE have decreased (Daucet et al., 2009; Nanes et al., 2014). However, the variation in the worldwide DDE data is high but the general trend of attenuation is obvious (Nanes et al., 2014).

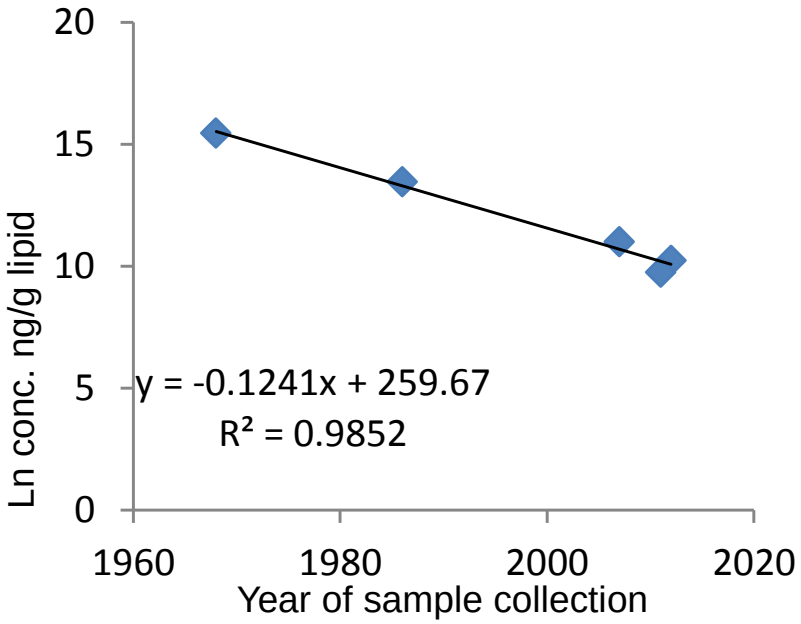


Figure 19. DDE concentration in placenta collected from 1968–2012 in the United States.

TABLE XXIV

CONCENTRATIONS* OF P,P'-DDE IN PLACENTA TISSUE

Population location	Collection Date	*n*	Mean Conc. (ng/g lw)	Median Conc. (ng/g lw)	Lipid content (%)	Mean Conc. (pg/g ww)	Median Conc. (pg/g ww)	Reference
USA	2011–2012	191	*27.2*	*18.2*	NA	271.8	181.6	This Study
USA	2011	42	*16.8*	*7.4*	NA	168	74	Nanes et al., 2014
USA	2009–2010	24	15.4	8.5	0.98	150.9	83.6	This Study, Chicago
India	2009	30	*390*	NA	NA	3900	-	Dewan et al., 2012
India	2009	30	*250*	NA	NA	2500	-	Dewan et al., 2012
USA	Before 2007	19	NA	58.3[#]	NA	-	*583*	Brooks et al., 2007
Brescia, Italy	2006	69	69.3	62.5	0.97	*693*	*625*	Bergonzi et al., 2009
Al-Kharj, Saudi Arabia	2005	1576	*6175*	*3653*	NA	61747	36526	Al-Saleh et al., 2012
Quebec, Canada	Before 2003	30	*5856*	4800	NA	58560	48000	Hamel et al., 2003
Southern Spain	2000	205	*201*	-	NA	2010	NA	Freire et al., 2011
Denmark	1997–2001	43	47.15	NA	NA	*472*	-	Shen et al., 2007
Finland	1997–2001	43	21.23	NA	NA	*212*	-	Shen et al., 2007
Murcia Province, Spain	1998–2000	102	*1770*	*0*	NA	17700	0	Falcón et al., 2004
Bratislava, Slovakia	Before 1999	57	-	*10*	NA	NA	100	Reichrtová et al., 1999
Stará Lubovna, Slovakia	Before 1999	63	-	*10*	NA	NA	100	Reichrtová et al., 1999
North Carolina, USA	Before 1986	790	NA	*677*	1–1.5	-	6770	Rogan et al., 1986
Lucknow, India (stillborn)	1979–1980	9	*1240*	*1150*	NA	12400	11500	Saxena et al., 1983
Lucknow, India (control)	1979–1980	27	*1830*	*1130*	NA	18300	11300	Saxena et al., 1983
Lucknow, India	1978	50	*5054*	-	NA	50540	NA	Saxena et al., 1980
California, USA	Before 1968	39	5000	NA	NA	*50000*	-	Rappolt and Hale, 1968

* lw = lipid weight-based, wwt = wet weight-based. *Italic values* (mean or median) are converted from the reported values (mean or median) using placenta lipid content of 1%# The reported value 58.3 in Brooks et al. (2007) is in the unit of pg/g lw, which could be a typo thus is changed to ng/g lw.

4.4.6 Clinical Data

A comprehensive clinical data abstraction form (Appendix A) was created to collect necessary clinical data for those pregnancies included in Project 18. The intended use of the data was to associate medical and pregnancy outcomes and histories with the analytical measurements. Unfortunately, the result of the clinical data collection was far from satisfactory. Clinical data were sparse and problematic with several variables of high importance (e.g., race, ethnicity, and body mass index) having high proportions of missing data. Nearly complete data were obtained for birth weight, gender, and gestational age at delivery. However, even for the variables with low rates of missing data, there was very little variation. For example, the ethnicity of the cohort is predominantly non-Hispanic, with only 5% of Hispanic (which includes five individuals), thus limiting the use in statistical analysis for any exposures (e.g., toxicants, genetic variants) to be identified as predictors of the clinical variables.

Of the placentas analyzed for environmental toxicants, 193 samples had location information. Birth weight, baby gender, and gestation age data were available for >97% of the participants. Response rates for mother's education, age at delivery, ethnicity, and race were 60%, 59%, 49%, and 50% respectively. Variables with less than 50% response rate were disregarded from further consideration. Race ethnicity data showed little variation with the vast majority being non-Hispanic whites. Among study participants, 53% had a higher educational level (college degree or post-graduate degree). Table XXV shows site-specific clinical data along with the number of participants from each site.

TABLE XXV

AVERAGES AND RANGES OF MATERNAL AGE, GESTATIONAL AGE, AND BIRTH WEIGHT BY COLLECTION SITE

Collection site	N	Age, y	Gestation age, weeks	Birth wt, g
NC-DC	10	25 (21–29)	39.4 (38.1–40.6)	3290 (2586–3845)
SD-BC	53	28 (18–36)	39.6 (37.7–43.7)	3556 (2466–4760)
PA-MC	23	31 (24–39)	39.3 (35.9–41.6)	3372 (2375–4440)
CA_OC	17	NA	39.2 (34.9–41.0)	3452 (2460–4400)
NY_QVC	8	31 (24–37)	39.7 (38.1–42)	3487 (3062–4196)
UT-SLC	31	30 (17–38)	39.3 (34.4–41.3)	3459 (1956–4255)
UT-CACHE	51	NA	39.2 (37.1–41.1)	3483 (2484–4450)

Given as average (range)

TABLE XXVI

PEARSON CORRELATION COEFFICIENTS BETWEEN MEASURED ANALYTE CONCENTRATIONS[#] AND CLINICAL VARIABLES

	Birth weight	Gestational age	Maternal age
Gestational age (wks.)	0.41***		
Maternal age (yrs.)	-0.03	-0.11	
DDE	-0.14	-0.09	0.01
Total PCBs	0.05	-0.001	0.12
Total PBDEs	0.06	0.12	-0.07
CB-138	-0.001	0.00	0.22*
CB-153	-0.03	-0.04	0.3**
CB-156	-0.11	-0.04	0.37***
CB-170	-0.16*	-0.06	0.49***
CB-180	-0.15*	-0.07	0.51***
CB-183	-0.09	-0.06	0.37***
CB-187	-0.12	-0.11	0.42***

#: pg/g wwt, * p <.05, ** p <.01, *** p <.001

Associations between selected clinical variables (maternal age at delivery, gestational age, and birth weight) and measured placental analyte concentrations (natural log transformed) were evaluated using Pearson correlation analysis (Table XXVI). As expected, gestational age and birth weight were positively and significantly correlated. Maternal age showed weak and negative associations with both birth weight and gestational age. Birth weight was negatively associated with DDE (Figure 20) and the association approaches statistical signficance (p=0.06). A regression analysis in a model with Total PCB and Total PBDEs, DDE showed a significant negative association with (unadjusted) birth weight (β=-118, SE=55, p=.03). Birth weight was not adjusted for possible confounders (e.g., maternal smoking, weight gain, parity) due to the unavailability of such information. None of the correlations for Total PCB or Total PBDE were found to be statistically significant. A similar relationship was observed upon comparing correlations on PBDE congener basis.In contrast to PBDE congeners, some of the PCB congeners (Table XXVI) showed strong positive associations especially with maternal age. Interestingly all these congeners were hexa- and heptaPCBs. Birth weight was negatively correlated with CB-170 and CB-180. Some of our findings are inconsistent with the study by Bergonzi et al. (2009 and 2011). They reported positive and significant correlations between maternal age and placenta DDE and Total PCB. A significant negative correlation between Total PCB and birth weight were also previously observed (Bergonzi et al., 2009, 2011).

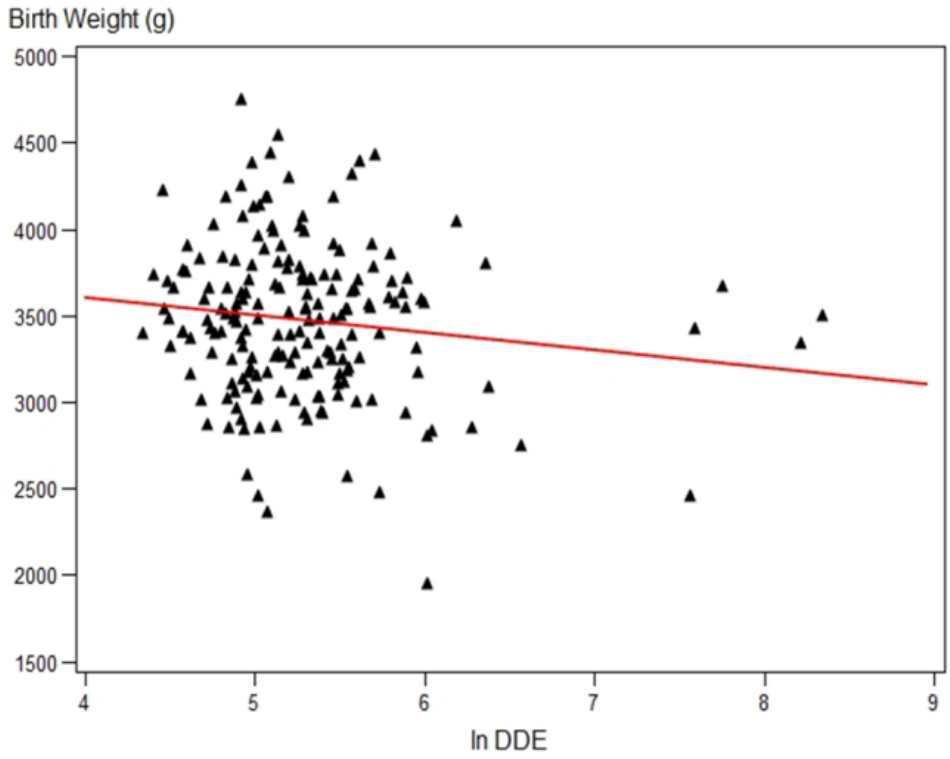


Figure 20. Scatter plot between natural logarithm DDE concentration in placenta and birth weight (with the regression line in red).

5. CHARACTERIZATION OF PLACENTAL TRANSFER OF PERSISTENT ORGANIC POLLUTANTS

5.1 Background

Chemicals made intentionally or unintentionally by man ultimately find their way into the natural environment. Some of these chemicals have properties that make them resistant to degradation or breakdown by natural processes and they therefore accumulate in the environment. Such chemicals are often called POPs. The Stockholm Convention on Persistent Organic Pollutants of the United Nations Environmental Program identified 12 initial POPs including a number of OCPs and PCBs. The later Amendments added nine new POPs to the list, including additional OCPs, some PBDEs, and others. The phase-outs of these chemicals have been accompanied by the syntheses and production of replacement chemicals that could also be environmentally persistent. For example, newer flame-retardant compounds such as BTBPE, DBDPE, and many others, which have chemical structures similar to PBDEs, have been introduced as replacements for PBDEs.

Humans are exposed to these chemicals at every stage of their lives via contaminated air, water, and food. Neonatal exposure predominantly occurs through breast milk (LaKind et al., 2004; Minh et al., 2004). While placenta is considered as a barrier that protects the fetus, research has documented that environmental chemicals in maternal blood can cross the placenta and thus cause prenatal exposures (Sala et al., 2011; Barr et al., 2005, 2007; Waliszewski et al., 2000). The developing fetus is more sensitive and vulnerable to toxicants because the defensive mechanisms against these chemicals are not fully developed in the fetus. Prenatal exposure most probably results in irreversible developmental defects in the newborn. Poor brain and neuro

development, sexual development, and cognitive ability, as well as childhood diabetes, asthma, and obesity are some of the adverse health effects that have been documented as associated with prenatal and neonatal POP exposure (Barouki et al., 2012; Schoeters et al., 2011; Sexton et al., 2014). Discussions on early human exposure to POPs caught much attention worldwide in the recent past. To date there is still a paucity of studies on human prenatal and neonatal exposure assessments. Due to the limitation on the number as well as standardization of studies that exist, evaluation of the evidence to understand the extent and the variety of exposure has been compromised.

The most common and the preferred method to assess the prenatal exposure in utero is to analyze levels of POPs in maternal blood, umbilical cord blood, and placenta and their sequential transfer to the fetus (Viscaino et al., 2014). The mechanisms and the extent of placental transfer of POPs are yet to be fully understood but proposed to be mainly driven by physicochemical characteristics of individual compounds (Pacifici and Nottoli, 1995; Bourget et al., 1995; Barr et al., 2005; Haddad et al., 2000). Studies using placenta and cord blood as matrices, maternal-fetal pairs, and transplacental transfer of POPs are comparatively less. The present study was designed to address the data gap as well as to apprehend the levels of prenatal exposure to POPs in the United States. We aimed to characterize concentration ratios and to gain potential insights into transfer of selected POPs and to understand the role and the efficiency of the placenta as a barrier to prevent prenatal exposure. The data generated by this study will strengthen the global and national databases, and be of significant importance to future investigations of human exposure to POPs, chemical risk assessments, and environmental policy-related decision making.

5.2 Study Design and Sample Collection

This study was designed to understand the current level of exposure in pregnant women to selected legacy and emerging environmental organic pollutants, and to characterize the transfer of such chemicals from mother to the unborn child through the placenta. Another purpose of the study was to evaluate the suitability of placental tissue to use as a surrogate for maternal and fetal (cord) bloods in human biomonitoring for prenatal exposure assessment purposes. Polychlorinated biphenyls and OCPs (including DDT and its metabolites) were included in the study as legacy POPs. As emerging POPs, PBDEs and a suite of novel (alternate) XFRs were selected.

A protocol to collect patient information (health and sociodemographic data), patient blood, umbilical cord blood, and placenta and to analyze the above-mentioned chemicals in these matrices was approved by the Institutional Review Board of UIC. Between October 2010 and November 2011, 122 normal healthy pregnant women (18 years and older, with no known bloodborne infectious disease such as HIV, AIDS, or hepatitis) who were admitted to the UIC Medical Center for child delivery, participated in the study by signing a consent form, donating their blood, umbilical cord blood, and placenta, and responding to questions in an interview. Twenty-four complete sets with maternal blood, cord blood, and placenta samples were randomly selected for the study presented in this chapter.

After a patient consented to participate in the study, 5 mL of her blood was drawn to Vacutainer* serum clear glass vials by venipuncture before the child delivery. The patient was interviewed to obtain demographics, dietary, and socioeconomical and lifestyle information. Cord blood was collected from the umbilical cord. The whole placenta was collected immediately after its delivery. Half of the placenta (including maternal and fetal side from the

middle as well as the periphery excluding calcified areas) and whole blood samples were refrigerated after collection and transferred under chilled condition to the analytical laboratory as soon as possible. Upon arrival, all samples were stored at -20°C until further processing. Placenta samples were later homogenized and freeze-dried using Labconco FreeZone 4.5 Liter Console Freeze Dry System (Labconco, Kansas City, Missouri). The dried placenta samples were covered with aluminum foil to protect from light and stored air-tight in a desiccator at room temperature (20˚C) until further processing. Birth information (birth weight, APGAR score, baby gender, gestational age) were collected from the patients' clinical records.

5.3 Target Analytes Extraction from Samples

5.3.1 Placenta

Placenta extraction and cleanup methods are described in detail in chapter 2. Briefly the method is as follows. Approximately 4 g freeze-dried sample was weighed into a solvent-washed aluminum pan. To the same pan, extraction sorbent Florisil was added in a 1:2 sample-sorbent mass ratio and transferred into a glass mortar. After adding a known amount of surrogate, the sample-sorbent mixture was thoroughly ground using a glass pestle for about 5 min to become a fine powder. A glass column (13 mm id and 30 cm in length) was packed from bottom to top with glass wool, prewashed anhydrous sodium sulfate (10 g), Florisil (4 g), and the prepared sample-sorbent mixture. The column was eluted with 120 mL of 4:1 (v/v) hexane:DCM solvent mixture. Excess solvent was evaporated and the sample was concentrated to about 2 mL. Multilayer silica gel columns were used for the sample cleanup with 100 mL 100% hexane to elute the analytes. Placenta lipid content was determined separately using the method of Folch (1959) on 1 g of dried placenta (see chapter 2 for details).

5.3.2 Maternal and Cord Blood

Both cord and maternal blood were extracted as whole blood. Paired samples (maternal and cord from the same person) were treated in the same batch to avoid analytical inconsistencies. Extraction was carried out by the method described by Hovander et al. (2000) with some modifications. Samples were brought to room temperature before weighing out 3 g of whole blood into a 50 mL screw cap glass centrifuge tube. Recovery standards (CB-52L 1 ng, FBDE-69 1 ng, and FBDE-208 2 ng) were added, and the samples were vortexed for about 15 seconds and left undisturbed for 1 hour for thorough stabilization of the surrogates with the matrix. Then 1 mL of 6M HCl and 4 mL of 2-propanol were added to the blood sample and the tube was vortexed again to denature the sample matrix. Into the mixture 5 mL of hexane:MTBE (1:1) was added and mixed by shaking the tube. Samples were centrifuged at 3,000 rpm for 20 min in a Beckman Avanti 30 Centrifuge (Beckman Instruments, Fullerton, California). The organic layer was pipetted into another centrifuge tube containing 4 mL of 1% KCl solution. The extraction was repeated twice using 3 mL of the solvent mixture and the organic layers were added into the same KCl solution. After gentle shaking to partition the coextracted non-lipids, the mixture was centrifuged again at 6,000 rpm for 20 min. The organic layer was collected into a pre-weighed glass tube. All the solvents were evaporated and the lipid was determined gravimetrically. The sample was reconstituted with hexane and the volume was brought to 2 mL by blowing a gentle stream of N_2. Cleanup procedure was similar to that of placenta described in chapter 2.

5.4 Instrumental Analysis

The PCBs and pesticide compounds were analyzed using an Agilent 7890 GC coupled with Agilent 7000 MS/MS in the EI mode. A 30 m Rxi-XLB column with 0.25 mm ID

and 0.1 um thickness (Restek Corporation, Bellefonte, Philadelpha) was used for separation in GC. The PBDEs and other emerging halogenated flame-retardants were analyzed by an Agilent 6890 GC coupled with an Agilent 5973 single quadrupole MS in the negative chemical ionization mode with methane as the reagent gas. A Restek Rtx-1614 column (15 m, 0.25 mm ID, 0.1 um thickness) was used for separating the compounds in the GC. Details regarding the temperature programs, ion selection, and injection parameters are given under materials and methods in the chapter 2.

5.5 Quality Control and Quality Assurance

One procedural blank analysis was performed with every eight sample-batches to monitor the background contamination. When/if the background level exceeded 30% of the sample concentration, background level was subtracted from the samples. Recovery standards were added to samples to monitor the analyte loss during the analytical procedure. The LOD was set as three times the ratio of signal to noise for each compound. Quantification was based on the IS method with a 5-point calibration curve. Retention time and qualifier-to-quantifier ion ratio was used for compound identification. Two types of SRM from NIST were analyzed in replicates: SRM1957 (non-fortified human serum) was selected to represent the blood samples and SRM1947 (Lake Michigan fish tissue) to represent placenta tissue. Detailed QA/QC procedure can be found in chapter 2.

5.6 Data Analysis

Concentrations of the target analytes were calculated both on fresh weight (pg/g wwt) and lipid (ng/g lwt) basis. Various comparisons were made mainly using the median concentration of the analyte. Basic statistics (mean, median, minimum, maximum, IQR) were

used to display the analyte distribution in each matrix. To evaluate the cross-placental transfer of the analytes, two types of ratios were calculated:

C/M ratio = Concentration in cord blood / concentration in maternal blood,

P/M ratio = Concentration in placenta / concentration in maternal blood.

Concentrations below the detection limits were replaced with a value equal to LOD/√2 before natural logarithm transformation of data to achieve normal distribution for further statistical analyses.

5.7 Results and Discussion

5.7.1 Survey Questionnaire

The average maternal age at delivery for the study population (N=24) was 25.3 years. The majority of the study population consisted of African American mothers (45%). Hispanic and non-Hispanic mothers were at 40% and 15% respectively of the cohort. More than 20% had a college degree or higher education while 30% had not finished high school. Almost all the participants (95%) were from Chicago. About 70% of them were living in apartments and more than 58% claimed that their residence were more than 25 years old. About 62% of the mothers in the study were unemployed at the time of the survey. None of the mothers were vegetarian and 71% declared that they ate fish during pregnancy. Twenty-one percent of the study cohort were smokers but only two people acknowledged smoking during pregnancy. Averages and the standard deviation of gestational age, and birth weight were at 39.6±1.9 weeks, and 3,366±511g. Average Apgar score after 1 hour and 5 hours were 8.3±1.3 and 8.9±0.3, respectively. All births were live, and female birth rate was 58%. Body mass index, parity, and breast feeding history were missing for about half of the study population.

5.7.2 **Polybrominated Dipheny Ethers**

The LOD ranged from 0.002 to 0.005 ng/mL for tri- to heptaBDEs and was 0.098 ng/mL for BDE-209. Separate blanks were examined for blood and placenta extraction. High levels (>30% of the sample) of BDEs 28, 183, and 209 were seen in blood blanks (the blank included all the solvents and the reagents pertaining to the analysis except the sample). The level of BDE-183 was high in placenta blanks as well. Therefore, the concentrations of the samples were corrected using the median blank concentration of the corresponding blanks. Average recovery for FBDE-69 was 90% for both placenta and blood. The FBDE-208 recovery showed variations between the two matrices. For blood, the average recovery was 100% (median 105%), while for placenta it was 129% (median 137%). Based on this finding, BDE-209 concentration in the placenta was corrected for FBDE-208 recovery. Analysis of SRM for both placenta and blood demonstrated good accuracy and precision for PBDEs.

Only BDEs 47 and 153 were detected 100% in all three matrices of all sample sets. The lowest detection rate was seen for BDEs 66 and 183 and a higher uncertainty was associated with these measurements as the concentrations were close to the detection limits. The detection rate in placenta was comparatively higher than maternal blood and cord blood. This might be a reflection of both the actual tissue burden as well as the sample quantity that was available for the analysis. While placenta was abundant, blood available for the analysis was limited in volume (3 mL). Detection rate for PBDEs are given in Table XXVII.

TABLE XXVII

DETECTION RATES (%)* OF PBDES IN PLACENTA, MATERNAL BLOOD UMBILICAL CORD BLOOD.

Congener	LOD ng/mL	Placenta	Maternal	Cord
28	0.003	96	58	29
47	0.002	100	100	100
66	0.005	57	4	0
100	0.004	100	96	88
99	0.002	100	100	92
85	0.005	100	58	33
154	0.003	100	100	96
153	0.003	100	100	100
183	0.004	87	13	0
209	0.098	100	79	67

*: percentage of samples above the detection limit

5.7.2.1 **Concentration Levels**

Concentrations of PBDEs measured in 24 matched maternal blood, cord blood, and placentas are reported in both unadjusted and lipid normalized forms in Table XXVI11 (detail results are given in Tables XLI–XLVII in Appendix A). On fresh weight basis, maternal blood had the highest absolute abundance of PBDEs followed by placenta and cord blood (median Σ_{10}PBDE 134.0, 83.2, 71.8 pg/g wwt, respectively). Except for BDE-209, which was higher in cord blood than in placenta, all the other congeners had a higher concentration in placenta (Figure 21). Different results were observed with lipid normalization of the concentration data. After such an operation, the placental concentrations were significantly

decreased ($p<.001$) compared to maternal and cord blood as a result of considerable differences of the lipid content of these matrices (average lipid content: maternal=.44%, placenta=.92%, cord blood=.2%). Median total PBDE concentration of maternal blood, cord blood, and placenta were at 35.8, 43.5, and 10.5 ng/g lipid. Total PBDE, BDE-47, and BDE-209 concentrations were higher in cord blood but did not differ significantly from those of maternal blood (paired t test, $p>.05$).

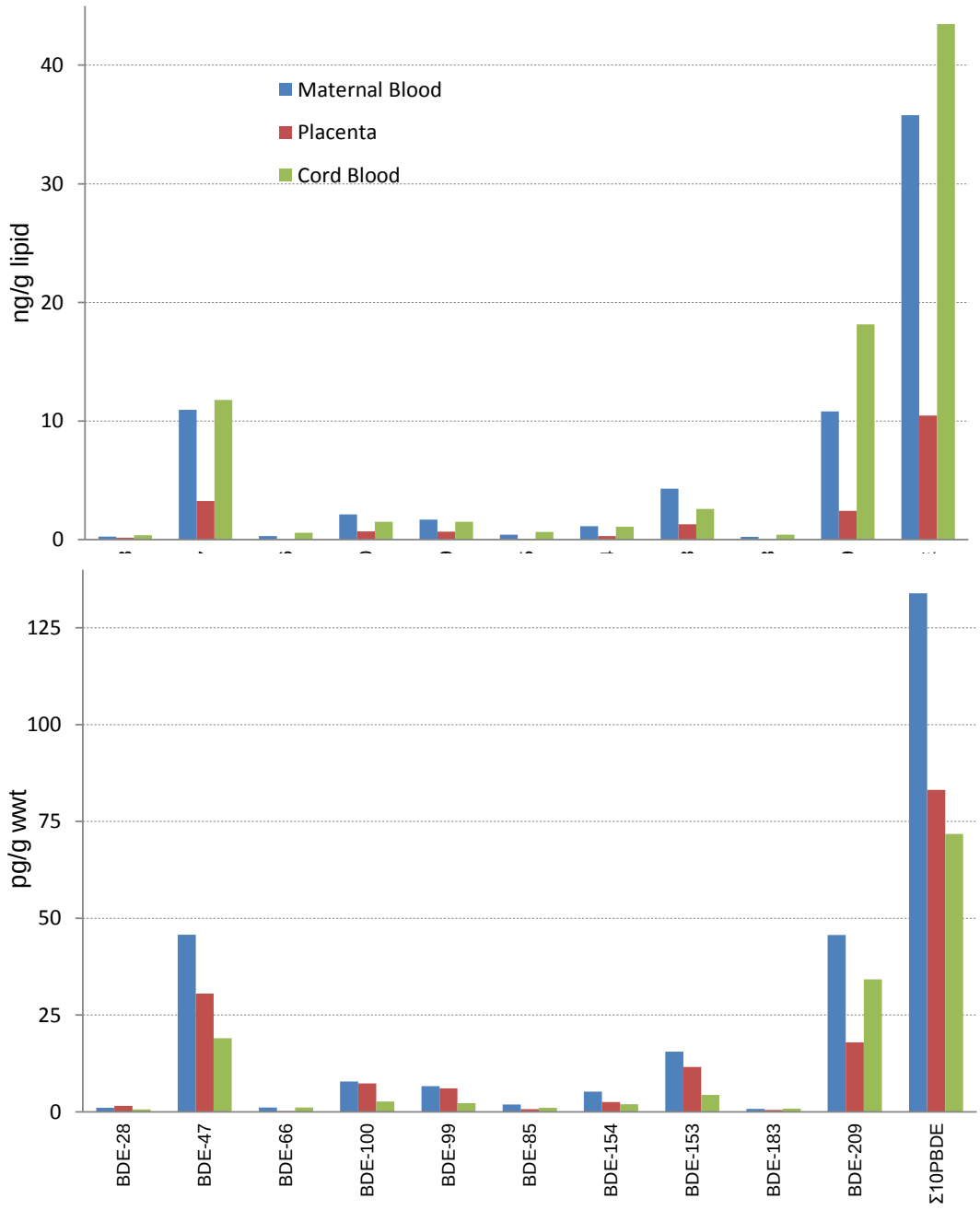


Figure 21. Concentrations (lipid-adjusted, above and wet weight-based, below) of PBDEs measured in matched (N=24) maternal blood, cord blood, and placenta samples.

TABLE XXVIII

SUMMARY STATISTICS FOR TOTAL PBDE (SUM OF 10 AND SUM OF TRI- TO HEPTABDE) AND INDIVIDUAL BDE CONGENER CONCENTRATIONS IN MATERNAL BLOOD, CORD BLOOD, AND PLACENTA

	MATERNAL BLOOD				**PLACENTA**				**CORD BLOOD**			
	Lipid-adjusted concentration, ng/g lipid											
	Mean	*Median*	*Min*	*Max*	*Mean*	*Median*	*Min*	*Max*	*Mean*	*Median*	*Min*	*Max*
BDE-28	0.4	0.2	0.1	1.7	0.2	0.2	0.0	0.9	0.5	0.4	0.2	2.2
BDE-47	20.1	10.9	4.2	71.8	5.6	3.3	0.1	23.9	20.9	11.8	4.3	108.3
BDE-100	3.7	2.1	0.2	18.7	1.2	0.7	0.2	5.2	2.9	1.5	0.4	23.5
BDE-99	3.0	1.7	0.2	12.7	1.1	0.7	0.3	4.5	3.5	1.5	0.2	26.4
BDE-85	0.6	0.4	0.2	2.0	0.1	0.1	0.0	0.5	1.0	0.6	0.3	4.6
BDE-154	1.3	1.1	0.6	2.6	0.4	0.3	0.1	1.4	1.4	1.1	0.5	4.0
BDE-153	5.4	4.3	1.1	22.2	1.8	1.3	0.3	6.9	3.5	2.6	0.8	21.6
BDE-183	0.3	0.2	0.1	2.1	0.1	0.0	0.0	0.2	0.5	0.4	0.2	1.2
BDE-209	12.8	10.8	3.8	35.9	24.1	2.4	0.9	286.2	23.6	18.1	7.7	53.9
Σ_{10}PBDE	47.9	35.8	13.7	149.1	34.6	10.5	2.6	304.7	58.6	43.5	20.2	247.5
$\Sigma_{3\text{-}7}$PBDE	35.1	20.9	7.4	120.5	10.5	6.5	1.7	40.4	35.0	20.3	8.9	193.6

TABLE XXIX

COMPARISON OF PBDE LEVEL IN US GENERAL FEMALE POPULATION WITH MATERNAL BLOOD LEVELS OF THE PRESENT STUDY)

	NHANES 03–04				This study
	GeoMean (95%CI)	75th (95%CI)	95th (95%CI)	LOD	GeoMean (95%CI)
BDE-28	1.17 (.990–1.38)			0.8	0.3 (0.2–0.4)
BDE-47	19.6 (16.4–23.5)			4.2	13.5 (9.0–20.1)
BDE-66	*		1.10 (<LOD–2.2)	1	0.3(0.2–0.4)[$]
BDE-85	*		3.60 (<LOD–5.2)	2.4	0.5 (0.4–0.7)
BDE-99	*	8.70 (6.60–10.6)		5	1.8 (1.1–2.7)
BDE-100	3.72 (3.15–4.40)			1.4	2.2 (1.5–3.3)
BDE-153	4.78 (4.20–5.43)			2.2	4.1 (3.0–5.5)
BDE-154	*	0.90 (.800–1.00)		0.8	1.2 (1.0–1.5)^
BDE-183	*		< LOD	1.7	0.2 (0.2–0.3)

$Higher measurement uncertainty
*Not calculated: proportion of results below limit of detection was too high to provide a valid result.
^ Coelute with BB153 (concentration in ng/g lipid)

The fourth National Report on Human Exposure to Environmental Chemicals (CDC, 2009) provides an ongoing assessment of the exposure of the US population to environmental chemicals by the use of biomonitoring. In Table XXIX, PBDE levels represent the exposure levels in the blood of the female participants in the 2003–2004 NHANES. Geo Means (GMs) of BDE-28, BDE-47, and BDE-100 found in this study were lower when compared to the NHANES 2003–2004 data. These results may reflect the effect of the ban imposed on penta commercial BDE mixtures in 2004 (Shaw et al., 2010). Our findings are consistent with previous studies reporting reduction in PBDE levels in humans and in furniture and house dust (Zota et al., 2013; Stapleton et al., 2012; Dodson et al., 2012; Sjödin et al., 2013).

5.7.2.2 **Correlations among Matrices**

Pearson correlation assessments on log-transformed, lipid-adjusted concentrations showed strong association among PBDEs in maternal cord blood and placenta. Placental PBDEs were significantly correlated to maternal PBDEs in terms of all but tri (BDE-28), hepta (BDE-183), and deca (BDE-209) BDEs (Table XXX). The most significant associations were seen between cord blood and maternal blood. Cord blood total PBDE, sum of tri-heptaBDEs, tri, tetra, penta, and hexaBDE levels were moderately correlated to maternal counterparts and the maternal total PBDEs (Table XXIX). Strong correlations between maternal and fetal concentration were reported in earlier studies by Mazdai et al. (2003), Meironyté Guvenius et al. (2003), and Vizcaino et al. (2014). Interestingly, cord blood BDE-209 showed no relation to any of the maternal PBDEs but significant correlation with placental BDE-209. Results of this study suggest maternal blood measurements to be the best alternative to predict fetal exposure for analytes other than BDE-209 . In the absence or scarcity of maternal blood, placental biomarker levels may be used as a surrogate for fetal exposure. According to our findings, maternal blood may not be the best as a surrogate to estimate fetal BDE-209 exposure. Instead the use of placental exposure with necessary corrections may be more appropriate. Placenta has been regarded as a dual biomarker to assess both maternal and fetal exposure to environmental chemicals (Iyeger and Rapp, 2001; Myllynen et al., 2005). According to our findings, placenta-cord correlations were weaker than the placenta-maternal correlations. This may be an indication of similarity of the processes (e.g., biotransformation) and distributions that are acting upon both maternal and placental PBDEs. We found significant correlations between maternal blood and placental PBDE measurements. Notably, the placental tetraBDEs were highly correlated with maternal tri, tetra, penta, and hexa homologs and total PBDEs. In this context, placenta is more suitable as a tissue to predict maternal exposure levels than fetal exposure.

TABLE XXX

PEARSON'S CORRELATION MATRIX BETWEEN PLACENTAL AND MATERNAL PBDE LEVELS.

	TriBDE	*TetraBDE*	*PentaBDE*	*HexaBDE*	*HeptaBDE*	*DecaBDE*	*3–7BDE*	*Total_BDE*
Placenta				Maternal				
TriBDE	0.277	**0.448**	**0.458**	0.377	-0.132	-0.006	**0.460**	0.391
TetraBDE	**0.425**	**0.541**	**0.573**	**0.503**	0.078	0.064	**0.577**	**0.519**
PentaBDE	0.251	**0.545**	**0.617**	**0.518**	-0.078	-0.160	**0.588**	**0.461**
HexaBDE	0.012	0.122	0.203	**0.588**	-0.243	-0.252	0.261	0.166
HeptaBDE	0.113	-0.061	-0.103	-0.082	0.373	0.295	-0.061	0.039
DecaBDE	0.320	0.276	0.242	0.414	-0.011	0.321	0.286	0.328
3–7_BDE	0.265	**0.488**	**0.555**	**0.581**	-0.087	-0.125	**0.560**	**0.450**
Total_BDE	0.340	0.403	0.420	**0.620**	-0.036	0.195	**0.462**	**0.447**

Statistically significant ($p<.05$) values are given in bold print. Natural log transformed lipid adjusted concentrations were used in the analysis.

TABLE XXXI

PEARSON'S CORRELATION I MATRIX BETWEEN CORD-MATERNAL AND CORD-PLACENTA PBDES.

	TriBDE	*TetraBDE*	*PentaBDE*	*HexaBDE*	*HeptaBDE*	*DecaBDE*	*1–7_BDE*	*Total_BDE*
Cord				Maternal				
TriBDE	**0.460**	**0.646***	**0.577**	**0.547**	0.250	0.288	**0.645**	**0.638**
TetraBDE	**0.497**	**0.796***	**0.767***	**0.643**	0.263	0.251	**0.805***	**0.767***
PentaBDE	0.381	**0.648**	**0.647**	**0.692**	0.229	0.281	**0.702***	**0.694**
HexaBDE	0.289	**0.542**	**0.540**	**0.670**	0.249	0.315	**0.587***	**0.593**
HeptaBDE	0.125	**0.387**	0.378	**0.428**	0.271	0.252	0.398	0.410
DecaBDE	0.122	0.305	0.303	0.380	0.119	0.384	0.306	0.364
1–7_BDE	**0.460**	**0.749***	**0.725***	**0.675**	0.259	0.275	**0.773***	**0.749***
Total_BDE	0.389	**0.671***	**0.650**	**0.631**	0.226	0.350	**0.686**	**0.691**
				Placenta				
TriBDE	0.348	0.280	0.248	0.112	0.131	**0.448**	0.248	**0.470**
TetraBDE	**0.470**	0.384	**0.502**	0.254	-0.078	0.322	**0.448**	**0.447**
PentaBDE	0.229	0.279	0.388	0.259	-0.009	0.406	0.337	**0.483**
HexaBDE	0.382	0.275	0.345	0.283	-0.030	**0.465**	0.336	**0.508**
HeptaBDE	0.242	0.178	0.157	0.039	0.069	0.386	0.151	0.336
DecaBDE	0.098	0.012	-0.074	-0.119	0.077	**0.515**	-0.068	0.358
1–7_BDE	0.418	0.360	**0.466**	0.268	-0.053	0.374	0.421	**0.479**
Total_BDE	0.337	0.276	0.311	0.146	0.001	**0.478**	0.282	**0.495**

Significant correlations ($p<.05$) are given in bold print. Highly significant values are marked with a star ($p<.0001$). Natural log transformed lipid adjusted concentrations were used in the analysis.

5.7.2.3 **Congener Profile**

Figure 22 represents the percentage contribution of each PBDE homolog (a slice) to the total PBDE concentration (whole pie, 100%) in each matrix. Maternal tetra, penta, and hexa homolog compositions were similar but slightly lower than that of the placental tissue. Higher percentage of BDE-28 was seen in placenta compared to both maternal and cord blood. In both maternal and placental compartments, BDE-47 contributed mostly to the overall PBDE concentration followed closely by BDE-209. The difference between 47 and 209 in maternal blood was only 4% while a difference of 10% was detected in placenta. Cord blood had the highest BDE-209 contribution, which was about half (46%) of the total PBDE concentration. The contribution of tetra, penta, and hexa homolog was less in cord blood comparative to that of both maternal blood and placenta.

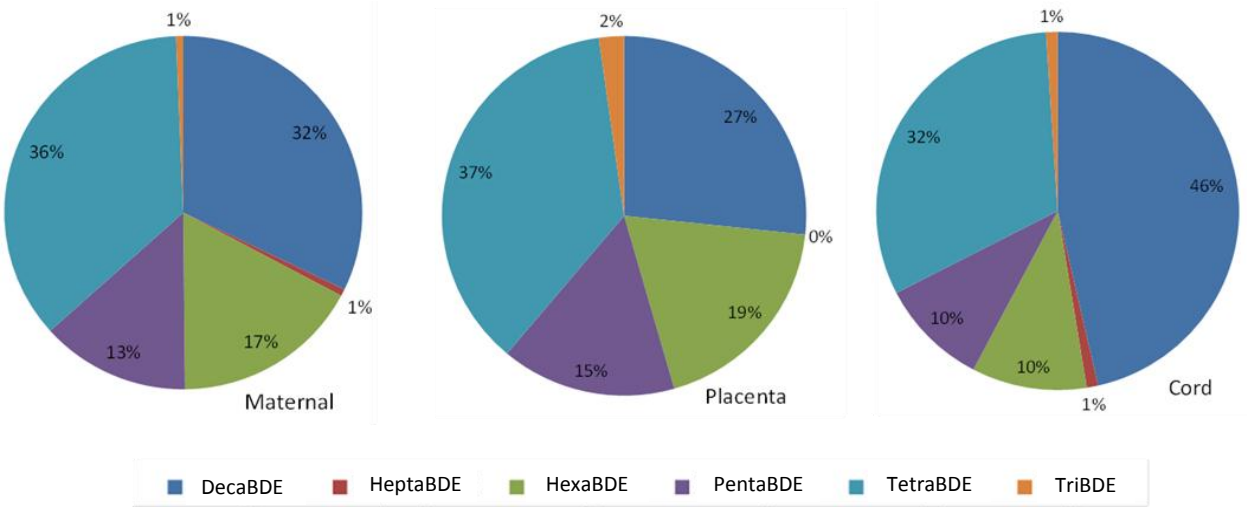


Figure 22. Comparison of PBDE congener profile (as a % of Total PBDE) in matched maternal blood, cord blood, and placenta.

5.7.2.4 **Concentration Ratios**

The C/M and P/M ratios for PBDEs were calculated using the lipid-adjusted PBDE concentration in cord blood, maternal blood, and placenta. The box and whisker plots in figure 24 show the median, 25th percentile, 75th percentile, and high and low values. Outliers are given as dots. Figure 25 was created by plotting the median values of the C/M and P/M distributions. These concentration ratios represent a "snap shot" of a dynamic system at or closer to the time of delivery.

The average C/M ratio for total PBDE was 1.2 with a range of 0.43–3.0 (N=24). The vast majority of the samples (87%) had C/M ratios at or above one. BDEs 28, 47, 85, 154, and 209 had median C/M ratios greater than one (>1), while it was below one (<1) for BDEs 100, 99, and 153. Accordingly at the time of delivery, lipid-adjusted PBDE concentrations were similar or higher than concentration in maternal compartments. In contrast to that, placenta had less than half of the maternal PBDE concentration at the time of delivery as shown by an average P/M ratio of less than 0.5 for all the BDE congeners except BDE-28. The P/M ratios showed a large variation with a range of 0.1–4.2. The number of outliers was also high for the P/M distribution compared to the C/M distribution. In figure 24, the BDE congeners and homologs were arranged in order of ascending molecular weights and number of bromine substitution. A clear declining trend from low to high number of bromine substitution was observed for both C/M (R^2=.9) and P/M (R^2=.4) ratios. An inverse relationship between C/M ratios and bromine substitution has been reported in previous studies (Viscaino et al., 2014; Frederiksen et al., 2009; Jakobsson et al., 2012; Meironyté Guvenius et al., 2003). The fully brominated BDE-209 exhibited a deviation from this trend by having the highest C/M ratio. A similar observation for BDE-209 has also been reported by Viscaino et al. (2014).

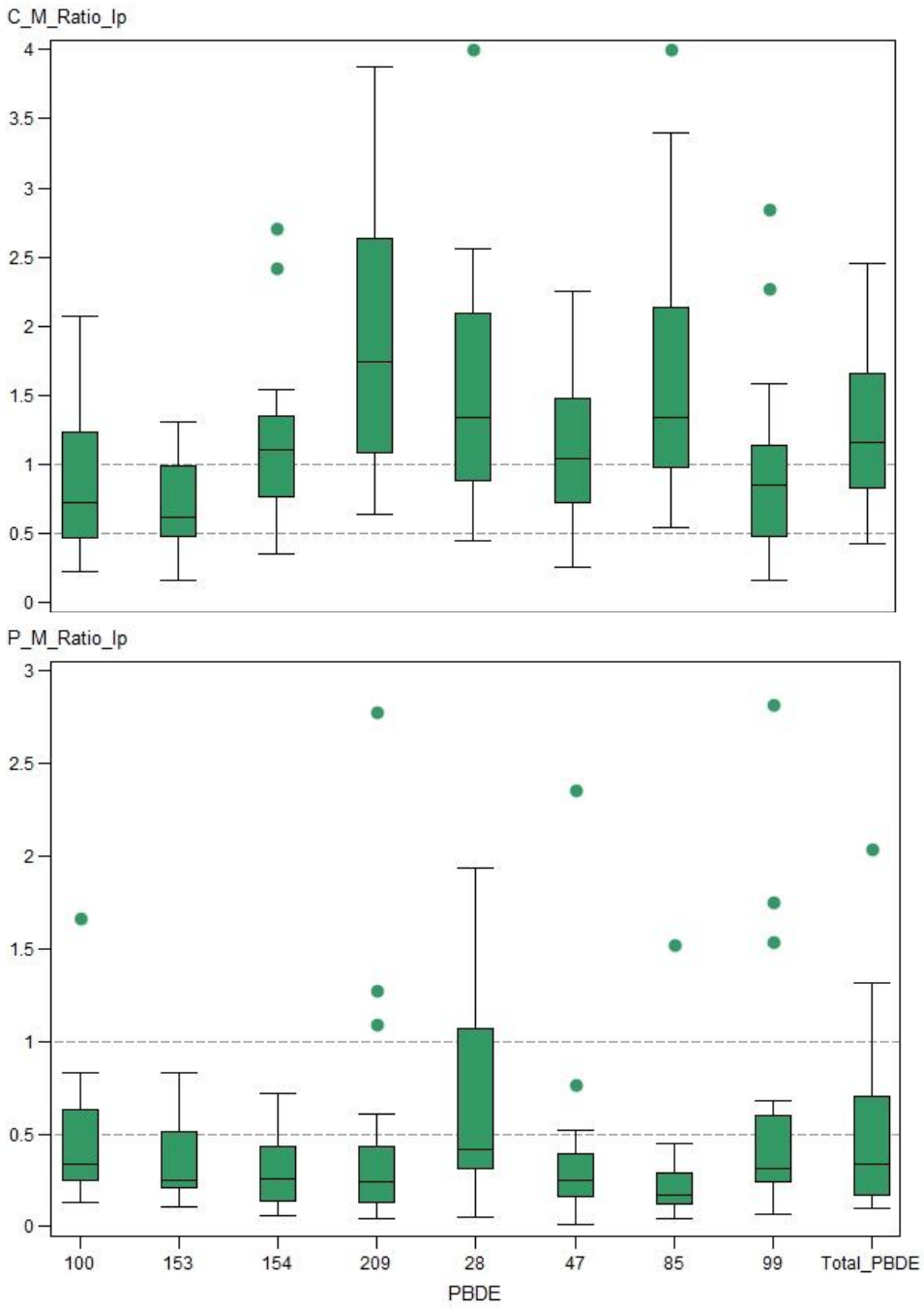


Figure 23. Distribution of PBDE concentration ratio (lipid-adjusted) between cord blood and maternal blood (C/M ratio, above) and placenta and maternal blood (P/M ratio).

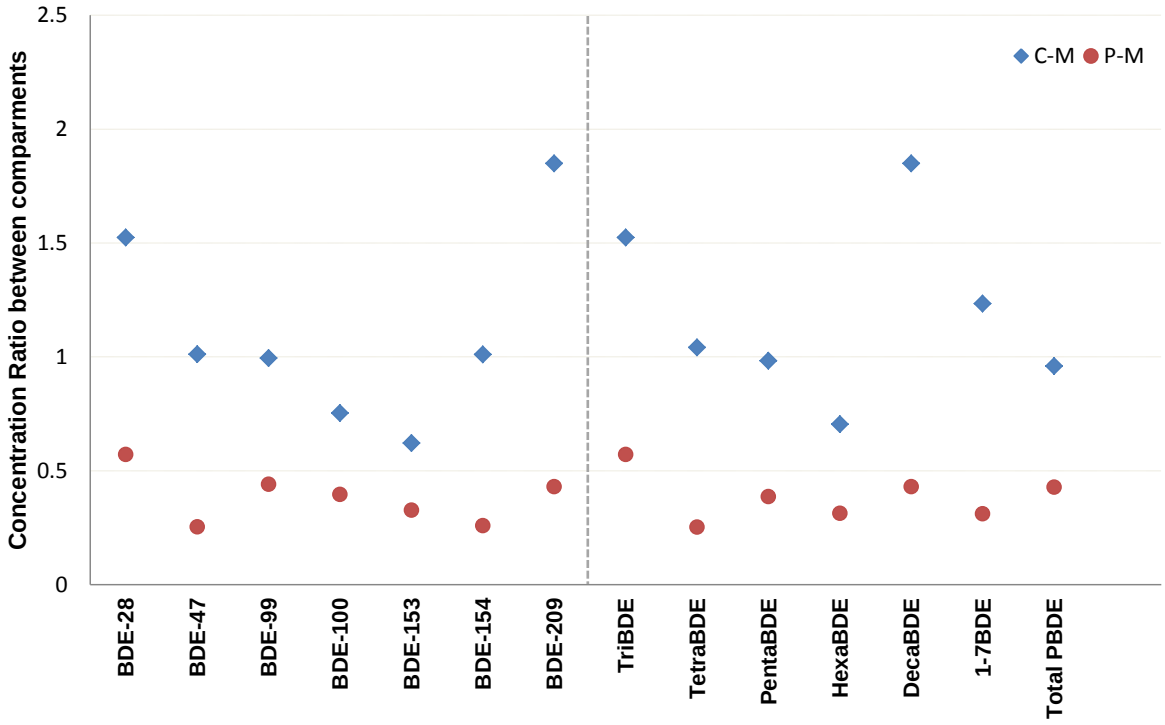


Figure 24. Scatter plot of concentration ratios (lipid-adjusted median) between maternal-cord (C-M) and maternal-placenta (P-M) (congeners and homologs are separated by a central line).

Placental accumulation and debromination of BDE-209 have been suggested by Frederiksen et al. (2010) based on an experimental *ex vivo* human placenta perfusion study. Placenta has the capacity to metabolize xenobiotic chemicals through a Cytochrome P450-dependent system (Wojtowics et al., 2011; Myllynen et al., 2004). Lack of evidence for placental accumulation of BDE-209 in our study may be attributable to a simultaneous but more rapid debromination. Frederiksen et al. (2009) also reported a rapid decrease of BDE-209 in the maternal system, which corroborates our finding of BDE-209 having an elevated C/M ratio. Despite the common acceptance of reduced bioavailability of large molecules due to their size,

the presence of BDE-209 in cord blood and placenta indicates its membrane permeability. Given BDE-209's expected high lipid affinity based on its reported log K_{ow} value of 9.97 (Wania et al., 2003), it is reasonable to assume that the hindrance caused by the structure is overcome by lipophilicity. In addition, the lipid flux and the thinning of placental membranes toward the end of the pregnancy might have contributed in facilitating the placenta crossing (Vahakangas and Myllynen, 2006). Vizcaino et al. (2014) suggest a possible involvement of some active transport mechanism in addition to passive diffusion BDE-209 (Vizcaino et al., 2014). Similar to few other studies, findings of our study support the notion of bioaccumulation of PBDE, especially BDE-209 in cord blood (Vizcaino et al., 2014; Gómara et al., 2007; Antignac et al., 2009; Needham et al., 2010; Wu et al., 2010). Unlike placenta, the fetus has a less-developed metabolic capacity and as a result, PBDEs that cross the placenta remain largely unaltered within the fetal system. Based on the percentage congener distribution, proportions of tetra, penta, and hexaBDE relative to decaBDE were calculated for each compartment. A variation in ratios between compartments was observed (Table XXXII). Compared to maternal compartment that can be considered as the original source, placenta had higher ratios of tetra, penta, and hexaBDEs to BDE-209. The low concentration of BDE-209 in placenta despite its higher P/M ratio compared to the other congeners raises the concern for placental metabolism or debromination of decaBDE. Those C/M ratios that are greater than one suggest that PBDEs are transferred to fetal circulation without much obstruction by the placenta. However, upon comparing the PBDE congener composition in the three matrices, it appeared that, at the time of child delivery, the more toxic congeners (tetra to hexaBDEs) tend to be retained in the placenta, relative to decaBDE, which is often considered as less toxic (RfDs BDE-47, 99=.0001, BDE-153=.0002 compared to BDE-209=.007 mg/kg-day, Wikoff & Birnbaum, 2011). In that sense it is

reasonable to assume that placenta protects the fetus by diluting the overall toxic effect of PBDEs. However, this view does not take into consideration the controversy on the relative toxicity among PBDE congeners and the metabolic debromination of BDE-209 into less brominated congeners on the fetus side.

TABLE XXXII

DECABDE NORMALIZED* BDE HOMOLOG VARIATION IN MATERNAL BLOOD, CORD BLOOD AND PLACENTA

Compartment	DecaBDE (BDE209)	TetraBDE (BDE47)	PentaBDE (BDEs 99, 100)	HexaBDE (BDEs 153, 154)
Maternal	1	1.1	0.4	0.5
Placenta	1	1.4	0.6	0.7
Cord	1	0.7	0.2	0.2

*: calculated by dividing the particular concentration (e.g., BDE-47) by the concentration of BDE209

5.7.3 **Polychlorinated Biphenyls**

The limit of detection ranged from 0.004 to 0.008 (ng/mL) for the 32 PCBs analyzed. Average surrogate recovery for PCB-52L was 94(±15)%. Separate procedural blanks were analyzed to represent blood and placenta sample preparation procedure. Background contamination was negligible for placenta blanks. Blood blanks showed signs of PCB contamination that may be attributable to the different chemicals used in the procedure, particularly the HCl and 2-proponol which had been purchased a few years back. To nullify the background effect, the mean concentration of each PCB congener in the blanks was calculated

and deducted from the concentration in the sample. The concentrations in placentas were not corrected for blanks. Repeated analysis shows good repeatability with RSD ranging from 1.8% to 16.8%. The SRM analysis for blood displayed evidence for overestimation for some PCB congeners 28, 99, 118, 105, 153, and 183. The PCB detection rate in placenta was higher, obviously as a result of the larger sample amount (24 g) used for the analysis of placenta compared to that of blood (3 g). Some PCB congeners (CBs 169, 189, 126, 179, 114, 77) were not seen in any of the cord or maternal blood sample.

Concentrations below the detection limits were replaced with a value equal to LOD/√2. Concentrations of PCBs were calculated both on fresh weight (pg/g wwt) and lipid (ng/g lwt) basis. Data analysis steps were similar to those used in PBDE analysis. Briefly, initial concentrations were assessed to check the distribution. Because data were non-normally distributed, median concentration levels were selected to compare matrices. Congeners detected in at least 50% of the samples in each matrix were selected for further analysis. Concentration ratios (C/M, P/M) were calculated for these selected congeners. Statistical comparisons were made on natural log transformed PCB concentrations.

5.7.3.1 **Concentration Levels**

Twenty-four complete sets of maternal blood, placenta, and cord blood samples were analyzed for 32 individual PCB congeners. The average, median, and range (minimum-maximum) of PCB concentrations are presented in Table XXXIII. The concentrations are given in ng/g lipid. Median Total PCB concentration calculated as the sum of 32 PCBs in maternal blood was 73.9 (range 21.2–208) unit. Cord blood PCB concentration (Total PCB median=111.9, range 14.7–523) was higher than that of both maternal blood and placenta. Lowest levels were found for placenta at median concentration of 32.1 with a range of 7.7–208 ng/g lipid. Fresh

weight-based (pg/g wwt) placental PCB concentration of this study, representing Chicago, Illinois, 2010–2011 period, was compared to NCS placental data from seven US locations for the 2011–2012 time period (chapter 4). Median Total PCB concentration of this study (274, range 110–783 pg/g wwt) was lower than the concentrations reported for both NCS and under the 10th percentile Main Study (341 pg/g wwt, see chapter 4). The reason for this trend is not clear, particularly when background contamination is not evident. Cord blood concentrations (lipid-adjusted) were comparable for CBs 180, 153, and (138+158) with the concentration reported by Herbstman et al. (2007) in 297 cord serum samples collected from Baltimore, Maryland during 2004–2005.

TABLE XXXIII

SUMMARY STATISTICS OF PCB CONCENTRATIONS IN MATERNAL BLOOD, CORD BLOOD, AND PLACENTA

	Cord				Maternal				Placenta			
	Mean	Min	Median	Max	Mean	Min	Median	Max	Mean	Min	Median	Max
CB-8	8.88	0.52	7.20	28.4	4.25	0.18	4.45	9.61	1.78	0.47	1.81	3.25
CB-28	10.52	0.41	8.34	38.0	4.94	0.23	4.47	12.02	2.02	0.50	1.90	4.51
CB-37	2.13	0.43	1.55	6.5	0.89	0.24	0.77	2.14	0.48	0.07	0.43	1.45
CB-44	7.20	0.43	5.43	24.7	3.27	0.24	3.07	8.11	1.96	0.31	1.66	5.30
CB-49	4.33	0.37	3.23	16.1	1.93	0.20	1.66	5.32	1.08	0.18	0.93	2.74
CB-52	10.55	0.35	8.64	36.5	5.05	0.20	4.70	13.97	3.15	0.57	2.74	7.76
CB-60	1.06	0.45	0.83	2.5	0.51	0.15	0.43	1.27	0.31	0.07	0.25	0.83
CB-66	4.41	0.42	3.27	15.4	2.13	0.23	1.97	4.96	1.26	0.22	1.09	3.30
CB-70	9.92	0.38	7.17	34.2	4.30	0.21	3.72	11.50	2.65	0.34	2.10	7.85
CB-74	3.62	0.43	2.82	12.0	2.21	0.43	2.13	4.50	1.08	0.29	0.86	2.39
CB-77	1.05	0.48	0.80	2.4	0.40	0.16	0.37	0.80	0.05	0.01	0.04	0.16
CB-82	2.11	0.42	1.48	6.9	0.86	0.23	0.73	2.63	0.40	0.05	0.27	1.28
CB-87	8.58	0.43	6.31	32.0	4.01	0.25	3.64	11.16	1.96	0.25	1.41	5.55
CB-99	7.33	0.45	5.46	26.2	3.93	0.45	3.56	8.83	1.96	0.40	1.51	4.84
CB-101	17.97	0.44	13.43	68.2	8.41	0.26	7.16	25.81	4.28	0.57	3.06	13.07
CB-105	3.85	0.51	2.76	13.9	2.28	0.98	2.00	5.91	1.05	0.25	0.95	2.76
CB-114	1.09	0.50	0.83	2.5	0.42	0.17	0.38	0.83	0.09	0.02	0.07	0.23
CB-118	13.65	0.50	9.58	52.4	7.92	3.38	6.48	21.21	3.19	0.72	2.53	7.72
CB-128	1.00	0.40	0.80	3.0	0.49	0.18	0.40	1.40	0.21	0.05	0.17	0.51
CB-138	5.74	0.48	4.88	16.9	5.14	1.54	5.07	11.22	2.01	0.65	1.87	5.15
CB-153	8.93	0.43	7.27	35.2	7.71	1.81	8.04	18.59	2.90	0.83	3.19	6.97
CB-156	1.20	0.55	0.91	2.7	0.77	0.18	0.63	1.63	0.28	0.08	0.22	0.68
CB-158	1.00	0.46	0.76	2.3	0.47	0.21	0.41	1.23	0.22	0.04	0.15	0.85
CB-166	1.04	0.47	0.79	2.4	0.40	0.16	0.36	0.79	0.03	0.01	0.02	0.05
CB-170	1.28	0.51	1.01	5.4	1.35	0.33	1.07	5.02	0.44	0.10	0.31	1.27
CB-179	0.91	0.41	0.69	2.1	0.36	0.14	0.32	0.69	0.09	0.01	0.07	0.24
CB-180	2.85	0.50	1.88	16.4	3.45	0.50	2.73	13.13	1.10	0.26	0.85	2.62
CB-183	1.06	0.48	0.81	2.4	0.62	0.16	0.54	2.02	0.24	0.07	0.23	0.48
CB-187	1.66	0.46	1.45	7.6	1.49	0.46	1.36	5.57	0.56	0.16	0.49	1.04
Σ_{32}PCB	148.6	14.7	111.9	523.4	81.3	21.2	73.9	208.0	36.9	7.7	32.1	83.3

Concentrations are in (ng/g lipid), Mean: arithmetic mean

TABLE XXXIV

COMPARISON OF PCB CONCENTRATION LEVELS (NG/G LIPID) BETWEEN NHANES (2003–2004, BLOOD, FEMALE ONLY) AND THE MATERNAL BLOOD OF THIS STUDY

	NHANES 2003–2004				This study
	GeoMean (95%CI)	75th (95%CI)	95th (95%CI)	LOD	GeoMean (95%CI)
CB-28	4.99 (4.66–5.35)			1.7	3.7 (2.5–5.5)
CB-44	1.99 (1.82–2.18)			0.4	2.5 (1.6–3.7)
CB-49	1.23 (1.12–1.35)			0.4	1.5 (1.0-2.0)
CB-52	2.57 (2.30–2.87)			0.8	3.3 (2.0–5.5)
CB-66	1.50 (1.42–1.58)			0.8	1.8 (1.3-2.5)
CB-74	5.65 (5.33–5.98)			0.8	2.0 (1.5–2.5)
CB-87	0.648 (.545-.771)			0.4	3.0 (2.0-4.5)
CB-99	4.35 (3.94–4.81)			0.6	3.3 (2.5-4.5)
CB-101	1.60 (1.41–1.81)			0.6	5.5 (3.7–9.0)
CB-105	1.40 (1.25–1.57)			0.4	2.0 (1.6–2.5)
CB-118	6.99 (6.32–7.73)			0.6	6.7 (5.5–8.2)
CB-126	17.8 (16.0–19.7)[#]			13.9[#]	0.41[$]
CB-128	*		.630 (.500-.800)	0.4	0.40 (0.3–0.5)
CB-138+158	15.3 (14.0–16.8)			0.4	4.5 (3.7–5.5)
CB-153	19.7 (18.4–21.1)			1.1	6.7 (5.5–8.2)
CB-169	16.0 (14.2–18.1)[#]			15.9[#]	0.44[$]
CB-170	5.14 (4.82–5.48)			0.4	1.0 (0.7–1.3)
CB-180	14.2 (13.4–15.0)			0.4	2.7 (2.0–3.7)
CB-183	1.44 (1.34–1.55)			0.4	0.5
CB-187	4.12 (3.84–4.41)			0.4	1.2 (1.0–1.6)
CB-189	*		1.39 (.890–2.18)	0.4	0.42[$]

[*] Not calculated: proportion of results below limit of detection was too high to provide a valid result.
[$] higher measurement uncertainty
[#] pg/g lipid

In Table XXXIV, the PCB levels in maternal blood were compared to those in women reported by the Fourth National Report on Human Exposure to Environmental Chemicals by the CDC (CDC, 2009) using NHANES 2003–2004 data. The sum of the geometric means of PCB (the congeners for which GM is available, same congeners in both studies) for the NHANES is 92.8 and is 54.9 for the present study. This is a decrease of 35% from 2003–2004 to 2010–2011. In the present study, the concentrations of lighter PCBs had higher contribution (than the heavier

ones) to the total PCB in contrast to the 2003–2004 NHANES study. For example, concentrations of hepta and hexa congeners (CBs 187, 180, 170, 153, and 138+158) were considerably lower in the present study while some tetra and penta congeners (CBs 44, 52, 101, 105) were higher. As less chlorinated congeners are known to have shorter half-lives, the presence of higher proportions of these congeners may be indicative of recent exposure (Megson et al., 2013; Gómara et al., 2012; Herrick et al., 2011). Several of these congeners (CBs 44, 49, 52, 87, and 149) have been reported to be associated with inhalation exposure (DeCaprio et al., 2005; Herrick et al., 2011). However, it is hard to identify and explain a specific source of PCBs to cause a recent exposure. In addition to the differences in exposure, age can also be considered as a factor determining the PCB congener profile in blood. Multivariate statistical analysis of the NHANES (2003–2004) data has shown an age-dependent concentration variation of PCBs in human sera. The results of this study indicated the enrichment of PCBs 44, 49, 52, 87, 101, 110, and PCB-149 in younger people (Megson et al., 2013)

5.7.3.2 **Congener and Homolog Profiles**

The CBs 153, 101, 118, 138, 52, 28, 8, 70, and 87 were among the dominant congeners regardless of the matrix analyzed (Figure 25). In the placenta, CB-153 was found as the most abundant congener (3.19 ng/g lipid) closely followed by CB-101 (3.06 ng/g lipid). A similar distribution was seen for maternal blood with CB-153 followed by CB-101 as dominant congeners. A slight variation in the congener arrangement was observed for cord blood. The congener dominancy decreased as CBs 101 > CB 118 > CB 52 > CB 28 > CB 153. Herbstman et al. (2007) reported a different congener profile in cord blood sampled in Maryland with CBs 153, 138, 118, and 180 as major congeners.

The variation in homolog contribution to the total PCB between matrices was found to be trivial. PentaPCBs (33%) followed by tertaPCBs (28%) served as dominant contributors to the total PCB profile. Tri and pentaPCB were slightly higher in cord blood in comparison to placenta and maternal blood. Higher percentages of hexa and heptaPCBs were seen in maternal blood. TetraPCBs showed a tendency to accumulate in placenta. A sequential decrease in hexaPCBs percentages was observed from maternal blood (21%) to cord blood (14%) through placenta (18%) such decreases; suggested restricted transfer.

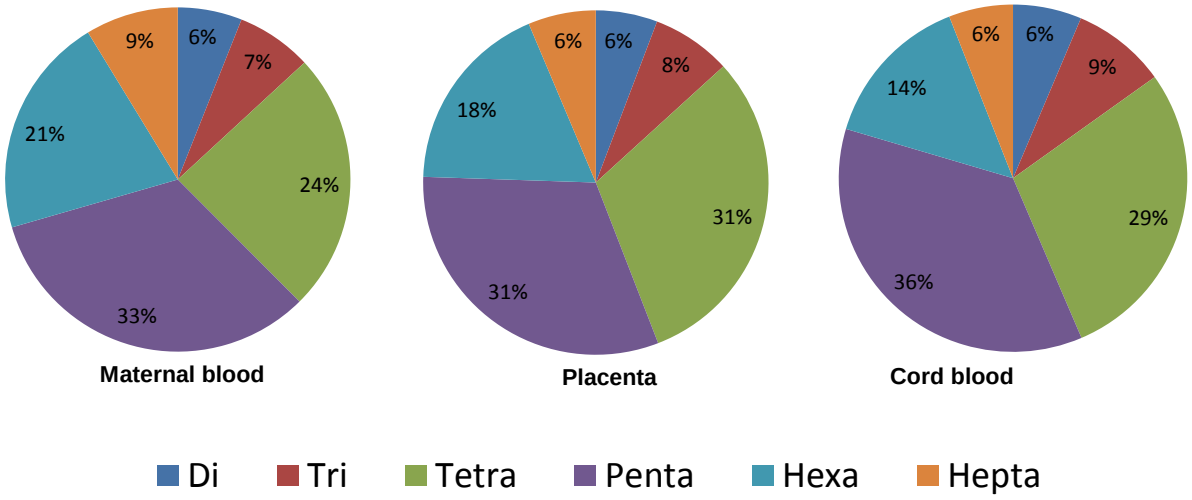


Figure 25. PCB homolog contribution (%) to the total PCB concentration in maternal blood, cord blood, and placenta.

Lipid-normalized PCB concentrations between compartments were found to be significantly different ($p<.0001$). Total PCB was most abundant in cord blood but the difference between cord and maternal blood was not statistically significant. Placental levels of PCB were significantly lower than that of both cord and maternal blood ($p<.05$). The CBs 118, 153, 105,

138, and 180 followed a similar pattern. The level of CB-101 was significantly higher in cord blood compared to both maternal blood and placenta. Concentration of most of the di, tri, and tetra congeners (CBs 8, 28, 37, 52, 44, 49, and 70) differed significantly in all three matrices. Similar or higher levels of PCB concentration in cord blood in comparison to maternal blood have been reported in some studies (Tsang et al., 2011; Jocobson et al., 1984; Gómara et al., 2007) while some studies have found higher levels of PCB in maternal blood (Vascaino et al., 2014; Adetona et al., 2013; Needham et al., 2010).

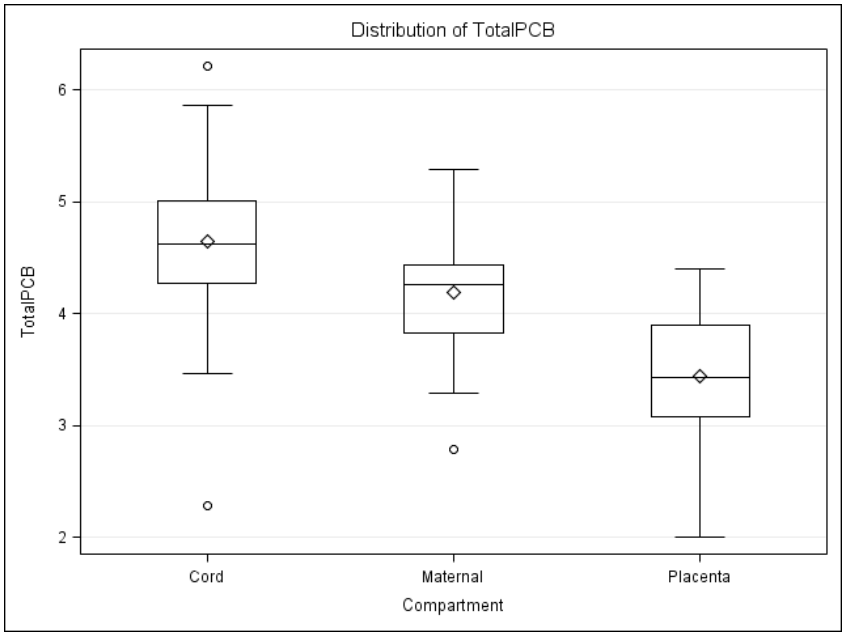


Figure 26. Box and whisker plot of total PCB concentration (ng/g lipid) in cord blood, maternal blood, and placenta.

5.7.3.3 **Correlations among Matrices**

Upon comparing homolog levels, highly significant correlations were observed between maternal and cord blood. Strong maternal-fetal correlations for PCBs have been observed (Bergonzi et al., 2011; Eik Anda et al., 2007; Meironyté Guvenius et al., 2003). Surprisingly for PBDEs, placental PCBs did not show any significant correlations with any of the maternal or cord homologs (Table XXXV). Between maternal blood and cord blood, penta and tetra homologs had higher correlation coefficients compared to heavier hexa and hepta homologs. Conversely, in Viscaino et al. (2014), significant correlations existed between all three matrices and higher correlation coefficients were seen for congeners with higher chlorination.

TABLE XXXV

PEARSON'S CORRELATION MATRIX I OF TOTAL PCB (Σ_{32}PCB) AND PCB HOMOLOGS BETWEEN MATERNAL AND FETAL COMPARTMENTS

Homolog	*MΣ_{32}PCB*	*Mdi*	*Mtri*	*Mtetra*	*Mpenta*	*Mhexa*	*Mhepta*	*PΣ_{32}PCB*	*Pdi*	*Ptri*	*Ptetra*	*Ppenta*	*Phexa*	*Phepta*
CΣ32PCB	**0.66**	**0.41**	**0.55**	**0.63**	**0.70**	**0.48**	**0.47**	-0.11	-0.20	-0.21	-0.19	-0.10	0.04	0.22
Cdi	**0.65**	**0.61**	**0.69**	**0.74**	**0.66**	**0.45**	0.32	-0.02	-0.04	-0.07	-0.04	-0.03	0.08	0.20
Ctri	**0.71**	**0.55**	**0.66**	**0.73**	**0.74**	**0.50**	**0.41**	-0.07	-0.17	-0.17	-0.12	-0.05	0.07	0.20
Ctetra	**0.71**	**0.51**	**0.64**	**0.72**	**0.74**	**0.50**	**0.43**	-0.11	-0.19	-0.19	-0.16	-0.10	0.03	0.20
Cpenta	**0.66**	**0.41**	**0.56**	**0.65**	**0.72**	**0.45**	**0.44**	-0.13	-0.19	-0.21	-0.20	-0.11	0.00	0.19
Chexa	**0.57**	0.27	**0.41**	**0.49**	**0.59**	**0.46**	**0.52**	-0.12	-0.23	-0.24	-0.22	-0.10	0.03	0.23
Chepta	0.27	-0.10	0.03	0.09	0.28	0.28	**0.52**	-0.14	-0.25	-0.28	-0.28	-0.12	0.06	0.31
MΣ_{32}PCB								-0.01	-0.24	-0.16	-0.06	0.03	0.07	0.11
Mdi								0.00	-0.13	-0.06	0.01	0.01	0.05	0.01
Mtri								-0.05	-0.15	-0.09	-0.04	-0.03	-0.02	-0.02
Mtetra								-0.03	-0.12	-0.08	-0.02	-0.01	-0.02	-0.01
Mpenta								-0.05	-0.20	-0.15	-0.08	0.00	-0.02	0.03
Mhexa								0.06	-0.26	-0.15	-0.02	0.08	0.21	0.22
Mhepta								0.03	-0.25	-0.20	-0.11	0.00	0.26	0.45

Concentrations in ng/g lipid, C=cord, M=maternal, P=placenta. Significant correlations are given in bold phase ($p<0.05$)

The reason for the nonexistence of placenta-maternal and placental-fetal correlations may be the placental metabolism of PCBs. Placental metabolic activity for environmental pollutants has been described by Shen et al. (2007). Accordingly, placental concentration may not reflect the fetal or maternal exposure and therefore may not be used as a surrogate measurement in biomonitoring.

5.7.3.4 **Concentration Ratios**

On lipid-weight basis, the average C/M ratio for total PCB was 1.65±0.89 and P/M was 0.53±0.29 (N=24). Generally, C/M ratios (range 0.80–2.64) were twice as high as the P/M ratios (range 0.44–0.92). On wwt basis, the C/M ratios were still higher than P/M ratios but the difference between the two ratios was less and mostly varied between 0.5 and 1 (average C/M=0.78±0.27 P/M=1.08±0.26). The PCB congeners showed a decreasing trend that was consistent with the number of chlorine substituents. The trend is stronger upon homolog comparison (Figure 27). Our results are consistent with previous studies reporting similar trends in transfer ratios (Needham et al., 2010; Jacobsen et al., 1984; Vizcaino et al., 2014).

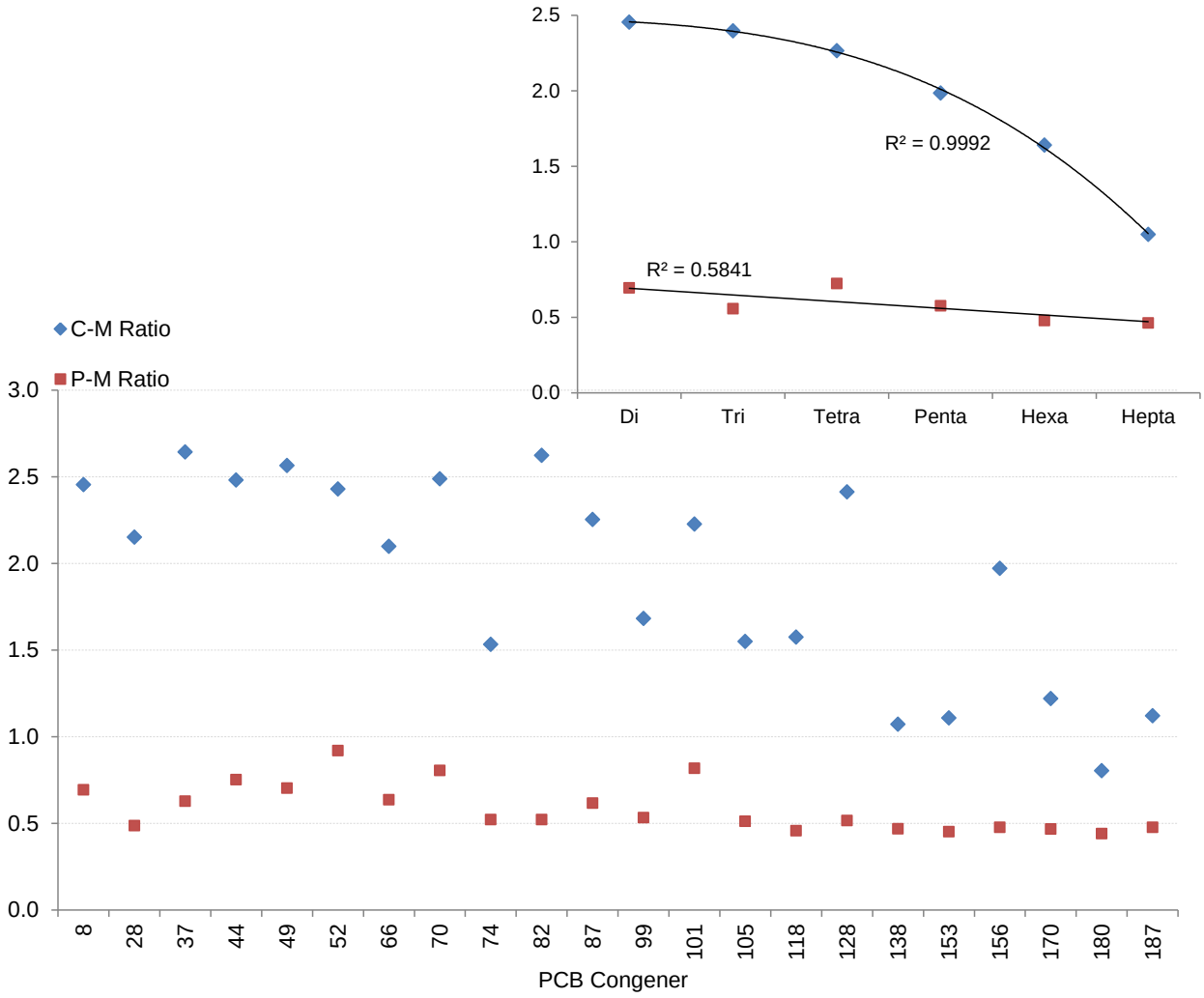


Figure 27. PCB transfer ratio (mean, lipid-adjusted) between placenta-maternal blood and cord-maternal blood.

Results of this study clearly show the prenatal exposure to PCBs. Placental barrier does not seem effective toward preventing the transfer of lighter PCB congeners into the placental circulation. The fetus to a certain extent is protected against the exposure to highly chlorinated PCBs.

5.7.4 Organochlorine Pesticides and Halogenated Flame-Retardants

Limit of detection was set as three times the signal-to-noise ratio of the instrument, and ranged from 0.21 to 11.5 pg/mL for individual OCPs and 0.14 pg/mL to 1 ng/mL for the XFRs analyzed. Average surrogate recoveries were 94(±15)% for PCB52L, 89(±20)% for FBDE69, and 111(±32)% for FBDE208.

Separate procedural blanks were analyzed to represent blood and placenta sample preparation procedure. The SRM 1957 (human serum) was analyzed for pesticides. No SRM was available for the target XFRs. For many of the OCPs and XFRs, sample concentrations were in the same range as the blank. After blank correction (by subtracting the blank from the sample) most of the compounds failed to meet the LOD. Repeated analysis showed good repeatability with RSD ranging from 0.5% to 10%. The SRM analysis accuracy ranged from 70% to 100%. Detection rate in placenta was higher compared to both maternal and cord blood. This may be caused by the procedural differences that existed between blood and placenta analysis. Guo at al. (2014) demonstrated the decomposition of some OCPs and XFR after acid treatment. In the analysis of blood, samples were treated with acid twice during the procedure.

Based on detection rate in all the three matrices, only HCB, p,p'-DDE, and p,p'-DDT were selected for further data analysis.

5.7.5 Concentration Levels of Selected Pesticides

As shown in Table XXXVI, the DDT metabolite DDE was much higher in all matrices compared to its parent compound. In placenta, DDE was 30 times higher than DDT. In maternal blood and cord blood DDE was six and three times higher. Higher DDE:DDT ratio in placenta may be indicative of placental metabolic break down of DDT and the accumulation of

DDE in placenta. On the contrary, in cord blood the DDE:DDT ratio is lower compared to both maternal blood and placenta.

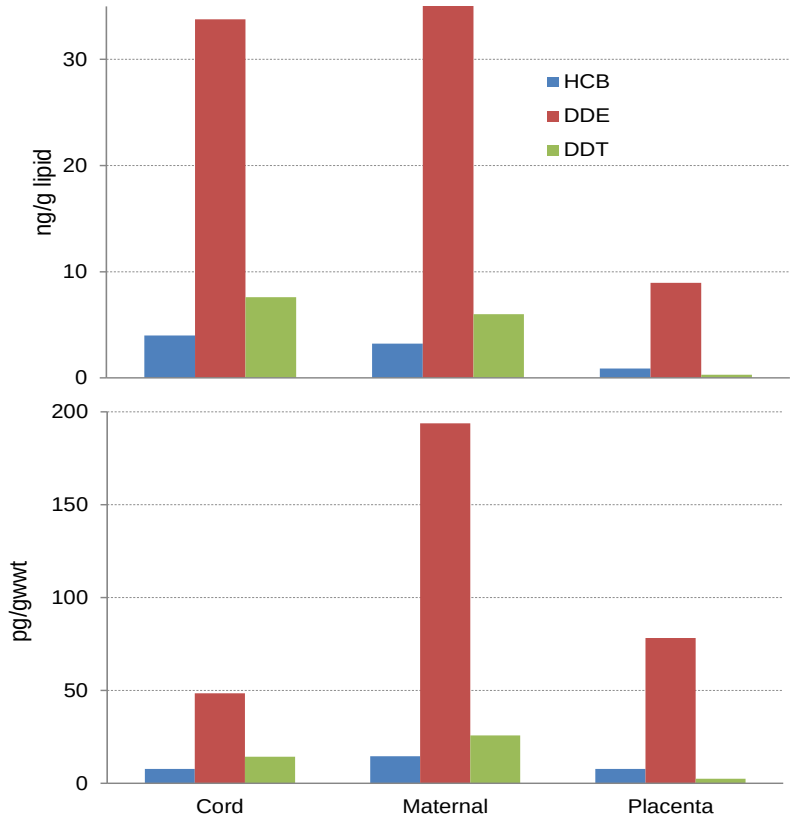


Figure 28. Selected OCP concentrations in cord blood, maternal blood, and placenta (above: lipid-adjusted median, ng/g lipid, below: unadjusted median, pg/g wwt).

TABLE XXXVI

SUMMARY STATISTICS OF CONCENTRATIONS OF SELECTED OCPS IN CORD BLOOD, MATERNAL BLOOD, AND PLACENTA

	Cord			Maternal			Placenta		
	HCB	p,p'-DDE	p,p'-DDT	HCB	p,p'-DDE	p,p'-DDT	HCB	p,p'-DDE	p,p'-DDT
					ng/g lipid				
Mean	5.52	93.35	14.68	3.88	84.92	15.24	0.97	16.56	0.50
Median	3.98	33.76	7.60	3.22	35.29	6.00	0.87	8.94	0.30
Min	1.76	6.46	3.59	1.94	18.62	2.43	0.37	4.12	0.13
Max	20.96	894.35	116.85	8.85	677.46	213.87	1.94	107.25	4.59
					pg/g wwt				
Mean	8.57	136.97	26.68	17.00	322.33	61.33	8.45	146.23	4.29
Median	7.70	48.44	14.36	14.59	193.78	25.76	7.73	78.24	2.50
Min	4.25	19.04	8.76	6.77	59.68	15.27	4.19	46.01	1.69
Max	18.43	1250.10	261.13	46.08	2597.30	819.93	23.47	900.93	38.54

5.7.5.1 **Correlation among Matrices**

Pearson's correlation analysis of HCB, DDE, and DDT revealed the existence of significant correlations within and between compartments (Table XXXVII). Highest correlation was seen between cord blood DDT and DDE (0.88) followed by maternal blood DDT and DDE (0.8). No significant relationships were found among HCBs in the three matrices but there were significant correlations between cord blood HCB and cord blood DDE and DDT, placental HCB and placental DDE, and maternal HCB and maternal DDE.

5.7.5.2 **Concentration Ratios**

Ratios for C/M and P/M were calculated both for wwt and lipid weight concentrations. Summary statistics for the lipid-adjusted concentration ratios are given in Table XXXVIII. Ratio distribution is also presented by using box plots with the median, quartile, 10th, and 90th percentiles and the outliers in figure 29.

Lipid-adjusted mean C/M ratios that were closer to 1 suggest the equal distribution of these OCPs between maternal and fetal blood circulations. On the other hand, it is an indication of passive diffusion (Vizcaino et al., 2014; Patayova et al., 2013). As per table XXXVIII, at the time of delivery, both HCB and DDT were present in the cord blood at higher propotions compared to DDE. The P/M ratios distribution of DDE and HCB were remarkably similar, while DDT P/M ratios were extremely low but interestingly also had the highest C/M ratio. Low P/M may be an indication of either low transfer into placenta or higher metabolism in placenta or both. Contrastingly, lower P/M ratio for DDT may be an indication of much faster metabolism of DDT into DDE in placenta. Comparatively higher P/M ratio of DDE is supportive for this notion.

TABLE XXXVII

PEARSON CORRELATION COEFFICIENTS AMONG CONCENTRATIONS OF HCB, DDE, AND DDT IN CORD BLOOD, MATERNAL BLOOD, AND PLACENTA *

	C_HCB	*C_DDE*	*C_DDT*	*M_HCB*	*M_DDE*	*M_DDT*	*P_HCB*	*P_DDE*	*P_DDT*
C_HCB									
C_DDE	**0.66**								
C_DDT	**0.53**	**0.87**							
M_HCB	0.23	0.20	0.08						
M_DDE	0.17	**0.73**	**0.65**	**0.48**					
M_DDT	-0.06	**0.53**	**0.68**	0.24	**0.79**				
P_HCB	0.38	0.35	0.23	0.33	0.25	0.02			
P_DDE	0.25	**0.71**	**0.63**	0.21	**0.77**	**0.59**	**0.58**		
P_DDT	0.10	**0.54**	**0.68**	0.08	**0.62**	**0.72**	0.24	**0.72**	

* Significant correlation coefficients ($p<.05$) are in bold phase, C=cord blood, M=maternal blood, P=placenta, N=24, lipid adjusted concentrations.

TABLE XXXVIII

SUMMARY STATISTICS OF DDE, DDT, AND HCB CONCENTRATION RATIOS

Compound	*Variable**	*Mean*	*sd*	*Min*	*Max*	*Median*	*Lower 95% CL*	*Upper 95% CL*
DDE	C/M_Ratio	1.1	0.9	0.1	3.5	0.8	0.7	1.5
	P/M_Ratio	0.3	0.2	0.1	0.8	0.2	0.2	0.4
DDT	C/M_Ratio	1.7	1.1	0.4	5.0	1.2	1.2	2.2
	P/M_Ratio	0.1	0.0	0.0	0.2	0.0	0.0	0.1
HCB	C/M_Ratio	1.6	1.2	0.3	5.1	1.2	1.1	2.1
	P/M_Ratio	0.3	0.2	0.1	0.8	0.2	0.2	0.4

CL: confidence limit,
sd = standard deviation
*: Lipid-adjusted concentration ratios

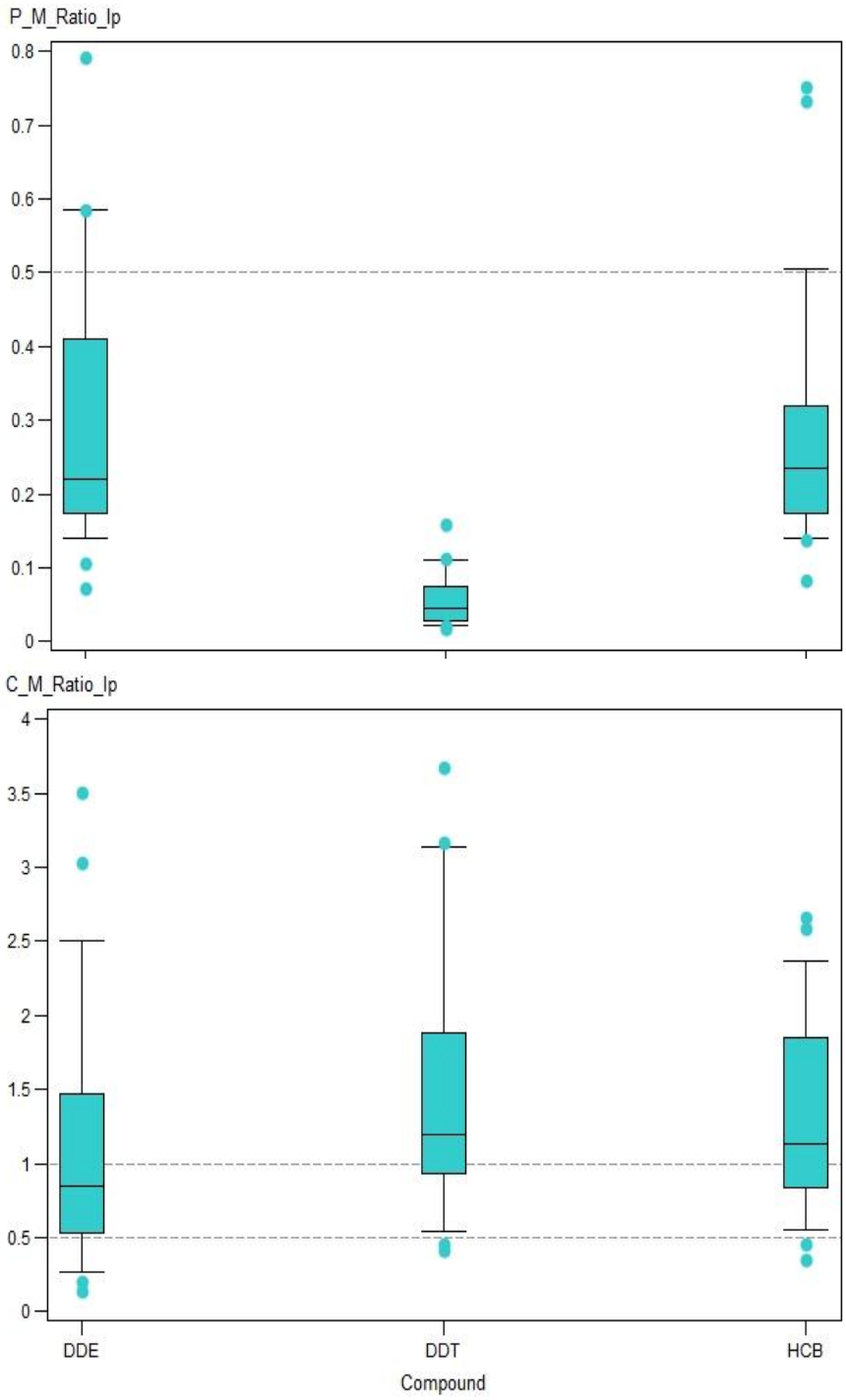


Figure 29. Box and whisker plots of C/M (below) and P/M (above) ratios distribution of DDE, DDT, and HCB (lp=lipid-adjusted).

5.7.6 **Halogenated Flame-Retardants**

A total of 31 non-PBDE flame-retardants (list of compounds is given in chapter 3) were analyzed in placenta, maternal blood, and cord blood (Table XXXIX). Most of the above compounds failed to show a response for selected ions or they were below the detection limit. Levels of TBECH-2, TBpX, TBCO, PBB, TboCT, PBT, PBEB, HBB, MIREX, PBB-101, BTBPE, TBPH, SYN-DP, ANTI-DP were detected at more than 40%. High background levels of HBB, TBPH, and BTBPE were revealed by procedural blanks. The data with blank-to-sample concentration ratios of >0.75 were discarded and those with the ratio 0.75 to 0.30 were blank-corrected by subtracting the mean blank level. Less than 30% were kept unaffected. When a target analyte was detected but the response was below its detection limit (3× signal-to-noise ratio), a value of LOD /√2 was used. Some compounds coeluted with PBDEs. For example, BB-153 coeluted with BDE-154, and TriBC with BDE-100. Most of the brominated compounds were analyzed using ECNI-MS with the most sensitive m/z values 79 and 81 as quantifier and qualifier ions respectively. Therefore, coeluting compounds could not be separated due to the lack of their own characteristic ions in the mass spectra. Concentrations for the correctly identified XFRs were calculated and are presented in Table XL. This is first attempt that has been taken to analyze most of these compounds in human placenta and cord blood.

TABLE XXXIX

XFR NONDETECTION RATE (%) IN CORD BLOOD, MATERNAL BLOOD, AND PLACENTA

XFR	LOD,pg/mL	Cord blood	Maternal blood	Placenta
TBECH-2	2.31	39	47	19
TBpX	0.15	17	35	50
TBCO	1.20	83	88	63
PBB	0.38	11	35	25
TboCT	0.56	72	76	100
PBT	0.14	11	12	25
PBEB	0.36	100	94	38
HBB	0.77	22	35	0
MIREX	6.00	94	88	6
PBB-101	0.46	72	71	38
BTBPE	2.14	0	0	50
TBPH	7.14	94	76	88
SYN-DP	3.75	100	100	56
ANTI-DP	3.53	50	35	0
DBDPE	1.0*	100	100	100

*ng/mL

TABLE XL

CONCENTRATION OF SELECTED HALOGENATED (NON-PBDE) FLAME-RETARDANTS (XFRS)

Compound	CORD BLOOD			MATERNAL BLOOD			PLACENTA		
	pg/g wwt								
	Mean	**Median**	**Range**	**Mean**	**Median**	**Range**	**Mean**	**Median**	**Range**
TBECH	2.24	1.02	<LOD–7.72	2.37	1.35	<LOD–7.88	0.90	0.94	<LOD–2.275
TBpX	9.48	6.21	<LOD–41.13	35.87	8.28	<LOD–201.86	0.27	0.11	<LOD–1.42
TBCO	1.78	1.49	<LOD–3.25	0.58	0.58	<LOD–0.65	0.14	0.08	<LOD–0.55
PBB	14.94	8.66	<LOD–78.14	60.79	13.88	<LOD–272.15	0.29	0.20	<LOD–0.91
TboCT	0.49	0.32	<LOD–1.22	0.38	0.36	<LOD–0.90	<LOD	<LOD	<LOD
PBT	0.50	0.46	<LOD–1.35	0.54	0.43	<LOD–1.80	0.20	0.13	<LOD–0.7
PBEB	<LOD	<LOD	<LOD	0.70	0.70	<LOD–0.70	0.23	0.12	<LOD–0.67
HBB	1.73	1.35	<LOD–4.30	2.65	2.91	<LOD–6.28	1.14	1.08	<LOD–1.91
MIREX	1.51	1.41	<LOD–3.06	3.39	1.41	<LOD–19.23	2.27	0.86	<LOD–15.07
PBB-101	0.14	0.11	0.08–0.35	0.18	0.11	0.09–0.53	0.03	0.02	<LOD–0.08
BTBPE	8.89	7.23	1.69–18.16	9.19	7.53	3.71–29.01	0.91	0.98	<LOD–2.06
TBPH	1.30	1.22	<LOD–2.93	2.62	2.31	<LOD–6.88	0.32	0.12	<LOD–0.72
SYN-DP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23.47	0.90	<LOD–133.17
ANTI-DP	11.13	10.56	<LOD–22.76	9.45	4.26	<LOD–38.48	98.70	4.34	<LOD–932.05
	ng/g lipid								
TBECH	1.56	0.63	<LOD–6.96	0.54	0.26	<LOD–2.27	0.12	0.09	<LOD–0.34
TBpX	5.49	2.84	<LOD–18.41	6.38	2.77	<LOD–28.84	0.06	0.01	<LOD–0.36
TBCO	0.67	0.61	<LOD–1.08	0.21	0.21	<LOD–0.33	0.02	0.01	<LOD–0.14
PBB	8.54	3.76	<LOD–34.97	11.05	4.83	<LOD–38.88	0.03	0.02	<LOD–0.10
TboCT	0.19	0.18	<LOD–0.41	0.09	0.09	<LOD–0.20	<LOD	<LOD	<LOD
PBT	0.38	0.24	<LOD–2.03	0.14	0.09	<LOD–0.60	0.02	0.01	<LOD–0.07
PBEB	<LOD	<LOD	<LOD	0.11	0.11	<LOD–0.11	0.03	0.02	<LOD–0.07
HBB	1.21	0.68	<LOD–6.44	0.54	0.48	<LOD–1.45	0.14	0.13	0.02–0.34
MIREX	1.05	0.71	<LOD–3.39	0.73	0.47	<LOD–3.91	0.26	0.12	0.02–1.60
PBB-101	0.09	0.06	0.01–0.32	0.04	0.03	0.01–0.11	0.00	0.00	<LOD–0.01
BTBPE	6.17	3.23	0.6–17.42	2.09	1.72	0.46–4.73	0.12	0.10	<LOD–0.30
TBPH	0.97	0.70	<LOD 1.87	0.70	0.56	<LOD–1.59	0.03	0.02	<LOD–0.06
SYN-DP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2.67	0.10	<LOD–11.89
ANTI-DP	6.35	4.11	<LOD–22.76	1.73	0.86	<LOD–9.77	12.13	0.55	0.2–102.03

6. CONCLUSIONS, SIGNIFICANCE, AND FUTURE WORK

This research has evaluated the human prenatal exposure to POPs, namely OCPs, PCBs, PBDEs, and alternative XFRs in the United States, with an emphasis on transplacental transfer of the chemicals. This section summarizes the results of the study and the conclusions reached based on such findings.

6.1 Conclusive Remarks

The optimized analytical methods were demonstrated to be reliable and efficient for the analyses of most targeted POPs in human placenta at trace levels. The major steps for the laboratory procedures included MSPD extraction with Florisil sorbent, silica gel chromatography cleanup, and instrumental analyses using large volume injection GC coupled with MS or MS/MS. The performance of the optimized method was validated by analyzing spiked placenta samples and an SRM of fish tissue, with the recovery ranging from 76% to 105% for the spiked samples and 89% to 115% for an SRM of fish tissue.

Human placenta has detectable and quantifiable levels of the POPs measured in this study, including the emerging flame-retardants. The PBDEs (sum of 10 congeners), PCBs (sum of 32 congeners), and DDE concentrations in 191 placentas ranged between 50 and 870, 220 and 1290, and 75 and 450 pg/g wwt, respectively. The BDE-209 and CB-101 were found to be the most abundant PBDE and PCB congeners in placenta. Collection-site-dependent PBDE concentrations were not significantly different. Total PCB, Total indicator PCB and Total dioxin-like PCB were significantly different for the placentas collected from Cache County and Salt

Lake County in Utah. The concentration of DDE that also show a significant difference was higher in the samples collected from Orange County, California.

The results of this work, in combination with the data obtained from a literature review, reveal that human prenatal exposure to most of the measured POPs in this study has generally decreased over time. However, an increasing trend was identified for the concentration of BDE-209 in placenta tissue. These observed time trends are in accordance with the commercial production and use histories and reflect the efficacy of the regulatory actions.

Together, maternal, placental, and fetal compartments represent a dynamic system where a number of physiological processes take place simultaneously. The POPs circulating within this system are subjected to one or more of the processes such as accumulation, transformation, elimination, diffusion, active transport, absorption, and storage. One-time sampling, similar to the at-birth sampling of this study, allows the generation of chemical concentration data that indicate only the net effect of above-mentioned processes present at the time of sampling. As it is difficult to isolate all the underlying processes and the magnitude of the effect (depending on the chemical), it may not be appropriate to discuss the concentration ratios measured in this study using a single term such as “transfer” or “accumulation” without the support of mechanistic studies, though it is the widely used strategy in the published literature. Rather, it is more suitable to compare concentration based on proportions and ratios. In the present study, the concentration ratios C/M and P/M were observed to be chemical-dependent. The concentration in the cord blood was higher than that in the placenta for all the chemicals analyzed. These proportions were inversely associated with the number of halogen substitutions for PBDE and PCB congeners. Though these findings favor some of the biological processes over the others (e.g., metabolism and accumulation), further studies such as placenta-perfusion models are

needed to correctly understand the findings. Though the placental barrier is inefficient in preventing transplacental transfer, the fetus is protected to a certain extent as placenta selectively retains some compounds, thus altering the relative concentration levels of the toxic chemicals in the maternal blood.

For chemicals analyzed in this work, concentration levels in placenta were weakly or moderately correlated with the concentrations in cord blood as well as maternal blood. Correlations between placenta and maternal blood were relatively stronger than the placenta-cord blood correlations. Cord blood BDE-209 was significantly correlated only to placental BDE-209. Placental PCB correlation with maternal and cord blood were extremely weak. Depending on the analyte, placenta may be used in human biomonitoring. Based on the result of this study placenta would be more appropriate as a surrogate for maternal exposure, where maternal blood is insufficient or not available.

6.2 **Significance of this Work**

Given the complexity of the placental tissue and the lack of standard method, establishment of a convenient and reliable analytical laboratory procedure for the analysis of PBDEs and other POPs in placenta tissues is a significant achievement of this research. The method was used to measure a selection of POPs in a large number of US placental tissue samples provided by the NCS as well as samples collected locally in Chicago, Illinois. To the best of our understanding, this is the largest study involving POPs analyses in placenta in the United States. Placenta is still an underutilized matrix in human biomonitoring. As a noninvasively collected tissue, placenta offers several advantages. This study demonstrated the versatility of using placental tissue (with regard to stability during storage and ease of collection), and the success when it is used in human biomonitoring.

As a part of this study, a large number of alternative flame-retardants were analyzed in placenta, maternal blood, and cord blood. Some of these chemicals have not been monitored in the human reproductive system before. In this study, we report prenatal exposure for most of the alternate flame-retardants for the first time. Results of this study are important to enrich the current global and US database and address the research needs. The results are also of assistance in better understanding of the current US human-exposure levels.

This study is one of the handfuls of studies performed to assess the sequential environmental pollutant transfer involving matched maternal blood, placenta, and cord blood. The study results strongly indicate the occurrence of in-utero exposure to mixtures of environmental pollutants. As evidence on toxicological effects of POPs accumulates, the findings of this study are important to persuade actions on chemical testing prior to mass production and release. Further these results can be used as evidence to reflect the effectiveness of imposed bans and restrictions.

6.3 Suggestions for Future Work

There is an urgent need for an improved laboratory analytical method particularly for the analysis of emerging environmental chemicals in blood. Enhancement of LODs is important because the amount of sample volume is often a limiting factor in blood analyses.

Some compounds, especially BDE-209, need dedicated new studies to evaluate the properties that govern the bioavailability and transfer among biological matrices. An experimental study involving a human placental perfusion system is recommended. The results of the study may be further strengthened by analyzing additional samples. Additionally, determination of metabolites concentration of parental compounds of POPs is strongly recommended. Sociodemographic information should be incorporated in future work, in order to

examine and predict potential associations between birth outcomes, maternal lifestyle, and chemical exposure.

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APPENDICES

APPENDIX A

TABLE XLI

DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (WWT.-BASED) IN UIC CORD BLOOD (CHAPTER 5)

	Cord blood							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
PCB	pg/gwwt							
8	14.53	0.95	4.07	10.30	12.47	18.53	22.71	37.57
28	16.42	0.99	4.77	12.63	16.57	21.20	25.28	35.01
52	17.10	0.86	4.42	13.48	16.87	21.64	25.51	37.33
49	6.97	0.90	2.75	5.17	6.29	9.45	11.13	14.07
44	11.79	1.06	4.63	7.66	10.54	16.07	17.33	29.24
37	3.49	1.04	1.52	2.40	3.23	4.38	5.34	7.04
74	5.87	1.05	2.06	4.24	5.70	8.04	9.09	10.84
70	15.87	0.93	3.28	10.49	14.35	21.31	27.25	38.50
66	7.00	1.02	2.30	5.01	6.53	8.98	10.81	14.77
60	1.82	1.11	1.22	1.35	1.49	1.57	2.47	7.22
101	28.49	1.07	7.78	17.03	23.91	37.54	49.81	83.57
99	11.59	1.10	3.99	7.43	9.91	16.87	19.44	27.60
87	13.68	1.05	4.10	7.46	12.71	17.96	24.11	35.49
82	3.43	1.01	1.46	1.99	2.87	4.62	6.04	7.77
77	1.83	1.17	1.28	1.42	1.57	1.59	2.13	7.59
118	21.17	1.23	8.06	12.26	15.31	31.38	34.95	53.28
114	1.90	1.21	1.33	1.48	1.63	1.66	2.21	7.89
153	14.14	1.06	5.15	8.16	12.64	20.15	22.88	31.28
179	1.58	1.01	1.11	1.23	1.36	1.38	1.84	6.55
105	6.21	1.24	2.01	4.25	5.40	8.17	9.80	14.11
138	9.33	1.16	2.78	6.85	9.07	11.48	15.81	18.97
158	1.75	1.11	1.22	1.35	1.50	1.52	2.03	7.24
187	2.70	1.12	1.24	1.44	2.17	3.45	4.86	7.32
166	1.81	1.15	1.27	1.41	1.55	1.58	2.11	7.51
183	1.85	1.18	1.30	1.44	1.59	1.61	2.15	7.68
126	2.03	1.29	1.42	1.57	1.74	1.77	2.36	8.41
128	1.68	0.97	1.07	1.23	1.33	1.91	2.16	6.34
156	2.09	1.33	1.47	1.62	1.80	1.82	2.43	8.68
180	4.26	1.23	1.40	1.68	4.11	6.10	7.75	10.96
170	2.12	1.24	1.36	1.51	1.69	1.97	2.99	8.05
169	2.19	1.39	1.53	1.70	1.87	1.90	2.54	9.06
189	2.08	1.32	1.45	1.61	1.78	1.81	2.42	8.61
Σ32PCB	238.8	35.9	96.9	172.6	226.1	312.2	361.5	514.7

APPENDIX A (continued)

TABLE XLII

DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (WET WT- BASED) IN UIC MATERNAL BLOOD (CHAPTER 5)

	Maternal blood							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
PCB	pg/gwwt							
8	19.19	0.86	2.40	15.11	18.11	22.36	26.95	67.25
28	22.35	1.11	5.05	16.45	22.21	26.90	30.27	66.80
52	22.80	0.97	2.33	17.36	21.80	23.37	33.70	97.82
49	8.69	1.00	1.56	6.26	8.03	9.10	12.82	37.26
44	14.46	1.19	2.50	11.30	13.30	16.27	21.26	54.66
37	3.90	1.17	1.39	2.87	3.77	4.21	5.15	13.68
74	10.37	1.32	4.32	6.71	8.80	11.82	17.46	29.54
70	18.85	1.04	3.63	13.37	18.47	22.13	26.66	68.97
66	9.57	1.15	4.00	6.94	9.10	10.58	13.43	32.27
60	2.34	1.24	1.32	1.45	1.73	2.43	3.04	8.90
101	37.21	1.33	8.09	22.94	34.70	43.38	51.77	161.59
99	18.13	1.37	7.91	11.24	16.16	22.71	27.28	61.63
87	17.73	1.31	4.36	12.64	17.70	21.02	22.78	68.76
82	3.78	1.13	1.57	2.44	3.23	4.33	5.22	14.05
77	1.75	1.31	1.36	1.46	1.53	1.59	2.07	4.78
118	36.05	10.29	20.55	22.90	31.30	41.08	51.72	135.29
114	1.85	1.36	1.42	1.52	1.60	1.66	2.26	4.97
153	35.26	5.51	15.47	23.54	32.42	44.74	62.72	80.94
179	1.54	1.13	1.18	1.26	1.32	1.38	1.92	4.13
105	10.26	4.16	5.60	6.46	9.32	12.00	14.79	33.05
138	23.38	6.16	11.80	13.10	21.62	30.92	42.40	50.63
158	2.04	1.25	1.33	1.45	1.73	2.49	2.98	4.56
187	6.76	1.41	2.51	3.07	6.08	7.81	12.17	19.00
166	1.73	1.29	1.35	1.45	1.51	1.58	2.04	4.73
183	2.73	1.33	1.50	1.56	2.42	3.52	4.66	5.42
126	1.94	1.45	1.51	1.62	1.69	1.77	2.29	5.30
128	2.19	1.09	1.16	1.27	1.84	2.65	2.99	8.11
156	3.53	1.50	1.69	1.81	2.98	4.55	6.54	9.11
180	15.21	1.53	4.82	7.86	13.40	21.67	26.67	33.31
170	5.89	1.39	1.62	2.96	5.25	8.72	9.96	13.72
169	2.09	1.56	1.63	1.75	1.83	1.90	2.47	5.71
189	1.99	1.48	1.55	1.66	1.74	1.81	2.34	5.42
Σ32PCB	365.5	64.5	176.3	241.3	321.3	448.7	499.6	1192.6

APPENDIX A (continued)

TABLE XLIII

DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (WET WT.-BASED) IN UIC PLACENTA (CHAPTER 5)

	Placenta							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
PCB	pg/gwwt							
8	14.78	6.78	8.79	10.85	12.34	18.11	23.10	28.22
28	17.05	7.20	9.52	11.12	15.10	21.16	27.24	42.36
52	27.06	8.20	11.60	13.79	21.90	32.92	55.56	72.93
49	9.27	2.58	4.06	4.56	7.35	11.27	18.30	25.71
44	16.84	4.38	7.24	8.07	13.25	19.41	34.48	49.81
37	4.15	0.96	1.69	1.93	3.75	5.10	7.37	13.65
74	9.33	3.26	4.67	5.26	7.78	10.89	15.61	22.51
70	22.92	4.91	7.42	9.85	18.72	25.62	48.02	73.82
66	10.85	3.16	3.96	5.14	8.62	13.19	20.34	31.02
60	2.68	0.98	1.09	1.29	2.07	3.38	5.02	7.84
101	37.48	8.14	11.81	16.30	28.60	35.70	84.87	122.90
99	17.09	5.74	6.94	9.47	14.41	16.69	34.02	45.51
87	17.28	3.53	4.92	7.33	12.60	17.94	41.97	52.19
82	3.60	0.72	1.01	1.49	2.36	3.83	8.42	11.99
77	0.47	0.20	0.20	0.20	0.34	0.53	1.01	1.47
118	28.18	10.23	11.52	16.56	20.56	28.66	58.38	72.58
114	0.79	0.21	0.36	0.51	0.66	0.91	1.32	2.16
153	25.08	9.27	13.15	14.80	23.05	30.44	37.04	65.51
179	0.83	0.17	0.30	0.44	0.59	1.08	1.92	2.22
105	9.23	3.53	4.25	5.38	6.70	10.88	18.68	25.96
138	17.54	6.22	9.43	12.43	14.82	20.94	25.63	48.37
158	1.87	0.58	0.78	0.88	1.16	2.53	3.19	7.99
187	4.81	1.64	2.00	2.51	4.32	6.67	8.69	9.17
166	0.21	0.20	0.20	0.20	0.20	0.20	0.26	0.32
183	2.05	0.78	0.91	1.33	1.76	2.51	3.68	4.53
126	0.24	0.22	0.22	0.22	0.22	0.22	0.29	0.35
128	1.81	0.74	0.87	1.12	1.34	2.35	3.10	4.81
156	2.37	0.87	1.15	1.40	2.00	2.83	4.21	6.41
180	9.30	2.59	3.52	4.79	6.98	14.12	17.52	20.07
170	3.71	1.08	1.25	1.61	2.64	5.78	7.32	8.96
169	0.38	0.24	0.24	0.24	0.32	0.50	0.59	0.85
189	0.26	0.23	0.23	0.23	0.23	0.30	0.35	0.36
Σ32PCB	319.5	109.8	154.5	213.6	274.1	311.5	591.6	783.0

APPENDIX A (continued)

TABLE XLIV

DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (LIPID-BASED) IN UIC CORD BLOOD (CHAPTER 5)

	Cord							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
	ng/g lipid							
8	8.88	0.52	1.84	5.07	7.20	10.47	17.71	28.42
28	10.52	0.41	2.16	5.64	8.34	10.75	23.50	37.98
52	10.55	0.35	2.00	6.89	8.64	11.30	22.54	36.50
49	4.33	0.37	1.53	2.45	3.23	4.77	9.27	16.13
44	7.20	0.43	2.12	4.12	5.43	7.97	14.83	24.73
37	2.13	0.43	1.09	1.28	1.55	2.17	4.75	6.53
74	3.62	0.43	1.44	1.91	2.82	4.08	7.28	12.00
70	9.92	0.38	1.52	5.37	7.17	10.77	22.26	34.24
66	4.41	0.42	1.40	2.38	3.27	4.95	9.99	15.40
60	1.06	0.45	0.52	0.63	0.83	1.52	2.05	2.52
101	17.97	0.44	3.19	8.68	13.43	20.88	35.33	68.17
99	7.33	0.45	1.98	3.47	5.46	8.05	15.05	26.19
87	8.58	0.43	1.88	4.20	6.31	9.85	17.14	31.99
82	2.11	0.42	0.88	1.23	1.48	2.22	4.01	6.92
77	1.05	0.48	0.55	0.66	0.80	1.59	1.59	2.39
118	13.65	0.50	4.90	6.62	9.58	15.01	24.01	52.44
114	1.09	0.50	0.57	0.69	0.83	1.66	1.66	2.49
153	8.93	0.43	3.33	4.31	7.27	10.45	15.05	35.16
179	0.91	0.41	0.48	0.57	0.69	1.38	1.38	2.06
105	3.85	0.51	1.64	1.69	2.76	4.30	6.93	13.92
138	5.74	0.48	1.59	2.95	4.88	7.24	12.14	16.91
158	1.00	0.46	0.53	0.63	0.76	1.52	1.52	2.28
187	1.66	0.46	0.58	0.88	1.45	1.69	2.23	7.62
166	1.04	0.47	0.55	0.65	0.79	1.58	1.58	2.37
183	1.06	0.48	0.56	0.67	0.81	1.61	1.61	2.42
126	1.17	0.53	0.61	0.73	0.88	1.77	1.77	2.65
128	1.00	0.40	0.46	0.55	0.80	1.33	1.80	3.00
156	1.20	0.55	0.63	0.76	0.91	1.82	1.82	2.73
180	2.85	0.50	0.63	1.13	1.88	2.98	5.47	16.44
170	1.28	0.51	0.58	0.70	1.01	1.69	1.69	5.43
169	1.26	0.57	0.66	0.79	0.95	1.90	1.90	2.85
189	1.19	0.54	0.63	0.75	0.90	1.81	1.81	2.71
Σ32PCB	148.6	14.7	63.6	80.7	111.9	156.3	301.6	523.4

APPENDIX A (continued)

TABLE XLV
DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (LIPID-BASED) IN UIC MATERNAL BLOOD (CHAPTER 5)

	Maternal Blood							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
	ng/g lipid							
8	4.25	0.18	0.50	2.65	4.45	5.32	7.89	9.61
28	4.94	0.23	1.31	3.55	4.47	5.99	9.41	12.02
52	5.05	0.20	0.76	2.98	4.70	5.84	10.37	13.97
49	1.93	0.20	0.50	1.03	1.66	2.11	4.08	5.32
44	3.27	0.24	0.77	1.82	3.07	3.59	7.02	8.11
7	0.89	0.24	0.40	0.49	0.77	0.99	1.75	2.14
74	2.21	0.43	0.85	1.46	2.13	2.87	3.59	4.50
70	4.30	0.21	0.85	2.75	3.72	4.91	9.13	11.50
66	2.13	0.23	0.83	1.39	1.97	2.51	4.00	4.96
60	0.51	0.15	0.26	0.34	0.43	0.56	0.90	1.27
101	8.41	0.26	1.71	5.02	7.16	8.46	18.26	25.81
99	3.93	0.45	1.83	2.34	3.56	4.58	7.17	8.83
87	4.01	0.25	0.83	2.60	3.64	4.38	8.30	11.16
82	0.86	0.23	0.41	0.59	0.73	0.78	1.80	2.63
77	0.40	0.16	0.22	0.27	0.37	0.49	0.66	0.80
118	7.92	3.38	4.17	5.07	6.48	8.54	14.48	21.21
114	0.42	0.17	0.24	0.29	0.38	0.51	0.68	0.83
153	7.71	1.81	3.43	4.50	8.04	9.37	11.48	18.59
179	0.36	0.14	0.19	0.23	0.32	0.42	0.59	0.69
105	2.28	0.98	1.18	1.51	2.00	2.43	4.14	5.91
138	5.14	1.54	2.04	3.19	5.07	6.44	7.18	11.22
158	0.47	0.21	0.25	0.34	0.41	0.56	0.65	1.23
187	1.49	0.46	0.58	0.82	1.36	1.76	2.32	5.57
166	0.40	0.16	0.22	0.26	0.36	0.49	0.65	0.79
183	0.62	0.16	0.37	0.40	0.54	0.69	0.90	2.02
126	0.44	0.18	0.25	0.29	0.41	0.54	0.73	0.88
128	0.49	0.18	0.23	0.33	0.40	0.52	0.91	1.40
156	0.77	0.18	0.42	0.52	0.63	0.96	1.32	1.63
180	3.45	0.50	1.17	1.83	2.73	4.57	5.50	13.13
170	1.35	0.33	0.44	0.55	1.07	1.75	2.58	5.02
169	0.48	0.19	0.27	0.32	0.44	0.59	0.78	0.95
189	0.46	0.18	0.25	0.30	0.42	0.56	0.75	0.90
Σ32PCB	81.3	21.2	37.0	51.5	73.9	89.9	144.7	208.0

APPENDIX A (continued)

TABLE XLVI
DESCRIPTIVE STATISTICS FOR PCB MEASUREMENTS (LIPID-BASED) IN UIC PLACENTA (CHAPTER 5)

	Placenta							
	Mean	Min	10%ile	25%ile	Median	75%ile	90%ile	Max
	ng/g lipid							
8	1.78	0.47	0.78	1.22	1.81	2.35	2.91	3.25
28	2.02	0.50	0.92	1.29	1.90	2.51	3.07	4.51
52	3.15	0.57	1.23	1.49	2.74	3.97	6.07	7.76
49	1.08	0.18	0.42	0.53	0.93	1.39	1.98	2.74
44	1.96	0.31	0.75	0.96	1.66	2.64	3.67	5.30
37	0.48	0.07	0.16	0.25	0.43	0.66	0.81	1.45
74	1.08	0.29	0.49	0.64	0.86	1.53	1.68	2.39
70	2.65	0.34	0.86	1.18	2.10	3.91	5.11	7.85
66	1.26	0.22	0.46	0.64	1.09	1.72	2.18	3.30
60	0.31	0.07	0.13	0.16	0.25	0.44	0.53	0.83
101	4.28	0.57	1.51	1.79	3.06	5.49	9.03	13.07
99	1.96	0.40	0.87	1.16	1.51	2.50	3.62	4.84
87	1.96	0.25	0.67	0.84	1.41	2.39	4.47	5.55
82	0.40	0.05	0.14	0.17	0.27	0.44	0.90	1.28
77	0.05	0.01	0.02	0.02	0.04	0.07	0.11	0.16
118	3.19	0.72	1.65	1.82	2.53	3.56	6.21	7.72
114	0.09	0.02	0.05	0.06	0.07	0.12	0.15	0.23
153	2.90	0.83	1.41	1.69	3.19	3.68	4.00	6.97
179	0.09	0.01	0.04	0.05	0.07	0.13	0.20	0.24
105	1.05	0.25	0.52	0.59	0.95	1.09	1.99	2.76
138	2.01	0.65	1.11	1.34	1.87	2.43	2.75	5.15
158	0.22	0.04	0.08	0.11	0.15	0.26	0.34	0.85
187	0.56	0.16	0.20	0.31	0.49	0.80	0.93	1.04
166	0.03	0.01	0.02	0.02	0.02	0.03	0.03	0.05
183	0.24	0.07	0.09	0.16	0.23	0.30	0.38	0.48
126	0.03	0.02	0.02	0.02	0.03	0.03	0.04	0.06
128	0.21	0.05	0.10	0.14	0.17	0.27	0.31	0.51
156	0.28	0.08	0.11	0.16	0.22	0.33	0.51	0.68
180	1.10	0.26	0.36	0.54	0.85	1.57	2.06	2.62
170	0.44	0.10	0.13	0.17	0.31	0.63	0.81	1.27
169	0.05	0.02	0.02	0.03	0.04	0.06	0.07	0.13
189	0.03	0.02	0.02	0.02	0.03	0.03	0.04	0.06
Σ32PCB	36.9	7.7	18.5	22.9	32.1	47.5	62.9	83.3

APPENDIX A (continued)

TABLE XLVII

FRESH WEIGHT-BASED CONCENTRATIONS OF PBDES IN MATERNAL BLOOD, PLACENTA, AND CORD BLOOD

		Maternal blood	**Fresh weight-based concentration, pg/g wet weight**									
					Pacenta				Cord blood			
BDE-28	1.6	1.0	0.5	6.9	1.9	1.5	0.1	8.3	0.9	0.6	0.4	2.8
BDE-47	78.6	45.7	14.6	273.0	46.1	30.6	0.5	224.7	30.2	19.0	9.5	107.4
BDE-100	13.7	7.8	0.8	45.7	10.2	7.3	1.1	36.6	3.9	2.7	0.9	15.7
BDE-99	11.8	6.6	0.7	41.9	8.5	6.1	1.8	34.0	5.2	2.2	0.5	18.5
BDE-85	2.4	1.9	0.9	5.4	1.0	0.7	0.3	3.0	1.6	1.1	0.8	5.1
BDE-154	5.4	5.2	2.0	11.4	3.2	2.6	0.6	12.8	2.4	2.0	0.8	8.1
BDE-153	21.7	15.5	3.6	52.4	15.3	11.6	2.4	64.8	5.2	4.4	2.4	14.4
BDE-183	1.2	0.8	0.7	6.2	0.5	0.5	0.1	2.0	1.0	0.8	0.6	3.9
BDE-209	51.1	45.7	19.7	128.1	279.9	17.9	9.2	3205.8	43.1	34.2	21.9	218.1
Σ_{10}PBDE	188.8	134.0	47.1	446.4	367.0	83.2	30.8	3412.7	94.7	71.8	41.9	314.2
$\Sigma_{3\text{-}7}$PBDE	137.7	95.1	25.5	394.6	87.1	63.7	13.5	326.5	51.6	35.2	18.9	139.9

APPENDIX B

TABLE XLVIII

BACKGROUND LEVELS ANALYZED BY BLANKS (NG/ML), (NCS STUDY, CHAPTER 4)

Analyte	Sample median	Blank median	Blank as a % of sample
PBDEs (N=19)			
BDE-28+-33	0.020	0.000	0
BDE-47	0.435	0.030	7
BDE-66	0.007	0.000	0
BDE-100	0.109	0.007	6
BDE-99	0.141	0.019	13
BDE-85	0.023	0.000	0
BDE-154	0.035	0.000	0
BDE-153	0.180	0.008	5
BDE-183	0.018	0.000	0
BDE-209	0.609	0.200	33
PCBs and DDE (N=23)			
CB-8	0.171	0.020	12
CB-28	0.238	0.018	7
CB-52	0.447	0.028	6
CB-49	0.178	0.012	7
CB-44	0.328	0.021	6
CB-37	0.111	0.006	5
CB-74	0.228	0.010	5
CB-70	0.588	0.036	6
CB-66	0.274	0.015	5
CB-60	0.070	0.003	4
CB-101	0.865	0.065	7
CB-99	0.371	0.025	7
CB-87	0.466	0.041	9
CB-82	0.092	0.010	11
CB-77	0.021	0.000	0
CB-118	0.686	0.070	10
CB-153	0.467	0.042	9
CB-179	0.018	0.003	16
CB-105	0.243	0.027	11
CB-138	0.367	0.044	12
CB-158	0.033	0.004	12
CB-187	0.085	0.008	9
CB-166	0.002	0.000	0
CB-183	0.033	0.004	13
CB-126	0.002	0.000	15
CB-128	0.041	0.009	23
CB-156	0.054	0.001	3
CB-180	0.192	0.007	3
CB-170	0.082	0.000	0
DDE	2.049	0.007	0

APPENDIX B (continued)

TABLE XLIX

LIMITS OF DETECTION (LOD), (NCS STUDY, CHAPTER 4)

Analyte	pg/mL	pg/g ww (for 10g sample)	RSD%, n=7
BDE-28+-33	0.86	0.09	7
BDE-47	7.18	0.72	62
BDE-66	2.43	0.24	21
BDE-100	4.91	0.49	28
BDE-99	11.85	1.19	68
BDE-85	5.45	0.54	31
BDE-154	5.06	0.51	22
BDE-153	4.07	0.41	17
BDE-183	4.31	0.43	15
BDE-209	27.53	2.75	24
CB-8	4.47	0.45	46
CB-28	5.73	0.57	59
CB-52	5.01	0.50	52
CB-49	5.19	0.52	53
CB-44	6.15	0.61	63
CB-37	6.05	0.60	62
CB-74	6.11	0.61	63
CB-70	5.38	0.54	55
CB-66	5.94	0.59	61
CB-60	6.43	0.64	66
CB-101	6.18	0.62	64
CB-99	6.36	0.64	65
CB-87	6.08	0.61	63
CB-82	5.87	0.59	60
CB-77	6.76	0.68	70
CB-118	7.14	0.71	74
CB-114	7.03	0.70	72
CB-153	6.14	0.61	63
CB-179	5.84	0.58	60
CB-105	7.19	0.72	74
CB-138	6.74	0.67	69
CB-158	6.45	0.64	66
CB-187	6.52	0.65	67
CB-166	6.69	0.67	69
CB-183	6.84	0.68	70
CB-126	7.49	0.75	77
CB-128	5.65	0.56	58
CB-156	7.73	0.77	80
CB-180	7.12	0.71	73
CB-170	7.17	0.72	74
CB-169	8.07	0.81	83
CB-189	7.67	0.77	79
DDE	6.50	0.65	67

APPENDIX B (continued)

TABLE L

ANALYTE DETECTION RATE IN PLACENTA SAMPLES COLLECTED IN THE NCS P18 MAIN STUDY (CHAPTER 4)

PCB	Detection rate,%		PBDE	Detection rate,%		DDE	Detection rate,%
CB-8	100		BDE-28+-33	100		DDE	100
CB-28	100		BDE-47	100			
CB-52	100		BDE-66	68			
CB-49	100		BDE-100	100			
CB-44	100		BDE-99	99			
CB-37	100		BDE-85	81			
CB-74	100		BDE-154	92			
CB-70	100		BDE-153	100			
CB-66	100		BDE-183	90			
CB-60	100		BDE-209	100			
CB-101	100						
CB-99	100						
CB-87	100						
CB-82	100						
CB-77	94						
CB-118	100						
CB-114	95						
CB-153	100						
CB-179	98						
CB-105	100						
CB-138	100						
CB-158	100						
CB-187	100						
CB-166	2						
CB-183	100						
CB-126	2						
CB-128	100						
CB-156	99						
CB-180	100						
CB-170	100						
CB-169	70						
CB-189	25						

APPENDIX B (continued)

TABLE LI

SURROGATE RECOVERY (NCS STUDY, CHAPTER 4)

Surrogate	Recovery %		
	Median	Average	sd(±)
^{13}**C PCB-52** for PCB, DDE	82	82	13
F – BDE-69 for tri-hepta PBDEs	90	91	17
F – BDE-208 for deca BDE	99	111	43

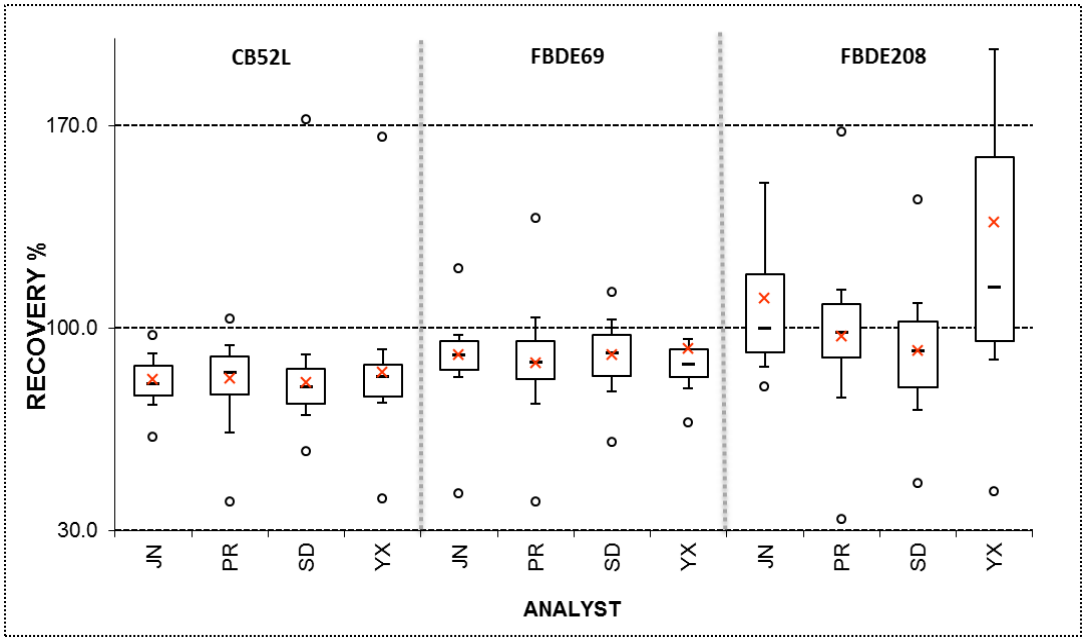

Figure 30. Surrogate recoveries by analyst (NCS study, chapter 4).

APPENDIX B (continued)

TABLE LII

ANALYTICAL RESULTS FOR NIST SRM 1957 (UNFORTIFIED HUMAN SERUM)
(UIC STUDY, CHAPTER 5)

	Replicate 1	Replicate 2	Replicate 3	Ave. Conc ng/kg	sd	RSD %	Certified conc. ng/kg	Accuracy %
PBDEs								
BDE-28	9.0	12.6	11.8	11.1	1.9	16.8	20.8 ± 2.4*	53
BDE-47	276.9	263.3	293.5	277.9	15.1	5.4	272 ± 14	102
BDE-66	3.9	1.6	2.3	2.6	1.2	46.4	7.14 ± 0.83*	36
BDE-100	66.8	51.2	56.4	58.1	8.0	13.7	50.5 ± 2.5	115
BDE-99	64.3	58.1	101.8	74.7	23.7	31.6	77.8 ± 1.7	96
BDE-85	10.9	11.8	7.6	10.1	2.2	21.5	8.8 ± 1.8*	115
BDE-154+ BB153	28.8	29.3	31.7	29.9	1.6	5.2	7.5 ±1.1* (+15.8±1.8)	129
BDE-153	71.1	55.1	67.7	64.6	8.4	13.0	62.1 ± 3.0	104
BDE-183	8.6	9.5	11.3	9.8	1.4	14.2	3.4 ± 2.3*	288
BDE-209	174.5	162.2	157.9	164.9	8.6	5.2	NA	_
PCBs								
CB-28	17.0	13.9	16.3	15.8	1.6	10.3	9.3 ± 1.2*	170
CB-74	14.6	20.0	16.2	16.9	2.8	16.6	14.3 ± 1*	118
CB-66	6.4	10.6	5.2	7.4	2.8	38.2	6 ± 1.8*	124
CB-99	17.3	23.8	18.0	19.7	3.6	18.3	12.1 ± 1.0*	163
CB-118	28.5	46.3	31.6	35.5	9.5	26.9	18.5 ± 2.7	192
CB-153	70.7	95.5	76.8	81.0	12.9	16.0	57.2 ± 3.3	142
CB-105	5.7	12.0	5.8	7.9	3.6	46.1	4.1 ± 3.1*	192
CB-138	37.9	56.6	40.8	45.1	10.1	22.4	36.9 ± 5.4	122
CB-183	6.8	11.2	6.7	8.2	2.6	31.3	6.2 ± 0.4*	133
CB-180	53.4	71.6	57.3	60.8	9.6	15.8	54.4 ± 1.3	112
CB-170	16.7	23.4	17.0	19.0	3.8	20.0	16.7 ± 2.6	114
CB-187	14.9	23.9	14.8	17.8	5.2	29.3	15.7 ± 0.9	114
CB-156	8.7	13.0	8.7	10.1	2.5	24.2	8.8 ± 1.1*	115
Pesticides								
HCB	23.7	20.4	20.3	21.5	1.9	9.0	30.3 ± 4.1	71
b-HCH	26.9	26.7	26.6	26.7	0.1	0.6	32.3 ±7.3*	83
t- Nonachlor	63.4	58.9	57.8	60.0	3.0	4.9	59.9 ±0.5	100
p,p' DDE	900.2	766.8	749.0	805.4	82.7	10.3	940 8	86

*Reference value

APPENDIX B (continued)

TABLE LIII

ANALYSIS OF NIST STANDARD REFERENCE MATERIAL SRM-1947 (LAKE MICHIGAN FISH TISSUE)

	SRM replicates			Replicate Average			Sample conc.	Certified conc.		Accuracy
Compound	1-SRM	2-SRM	3-SRM	ng/mL	sd±	RSD%	ug/kg wwt	ug/kg wwt	sd±	%
CB-18	0.69	0.67	0.67	0.68	0.02	2.31	2.71	2.72*	0.95	100
CB-28	5.90	5.53	5.80	5.74	0.19	3.36	22.98	14.10	1.00	163
CB-52	9.29	9.14	10.30	9.58	0.63	6.61	38.31	36.40	4.30	105
CB-44	5.85	5.47	5.65	5.66	0.19	3.28	22.62	20.40	1.70	111
CB-74	7.47	6.86	7.16	7.16	0.30	4.23	28.65	33.70	3.10	85
CB-66	16.64	15.91	15.79	16.11	0.46	2.84	64.45	69.40	5.30	93
CB-101	21.66	20.29	21.55	21.17	0.76	3.58	84.67	90.80	0.30	93
CB-99	19.70	21.86	23.49	21.68	1.90	8.78	86.73	78.00	6.00	111
CB-87	7.46	8.88	8.50	8.28	0.73	8.88	33.12	27.90	1.50	119
CB-110	22.55	26.16	24.88	24.53	1.83	7.46	98.12	94.60	4.30	104
CB-82	0.78	0.88	0.91	0.85	0.07	7.89	3.41	3.87*	0.67	88
CB-153	60.28	67.67	67.34	65.10	4.17	6.41	260.40	201.00	3.00	130
CB-138	43.78	46.72	53.11	47.87	4.77	9.96	191.48	162.00	6.90	118
CB-187	16.73	18.38	19.89	18.33	1.58	8.62	73.32	54.80	2.60	134
CB-174	5.27	6.16	6.03	5.82	0.48	8.23	23.29	18.60	1.70	125
CB-180	22.31	25.27	24.17	23.91	1.50	6.25	95.66	80.80	5.00	118
p,p'-DDE	127.42	127.87	127.17	127.48	0.36	0.28	509.94	720.00	43.00	71
BDE-(28 +33)	0.55	0.54	0.53	0.54	0.01	1.22	2.17	2.26*	0.46	96
BDE-49	1.23	1.24	1.23	1.23	0.00	0.30	4.93	4.01	0.10	123
BDE-47	22.09	21.97	22.22	22.09	0.12	0.56	88.37	73.30	2.90	121
BDE-100	5.10	4.97	5.01	5.02	0.07	1.37	20.10	17.10	0.60	118
BDE-99	4.99	4.90	4.95	4.95	0.04	0.90	19.79	19.20	0.80	103
BDE-154	1.35	1.37	1.38	1.37	0.01	0.92	5.47	6.88	0.52	80
BDE-153	1.18	1.15	1.12	1.15	0.03	2.58	4.60	3.83	0.04	120

*: a reference value

APPENDIX C

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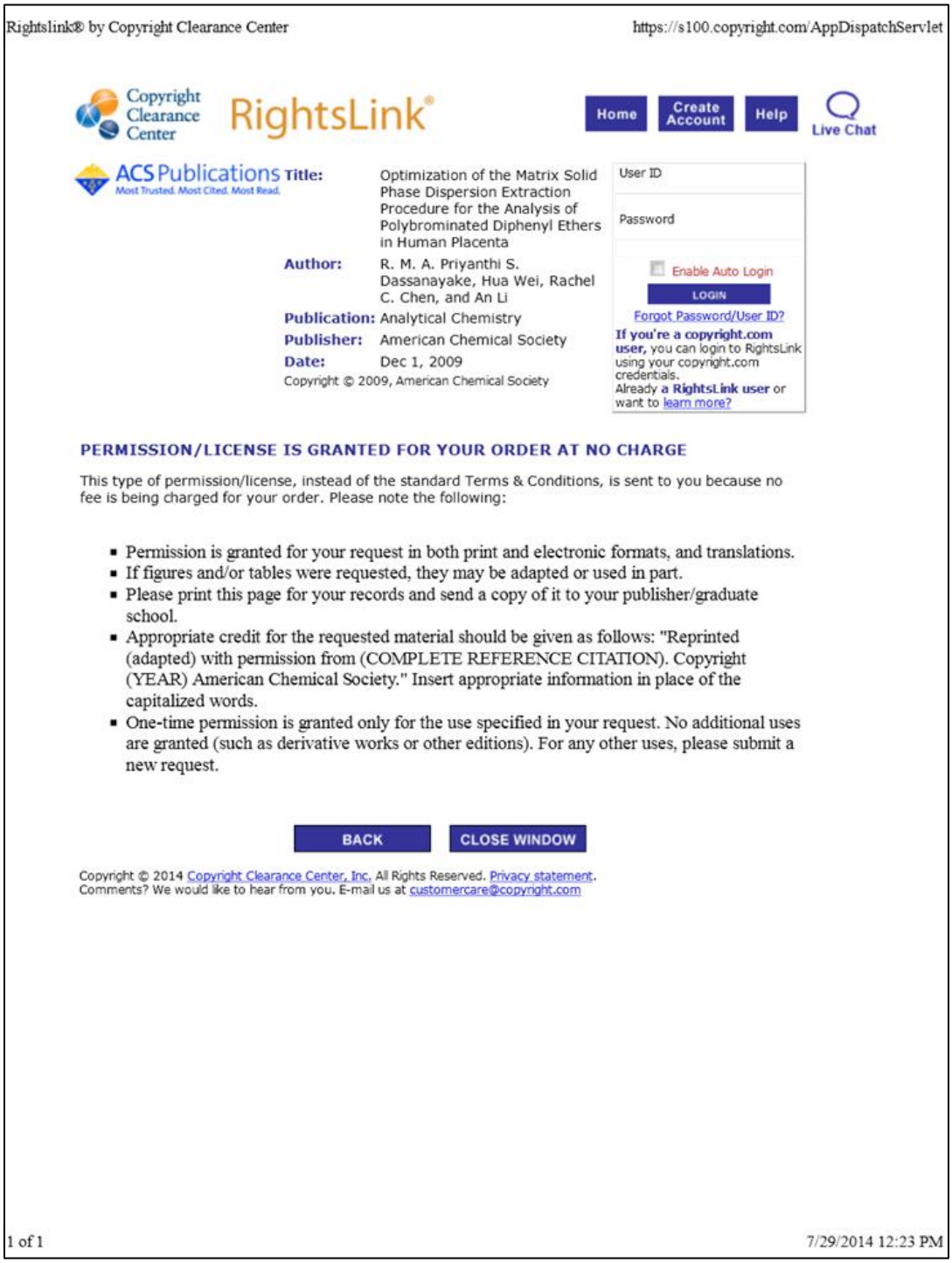

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Title: Optimization of the Matrix Solid Phase Dispersion Extraction Procedure for the Analysis of Polybrominated Diphenyl Ethers in Human Placenta

Author: R. M. A. Priyanthi S. Dassanayake, Hua Wei, Rachel C. Chen, and An Li

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