Polybrominated Diphenyl Ethers in Human Placentas in the United States

BY

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THESIS

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May this piece of work be desirable to You and find favor in Your eyes.

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This thesis marks the completion of the pursuit of a degree, and from here more pursuits have just begun.

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LIST OF ABBREVIATIONS

AIC	Akaike Information Criterion
BDE	Brominated Diphenyl Ether
CI	Chemical Ionization
DCM	Dichloromethane
ECD	Electron Capture Detector
ECNI	Electron Capture Negative Ionization
EI	Electron Impact Ionization
EPA	Environmental Protection Agency
FBDE	Fluorinated Polybrominated Diphenyl Ether
FD	Freeze Dry
GC	Gas Chromatography
GPC	Gel-Permeation Chromatography
ID	Identification Number
IEPA	Illinois Environmental Protection Agency
IRIS	Integrated Risk Information System
IUPAC	International Union of Pure and Applied Chemistry
K-D	Kuderna-Danish
K_{OW}	Octanol-Water Partition Coefficient
LOD	Limit of Detection
LOQ	Limit of Quantification
MAE	Microwave-Assisted Extraction

LIST OF ABBREVIATIONS (continued)

MCW	Medical College of Wisconsin
MDL	Method Detection Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
MSPD	Matrix Solid-Phase Dispersion
NCSAC	The National Children's Study Federal Advisory Committee
NICHD	The National Institute of Child Health and Human Development
NCS	National Children's Study
PBDE	Polybrominated Diphenyl Ether
PCB	Polychlorinated Biphenyl
PFE	Pressurized Fluid Extraction
PTV	Programmable Temperature Vaporization
QA/QC	Quality Assurance and Quality Control
QQQMS	Triple Quadrupole Mass Spectrometer
SLE	Solid Liquid Extraction
SPME	Solid Phase Microextraction
UCD	University of California Davis
UR	University of Rochester

SUMMARY

As a group of synthetic brominated organic chemicals, polybrominated diphenyl ethers (PBDEs) are widely used in consumer products as flame retardants to enhance fire safety. On the other hand, their detection in various matrices of the environment and human body has raised many concerns. Research has identified PBDEs as potential endocrine disrupters that interfere with thyroid hormones, which may lead to irreversible cognitive defects in newborns. However, there is not enough data to know the prenatal exposure to PBDEs and its association with health effects among children. As part of a formative research project under the National Children's Study (NCS), this study aims to produce a large data set on the levels of PBDEs in human placenta in the United States. It also intends to evaluate collection time and site effects on measured PBDE levels. A total of 43 placentas were collected at University of Rochester (UR), University of California Davis (UCD), and Medical College of Wisconsin (MCW). Each placenta was sampled at different collection times up to 96 hours after the delivery, resulting in a total of 169 tissue samples. Analytical procedure included matrix solid-phase dispersion technique for extractions of PBDEs from human placentas, multi-layer silica gel column chromatography for cleaning up the extract, and gas chromatography coupled with mass spectrometry to determine the concentrations of BDEs 28 + 33, 47, 66, 83, 99, 100, 153, 154, 183, and 209. Quality control and quality assurance procedures including spikes, surrogates, and blanks were applied. The average surrogate recovery of FBDE 69 is 106 ± 19.4% and that of FBDE 208 is 97.3 ± 25.5 %. The recoveries of the ten BDEs from the matrix spike range from 56% to 98%. The median of the Σ_{10}BDEs in the 42 placentas is 330 pg/g wet wt (42.6–1723 pg/g wet wt). The total concentration of tri- to heptaBDEs is lower than that from Dassanayake et al.,

(2009) who reported a range of 355–2303 pg/g wet wt for the same nine congeners in five placentas collected in 2007–2008 in Chicago, Illinois, yet still significantly higher than those reported from Europe and Japan. The level of BDE 209, however, is 56% higher than that from Dassanayake et al., (2009). The PBDE levels approximately follow a log-normal distribution. Among the ten congeners measured, BDE 47 is the most abundant, followed by BDEs 153, 99, 100, and 209. The congener distribution pattern is similar in placentas collected from all three collection sites. Among the three sites, the concentration of Σ_{10}BDEs from UCD is statistically significantly higher than that from UR and MCW at $p = 0.1$ level. With regard to collection time effect, the percent change in Σ_{10}BDEs is in the range of -9.0% to 15.8% up to 72 hours after the initial sample collection. Storing a placenta for 96 hours has led to more significant changes in PBDE levels. Mixed effects models with placenta chosen as a random effect to count for its uniqueness were developed in this thesis. The models demonstrate no linear relationship between the Σ_{10}BDEs concentration and the collection time. In addition, no interaction between collection time and location is observed. These findings along with the large data set provide opportunities to further study the association between prenatal exposure to PBDEs and health effects in children.

1. INTRODUCTION

1.1 Introduction

Flame retardants are required to be added to consumer products to suppress ignition and limit the spread of fire if ignition takes place in order to save lives and prevent property loss. Among various flame retardant groups, compounds that contain a high portion of bromine are widely used because of their high efficiency in suppressing ignition and relatively low cost (IEPA 2006). Polybrominated diphenyl ethers (PBDEs), one of the main classes of brominated flame retardants, have been manufactured since the 1970s. They have been added to a wide range of products, including furniture, electronic casing, plastics, textiles, nylon, and adhesives (Frederiksen et al., 2009b).

While PBDEs have been widely used as flame retardants, they have also caused problems as persistent organic pollutants. Because of their high affinity to lipids and low water solubility, PBDEs tend to accumulate in lipids of biota and have the potential to biomagnify in the food web (Frederiksen et al., 2009b). They have been found in various matrices of the environment, wildlife, and human. When some legacy persistent organic pollutants such as organochlorine pesticides and polychlorinated biphenyls (PCBs) are declining, PBDEs have been rapidly increasing in human tissues from the 1970s to the mid-2000s in the United States (Schecter et al., 2005), doubling every 3–5 years (Hites 2004). The levels of PBDEs in many matrices, including in-house dust, breast milk, and human blood, are much higher in the North America than in Europe, Asia, and Oceania (Frederiksen et al., 2009b).

Due to the increasing detection and levels of PBDEs in human bodies, concerns have been raised whether this synthetic chemical would bring health concerns. Animal studies have shown that the acute toxicity of commercial PBDEs were low (Darnerud 2003). However, there

are also strong reasons to suspect PBDEs as endocrine disrupters that interfere with thyroid hormones (Darnerud 2008). Since thyroid hormones are crucial to brain development, PBDEs may potentially cause irreversible cognitive defects in newborns (Darras 2008). Studies have already found a strong correlation between elevated PBDE levels in breast milk and cryptorchidism in the newborns of those mothers (Main et al., 2007).

The potential endocrine disruption caused by PBDEs raises special attention to their impact on infants and children as brain development is vital during those ages. Infants are primarily exposed to PBDEs through breast milk, and young children through incidental ingestion of dust (Schecter et al., 2006). However, the prenatal exposure to PBDEs has not been well assessed. Previous prenatal exposure studies were often based on data from maternal blood and cord blood collected at the time of birth. Human placenta may have the advantage of better reflecting the exposure for a longer time period during the pregnancy. It is also a good matrix in which to study the mechanisms and kinetics of cross-placental transfer of toxics and nutrients (Dassanayake et al., 2011).

Dassanayake et al. (2009) had successfully analyzed PBDEs in human placentas in the United States, using matrix solid-phase dispersion extraction followed by multi-layer silica gel column chromatography cleanup and gas chromatographic separation coupled with mass spectrometric detection. It revealed that PBDE levels in human placenta from the mothers in the United States were about a hundred times higher than European or Japanese mothers (Dassanayake et al., 2009). However, the number of samples involved in the 2009 study was small (N = 5). A much larger number of samples is needed in order to produce reliable data for a quantitative assessment of prenatal exposure to PBDEs, and further studies on the association between neonatal exposure and health outcomes among children.

1.2 Introduction to the National Children's Study

Owing to concern that children are more vulnerable to environmental hazards and may suffer lifelong from prenatal exposures, the US Congress has instructed the National Institute of Child Health and Human Development (NICHD) to launch the National Children's Study (NCS) through the Children's Health Act of 2000 (Branum et al., 2003). The NCS is a large-scale longitudinal cohort study on children's health in the United States. It is designed to follow approximately 100,000 children born in the United States, starting before their birth until they are 21 years old (NCSAC 2005). The NCS investigates a wide range of environmental exposures, including chemical, biological, physical, psychosocial, and gene environment (NCSAC 2005). It also monitors many types of diseases that children are likely to encounter, such as birth defects, asthma, autism, schizophrenia, and obesity (Landrigan et al. 2006). The aim of the NCS is "to provide information that will ultimately lead to improvements in the health, development, and well-being of children" (NCSAC 2005, page 44).

In order to ensure the successful deployment of its main study, NCS has initiated a formative research stage to evaluate the feasibility, acceptability, and cost of various elements including recruitment and retention strategies, data collection and analysis, and operational and logistical models. There are a few dozens of approved formative research projects, which have been carried out under the NCS Vanguard Centers across the country.

This project, specifically, is part of the NCS Formative Research Project 2-18—Placenta Study: Stem Cells, Genetics/Epigenetics, Environmental Exposures, and Morphology/Pathology. One of the major purposes of Project 2-18 is to evaluate and optimize parameters for the collection, storage, utilization, and analysis of placenta samples so that accurate and reproducible data would be produced in the studies of stem cells, genetics/epigenetics, morphology/pathology,

as well as environmental exposures and effects (Miller 2011). Project 2-18 has been conducted with its own preliminary (the Nine Study), pilot and main study stages, which have different recruitment methods and scopes of data collection. One of the components of Project 2-18 is the environmental contaminant assessment, which includes the analyses of selected toxic metals, persistent organic pollutant groups, and the plasticizer bisphenol A.

The specific goal of this thesis is to accurately report the levels of the selected PBDEs in human placentas collected in the Project 2-18 preliminary and pilot stages. The result of this work will provide insight into the specimen collection techniques, with regard to the maximal time length allowed for tissue sampling after the child delivery. In addition, it explores whether there exists any regional differences, as the placentas analyzed in this thesis project were collected from three regions in the United States, by the NCS centers located at University of Rochester (UR), the University of California Davis (UCD), and the Medical College of Wisconsin (MCW).

1.3 Thesis Objectives

The objectives of this thesis are:

1. Use a previously developed analytical method to measure the levels of 10 PBDE congeners in 169 human placenta samples collected in the United States.

2. Verify the quality of the experimental data based on the results of quality control procedures.

3. Compare the levels of PBDEs in human placentas measured in this work to findings in the literature.

4. Summarize the PBDE congener distribution pattern and congener correlation statistically.

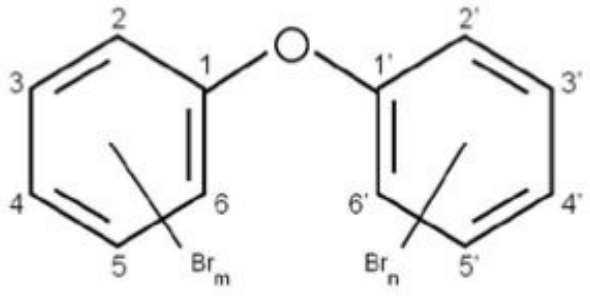
5. Systematically examine the relationship between PBDE levels and collection factors, including collection site and time.

2. LITERATURE REVIEW

2.1 Polybrominated Diphenyl Ethers

2.1.1 Structure and Nomenclature

As a group of synthetic aromatic chemicals, PBDEs have a formula of $C_{12}H_{(10-x)}Br_xO$ (x = 1 to 10). The structure contains two phenyl rings bridged by an oxygen atom, which makes it ether (Figure 1). Up to ten bromine atoms can replace the hydrogen atoms on the two phenyl rings of a diphenyl ether. Although the phenyl ring is of plane structure, the two bonds linking the oxygen atom are rotatable, making PBDEs not planar.



m + n = 1 to 10

Figure 1. General chemical structure of PBDEs.

According to the number of bromines on the phenyl rings, each individual PBDE belongs to a specific homologue group, distinguished by the number of bromine atoms. There are altogether ten homologue groups, namely mono-, di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decaBDEs.

Theoretically, there can be 209 different structures of PBDEs, depending on the number and placement of bromine atom(s). Each unique structure is called a congener. Therefore, there are 209 congeners. Their nomenclature follows the International Union of Pure and Applied Chemistry (IUPAC). When naming a specific congener, both the number and the placement of bromine atom(s) on each phenyl ring are indicated. Table I lists the IUPAC name and the homologue group of ten congeners that are selected for the project.

TABLE I

NOMENCLATURE OF PBDES OF INTEREST

Congener	IUPAC Name	Homologue Group
BDE 28	2,4,4'-tribromodiphenyl ether	Tri-BDE
BDE 47	2,2',4,4'-tetrabromodiphenyl ether	Tetra-BDE
BDE 66	2,3',4,4'-tetrabromodiphenyl ether	Tetra-BDE
BDE 85	2,2',3,4,4'-pentabromodiphenyl ether	Penta-BDE
BDE 99	2,2',4,4',5-pentabromodiphenyl ether	Penta-BDE
BDE 100	2,2',4,4',6-pentabromodiphenyl ether	Penta-BDE
BDE 153	2,2',4,4',5,5'-hexabromodiphenyl ether	Hexa-BDE
BDE 154	2,2',4,4',5,6'-hexabromodiphenyl ether	Hexa-BDE
BDE 183	2,2',3,4,4',5',6-heptabromodiphenyl ether	Hepta-BDE
BDE 209	2,2',3,3'4,4',5,5',6,6'-decabromodiphenyl ether	Deca-BDE

2.1.2 Properties

Polybrominated diphenyl ethers have relatively low aqueous solubility, and solubility decreases as PBDE congeners increase in mass. As a result, PBDEs are not easily distributed by the hydrologic cycle. The vapor pressure of PBDEs is also relatively low compared to many other groups of organic compounds. Nonetheless, most PBDE congeners are considered as

semivolatile, and partition into the atmosphere to some extent. Strongly attach to particles suspended in water and air, PBDEs can in-this-form be transported by water and air for long distances. As can be predicted from their high octanol-water partition coefficients (K_{ow}), PBDEs have high affinity to lipids. Therefore, PBDEs tend to bioaccumulate in biota in the environment and in humans. Some important physical and chemical properties of selected PBDE congeners are summarized in Table II.

TABLE II

PHYSICOCHEMICAL PROPERTIES OF PBDES OF INTEREST [a]

Congener	Number of Br	Molecular Weight	Melting Point (°C)	S_w [b] (mg/L)	Log K_{oa} [c]	Log K_{ow} [d]	Vapor Pressure (Pa)
BDE 28	3	406.9	64	7×10^{-1}	9.46	5.94	2.19×10^{-3}
BDE 47	4	485.8	84	1.5×10^{-2}	10.53	6.81	1.86×10^{-4}
BDE 66	4	485.8	N/A	N/A	N/A	6.90	N/A
BDE 85	5	564.7	123	7.86×10^{-5}	11.66	7.37	5.11×10^{-5}
BDE 99	5	564.7	92	9.4×10^{-3}	11.32	7.32	1.76×10^{-5}
BDE 100	5	564.7	100	4×10^{-2}	11.18	7.24	2.09×10^{-6}
BDE 153	6	643.6	162	8.7×10^{-4}	11.86	7.90	2.09×10^{-6}
BDE 154	6	643.6	132	8.7×10^{-4}	11.93	7.82	3.80×10^{-6}
BDE 183	7	722.5	172	1.5×10^{-3}	11.96	8.27	4.68×10^{-7}
BDE 209	10	959.2	~300	1.3×10^{-8}	N/A	8.70	5.42×10^{-11}

[a] Tittlemier et al., 2002; Braekevelt et al., 2003; Chen et al., 2003; Wania and Dugani, 2003; Guan et al., 2009.
[b] S_w = Water Solubility.
[c] K_{oa} = n-Octanol-air partition coefficient at 25 °C.
[d] K_{ow} = Octanol-water partition coefficient at 25 °C.

2.1.3 Industrial Use and Regulations

Production of PBDEs as additive flame retardants in the United States began in the 1970s. The demand for brominated flame retardants doubled during the 1990s, and production peaked in the late 1990s (Hardy 2002; Alaee et al., 2003).

Three types of commercial PBDEs are manufactured: pentaBDEs, octaBDEs, and decaBDEs. Each of these commercial products is not of one pure compound, but a mixture of different congeners.

The commercial pentaBDEs, under product names such as DE-71 and Bromkal 70-5DE, are composed of tri- to hexaBDE congeners, among which tetraBDE congeners (especially BDE 47) and pentaBDE congeners (especially BDE 99 and BDE 100) predominate (EPA 2006; La Guardia et al., 2006). Commercial pentaBDEs are primarily used in polyurethane foam in mattresses and padding beneath carpets and furniture (Kimbrough et al., 2009).

The commercial octaBDEs, under product names such as DE-79 and Bromkal 790-8DE, contain mainly hepta- and octaBDE congeners. It also has relatively small quantities of hexa- and nonaBDEs, along with even smaller quantities of penta- and decaBDEs. The commercial octaBDEs are mainly used in acrylonitrile-butadiene-styrene plastics found in many electronic casings, such as computers, monitors, etc. (EPA 2006; La Guardia et al., 2006).

The commercial decaBDEs, under product names such as DE-83R, Saytex 102E and Bromkal 82-0DE, are mainly composed of the decaBDE congener BDE 209 (97%–99%) (EPA 2006; La Guardia et al., 2006). The rest of the components in commercial decaBDE are nonaBDEs. The primary use of BDE 209 is in the black plastic high impact polystyrene (HIPS) electronic enclosures found in the rear of television sets, accounting for 80% of the use of commercial decaBDE (IEPA 2006). Among the three commercial products, decaBDE has been

the most widely used both in the United States and worldwide. In 2006, production of decaBDE exceeded 60,000 metric tons internationally, among which over 40% found their final destination in North America (IEPA 2006).

In addition to the specific use of each PBDE commercial products, pentaBDEs, octaBDEs, and decaBDE are also used in textiles, nylon, and adhesives (Kimbrough et al., 2009).

As flame retardants, PBDEs are added to consumer products in the range of 5%–30% by weight to meet fire safety standards (IEPA 2006). Thus, PBDEs may have saved lives and properties by inhibiting ignition and subsequent burning of consumer products. However, the increasing detection of PBDEs in the environment, in biota, and even in human bodies has raised attention from policymakers globally and domestically. In August 2004, the European Union banned the use of penta- and octaBDEs. In the same year, the only manufacturer of the commercial penta- and octaBDEs in the United States, the Great Lakes Chemical Corporation (now Chemtura), voluntarily stopped the production of these two commercial products (EPA 2006). The state of California (followed by Hawaii, Illinois, New York, Maine, Maryland, Michigan, and Oregon) adopted laws to ban the manufacture or distribution of products that contain penta- or octaBDEs (EPA 2006). The Environmental Protection Agency (EPA) also proposed a Significant New Use Rule that provides EPA with authority to review the intended reproduction or import of penta- and octaBDEs (EPA 2006). Subsequently in 2008, the European Union further banned the production of decaBDEs (Kimbrough et al., 2009). Currently, commercial decaBDEs are still being produced in the United States, although the two US producers of decaBDEs (Albemarle Corporation and Chemtura Corporation) along with the largest US importer (ICL Industrial Products Inc.) have made commitments to phase out decaBDEs by the end of 2013 (EPA 2010).

2.1.4 Toxicity

Increasing detection and levels of PBDEs in the wildlife (Ikonomou et al., 2002) and human breast milk (Frederiksen et al., 2009b) has led to more efforts to understand the biological effects of PBDEs. Animal studies on rats and rodents have revealed that commercial pentaBDEs could give a low acute toxicity, with the oral LD-50 in rats being 0.5–5 g/kg body wt (IPCS 1994). Various studies have shown that commercial pentaBDEs could cause impaired thyroid, neurobehavioral development, and maternal and fetal toxicity (Darnerud 2003). The critical effect of commercial pentaBDEs is developmental neurotoxicity (Darnerud 2003). Although the mechanism of neurotoxicity in mice is not well understood, studies have suggested that pentaBDEs have the capacity to induce cerebellar granule cell death (Reistad et al., 2002) and release arachidonic acid that disrupts signal transmission in brain (Kodavanti and Derr-Yellin 2002). At higher doses, pentaBDEs may lead to altered thyroid hormone homeostasis. The suggested mechanism from the rodent model is that PBDEs lower the thyroxin levels in blood and peripheral organs after they bind the thyroxin-transporting protein TTR (Brouwer et al., 1998). Another proposed explanation is that thyroxin will degrade and be excreted faster because of the phase II enzyme UDP-GT induced by PBDEs (Zhou et al., 2001). It has been concluded that during early developmental stages, thyroid effects, such as impaired learning and memory functions, can occur at lower PBDE exposure levels because the development of vital organs, including the brain, highly depend on these hormones (Darnerud 2003). Based on the most sensitive effects observed, the LOAEL for the commercial pentaBDE may be 0.6–0.8 mg/kg body wt. (Eriksson et al., 2001). Carcinogenic studies of pentaBDE have not been performed (EPA 2008).

Similar to pentaBDEs, the acute toxicity of octaBDEs was observed to be low (IPCS 1994). However, morphological effects on liver were already present at 10 mg/kg body wt in adult rats (IPCS 1994). Increasing dosage led to an observation of impacted thyroid, kidney, and hematological system (Darnerud 2003). Fetal effects, such as weight decrease, reduced ossification, and bent ribs, started to manifest at 2 mg/kg body wt. in rabbits (Breslin et al., 1989) and at higher dosage in rats. Maternal toxicity was also observed in the animal tests. It was concluded that the toxicity of octaBDEs would be seen at the lowest doses from exposures during developmental stages (Darnerud 2003).

Due to its bulky and highly brominated structure, decaBDE is more hindered in passing through membranes of cells than lighter congeners (IEPA 2006). In addition, its large structure makes it more easily to be excreted, resulting in shorter retention in the body. Animal studies have shown that more than 99% of the administered decaBDE was eliminated after 24–72 hours (El Dareer et al., 1987). DecaBDE still has liver effects, although it is less toxic than lighter BDE congeners (IEPA 2006). It also has neurological effects (Viberg et al., 2003) and decreases thyroid hormone level (Norris et al., 1973; Norris et al., 1975a; Norris et al., 1975b; NTP 1986). While decaBDE was observed to be associated with these effects, the effects might come from lower BDE congeners that are the in vivo degradation products of decaBDE. The EPA classified decaBDE as “a possible human carcinogen” (IRIS 2012). However, the US National Toxicology Program, the Occupational Safety and Health Administration, and the International Agency for Research on Cancer did not categorize decaBDE as carcinogenic (IEPA 2006).

In sum, most studies looked at the toxicity of three types of commercial PBDEs, rather than at individual congeners. Among the three commercial types, pentaBDEs showed toxicological effects at the lowest dosage. Studies suggested that all commercial PBDEs could be

endocrine disrupters that interfere with thyroid hormone homeostatis (Darnerud 2008). As thyroid hormones play a vital role in brain development, PBDEs may cause irreversible defects in cognitive performance and motion skills in the offspring (Darras 2008).

In humans, thyroid and neurodevelopmental effects, as well as cryptorchidism have been found. Exposure to PBDEs (mainly pentaBDEs) via dust may lead to altered hormone levels in adult males (Meeker et al., 2009); and increased thyroglobulin antibodies and thyroid hormones thyroxine in men may be associated with general PBDE exposure (Turyk et al., 2008). Children with higher concentrations of BDEs 47, 99, or 100 in their cord blood did poorer on mental and physical development test at 12–48 and 72 months (Herbstman et al., 2010). A strong correlation between elevated PBDE levels in breast milk and cryptorchidism in newborns has also been observed (Main et al., 2007).

2.1.5 Environmental Releases, Occurrences, and Levels

As an additive flame retardant, PBDEs are mixed with the polymer yet not covalently bound to it. Therefore, they are more likely to leach out from the products to the environment than reactive flame retardants chemically bound to the product. In addition, PBDEs are likely to be directly released from manufacturing and processing plants that add PBDEs to consumer products (EPA 2006). Moreover, recycling, land filling, and incineration of products that contain PBDEs may add more to PBDEs in the environment. Once released to the environment, due to its low water solubility and high affinity to lipids, PBDEs tend to accumulate in the environment. Therefore, PBDEs are categorized as persistent organic pollutants (D'Silva et al., 2004).

Since their first detection in the environment in 1979 and in biota in the 1980s (EPA 2006), PBDEs have been found in almost every possible matrix. In the environment, PBDEs have been detected in sediments, sewage sludge, soil, water, indoor dust, and indoor and outdoor

air (Hale et al., 2003; Li et al., 2006). They have also been found in various animals, including fish, seals, aquatic birds, marine mussels, and falcons (Johansson et al., 2006). The bioaccumulation and biomagnification of lower BDE congeners are revealed in aquatic and aquatic-terrestrial food webs (IEPA 2006). Rapid increase of PBDE levels in North America wildlife is well documented, as PBDEs bioaccumulate and biomagnify in food chains (Darnerud et al., 2001). Even Arctic biota are not exempted, which indicates the long range transport capability of PBDEs (de Wit et al., 2006).

It is also well established that PBDEs are present in human bodies. Studies have detected PBDE levels in human blood, breast milk, adipose tissue, umbilical cord blood, and placenta (Antignac et al., 2009; Dassanayake et al., 2009). It was reported that in human tissues, the level of PBDEs had increased about 100 fold during the past 30 years (Hites 2004). Worldwide, PBDE levels in blood samples from the United States were the highest, reaching 79.7 ng/g lipid (Schecter et al., 2005). When other persistent organic pollutants, such as dioxins, dibenzofurans, and polychlorinated biphenyls were declining from 1973 to 2003, PBDEs were rapidly increasing in human serum in the United States. During this period, PBDE concentrations in serum have increased 60 fold (Schecter et al., 2005). The first study analyzing PBDEs in human placenta in the United States revealed the sum of the tri- to heptaBDEs had a median of 1205 pg/g wet weight (Dassanayake et al., 2009).

Among various congeners detected, BDE 47 has been found to be the most abundant in human serum, followed by BDEs 99, 100, 153, and 154 (Frederiksen et al., 2009a). In sediments, BDE 209 is the most abundant congener, accounting for more than 90% of the total concentrations detected (Song et al., 2005a; Song et al., 2005b). In house dust, BDE 209 was

also predominant, occupying 32%–97% of the total BDEs (Frederiksen et al., 2009b). Similar dominance of BDE 209 was also found in indoor air (Karlsson et al., 2007).

When comparing BDE levels among continents, the levels of PBDEs in many matrices are much higher in North America than in Europe, Asia, and Oceania. With regard to in-house dust, concentrations of tetra- to hexaBDEs as well as BDE 209 were observed to be one order of magnitude higher in North America than in Europe (Frederiksen et al., 2009b). For instance, the mean concentration of BDE 47 was 32 ng/g dry weight in Europe, compared to 429 ng/g dry weight in the North America (Frederiksen et al., 2009b). However, there was no statistically significant difference observed between the two continents with respect to the level of BDE 183, a congener in the commercial octaBDEs (Frederiksen et al., 2009b). With regard to breast milk and human blood, the concentration of BDE 47 was also measured to be one order of magnitude higher in the North America than other continents (Frederiksen et al., 2009b). A similar difference was revealed in adipose tissue (Frederiksen et al., 2009b).

2.1.6 Human Exposure

Found in many consumer products used on a daily basis in homes, vehicles, and work places, most of the population are exposed to PBDEs via ingestion, inhalation, and dermal contact. Ingestion of fatty animal foods and dust are major routes of exposure to PBDEs. Inhalation and dermal contact with house dust, and contact with furniture, electronic devices, and textiles also add to the overall exposure.

The primary exposure route to PBDEs for most adults is ingestion of foods that contain PBDEs, such as fish, meat, poultry, eggs, and dairy products (IEPA 2006). A study done in Dallas, Texas, revealed that 30 types of food of animal origin from major supermarket chains had elevated PBDE levels (Schecter et al., 2004). Compiling all the study data, (Frederiksen et

al., 2009b) graphically presented the level of BDE 47 in different food groups (Figure 2). Compared to meat, dairy products, and vegetables, BDE 47 concentrations were highest in the fish samples. DecaBDE accounted for more than half of the total BDE concentrations in food (Schecter et al., 2004). In the Great Lakes region, human body burden of PBDEs was found to be positively associated with the years of sport fish consumption, particularly shellfish and catfish intake (Anderson et al., 2008; Turyk et al., 2010). Market-basket studies have estimated the dietary intake of total BDEs to be 23–88 ng for people in the United States and European countries (Frederiksen et al., 2009b). The PBDEs taken in through food consumption depends on dietary habits. For Americans, 60%–70% of PBDE dietary exposure comes from meat products, particularly poultry and processed meat, while only 10%–20% comes from consumption of fish (Schecter et al., 2006). This profile differs from that in Scandinavian countries and Japan where fish consumption accounted for 50% of the total dietary exposure to PBDEs (Ohta et al., 2002; Kiviranta et al., 2004; Darnerud et al., 2006).

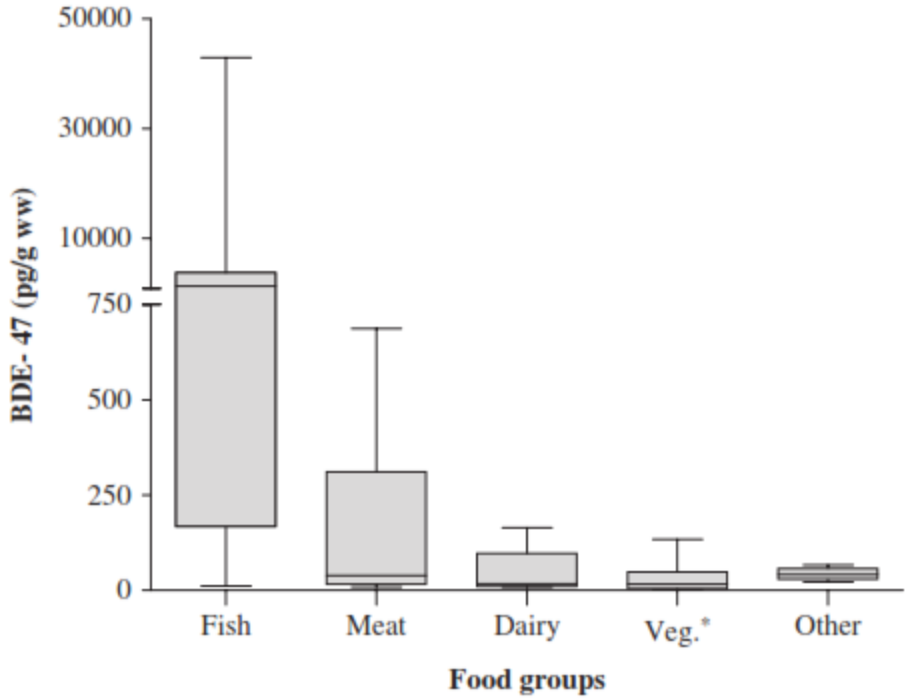


Figure 2. Level of BDE 47 in different food groups (Frederiksen et al., 2009b). The level of BDE 47 for vegetables was not available, thus total BDE was used instead. "Other" food items include fast food and beverages. The dash line in the box plot is the mean.

Another major exposure route to PBDEs is incidental ingestion of dust. As PBDEs are added as flame retardants to carpets, furniture, draperies, electronic casings, and other items in daily use, PBDEs leached from these products are present at homes and work places. Therefore, PBDE-laden dust may be ingested or inhaled. Studies from various countries have shown increasing levels of PBDEs in house dust (Stapleton et al., 2005; Wilford et al., 2005). This becomes a serious issue for children as they are more vulnerable to dust exposures than adults. A study estimated that for Canadian toddlers, 90% of the exposure to PBDEs was via dust (Jones-Otazo et al., 2005). Another piece of evidence comes from the contrast between PBDEs and other persistent organic pollutants such as PCBs. While it was observed that as age goes up, the

concentrations of PCBs also increased, the highest concentrations of PBDEs were detected in serum from small children (Thomsen et al., 2002; Thomsen et al., 2007). Incidental ingestion of dust also explains why levels of PBDEs were detected to be much higher in North Americans than Europeans when exposure through food was comparable between the two continents. Higher levels of PBDEs in dust in North America homes has led to more incidental ingestion of PBDEs (Frederiksen et al., 2009b).

For infants (0–6 months), the major ingestion source of PBDEs is breast milk. The level of PBDEs found in breast milk in Dallas, Texas, was 6.2–419 ng/g lipid, much higher than the European levels (Schecter et al., 2003). The estimated daily intake of PBDEs for nursing infants was 307 ng/kg/day (Schecter et al., 2006).

Occupational exposure to PBDEs also deserves attention. Industries such as electronics production and dismantling as well as household furnishing material manufacturing are highly likely to expose their workers to elevated levels of PBDEs. A study in south China found high concentrations of BDEs 47, 99, 153, 183, and 209 (those that are dominant in electronic products) in the serum of electronics dismantling workers (Bi et al., 2007). Incineration workers and those handle sewage sludge are also greatly exposed to PBDEs (Kim et al., 2005). Routes of occupational exposure include inhalation and incidental ingestion (IEPA 2006). Although there are existing regulations that control the production and use of PBDEs, occupational exposure can continue given the fact that PBDEs can be found in many items in work places. However, different environmental safety standards in work place may lead to different extent of occupational exposure to PBDEs.

2.2 Human Placenta

2.2.1 The Development and Function of Human Placenta

The human placenta is a discoid organ that weighs about 470 grams at term, which is about 1/7 of the fetus weight. Its diameter is about 20 cm and its thickness 2.0–2.5 cm (Rampersad et al., 2011). The human placenta is evolved from the trophectoderm as the outer layer of the blastocyst is formed from a fertilized egg. The placenta has two surfaces: the maternal surface (basal plate) and the fetal surface (chorionic plate) where the umbilical cord inserts (Figure 3). Where the basal and the chorionic plates meet, the edges of the placenta converge to form the chorioamnion membrane that contains the amniotic fluid, which normally begins on the fourth day after conception (Rampersad et al., 2011).

The human placenta develops villi, which are finger-like projections that form a treelike structure and extend to the uterus. In the villi develop blood vessels. The placenta maintains a low oxygen and nutrient environment until the end of the first trimester when maternal blood perfuses the intervillous space (Rampersad et al., 2011). The fetal blood in the villi is normally separated from the maternal blood in the intervillous space by a membrane, although materials exchange between them can still take place.

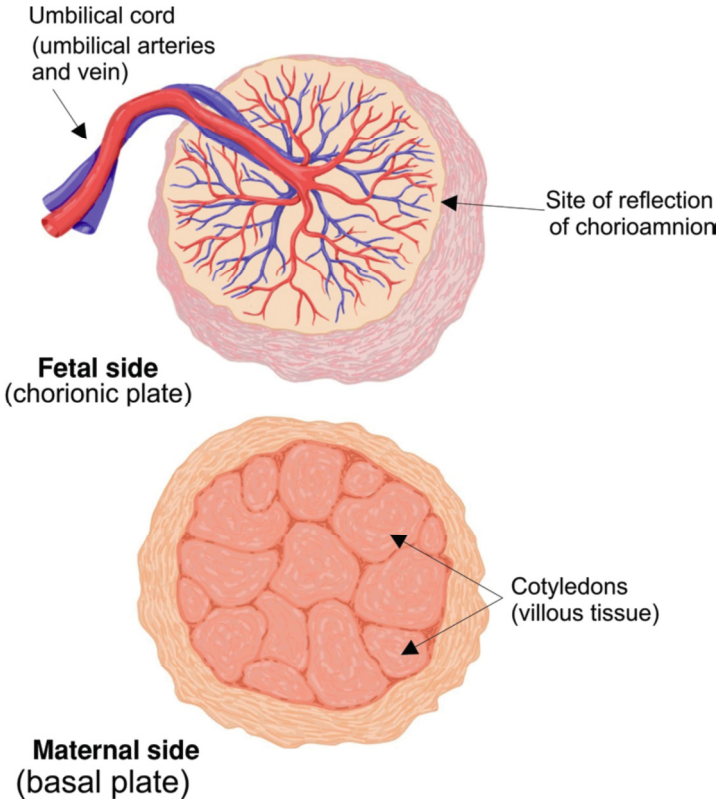


Figure 3. Illustration of the human placenta at term. (Rampersad et al., 2011)

The human placenta grows slowly in the first trimester, but then rapidly during the second and the third trimesters. During these stages, it uptakes and metabolizes large quantities of nutrients in order to generate cellular energy and synthesize components critical to cellular growth, such as DNA and RNA (Illsley 2011). Capable of measuring the concentration of nutrients, the placenta plays a dynamic and vital role to adapt placental metabolism to sustain fetal growth (Illsley 2011). It transfers and metabolizes oxygen and glucose—the primary source for placental energy generation—as well as amino acids when there is an increasing concentration gradient from maternal to fetal circulation. The placenta also serves as the organ by which wastes are removed. While the placenta is permeable to hydrophilic molecules, it can

also be a barrier to other molecules. Studies have shown that human placenta highly restricts the transfer of protein (Illsley 2011).

2.2.2 Placental Transfer of Polybrominated Diphenyl Ethers

The human placenta does not only transfer vital nutrients from the mother to the fetus, it also transfers unwanted chemicals. As pregnant women are exposed to potentially harmful chemicals in the environment on a daily basis, concerns have been raised whether such chemicals can be transferred to the fetus via the placenta. Of particular concern are endocrine disruptors, such as PBDEs, PCBs, pesticides, and dioxins, because they can affect the hormone balance in the placenta when it synthesizes steroid and protein hormones.

The tragedy of the thalidomide catastrophe had already given enough evidence that certain chemicals could be transferred from the mother to the fetus. Many children born in the early 1960s had severe birth defects because their mothers took the anti-anxiety drug during pregnancy. Placental transfer of PCBs has been known for a long time (Wang et al., 2006). More recent studies have found PBDEs in umbilical cord blood, indicating placental passage of PBDEs (Gomara et al., 2007). More specifically, the fetus in utero have been found to be exposed to BDE 47 and BDE 99, the two most abundant BDE congeners in human bodies (Frederiksen et al., 2010b). Although the exposure to BDE 209 was limited partly due to its bulky structure, its harmfulness should not be neglected because its degradation products could be more toxic and bioaccumulative, leading to continuous exposure.

The major mechanism for chemicals, including PBDEs, to pass through the human placenta is passive diffusion. Other possible mechanisms include facilitated diffusion, active transport, and pinocytosis (Myren et al., 2007). The placental transfer rate and extent vary for different BDE congeners. It was observed that BDE 47 had greater transfer extent and rate than

BDE 99 (Frederiksen et al., 2010b). Research has indicated that placental transfer tends to decrease when more bromines are on the diphenyl ether structures (Meijer et al., 2008; Frederiksen et al., 2010a). In addition, molecular size plays a role: bulky congeners may be hindered in placental transfer. It is also suggested that lipid solubility and affinity with carrier proteins may determine the transport of BDE congeners (Myren et al., 2007). The transfer rate and extent are not only determined by the physiochemical characteristics of the chemical, they also rely on the physical characteristics of the maternal-placental unit, such as the thickness of the membrane, the surface area of the exchange membrane, the maternal blood flow, and the hydrostatic pressure in the intervillous chamber. (Myren et al., 2007). This explains why the transfer rate can be faster when the pregnancy is closer to term, as the membrane becomes thinner.

2.2.3 Placental Studies on Polybrominated Diphenyl Ethers

The studies of PBDEs in humans did not begin with placenta or even cord blood. In fact, it took years until researches realized the value of studying placenta. Because the placenta retains hydrophobic compounds, it better reflects exposure during the entire pregnancy than blood. The human placenta is now considered the primary matrix in which to study mechanisms and kinetics of cross-placental transfer of toxics and nutrients (Dassanayake et al., 2011). Studies have found larger percentage of BDE 209 in human placenta than in maternal blood although BDE 209 concentrations were similar in the two matrices (Frederiksen et al., 2009b). The concentration of BDE 47, however, was one order of magnitude lower in placenta than in maternal blood (Frederiksen et al., 2009b). A shift in congener distribution from maternal to umbilical cord blood was also observed. Some found that the percentage of higher brominated PBDE congeners, especially of hexaBDEs, was higher in maternal blood than umbilical cord blood (Guvenius et al.,

2003; Weiss et al., 2004; Bi et al., 2006). It can be that higher brominated PBDE congeners encounter more hindrance in trans-placental transfer. Another possible reason is that the less-lipophilic congeners, such as BDE 47, are favored more by the umbilical cord that contains low lipid content (Frederiksen et al., 2009b). Unfortunately, published studies do not give agreed observations on congener distribution patterns and shifts (Guvenius et al., 2003; Mazdai et al., 2003; Bi et al., 2006; Gomara et al., 2007). More thorough studies on placenta may help to obtain more conclusive results and explain different observations.

The collection of placentas is noninvasive to the human body, thus avoiding any health consequences from sample collection. In addition, placenta can be donated for multiple research purposes. Compared with the cord blood, the placenta weighs hundreds of grams, providing sufficient amount for various laboratory analyses.

There are, however, difficulties associated with laboratory dissection and analysis of placenta. As introduced above, human placenta is a complicated organ. Due to the heterogeneity of placenta, it is desirable to either analyze aliquots of homogenized entire placenta, or an accurately defined portion of it, if comparison among different placentas is to be made. Reliable quantitative analysis of trace-level chemicals in placenta also calls for sufficient mass of each specimen, because the concentrations of toxins including PBDEs are usually very low (at parts per billion or even parts per trillion levels). The low concentration also challenges the analytical technique. Among studies that looked at BDE 209 in human bodies, some of them were not able to find BDE 209 because the level was below the limit of quantification (Herbstman et al., 2007; Kawashiro et al., 2008; Frederiksen et al., 2010a). In addition, since studying human placenta for exposure is relatively new, there are no standard reference materials available for quality control (Dassanayake et al., 2011). The number of studies available for comparison with this study is

also small, and most were done outside of the United States (Table III). Comparison is made more difficult because investigations did not follow the same analytical method, or report the level in the same way. However, these issues should not hinder researchers from taking advantage of human placenta, but rather call for more attention. This is especially so in the United States, as limited studies have already indicated PBDE levels to be alarmingly high.

TABLE III

PLACENTAL STUDIES ON PBDES REPORTED IN THE LITERATURE

Country	Year	Sam-ple Size	Number Of Conge-ners	Median ∑BDE (Tri- to HeptaBDEs) (ng/g lw)	Median BDE 209 (ng/g lw)	Median Lipid %	Median ∑BDE (≤ HeptaBDE)[a] (pg/g ww)	Median BDE 209[a] (pg/g ww)	Predominant Congeners	References
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	14.76	14	209>47≈153	(Frederiksen et al., 2009b)
Spain	2003–2004	30	15	0.65	1.0	0.7	4.55	7.0	209>47>153	(Gomara et al., 2007)
Denmark	1997–2001	129	14	1.31	NA	1.09	14.28	NA	153≈47	(Main et al., 2007)
Finland	1997–2001	56	14	1.18	NA	1.21	14.28	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)

[a] All the data under Median ∑BDE (≤ HeptaBDE) (pg/g ww) and Median BDE 209 (pg/g ww) except the data from Dassanayake et al. (2009) and the Median BDE 209 (pg/g ww) from Frederiksen et al., (2009) were calculated following the formula: ww (pg/g) = lw (ng/g) × 1000 × Median Lipid% / 100.

2.3 Analytical Methods for Polybrominated Diphenyl Ethers in Human Samples

2.3.1 Extraction

The extraction process transfers analytes of interest in a sample to the extracted effluent. For hydrophobic organic analytes such as PBDEs in semi-solid biological samples such as placenta specimen, there are a variety of extraction methods, such as the traditional Soxhlet extraction and solid liquid extraction (SLE), and more recently developed methods including pressurized fluid extraction (PFE), microwave-assisted extraction (MAE), and solid phase microextraction (SPME). Soxhlet extraction is the technique recommended in EPA Standard Methods 1614 as well as SW846 Section 3540C (EPA 1996). One of the advantages of Soxhlet extraction is its ability to process a large volume of sample. Its major disadvantage lies in the relatively high consumption of time and solvent.

Matrix solid-phase dispersion (MSPD) was first developed in the late 1980s to extract solid and semi-solid biotic samples. By mechanically blending and pulverizing samples with sorbents, such as Florisil that has sharp edges and rough surface to provide shearing force, the sample architecture is disrupted and it is dispersed over the solid phase sorbents (Barker 2000). With column packing and solvent elution, the target compounds are thus extracted from the dispersed tissues. The advantages of MSPD over Soxhlet include less solvent and time consumptions. Normally, the Soxhlet extraction would need 12 to 24 hours while MSPD only takes 2 to 3 hours. In addition, MSPD has the potential to produce a cleaner extract with much fewer lipids, thus reducing efforts for the subsequent cleanup processes (Dassanayake et al., 2009). When the sample quantity is small, MSPD is particularly advantageous.

As the MSPD method requires sorbents, it needs to be properly determined which and what amount to use to make sample dispersion effective. A sorbent-to-dry-placenta weight ratio

of 1 to 4 was tried in previous studies to optimize the amount (Kristenson et al., 2006). It was found that for dry placenta samples, the 2:1 ratio performed best as extraction efficiency was not improved by increasing the amount of sorbent used (Valsamaki et al., 2006). In terms of which sorbent to use, silica gel, C18, and Florisil are the most common. According to previous research results, C18 had the highest extraction efficiency, followed by Florisil (Dassanayake et al., 2009). However, with C18, the co-extracted lipids were also higher than with other sorbents, and would demand more cleanup steps later on. Elevated baseline was also observed in instrumental analysis with regard to C18 (Dassanayake et al., 2009). Compared to C18, although the Florisil yielded less extraction efficiency, it produced much cleaner extracts. With its sharp edges, Florisil may even provide better shearing forces in placenta sample dispersion.

The MSPD method requires chromatographic elution. Therefore, the eluting solvent or solvent mixture needs to be carefully chosen so that the target compounds, i.e., PBDE analytes, would be extracted. Nonpolar organic solvents, such as hexane, have a high solubility of PBDEs but cannot penetrate to the inner part of biotic tissues. Polar organic solvents, such as acetone, can more easily access the inner part of biotic tissues although they dissolve fewer PBDEs. Therefore, in order to reach higher extraction efficiency, a combination of nonpolar and polar organic solvent is usually used. Previous experiments showed that a mixture of hexane and dichloromethane (DCM) with a volume ratio 8:2 was the optimal solvent mixture to elute PBDEs from placenta samples (Dassanayake et al., 2009).

2.3.2 Cleanup

The extraction process usually lacks selectivity. In other words, not only analytes of interest are extracted, but also some other compounds that may interfere with the analysis of the targeted analytes during the instrumental analysis. In order to avoid, or at least minimize, such

interferences, the extract from the extraction process needs to be cleaned up so that few interfering materials remain in the final solution to go through instrumental analysis.

For biological samples, the most common co-extracts are lipids (i.e., fatty acids and their derivatives). Due to their low volatility and affinity to glassware, lipids tend to attach to the inside of the inlet liner as well as the capillary column in gas chromatograph (Wei 2007). Then they are likely to cause retention time shift and peak tailing in the chromatograph. They may also decrease instrument sensitivity and signal to noise ratio and raise the baseline. Thus, efforts should be invested to remove lipids in the cleanup process.

Generally there are two types of cleanup methods—i.e., non-destructive and destructive. In the category of non-destructive methods, gel-permeation chromatography (GPC) is a common method endorsed by EPA. It uses organic solvent and hydrophobic gels to separate various macromolecules (EPA 1994). The GPC method is especially widely used for the primary cleanup of biological samples (Covaci et al. 2003). It works well when the interferences have different molecular sizes than the analytes of interest because the principle for GPC is size exclusion, which separates compounds based on different molecular sizes. However, it does not work well when interferences have similar molecular size to the analytes of interest. In this situation, further cleanup methods will need to be applied.

In the category of destructive cleanup methods, acidic or basic treatment of the extract to break down large molecules is the main idea. Sulfuric acid is widely used. Since PBDEs are not broken down by strong acids or bases (de Boer et al. 2001), a multi-layer silica gel column packed with the combination of neutral, acidified, and basified silica gel would be the best method to clean up the extract. The uniform stationary phase formed by the multi-layer silica gel eluted by solvents has the potential for better cleanup effectiveness because it adsorbs and breaks

down interferences at the same time. The adsorption performs based on the polarity and surface area of the sorbents and sorbates. The break down takes place due to the acidic and basic treatment.

The eluting solvent should be carefully chosen, taking into consideration the polarity of solvent and chemicals in the extract. Normally, the eluting solvent whose polarity is less than the chemicals in the extract is selected.

2.3.3 Sample Concentration

The eluate from the cleanup may be ready for instrumental analysis as it is clean. However, since PBDE levels in human placenta may be too low to be detected when they are dissolved in a large amount of solvent, concentration of the eluate is frequently necessary. Through solvent evaporation, the volume of the eluate is reduced so that the concentrations of BDE analytes are raised.

There are two types of concentration. The first type is macro-concentration, which means the volume to reduce is large. One of the most common methods is rotary evaporation. Rotary evaporation utilizes vacuum and heat at the same time. The evaporation flask that contains the sample spins at a speed between 0 and 220 rpm in a water bath that provides the heat. The temperature of the water bath is set below the boiling point of the solvent. As the solvent vapor cools in a condenser above the evaporation flask, the reflux of the solvent helps prevent the loss of chemicals with higher boiling points such as PBDEs. When both heat and vacuum are applied, some solvents, such as DCM, will evaporate rather quickly, causing bubbles in the evaporation flask. This bubbling should be prevented, otherwise there might be potential loss in target chemicals as they are carried away along with the solvent. Therefore, heat and pressure should be carefully applied during the evaporation process. Generally, the volume of the sample is not

reduced to less than 1 mL, otherwise potential loss of the analytes may occur. The evaporation flask is let cool before removal from the rotary evaporator.

One of the other common macro-concentration methods is the Kuderna-Danish (K-D) technique. The detailed procedure for K-D concentration can be found in EPA method 3540C (EPA 1996). Basically a three-ball Snyder column is used along with a water bath. Unlike rotary evaporation where the temperature of the water bath is to set below the boiling point of the solvent, with K-D concentration, the temperature of the water bath is 15°C to 20°C above the boiling point of the solvent. This produces hot vapor. For a solvent whose boiling point is above 90°C, an oil bath can be used instead of the water bath. The bath temperature and the vertical position of the column can be adjusted during the concentration. Flooding needs to be avoided during the concentration process to avoid potential loss of target chemicals. Similar to rotary evaporation, the K-D concentration is stopped when the volume reaches 1 to 2 mL.

The second type of concentration is micro-concentration, which refers to the event that only a small volume needs to be reduced. Generally, micro-concentration follows macro-concentration to further reduces the volume of organic solvent. While macro-concentration has the limit that it cannot reduce the volume to below 1 mL without the risk of losing target compounds, micro-concentration handles small volume reduction gently. A gentle stream of nitrogen is often used to perform micro-concentration.

2.3.4 Instrumental Analysis

After the time-consuming sample treatment, the sample extract is finally ready for instrumental analysis. Just as most volatile and semi-volatile organic compounds are analyzed by gas chromatography (GC), PBDEs are also predominately studied by GC that separates congeners based on their different interactions with the stationary phase due to their physical and

chemical properties. After the PBDE congeners are separated in the column, they are identified by a detector. For this purpose, mass spectrometric (MS) detector is widely used for analyzing PBDEs.

Although both GC and MS are common instruments for PBDE study, their configurations and operational parameters vary a lot. In order to ensure the reliability of the instrumental analysis, many factors need to be taken into consideration as discussed below.

2.3.4.1 Injection Type

First of all, the injection type requires careful selection. There are four injection techniques commonly used for analyzing PBDEs in liquid samples. They are on-column, splitless/split, pulsed splitless, and programmable temperature vaporization (PTV) injections (Björklund et al. 2004).

As suggested by the name, in the on-column method the sample is directly introduced to the column. When the sample is transferred to the column, its temperature is much lower than the boiling points of analytes of interest. The liquid in the column is evaporated and separated when the oven temperature gradually increases. The advantage of on-column injection is that it avoids thermal degradation to the most possible extent. The disadvantage is that the sample injection volume is confined to 1 μL to 2 μl to protect the instrument from being overwhelmed by the excess solvent (Stapleton et al., 2006).

Unlike on-column injection, splitless/split and pulsed splitless injections transfer the sample first to the injection port. The liquid sample immediately evaporates at a certain temperature in the injection port liner, and the vapor of compounds then goes into the GC column. The strength of these injection techniques is their potential to treat dirty samples (Stapleton et al. 2006). However, these methods also have weaknesses. Because only the vapor is

transferred to the GC column, incomplete vaporization of compounds with high boiling points will result in loss of detection. The temperature in the inlet cannot be too high in order to avoid thermal degradation. In addition, the inlet may be a source of contamination. Similar to on-column injection, the injection volume is limited to 1 µL to 2 µL. This volume limitation makes the split technique even more difficult because it requires high concentrations of analytes for reliable detection.

With PTV injection technique, a liquid sample is also vaporized in the injection port inlet, but at a temperature below the boiling point of the solvent. The PTV technique supports multiple injections and the total volume can reach 500 µl when the solvent vent mode is in use. The large volume is allowed because the solvent is first evaporated and vented under a high flow of an inert gas. After the desired volume of sample is injected, the inlet temperature is increased rapidly to facilitate the transfer of the sample from the inlet to the GC column. The large volume capacity becomes the advantage of this technique since it greatly enhances the detection sensitivity. The potential problem with PTV is the loss of relatively volatile analytes during the solvent vaporization. In addition, as analytes are retained in the inlet while the solvent is vented, compounds with high boiling points may attach to the liner material so that they are not fully evaporated and transferred to the column. This may cause carryover from sample to sample, affecting the accuracy of the analysis.

2.3.4.2 Column Selection

An ideal column for GC analysis is characterized by its capability to separate all detectable compounds within a short period of time. To achieve this goal, the type and thickness of the stationary phase as well as the dimension (length and diameter) of the column play important roles.

Thorough research has characterized those important parameters for PBDEs (Björklund et al., 2004). Studies have shown that non-polar stationary phase dimethylpolysiloxane (such as DB-1) and phenyl-methylpolysiloxane (such as DB-5) performed well for analysis of PBDEs. With regard to stationary phase thickness, a thinner stationary phase (i.e., 0.1 μm) generally gives better response than a thicker one (i.e., 0.25 μm), especially with heavier PBDEs such as BDE 209. However, co-eluting phenomena is more likely to take place in a thin stationary phase coupled with a short column (Björklund et al., 2004). As for column length, shorter column results in higher response and less discrimination because PBDEs have less time to thermally degrade in a shorter column. However, a shorter column increases the chance of compounds to co-elute.

2.3.4.3 Detection Method

The criteria for good detectors are sensitivity and selectivity. Electron capture detectors (ECD) produce signals based on electron-capturing functional groups, thus responding to all compounds with hetero-atoms, including all halogens. Its low selectivity makes it not the best choice for analyzing PBDEs, although it is relatively inexpensive and easy to maintain and operate. Numerous compounds such as PCBs and organohalogen pesticides, which may have higher concentration than PBDEs, may interfere with the correct detections of PBDEs.

Compared to ECD, MS that measures the mass-to-charge ratio (m/z) of ionized particles is a better detector to analyze PBDEs. Within MS, the compounds are ionized by two different mechanisms: electron impact ionization (EI) and electron capture negative ionization (ECNI). Electron impact ionization is a hard ionization technique in which the molecule is vaporized and then bombarded by a beam of energized electrons, generating fragment ions. The major fragment ions of PBDEs for qualification and quantification are M^+ and $[M-2Br]^+$. While EI can be used in

both high and low resolution MS, EI in high resolution MS has much better sensitivity and selectivity than EI in low resolution; however, at a much greater cost. Low resolution EI-MS has a drawback with PBDEs because of its relatively lower sensitivity compared with ECNI-MS. This drawback is more of a problem with heavier PBDE congeners, especially with hepta- to decaBDEs, as the responses decrease with increasing number of bromines in PBDEs. The major advantage with EI is its ability to distinguish PBDEs in different homologs due to their different molecular mass and ionization patterns. It also allows for the use of C-13 labeled compounds, so that C-13 labeled PBDEs can be chosen as surrogates or internal standards for more accurate recoveries calculation.

Electron capture negative ionization, on the other hand, is a soft chemical ionization technique in which the low energy ions (thermal electrons) produced by high energy electron bombardment of reagent gas collide with gaseous molecules of the sample, resulting in negatively charged ions. The bromide ions (m/z = 79 and 81) are monitored for qualification and quantification of all PBDEs, except the fully brominated BDE 209 (Wei 2007), because of their much higher abundances than other fragment ions. Research has shown that the sensitivity of ECNI was more than 10 times better than ECD (Covaci et al., 2003) and its limit of detection was an order of magnitude lower than low resolution EI (Stapleton et al., 2006). However, the selectivity of ECNI on PBDEs is lower than EI because for such a wide range of PBDE congeners, most of them are only monitored by bromide ions in ECNI, while in EI, $[M-2Br]^+$ can be specified for different homologue groups. Improvement on selectivity of ECNI can be achieved by increasing the relative abundance of molecular fragment ions $[M-xH-yBr]^-$ through optimizing electron energy, emission current, source temperature, and etc. (Ackerman et al., 2005).

More recently, triple quadrupole mass spectrometer (QQQMS) has caught more attention. Able to achieve better signal to noise ratio in complex matrices, the triple quadrupole technique further increases the detection sensitivity and lowers the detection limit. In addition, it also allows for better selectivity in dirty matrices (Agilent 2011). Instead of one quadrupole that sorts ions based upon the mass-to-charge ratio as in the single quadrupole MS, the QQQMS has two sets of quadrupole that perform different functions. The first set of quadrupole (Q1) selects the precursor ion, also called parent ion. The selected parent ions pass through a collision cell (Q2) where selected ions collide with a gas (e.g., nitrogen) to produce product ions, also called daughter ions. The second set of quadrupole (Q3) then allows the selected product ions to be passed to the detector. Various scan types can be applied in Q1 and Q3. Multiple reaction monitoring (MRM) is commonly used in which Q3 looks for multiple product ions for qualification and quantification purposes. With the ability to produce highly specific and sensitive results, MRM was crowned as “the ultimate target compound analysis tool” (Agilent 2011, page 26).

3. METHODS AND PROCEDURES

3.1 Chemicals and Equipment

3.1.1 Chemicals

The mixture of 39 PBDE standards (BDEs 1, 2, 3, 7, 8, 10, 11, 12, 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, 154, 155, 166, 181, 183, and 190), 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 (BB 209), and C-13 labeled 2,3,3',4,4',5,5',6-octachloriobiphenyl (CB 205L) were purchased from AccuStandard (New Haven, Connecticut). Individual PBDE standards (BDEs 28, 47, 66, 85, 99, 100, 153, 154, 183, and 209) were purchased from Cambridge Isotope Laboratories (Andover, Massachusetts).

Major solvents used for the project were n-hexane (Fisher H303-4, Optima grade, >99.9%, Hanover Park, Illinois); methylene chloride (DCM) (Fisher D150-4, HPLC GC/MS grade, Hanover Park, Illinois), and acetone (GC grade, Fisher Scientific, Hanover Park, Illinois). Other solvent includes sodium hydroxide (1N) and concentrated sulfuric acid.

Sorbents used in the project are Florisil (60-100 mesh, Fisher Scientific, Hanover Park, Illinois) and silica gel (100-200 mesh, Davisil Grade 644, Fisher Scientific, Hanover Park, Illinois). Anhydrous sodium sulfate (Certified A.C.S., Fisher Scientific, Hanover Park, Illinois) was used for moisture removal from sample extracts.

Deactivated borosilicate glass wool (Restek Corporation, Bellefonte, Pennsylvania) was used in the extraction and cleanup columns.

The laboratory reagents were prepared as following:

Sodium sulfate and silica gel were prepared at 150°C in the oven overnight. Florisil was activated in the oven at 150°C for 12 to 14 hours. All the sorbents were stored in glass bottles with screw-caps in the desiccator to avoid moisture. Acidic silica gel (30% by weight) was prepared by mixing 100 grams of activated silica gel with 44 grams of concentrated sulfuric acid. Basic silica gel was prepared by mixing 10 grams of activated silica gel with 3 grams of sodium hydroxide (1N). During the process of making acidic and basic silica gel, the container was well shaken so that aggregates were broken up and a uniform mixture was obtained.

3.1.2 Equipment

Laboratory equipment includes an oven maintained at 150˚C, a furnace set at 500˚C, a freeze dryer with 60 mL freeze dry flasks (FreeZone 4.5L, Labconco, Kansas City, Missouri), a rotary evaporator (Hei-VAP Advantage, Heidolph Instruments GmbH & Co.KG, Schwabach, Germany), and nitrogen blow devices.

Laboratory glassware included mortars, pestles, pear-shaped evaporator flasks (200mL), KD tubes, 1 mL volumetric flasks, disposable Pasteur pipettes (glass), amber storage vials, amber auto-sampler vials, extraction columns (glass, 13.4 mm inner diameter, 305 mm length), and cleanup columns (glass, 11 mm inner diameter, 400 mm length).

Instrumental analyses were carried out by GC/MS (Agilent 6890+/5973 GC/MS) and GC/QQQMS (Agilent Technologies 7890/7000 GC/QQQMS).

3.2 Sample Collection

Placenta samples were collected at three sites, namely UR, MCW, and UCD, representing three regions: the East Coast, the Midwest, and the West Coast. The tissues were collected at different time points, ranging from 0 to 96 hours after the consented women gave birth. (Only one sample was accidentally collected 120 hours after birth.)

A sample collection matrix was designed to test the collection time and site effects. Samples from the same placenta were collected at three or more times. In addition, at least two sites collected samples at each identified time point. Table IV displays the placenta sample collection layout. Altogether, 42 women were recruited for the study, with one woman giving birth to twins.

For the purpose of testing the collection and laboratory procedures, the first nine placentas form a preliminary study, nick-named the Nine Study, with a total of 46 samples. The other 34 placentas form the pilot study, with a total of 123 samples.

TABLE IV

PLACENTA SAMPLES COLLECTION LAYOUT

Time Points (Hrs)	Placenta Numbers						
	1–6	7–12	13–18	19–24	25–30	31–36	37–42
	UR[a]	MCW[a]	UCD[a]	UR	MCW	UCD	UR
0	X[b]	X	X	X	X	X	X
1	X			X			X
2	X		X				
4		X	X				
8	X	X					
12		X	X				
24			X	X			
36				X	X		
48	X					X	
72					X		X
96						X	X

[a] UR = University of Rochester; MCW = Medical College of Wisconsin; UCD = University of California Davis.
[b] Each X represents six samples.

3.3 Sample Preparation

Placenta samples were delivered to this laboratory by overnight shipping. Upon arrival, samples were immediately retrieved and stored in the freezer at -20 °C.

Once a sample was about to be processed, it was taken out of the freezer and left in the refrigerator to thaw. Once thawed, it was weighed and transferred to a clean and solvent-washed freeze-dry (FD) flask. The wet weight was recorded in the Chain of Custody (Appendix A). The sample tube was rinsed with minimum amount of deionized water, and both the blood and deionized water were transferred to the FD flask. The flask was covered with aluminum foil and placed in the freezer again to freeze overnight. The FD flask was labeled with the sample identification number (ID).

During the transfer, the placenta sample was cut into very small pieces by a pair of scissors to homogenize the sample. In the past, the sample was ground in the FD flask by a homogenizer for less than one minute. However, it was decided that since the samples all came in small amounts, cutting the samples to very small pieces would be sufficient.

After the placenta sample was completely frozen, it was freeze-dried for at least 12 hours at -53°C and 0.060 mBar. If extraction was not immediate, the dried sample would be weighed, wrapped with aluminum foil, and stored in a desiccator.

3.4 Extraction

Based on the strengths of MSPD mentioned in Section 2.3.1 and the fact that the placenta samples were in small amounts (mostly 10 to 40 grams wet weight), the MSPD method was chosen for the project.

After the sample was taken from the freeze dryer, it was weighed and its dry weight recorded in the Chain of Custody. The dried sample was then transferred to a pre-cleaned glass

mortar. The FD flask that contained the sample was rinsed with minimum amount of hexane, and the hexane was added to the sample. After the sample was dried, 10 mL of FBDE 69 (100 ng/mL) and 20 mL of FBDE 208 (100 ng/mL) were added to the sample using a 25 μL micro-syringe. Then Florisil was poured onto the sample. The amount of the Florisil added equaled two times the amount of the dry weight of the placenta sample. The placenta sample was mixed and ground with Florisil using a glass pestle for approximately five minutes until a fine powder was formed. It is important that there are no chunks or long fibers in the placenta powder to ensure extraction effectiveness. The mortar and pestle set was covered while the extraction column was being prepared for sample packing.

A small amount of glass wool was plugged into the pre-cleaned and solvent-washed extraction column. The extraction column was packed with ten grams of anhydrous sodium sulfate and four grams of Florisil from bottom to top (the sorbents were prepared following the procedure described in Section 3.1.1). The extraction column was tapped to ensure tight packing. Twenty mL of 8:2 hexane to DCM solvent mixture was used to pre-elute the packed column to clean the Florisil and sodium sulfate. After the flow ceased, vacuum was applied to remove any remaining solvent in the extraction column.

The ground mixture of placenta and Florisil was transferred into the column through a funnel. The column was tapped during the transfer to remove air and increase compactness. A solvent mixture of 120 mL of 8:2 hexane to DCM was prepared. Some volume was used to rinse the mortar, the pestle, and the funnel and then added to the column while the stopcock was at the "open" position. The remaining solvent was poured to the extraction column slowly to prevent disturbance to the column bed. The gravitation flow rate was adjusted to one drop per second. The flow was controlled to be slow to allow sufficient time for the solvent mixture to interact

with samples so that BDE analytes of interest could be effectively extracted. The effluent was collected directly into a pre-cleaned and solvent-rinsed pear-shape evaporator flask that was covered with aluminum foil to protect BDE analytes from exposure to light. After the gravitation flow ceased, the pear-shape evaporation flask was removed from the extraction column and covered with aluminum foil on the top.

After the chromatographic elution, concentration was needed to reduce the large volume collected from the elution to prepare for the clean-up procedure. As this was macro-scale solvent evaporation, the rotary evaporation utilizing heat and vacuum was applied. The rotary evaporator bath was filled with deionized water. The temperature was set at 50°C and the rotator at 80 rpm. The rotary evaporator was cleaned with 20 mL hexane before and after each sample. Then the pear-shape evaporation flask was attached and the vacuum was applied. During the evaporation process, boiling was avoided to prevent losing analytes of interest. The rotary evaporation was stopped when there was about 2 mL of extract left. It is very critical that the extract must never be evaporated to dryness; otherwise, the recovery would be significantly decreased. The flask was removed from the evaporator after cooling for 2–3 minutes. The vapor tube end inside the flask was rinsed with approximately 0.5 mL of hexane, and the hexane was collected into the flask. The flask was covered with aluminum foil again until the cleanup procedure.

3.5 Cleanup

For this project, because of the merit of the matrix solid-phase dispersion extraction method that produces a cleaner extract with much fewer lipids, the initial cleaning of the extract using GPC is no longer needed. Instead, a multi-layer silica gel column packed with the combination of neutral, acidified, and basified silica gel was selected to clean up the extract.

Hexane, a nonpolar solvent with polarity much less than other common solvents such as toluene, DCM, acetone, and methanol, was chosen to be the eluting solvent.

A small amount of glass wool was plugged into the pre-cleaned and solvent-washed cleanup column. The cleanup column was first filled with 40 mL of hexane. Then it was packed with, from bottom to top, one gram of anhydrous sodium sulfate, one gram of neutral silica gel, one gram of basic silica gel, one gram of neutral silica gel, four grams of acidic silica gel, one gram of neutral silica gel, and five grams of anhydrous sodium sulfate (the sorbents were prepared following the procedure described in Section 3.1.1). The cleanup column was tapped while the sorbents were settling down so that they were packed without air bubbles to avoid channeling. The excess hexane in the cleanup column was drained until approximately 1 mL above the sorbent bed. Special care was devoted to assure that the column was not dried out. After the initial cleanup column packing, 20 mL of hexane was added to pre-elute the column to clean the sorbents.

When the pre-eluting hexane came close to the upper layer of anhydrous sodium sulfate (i.e., approximately 1 mm above), the extract that was reduced by the rotary evaporation to about 2 mL was loaded into the cleanup column with a Pasteur pipette. The stopcock was adjusted so that the column eluted at about one drop per second. A pre-cleaned and pre-solvent-rinsed pear-shape evaporation flask was set beneath the cleanup column to collect the eluate. When the extract was close to the sorbent bed again, 50 mL of hexane was first used to rinse the pear-shape evaporation flask that contained the extract and then poured into the cleanup column. All the eluate was collected until the column stopped dripping.

3.6 Sample Concentration

For this project, rotary evaporation was chosen to reduce the extract volume as well as that of the eluate from the cleanup process. The last paragraph in Section 3.4 details how the rotary evaporator was operated to reduce the extract to around 2 mL. The same procedures were applied to concentrate the eluate from the cleanup process to 1 to 2 mL. This was followed by micro-concentration using nitrogen blow.

After the sample was reduced to about 2 mL by the rotary evaporator, it was transferred to a K-D tube by a clean disposable Pasteur pipette. The flask was rinsed with 0.5 mL hexane three times and the hexane was also transferred to the K-D tube. The K-D tube was then set under a gentle nitrogen stream blown from a nitrogen tank. The flow of nitrogen was controlled to the point that the surface of the solvent was gently disturbed. A large vortex in the solvent should be avoided to prevent loss of targeted analytes.

When the sample volume was reduced to about 0.5 mL, it was transferred to a 1 mL volumetric flask by the same pipette that was used to transfer the sample to the K-D tube. The K-D tube was rinsed with hexane three times using less than 0.5 mL hexane in total. The hexane was added to the 1 mL flask as well. Finally, the volume was adjusted to 1.0 mL in the 1 mL volumetric flask. It was then transferred by the same pipette again to an amber 2 mL storage vial. The vial was carefully labeled, sealed, and kept in the refrigerator until instrumental analysis.

3.7 Instrumental Analysis

3.7.1 Injection Type

For this project, an Agilent Model 6890 GC was used. The PTV injection technique was chosen mainly because of its large volume injection capacity. As some PBDE congeners in human placentas are of trace level, a method that enhances detection sensitivity is highly

desirable. The operational parameters of the PTV, including injection rate, temperature, volume, and vent flow, had been optimized for PBDE analysis (Wei et al., 2010), and are summarized in Table V.

TABLE V

SELECTED PARAMETERS FOR GAS CHROMATOGRAPH

Injection mode	solvent vent
Front inlet initial temperature	40 °C
Front inlet initial time	1 minute
Front inlet temperature ramp	The temperature is increased at a rate of 600°C per minute from 40°C to 300°C and holds at 300°C for 5 minutes.
Front inlet vent time	1 minute
Front inlet vent flow	100.0 mL per minute
Front inlet purge flow	50.0 mL per minute
Carrier gas type	Helium
Initial oven temperature	50°C
Holding time	3 minutes
Oven temperature ramps	The temperature is first increased at 10°C per minute to 150°C, then 5°C per minute to 225°C, and finally 15°C per minute to 300°C and holds at 300°C for 10 minutes.
Column flow	1.0 mL/min constant flow
Injection volume	25.00 µL repeated 3 times with a total of 75.00 µL

3.7.2 Column Selection

Balancing many factors in column selection, such as stationary phase material and thickness, column width and length, it was recommended that for analyzing a wide range of PBDE congeners, a short, non-polar column with a thin (0.1 µm) stationary phase should be used (Björklund et al., 2004). Therefore, for this project, a 15-meter Rtx 1614 capillary column (Restek Corp.) with an internal diameter of 0.25 mm and stationary thickness 0.10 µm was

chosen. This column was specially designed to use with EPA Standard Method 1614—Brominated Diphenyl Ethers in Water Soil, Sediment and Tissue by HRGC/HRMS (EPA 2007).

3.7.3 Detection Method

Based on the comparison among the four detection techniques (Section 2.3.4.3), the ECNI-MS and QQQMS with chemical ionization (CI) source were chosen to monitor PBDEs for this project. These methods were determined to be the most appropriate detection methods because the PBDE concentrations in placenta samples are low, requiring high sensitivity. The Agilent 7000 QQQMS was used for the Nine Study while the Agilent 5973 MS was used for the Pilot Study of the Project 2-18. With the QQQMS, although MRM was set, the monitoring ions for Q3 were not different from those for Q1 because the characteristic fragment ions for PBDEs in CI mode are isotope bromide ions 79 and 81, which cannot be further fragmented. In this sense, the strength of MRM is not taken advantage of.

For both instruments, the MS ion source temperature was set at 200°C and the quadrupole temperature was 150°C. The selected ion monitoring mode was used for both Q1 and Q3 in QQQMS and the single quadrupole MS. The ions monitored are recorded in Table VI.

TABLE VI

IONS MONITORED WITH ELECTRON CAPTURE NEGATIVE IONIZATION ON MASS SPECTROMETER

Analyte of Interest	Ions
Tri- to heptaBDEs	79, 81
BDE 209	484.6, 486.6
CB 205 L (Internal standard)	441.8, 430.8, 405.8
BB 209 (Internal standard)	79, 81
FBDE 69; FBDE 208 (Surrogates)	79,81

3.7.4 Experimental Procedures

One hundred and fifty μL of sample (such as placenta, blank, reference) stored in the amber vial was withdrawn by a 250 μL micro-syringe and injected into a vial insert. Internal standards, of 7.5 μL decabromobiphenyl 209 (BB209, 101.4 ng/mL) and 1.5 μL C-13 labeled chlorinated biphenyl 205 (CB205L, 40.0 ng/mL) were also injected into the insert by dedicated micro-syringes. The insert was stored in the GC amber vial and the vial was placed on the GC tray. An injection sequence was written in which four samples were followed by a hexane blank run for a constant check on the instrument background.

The GC/MS operation was controlled through Agilent software ChemStation (version G1701EA E.02.01.1177), and the GC/QQQMS through Agilent software MassHunter Acquisition (B.05.01.0696.6). After the acquisition method was loaded, the first two runs were instrumental blanks—hexane runs. When the chromatograms of the hexane did not contain peaks of targeted analytes and the baselines were as low as normal, the samples were then injected. If the chromatograms had unexpected peaks or carryover, the instrument conditions and possible source of contamination were checked. For example, the instrument injection port, the liner, or the MS ion source might have needed cleaning. The machine was adjusted until the solvent blank checks returned to normal. Then the sample sequence was run.

After the samples were all injected and the data acquisition completed, the data analysis was performed on each sample. Although an automated calculation procedure was set based on calibration curves, each sample was manually checked by verifying peaks (e.g., whether there was a tailing or split in any peaks) and matching the retention times. The results were saved for data analysis. Figure 4 is an example of a total ion chromatogram obtained by GC/MS. The

peaks are sharp and separated, and there is little trailing. BDE congeners are recognized by retention times and quantified by comparison to the internal standards.

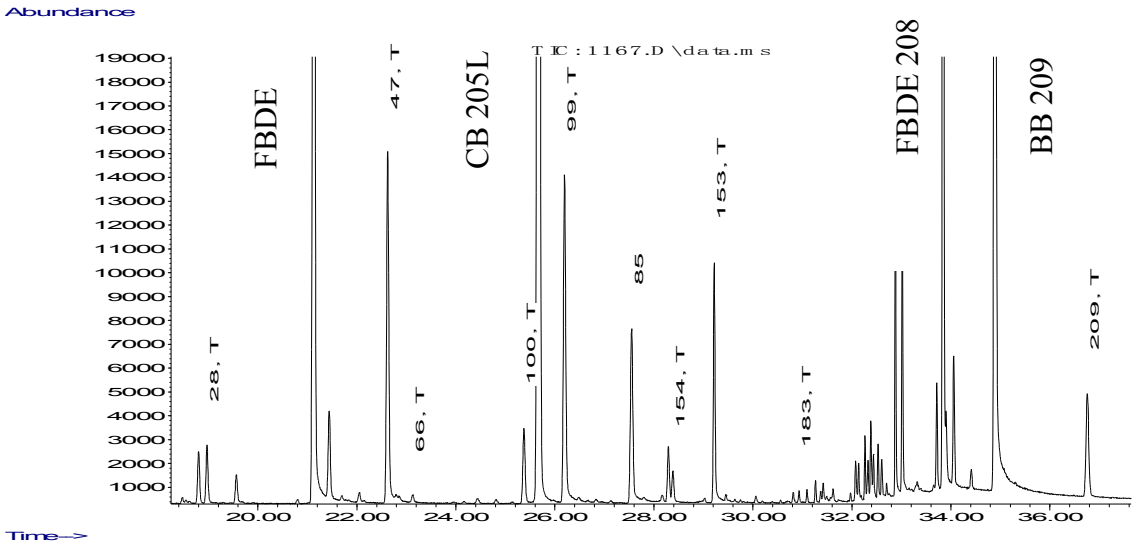


Figure 4. Example GC/MS total ion chromatogram of a placenta sample. The PBDE congeners of interest are labeled by their congener numbers.

3.7.5 Concentration Calculation

The internal standard method in EPA Method 1614 was used to calculate PBDE concentrations (EPA 2007). The response factor of each BDE analyte and surrogate was obtained through series of five calibration standards. The concentrations of the calibration standards spanned a range of 50-fold for GC/QQQMS (for the Nine Study) and a range of 100-fold for GC/MS (for the Pilot Study), covering the range of the analytes in the placenta samples. Calibration standards were injected before the sample batch analysis began. The correlation coefficients (R^2) of all the calibration curves are > 0.99. This suggests a linear model is

appropriate for calculating concentrations of the analytes in the placenta samples. The BDE concentration of a sample (C_s) is calculated following the equation:

$$C_s = \frac{C_{is} A_s}{A_{is} \mathrm{RF}}$$

where A_s is the measured area for the BDE analyte; A_{is} is the measured area for the internal standard; and C_{is} is the concentration of the internal standard.

4. QUALITY ASSURANCE AND QUALITY CONTROL

Quality assurance and quality control (QA/QC) are essential to the success of the project. Well-developed and well-implemented QA/QC procedures not only enhance confidence in the final analytical data, but also make good data interpretation possible. While errors are unavoidable, good QA/QC programs help to ensure that the errors in the analytical data are of an acceptable magnitude. Thus strict QA/QC procedures were designed and routinely carried out to produce reliable data with minimum errors.

The QA/QC measures in this project include: Chain-of-Custody forms, method detection limits, spikes, blanks, surrogates, and references.

4.1 Chain of Custody

A Chain-of-Custody form (Appendix A) was designed to track the activities in receiving and processing samples. The Chain-of-Custody form has four sections.

The first section was about sample receiving. Once a sample was received, its ID, wet weight, receiving time and the storage place were recorded, and this section was signed by the researcher who received the samples.

The second category was about sample pretreatment including freeze drying. When a sample was transferred to the FD flask, its ID along with the wet weight was recorded. Then the date and exact time when the sample was put onto the FD machine was written down next to the Start Date. Finally, the date and time when the sample was taken off the freeze dryer was recorded together with their dried weights. The most important content in this section was the wet weight, as the final results were to be reported in the form of pg/g wet wt.

The third section was about laboratory procedures—i.e., extraction and cleanup. With respect to extraction, major contents included the volumes of surrogates spiked and the amount

of Florisil added to the samples. The form also required information on the start and end dates of the extraction process. With respect to cleanup, the dates of the cleanup and final concentration were required along with the analyst's name.

The fourth section was on instrumental analysis. The volume of the internal standards added and the date when the samples were analyzed by the instrument were recorded.

4.2 Method Detection Limit

The method detection limit (MDL) was determined according to the procedures established by EPA (EPA 1996). The Limit of Detection (LOD) is an instrumental detection limit that is determined to be a concentration able to produce a response that is three times greater than the noise signal. The Limit of Quantification (LOQ) is ten times the noise signal. The LOD and LOQ for each analyte are reported along with the sample results for GC/QQQMS (Table VII). The machine has very low LODs and LOQs for all the BDE analytes of interest. This makes it possible to detect PBDEs in human placentas when the concentrations are low.

TABLE VII

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION (Unit: pg)

Compounds	LOD (pg/ml)	LOQ (pg/ml)	LOQ[a] (pg/g wet wt)	LOQ[b] (pg/g wet wt)
BDE 28+33	0.14	0.47	0.05	0.23
BDE 47	0.35	1.18	0.12	0.59
BDE 66	0.36	1.21	0.12	0.61
BDE 100	0.34	1.12	0.11	0.56
BDE 99	0.40	1.34	0.13	0.67
BDE 85	0.70	2.33	0.23	1.16
BDE 154	0.38	1.26	0.13	0.63
BDE 153	0.38	1.28	0.13	0.64
BDE 183	0.78	2.60	0.26	1.30
BDE 209	4.71	15.70	1.57	7.85

[a] For a sample weight of 10g.
[b] For a sample weight of 2g.

4.3 Spike Results

Spiking the sample with known amount of analytes is a typical way to validate methodology. In this project, two types of spikes were used: blank spikes and matrix spikes.

4.3.1 Blank Spikes

In order to validate the method, two types of spikes were used. The first type was blank spikes. Before grinding the samples, known amounts of BDE analytes were added to Florisil, which had negligible levels of those BDE analytes. The spiked samples then went through the same experimental procedures and instrumental programs as the regular placenta samples. Three blank spiked samples were performed. Table VIII presents the detected levels of BDE analytes of interest, from which recoveries are calculated and the validity of the method evaluated.

TABLE VIII

BLANK SPIKES

Compounds	S1[a]	S2	S3 (ng/mL)	Reference	R1[a,b]	R2	R3 (%)	Mean R	SD
BDE 28+33	3.0	3.0	2.8	3.6	84.4	82.4	77.1	81.3	3.8
BDE 47	2.8	2.7	2.6	3.1	87.9	86.3	81.9	85.4	3.1
BDE 66	2.8	2.8	2.6	3.3	86.0	84.8	80.2	83.6	3.1
BDE 100	4.4	4.3	4.1	4.9	88.8	87.6	82.3	86.3	3.5
BDE 99	4.1	4.1	3.8	4.7	87.3	86.8	80.9	85.0	3.6
BDE 85	4.1	4.0	3.8	4.6	90.4	88.6	82.5	87.1	4.2
BDE 154	5.8	5.6	5.2	6.4	91.0	87.6	82.5	87.0	4.3
BDE 153	5.6	5.5	5.1	6.0	93.8	91.8	85.7	90.4	4.2
BDE 183	6.8	6.7	6.2	6.9	99.6	98.1	90.4	96.0	4.9
BDE 209	6.1	4.2	5.0	6.0	101	70.5	84.0	85.3	15.5
FBDE 69	2.7	2.6	2.5	2.8	95.0	92.9	90.1	92.7	2.5

[a] S – Spike; R – Recovery.
[b] Recovery (%) = Spike / Reference × 100%.

Because the BDE standard solutions had been stored for years and their concentrations could be slightly altered, a reference was carried out to be compared to the detected levels for more accurate recovery calculations (with the exception of BDE 209). The same amounts of BDE analytes used as spikes were added to a 1 mL volumetric flask. This was the reference. By comparing the detected levels of BDE analytes to the references, the recoveries are calculated.

The recovery of FBDE 69, the surrogate, is 92.7%. This good recovery makes the interpretation of blank spikes recovery data meaningful. The recoveries of BDE analytes in the blank spikes range from 81% to 96%, with an average recovery of 87.3% and a standard deviation of 4.2%. These are well in the range of 60% to 140%, which is considered acceptable. Worth noticing is that the recoveries of the heavier BDEs (i.e., BDE 154, 153, 189, and 209) are not lower than the recoveries of the lighter BDEs, suggesting the heavier PBDEs were well

preserved during the entire procedure without degradation. In summary, the recovery data of blank spikes suggest that the methods and procedures are accurate and reliable.

4.3.2 Matrix Spikes

The second type of spikes done in the project is matrix spikes. Matrix spikes are necessary in addition to blank spikes because of the possible interaction between PBDEs and the matrix substances. Therefore, the method cannot be entirely validated without knowing the matrix effects on the analytes of interest.

The placenta sample used in the matrix spikes is a placenta collected at the University of Illinois hospital. The placenta was first analyzed following the same procedure as NCS project samples. Then, it was reanalyzed in duplicates, spiked with PBDEs. Recoveries are calculated by subtracting the PBDE levels in the unspiked placenta from the spiked results, divided by the known amount of BDEs spiked. The results are presented in Table IX.

TABLE IX

MATRIX SPIKES

Compounds	Spike1	Spike2	Reference (ng/mL)	Placenta	Recovery1[a]	Recovery2 (%)	Mean	SD
BDE 28+33	1.6	2.0	3.3	0.0	49.8	63.0	56.4	9.3
BDE 47	3.3	3.0	3.0	0.6	90.0	78.4	84.2	8.2
BDE 66	2.6	2.4	3.1	0.0	84.6	79.7	82.1	3.5
BDE 100	4.5	4.0	4.5	0.2	94.3	84.1	89.2	7.2
BDE 99	6.2	4.6	4.5	1.0	114	80.9	97.7	23.8
BDE 85	3.3	3.4	4.5	0.0	74.4	76.9	75.7	1.7
BDE 154	5.7	5.3	6.0	0.1	93.5	86.4	89.9	5.0
BDE 153	6.1	5.3	6.0	0.2	99.0	84.9	91.9	10.0
BDE 183	6.8	6.2	7.3	0.0	92.6	84.5	88.5	5.8
BDE 209	7.6	6.0	5.7	1.1	112	84.4	98.3	19.5
FBDE 69	2.9	2.7	3.0	0.0	95.7	90.6	93.1	3.6
FBDE 208	6.3	5.9	5.8	0.0	110	103	106	4.9

[a] Recovery (%) = (Spike – Placenta) / Reference × 100%.

Similar to blank spikes, matrix spikes also use the reference for the same reason. FBDE 69 and FBDE 208 are the two surrogates. Their recoveries were 93.1% and 106.3% respectively, laying a good ground for interpreting recoveries of BDE analytes of interest.

Table IX shows that the recoveries for most of the BDE analytes fall in the range of 80% to 100%. The recovery of BDE 85 is 75.7%, which is slightly lower than others. However, the recovery of BDE 28 + 33 is only 56.4%. It is observed that although recoveries of various BDE analytes were similar in the blank spikes, the recoveries in the matrix spikes vary among analytes. It appears that the heavier BDE congeners had recoveries closer to 100%. It could be because lighter BDE analytes are more volatile, since the recovery for BDE 28 + 33 in blank spikes is also the lowest. However, in matrix spikes, it is almost 40% lower than recoveries for other BDE analytes. Thus, it could be concluded that the matrices have a stronger effect on lighter BDE

analytes. With the current method, the concentrations of lighter BDE analytes, especially BDE 28 + 33, are likely to be underestimated.

In sum, blank spikes and matrix spikes have been used to evaluate the analytical method. The blank spikes demonstrate the method to be accurate and reliable. The matrix spikes yields satisfying recoveries, except for the lightest BDE analyte of interest, namely BDE 28 + 33. This indicates stronger matrix effects on lighter BDE congeners.

4.4 Blanks

The blank test is an analysis in which all steps of the analytical procedure implemented without a real sample, using only the reagents. Test of blanks reveals background levels of contamination of the solvents, sorbents, and equipment. The low levels of some PBDEs in human placenta demand the blanks to be clean so that the background does not interfere with samples. In the project, two types of blanks—procedural blank and instrumental blank—were developed for different sub-procedures of the entire analytical method.

4.4.1 Procedural Blanks

The goal of procedural blanks is to monitor the degree of background contamination during the laboratory sample preparation. Procedural blanks were applied from sample grinding to the final storage in the amber vial. Instead of grinding the freeze-dried placenta samples, various amounts of Florisil, similar in weight to the dried placenta sample weights, were ground. The ground Florisil then went through the entire extraction, concentration, cleanup, and further concentration processes, until the final transfer to the amber vials for storage. Later in the instrumental analysis, it was analyzed with other regular samples.

In the project, one procedural blank was run with every 10 samples following the entire analytical process. For each PBDE analyte, the concentration in the blanks was recorded and

reported along with samples. The mean and standard deviation of those concentrations in the blanks were computed periodically to monitor the level and variation of laboratory contamination. Table X presents the procedural blank results of both the Nine Study and of the Pilot Study.

In Table X, the blank levels for the Nine Study were low, indicating sound experimental procedure and good lab conditions. However, the blanks performed during the next couple of months yielded much higher values for almost all the PBDE analytes, especially for BDE 209, the mean showing an increase of more than one order of magnitude. This raises concerns about whether sorbents, glassware, or lab environment was contaminated. A thorough laboratory cleaning was immediately performed. In order to further figure out why the blank levels went much higher within months, and more importantly, to reduce the blank levels, more blank checks were carried out.

TABLE X

BLANK RESULTS OF THE NINE STUDY AND THE PILOT STUDY (Unit: pg/g)[a]

	The Nine Study (N = 5)		The Pilot study (N = 9)	
Compounds	Mean	SD	Mean	SD
BDE 28+33	0.0	0.0	0.9	0.8
BDE 47	4.5	9.0	27.4	12.4
BDE 66	0.0	0.0	0.6	0.5
BDE 100	3.7	7.3	3.2	1.7
BDE 99	4.3	8.7	5.1	5.4
BDE 85	0.0	0.0	1.8	1.7
BDE 154	1.6	3.1	3.0	1.8
BDE 153	3.6	7.2	4.1	1.6
BDE 183	0.0	0.0	5.2	1.9
BDE 209	2.3	0.6	78.4	42.4
Σ_{10}BDEs	19.9	35.4	130	50.8
FBDE 69 %	98.8	17.8	92.2	18.3
FBDE 208 %	N/A	N/A	75.2	22.0

[a] The unit is picograms of PBDE per grams of Florisil.

Firstly, experiments were designed to check sorbent clean-up effectiveness. More solvent was added in stages to check the effectiveness of sorbent cleanup. The experiment showed that the initial experimental procedure is effective for cleaning up the sorbents, although more washes of silica gel might help to further reduce the background levels. In consideration with solvent consumption, it was decided that the initial sorbent clean-up steps will be maintained.

Secondly, experiments were designed to compare blank results from different sorbent preparation methods. It is possible that sorbents are contaminated, and being baked at 150°C is not sufficient to clean them up. Therefore, some sorbents were baked under a temperature program that gradually increases the temperature to 500°C and remains at 500°C for five hours. Results showed that using silica gel prepared at 500°C significantly lowered the blank levels for

the cleanup procedures. On the other hand, baking Florisil did not reduce background levels. The methods of solvent-washing Florisil, as well as baking Florisil at 500°C followed by solvent washing were further tested. However, neither approach lowered the blanks. It was therefore decided that Florisil would continue to be baked at 150°C for 12–14 hours while silica gel will be prepared in the furnace at 500°C to keep the background levels low.

Thirdly, experiments were designed to evaluate glassware cleaning procedures. Concerns were raised about whether BDE analytes were attached to the glassware so that latter batches yielded higher levels than former ones. Some operations involving instruments were also checked to determine if they brought in contamination. Extraction columns, cleanup columns, 1 mL volumetric flasks, mortars and pestles, rotary evaporation, and nitrogen blow were tested. Results showed that the normal cleaning of mortars and pestles and the ultrasonication of 1 mL volumetric flasks was sufficient. The use of nitrogen blow did not increase background BDE levels. When the rotary evaporation process was checked, it was found that hexane rather than DCM should be used between samples to clean the rotary evaporator.

Fourthly and lastly, fume-hood differences were given consideration as samples were processed in three different hoods within the same laboratory room. In order to check whether those three hoods had different conditions, three blank samples were processed in each hood by one person in one day to minimize intrapersonal differences. The results of these nine blanks are almost the same, indicating no differences among the three hoods.

In summary, this section reports the procedural blank results for the Nine Study and the Pilot Study (Table X). There were concerns whether lab environment, sorbents, or solvents were contaminated because the background levels were rising significantly after a few months after the project started. A thorough lab cleaning was carried out. In addition, sets of experiments were

designed to look for root problems so that corresponding measure can be taken to reduce the background levels. Sorbent clean-up effectiveness, sorbent preparation methods, glassware and instruments cleaning procedures, and hood differences were examined. There are three major conclusions from these sets of experiments. First, among all the efforts done to reduce background levels, laboratory cleaning is the most effective one. The heavy use of sorbents in laboratory procedures makes the laboratory environment vulnerable. Silica gel can be accidentally dropped into pear-shape flasks and it can even find its place in the final storage vial, which, if injected, may potentially skew the levels to a large extent. Therefore, more attention was paid before, during, and after the laboratory procedures to keep benches clean and free of sorbents so that the background can be kept low. Second, the temperature for the preparation of silica gel was increased to 500°C in the furnace. Third, building upon the previous two conclusions, three blank samples using Florisil burned at 150°C and silica gel burned at 500°C were carried out following the analytical procedures. The results in Table XI reveal that the background has returned to a low level. The average Σ_{10}BDEs concentration is 0.47 pg/g with a standard deviation of 0.07 pg/g (Table XI). This blank level is 1.5% of the lowest reported value on placenta samples.

For this thesis, the blank results are reported along with the PBDE levels from placentas. In other words, no further modification was made to the detected PBDE levels in placentas. A concern was raised whether variation in blank contamination levels would invalidate the comparisons among samples. If there is a relationship between the samples done on one day and the blank done on the same day, the relationship among samples is then dependent on the blanks. In order to address this concern, a Pearson correlation test was performed to investigate the association between the natural log of blanks and the natural log of samples. The Pearson's

correlation coefficient turned out to be -0.068, which is much smaller than the critical value of 0.260 for N = 9 at p = 0.25 (one-tail). Such a small correlation coefficient alleviates the concern that there are associations between the blanks and samples.

TABLE XI

BLANK CHECK (Unit: pg/g)[a]

Compounds	Blank 1	Blank 2	Blank 3	Mean	SD
BDE 28+33	0.00	0.00	0.00	0.00	0.00
BDE 47	0.12	0.11	0.11	0.11	0.01
BDE 66	0.00	0.00	0.00	0.00	0.00
BDE 100	0.01	0.01	0.00	0.01	0.01
BDE 99	0.13	0.08	0.07	0.09	0.03
BDE 85	0.00	0.00	0.00	0.00	0.00
BDE 154	0.01	0.01	0.00	0.01	0.01
BDE 153	0.01	0.00	0.01	0.01	0.01
BDE 183	0.00	0.00	0.00	0.00	0.00
BDE 209	0.27	0.24	0.23	0.25	0.02
Σ_{10}BDEs	0.55	0.45	0.42	0.47	0.07

[a] The unit is picograms of PBDE per grams of Florisil.

4.4.2 Instrumental Blanks

Instrumental blanks were carried out to check the background of the GC/MS instruments. Under the same configuration and operational conditions, hexane runs were performed before sample analysis to verify that the analytical instruments were in good condition. Hexane was also run between every fifth or sixth sample. Throughout the entire project, the results of hexane runs did not show unexpected peaks and the baselines were low, indicating good instrumental background.

4.5 Surrogates and References

Known amounts of surrogates were added to the placenta and blanks samples to monitor any loss of analytes in the analytical procedure. A good surrogate has similar physical and chemical properties to the analytes of interest. It does not interfere with the target compounds and can be distinguished separately in the chromatogram. For the Nine Study, the surrogate chosen to monitor BDE analytes was FBDE 69 (10 mL of 100 ng/mL), a fluorinated BDE. For the Pilot Study, FBDE 69 (10 mL of 100 ng/mL) along with FBDE 208 (40 mL of 100 ng/mL or 20 mL of 200 ng/mL) was added to the samples. In order to monitor the recovery of BDE 209, FBDE 208 was additionally selected, as both of them have relatively high molecular weights.

References refer to the procedure in which same amounts of surrogates were added to a 1 mL volumetric flask. The purpose of references is to examine the bias caused by the micro-injection process involving the micro-syringe and some other unknown conditions (such as evaporation during storage). Therefore, reference is also called the surrogates check. By comparing the detected concentrations of the surrogates in samples to those of the references, recoveries were calculated.

For the Nine Study, the average recovery of FBDE 69 is 112%. For the Pilot Study, the average recovery for FBDE 69 is 104%, while that for FBDE 208 is 97.3%. They are all acceptable recoveries according to the criteria of EPA Method 1614 (EPA 2007). Table XII summarizes the surrogate recovery results. Since all the recovery results are close to 100%, no back calculation is performed on BDE analytes in the placenta samples.

TABLE XII

STATISTICAL SUMMARY OF SURROGATES (UNIT: %)

	N	Mean	SD	RSD	Median	Min	10th %	Max	90th %
FBDE 69	165	106	19.4	18.3	102	1.2	89.9	176	131
FBDE 208	119	97.3	25.5	26.2	95.4	41.3	80.4	264	113

5. RESULTS AND DISCUSSION

5.1 Concentrations of Polybrominated Diphenyl Ethers in Human Placentas

Altogether, 169 tissue samples were collected from 43 placentas following the sample collection matrix (Table IV). One placental sample (Placenta ID 1040 with Sample ID 1083) was lost during the analytical procedure due to the storage vial breaking. Another three samples (Placenta ID 1022 with Sample ID 1098; Placenta ID 3029 with sample ID 1159; and Placenta ID 1023 with Sample ID 1165) had unexpected interruptions during injections. Therefore, PBDE levels are reported for the 165 placenta samples.

5.1.1 Statistical Summary

This section first summarizes the statistical results for all 165 samples collected at three study sites at all collection times. It then presents the summary for 42 samples that were collected at time t = 0, which represents the 42 placentas.

5.1.1.1 Samples Collected at All Locations at All Collection Times

Forty-six samples in the Nine Study and 119 samples in the Pilot Study were analyzed following the validated laboratory and instrumental procedures. The entirety of the data is recorded in Appendix B (for the Nine Study) and Appendix C (for the Pilot Study), and serves as the basis for the analysis presented herein.

Table XIII and Figure 5 summarize descriptive statistical results for the 165 samples that were collected at all three locations and at all collection times. The median concentration of Σ_{10}BDEs is 313 pg/g wet wt (range 30.9–6083 pg/g wet wt). The detailed information of each quartile can be found in Appendix D.

TABLE XIII

STATISTICAL SUMMARY FOR THE SAMPLES COLLECTED AT ALL LOCATIONS AND AT ALL TIMES (N = 165) (Unit: pg/g wet wt)

Compounds	Median	Min	Max	Average	SD	RSD (%)
BDE 28+33	5.6	0.6	41.5	8.0	6.5	81.8
BDE 47	111	8.0	1930	199	257	129
BDE 66	1.6	0.0	23.4	2.5	3.1	123
BDE 100	24.8	0.0	555	48.0	69.2	144
BDE 99	40.9	0.0	3236	101	286	284
BDE 85	4.3	0.0	192	9.5	22.0	230
BDE 154	6.1	0.6	236	11.2	23.0	204
BDE 153	48.3	0.0	630	84.4	104	123
BDE 183	2.3	0.0	59.6	4.9	8.8	181
BDE 209	32.2	0.5	301	36.7	37.0	101
Σ_{10}BDEs	313	30.9	6083	505	659	131

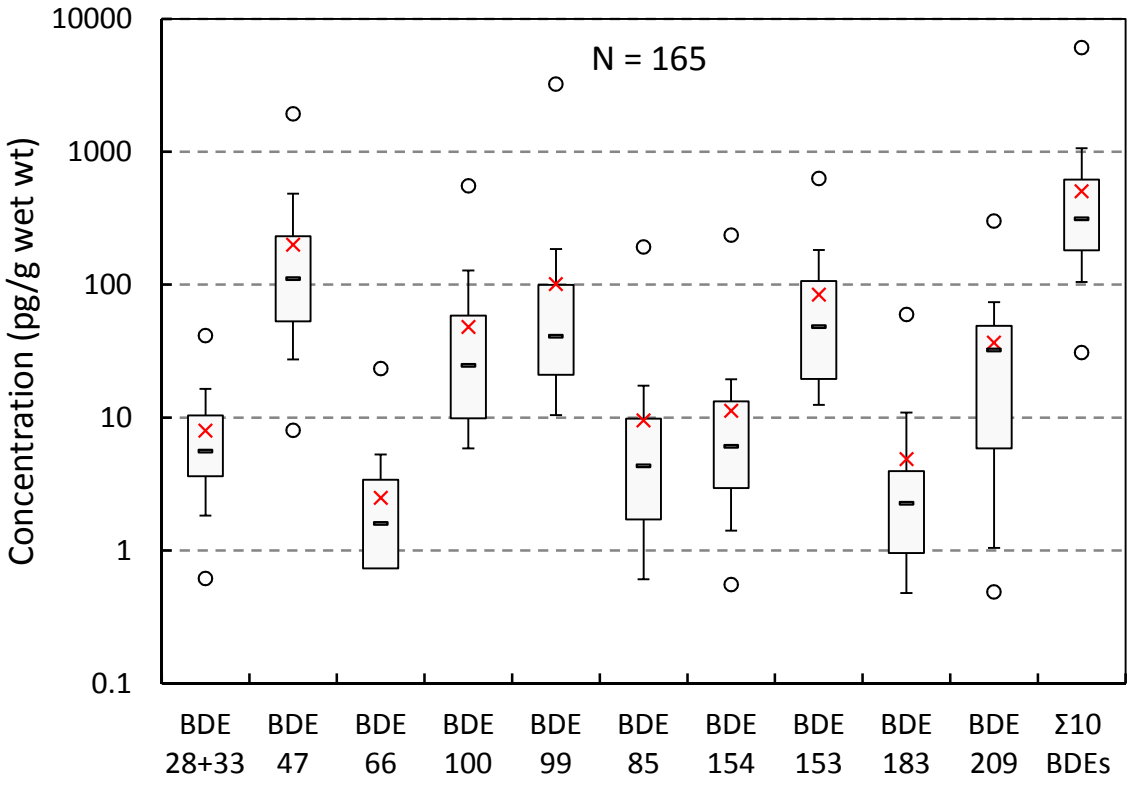


Figure 5. Box plot for the 165 samples collected at all sites and at all times. The line in the box represents the median; the cross represents average; the box covers the range from the first to the third quartile; the bars outside the box represent the 10th and 90th percentile values; the circles outside the box represent the maximum and the minimum values.

5.1.1.2 Samples Collected at All Locations at Time T = 0

The 165 samples discussed in Section 5.1.1 are not all independent because the same placenta was sampled at different times to investigate the collection time effect, and not all placentas were sampled at the same times. In fact, the 165 samples come from only 43 placentas. All the placentas have one specimen sample collected at time t = 0. (One placenta sample at time t = 0, Placenta ID 1040 with Sample ID 1083 was lost during the analytical procedure as the storage vial was broken.) Table XIV and Figure 6 summarize BDE results measured at collection time t = 0 for 42 placentas.

The median of the Σ_{10}BDEs for these 42 placentas is 330 pg/g wet wt (range 42.6–1723 pg/g wet wt). This value is similar to the median value obtained from all the 165 samples. The detailed information of each quartile can be found in Appendix E. More qualitative and quantitative analysis on collection time effect is discussed in Section 5.3.

TABLE XIV

STATISTICAL SUMMARY FOR THE PLACENTAS COLLECTED AT TIME = 0 (N = 42) (Unit: pg/g wet wt)

Compounds	Median	Min	Max	Average	SD	RSD (%)
BDE 28+33	5.5	0.6	21.8	7.5	5.8	76.9
BDE 47	108	8.0	725	169	180	107
BDE 66	1.5	0.0	9.6	1.9	1.8	96.2
BDE 100	26.5	1.9	195	42.3	48.1	114
BDE 99	41.2	3.7	726	81.6	122	150
BDE 85	3.9	0.0	157	9.8	24.5	251
BDE 154	4.8	0.6	145	11.1	22.7	205
BDE 153	51.2	2.5	514	86.7	103	119
BDE 183	1.5	0.0	29.5	2.9	4.74	163
BDE 209	29.4	0.7	90.1	32.5	23.7	72.9
Σ_{10}BDEs	330	42.6	1723	446	408	91.6

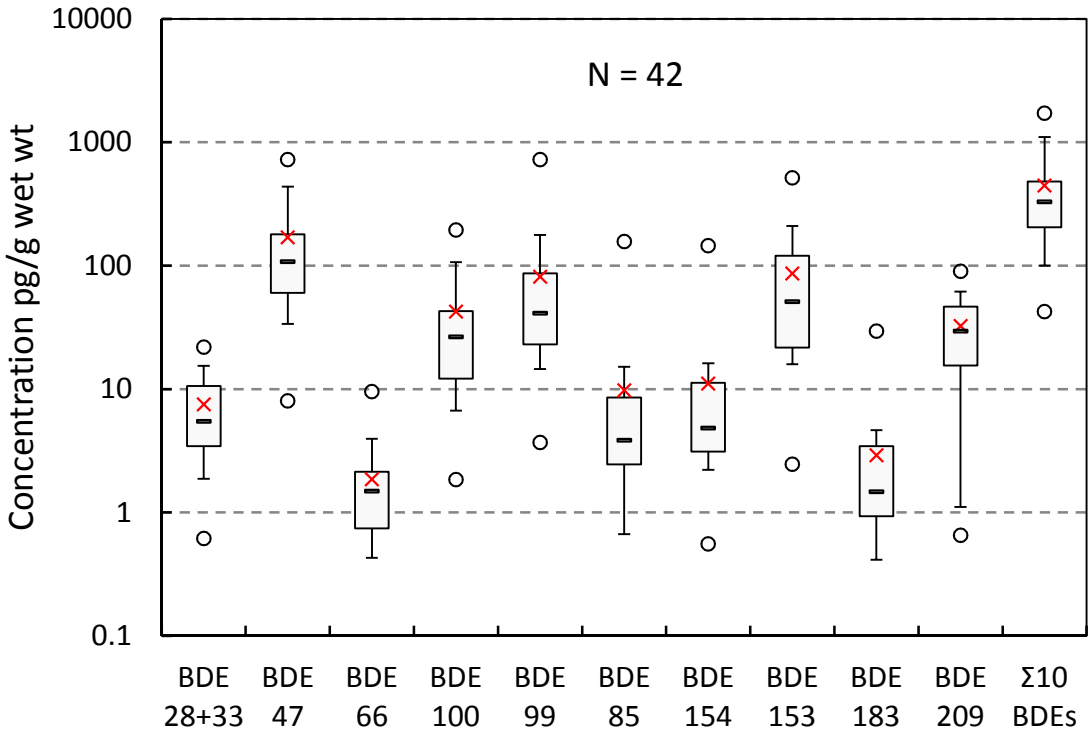


Figure 6. Box plot for the 42 placentas collected at time t = 0. The line in the box represents the median; the cross represents average; the box covers the range from the first to the third quartile; the bars outside the box represent the 10th and 90th percentile values; the circles outside the box represent the maximum and the minimum values.

5.1.2 Distribution of Polybrominated Diphenyl Ether Levels

The box plot in Figure 6 shows that the average concentration of each congener (represented by the cross) is higher than the median (represented by the solid line in the box). The same conclusion applies to Σ_{10}BDEs. These results indicate that the concentration distribution is skewed. The histograms (Figure 7) give a clearer picture on the frequency distribution of the Σ_{10}BDEs concentrations. When the concentration is transformed to logarithm scale, it follows a normal distribution.

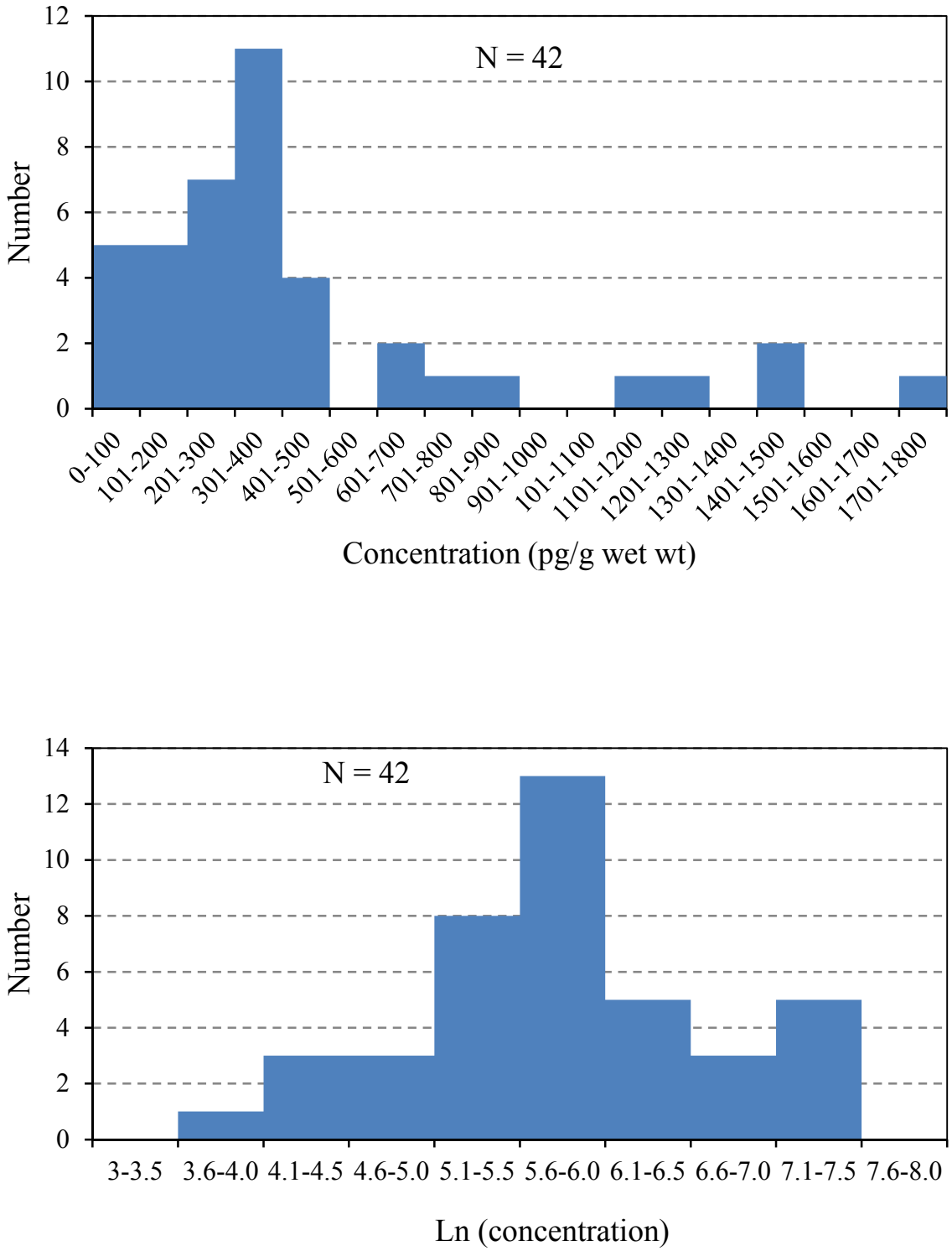


Figure 7. Frequency distribution of the Σ_{10}BDEs concentration of placentas collected at time t = 0. (Top: Original unit. Bottom: Natural log unit)

5.1.3 Comparison of Polybrominated Diphenyl Ether Levels among Studies

Compared to European countries and Japan, the PBDE levels in human placentas in the United States are alarmingly high (Table XV). The only published studies from the United States for PBDEs in human placentas focused on analytical method development and involved only five placenta specimens collected in Chicago during 2007 to 2008 (Dassanayake et al., 2009). The small number of samples makes a direct comparison with this work highly inclusive. Nonetheless, the range of the total concentration of the same ten congeners reported in Dassanayake et al. (2009), 367–2317 pg/g wet wt, overlapped with the ranges observed from this study (Table XIII and Table XIV). Compared to Dassanayake et al. (2009), the median of the total tri- to heptaBDE concentrations in this study is significantly lower, while the concentration of BDE 209 measured in this study is 56% higher than that measured by Dassanayake et al. (2009). These differences may reflect the phaseout of commercial pentaBDEs, which began in 2004, and the continued use of commercial decaBDEs. Due to the limited number of sample involved in Dassanayake et al. (2009), caution is needed in generalizing these comparisons and accepting the interpretations above. There could also be some location effects, as well as inter-individual variability.

TABLE XV

COMPARISON OF PBDE LEVELS IN HUMAN PLACENTAS AMONG LITERATURE

Country	Year	Sample Size	Number of Congeners	Median ∑BDEs (Tri- to HeptaBDEs) (pg/g wet wt)	Median BDE 209 (pg/g wet wt)	References
USA	2007–2008	5	42	1205 (369–2353)	18.8 (<MDL–29)	(Dassanayake et al., 2009)
Denmark	2007	50	12	14.8 (4.8–192)[a]	14 (3.3–68)	(Frederiksen et al., 2009a)
Spain	2003–2004	30	15	4.6 (1.0–16)[a]	7.0 (0.4–59)[a]	(Gomara et al., 2007)
Denmark	1997–2001	129	14	14.3 (6.7–36)[a,b,c]	NA	(Main et al., 2007)
Finland	1997–2001	56	14	14.3 (4.2–120)[a,b,c]	NA	(Main et al., 2007)
Japan	NA	10	25	9.0 (6.4–18)[a,b]	11.5 (9–20)[a]	(Takasuga et al., 2006)
USA	2011	42	10	291 (19–1647)	29.4 (0.7–90)	This study

[a] Figures were calculated from the concentration per lipid weight reported in the literature.
[b] The concentrations per lipid weight were the best estimations from the literature.
[c] The range was from the 2.5th percentile to the 97.5th percentile.

5.2 Polybrominated Dipheny Ether Congener Distribution Pattern

5.2.1 Polybrominated Diphenyl Ether Congener Distribution

The results presented in Sections 5.1.1 demonstrate that BDE congeners have various abundances. This section gives more detailed information on congener distribution. The average percentage of Σ_{10}BDEs mass contributed by each congener measured in placentas at collection time t = 0 is displayed in Figure 8. The most abundant congeners are BDEs 47, 153, 99, 100, and 209. The variation between placentas in the percentage contribution of these most abundant congeners is shown in Figure 9.

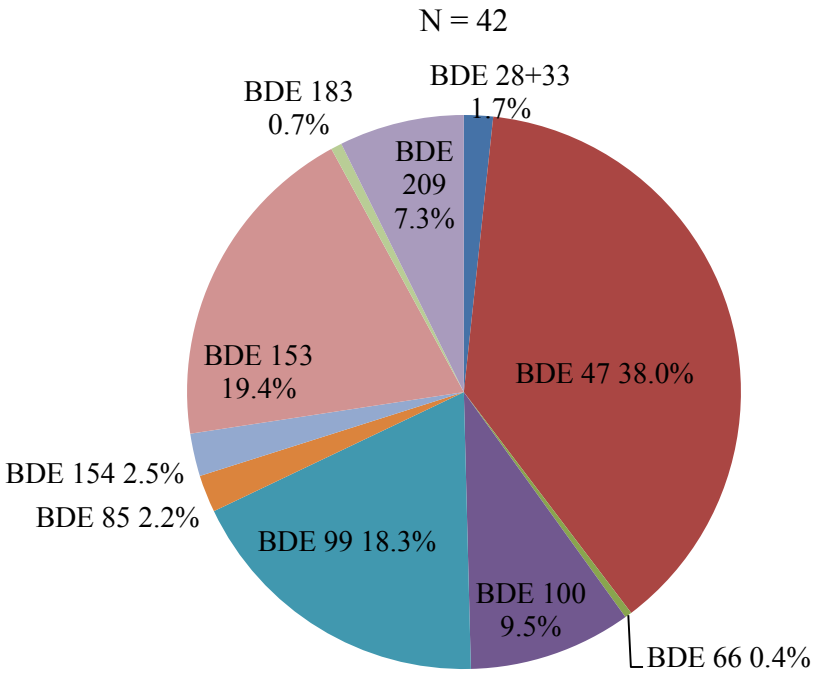


Figure 8. Average percentage of BDE congeners collected at time t = 0.

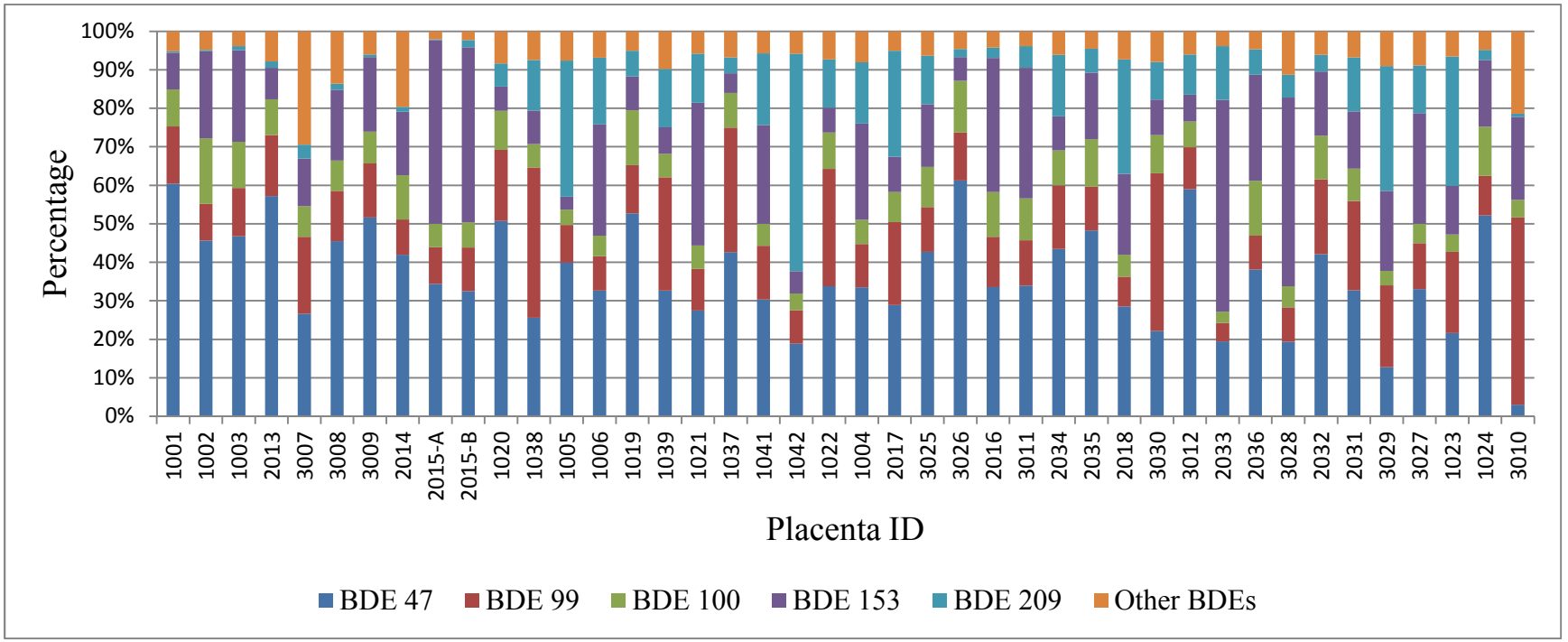


Figure 9. Distribution of major BDE congeners in placenta samples collected at time t = 0.

The relative abundance of congeners varies around the world (Table XVI). In Europe and Japan, BDE 209 is the most abundant congener, accounting for more than 50% of the total concentrations in human placentas. In the United States, however, BDE 47 is the most prevalent congener. The concentration of BDE 47 in placentas is about one order of magnitude higher in the United States than in Europe, which is consistent with the relatively high concentrations of BDE 47 in dust in the United States (Frederiksen et al., 2009b). The differences in congener distribution between the continents are not surprising, given the fact that the Americans consumed 98% of pentaBDEs on the global market, while only 44% of decaBDE (Hites 2004).

TABLE XVI

COMPARISON OF CONGENER DISTRIBUTION IN HUMAN PLACENTAS AMONG LITERATURE

Country	Year	Predominant Congeners	References
USA	2007–08	47>99>100>153>209	(Dassanayake et al., 2009)
Denmark	2007	209>47≈153>99>100	(Frederiksen et al., 2009a)
Spain	2003–04	209>47>99>100>153	(Gomara et al., 2007)
Japan	NA	209>47>153>99>100	(Takasuga et al., 2006)
USA	2011	47>153≈99>100>209	This study

5.2.2 Correlations of Polybrominated Diphenyl Ether Congeners

The Pearson correlation test on concentrations is used to examine the congener correlations. The results in Table XVII show that lower BDE congeners, namely BDEs 28+33, 47, 66, 99, 100, and 153 are significantly positively correlated at $p = 0.05$ level. The positive coefficient is in alignment with the grouping of congeners from commercial pentaBDEs.

TABLE XVII

PEARSON'S CORRELATION OF BDE CONGENERS

	BDE 28+33	BDE 47	BDE 66	BDE 100	BDE 99	BDE 85	BDE 154	BDE 153	BDE 183	BDE 209
BDE 28+33	1									
BDE 47	0.83**	1								
BDE 66	0.71**	0.84**	1							
BDE 100	0.75**	0.95**	0.85**	1						
BDE 99	0.28*	0.33*	0.46**	0.51**	1					
BDE 85	0.10	0.14	0.26*	0.33*	0.95**	1				
BDE 154	0.11	0.10	0.19	0.28*	0.90**	0.95**	1			
BDE 153	0.44**	0.48**	0.52**	0.66**	0.55**	0.49**	0.50**	1		
BDE 183	0.00	-0.01	0.17	0.07	0.22	0.29*	0.28*	0.12	1	
BDE 209	0.52**	0.30*	0.38**	0.28*	0.11	-0.01	-0.04	0.12	-0.18	1

* indicates the correlation coefficient is statistically significant at p = 0.05 level. Upper critical value for Pearson correlation coefficient is 0.257 for N = 42 at p = 0.05 level (one-tailed test).

** indicates the correlation coefficient is statistically significant at p = 0.01 level. Upper critical value for Pearson correlation coefficient is 0.358 for N = 42 at p = 0.01 level (one-tailed test).

5.3 Collection Time Effect

One of the major purposes of this project is to evaluate whether the time of sample collection after delivery influences PBDE levels in human placentas. In order to achieve this goal, a collection matrix was designed by NCS (Table IV). Each placenta had samples collected at multiple times, up to 96 hours after the first tissue sampling (time = 0).

5.3.1 Qualitative Analysis

Since every placenta was measured at different time intervals and every placenta has different Σ_{10}BDEs at collection time t = 0 hours, the concentration change for each placenta at the time interval is calculated. The summary of those differences are presented in Table XVIII for both individual collection sites and all sites pooled. The concentration change equals to the Σ_{10}BDEs of a placenta at a later collection time t_x minus the Σ_{10}BDEs from the same placenta collected at t = 0, divided by the initial Σ_{10}BDEs. Figure 10 displays the concentration change in a graphic form.

Figure 10 shows that, with all sites pooled, the percent change in Σ_{10}BDEs is in the range of -9.0% to 15.8% up to 72 hours after the initial sample collection. Storing a placenta for 96 hours has led to more significant changes in PBDE levels. For each individual collection site, the trend of concentration change is not the same. Further statistical analysis is presented in Section 5.3.2.

Variation of PBDE concentration with placenta storage time may be caused by contamination from air and containers, loss of blood, and other factors. It may also be related to the tissue heterogeneity of the placenta.

TABLE XVIII

MEDIAN CHANGE IN Σ_{10}BDEs PAIRED FOR EACH PLACENTA AT DIFFERENT COLLECTION TIMES [a] (Unit: %)

Time (hr)	1	2	4	8	12	24	36	48	72	96
UR	8.7	19.1	N/A	16.3	N/A	2.6	35.7	16.0	-50.2	-55.1
UCD	N/A	4.6	-4.1	N/A	20.3	20.4	N/A	-21.3	N/A	137
MCW	N/A	N/A	-29.5	12.3	14.6	N/A	13.9	N/A	37.2	N/A
All Sites	8.7	10.2	-4.1	12.3	14.6	11.5	15.8	11.2	-9.0	-34.2

[a] Concentration change (%) = Σ_{10}BDEs at $(t_x - t_0) / t_0 \times 100\%$

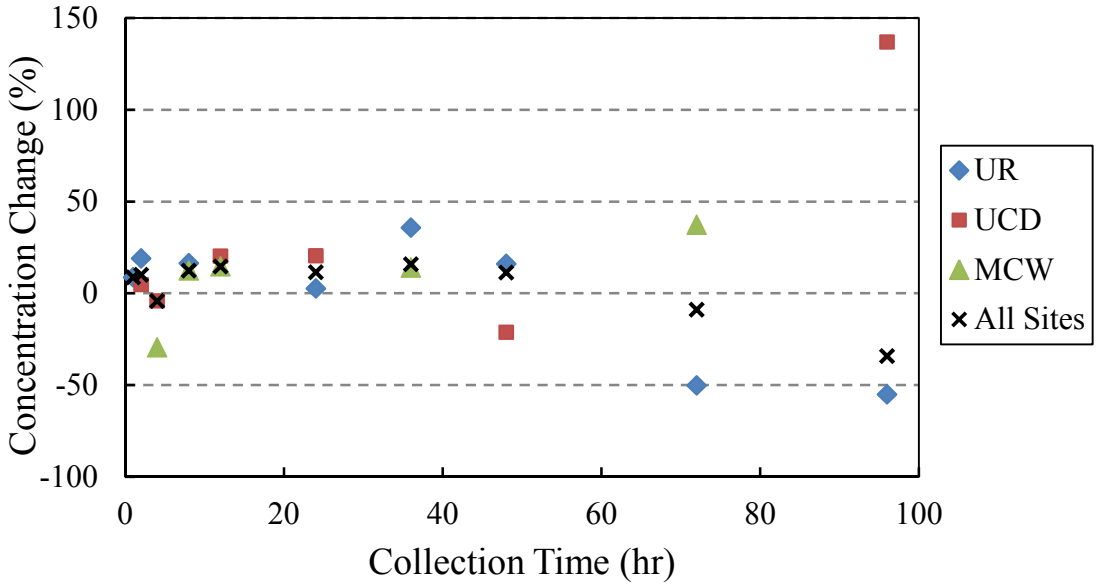


Figure 10. Median concentration change for Σ_{10}BDEs at different collection times.

5.3.2 Quantitative Analysis

Qualitative evaluation (Table XVIII and Figure 10) indicates that storing a placenta up to 72 hours would not greatly alter the PBDE levels, while storing it longer could result in a larger reduction from initial PBDE levels. To evaluate this finding statistically, a model with collection time as a dependent variable is proposed. Since repeated samplings from the same placenta were performed at different time points, but not all placentas were sampled at every time point, an ANOVA test would fail to reveal reliable associations between time and the change in PBDE concentrations. Therefore, a random-effect model is used.

The logarithm of Σ_{10}BDEs is plotted against the time of sample collection for each placenta in Figure 11. A linear relationship is observed in many panels. However, there is indication that the slope and intercept may differ between placentas. These give inspiration to use a random-effect linear model that includes these factors.

The proposed model is:

$$\ln(C_{i,t}) = b_0 + b_1 t + b_i + \varepsilon_{i,t}, \quad (1)$$

where $C_{i,t}$ is the Σ_{10}BDEs concentration of the i-th placenta collected at time = t; b_0 and b_1 are regression coefficients for the fixed part of the linear model; b_i is a random intercept that accounts for the shift from each placenta; $\varepsilon_{i,t}$ is the random error of each observation.

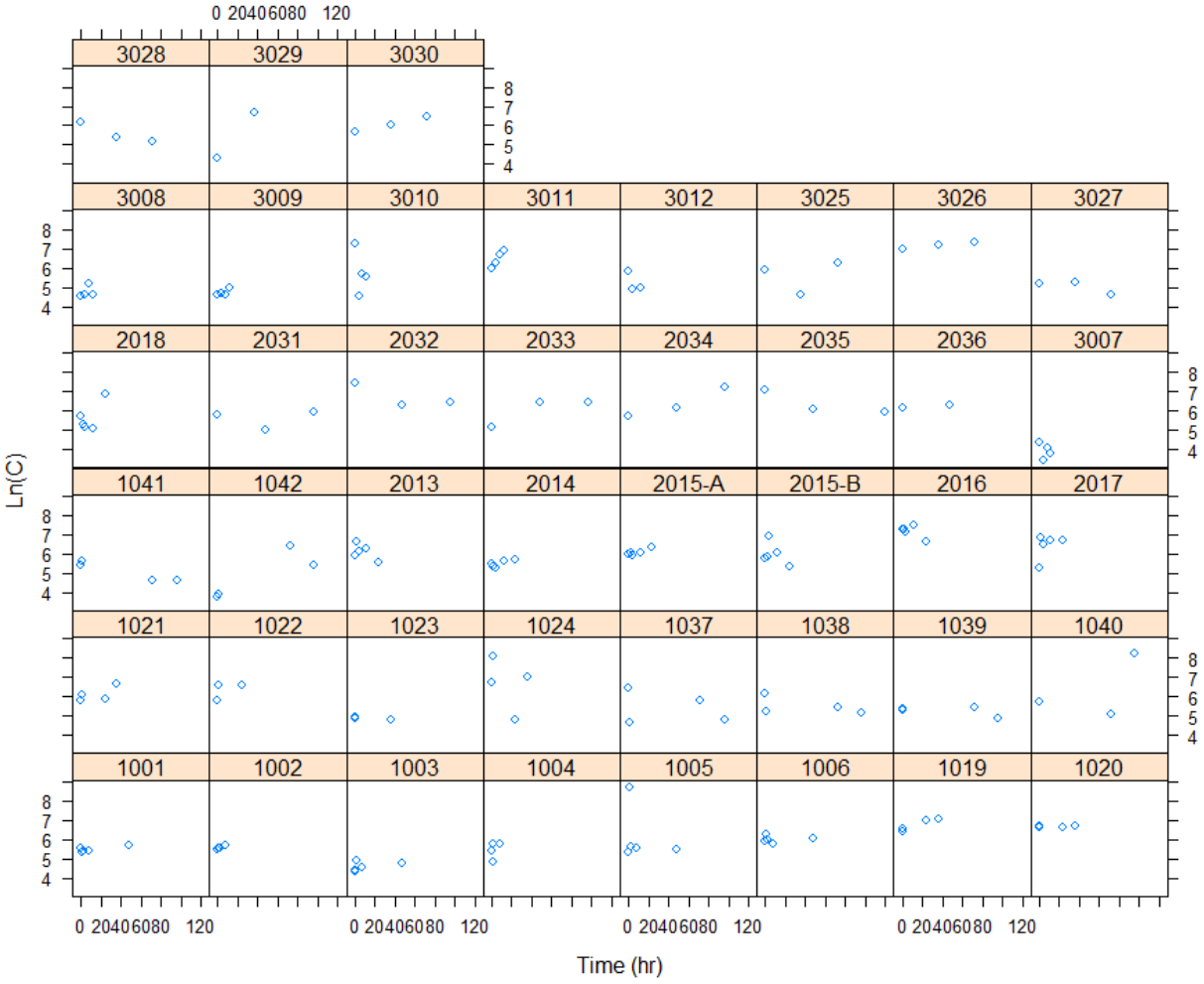


Figure 11. Ln(Σ_{10}BDEs) as a function of collection time for each placenta.

The benefit of adding the random effect is that all the observations now share the same coefficients for the fixed variables. In addition, the uniqueness of each placenta is captured by the random effect b_i which follows a Gaussian distribution. There are many other properties from each placenta that can result in different PBDE levels, such as placenta donors' physical conditions and the manner in which a placenta is stored. These properties differ by placenta, but not by collection time of the same placenta, thus carrying a subscript of i only, but not t. Therefore, this is a succinct model that describes the universal properties as well as the distinctiveness of each placenta.

Table XIX gives statistics of the random effect and the regression coefficients of Model 1.

TABLE XIX

REGRESSION RESULTS OF MODEL 1

Random Effects

	Intercept	Residual
SD	0.627	0.655

Fixed Effects

	Value	Std Error	DF	t-value	p-value
b_0	5.75	0.118	121	48.9	0.0000
b_1	0.00180	0.00200	121	0.897	0.3714

Table XIX shows that the regression coefficient for time, b_1, in Model 1 is not statistically significant. This indicates that the natural log concentration may not have a linear relationship with collection time for each placenta. To verify the model, the fitted Σ_{10}BDEs is

plotted against collection time for each placenta in Figure 12. Note that for most placentas, the fitted concentration matches well with the observed values.

The validity of Model 1 is further tested by the distribution of residuals. Figure 13 plots respectively the standardized residuals against the fitted values and the sample quantiles versus the theoretical quantiles. In Figure 13A, no specific patterns or trends are observed between the residuals and the fitted values. In other words, the residuals do not depend on the fitted values. In Figure 13B, the observed residual $\varepsilon_{i,t}$ (circle) is compared to a standard Gaussian distribution (solid line). The result shows that the residuals generally follow a Gaussian distribution. These two figures justify the assumptions of a linear regression model.

In addition, comparison has been made between the random effect model (Model 1) and the fixed effect model (Model 1 without the b_i term). The Akaike information criterion (AIC) results are in Table XX. It shows that the random effect model has a smaller AIC. Therefore, Model 1 is believed to be a more advantageous model.

TABLE XX

COMPARISON BETWEEN THE RANDOM EFFECT MODEL AND THE FIXED EFFECT MODEL

	AIC	ANOVA
Random effect model	412.1771	
Fixed effect model	454.0799	< 0.0001

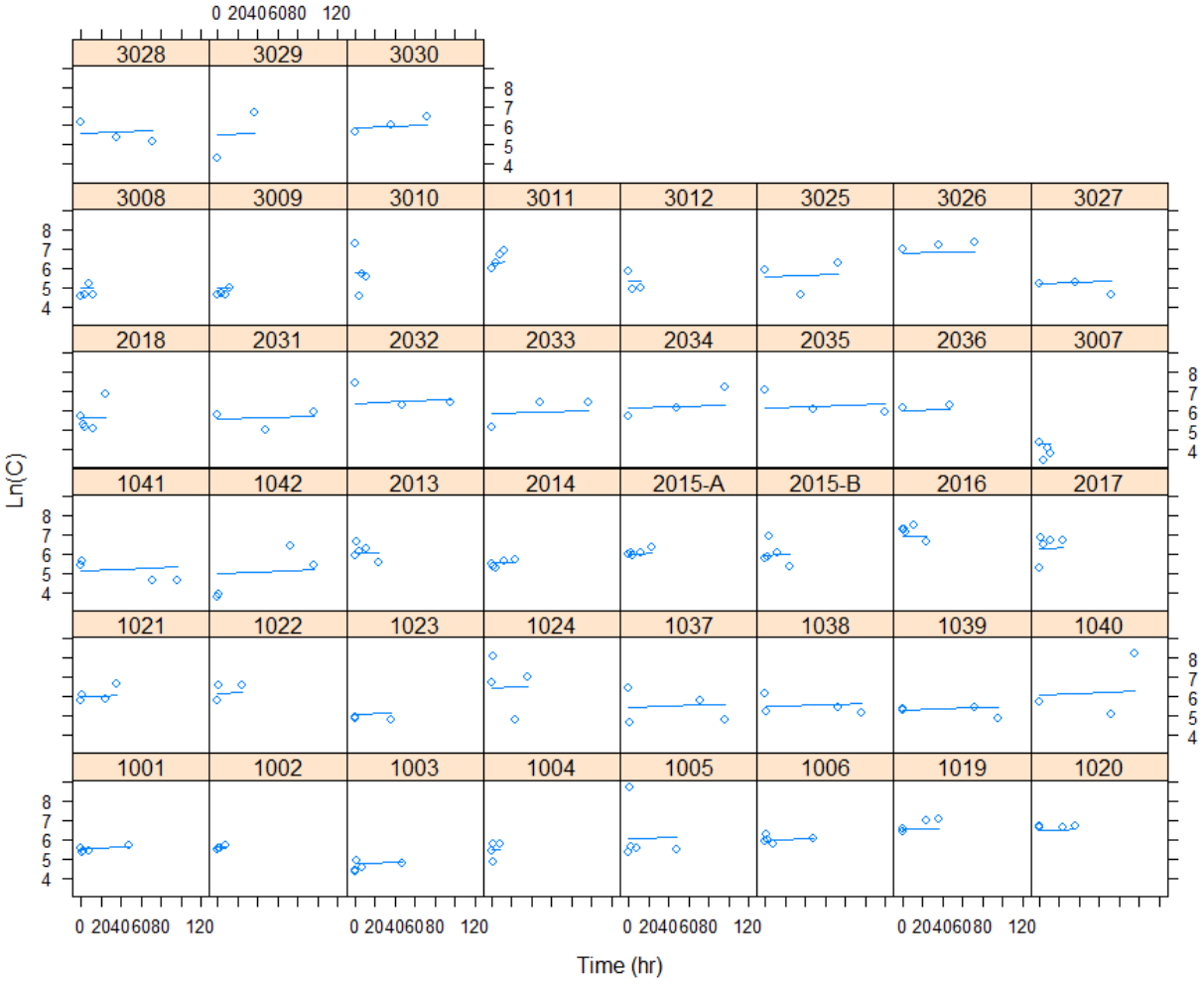
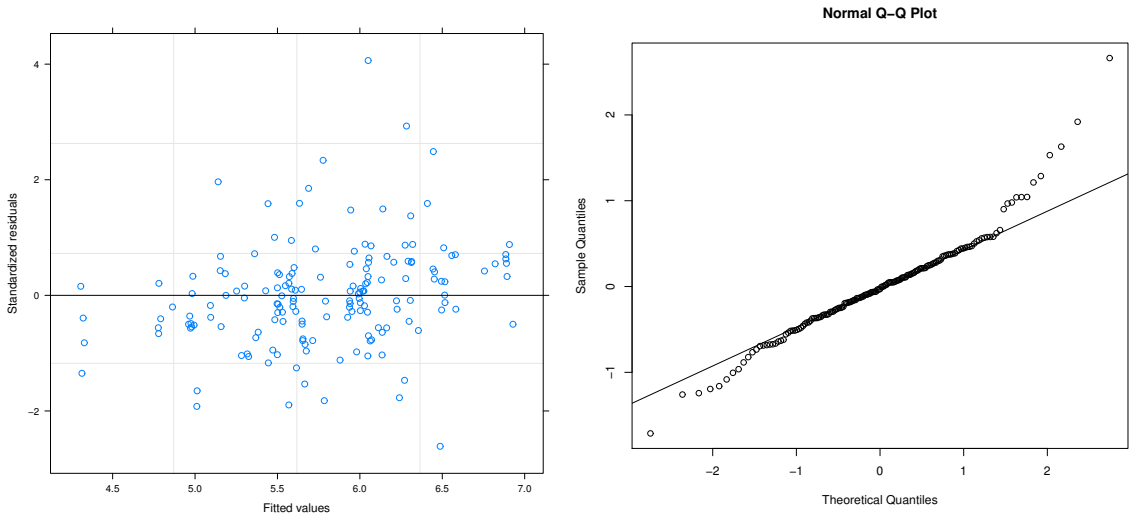


Figure 12. Fitted Ln(Σ_{10}BDEs) as a function of collection time for each placenta.



A B

Figure 13. The standardized residuals against fitted values and the normal Q-Q plot for Model 1.

5.4 Collection Site Effect

A total of 165 samples were collected at three sites: UR, UCD, and MCW. Only samples collected at time t = 0 are used to compare among collection sites. In addition, congener distribution pattern is also compared among the three sites in this section.

5.4.1 Qualitative Analysis

Table XXI displays the medians of individual and Σ10BDEs among three different collection sites. For all the ten BDE congeners and Σ_{10}BDEs, the median concentration values are the highest at UCD. Concentrations of BDE 47 and BDE 153 are particularly elevated at UCD relative to the other study sites. Only the median concentration of BDE 209 at UCD is similar to that detected from UR. The median concentrations from MCW, on the other hand, are lower than the pooled median, except BDE 154, which is slightly higher.

TABLE XXI

THE MEDIAN CONCENTRATION FOR BDE CONGENERS AT THREE SITES AT TIME T = 0 (Unit: pg/g wet wt)

	Median BDE Concentration (pg/g wet wt)			
	UR	UCD	MCW	All Sites
Sample #	17	13	12	42
BDE 28+33	4.9	5.6	4.2	5.5
BDE 47	108	132	61.8	108
BDE 66	1.3	1.9	1.5	1.5
BDE 100	20.5	28.7	25.1	26.5
BDE 99	36.9	43.5	40.2	41.2
BDE 85	3.1	5.6	3.5	3.8
BDE 154	3.6	5.0	5.1	4.8
BDE 153	40.3	94.4	39.3	51.2
BDE 183	1.3	2.5	1.3	1.5
BDE 209	41.3	40.4	23.2	29.4
Σ_{10}BDEs	266	343	317	330

Interestingly, the distribution of the Σ_{10}BDEs concentrations at collection time t = 0 at UCD is narrower than that at the other two locations (Figure 14). The distribution of the Σ_{10}BDEs concentrations at MCW has a particularly wide range between the 25th and the 75th percentiles. This observation may indicate individual variability in exposure.

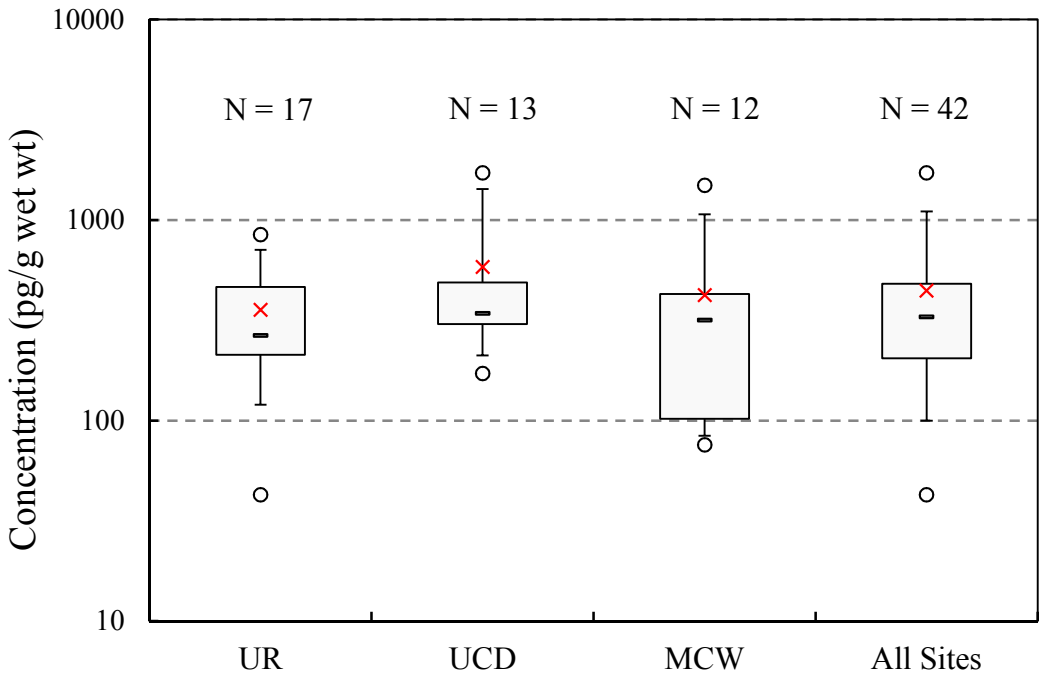


Figure 14. Distribution of the Σ_{10}BDEs concentrations at three sites at time t = 0. The line in the box represents the median; the cross represents average; the box covers the range from the first to the third quartile; the bars outside the box represent the 10th and 90th percentile values; the circles outside the box represent the maximum and the minimum values.

5.4.2 Statistical Analysis

The observations in Section 5.4.1 indicate there might be collection site effects. However, whether the differences were significant or not needs more statistical verification.

An unpaired t-test is performed using the natural log of the Σ_{10}BDEs concentration among the three collections sites. The t-statistics are given in Table XXII along with the one-tail p values (in parenthesis). Results show that samples from UCD have statistically significantly higher Σ_{10}BDEs than UR and MCW at p = 0.1 level. This agrees with the observations made in Section 5.4.1.

TABLE XXII

T-TEST STATISTIC (P VALUE) OF LN(Σ_{10}BDEs) AMONG THREE SITES

	UR	UCD	MCW
UR	0		
UCD	-1.59 (0.0621)*	0	
MCW	-0.0975 (0.467)	-1.36 (0.0942)*	0

5.4.3 Congener Distribution Pattern Among Collection Sites

Table XXIII shows that all sites have similar percentage of BDE 47, the most abundant congener in Σ_{10}BDEs. A higher percentage of BDE 209 is found in UR, while BDE 153 from UCD is higher than the other two sites, and MCW has the highest percentage of BDE 99. However, the sum of BDE 99, 153, and 209 is almost the same among three sites.

Lastly, Figure 15 presents the Σ_{10}BDEs concentration as well as congener distribution of individual placenta.

TABLE XXIII

AVERAGE PBDE CONGENER DISTRIBUTION COMPARISON BY SITES AT COLLECTION TIME T = 0 (Unit: %)

	UR	UCD	MCW
BDE 28+33	2.2 ± 1.5	1.7 ± 0.9	2.0 ± 1.4
BDE 47	38.1 ± 12.0	37.0 ± 9.7	34.5 ± 18.2
BDE 66	0.4 ± 0.2	0.4 ± 0.2	0.7 ± 0.8
BDE 100	8.4 ± 3.8	9.0 ± 3.2	8.2 ± 2.9
BDE 99	17.3 ± 9.6	13.3 ± 5.7	21.5 ± 13.8
BDE 85	1.4 ± 0.8	1.4 ± 0.9	2.7 ± 2.8
BDE 154	2.0 ± 1.2	1.3 ± 0.6	3.5 ± 2.8
BDE 153	14.9 ± 10.2	24.8 ± 15.9	16.9 ± 8.7
BDE 183	0.6 ± 0.5	1.3 ± 3.2	1.5 ± 2.7
BDE 209	14.8 ± 14.9	9.7 ± 9.9	8.5 ± 8.8

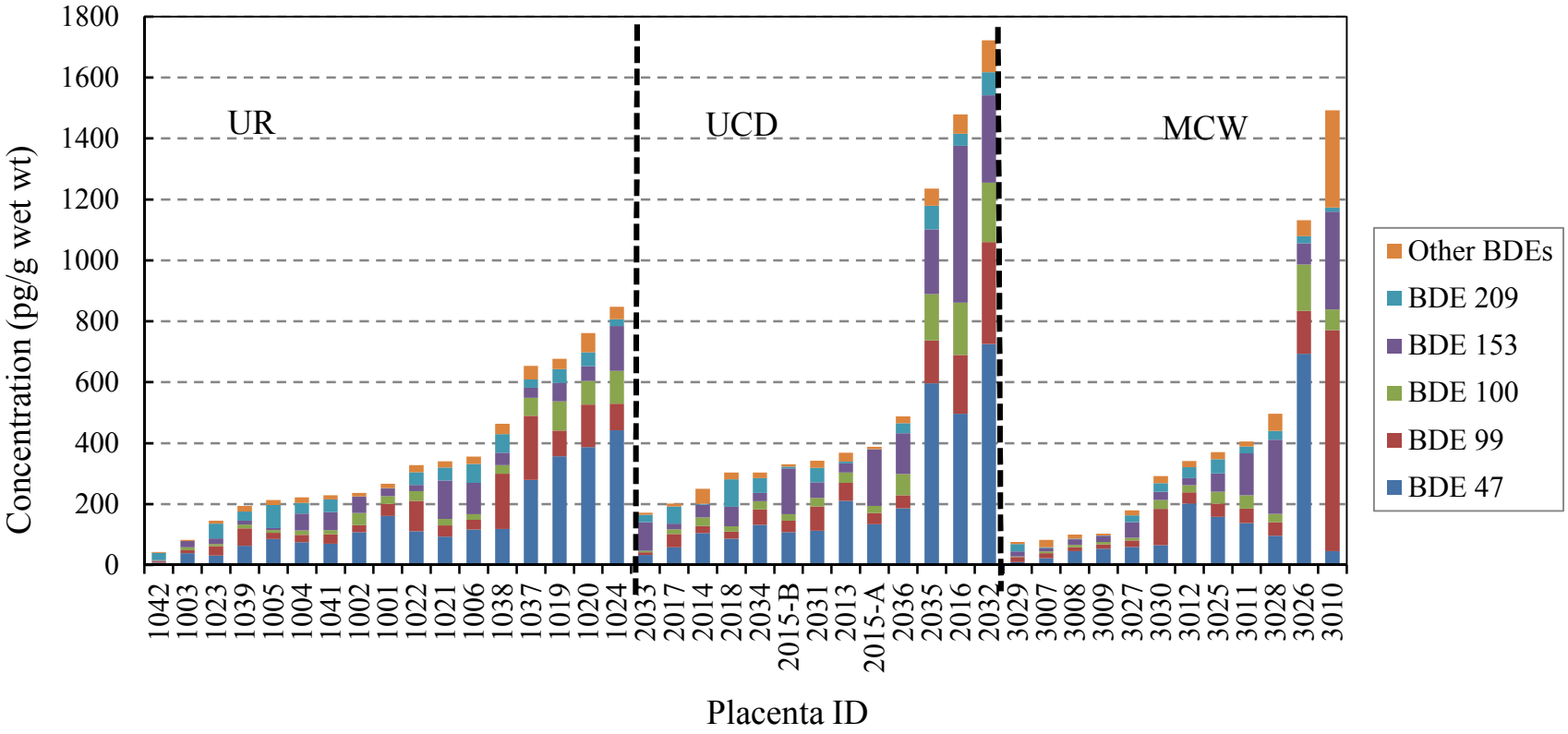


Figure 15. Distributions of major BDE congeners in individual placentas at three sites collected at time t = 0.

5.5 Combined Effects of Collection Time and Collection Site

In Sections 5.3 and 5.4, comparisons are made independently among various collection times as well as three collection sites. In Model 1, collection site differences are not taken into consideration. In this section, the combined effects of collection time and collection site are examined.

Similar to Model 1, a random effect model is proposed here as Model 2:

$$\ln(C_{i,t}) = b_0 + b_1 t + b_2 \mathrm{UR}_i + b_3 \mathrm{MCW}_i + b_4 \mathrm{UR}_i t + b_5 \mathrm{MCW}_i t + b_i + \varepsilon_{i,t}, \quad (2)$$

where $C_{i,t}$ is the Σ_{10}BDEs concentration of the i-th placenta collected at Time = t; UR_i and MCW_i are dummy variables representing collection sites (UCD is not in the model because it is chosen as a reference site); $\mathrm{UR}_i t$ and $\mathrm{MCW}_i t$ represent the coupling effect of collection time and collection site; b_i $(i = 0 \ldots 5)$ are regression coefficients for the fixed part of the linear model; b_i is a random intercept that accounts for the shift from each placenta; $\varepsilon_{i,t}$ is the random error of each observation.

Table XXIV gives statistics of the random effect and the regression coefficients of Model 2. Table XXIV shows the coefficients of b_4 and b_5 are not statistically significant, indicating no interaction between collection time and location. The negative value of b_2 and b_3 in Model 2 suggests that samples from UR and MCW have lower Σ_{10}BDEs concentrations than those from UCD. However, only the regression coefficient for MCW in Model 2, b_3, is statistically significant at the p = 0.05 level. This result is consistent with the observation in Section 5.4.1.: Σ_{10}BDEs concentrations are lower at MCW than the other locations.

TABLE XXIV

REGRESSION RESULTS OF MODEL 2

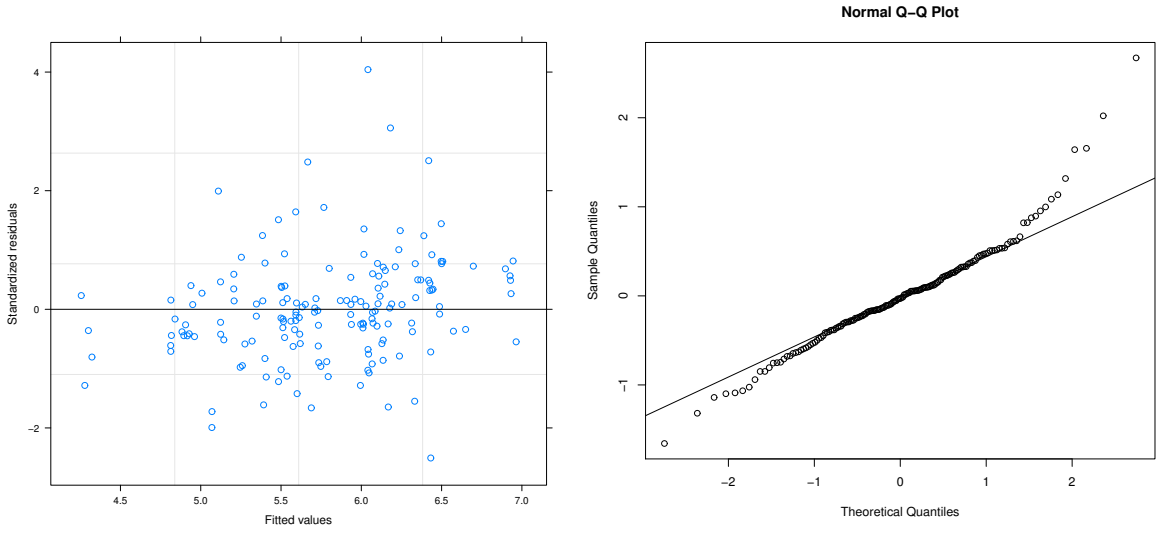
Random Effects

	Intercept	Residual
SD	0.578	0.661

Fixed Effects

	Value	Std Error	DF	t-value	p-value	
b_0	6.126	0.205	119	29.8	0.0000	
b_1	0.00158	0.00357	119	0.444	0.6580	
b_2	-0.419	0.266	40	-1.58	0.1227	
b_3	-0.750	0.301	40	-2.49	0.0171	*
b_4	-0.00102	0.00451	119	-0.227	0.8207	
b_5	-0.00397	0.00627	119	0.633	0.5277	

The validity of Model 2 is tested by the distribution of residuals. Figure 16 displays the standardized residuals plot and the Q-Q plot. These two figures show the normality of the residual distributions.



A B

Figure 16. The standardized residuals against fitted values and the normal Q-Q plot for Model 2.

Similarly, comparison is made between the random effect model (Model 2) and the fixed effect model (Model 2 without the b_i term). The AIC results are in Table XXV. Again, the random effect model is favored with a smaller AIC.

TABLE XXV

COMPARISON BETWEEN THE RANDOM EFFECT MODEL AND THE FIXED EFFECT MODEL

	AIC	ANOVA
Random effect model	432.8388	
Fixed effect model	464.4964	< 0.0001

In summary, Model 2 investigates the combined effect of collection time and collection site. While no combined effect is found, it is observed that samples collected from MCW have statistically significantly lower Σ_{10}BDEs levels than UCD.

6. CONCLUSIONS AND SIGNIFICANCE

6.1 Conclusions

In this project, a total of 43 placentas were collected by three study sites: UR, UCD, and MCW in 2011. Following a sample collection matrix, a total of 169 placenta samples were collected at various time intervals up to 96 hours after the delivery. The samples were sent to the University of Illinois at Chicago for the analysis of selected organic pollutant chemicals including ten PBDE congeners (BDEs 28 + 33, 47, 66, 85, 99, 100, 153, 154, 183, and 209).

Analytical method was developed to analyze a large number of placenta specimen samples. The MSPD method was used to extract PBDEs from the placenta tissue. Multi-layer silica gel was chosen to clean up the extract. Rotary evaporation and nitrogen blow were used to reduce the extract volume. Finally, GC/MS and GC/QQQMS were used to detect the selected congeners and determine their concentrations.

Quality assurance and quality control protocols were implemented to ensure data quality. Surrogates were applied to test the recovery of the analytical procedural. The average recovery of FBDE 69 is 106 ± 19.4% and the average recovery of FBDE 208 is 97.3 ± 25.5 %. Blanks were analyzed along with placenta samples to monitor background contamination.

All placenta samples were analyzed following the validated method. The entire analytical process was strictly recorded in the Chain of Custody. The median of the Σ_{10}BDEs level is 330 pg/g wet wt among all the placentas with the range from 42.6 to 1723 pg/g wet wt. The median of the total concentration of tri- to heptaBDEs is lower while the concentration of BDE 209 is higher than those reported by Dassanayake et al. (2009) in five placentas collected in 2007–2008 in Chicago, Illinois. The range of Σ_{10}BDEs overlaps with Dassanayake et al. (2009). The PBDE

concentrations reported in this thesis are significantly higher than the PBDE levels from European countries and Japan.

The PBDE levels approximately follow a natural log-normal distribution. Among the ten congeners tested, BDE 47 has the highest abundance, followed by BDEs 153, 99, 100, and 209. The congener distribution pattern is similar in samples collected at all three study sites.

Among three collection sites, the placentas collected from UCD have the highest PBDE levels. The Σ_{10}BDEs concentration from UCD is statistically significantly higher than UR and MCW at $p = 0.1$ level.

With regard to collection time effect, the percent change in Σ_{10}BDEs is in the range of -9.0% to 15.8% up to 72 hours after the initial sample collection. Storing a placenta for 96 hours has led to more significant changes in PBDE levels. In order to further explore the collection time effect, mixed effects models were developed in this work. Placenta is chosen to be a random effect to count for its uniqueness. The models show no linear relationship between the Σ_{10}BDEs concentration and the collection time. In addition, no interaction between collection time and location is found.

6.2 Significance and Future Work

This project is the first study that reports PBDE levels in human placenta in the United States based on a reasonably large number of samples. This data set is helpful in further studies on prenatal exposure to PBDEs and its impacts on children. This study is also the first one that compares PBDE levels among different regions in the United States. The modeling effort enhances understanding on the influence of collection time on detected PBDE levels.

The goal of producing a large amount of quality data on PBDE levels in human placenta has been achieved. From here, further studies on prenatal exposure to PBDEs can be done. It is

worth exploring whether there is a certain type of exposure route that would lead to elevated PBDE levels in the placenta. In addition, as researchers follow on those children whose mother donated their placentas for the NCS, more research will be carried out to understand the association between the elevated PBDE levels in placenta and health effects in those children's development. It is desirable that this project contributes to more findings and policies to better protect the environment and human health.

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APPENDICES

APPENDIX A

CHAIN OF CUSTODY RECORD

Project: NCS Project 18 Chemical Analysis **Analyst Name:**_____
Sample: Placenta **Start Date:**_____

Sample Pretreatment: Freeze Dry Start Date:_____ End Date:_____

Sample ID	Wet Wt (g)	Dry Wt (g)	Notes	Analyst Initials

Sample Extraction: Start Date:_____ End Date:_____

Sample ID	Surr-1 (uL)	Surr-2 (uL)	Surr-3 (uL)	Surr-4 (uL)	Florisil (g)	Notes	Analyst Initials

Surr-1: PCB 52L (initial concentration 100ng/ml)
Surr-2: F-BDE 69 (initial concentration 100ng/ml)
Surr-3: F-BDE 208 (initial concentration 100ng/ml)
Surr-4:_____

Cleanup, Concentration, and Analysis (dates):

Sample ID	Silica Gel Cleanup	Final Concentration	Notes	Analyst Initials

APPENDIX A (continued)

Spiking Internal Standard and GC/MS Analysis:

Sample ID	PBDE IS-1 (uL)	PBDE IS-2 (uL)	PBDE Analysis Date	PCB IS-1 (uL)	PCB IS-2 (uL)	PCB Analysis Date	Notes	Analyst Initials

PBDE IS-1: BB 209 (initial conc. 101.4ng/mL)
PBDE IS-2: CB 205L (initial conc. 40ng/mL)

PCB IS-1: CB 47L (initial conc. 15ng/mL)
PCB IS-2: _____

APPENDIX B

PBDE CONCENTRATIONS FOR THE NINE STUDY (Unit: pg/g wet wt)

Sample ID	Blank	1001	1002	1003	1004	1005	1006	1007	1008	1009
Sample wet weight (g)		9.9	11.2	9.6	10.4	14.5	12	13.5	10.1	2.4
Placenta ID		1001	1001	1001	1001	1002	1002	1002	1002	1001
Collection Site		UR	UR	UR	UR	UR	UR	UR	UR	UR
Collection Time (hr)		0	1	2	8	0	1	2	8	48
BDE 28+33	0.00	4.41	4.71	4.84	5.59	3.69	2.89	3.64	5.58	8.82
BDE 47	4.50	161.04	119.17	126.77	124.75	107.89	113.59	118.83	104.32	137.03
BDE 66	0.00	1.26	0.00	0.00	2.05	0.66	0.75	0.69	5.68	6.55
BDE 100	3.65	25.63	19.40	22.00	21.25	40.24	44.87	43.99	43.40	27.35
BDE 99	4.33	39.45	26.60	31.10	29.01	22.58	21.68	23.50	28.42	38.66
BDE 85	0.00	4.26	3.95	4.95	4.70	3.76	4.16	4.33	0.00	0.00
BDE 154	1.56	2.73	3.47	5.32	4.37	3.39	2.95	2.90	15.99	11.13
BDE 153	3.61	25.41	19.39	21.59	20.57	53.51	59.68	61.99	58.32	36.11
BDE 183	0.00	1.29	4.15	4.68	3.67	0.00	0.00	0.00	26.05	15.94
BDE 209	2.30	0.89	0.79	0.92	0.73	0.80	0.88	0.67	1.29	4.66
Σ_{10}BDEs	19.94	266.38	201.63	222.18	216.69	236.52	251.46	260.53	289.04	286.25
FBDE 69 (%)	109	131	102	101	98	115	132	116	100	104

APPENDIX B (continued)

Sample ID	1010	1011	1012	1013	1015	1016	1017	1018	1019	1020
Sample wet weight (g)	12	14.1	12.3	11.1	8.1	3.8	5.4	6.1	8.5	6.2
Placenta ID	1003	1003	1003	1003	2013	2013	2013	2013	2013	1003
Collection Site	UR	UR	UR	UR	UCD	UCD	UCD	UCD	UCD	UR
Collection Time (hr)	0	1	2	8	0	2	4	12	24	48
BDE 28+33	1.34	0.85	1.82	1.70	5.56	17.23	7.22	8.48	4.52	1.90
BDE 47	38.42	36.01	64.37	35.95	210.69	375.72	290.45	299.14	73.52	43.58
BDE 66	0.42	0.00	0.00	1.61	2.95	13.11	3.04	4.80	3.52	1.05
BDE 100	9.86	8.84	14.94	10.44	34.18	62.75	44.85	48.00	15.81	11.60
BDE 99	10.35	9.54	15.98	10.70	58.54	99.49	75.25	78.02	23.36	12.64
BDE 85	0.00	1.36	2.67	0.00	9.48	28.08	10.07	15.91	7.72	3.99
BDE 154	1.44	1.52	2.13	4.75	6.17	21.97	5.91	10.43	7.75	3.94
BDE 153	19.54	16.76	32.93	19.86	30.13	66.16	37.74	46.02	97.84	30.74
BDE 183	0.00	0.99	0.00	5.90	4.68	54.50	0.00	8.83	25.87	3.33
BDE 209	0.86	1.19	1.71	1.44	6.22	4.71	1.62	4.93	1.74	0.67
Σ_{10}BDEs	82.23	77.06	136.56	92.34	368.61	743.71	476.15	524.54	261.64	113.44
FBDE 69 (%)	128	113	120	98	96	121	119	102	105	101

APPENDIX B (continued)

Sample ID	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030
Sample wet weight (g)	11	11.1	9.2	11	14.5	14.8	16.3	18.3	12.4	12.9
Placenta ID	3007	3007	3007	3007	3008	3008	3008	3008	3009	3009
Collection Site	MCW	MCW	MCW	MCW	MCW	MCW	MCW	MCW	MCW	MCW
Collection Time (hr)	0	4	8	12	0	4	8	12	0	4
BDE 28+33	3.26	1.06	3.96	1.58	2.46	3.79	3.02	3.74	2.13	3.55
BDE 47	21.88	18.19	26.92	16.37	45.30	44.75	51.66	52.29	53.09	51.57
BDE 66	2.43	0.26	1.73	1.42	1.50	1.44	2.25	0.57	0.56	1.62
BDE 100	6.51	2.23	3.86	3.50	7.83	9.10	14.88	9.61	8.49	8.82
BDE 99	16.44	4.63	7.66	5.51	12.99	10.12	18.29	11.19	14.37	14.90
BDE 85	4.67	0.00	0.00	3.11	3.06	2.92	8.27	1.51	1.51	3.04
BDE 154	5.85	0.60	2.72	2.62	2.96	2.90	13.54	1.09	1.07	3.06
BDE 153	10.21	2.15	5.60	5.58	18.31	22.77	41.65	24.12	19.93	21.71
BDE 183	8.00	0.42	3.76	3.61	3.55	3.28	27.25	0.46	0.96	3.96
BDE 209	2.94	1.32	1.93	0.97	1.57	0.58	1.03	0.71	0.65	1.35
Σ_{10}BDEs	82.19	30.86	58.16	44.27	99.52	101.65	181.83	105.29	102.77	113.59
FBDE 69 (%)	90	114	104	107	110	107	128	134	139	121

APPENDIX B (continued)

Sample ID	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040
Sample wet weight (g)	14.6	13.2	9.6	8	6.8	11.6	5.2	8.4	8.1	4.9
Placenta ID	3009	3009	2014	2014	2014	2014	2014	2015-A	2015-B	2015-A
Collection Site	MCW	MCW	UCD	UCD	UCD	UCD	UCD	UCD	UCD	UCD
Collection Time (hr)	8	12	0	2	4	12	24	0	0	2
BDE 28+33	3.43	4.65	6.11	5.45	3.36	7.13	9.27	5.41	3.39	3.56
BDE 47	46.94	47.05	104.91	87.90	89.82	109.80	119.86	133.56	107.56	139.86
BDE 66	1.60	3.78	2.10	2.94	0.68	3.55	5.43	0.00	0.00	0.00
BDE 100	8.32	13.94	28.78	29.61	28.37	33.94	34.74	23.53	21.63	24.23
BDE 99	13.13	15.96	22.81	20.94	19.86	25.65	30.02	36.98	37.46	44.12
BDE 85	3.13	7.89	5.13	6.69	2.33	8.77	10.70	0.00	0.00	0.00
BDE 154	2.97	6.90	6.07	6.22	2.78	8.11	10.03	2.46	4.23	4.15
BDE 153	20.98	35.55	41.15	38.60	34.40	47.87	56.30	185.04	150.50	182.15
BDE 183	3.71	10.69	29.54	9.54	1.75	38.35	18.53	0.00	0.00	0.00
BDE 209	0.92	2.38	3.28	0.49	4.84	3.23	5.88	1.06	6.18	19.88
Σ_{10}BDEs	105.13	148.77	249.88	208.38	188.19	286.40	300.76	388.04	330.95	417.96
FBDE 69 (%)	109	130	99	114	111	116	91	128	131	123

APPENDIX B (continued)

Sample ID	1041	1042	1043	1044	1045	1046	1047
Sample wet weight (g)	5.5	4.1	4.9	13.1	8.2	4.7	7.8
Placenta ID	2015-B	2015-A	2015-B	2015-A	2015-B	2015-A	2015-B
Collection Site	UCD	UCD	UCD	UCD	UCD	UCD	UCD
Collection Time (hr)	2	4	4	12	12	24	24
BDE 28+33	5.74	7.04	19.95	5.43	5.08	9.41	5.24
BDE 47	103.70	107.44	523.30	119.12	150.22	208.20	137.78
BDE 66	4.55	5.19	12.64	3.28	0.00	5.56	0.00
BDE 100	22.46	24.98	86.70	25.17	26.38	32.99	22.34
BDE 99	32.65	32.68	140.94	43.08	49.22	56.62	37.59
BDE 85	9.35	9.83	32.71	7.08	4.24	10.66	0.00
BDE 154	8.19	8.54	25.58	8.29	3.91	11.66	4.35
BDE 153	146.60	161.80	97.59	181.73	189.50	195.24	0.00
BDE 183	11.04	12.34	59.61	36.20	0.00	28.40	0.00
BDE 209	1.97	2.19	5.09	1.19	1.73	8.24	0.99
Σ_{10}BDEs	346.24	372.02	1004.11	430.56	430.28	566.98	208.29
FBDE 69 (%)	99	96	102	97	127	115	119

APPENDIX C

PBDE CONCENTRATIONS FOR THE PILOT STUDY (Unit: pg/g wet wt)

Sample ID	Blank	1048	1049	1050	1051	1053	1054	1055	1056	1057
Sample wet weight (g)		17.3	13.0	12.4	14.9	27.0	12.1	13.9	18.7	13.2
Placenta ID		1020	1020	1020	1020	3010	3010	1038	1038	1038
Collection Site		UR	UR	UR	UR	MCW	MCW	UR	UR	UR
Collection Time (hr)		36	24	1	0	12	8	0	96	72
BDE 28+33	0.87	1.73	17.69	16.13	20.13	8.15	8.26	10.79	5.35	6.82
BDE 47	27.36	391.91	379.23	341.94	386.58	74.07	77.69	118.71	52.41	68.18
BDE 66	0.59	5.20	5.38	3.23	4.03	1.11	0.83	2.16	0.00	1.52
BDE 100	3.24	71.10	68.46	72.58	77.18	14.81	12.40	28.06	10.16	10.61
BDE 99	5.05	184.97	146.15	218.55	140.27	71.11	50.41	181.29	27.81	40.15
BDE 85	1.83	10.98	9.23	13.71	8.72	4.81	2.48	10.07	2.14	1.52
BDE 154	2.95	22.54	29.23	18.55	29.53	17.04	19.83	9.35	2.67	2.27
BDE 153	4.11	41.62	46.15	53.23	48.32	34.81	43.80	40.29	28.88	31.82
BDE 183	5.24	2.31	3.08	2.42	1.34	0.37	19.01	2.16	1.60	2.27
BDE 209	78.41	56.07	71.54	87.90	45.64	32.22	71.90	61.15	43.32	62.88
Σ_{10}BDEs	129.65	788.44	776.15	828.23	761.74	258.52	306.61	464.03	174.33	228.03
FBDE 69 (%)	92	145	86	94	105	100	99	105	90	103
FBDE 208 (%)	75	84	42	41	43	41	90	91	88	89

APPENDIX C (continued)

Sample ID	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067
Sample wet weight (g)	14.4	12.3	11.5	11.5	11.0	15.4	12.3	13.3	10.5	9.9
Placenta ID	1038	1005	1005	1005	1005	1005	1006	1006	1006	1006
Collection Site	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Collection Time (hr)	1	8	2	48	1	0	48	8	2	1
BDE 28+33	6.25	15.45	15.65	4.35	8.18	11.04	17.89	12.03	8.57	12.12
BDE 47	48.61	113.82	100.87	101.74	1470.00	85.06	135.77	92.48	105.71	130.30
BDE 66	0.00	0.00	1.74	0.00	18.18	0.65	1.63	0.75	1.90	2.02
BDE 100	9.03	11.38	10.43	13.04	554.55	8.44	25.20	19.55	24.76	35.35
BDE 99	30.56	35.77	33.91	34.78	3235.45	20.78	42.28	24.81	59.05	108.08
BDE 85	0.00	0.81	1.74	0.87	191.82	0.00	3.25	1.50	3.81	7.07
BDE 154	1.39	6.50	19.13	6.96	235.45	3.25	4.88	3.76	5.71	11.11
BDE 153	29.86	8.13	9.57	8.70	280.91	7.14	138.21	109.77	132.38	156.57
BDE 183	2.08	1.63	2.61	1.74	7.27	1.30	0.81	0.00	2.86	2.02
BDE 209	54.86	62.60	73.04	62.61	80.91	75.32	64.23	55.64	54.29	74.75
Σ_{10}BDEs	182.64	256.10	268.70	234.78	6082.73	212.99	434.15	320.30	399.05	539.39
FBDE 69 (%)	94	96	100	92	109	101	97	99	110	116
FBDE 208 (%)	89	81	99	90	95	90	85	85	96	103

APPENDIX C (continued)

Sample ID	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077
Sample wet weight (g)	11.0	14.9	13.7	14.0	13.8	18.6	19.0	18.1	19.4	14.9
Placenta ID	1006	1019	1019	1019	1019	1039	1039	1039	1039	1021
Collection Site	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Collection Time (hr)	0	1	24	36	0	72	96	1	0	24
BDE 28+33	15.45	18.79	16.06	25.00	10.14	7.55	3.68	9.39	3.09	4.70
BDE 47	116.36	348.32	552.55	652.14	356.52	90.03	40.00	77.90	63.40	91.95
BDE 66	1.82	2.01	4.38	5.00	2.17	1.08	1.05	1.66	1.03	1.34
BDE 100	19.09	87.92	143.80	138.57	96.38	14.56	5.79	12.15	11.86	22.15
BDE 99	31.82	57.72	225.55	137.86	84.78	66.85	13.16	58.56	57.22	40.94
BDE 85	2.73	5.37	16.06	11.43	7.25	4.85	0.00	4.42	3.09	3.36
BDE 154	3.64	8.72	21.17	27.14	11.59	9.70	11.05	8.29	11.34	6.04
BDE 153	102.73	52.35	81.02	80.00	59.42	16.71	11.58	17.13	13.40	137.58
BDE 183	0.91	2.68	3.65	5.00	2.90	1.08	1.58	1.10	0.52	5.37
BDE 209	61.82	38.93	42.34	60.00	45.65	27.49	40.00	32.04	29.38	35.57
Σ_{10}BDEs	356.36	622.82	1106.57	1142.14	676.81	239.89	127.89	222.65	194.33	348.99
FBDE 69 (%)	102	100	115	121	110	108	128	114	100	98
FBDE 208 (%)	92	90	100	96	77	99	90	74	82	85

APPENDIX C (continued)

Sample ID	1078	1079	1080	1081	1082	1084	1085	1086	1087	1088
Sample wet weight (g)	12.3	13.1	12.2	12.4	14.1	23.2	13.6	25.4	14.2	15.8
Placenta ID	1021	1021	1021	1040	1040	1040	1037	1037	1037	1037
Collection Site	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Collection Time (hr)	1	36	0	96	1	72	96	72	0	1
BDE 28+33	8.94	11.45	4.92	15.32	4.96	4.81	4.41	11.42	8.45	3.80
BDE 47	122.76	183.97	93.44	1221.77	136.88	73.08	59.56	198.82	278.87	49.37
BDE 66	1.63	2.29	1.64	23.39	1.42	0.00	0.74	1.57	4.23	0.63
BDE 100	25.20	35.88	20.49	318.55	38.30	15.38	8.82	35.43	58.45	7.59
BDE 99	60.98	100.76	36.89	1594.35	58.16	18.27	7.35	56.30	211.27	9.49
BDE 85	3.25	5.34	2.46	129.84	7.09	2.88	0.74	4.72	15.49	1.27
BDE 154	7.32	12.21	5.74	100.00	5.67	1.92	0.74	3.54	11.97	0.63
BDE 153	126.02	158.02	126.23	190.32	34.04	14.42	13.97	0.79	33.80	12.03
BDE 183	5.69	9.92	4.92	4.84	1.42	0.96	1.47	0.79	4.23	1.27
BDE 209	76.42	236.64	43.44	53.23	24.82	28.85	22.79	11.81	26.76	21.52
Σ_{10}BDEs	438.21	756.49	340.16	3651.61	312.77	160.58	120.59	325.20	653.52	107.59
FBDE 69 (%)	108	102	100	98	70	77	110	100	99	106
FBDE 208 (%)	86	89	86	113	100	114	97	264	113	98

APPENDIX C (continued)

Sample ID	1089	1090	1091	1092	1093	1094	1095	1096	1097	1099
Sample wet weight (g)	22.9	10.4	12.8	16.0	13.4	15.4	16.2	16.7	13.8	12.6
Placenta ID	1041	1041	1041	1041	1042	1042	1042	1042	1022	1022
Collection Site	UR	UR	UR	UR	UR	UR	UR	UR	UR	UR
Collection Time (hr)	96	72	1	0	72	96	0	1	0	1
BDE 28+33	2.18	2.23	5.47	3.75	9.70	10.39	0.62	1.20	3.62	12.70
BDE 47	29.69	22.91	82.81	69.38	214.93	90.26	8.02	11.98	110.87	337.30
BDE 66	0.44	0.56	1.56	1.25	3.73	1.30	0.00	0.00	1.45	3.97
BDE 100	4.80	3.91	16.41	13.13	47.76	13.64	1.85	2.40	31.16	91.27
BDE 99	13.54	10.61	42.19	31.88	235.07	49.35	3.70	5.99	100.00	159.52
BDE 85	1.31	1.12	3.91	3.13	18.66	3.90	0.62	0.60	9.42	19.84
BDE 154	0.87	1.12	3.91	3.13	18.66	5.19	0.62	0.60	7.97	14.29
BDE 153	18.34	16.76	66.41	58.75	35.82	18.18	2.47	3.59	21.01	62.70
BDE 183	0.44	0.56	1.56	1.88	2.24	1.95	0.62	0.60	1.45	3.17
BDE 209	31.00	39.66	45.31	42.50	32.09	33.77	24.07	23.95	41.30	34.92
Σ_{10}BDEs	102.62	99.44	269.53	228.75	618.66	227.92	42.59	50.90	328.26	739.68
FBDE 69 (%)	108	105	106	114	101	95	91	89	100	99
FBDE 208 (%)	96	101	98	98	122	106	88	91	96	115

APPENDIX C (continued)

Sample ID	1100	1101	1102	1103	1104	1106	1107	1108	1109	1110
Sample wet weight (g)	16.7	10.5	12.7	12.8	12.8	9.4	13.0	9.7	10.8	10.8
Placenta ID	1022	1004	1004	1004	1004	2017	2017	2017	2017	2017
Collection Site	UR	UR	UR	UR	UR	UCD	UCD	UCD	UCD	UCD
Collection Time (hr)	24	1	8	0	2	24	4	2	12	0
BDE 28+33	11.98	1.90	8.66	7.81	4.69	14.89	10.00	9.28	16.67	1.85
BDE 47	355.69	23.81	90.55	74.22	92.19	346.81	286.15	378.35	337.04	58.33
BDE 66	3.59	0.95	1.57	0.78	2.34	5.32	3.85	5.15	4.63	0.93
BDE 100	89.82	6.67	18.90	14.06	19.53	78.72	70.00	84.54	81.48	15.74
BDE 99	132.34	28.57	59.06	25.00	92.19	186.17	134.62	297.94	183.33	43.52
BDE 85	17.37	2.86	7.09	3.13	6.25	13.83	8.46	15.46	12.04	3.70
BDE 154	13.77	1.90	7.09	3.13	11.72	13.83	10.00	19.59	14.81	2.78
BDE 153	64.07	14.29	42.52	55.47	43.75	94.68	88.46	78.35	100.00	18.52
BDE 183	2.99	0.95	2.36	3.13	1.56	3.19	3.85	3.09	3.70	0.93
BDE 209	29.94	42.86	75.59	35.16	42.19	53.19	29.23	46.39	42.59	55.56
Σ_{10}BDEs	721.56	124.76	313.39	221.88	316.41	810.64	644.62	938.14	796.30	201.85
FBDE 69 (%)	92	86	150	112	135	91	103	118	95	86
FBDE 208 (%)	138	91	222	103	90	105	100	80	104	91

APPENDIX C (continued)

Sample ID	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120
Sample wet weight (g)	11.1	9.5	17.8	20.6	10.9	11.8	10.4	10.4	13.1	8.6
Placenta ID	3025	3025	3025	3026	3026	3026	2016	2016	2016	2016
Collection Site	MCW	MCW	MCW	MCW	MCW	MCW	UCD	UCD	UCD	UCD
Collection Time (hr)	0	72	36	0	72	36	0	4	12	24
BDE 28+33	9.90	9.44	2.25	21.84	32.14	26.27	19.23	14.42	25.19	11.63
BDE 47	158.41	231.78	42.13	693.20	915.44	787.13	496.15	478.85	616.79	330.23
BDE 66	1.80	3.15	0.56	3.40	4.59	5.08	5.77	3.85	7.63	3.49
BDE 100	38.70	51.39	8.99	152.43	202.00	174.54	172.12	151.92	196.95	89.53
BDE 99	43.20	98.58	8.43	140.78	168.03	140.65	193.27	164.42	225.19	139.53
BDE 85	4.50	9.44	1.12	12.14	22.95	17.79	17.31	9.62	19.08	15.12
BDE 154	4.50	7.34	1.12	14.08	17.45	13.56	16.35	14.42	20.61	13.95
BDE 153	60.30	70.27	13.48	69.42	136.81	104.22	514.42	336.54	629.77	84.88
BDE 183	2.70	4.20	1.12	0.97	19.28	5.08	3.85	1.92	4.58	2.33
BDE 209	46.80	35.66	26.40	23.79	34.89	36.43	40.38	44.23	32.82	45.35
Σ_{10}BDEs	370.81	521.23	105.62	1132.04	1553.58	1310.76	1478.85	1220.19	1778.63	736.05
FBDE 69 (%)	90	86	89	138	94	88	95	126	96	87
FBDE 208 (%)	111	98	95	83	166	102	106	90	105	105

APPENDIX C (continued)

Sample ID	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130
Sample wet weight (g)	11.0	20.3	17.2	19.1	20.8	14.3	13.4	12.4	11.1	10.2
Placenta ID	2016	3011	3011	3011	3011	2034	2034	2034	2035	2035
Collection Site	UCD	MCW	MCW	MCW	MCW	UCD	UCD	UCD	UCD	UCD
Collection Time (hr)	2	0	12	4	8	96	0	48	0	120
BDE 28+33	20.91	3.94	10.47	6.27	8.17	23.78	5.22	4.84	15.32	5.88
BDE 47	485.45	137.44	332.56	170.85	275.96	690.91	132.09	162.10	596.40	177.45
BDE 66	5.45	1.48	4.65	1.57	2.88	9.09	1.49	2.42	5.41	0.98
BDE 100	161.82	43.84	90.70	52.77	78.85	146.85	27.61	39.52	152.25	39.22
BDE 99	128.18	47.78	280.23	87.77	209.62	229.37	50.00	116.13	140.54	44.12
BDE 85	13.64	3.94	18.60	0.00	14.42	28.67	5.97	12.10	16.22	5.88
BDE 154	13.64	5.42	18.60	8.36	15.87	20.28	4.48	9.68	14.41	3.92
BDE 153	530.91	137.44	176.16	156.74	167.79	151.75	26.87	36.29	212.61	51.96
BDE 183	3.64	0.99	2.91	2.09	2.88	5.59	1.49	1.61	4.50	0.98
BDE 209	47.27	22.66	55.23	30.83	24.04	46.15	48.51	88.71	77.48	55.88
Σ_{10}BDEs	1410.91	404.93	990.12	517.24	800.48	1352.45	303.73	473.39	1235.14	386.27
FBDE 69 (%)	93	162	140	115	116	113	94	96	104	95
FBDE 208 (%)	110	116	93	77	80	106	87	90	110	94

APPENDIX C (continued)

Sample ID	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140
Sample wet weight (g)	14.7	16.2	11.6	11.7	10.9	12.2	12.6	21.1	17.2	18.2
Placenta ID	2035	2018	2018	2018	2018	2018	3030	3030	3030	3012
Collection Site	UCD	UCD	UCD	UCD	UCD	UCD	MCW	MCW	MCW	MCW
Collection Time (hr)	48	0	4	12	24	2	36	0	72	4
BDE 28+33	9.52	13.58	1.72	2.56	10.09	0.82	7.15	1.42	10.47	4.94
BDE 47	219.73	86.42	19.83	25.64	497.25	31.15	182.03	64.58	271.76	70.21
BDE 66	2.04	1.23	0.00	0.85	1.83	0.82	1.59	1.42	4.07	0.55
BDE 100	45.58	17.28	5.17	5.98	144.04	9.02	40.54	28.96	59.36	7.68
BDE 99	41.50	23.46	10.34	17.09	98.17	36.89	56.44	119.66	177.49	9.87
BDE 85	6.80	2.47	0.86	1.71	7.34	3.28	5.56	8.07	11.06	1.10
BDE 154	4.08	2.47	0.86	1.71	14.68	2.46	3.97	10.92	11.06	1.10
BDE 153	66.67	63.58	16.38	16.24	181.65	17.21	65.18	27.07	57.03	9.87
BDE 183	1.36	2.47	0.86	0.85	2.75	0.82	2.38	1.42	2.91	0.55
BDE 209	34.69	90.12	114.66	93.16	36.70	102.46	41.34	28.49	45.97	26.88
Σ_{10}BDEs	431.97	303.09	170.69	165.81	994.50	204.92	406.20	292.02	651.18	132.75
FBDE 69 (%)	105	99	102	96	87	79	91	84	143	107
FBDE 208 (%)	104	137	102	90	82	79	96	107	91	102

APPENDIX C (continued)

Sample ID	1141	1142	1143	1144	1145	1146	1147	1149	1150	1151
Sample wet weight (g)	18.9	20.9	18.0	17.6	15.1	11.9	16.1	17.9	24.1	19.7
Placenta ID	3012	3012	2033	2033	2033	2036	2036	3028	3028	3028
Collection Site	MCW	MCW	UCD	UCD	UCD	UCD	UCD	MCW	MCW	MCW
Collection Time (hr)	0	12	0	48	96	48	0	72	36	0
BDE 28+33	11.66	3.89	3.89	5.68	3.97	10.08	6.21	2.65	2.49	7.63
BDE 47	201.48	78.15	33.33	121.59	115.23	218.49	186.34	22.57	23.67	96.09
BDE 66	1.59	0.86	0.56	1.70	3.31	1.68	1.86	0.00	0.42	1.53
BDE 100	22.80	9.07	5.00	23.30	0.00	76.47	69.57	7.52	8.31	27.45
BDE 99	37.12	14.25	8.33	40.34	0.00	43.70	42.86	4.42	5.40	44.23
BDE 85	2.12	1.30	1.11	4.55	0.00	5.88	5.59	0.44	0.83	3.05
BDE 154	4.24	0.86	0.56	3.41	13.91	5.04	4.97	13.27	14.12	40.16
BDE 153	23.86	10.79	94.44	410.80	444.37	140.34	134.16	106.64	114.61	243.01
BDE 183	1.06	0.86	0.56	2.27	2.65	5.04	4.35	2.21	18.27	3.56
BDE 209	35.52	23.32	23.89	29.55	34.44	41.18	32.30	21.68	24.08	29.49
Σ_{10}BDEs	341.46	143.35	171.67	643.18	617.88	547.90	488.20	181.42	212.19	496.19
FBDE 69 (%)	131	102	111	111	90	104	109	118	122	142
FBDE 208 (%)	80	99	86	86	122	89	96	103	109	95

APPENDIX C (continued)

Sample ID	1152	1153	1154	1155	1156	1157	1158	1160	1161	1162
Sample wet weight (g)	11.5	11.1	14.1	11.1	10.8	18.2	17.1	24.9	20.9	22.2
Placenta ID	2032	2032	2032	2031	2031	2031	3029	3029	3027	3027
Collection Site	UCD	UCD	UCD	UCD	UCD	UCD	MCW	MCW	MCW	MCW
Collection Time (hr)	0	96	48	48	0	96	36	0	72	36
BDE 28+33	20.00	5.41	7.09	1.80	5.56	3.30	7.03	0.80	3.35	4.05
BDE 47	725.22	244.14	223.40	42.34	112.04	36.26	414.42	9.66	28.25	61.60
BDE 66	9.57	2.70	2.84	0.90	1.85	0.55	2.34	0.00	0.00	0.90
BDE 100	194.78	60.36	54.61	9.01	28.70	8.24	129.54	2.82	5.75	11.24
BDE 99	334.78	114.41	96.45	29.73	79.63	21.43	97.30	16.09	19.15	21.13
BDE 85	46.09	17.12	14.89	2.70	7.41	2.20	21.10	1.21	1.44	1.80
BDE 154	26.96	9.01	7.80	1.80	6.48	1.65	8.79	4.43	1.44	12.14
BDE 153	286.96	90.09	82.27	14.41	50.93	14.84	80.30	15.69	23.46	56.21
BDE 183	2.61	0.90	0.71	0.90	1.85	0.55	2.34	0.40	0.96	1.35
BDE 209	75.65	72.97	70.92	51.35	48.15	301.10	31.07	24.54	21.07	30.13
Σ_{10}BDEs	1722.61	617.12	560.99	154.95	342.59	390.11	794.26	75.65	104.87	200.54
FBDE 69 (%)	107	92	102	94	91	96	99	1	43	156
FBDE 208 (%)	101	92	97	90	99	97	101	80	113	93

APPENDIX C (continued)

Sample ID	1163	1164	1166	1167	1168	1169	1170	1171	1172	1173
Sample wet weight (g)	27.6	13.7	12.5	13.7	11.2	13.5	14.6	17.6	13.5	13.0
Placenta ID	3027	1023	1023	1023	1024	1024	1024	1024	3010	3010
Collection Site	MCW	UR	UR	UR	UR	UR	UR	UR	MCW	MCW
Collection Time (hr)	0	36	1	0	36	1	24	0	0	4
BDE 28+33	7.97	2.92	0.80	2.92	13.39	41.48	1.37	14.20	4.44	1.54
BDE 47	59.06	24.82	12.80	31.39	522.32	1930.37	20.55	442.05	45.19	18.46
BDE 66	0.72	0.73	0.00	0.73	3.57	9.63	0.68	2.27	1.48	0.77
BDE 100	9.06	3.65	3.20	6.57	125.00	385.19	4.79	107.95	68.15	5.38
BDE 99	21.38	11.68	13.60	30.66	198.21	306.67	13.01	87.50	725.93	25.38
BDE 85	1.45	1.46	0.80	2.92	18.75	25.19	0.68	10.23	157.04	2.31
BDE 154	4.71	0.73	1.60	2.19	18.75	40.00	1.37	11.93	145.19	2.31
BDE 153	51.45	17.52	14.40	18.25	152.68	422.96	10.96	147.16	320.74	13.08
BDE 183	1.09	0.73	0.80	0.73	2.68	6.67	0.68	2.27	11.11	0.77
BDE 209	22.10	57.66	79.20	48.91	94.64	48.89	64.38	22.16	13.33	28.46
Σ_{10}BDEs	178.99	121.90	127.20	145.26	1150.00	3217.04	118.49	847.73	1492.59	98.46
FBDE 69 (%)	176	105	91	101	90	153	92	108	113	92
FBDE 208 (%)	126	102	85	93	82	96	86	101	77	82

APPENDIX D

STATISTICAL SUMMARY FOR THE 165 SAMPLES COLLECTED AT ALL LOCATIONS AND AT ALL TIMES

(Unit: pg/g wet wt)

Compounds	Average	SD	RSD (%)	Min	10%	25%	Median	75%	90%	Max
BDE 28+33	7.99	6.54	81.80	0.62	1.83	3.62	5.59	10.39	16.45	41.48
BDE 47	199.31	257.11	129.00	8.02	27.46	53.09	110.87	231.78	482.81	1930.37
BDE 66	2.49	3.06	122.81	0.00	0.00	0.74	1.60	3.40	5.27	23.39
BDE 100	48.00	69.18	144.11	0.00	5.87	9.86	24.76	58.45	127.73	554.55
BDE 99	100.80	285.96	283.70	0.00	10.45	20.94	40.94	99.49	185.69	3235.45
BDE 85	9.53	21.96	230.28	0.00	0.61	1.71	4.33	9.83	17.34	191.82
BDE 154	11.23	22.96	204.44	0.56	1.41	2.95	6.07	13.27	19.40	235.45
BDE 153	84.39	104.12	123.38	0.00	12.45	19.54	48.32	106.64	181.99	629.77
BDE 183	4.86	8.80	180.91	0.00	0.48	0.96	2.27	3.96	10.90	59.61
BDE 209	36.67	36.97	100.82	0.49	1.04	5.88	32.22	48.91	74.07	301.10
Σ_{10}BDEs	505.27	659.16	130.46	30.86	104.98	181.42	313.39	618.66	1065.59	6082.73
FBDE 69 (%)	106.05	19.39	18.29							
FBDE 208 (%)	97.29	25.45	26.16							

APPENDIX E

STATISTICAL SUMMARY FOR THE 42 PLACENTAS COLLECTED AT TIME T = 0 (Unit: pg/g wet wt)

Compounds	Average	SD	RSD (%)	Min	10%	25%	Median	75%	90%	Max
BDE 28+33	7.50	5.77	76.9	0.62	1.88	3.45	5.48	10.6	15.4	21.8
BDE 47	169	180	107	8.02	33.8	60.1	108	180	436	725
BDE 66	1.86	1.79	96.2	0.00	0.43	0.74	1.49	2.14	3.96	9.57
BDE 100	42.3	48.1	114	1.85	6.70	12.2	26.5	42.9	107	195
BDE 99	81.6	122	150	3.70	14.5	23.0	41.2	86.8	177	726
BDE 85	9.77	24.5	251	0.00	0.67	2.46	3.85	8.56	15.2	157
BDE 154	11.1	22.7	205	0.56	2.22	3.13	4.84	11.2	16.2	145
BDE 153	86.7	103	119	2.47	15.9	21.7	51.2	120	210	514
BDE 183	2.91	4.74	163	0.00	0.41	0.93	1.47	3.44	4.66	29.5
BDE 209	32.5	23.7	72.9	0.65	1.11	15.5	29.4	46.5	61.8	90.1
Σ_{10}BDEs	446	408	91.6	42.6	99.8	205	330	482	1104	1723

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