Legacy Persistent Organic Pollutants in Human Placental Tissue from the United States

BY

JESSICA ANNE NANES
B.S., Colorado State University – Pueblo, 2008

THESIS

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Defense Committee:
An Li, advisor
Mary Turyk
Rachael Jones

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LIST OF ABBREVIATIONS

AIC	Akaike information criterion
ANOVA	Analysis of variance
BCF	Bioconcentration factor in fish
BFR	Brominated flame–retardants
BPA	Bisphenol A
COC	Chain of Custody
DCM	Dichloromethane (Methylene chloride)
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene (1,1–*bis*–(4–chlorophenyl)–2,2–dichloroethene)
DDT	Dichlorodiphenyltrichloroethane (1,1–*bis*–(4–chlorophenyl)–2,2,2–trichloroethane)
DI	Deionized
ECD	Electron capture device
EI	Electron impact ionization mode
EPA	Environmental Protection Agency
GC	Gas chromatography
IARC	International Agency for the Research on Cancer
K_{ow}	Octanol–water partition coefficient
LOAEL	Lowest observable adverse effect level
LOQ	Level of quantification

LIST OF ABBREVIATIONS (continued)

MCW	Medical College of Wisconsin
MRM	Multiple reaction monitoring
MS	Mass spectrometer
MSPD	Matrix solid phase dispersion
NCS	National Children's Study
NIH	National Institute of Health
NOAEL	No observable adverse effect level
OC	Organochlorine
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutants
QA/QC	Quality assurance and quality control
QQQMS	Triple quadruple mass spectrometer
TCDD	2,3,7,8–tetrachlorodibenzo–*p*–dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
UCD	University of California – Davis
UR	University of Rochester
USDA	United States Department of Agriculture

SUMMARY

Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) are considered legacy environmental pollutants because they have become ubiquitous around the world even after being banned for decades in most countries. These chemicals are persistent and accumulate in biological tissues. Dichlorodiphenyldichloroethylene (DDE) is a common degradation product of DDT that is often found in higher concentrations than DDT. Pollutants such as DDE and different PCB isomers are commonly found in maternal blood, cord blood, and breast milk of humans, indicating that prenatal exposure to these chemicals is occurring.

The purpose of this study was to determine the levels of these pollutants in human placental tissue so as to: (1) determine the PCBs and DDE concentrations in placental tissue specimens, (2) compare pollutant levels among three placenta collection locations in the United States, (3) evaluate the potential change of pollutant levels with the time between the initial tissue collection after child delivery and tissue collection at times thereafter, and (4) examine the relative levels of PCBs and DDE, and of PCB congeners. Human placental tissue samples were collected in conjunction with the National Children's Study (NCS) and extracted using the matrix solid phase dispersion (MSPD) method that was optimized for placental tissue analysis. The extract was cleaned using a multi–layer silica gel column. The PCBs and DDE were determined using a gas chromatograph, coupled with a triple quadruple mass spectrometer (GC/QQQMS).

Results show detectible levels of most PCBs and of DDE in the placental tissue samples. The individual compound with the overall highest concentration in the tissue was DDE. Concentrations of DDE ranged from 9.8 to 3,220 pg/g wet tissue weight, with a median of 82.2 pg/g and an average of 208 pg/g. The sum of the 32 PCBs analyzed ranged from 76 to 1570 pg/g

wet tissue weight. The average $\sum_{32}$ PCB was 442 pg/g and the median was 395 pg/g. Placenta samples collected in Wisconsin had significantly lower PCB concentrations than those collected in California indicating regional differences in exposures. There were not statistically significant differences in chemical concentration among tissues collected at different times up to 96 h from the same placenta after the child delivery, although paired t–tests indicated that comparisons between initial collection and collection 72 h after delivery may be significant. The compound DDE was the most abundant analyte detected in the tissues. The tissues had higher levels of lighter PCB congeners with PCB 52 and PCB 28 being the most abundant (medians were 45.6 and 39.2 pg/g wwt respectively). The least detected congener was PCB 126, which also had the overall lowest concentration in the samples ranging from below the detection limit to 0.1 pg/g wwt.

I. INTRODUCTION

A. Background

The placenta is a temporary organ that develops as an interface between a mother and her developing fetus. Nutrients and gases are passed from the maternal blood through the placenta to the fetus, and fetal waste products are transferred to the maternal blood (Rampersad et al., 2011). Molecules other than nutrients can pass through the placental barrier from the mother to the fetus during development. This makes the placenta a unique tool to assess fetal exposure to environmental contaminants.

The advantages of using placenta over the use of other tissues and fluids include the non–invasive collection, which brings no health concerns to the mother or child, and the organ's large tissue size, which allows multiple laboratory procedures for various characterization and analyses. An additional benefit is that the placenta may provide information about exposure over the course of fetal development. In contrast, cord blood and breast milk reflect relatively short–term exposures (Iyengar and Rapp 2001; Myllynen et al., 2005; Myren et al., 2007). The placenta has been used as a matrix for analysis in several studies to determine levels of other pesticides, flame–retardants, therapeutic and illegal drugs, and metals (Dassanayake et al., 2009; Shen et al., 2007; Myren et al., 2007; Iyengar and Rapp, 2001).

The PCB chemicals and DDT belong to a group of persistent halogenated organic pollutants. Due to certain chemical characteristics such as lipophilicity, bioaccumulative tendencies, and resistance to biodegradation, DDT and PCBs are part of the "dirty dozen" pollutants of worldwide concern and are monitored, along with other selected persistent organic pollutants (POPs) under the Stockholm Convention in order to reduce or eradicate their worldwide use. These compounds are also classified as priority pollutants under the Clean Water

Act and monitored by the U.S. Environmental Protection Agency (USEPA, 2009). Their legacy of longevity and persistence is illustrated by the fact that these compounds are still found in humans and the environment today, over thirty years since their production was banned in the United States and other industrialized countries (ATSDR, 2008; ATSDR, 2011; Diamond et al., 2009; Li et al., 2010; Patterson et al., 2009). In addition to their long–lasting presence in the environment, these chemicals have been shown to cause various adverse human and ecological affects. Acute effects of these chemicals include skin conditions, irritations, and neurotoxic effects. However due to the decreased use of these compounds in the modern age, chronic low dose exposure is of more concern. Both PCBs and DDE are considered endocrine disrupters, and adverse health effects can be observed at low doses (ATSDR, 2000; ATSDR, 2002; Norris and Carr, 2006).

The National Children's Study (NCS) is a nationwide longitudinal epidemiological study created to analyze the effects of the environment on children's health and development. More than 100,000 children across the United States will be followed from before birth until age 21. Information about children evaluated in the NCS study could provide important information about how these environmental factors alter human health, specifically children who may be particularly sensitive to certain environmental pollutants. The project "Placenta Study" is part of the NCS formative research. The objective of this project is to develop reliable procedures for various evaluations including stem cells, genetics, epigenetics, morphology, pathology, and environmental exposures to toxic chemicals. The project has been conducted in two stages: the pilot study and the main study. Placental samples were collected from three sampling sites to represent different areas of the United States. The sites were located at Rochester in New York, Milwaukee in Wisconsin, and Davis in California. For environmental exposure assessment, toxic

metals, bisphenol A (BPA), and selected persistent organic pollutants have been measured in the collected placenta tissue samples. The organic pollutant chemicals selected included both the legacy pollutants, such as PCBs and DDE, and representative emerging pollutants such as polybrominated diphenyl ethers (PBDEs). This work presented in this thesis focuses on PCB and DDE levels in the placenta tissue collected in the pilot stage of the Placenta Study.

B. **Statement of Problem**

Legacy pollutants such as PCB and the DDT metabolite DDE continue to be ubiquitous in the environment and present in human blood and tissue levels. They have been linked to adverse health outcomes including impaired neurodevelopment due to prenatal exposure. Previous studies have focused on maternal and cord blood analysis to determine prenatal exposure to the compounds.

There are only a few studies that analyze PCB and DDE concentrations in human placental tissue samples. No information was available on how the procedure and time of tissue collection can influence results. Many studies also focus on one geographical area. Concentrations of these pollutant chemicals in such samples must be determined using reliable laboratory procedures, in order to assess the human exposure and associated risks.

C. **Objectives**

The objective of this project was to determine the concentrations of specific PCB congeners and DDE in collected human placental tissue. The specific tasks were to:

1. Determine the PCBs and DDE concentrations in a total of 169 placental tissue specimens (from 43 placentas), which were received from the NCS Placenta

Processing Center (Rochester, New York), using a previously developed laboratory procedure;

2. Compare the concentration levels in the placenta tissues collected at three NCS study centers located at different regions in the United States;
3. Evaluate the potential change of concentrations with the time between the initial tissue collection after child delivery and the and the subsequent tissue collection up to 96 h after the initial collection; and
4. Examine the relative contamination levels between PCBs and DDE and among PCB congeners.

II. LITERATURE REVIEW

A. Chemical Descriptions

1. Dichlorodiphenyldichloroethylene

a. Structure and Nomenclature

Dichlorodiphenyldichloroethylene (DDE) is a metabolite of the once widely used pesticide dichlorodiphenyltrichloroethane (DDT). The chemical structure for p,p'–DDE is pictured in Figure 1.

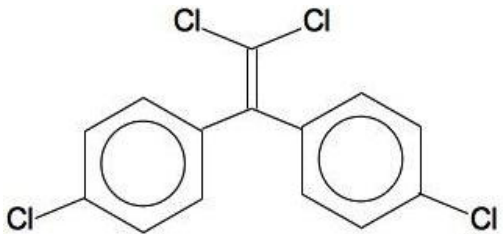


Figure 1. Chemical structure of dichlorodiphenyldichloroethylene (DDE).

The pesticide DDT is broken down to DDE through the removal of hydrogen chloride from the structure as shown in Figure 2. The most common forms of DDT and DDE feature two chlorine atoms attached at the *para*–position of the phenyl rings. Other forms of DDT and its metabolites are shown in Figure 3. Another metabolite of DDT is dichlorodiphenyldichloroethane (DDD), which is formed through the reductive dechlorination of DDT. This paper focuses on p,p'–DDE which is the most abundant and persistent metabolite of DDT found in the human tissues (Falcón et al., 2004; Snedeker, 2001). From this point forward p,p'–DDE will be referred to as DDE unless otherwise noted.

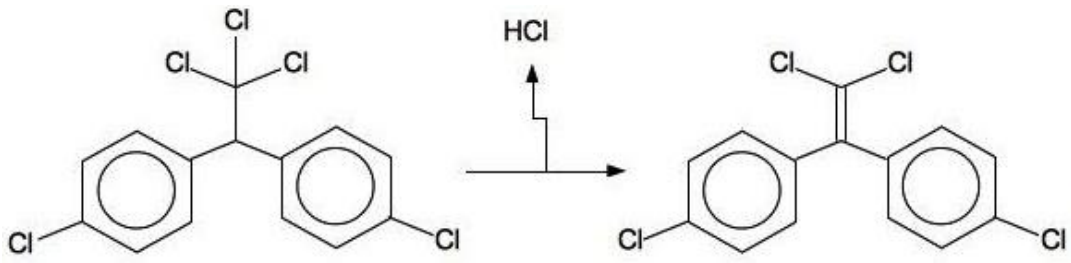


Figure 2. DDT loses a hydrogen chlorine molecule to produce DDE.

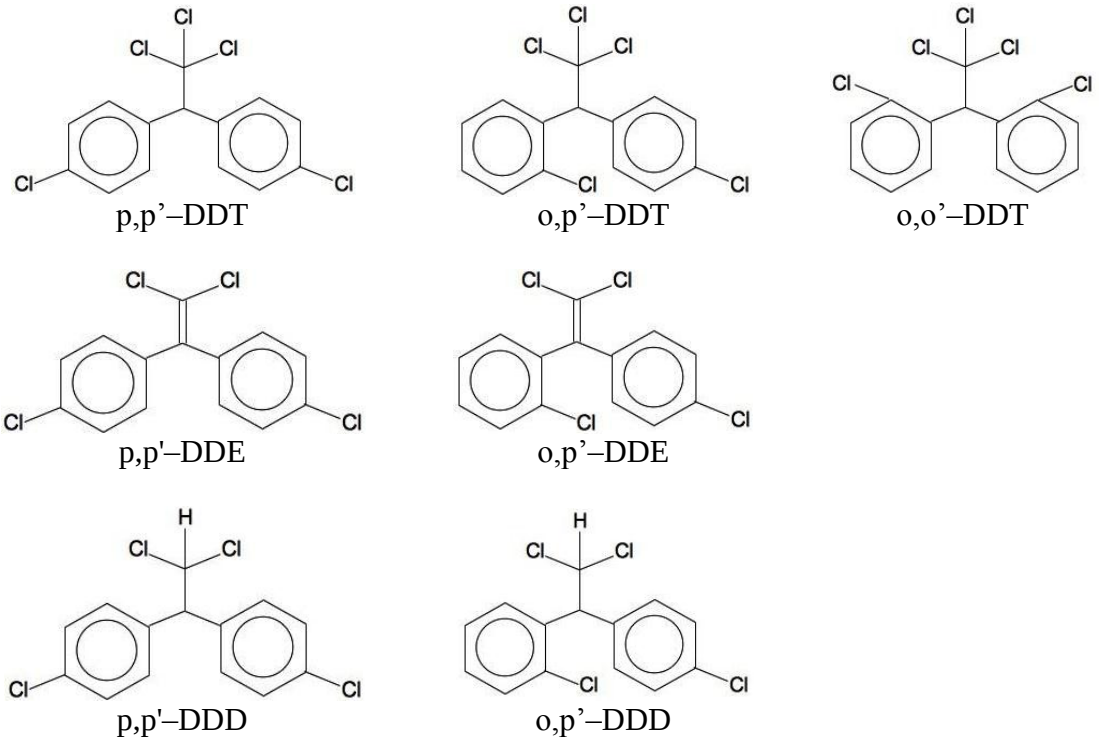


Figure 3. Chemical structure of important isomers and metabolites of DDT.

b. **History of production and use**

Although DDT was first synthesized in the late 1800s, its potential as an insect repellant was not discovered until 1939. In 1943 massive production of DDT began. Due to its low price and accessibility it was used worldwide for insect control (Turusov et al., 2002; Norris and Barr, 2006). Technical grade DDT is produced by the condensation of chlorobenzene with dichloroacetaldehyde. It typically includes impurities including the other DDT isomers, o,p'–DDT and o,o'–DDT, as well as the different isomers of the breakdown products DDD and DDE as shown in Figure 3.

In the United States, this pesticide was used primarily on cotton crops. It was also used to control pests such as moths, beetles and lice, and was used to prevent the spread of malaria through mosquitoes. The massive usage of DDT throughout the world had unintended consequences. Not only were many pests becoming resistant, it was found that DDT caused eggshell thinning in birds of prey. This was particularly severe in fish eating birds as DDT biomagnifies up the food chain. Though DDT it has been banned since 1972, it is still presently measurable in natural bodies of water (Kwong et al., 2008, Norris and Carr, 2006).

Global attention was focused on this phenomenon and the consequences of excessive pesticide use when the biologist Rachel Carson published her book *Silent Spring* in 1962. This resulted in the banning of DDT application in the United States in 1972, though its production in the United States continued through the 1980s for export. Now most countries around the world have banned DDT with an exception for indoor use to prevent malaria and other vector–borne diseases in places such as Africa and India (ATDSR, 2002).

c. **Chemical properties**

The chemical properties of DDT and its derivatives are listed in Table I. The octanol–water partition coefficient (K_{ow}) and overall low water solubility indicate that all of the derivatives are lipophilic and hydrophobic. The soil organic carbon/water partition coefficient (K_{oc}) also indicates that DDT compounds tend to accumulate in soils and sediments. They are semi–volatile compounds that have the tendency to vaporize and deposit, lending to their long–range mobility. The half–life has been estimated to be 1.5–3 days as atmospheric vapors (ATDSR, 2002).

TABLE I
CHEMICAL PROPERTIES OF DDT AND ITS DERIVATIVES

Property	p,p'–DDT	p,p'–DDE	p,p'–DDD	o,p'–DDT	o,p'–DDE	o,p'–DDD
Chemical Formula	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$	$C_{14}H_9Cl_5$	$C_{14}H_8Cl_4$	$C_{14}H_{10}Cl_4$
Molecular Weight	354.49	318.03	320.05	354.49	318.03	320.05
Melting Point (°C)	109	89	109–110	74.2	–	76–78
Boiling Point (°C)	Decomposes	336	350	–	–	–
Vapor Pressure (torr)	$^{*}1.60x10^{-7}$	$6.00\ x10^{-6}$	$1.35\ x10^{-6}$	$^{*}1.10\ x10^{-7}$	$6.20\ x10^{-6}$	$1.94\ x10^{-6}$
Water Solubility (g/m^3)	0.025	0.120	0.090	0.085	0.140	0.100
Log K_{ow} Coefficient	6.91	6.51	6.02	6.79	6.00	5.87
Log K_{oc} Coefficient	5.18	4.70	5.18	5.35	5.19	5.19
Henry's law constant ($atm–m^3/mol$)	$8.3x10^{-6}$	$2.1x10^{-5}$	$4.0x10^{-6}$	$5.9x10^{-7}$	$1.8x10^{-5}$	$8.17x10^{-6}$

Note: Data based on standard temperature (25°C); * Vapor pressure at 20°C; – No data available.
Source: ATSDR, 2002.

The Convention on Long–Range Transboundary Air Pollution defined criteria for persistent organic pollutants (POPs). According to these criteria, POPs must show evidence of transboundary atmospheric transport to remote regions, bioaccumulation in organisms,

persistence in the environment, and some form of toxicity to human health or the environment. Specific criteria are listed in Table II below. The metabolite DDE has been identified in remote areas of the world such as the Arctic and Antarctic and has been subsequently classified as a POP (Ballschmiter et al., 2002; ATDSR, 2002).

TABLE II
CRITERIA FOR DEFINING PERSISTENT ORGANIC POLLUTANTS

Criteria Description	Level
Vapor Pressure	< 7.5 torr
Atmospheric Half–life	> 2 days
Water Half–life	> 2 months
Soil and Sediment Half–life	> 6 months
Fish Bioaccumulation Factor	> 5,000
Log K_{ow}	> 5

Source: Ballschmiter et al., 2002.

d. **Environmental release**

It is estimated that over two million tons of DDT have been produced worldwide (ATSDR, 2002). As of 2008, quantities of DDT were still being produced in India, China and the Democratic People's Republic of Korea. In 2007 India alone produced over six thousand metric tons of the pesticide for export and for local use to defend against malaria and leishmaniasis diseases (Van den Berg, 2008). Mexico discontinued its production and use of DDT for vector control in 2000 (ATDSR, 2008). In most countries the use and production of DDT has been banned.

e. **Human exposure**

When DDT was used regularly for vector control— sprayed across crops, over forests, and directly onto waters—there were numerous routes of exposure: ingestion, inhalation, and dermal. In most places throughout the world this insecticide has been banned from use for decades, but due to transcontinental air transportation, DDT, currently being used in other countries, can be found in North America (Snedeker, 2001).

Presently the primary route of exposure for DDT and its metabolite DDE is through food consumption. Bioaccumulation occurs in fish because DDE has a lipophilic nature. Biomagnification up the food chain occurs so that predatory fish and birds show much higher concentration levels than the water in which their food came from (Norris and Barr, 2006; Kwong et al., 2008). The chemical DDE adsorbs to and accumulates in soil. There, growing plants absorb it through root structures and it concentrates in their tissues. The primary route of human exposure is by the consumption of food, plant or animal, which contains trace levels. The metabolites of DDT are commonly found in fatty foods such as fish, meats, and dairy products, as well as in fruits and vegetables. A review of persistent toxic chemicals in the United States food supply found that DDE was the most common chemical detected in foods (Schafer and Kegley, 2002). The safe intake and regulatory levels for all DDT compounds can be found in Table III.

TABLE III
SAFE INTAKE AND REGULATORY LEVELS FOR ∑DDT

Name	Level	Established by	Date
Acceptable Daily Intake (ADI)	20 µg/kg bw*	FAO/WHO	1984
Provisional Tolerable Daily Intake (PTDI)	10 µg/kg bw*	JMPR	2000
Maximum Residue Limits (MRL):		Codex Alimentarius	
Milk	20 µg/kg		1997
Eggs	100 µg/kg		1997
Cereal grains	100 µg/kg		–
Carrot	200 µg/kg		1997
Poultry meat	300 µg/kg		2003
Meat (non–marine)	5,000 µg/kg		2001

*bw = body weight
Source: ATDSR, 2008; Codex Alimentarius, 2010.

Surveys on DDT contamination in foods have shown a dramatic decrease in concentration levels since the 1970s but detectable levels are still present in our food supplies (ATDSR, 2002; Snedeker, 2001). A 2008 inspection of catfish by the USDA found detectable levels of DDE in 97% of the 435 samples collected from U.S. water sources, although the concentrations were below regulatory levels (USDA, 2008). An assessment of DDE concentrations in butter from around the globe was performed on samples collected in 1998–1999. Large concentration variations were observed ranging from 380–180,000 pg/g lipid. The mean DDE concentration found in the United States was 24,070 pg/g lipid (ranging from 1620–140,380 pg/g lipid) with highest concentrations in the southern states (Kalantzi et al., 2001). These surveys suggest that DDE is present in a variety of dietary sources, exposing the average American from multiple avenues.

f. **Placental transfer and prenatal exposure**

It was first discovered that DDT could pass through the placental barrier in 1949 in a series of canine studies. Later research confirmed DDT presence in the tissues of other

mammals such as mice, rabbits, deer, and ultimately in humans. The most stable metabolite of DDT found in human tissues is DDE (Rogan et al., 1986; Falcón et al., 2004). Detectable levels of DDE were found in various tissues of neonates and stillborn babies as early as 1968, indicating the occurrence of prenatal exposure (Rappolt and Hale, 1968; Curley, 1969).

Maternal blood levels of DDT compounds are generally higher than those found in cord blood and placental tissue, with DDE being the most frequently observed derivative metabolite (Al–Saleh et al., 2012). The fetus is exposed to these compounds through maternal blood via the placental interface. Maternal diet and changes in maternal fat distribution during pregnancy are major contributors to *in utero* exposure (Saxena 1983; Falcón et al., 2004). During pregnancy increased maternal metabolism causes fat stores to fluctuate, potentially releasing lipid soluble organic pollutants into the bloodstream where they have the opportunity to transfer to the fetus after passing through the placenta (Bergonzi et al., 2009).

g. **Trends and levels**

The concentrations of DDT in humans have shown a decrease over the years, but levels of DDE have remained steady, most likely due to its lipophilicity (Turusov et al., 2002; ATSDR, 2002). The half–life of DDT in human serum is 10 years (Jaga and Dharmani, 2003) and its metabolite DDE is commonly detected in human samples, often found in 100% of the human samples analyzed (Doucet et al., 2009; Herbstman et al., 2007; Shen et al., 2007; Woodruff et al., 2011). The chemical DDE is also present in breast milk, generally at higher concentrations than in placenta tissues or blood samples. Overall, breast milk concentrations have decreased over the years (LaKind et al., 2001; Shen et al., 2007). Breast milk samples have higher lipid content than placental samples, and the placenta contains more lipids than serum (DeKoning and Karmaus, 2000; Bergonzi et al., 2009). Breast milk represents an ideal sampling

avenue for neonatal exposure due to its ease of collection and high lipid content where lipophilic compounds are of concern. For *in utero* exposure, the placenta may be a better matrix than cord blood because its higher fat content allows for better detection of lipid soluble analytes.

Table IV lists all of the studies this author found on DDE levels in human placental tissues. The data have been adjusted based on an average placenta lipid content of 1.25% utilized a previous review study (DeKoning and Karmaus, 2000). The original data can be found in Appendix A. Some of the studies evaluated only reported either the concentration without the lipid content of the placenta or the concentration per weight in lipids without individual lipid values. The original values are difficult to compare based on these differences in reporting units. To account for such discrepancies, they were normalized to pg/g lipid in the table below. The EPA method for determining PCB levels in tissue samples suggests reporting data as concentration per wet tissue weight with the lipid content values listed separately (USEPA, 2008).

Given these data there appears to be a downward trend in DDE levels after they are lipid adjusted, with the exception of high levels noted in the Spain and Italy reports. No mention of extensive DDT pollution was reported in the Italian study to account for the elevated DDE levels in placental tissues (Bergonzi et al., 2009) and the study in Spain did not specify an exposed population (Falcón et al., 2004). It is unclear why these populations have elevated DDE levels compared to the other studies performed in different regions during that time frame.

TABLE IV
TRENDS AND LEVELS OF DDE IN PLACENTAL TISSUES

Population	Collection Date	*n*	Mean Conc.	Median Conc.	Detection Method	Reference
California, USA	Before 1968	39	5000000		GC/ECD	Rappolt, 1968
Lucknow, India	1978	50	4043200*		GC with 3H+ detector	Saxena et al., 1980
Lucknow, India	1979–1980	9	992000*	920000*	GC/ECD	Saxena et al., 1983
Lucknow, India	1979–1980	27	1464000*	904000*	GC/ECD	Saxena et al., 1983
North Carolina	Before 1986	790		541600*	GC/MS	Rogan et al., 1986
Denmark	1997–2001	43	47150		GC/MS	Shen et al., 2007
Finland	1997–2001	43	21230		GC/MS	Shen et al., 2007
Murcia Province, Spain	1998–2000	102	1416000*		GC/ECD	Falcón et al., 2004
Bratislava, Slovakia	Before 1999	57	8000*		GC/ECD	Reichrtová et al., 1999
Stará Lubovna, Slovakia	Before 1999	63	8000*		GC/ECD	Reichrtová et al., 1999
Brescia, Italy	2006	69	69300	62500	GC/MS (EI)	Bergonzi et al., 2009
USA	Before 2007	19	58.3		GC/ECD	Brooks et al., 2007

Units adjusted to pg/g lipid.
*Adjusted based on lipid content of 1.25%.

h. **Toxicity**

The toxic effects of DDT and its metabolites were observed as early as the 1950s in various animal studies. Its use was initially banned in the United States because it was thought to be carcinogenic in humans and in light of its implication in eggshell thinning in many important predatory bird species such as the bald eagle (Norris and Carr, 2006). Acute exposure to DDT has produced various nervous system symptoms from excitability to seizures (ATSDR, 2002). Presently, in light of the ban, most people are only exposed to low levels of residual DDT and DDE, suggesting toxicity may not be readily manifested.

Many studies have attempted to link DDT exposure to certain cancers such as breast carcinoma but, to date, significant correlations between the two have not been consistently established (ATSDR, 2002). The International Agency for Research on Cancer has classified DDT as a possible human carcinogen. The EPA has classified DDT and all of its metabolites as probable human carcinogens (ATSDR, 2002; Snedeker, 2001). Table V lists the no observable adverse effect level (NOAEL) and lowest observable adverse effect levels for DDT and its metabolites. Based on the observed levels in placenta tissue from table IV, eight times the highest reported level would need to pass through the placenta a day to reach the lowest observable adverse effect level. This suggests acute effects of *in utero* exposure would be rare based on measured values in placenta tissue.

TABLE V
NOAEL AND LOAEL LEVELS FOR ∑DDT

Exposure Route	Effect	Level	Species	Details
Acute Oral	LOAEL	0.5 mg/kg/day	Mice	Neurodevelopment effects
Intermediate Oral	NOAEL	0.05–0.09 mg/kg/day	Osborne–Mendel rats	Administered 0–50ppm dose over 15–27 weeks

Source: ATDSR, 2002.

The pesticide DDT and its metabolites also have endocrine disruption potential. They have been linked to reproductive effects in animals such as eggshell thinning, vitellogenesis or yolk formation in fish, and altered sex ratios (Norris and Carr, 2006). Altered sex ratios in humans have also been assessed with the suggestion that DDT and DDE exposure, as well as select PCB exposures, tend to shift the sex ratio in humans toward male offspring (Tan et al., 2009). The metabolite DDE has been associated with antiandrogenic actions, progesterone activities, and possesses an affinity for sex steroid receptors in various animal species (Norris and Carr, 2006). There is limited evidence that DDT is associated with a decrease in gestational size, preterm birth, and accessory nipples (Wigle et al., 2007). These results suggest the potential significance of prenatal exposure to DDT and DDE as this is a time of rapid development for the endocrine system.

In addition, prenatal exposure to DDE may impact neurodevelopment in children, and DDE has been associated with impaired mental and psychomotor development (Jurewicz et al., 2006). It has also been linked to poor memory and verbal skills as well as childhood ADHD–like symptoms (Ribas–Fitó et al., 2006; Sagiv et al., 2009). Prenatal exposure is associated with allergy susceptibility biomarkers and increased incidence of asthma in children (Brooks et al.,

2007). There is evidence that DDE exposure in utero can result in obesity as the child ages with rapid weight gain in early childhood (Mendez et al., 2010; Valvi et al., 2012). These results highlight the implications of prenatal exposure to human health outcomes and point out the need for continuous monitoring of these exposures.

2. Polychlorinated biphenyls

a. Structure and nomenclature

Polychlorinated biphenyls (PCBs) are chlorinated hydrocarbons with a general structure as shown in Figure 4. These chemicals consist of two connected phenyl rings with chlorine atoms attached the rings. There are 10 homologs and 209 congeners of PCBs, ranging from one to ten chlorine atoms at various substitution positions on the two phenyl rings of the molecule.

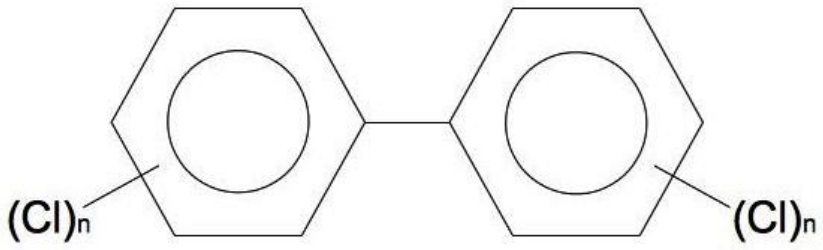


Figure 4. Chemical structure of polychlorinated biphenyls (PCBs).

b. Chemical properties

The PCB analytes are chemically stable compounds that do not react readily with other compounds. They are resistant to degradation, non-water soluble, and fire

resistant. It is because of these attributes that PCBs were remarkable compounds for industrial uses. The chemical properties of the various isomers of PCBs are listed in Table VI. The octanol-water partition coefficient (K_{ow}) is high for PCBs (log K_{ow} ranging from 4.7–8.3) signifying a strong affinity to lipids. The bioconcentration factor in fish (BCF) is also very high, allowing PCBs to bioaccumulate in fat tissues with subsequent biomagnification up the food chain.

TABLE VI
PCB HOMOLOGS AND THEIR RESPECTIVE CHEMICAL PROPERTIES.

Homolog Group	Chemical Formula	Molecular Weight	# of Isomers	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (Pa)	Water Solubility (g/m^3)	Log K_{ow} Coefficient	Approximate BCF
Monochlorobiphenyl	$C_{12}H_9Cl$	188.7	3	25–77.9	285	1.1	4	4.7	2500
Dichlorobiphenyl	$C_{12}H_8C_{l2}$	223.1	12	24.4–149	312	0.24	1.6	5.1	6300
Trichlorobiphenyl	$C_{12}H_7Cl_3$	257.6	24	28.87	337	0.054	0.65	5.5	$1.6x10^4$
Tetrachlorobiphenyl	$C_{12}H_6Cl_4$	292.0	42	47–180	360	0.012	0.26	5.9	$4.0x10^4$
Pentachlorobiphenyl	$C_{12}H_5Cl_5$	326.4	46	76.5–124	381	$2.6x10^{-3}$	0.099	6.3	$1.0x10^5$
Hexachlorobiphenyl	$C_{12}H_4Cl_6$	360.9	42	77–150	400	$5.8x10^{-4}$	0.038	6.7	$2.5x10^5$
Heptachlorobiphenyl	$C_{12}H_3Cl_7$	395.3	24	122.4–149	417	$1.3x10^{-4}$	0.014	7.1	$6.3x10^5$
Ochtachlorobiphenyl	$C_{12}H_2Cl_8$	429.8	12	159–162	432	$2.8x10^{-5}$	$5.5x10^{-3}$	7.5	$1.6x10^6$
Nonachlorobiphenyl	$C_{12}H_1Cl_9$	464.2	3	182.8–206	445	$6.3x10^{-6}$	$2.0x10^{-3}$	7.9	$4.0x10^6$
Decachlorobiphenyl	$C_{12}Cl_{10}$	498.7	1	305.9	456	$1.4x10^{-6}$	$7.6x10^{-4}$	8.3	$1.0x10^7$

Note: Data based on standard temperature (25°C). Most values are approximate ranges for all isomers in the group
Source: Robertson and Hansen, 2001.

c. History of production and use

A description of the synthesis of PCBs was first published in an article written by H. Schmidt and G. Schultz in the *Liebigs Annalen der Chemie Journal* in 1881 (Schmidt et al., 1881). Production for industrial applications did not begin until 1929 by the Swan Chemical Company, which was subsequently sold to Monsanto Chemical Corporation (Cairns et al., 1981; Risebrough et al., 1970; USEPA 1979). Monsanto was the primary PCB manufacturer in the United States and is estimated to have produced around 60% of total worldwide PCBs (Robertson and Hansen, 2001). The trade name used by Monsanto for the various PCB mixtures was Aroclor, which was manufactured out of two plants in the United States located in Sauget, Illinois and Anniston, Alabama (Risebrough et al., 1970). A second U.S. manufacturer was the General Electric Company who marketed their product under the trade name Pyranol (ATDSR, 2010). Pyranol was manufactured in Rome, Georgia from 1953 to 1977.

Due to their non-flammable, chemically stable characteristics, PCBs were ideal coolants, insulators, and lubricants. They were used in a variety of products such as electrical transformers and capacitors, in paints and pigments, as plasticizers, fluorescent lighting fixtures, cable insulation, adhesives, floor finishes, copy paper, caulking, hydraulic oils and even microscope oil (ATSDR, 2000). This diversity of applications made them ubiquitous in the surrounding environment of the modern world.

The compounds PCBs are structurally similar to the pesticide DDT. Both contain biphenyl rings and attached chlorine atoms. While analyzing DDT and DDE in environmental and human samples, other chlorinated compounds similar to DDT were found but not identified as PCBs until 1966. In this year PCBs were found to be ubiquitous in 200 pike fish indicating

widespread environmental contamination (Risebrough et al., 1970). A retrospective analysis of preserved eagle feathers, dating back to 1880, identified the emergence of PCBs in 1944 (Risebrough et al., 1970). Due to concerns over the toxic potential of PCBs, manufacturing was voluntarily suspended in 1977 in the United States. The EPA, established in 1970, began regulating the disposal of PCBs in 1978, and outright banned their production in 1979 (USEPA, 1979).

d. **Environmental release**

The PCB chemicals were produced worldwide under various trade names but production volumes and composition data is scarce. This makes determination of total global production difficult to assess. The World Health Organization estimated that over one million metric tons were produced worldwide (Robertson and Hansen 2001; Hansen 1999). Though the production of PCBs has been banned throughout the world, their historical use in numerous applications indicates they may still be present in older products and machinery.

A recent study of building sealants in Canada found measurable levels of PCBs in 14% of the buildings tested (Robson et al., 2010). An inventory of all PCBs in Toronto identified 437 metric tons of PCBs, mostly in closed sources such as insulating fluids and oils. This is most likely an underestimate due to the unknown amounts of PCBs in waste facilities and other minor products (Robson et al., 2010). Models estimate up to 880 kg of PCBs could be emitted from the city of Toronto alone each year from known sources (Diamond et al., 2010).

Varying concentrations of PCBs have been found around the world, including places of limited or no PCB use, such as Antarctica (Connell et al., 1999). Those PCBs with fewer chlorines and lower molecular weight are more volatile and exhibit increased mobility through the air (Li et al., 2010; Hansen, 1999). Plants also more readily absorb these lower congeners

giving them an opportunity to enter the food chain with biomagnification potential (Hansen, 1999).

e. **Human exposure**

Fish and seafood are important sources of PCB exposure in humans (ATDSR, 2000; Berntssen et al., 2011; Gasull et al., 2011). A study of pregnant Taiwanese women found that high levels dioxin-like PCBs in maternal blood and placental tissues were associated with saltwater fish and fish oil consumption (Huang et al., 2007). High PCB carry-over has been observed in farm-raised fish fed with high fish oil diets (Berntssen et al., 2011).

Milk and dairy product consumption has been associated with high body burden of PCBs in humans (Gasull et al., 2011). The PCB levels in milk samples in the United States ranged between 410–3500 pg/g lipid for samples collected between 1998 and 1999. Overall samples from the Czech Republic had highest PCB concentrations (maximum 14,090 pg/g lipid), and New Zealand had the lowest (minimum 230 pg/g lipid), with PCBs 138, 153, 180, and 118 being most abundant (Kalantzi et al., 2001). In a 2003 study, the sum of the dioxin-like PCBs found in milk samples was 14,133.24 pg/liter of milk. The highest levels were found in the mid-Atlantic region of the United States. Upon comparison of TEQ concentrations, the authors reported a 50% decrease in PCB concentrations from a previous study performed in 1996. However, milk could be an important exposure pathway for PCBs since adults consume around 12% of their daily fat from milk (Schaum et al., 2003).

f. **Placental transfer and prenatal exposure**

Like DDT and its metabolites, PCBs have been shown to readily cross the human placenta due to their lipophilicity. They have been detected in maternal blood, placental tissue, cord blood and fetal tissues (Ando, 1986; Bergonzi et al., 2009; DeKoning and Karmaus,

2000; Doucet et al., 2009; Shen et al., 2007; Wang et al., 2004). Cord blood concentrations are generally lower than maternal blood concentrations (Ando, 1986; ATSDR, 2000). One review of neonate PCB exposure prior to and after birth estimated that around 60% of the PCBs in maternal blood could be found in the neonate (DeKoning and Karmaus, 2000). The fetus is exposed to PCBs as these compounds pass from the maternal blood through the placental barrier. The concentration gradient produced from of low cord blood concentrations and higher maternal blood concentrations is assumed to facilitate this process (Myren et al., 2007).

g. **Trends and levels**

Levels of PCBs in human placental tissues are difficult to compare among studies. This is due to differences in study designs and reporting methods (DeKoning and Karmaus, 2000). The major reporting issues noted were the use of the mean versus median and differing units of measure. In particular, some studies report on a concentration per lipid basis while others report on a concentration per weight of tissue (DeKoning and Karmaus, 2000). Researchers also measure selected congeners or report only the sum of detected PCB congeners rather than individual congener concentrations.

Table VII lists some of the placental studies with PCB analyses. As with the DDE trend data (Table IV), the PCB data have been adjusted to picogram (pg) of PCB per gram of lipid based on a placental lipid content of 1.25%. Focusing of one specific congener, PCB 153, from the data below we can see concentrations span between 8000–26000 pg/g lipid over a 10-year period. The largest value came from a population in Italy with a history of high environmental PCB contamination (Bergonzi et al., 2009). The smallest value came from a rural population in the mountains of Slovakia where PCB consumption is assumed to be minimal (Reichrtová et al., 1999). It appears from this data that location of residence can have a great effect on placental

concentrations. Total PCB levels from these studies are difficult to compare due to the discrepancies in study methodologies as mentioned previously.

TABLE VII
TRENDS AND LEVELS OF PCBS IN PLACENTAL TISSUES

Population	Collection Date	Analyte	n	Mean	Median	Detection Method	Reference
Brescia, Italy	2006	Σ_{30} PCBs	70	98700	92500	GC/MS (EI)	Bergonzi et al., 2009
		PCB 28			ND		
		PCB 52			ND		
		PCB 153			26000		
Madrid, Spain	2003–2004	Σ_{15}PCBs	17	203680*	183360*	GC/ECD	Gómara et al., 2012
		PCB 28		13520*	13520*		
		PCB 52		51040*	52640*		
		PCB 153		24720*	20640*		
Bratislava, Slovakia	Before 1999	PCB 28	57		8000*	N.P.	Reichrtová et al., 1999
		PCB 52			8000*		
		PCB 153			16000*		
Stará Lubovna, Slovakia	Before 1999	PCB 28	63		ND	N.P.	Reichrtová et al., 1999
		PCB 52			ND		
		PCB 153			8000*		
N.P.	before 1977	PCBs	19	5027000		CCGC/ECD	DeKoning and Karmaus, 2000
Germany	before 1994	PCBs	46		248000–373000		
N.P.	before 1996	PCBs	25		950000		
Central Taiwan	2000–2001	Dioxin–like PCBs	20	5292	4848	GC/MS	Wang et al., 2004
		Non–ortho PCBs		67.6	44.05		
		Mono–ortho PCBs		5224	4824		
		PCB 138, 153, 180		28837	26228		
Upstate New York, USA	1995–1996	Coplanar PCBs	5	18.2		GC/MS	Schecter et al., 1998
		PCB 77		4			
		PCB 126		10.1			
		PCB 169		4.1			

Units adjusted to pg/g lipid. *Adjusted based on lipid content of 1.25%.
Notes: Not Provided (N.P.); Not Detected (ND); Non–ortho PCBs 81, 77, 126, 169; Mono–ortho PCBs 105, 114, 118, 123, 156, 157, 167, 189.

h. **Toxicity**

The toxicity of PCBs depends on the chemical structure. Dioxin-like PCBs are those that have a similar structure to 2,3,7,8–tetrachlorodibenzo–*p*–dioxin (TCDD). The TCDD compound is used as a reference to determine toxic equivalency factors (TEF) for other TCDD–like compounds. The TEF value therefore indicates the relative magnitude of toxicity. The total toxic equivalent (TEQ), the sum of each compound's concentration multiplied by its TEF value, is a standardized measure of toxicity for risk assessment. Table VIII shows the current and past TEF values for the dioxin-like PCB congeners.

TABLE VIII
TEF VALUES FOR PCBS FROM THE WORLD HEALTH ORGANIZATION

PCB Congener	**2005 TEF**	**1998 TEF**	**1994 TEF**
non–ortho substituted			
77	0.0001	0.0001	0.0005
81	0.0003	0.0001	–
126	0.1	0.1	0.1
169	0.03	0.01	0.01
mono–ortho substituted			
105	0.00003	0.0001	0.0001
114	0.00003	0.0005	0.0005
118	0.00003	0.0001	0.0001
123	0.00003	0.0001	0.0001
156	0.00003	0.0005	0.0005
157	0.00003	0.0005	0.0005
167	0.00003	0.00001	0.00001
189	0.00003	0.0001	0.0001

Source: Van den Berg et al., 2006.

Although there are many PCBs that are structurally similar to TCDD, those listed above are of particular importance because of their ability to bind to the aryl hydrocarbon receptor,

causing adverse health effects similar to TCDD, and their ability to persist in the environment for extensive periods of time (USEPA, 2010; Hansen, 1999; Safe et al., 1985). Additionally, the EPA has classified PCBs as probable human carcinogens. There is evidence of increased cancer incidence in animal studies and epidemiological studies in humans have found an increase incidence in certain cancers among workers with occupational exposures to PCBs (USEPA, 2012).

Acute toxicity to PCBs, along with toxic polychlorinated dibenzofurans, from *in utero* exposure was seen in the Yushō and Yu-Cheng incidents in Japan and Taiwan. In these cases cooking oil was contaminated with PCBs and consumed by the public. Fetuses exposed *in utero* were born with tooth eruption prior to birth, as well as skin and nail abnormalities. They were small for gestational size and follow-up studies found that these children performed poorly on standardized intelligence tests (Swanson et al., 1995; Jacobson et al., 1990; Wigle et al., 2007). Another study found associations between high PCB exposure and decreased gestational age with decreased birth weights of children born to occupationally exposed mothers (Taylor et al., 1984).

Chronic exposures to low levels of PCBs can also have detrimental effects on children who were exposed *in utero*. Neurodevelopmental deficiencies such as poor memory and lower intelligence scores have been correlated with elevated cord blood levels of PCBs (Jacobson et al., 1990; Wigle et al., 2007). These compounds have been linked to reduced reflexes and muscle tone in newborns and there is evidence that prenatal PCB exposure can lead to an increased incidence in ear infections (Wigle et al., 2007).

B. Human Placenta

1. Description and function

The human placenta is a discoid shaped temporary organ, which grows during gestation and at full term weighs an average of 470g. The size of the placenta is approximately 1/7th the size of the fetus. During the beginning stages of pregnancy, the fertilized egg cells divide to form the trophectoderm and the blastocyst layers containing a cell mass called the embryoblast. The embryoblast develops into the fetus whereas the blastocyst develops into the placenta after implantation into the uterine epithelium. This site of implantation is known as the decidua basalis or the basal plate. The side of the placenta facing the fetus is called the chorionic plate. An illustration of a full term placenta can be seen in Figure 5 (Rampersad et al., 2011).

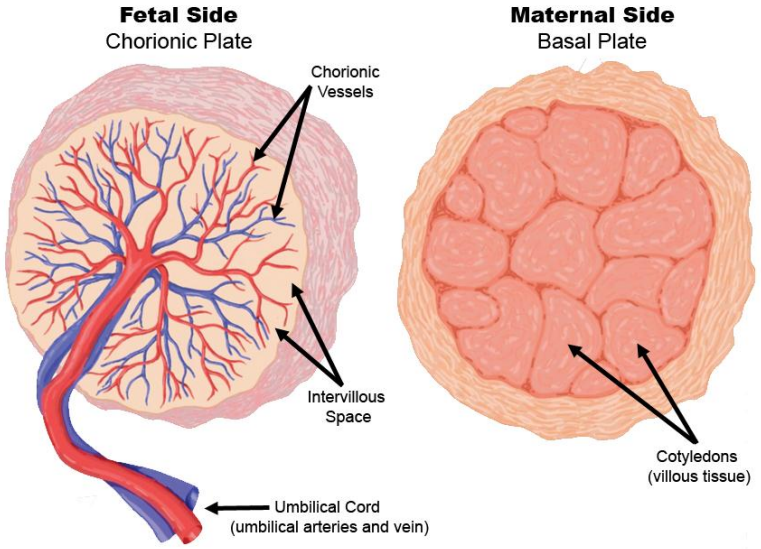


Figure 5. Image of a full term human placenta with views from both sides. (Image source: Rampersad et al., 2011)

The maternal and fetal blood circulation systems remain separate throughout pregnancy. It is the job of the placenta is to provide an intermediate site for nutrient and waste exchange. Fetal circulation is comprised of the two umbilical arteries and the umbilical vein, which breaks off into chorionic vessels that extend throughout the villous tree through the umbilical cord and to the fetus. An illustration of the villous tree can be found in Figure 6. Nutrients and gases are exchanged between the fetal and maternal systems at the intervillous space.

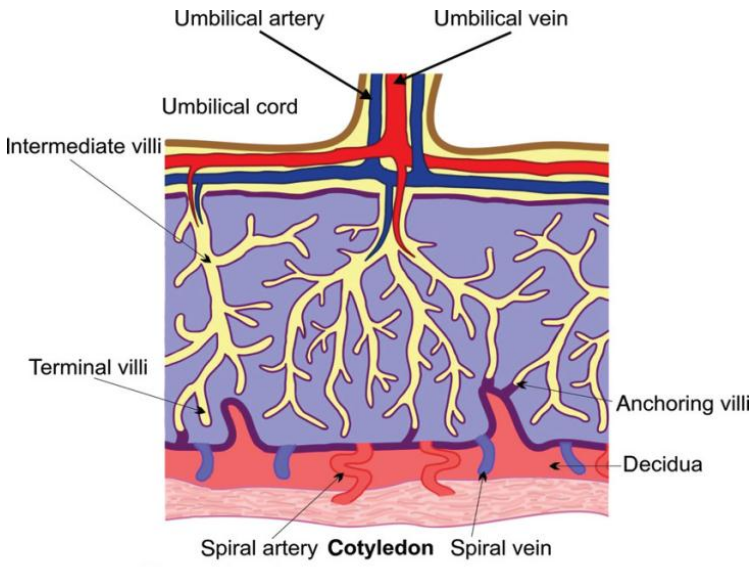


Figure 6. Illustration of the villous tree and the intervillous space of the placenta. (Image source: Ramersad et al., 2011)

After approximately 10 to 12 weeks gestation, spiral arterioles release maternal blood into the intervillous space. The villous core then acts as a diffusion barrier between the two circulatory systems allowing the transfer of gasses and nutrients (Rampersad et al., 2011; Fox and Sebire, 2007). The placental structure and function differs considerably between different

species of mammals, complicating our interpretation of animal models to predict xenobiotic effects (Myllynen et al., 2005).

2. Placental transfer of organochlorines

The most important properties that influence the transfer of compounds across the placenta are the molecular weight of the compound, lipid solubility, and the ionization. Other factors that influence permeability include the presence of a concentration gradient, amount of blood flow, protein binding, potential maternal and placental metabolism of the compound and the mechanism of transportation across the membrane (Klaassen et al. (eds.), 2010; Myllynen et al., 2005). Organochlorine (OC) chemicals such as DDE and PCBs are light, lipophilic compounds. This allows them to passively diffuse through the placenta.

C. Methods for Measuring PCBs and DDE

1. Tissue analysis

The placenta offers a unique view of prenatal exposure that differs from that given by cord blood analysis. Blood analysis shows a snapshot of current exposure levels, whereas placental tissue offers a measure of long-term exposure levels of bioaccumulative compounds (Iyengar and Rapp, 2001; Myllynen et al., 2005). This is further useful because it limits the timeframe of exposure to that which occurs during fetal development. Other benefits of placental tissue analysis include the ability to collect the sample non–invasively and a yield of a large amount of tissue, which can be used for multiple analyses. This is of particular importance for projects such as the NCS where multiple analyses are required with limited matrix available (Barr et al., 2005).

The placenta is a complex mix of fetal and maternal cells and blood. Collection procedures vary depending on the study. Certain parts of the placenta may be highly vascularized or have higher lipid concentrations so determination of which part of the tissue to collect is essential (Iyengar and Rapp, 2001). Handling excess blood from the placenta is another factor to consider. Investigators need to determine how to remove excess blood without contamination of tissue samples. Some procedures include rinsing the tissue with deionized (DI) water (Ando, 1986; Al–Saleh et al., 2012). Previous studies suggest that all samples should be frozen prior to use. Table IX lists collection procedures from the literature review.

As shown, there are various procedures for tissue collection. Some involve rinsing the placenta to remove any excess blood while others do not. Different parts of the placenta were collected, as detailed in the tissue collection notes of Table IX. All studies indicated that the samples were frozen upon collection. Only three studies dried the tissues prior to analysis, one by centrifugation and the others via lyophilization (Ando, 1986; Gómara et al., 2012; Al–Saleh et al., 2012).

TABLE IX
PLACENTA TISSUE COLLECTION TECHNIQUES

Date	Analyte(s)	Storage Temperature	Excess Blood	Tissue Collection Notes	Source
Before 1986	PCBs	–20°C	Rinsed DI	Chorionic tissue was used, centrifuged for dehydration, homogenized in ice bath	Ando, 1986
1995–1996	PCBs	N.P.	N.P.	400g tissue collected, frozen and shipped to a certified laboratory	Schecter et al., 1998
1998–2000	DDE	–50°C	N.P.	1g tissue collected, choosing tissue without calcification and avoiding decidua basalis and chorionic plate. Homogenized for 5 minutes.	Falcón et al., 2004
1998–2006	PCBs/DDE	–80°C	N.P.	Villous region of placenta collected after pregnancy terminations, tissue flash–frozen.	Doucet et al., 2009
Before 1999	PCBs/DDE	–20°C	N.P.	10g tissue collected, cut from periumbilical zone through intermediate zone to the marginal zone, homogenized with anhydrous sodium sulfate.	Reichrtová et al., 1999
2000–2001	PCBs	N.P.	N.P.	100g tissue collected, frozen and shipped to a certified laboratory	Wang et al., 2004
2003–2004	PCBs	N.P.	N.P.	Freeze dried then stored at room temperature	Gómara et al., 2012
2005–2006	DDE	–20°C	Rinsed DI	15g tissue collected (5g each from the center, paracenter, and margin), cut and freeze dried. The three samples were then combined.	Al–Saleh et al., 2012
2006	PCBs/DDE	–30°C	Removed	2g tissue collected, about 3–4 cm cut from near umbilical cord	Bergonzi et al., 2009

Notes: Information Not Provided (N.P.)

The EPA method for evaluating PCBs in tissues recommends reporting all results as concentration per weight of wet tissue with separate reporting of the lipid content (USEPA 2008). However, most of the articles reviewed did not provide a separate lipid content value: the concentrations of PCBs and DDE were reported at either concentration per weight lipid or concentration per wet tissue weight. The papers that did report placental lipid content had ranges between 0.4 and 1.5% (Rappolt 1968; Rogan et al., 1986; Bergonzi et al., 2009; Gómara et al., 2012). This indicates that the full term human placenta has low lipid content without much variation between samples.

2. **Extraction and analysis**

Extraction methods varied by study, however most consisted of sample homogenization, a solvent extraction, cleanup of the extract and analysis using gas chromatography (GC) coupled with an electron capture device (ECD) or a mass spectrometer (MS). The tissue extraction method suggested by EPA method 1668B is the traditional Soxhlet extraction (USEPA, 2008). Table X provides details of extraction techniques utilized by other studies. Cleanup and analysis techniques utilized for placenta analysis are listed in Table XI.

All of the techniques reviewed were unique. None used the same extraction solvent, technique, nor the same analytical instrument as was performed in this study. The Gómara (2012) study used a similar cleanup technique as this study.

TABLE X
EXTRACTION TECHNIQUES FOR PLACENTAL TISSUES

Date	Analyte(s)	Extraction Solvent	Extraction technique	Source
Before 1986	PCBs	1) 50 mL 0.45 N KOH, 5 mL n–heptane. 2) n–hexane and 4% ethyl ether–n–hexane	6g tissue with solvent #1 was heated for 2 hours using a heat transfer medium collector with reflex condensers. Dehydrated, and transferred to Florisil column eluted with solvent #2.	Ando, 1986
1998–2000	DDE	30 mL n–hexane	Extracted with hexane, sonicated for 10 minutes, filtered through anhydrous sodium sulfate	Falcón et al., 2004
1998–2006	PCBs/DDE	2:1 acetone:hexane	1 g thawed, homogenized in 20 mL 2:1 acetone/hexane solution for 1 min. Supernant filtered through glass wool, dried with anhydrous sodium sulfate	Doucet et al., 2009
Before 1999	PCBs/DDE	250 cm^3 n–hexane	Soxhlet extraction for 5 hours	Reichrtová et al., 1999
2003–2004	PCBs	1:1 cyclo–hexane/methyl–tert–butyl ether (v:v)	Proteins were denaturalised with hydrochloric acid and 2–propanol	Gómara et al., 2012
2005–2006	DDE	10 mL (1:2:2) ethyl acetate–methanol–acetone	Extracted with solvent and shaken vigorously 4 minutes. Ultrasonic bath for 20 minutes, centrifuged at 2000 rpm for 20 min at 20C	Al–Saleh et al., 2012
2006	PCBs/DDE	2:1 acetone:n–hexane (v:v)	2 min homogenization in a glass tube, 3000 rpm centrifuge for 5 minutes, upper layer transferred, dried, dissolved in hexane with anhydrous sodium sulfate, rinsed twice and concentrated to 1 mL	Bergonzi et al., 2009

TABLE XI
CLEANUP AND ANALYSIS TECHNIQUES FOR PLACENTAL TISSUES

Date	Analyte(s)	Cleanup	Analysis	Source
Before 1986	PCBs	Concentrated to 4 mL, then passed through a Florisil column chromatography and silica gel for column chromatography	GC/MS and GC/ECD	Ando, 1986
1998–2000	DDE	Concentrated to 5 mL, then eluted through Florisil with 85:15 petroleum ether:ethilic ether. Dried and reconstituted with 2mL hexane.	GC/ECD	Falcón et al., 2004
1998–2006	PCBs/DDE	2% water–deactivated Florisil column, eluted with 70 mL hexane.	GC/MS (EI)	Doucet et al., 2009
2003–2004	PCBs	Multi–layer silica gel column	GC/ECD	Gómara et al., 2012
2005–2006	DDE	Supernant diluted with 13 mL DI water, passed through Bond Elut–C13 cartridges at rate of 4–5 mL/minute. Cartridges washed with 2x1mL 25% acetonitrile–water. Eluted with 2x0.5mL isooctane.	GC/ECD	Al–Saleh et al., 2012
2006	PCBs/DDE	SPE by LC–Florisil and LC–Si tubes in serial connection, eluted with hexane	GC/MS (EI)	Bergonzi et al., 2009

D. The National Children's Study

1. Background and purpose

The NCS is an observational longitudinal study of over 100,000 children in the United States. This study will follow these children from before birth until the age of 21 to examine health related outcomes due to environmental exposures. Congress, with the passing of the Children's Health Act of 2000, authorized the National Children's Study. The purpose of this study is to evaluate the effects of the environment, everything from pollution to physical activity, on child health and development. This will be the longest cohort study of this kind in the United States and will shed light on factors that influence health as well as health disparities throughout the nation (National Children's Study, 2011). The purpose of the pilot study, performed prior to the main study, was to determine study feasibility and to work out and fine tune any procedural issues that may occur. The data presented in this thesis is part of the pilot study.

2. Project 18 placental study

Formative research project 18 of the NCS focuses on human placentas. Analyses performed for Project 18 include stem cell and genetic research, morphology studies, and environmental exposures. Sixteen institutions from across the United States participated in this project. A total of 43 placentas were collected for the pilot study from three collection sites: University of Rochester (UR), the University of California, Davis (UCD), and the Medical College of Wisconsin (MCW).

The purpose of the pilot study was to determine how collection time influences analysis of the placentas. For the pilot, multiple placental tissue samples were collected at different time periods after birth and sent to laboratories for analysis (NCS Project 18, 2011). Maternal data and fetal characteristics were not collected for this portion of the study. Our laboratory was

responsible for the analysis of 41 organic compounds: 32 PCB congeners, DDE, as well as 10 PBDE congeners not presented in this paper.

III. MATERIALS AND METHODS

A. Method Background

The method for this experiment was developed by Dassanayake et al. (2009). The matrix solid phase dispersion (MSPD) method of extraction was optimized for the extraction of PBDEs in human placental tissues. This method was found to be comparable to the standard Soxhlet extraction procedure and had better efficiency than a liquid extraction method (Dassanayake et al., 2009). Figure 7 shows the sequence of steps followed for this analytical procedure.

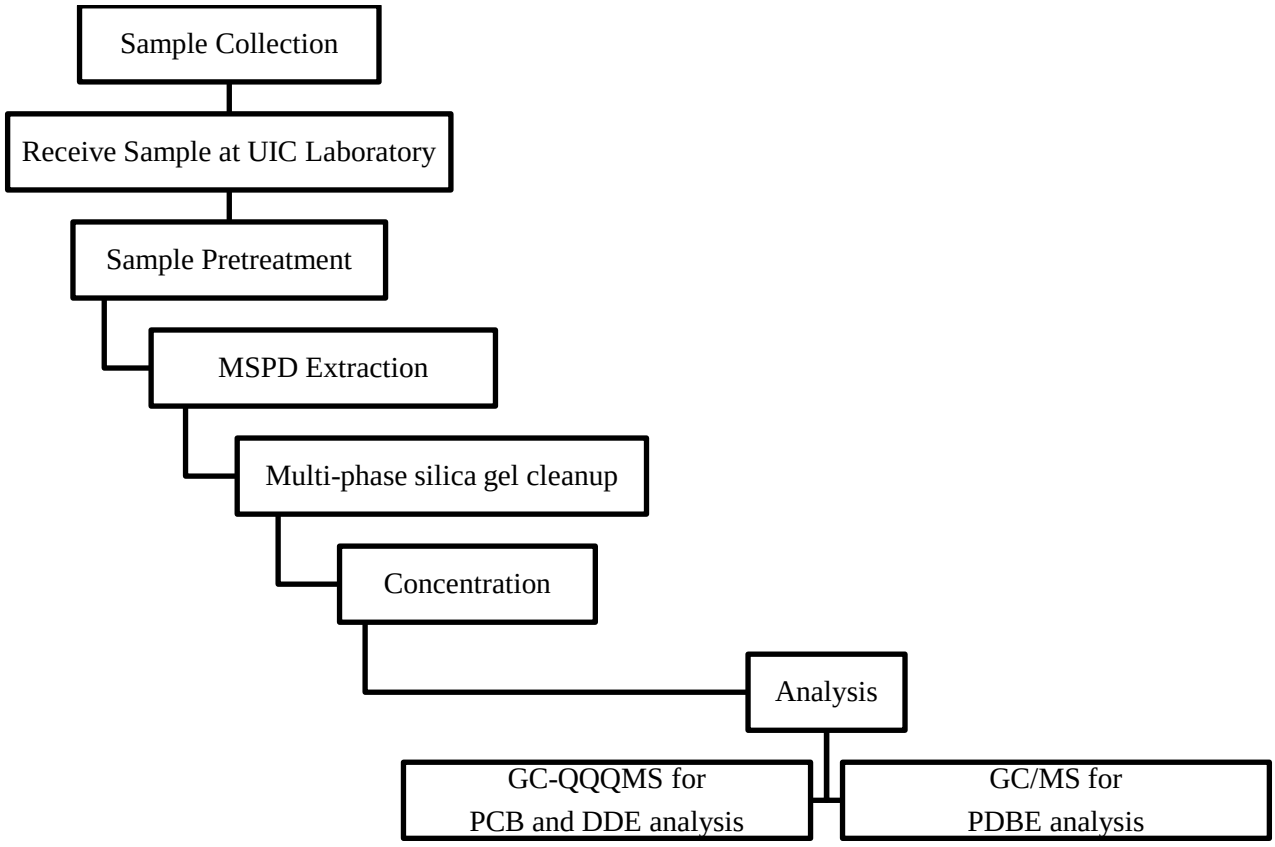


Figure 7. Sequence of steps for placental analysis.

B. **Chemicals**

A standard mix of 32 chlorinated biphenyls was used for this project along with DDE. The PCB Congeners Mix #6 (food and human tissue analysis standard mix), DDE, ^{13}CB52, and ^{13}CB47 standards were purchased from AccuStandard (New Haven, Connecticut). The 32 PCB congeners in the Mix #6 (AccuStandard catalog number C–SCA–06) were selected based on the review of PCB occurrence in human tissues and food products, as well as for the persistence of the congeners in the environment and their perceived toxicity (Jones, 1988). A list of the PCB congeners analyzed in order of their molecular weight is found in Table XII. Those compounds that are predicted to account for more than 70% of total PCB tissue burden are bolded. The other PCBs were selected due to their toxicity in humans and their relative persistence (Jones, 1998).

Optima grade > 99.9% hexane and HPLC GC/MS grade DCM (Fisher Scientific, Pittsburgh, Pennsylvania) were used for the extraction. The 60–100 mesh Florisil, anhydrous sodium sulfate (Certified A.C.S.), and silica gel (100–200 mesh, Davisil, Grade 644) were also purchased from Fisher Scientific.

TABLE XII

COMPOUNDS ANALYZED SORTED BY MOLECULAR WEIGHT.

Compound ID	Scientific Name	CAS	Formula	Molecular Weight
PCB 8	2,4'–Dichlorobiphenyl	34883–43–7	C12H8Cl2	223.1
PCB 28	**2,4,4'–Trichlorobiphenyl**	**7012–37–5**	**C12H7Cl3**	**257.5**
PCB 37	3,4,4'–Trichlorobiphenyl	38444–90–5	C12H7Cl3	257.5
PCB 44	2,2',3,5'–Tetrachlorobiphenyl	41464–39–5	C12H6Cl4	292.0
PCB 49	2,2',4,5'–Tetrachlorobiphenyl	41464–40–8	C12H6Cl4	292.0
PCB 52	2,2',5,5'–Tetrachlorobiphenyl	35693–99–3	C12H6Cl4	292.0
PCB 60	2,3',4',5–Tetrachlorobiphenyl	32598–11–1	C12H6Cl4	292.0
PCB 66	2,3',4,4'–Tetrachlorobiphenyl	32598–10–0	C12H6Cl4	292.0
PCB 70	2,3,4,4'–Tetrachlorobiphenyl	33025–41–1	C12H6Cl4	292.0
PCB 74	**2,4,4',5–Tetrachlorobiphenyl**	**32690–93–0**	**C12H6Cl4**	**292.0**
PCB 77	**3,3',4,4'–Tetrachlorobiphenyl**	**32598–13–3**	**C12H6Cl4**	**292.0**
p,p'–DDE	4,4'–dichlorodiphenyldichloroethylene	72–55–9	C14H8Cl4	318.0
PCB 82	2,2',3,3',4–Pentachlorobiphenyl	52663–62–4	C12H5Cl5	326.4
PCB 87	2,2',3,4,5'–Pentachlorobiphenyl	38380–02–8	C12H5Cl5	326.4
PCB 99	**2,2',4,4',5–Pentachlorobiphenyl**	**38380–01–7**	**C12H5Cl5**	**326.4**
PCB 101	2,2',4,5,5'–Pentachlorobiphenyl	37680–73–2	C12H5Cl5	326.4
PCB 105	**2,3',4,4',5–Pentachlorobiphenyl**	**31508–00–6**	**C12H5Cl5**	**326.4**
PCB 114	2,3,3',4,4'–Pentachlorobiphenyl	32598–14–4	C12H5Cl5	326.4
PCB 118	**2,3,4,4',5–Pentachlorobiphenyl**	**74472–37–0**	**C12H5Cl5**	**326.4**
PCB 126	**3,3',4,4',5–Pentachlorobiphenyl**	**57465–28–8**	**C12H5Cl5**	**326.4**
PCB 128	**2,2',3,3',4,4'–Hexachlorobiphenyl**	**38380–07–3**	**C12H4Cl6**	**360.9**
PCB 138	**2,2',3,4,4',5'–Hexachlorobiphenyl**	**35065–28–2**	**C12H4Cl6**	**360.9**
PCB 153	**2,2',4,4',5,5'–Hexachlorobiphenyl**	**35065–27–1**	**C12H4Cl6**	**360.9**
PCB 156	**2,3,3',4,4',5–Hexachlorobiphenyl**	**38380–08–4**	**C12H4Cl6**	**360.9**
PCB 158	2,3,3',4,4',6–Hexachlorobiphenyl	74472–42–7	C12H4Cl6	360.9
PCB 166	2,3,4,4',5,6–Hexachlorobiphenyl	41411–63–6	C12H4Cl6	360.9
PCB 169	**3,3',4,4',5,5'–Hexachlorobiphenyl**	**32774–16–6**	**C12H4Cl6**	**360.9**
PCB 170	**2,2',3,3',4,4',5–Heptachlorobiphenyl**	**35065–30–6**	**C12H3Cl7**	**395.3**
PCB 179	**2,2',3,3',5,6,6'–Heptachlorobiphenyl**	**52663–64–6**	**C12H3Cl7**	**395.3**
PCB 180	**2,2',3,4',5,5',6–Heptachlorobiphenyl**	**52663–68–0**	**C12H3Cl7**	**395.3**
PCB 183	2,2',3,4,4',5',6–Heptachlorobiphenyl	52663–69–1	C12H3Cl7	395.3
PCB 187	2,2',3,4,4',5,5'–Heptachlorobiphenyl	35065–29–3	C12H3Cl7	395.3
PCB 189	2,3,3',4,4',5,5'–Heptachlorobiphenyl	39635–31–9	C12H3Cl7	395.3

Note: Congeners bolded are predicted to account for >70% total PCB tissue burden (Jones, 1998)

C. Glassware Preparation

All glassware in contact with the samples was cleaned thoroughly with Contrad 70 soap and water followed by several rinses with DI water and allowed to dry. Periodically the glassware were placed in a furnace at 500°C overnight to further remove possible contaminants. Before use, the glassware was solvent–washed three times each with acetone, DCM, and finally with hexane.

D. Sorbent Preparation

Silica gel was activated in an oven at 150°C for at least 15 hours prior to use. It was then cooled in a desiccator and stored in a pre-cleaned screw-top bottle. Acidic silica was prepared by mixing 100 g activated silica gel with 40 g concentrated sulfuric acid. This was thoroughly mixed and stored in a sealed container. Basic silica was prepared by thoroughly mixing 100 g activated silica gel with 30 g 1N sodium hydroxide.

Granulated sodium sulfate was activated in the oven at 150°C for at least 12 hours. Florisil was activated in the oven at 150°C for between 12–14 hours. Both the Florisil and sodium sulfate were cooled and stored in a desiccator in airtight containers to prevent moisture exposure.

E. Tissue Collection

Placentas were collected from consenting women after childbirth from three locations: UR, UCD, and MCW. The collection procedure is outlined in the NCS Project 18 revised standard operating procedure for pilot study collections revised on August 31, 2011 (NCS Project 18, 2011). Placentas were received shortly after childbirth, ideally within 10 minutes

after delivery of the placenta. Placental weight was obtained if possible and the time received was recorded. Cord blood was collected for other Project 18 studies.

For collection of the placental villous tissue, the maternal tissue flaps were expanded and approximately 45 grams or tissue removed, avoiding maternal decidua. The tissue sample was then blotted on a gauze pad and stored in 50 mL collection tubes, with the tissue volume reaching at least the 10 mL mark on the tube. The samples were then placed on ice until they could be frozen at -20°C and the time of collection was recorded.

The remaining placenta was stored in a refrigerator at 4°C until the next sampling time. A total of 169 specimens were collected for analysis from 43 placentas. Collection times ranged from 0 hours from delivery to 120 hours from delivery. All specimens for this study were then shipped overnight to UR with temperature monitors and dry ice. Upon retrieval at UR, the shipment was inspected for damages and temperature requirements. The tissue was then divided and labeled with an analytical ID number to ensure the analytical sites were blinded as to which sample came from which placenta (NCS Project 18, 2011).

The samples were then packaged and shipped on dry ice to UIC. Table XIII shows the placenta sample IDs corresponding to the analytical ID and the hour the tissue was collected. Placenta IDs beginning with 10– were collected at UR, those beginning with 20– were collected at UCD, and those beginning with 30– were collected at MCW.

TABLE XIII

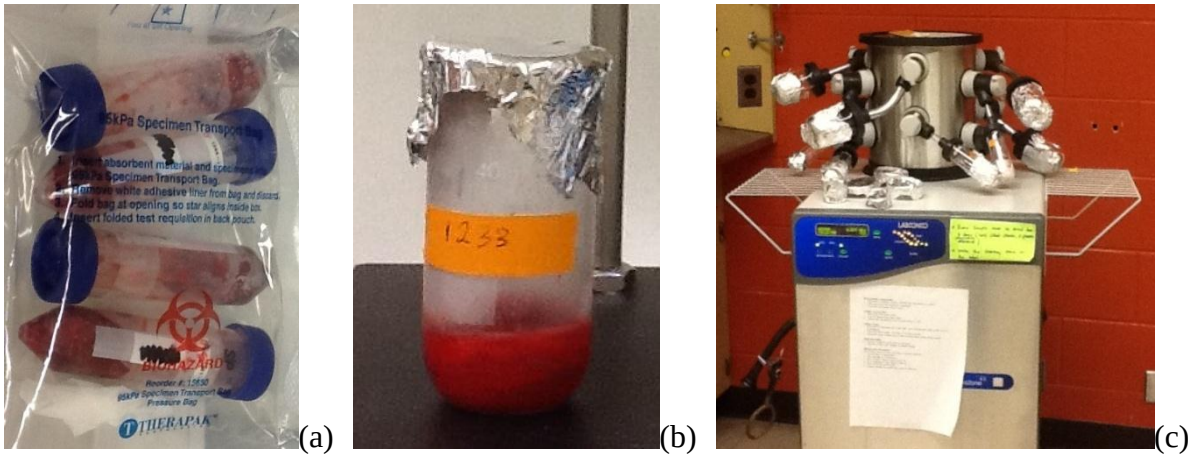
PLACENTA SAMPLE IDS AND ANALYTICAL SAMPLE IDS

	Hour of Sample Collection Following Delivery											
Placenta ID	0	1	2	4	8	12	24	36	48	72	96	120
1001	1001	1002	1003		1004				1009			
1002	1005	1006	1007		1008							
1003	1010	1011	1012		1013				1020			
1004	1103	1101	1104		1102							
1005	1063	1062	1060		1059				1061			
1006	1068	1067	1066		1065				1064			
3007	1021			1022	1023	1024						
3008	1025			1026	1027	1028						
3009	1029			1030	1031	1032						
3010	1172			1173	1054	1053						
3011	1122			1124	1125	1123						
3012	1141			1140		1142						
2013	1015		1016	1017		1018	1019					
2014	1033		1034	1035		1036	1037					
2015–A	1038		1040	1042		1044	1046					
2015–B	1039		1041	1043		1045	1047					
2016	1117		1121	1118		1119	1120					
2017	1110		1108	1107		1109	1106					
2018	1132		1136	1133		1134	1135					
1019	1072	1069					1070	1071				
1020	1051	1050					1049	1048				
1021	1080	1078					1077	1079				
1022	1097	1099					1100	1098				
1023	1167	1166					1165	1164				
1024	1171	1169					1170	1168				
3025	1111							1113		1112		
3026	1114							1116		1115		
3027	1163							1162		1161		
3028	1151							1150		1149		
3029	1160							1158		1159		
3030	1138							1137		1139		
2031	1156								1155		1157	
2032	1152								1154		1153	
2033	1143								1144		1145	
2034	1127								1128		1126	
2035	1129								1131			1130
2036	1147								1146			
1037	1087	1088								1086	1085	
1038	1055	1058								1057	1056	
1039	1076	1075								1073	1074	
1040	1083	1082								1084	1081	
1041	1092	1091								1090	1089	
1042	1095	1096								1093	1094	
	Analytical ID displayed above in boxes											

Note: Corresponding to the hour the tissue was collected. Placentas 2015–A and 2015–B came from one mother who had twins with 2 separate placentas.

F. **Sample Pre–treatment**

Samples were received frozen on dry ice and were immediately transferred to a -20°C freezer until processing time. The analytical ID, date, time, and sample condition were recorded. Prior to analysis, the samples were defrosted, homogenized using solvent rinsed scissors, and transferred to pre-cleaned freeze-drier flasks. The initial wet weights were recorded. The samples were re–frozen overnight at -20°C. The flasks were then attached to the freeze-drier where they were lyophilized for between 1–4 days depending on the number of samples attached to the freeze dryer.



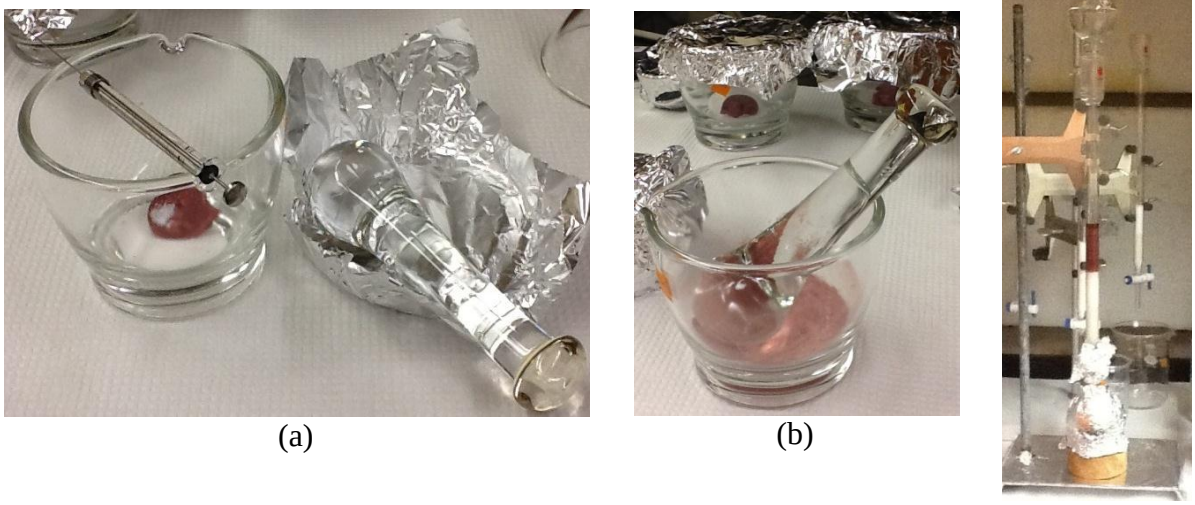
(a) (b) (c)

Figure 8. Pre-treatment process of samples: (a) samples received at UIC, (b) homogenized sample, and (c) samples on the freeze drier.

The starting and ending time of lyophilization was recorded on the Chain of Custody form (COC). Once the samples were completely dry, the dry weight of the sample was taken. If the samples were not processed at once they were covered in foil and stored in desiccators.

G. **Sample Extraction**

Dried samples were transferred to pre–cleaned mortar The surrogate, 1 ng of carbon-13 labeled CB52 (^{13}C-CB52), was added using a glass syringe directly to the dried tissue Florisil sorbent was added in a 2:1 sorbent to sample ratio. The tissue and sorbent mixture was then ground with a pestle for approximately five minutes (Figure 9).



(a) (b) (c)

Figure 9. Preparation of dried tissue for (a) surrogate addition, (b) grinding and (c) extraction.

Extraction columns were prepared in pre-cleaned glass columns (13.4 mm inner diameter, 305 mm long) by adding a glass wool plug to the bottom and loading it with 10 g anhydrous sodium sulfate and 4 g activated Florisil. The column was rinsed with 20 mL of 4:1 hexane to DCM mixture and vacuum dried. The tissue-sorbent mixture was then added to the top of the Florisil layer with intermittent tapping to increase compactness.

A 120 mL mixture of 4:1 hexane to DCM mixture was used to rinse the mortar and pestle and extract the analytes from the column. Extract was collected at a flow rate of 1–2 drops per second in a pre-cleaned pear-shaped flask under gravitational flow. After collection each sample was concentrated to approximately 2 mL using a rotary evaporator (Hei-Vap Advantage). Foil was used to cover sample extract whenever possible to prevent light exposure and the contamination by falling dust.

H. **Sample Cleanup**

Glass cleanup columns with 11 mm inner diameter and 400 mm length were plugged with glass wool and filled with hexane. The columns were filled from bottom to top with 1 g anhydrous sodium sulfate, 1 g activated silica, 1 g basic silica, 1 g activated silica, 4 g acidic silica, 1 g activated silica, and 5 g anhydrous sodium sulfate while tapping the column constantly to prevent air bubbles. The hexane was drained from the column until it was approximately 1 mm from the top sodium sulfate layer. The column was then rinsed with an additional 20 mL of hexane and again drained to 1 mm from the top layer.

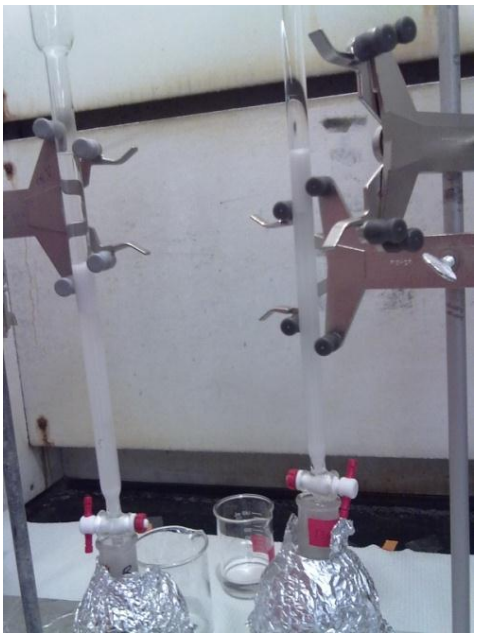


Figure 10. Multi-phase silica gel cleanup column.

The sample extract was carefully transferred onto the top layer of the column. It was eluted with 50 mL hexane, after using a few milliliters to rinse the pear-shaped flask, which was then added to the column. Extract was collected in a pre-cleaned pear-shaped flask at a flow rate of 1–2 drops per second until the column stopped dripping as shown in Figure 10.

I. **Final Concentration**

The final extract was concentrated using a rotary evaporator to approximately 0.5–2.0 mL. The flask was allowed to cool for several minutes before releasing the vacuum of the rotary evaporator. After the flask was removed, the vapor tube end of the rotary evaporator that was inside the collection flask was rinsed with approximately 0.5 mL of hexane into the flask.

The sample was then transferred to a K-D tube. The collection flask was rinsed 3 times with hexane, and combined with the sample in the K-D tube. The extract was concentrated using a nitrogen evaporation device that was adjusted so the solvent surface was visibly disturbed but no vortexes were formed. Once concentrated to 0.5 mL, the sample was transferred to a 1 mL volumetric flask. The K-D tube was rinsed 3 times using less than 0.5 mL hexane and this rinse solution was added to the final sample and brought to 1 mL. This was transferred to an amber glass storage vial, sealed and stored in the refrigerator prior to analysis.

J. **Instrumental Analysis**

Immediately before analysis, the internal standard ^{13}C-CB47 was added to the sample. The samples were analyzed using an Agilent 7890A gas chromatograph (GC) coupled with an Agilent 7001B triple quadrupole mass spectrometer (QQQMS). The GC/QQQMS was equipped with a multi-mode injection port and a 7693 auto sampler. A Restek Rxi-XLB column (30 m length x 0.25 mm inner diameter x 0.1 um film thickness) was used for compound separation.

The operational condition of the GC was as follows: The initial inlet temperature was held for one minute at 60ºC then ramped at a rate of 600ºC/min to 300ºC. The vent flow was 100 mL/min and the purge flow was 50 mL/min. Three injections of 25 uL each, total of 75 uL, were made in each run. The column flow was set at 1.1 mL/min using helium (He) as the carrier gas. The oven conditions were as follows: initial temperature of 45ºC held for two minutes, increased to 150ºC at 10ºC/min, increased to 200ºC at 2ºC/min and held for five minutes, followed by an increase to 300ºC at 10ºC/min and held for 1.5 minutes. The total run time was 54 minutes. The interface temperature was set at 300ºC.

The operational condition of the QQQMS was as follows. The electron impact (EI) ionization source temperature was set at 230°C, and the ionization voltage was -70 V. The temperature of the MS-1 analyzer was set at 150°C, and that of the MS-2 at 150°C. The collision cell gases were helium at 2.25 mL/min and nitrogen (N_2) at 1.5 mL/minute. Multiple-reaction monitoring (MRM) was used for data acquisition. The collision energy was optimized, and is summarized in Table XIV, along with the precursor and product ions for each PCB homolog and for DDE.

Agilent computer software MassHunter (version B04.00) was used for controlling GC/QQQMS operation, data acquisition and quantification of the concentrations. Quantification was performed using internal standards with linear response factors.

TABLE XIV
DATA QUANTITATION USING MRM TRANSITIONS

Analyte	Precursor ion	Product ion (Q, q)	Collision energy
Di PCB	221.9	187.1, 152.0	10, 32
Tri PCB	255.9	186.0, 221.0	28, 10
Tetra PCB	291.9	220.0, 222.0	32
Penta PCB	325.8	256.0, 254.0	31
Hexa PCB	359.8	289.9, 287.9	31
Hepta PCB	393.8	323.9, 321.9	31
DDE	246.0, 317.9	176.0, 246.0	30, 24
CB 47L	303.9	234.0, 232.0	31
CB 52L	303.9	234.0, 232.0	31

Note: MRM transitions in EI ionization mode.

All PCBs, one precursor ion and two product ions (quantifier, Q and a qualifier, q) were selected. The compound DDE had two separate transitions for quantifier ($246.0 \rightarrow 176.0$) and qualifier ($317.9 \rightarrow 246.0$).

K. **Quality Assurance and Control**

Procedural blanks were run with each batch of 10–15 samples, following the same procedure as sample extraction. Matrix and blank spike recovery experiments were also performed. Recovery standards (surrogates) were added to each sample before extraction, and their recoveries were used to determine the efficiency and accuracy of the analytical procedure. For PCBs and DDE the surrogate used was ^{13}C-CB52. Duplicate analyses could not be performed due to of a lack of tissue volume that would be required to split the sample. Most samples received were around 13 g wet weight, which when freeze dried was generally less than 4 g dry weight. The extraction procedure was optimized for 4 g of dry tissue.

IV. RESULTS AND DISCUSSION

A. Site and Analyst Data

A total of 43 placentas were collected from three locations (Table XV). Three analysts processed a total of 167 samples from the 43 placentas, analyzing each sample from extraction to final concentration measurement using GC/QQQMS (Table XVI). The gas chromatography column was changed after running the first 47 samples received (analytical IDs 1001 through 1047) due to sensitivity issues.

TABLE XV
NUMBER OF SAMPLES FROM EACH SITE

Site Location	University	# of placentas	# of samples
Rochester, NY	University of Rochester (UR)	18	74
Davis, CA	University of California – Davis (UCD)	13	52
Milwaukee, WI	Medical College of Wisconsin (MCW)	12	41
Total		43	167

TABLE XVI
NUMBER OF SAMPLES EXTRACTED BY ANALYST

Analyst Initials	# of samples
SD	25
JN	69
YX	73
Total	167

One sample, analytical sample ID 1083 from placenta ID 1040, was lost during analysis due to the storage vial cracking with spilling of the contents. This sample was collected in

Rochester, New York at collection time 0 hours. Analytical sample ID 1165 from placenta ID 1023 was missed during GC/MSQQQ analysis and is not included in this dataset. Therefore only 42 of 43 placentas will have results from the initial collection time (time = 0) and only 167 of the 169 analytical samples are reported in this paper.

B. PCB Congener Coelution

To determine if any other PCB congeners coelute with the 32 congeners of interest, a control sample containing all 209 PCB congeners was analyzed by GC/QQQMS. Only one congener 2,2',3,3',4,4'–hexachlorobiphenyl (PCB 128) was not separated from 2,3,3',4,5,5'-hexachlorobiphenyl (PCB 159) owing to the similar retention times (39.74 and 39.73 respectively).

C. Pooled Sample Concentrations

The data in this section present analysis of all 167 analytical samples combined.

1. DDE concentrations

Concentrations of DDE were detected in all samples analyzed (n=167) with concentrations ranging from 9.8 pg/g wet tissue weight (wwt) to 3,220 pg/g wwt. The summary statistics are listed in Table XVII and the data for each sample can be found in Appendix A. Most (86%) of the samples had DDE concentrations below 200 pg/g wwt (Figure 11).

TABLE XVII

DDE STATISTICS IN ALL PLACENTA SAMPLES AT ALL COLLECTION TIMES (N=167)

Average	STD	RSD, %	Minimum	10%	Q1	Median	Q3	90%	Maximum
208	470	226	9.8	32.5	48.3	82.2	151	269	3220

Notes: Standard Deviation (STD); Relative Standard Deviation (RSD) % = Average/Standard Deviation; % Non–detect (N.D.) = Number of samples with concentration = 0 / total number of samples; Quartile 1 (Q1) = Median of the first half of data points; Quartile 3 (Q3) = Median of the second half of data points.

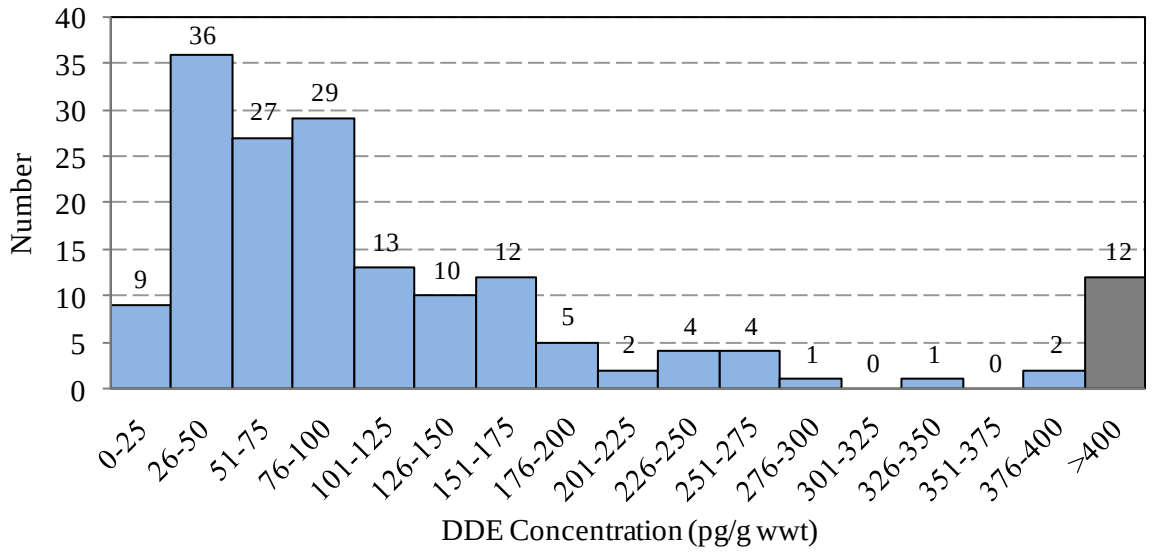


Figure 11. Frequency distribution of DDE concentrations in all samples.

The lipid content of the placenta samples could not be determined due to the limited amount of tissue provided. In order to compare DDE concentrations from this study with other studies (Table IV), a placenta lipid content of 1.25% (DeKoning and Karmus, 2000) was assumed. Thus, the median DDE concentration from this study, 82.2 pg/g wwt was estimated to be 6,573 pg/g lipid. The median values reported in the literature ranged from 58.3 pg/g lipid to 5,000,000

pg/g lipid (Brooks et al., 2007; Rappolt, 1968). The median value measured in this study was almost 100 times higher than the median of 58.3 pg/g (N = 19) reported in the United States by Brooks et al. (2007), but was comparable with concentrations measured in Slovakia in the 1990s (Reichrtová et al., 1999). Since complete data about the mothers who participated in this study are not available, it is difficult to compare the populations further to understand the differences between DDE levels.

2. **PCB concentrations**

Concentrations of PCB were detected in all samples analyzed. The concentration of the sum of the 32 PCB congeners analyzed, denoted Σ_{32} PCBs, ranged from 76.2 pg/g wwt to 1570 pg/g wwt. The summary statistics for individual PCB congeners and the Σ_{32} PCBs are presented in Table XIX. Individual sample results are in Appendix A. Certain congeners—82, 77, 114, 179, 138, 158, 166, 183, 126, 156, 180, 170, 169, and 189—were not present at detectable concentrations in some placentas (Table XVIII). The PCB congener 126 was detected with the lowest frequency.

TABLE XVIII

PCB CONGENER STATISTICS IN ALL PLACENTA SAMPLES AT ALL COLLECTION TIMES (N=167)

PCB	Average	STD	RSD, %	Min.	10%	Q1	Median	Q3	90%	Max.	% N.D.
8	31.9	13.5	42.4	3.7	15.9	21.3	31.1	40.7	47.8	72.9	0.0
28	47.7	30.1	63.1	4.9	21.2	28.8	39.2	57.5	88.1	193.2	0.0
37	11.5	8.3	71.9	0.3	4.5	6.4	9.1	13.9	21.4	61.6	0.0
44	27.9	11.5	41.3	4.0	15.0	19.7	27.2	34.8	41.6	84.5	0.0
49	17.4	7.3	42.1	2.5	9.8	12.5	16.6	21.1	25.9	59.3	0.0
52	46.9	19.3	41.2	6.5	25.6	34.4	45.6	58.3	68.2	135.0	0.0
60	33.3	21.4	64.2	1.6	7.0	15.6	31.2	45.7	64.6	94.3	0.0
66	14.6	6.9	47.3	2.2	6.9	9.6	13.9	18.2	24.3	38.7	0.0
70	33.8	17.3	51.1	4.3	15.5	21.6	30.6	43.6	56.2	110.8	0.0
74	13.3	7.5	56.3	2.7	7.0	9.1	12.1	16.2	20.2	81.7	0.0
77	14.3	29.4	206.0	0.0	0.3	0.5	0.7	12.7	55.0	174.5	0.6
82	2.6	2.2	81.8	0.0	0.0	1.2	2.4	3.7	5.4	13.8	21.0
87	19.7	15.7	79.8	2.6	8.2	10.8	16.0	24.3	33.3	151.3	0.0
99	17.7	8.3	47.0	4.0	8.8	11.7	16.2	22.7	28.0	60.9	0.0
101	16.7	27.4	164.3	0.6	2.0	2.8	4.0	15.0	55.3	157.7	0.0
105	7.0	5.1	72.2	0.9	2.9	4.1	5.7	8.5	12.1	41.7	0.0
114	0.5	0.4	81.8	0.0	0.0	0.0	0.5	0.7	0.9	2.0	26.4
118	26.0	18.5	71.3	2.9	10.3	14.2	20.6	32.3	47.0	131.1	0.0
126	0.0	0.0	131.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	52.1
128	0.9	1.1	126.5	0.0	0.0	0.0	0.8	1.2	1.7	9.0	26.4
138	16.9	16.5	97.5	0.0	5.6	7.7	11.4	19.6	33.8	100.0	0.6
153	22.4	17.5	78.0	3.6	8.6	12.3	17.0	26.3	40.4	103.9	0.0
156	1.5	1.8	117.9	0.0	0.0	0.5	0.9	2.1	3.6	10.4	22.2
158	0.7	0.6	90.0	0.0	0.0	0.0	0.6	1.0	1.3	4.3	26.4
166	0.1	0.1	168.7	0.0	0.0	0.0	0.1	0.1	0.1	1.2	35.3
169	0.3	0.3	93.0	0.0	0.0	0.0	0.2	0.4	0.7	1.1	28.1
170	2.6	3.7	143.8	0.0	0.0	0.7	1.6	3.0	5.1	26.3	18.6
179	1.0	1.4	143.8	0.0	0.0	0.4	0.6	1.0	2.1	12.7	13.2
180	8.1	8.6	105.5	0.0	2.1	3.4	5.9	9.5	13.9	54.4	1.2
183	1.2	2.5	209.2	0.0	0.0	0.4	0.9	1.4	2.1	30.6	23.4
187	3.7	3.7	99.1	0.0	1.4	1.9	2.7	4.1	6.2	25.3	3.6
189	0.1	0.2	155.3	0.0	0.0	0.0	0.1	0.2	0.3	2.1	27.5
$\sum_{32}$ PCB	442	228	52	76	227	300	395	543	700	1572	0

Note: Standard Deviation (STD); Relative Standard Deviation (RSD) % = Average/Standard Deviation; % Non–detect (N.D.) = Number of samples with concentration = 0 / total number of samples; Q1 = first quartile and Q3 = third quartile.

For comparison with previous studies, the concentrations of PCBs 28, 52, and 153 were converted to estimate per lipid concentrations, as was done for DDE. The estimated median concentrations on a per lipid basis are: 3,132 pg/g lipid, 3,650.4 pg/g lipid, and 1,361.6 pg/g lipid, respectively. Previous placenta studies measured PCB 52 concentrations in placental tissue to range from below the detection limit to 52,640 pg/g lipid (Table VII). Most PCB concentrations measured in this study fall within the range of previous studies. Interestingly PCB 28 and 52 were the most prevalent congeners in this study, yet they were not detected in one population–based survey of PCB placental burden (Bergonzi et al., 2009), and were below the detection limit in nearly half of the samples (45% for PCB 28, 41% for PCB 52) from another study (Reichrtová et al., 1999). The level of quantitation (LOQ) was not reported for the Reichrtová (1999) study, but in the Bergonzi (2009) study the LOQ was much higher than that of this study. The LOQ in that study was between 25–50 pg/g placenta. In this study, the median concentrations of PCB 28 and PCB 52 (39.15 and 45.63 pg/g respectively) are right between the LOQ values reported by Bergonzi (2009). Concentrations of PCB 153 measured in this study were 10-fold lower that was found by Bergonzi et al. (2009).

The variation between the distributions of individual PCB congener is illustrated in Figure 12. For some congeners, such as PCB 101 and 77, the distributions are visibly skewed. Due to the asymmetry of the data, statistical analyses were performed on log-transformed data. The Σ_{32} PCBs appear to be normally distributed, slightly skewed to the right (Figure 13).

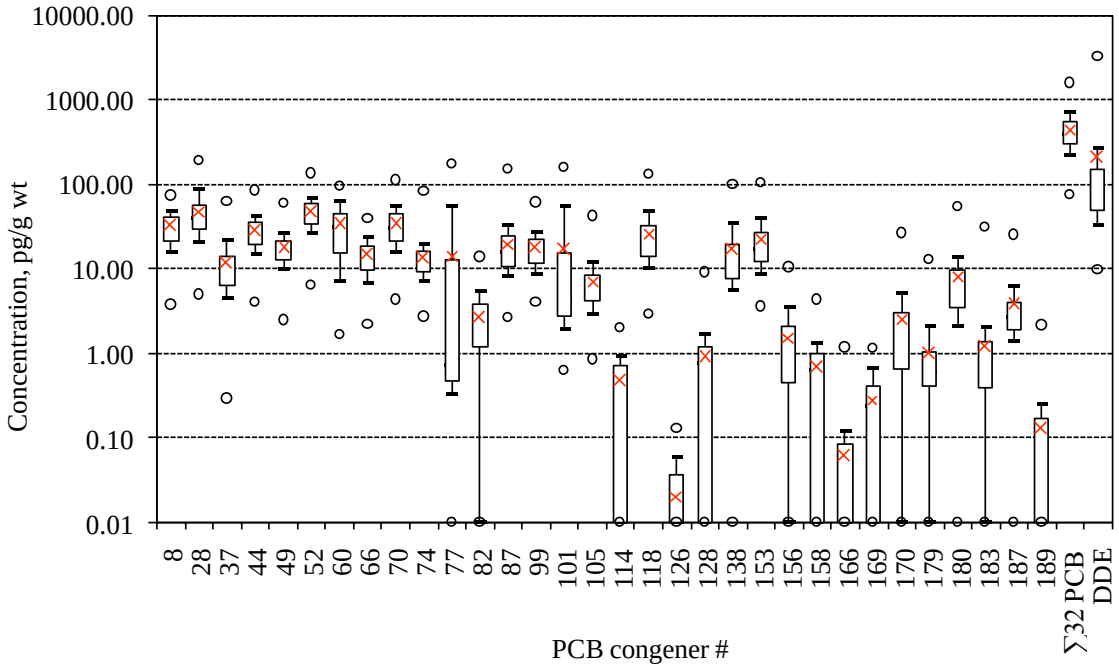


Figure 12. PCB congener distribution in the pooled samples.

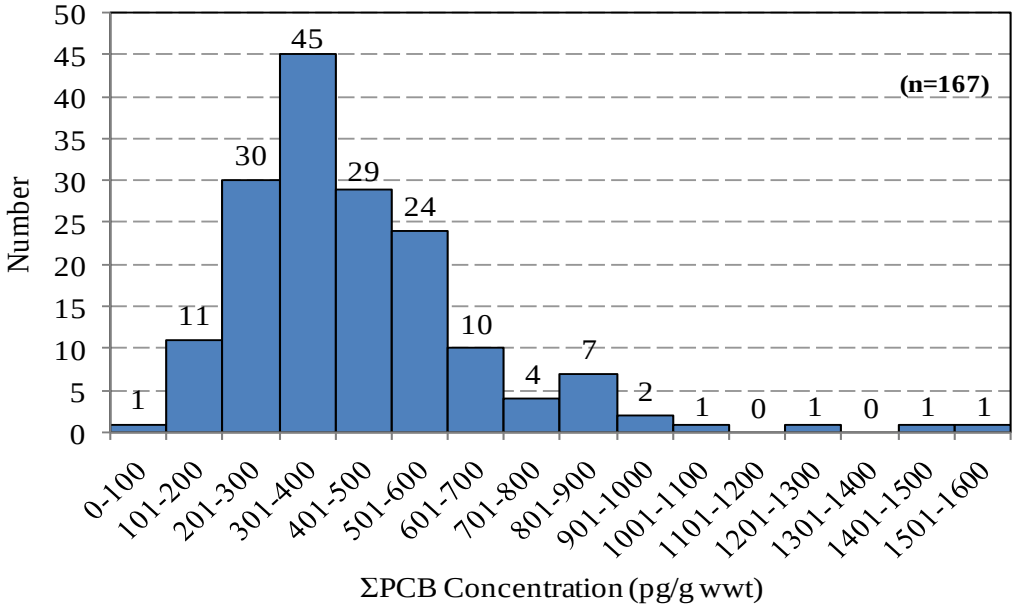


Figure 13. Frequency distribution of Σ_{32} PCB concentrations in all samples.

a. **PCB Congener Distribution**

Based on congener concentrations, PCB 52 has the highest concentration found in placenta samples, followed by other lower molecular weight PCBs such as 28, 60, and 70 (Figure 14). The PCB 126 congener was detected least frequently and was present in only 48% of the samples.

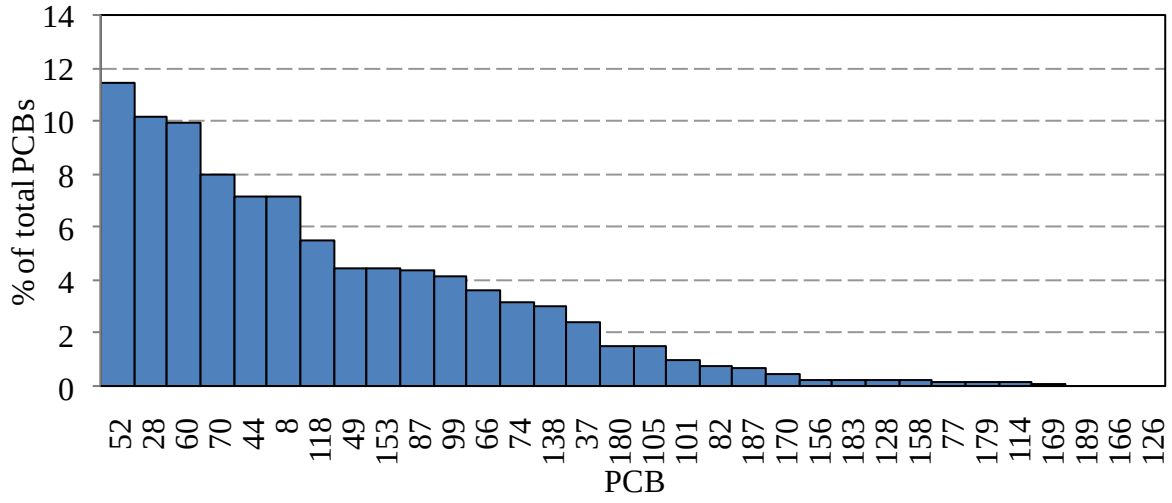


Figure 14. PCB congener density relative to $\sum_{32}$ PCB concentration, based on median values.

The relative levels of each PCB congener differ from other reviewed placenta studies (Table XIX). One other study (Gómara et al., 2012) found PCB 52 had the highest concentration among other PCBs analyzed. This congener along with PCB 101 accounted for 44% of the total PCBs mass found in that study. Other results found PCB 153 to be the most abundant congener (Bergonzi et al., 2009; Reichrtová et al., 1999). This is consistent with a 2003–2004 NHANES comparison study performed on pregnant and non-pregnant women using serum as a matrix

(Woodruff et al., 2011), in which the relative order of congener abundance was: 153 > 138/158 > 28 > 180 > 118 > 52 > 101 >> 126.

TABLE XIX
PCB CONGENER PATTERN FROM PREVIOUS PLACENTA STUDIES

Reference	Congener Pattern
Bergonzi et al., 2009	153 > 138 = 180 > 170 > 118 = 187 (52 and 28 N.D.)
Gómara et al., 2012	52 > 101 > 153 > 138 > 28
Reichrtová et al., 1999	
Urban population*	153 > 101 > 118 > 138 > 28 > 180 > 52
Rural population*	153 > 101 > 138 > 52 > 118 > 28 > 180

Note: *Rank based on maximum values; Not Detected (N.D.).

In the Reichrtová et al. (1999) study there was a difference in congener distribution between urban and rural populations. Similarly, upon visual comparison of the three collection sites in this study (Figure 15), the congener pattern varies slightly by location. Notably, MCW samples have a lower proportion of PCB 153 and a higher proportion of PCB 126 compared to UR and UCD. This may reflect regional differences in PCBs used in the past as well as regional differences in weathering and persistence of PCBs. The significance of these differences will be addressed in sections D and F of this section.

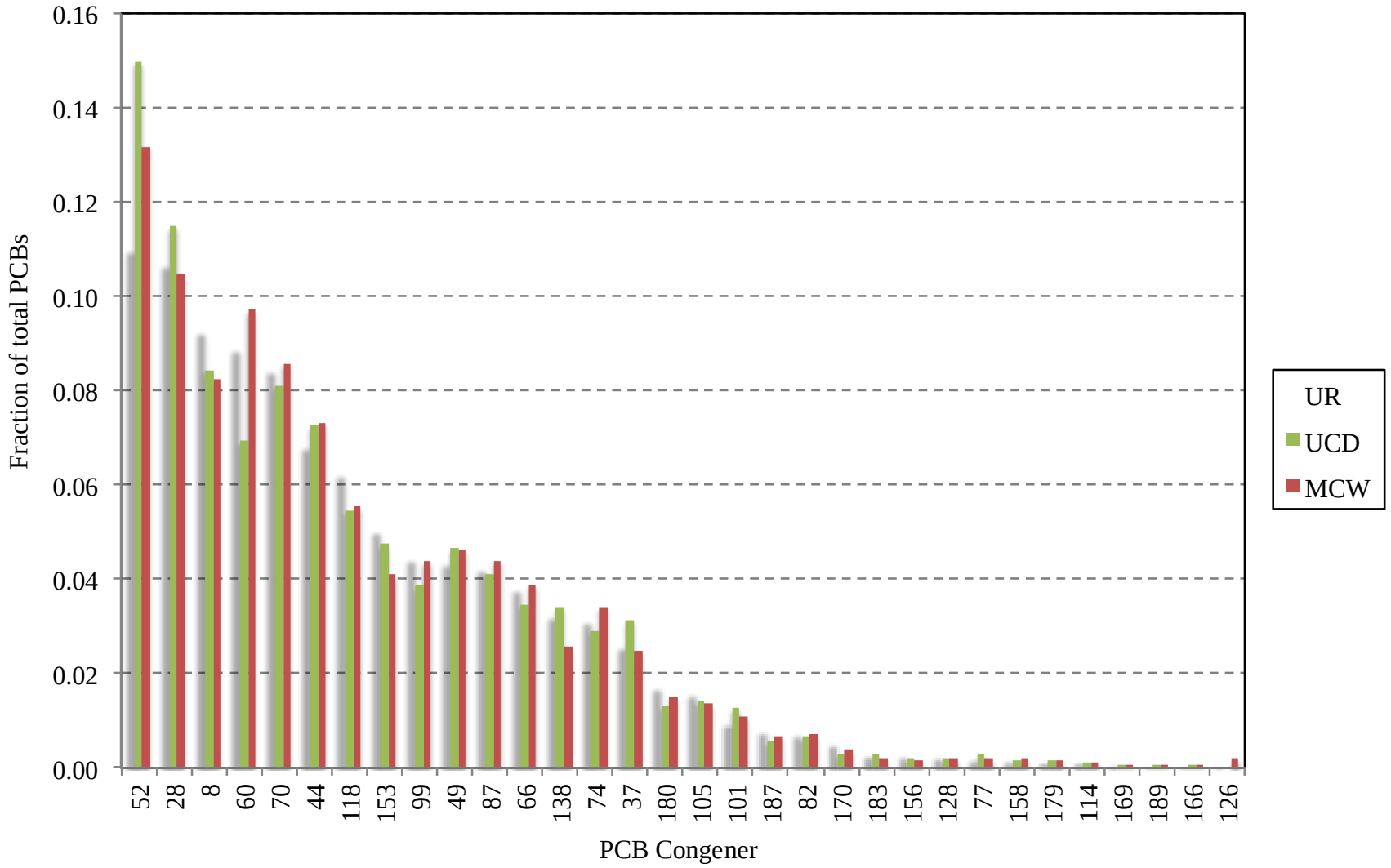


Figure 15. PCB congener pattern in samples by location and sorted by abundance from UR samples (time = 0, median values).

b. **Correlation**

The relationships between selected PCB congeners and DDE were explored using Spearman's rank correlation coefficient (Table XX). These congeners were selected because they were found in high concentrations in this study and those reviewed. The congener PCB 126 was included because it was the least frequently detected congener in this research. It appears that all PCB congeners and DDE are positively and statistically significantly correlated except PCB 126. There is a negative association between PCB 126 and both DDE and PCB 138. All other analytes, excluding PCB 126, showed positive associations indicating that when one congener increases in concentration the others do as well.

TABLE XX
SPEARMAN'S CORRELATION COEFFICIENTS FOR SELECTED CONGENERS MEASURED AT INITIAL COLLECTION TIME.

	28	52	101	118	126	138	153	180	Σ_{32}PCBs	DDE
28	1.00									
52	0.66	1.00								
101	0.91	0.57	1.00							
118	0.81	0.67	0.87	1.00						
126	–0.31	0.04*	–0.22*	–0.15*	1.00					
138	0.78	0.42	0.74	0.81	–0.31	1.00				
153	0.75	0.54	0.73	0.84	–0.19*	0.94	1.00			
180	0.38	0.08	0.39	0.34	–0.01*	0.61	0.65	1.00		
Σ_{32}PCBs	0.85	0.88	0.83	0.90	–0.10*	0.71	0.80	0.34	1.00	
DDE	0.52	0.26	0.48	0.49	–0.29	0.67	0.57	0.43	0.44	1.00

Notes: *Values are not statistically significant at p=0.05 level. Upper critical value for Spearman's Rank (p=0.05) is 0.257 for 42 placenta samples (at initial collection time).

D. **Collection Site Comparisons**

Figure 16 illustrates the concentrations of different PCB homologs with regard to individual placentas grouped by collection site. Each placenta showed visible differences in the type and level of PCB concentrations. A trend for greater levels of the less chlorinated PCB homologs present was observed along with lower measured levels of hexa- and hepta-chlorinated biphenyls (Figure 17).

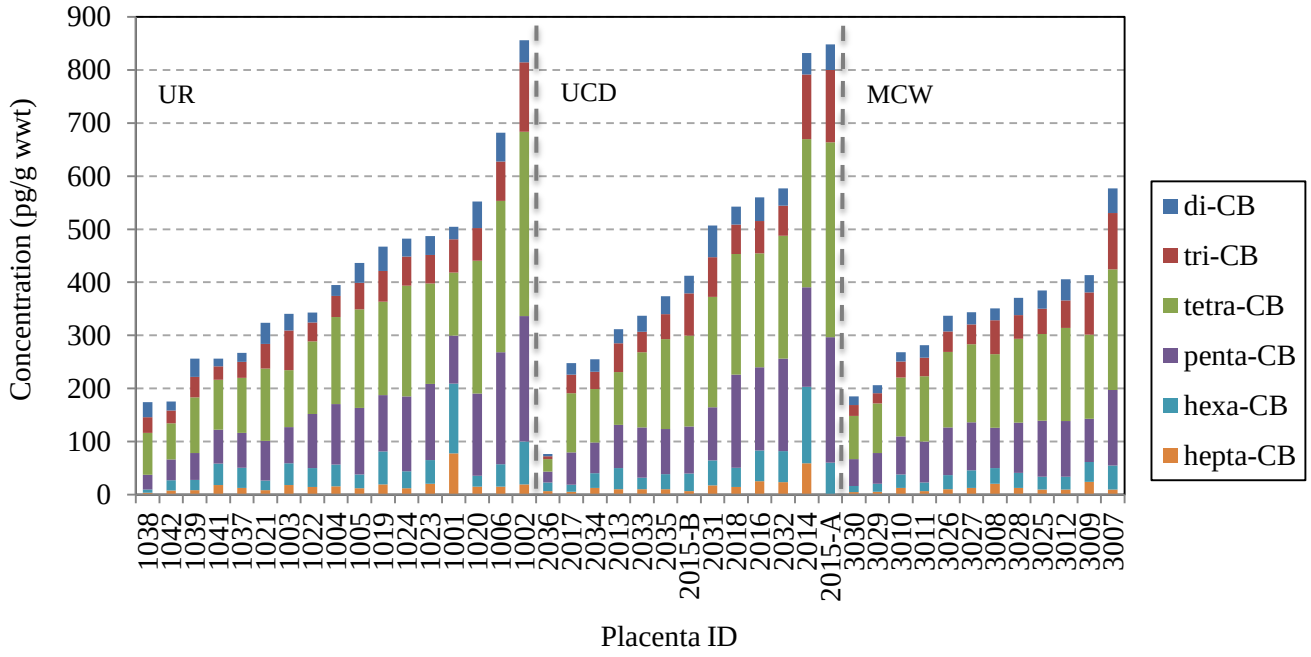


Figure 16. PCB homolog distribution in each placenta (time = 0).

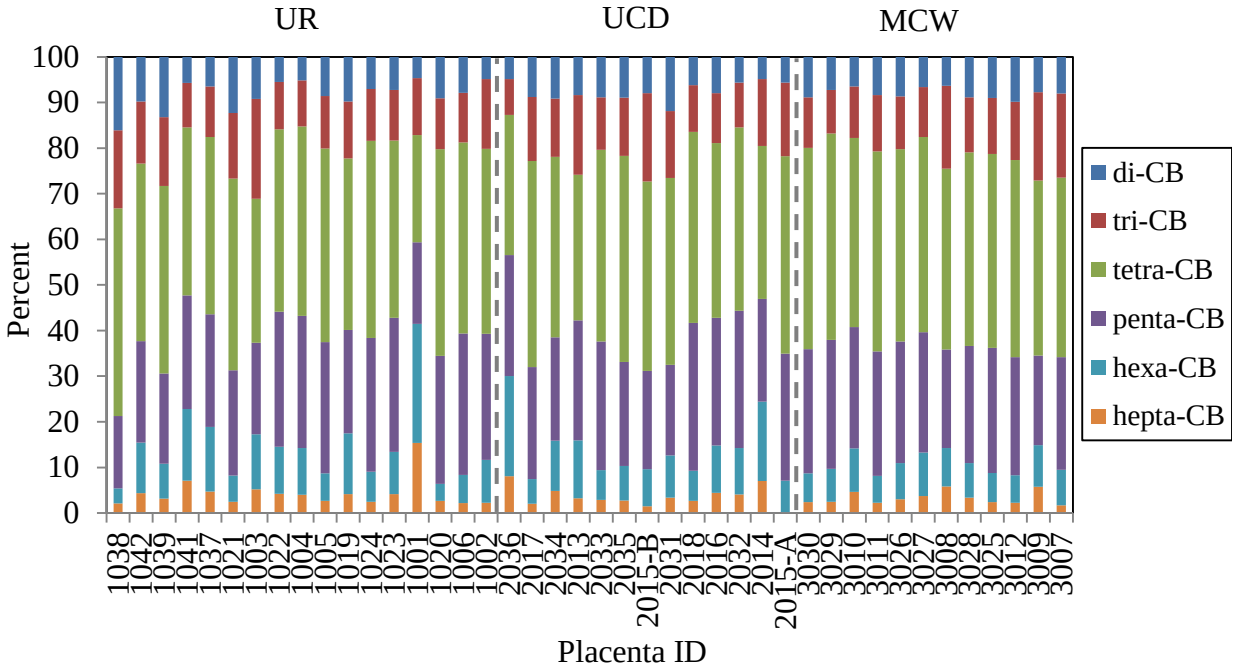


Figure 17. PCB homolog distribution as a percentage of total PCBs by placenta (time = 0).

We hypothesized that there would not be a statistical difference in PCB or DDE concentrations between collection sites. This hypothesis was tested using an ANOVA single factor test on the log–transformed means of selected analytes (PCBs 28, 52, 101, 118, 153, 138, 126, 180, Σ_{32} PCBs, and DDE) from the initial collection time. Indeed, the differences between collection sites were not statistically significant (p-value = 0.94 for all congeners).

E. **Collection Time Comparisons**

To evaluate how time of collection influences the concentrations in each placenta, paired t-tests were performed for each collection interval (e.g. times 0 and 1, times 0 and 48, etc.).

Between initial collection and collection hour 72 there was a statistically significant difference (paired t-test, 2-tail distribution, $p < 0.05$) for both Σ_{32} PCBs and DDE (Table XXI).

TABLE XXI

PAIRED T–TEST P–LEVELS COMPARING MEANS BY COLLECTION HOUR

Hour	1	2	4	8	12	24	36	48	72	96
Σ_{32}PCBs	0.579	0.556	0.478	0.283	0.618	0.266	0.743	0.682	**0.008**	0.901
DDE	0.549	0.481	0.131	0.200	0.207	0.404	0.062	0.975	**0.001**	0.150
# Samples	18	13	13	11	13	12	12	10	12	10

Note: Bolded values statistically significant at the $p < 0.05$ level.

Two sites collected at hour 72: UR and MCW. There was a noticeable increase in concentrations for both Σ_{32} PCBs and DDE at both sites. The Σ_{32} PCBs increased by 102.2% at UR and by 24.2% at MCW (Table XXII). The DDE increased by 64.4% at UR while increasing by 35.5% at MCW (Table XXII). These observations may suggest that extended handling time influences PCB and DDE concentrations, and it is suggested that this time be reduced to prevent significant increases in analyte concentrations.

TABLE XXII

COMPARING MEANS BY COLLECTION SITE AND HOUR

A.)

$\sum_{32}$ PCB	Hour Collected	
Site	0	72
UR	226	457
MCW	304	378

B.)

DDE	Hour Collected	
Site	0	72
UR	44.9	73.8
MCW	79.9	108

Note: Concentrations in pg/g wwt.

F. **Random Effects Model**

Since there were multiple samples from each placenta, a random effects model was used to explore the influence of sample time and location on Σ_{32} PCBs and DDE concentrations as well as to verify the previous analyses. The random effects model accounts for the correlation between multiple samples collected from the same placenta.

First the Σ_{32} PCBs were plotted against time to determine if there are visually noticeable differences among collection sites over time (Figure 18). More so than the UR and MCW placentas, the UCD placentas appear to show an increase in concentration over time.

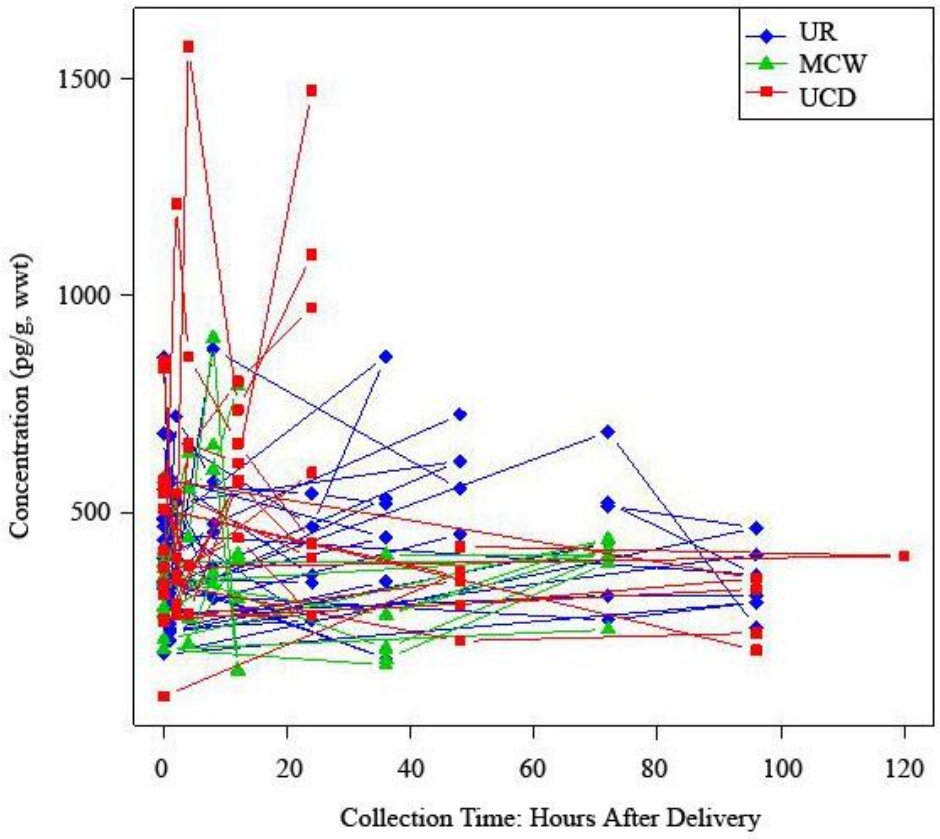


Figure 18. Σ_{32} PCBs at different collection times by site.

Though the Σ_{32} PCBs concentrations are not strongly skewed (Figure 13), initial model fitting suggested that natural logarithm–transformation was required to ensure independently identically distributed residuals. Three models were built:

1. $\text{Ln}(C_{ij}) = \beta_0 + \beta_1\text{time} + \beta_2\text{locUR} + \beta_3\text{locMCW} + \beta_4\text{time(locUR)} + \beta_5\text{time(locMCW)} + b_i + e_{ij}$.
2. $\text{Ln}(C_{ij}) = \beta_0 + \beta_1\text{time} + \beta_2\text{locUR} + \beta_3\text{locMCW} + b_i + e_{ij}$.
3. $\text{Ln}(C_{ij}) = \beta_0 + \beta_1\text{time} + \beta_2\text{time}^2 + \beta_3\text{locUR} + \beta_4\text{locMCW} + b_i + e_{ij}$.

Where C_{ij} is the concentration of $\sum_{32}$ PCB in the j^{th} measurement of the i^{th} placenta;
β_0 is the natural logarithm mean concentration at UCD at initial collection (t = 0);
β_1 is the projected change in $Ln(C_{ij})$ for each unit change of time when all other variables are held constant;
b_i is the random effect for placenta ID and has a Normal(0, σ_b) distribution; and
e_{ij} is the residual error and has a Normal (0, σ) distribution.

The Akaike information criterion (AIC) values were used to assess the appropriateness of each model. Model results for PCBs are displayed in Table XXIII: the MCW location coefficients achieve statistical significance at the p = 0.05 level for models 1 and 3. None of the time or interaction coefficients are significant. All models have similar AIC values, indicating no preference for any model. These results suggest that there is a noteworthy difference in $\sum_{32}$ PCBs from placentas collected at the MCW location when compared to the UCD location. The difference between UCD and UR is insignificant. Based on this model we can conclude that collection time does not influence $\sum_{32}$ PCB concentrations and the $\sum_{32}$ PCB levels from MCW are significantly lower than those from UCD. This may be due to different exposures based on geographical location, such as different quantities and unique mixtures of PCBs used regionally in previous years.

There is not significant interaction between collection locations and times. Based on model 1, the increase in $\sum_{32}$ PCB concentration per hour would be: 0.973 pg/g at UCD [$e^{-0.0027}$], 1.0021 pg/g at UR [$e^{(-0.0027+0.0048)}$], and 1.0006 pg/g at MCW [$e^{(-0.0027+0.0033)}$]. Each site shows a

slight increase in total PCBs by hour, however the p-values ($p > 0.05$) for the variables in the model indicate that this increase is due to chance.

TABLE XXIII
PCB MODEL STATISTICS

	Model 1		**Model 2**		**Model 3**	
Variable	Value	p–value	Value	p–value	Value	p–value
intercept	6.1185	0.0000	6.0466	0.0000	6.0264	0.0000
time	–0.0027	0.1945	0.0003	0.7684	0.0051	0.1497
$time^2$	–	–	–	–	–0.0001	0.1526
Location						
locUR	–0.1849	0.1577	–0.0746	0.5183	–0.0756	0.5092
locMCW	–0.3286	**0.0313**	–0.2519	0.0568	–0.2675	**0.0427**
Interaction						
time*locUR	0.0048	0.0718	–	–	–	–
time*locMCW	0.0033	0.3482	–	–	–	–
AIC	213.7888		213.1842		213.0646	
σ_b	0.2328		0.2380		0.2342	
σ	0.3918		0.3950		0.3932	
R^2	0.4608		0.4591		0.4592	

Note: Bolded values are statistically significant at $p < 0.05$; σ_b is the standard deviation of the intercept; σ is the standard deviation of the residual.

Model results for DDE are displayed in Table XXIV: none of the coefficients achieve statistical significance at the $p = 0.05$ level. This suggests that neither collection location nor collection time considerably change the DDE concentration of each placenta. As with the PCB model, interactions between collection site and time are not significant for DDE. For DDE, there is an increase of approximately 1 pg/g each hour after delivery for all collection sites.

TABLE XXIV
DDE MODEL STATISTICS

	Model 1		**Model 2**		**Model 3**	
Variable	Value	p–value	Value	p–value	Value	p–value
intercept	4.8978	0.0000	4.8255	0.0000	4.8267	0.0000
time	–0.0009	0.7132	0.0020	0.1147	0.0017	0.6403
$time^2$	–	–	–	–	0.0000	0.9302
Location						
locUR	–0.6700	0.0576	–0.5631	0.0999	–0.5630	0.1010
locMCW	–0.5207	0.1783	–0.4423	0.2371	–0.4412	0.2401
Interaction						
time*locUR	0.0044	0.1301	–	–	–	–
time*locMCW	0.0032	0.4183	–	–	–	–
AIC	309.0464		307.4466		309.4386	
σ_b	0.8821		0.8839		0.8842	
σ	0.3960		0.3996		0.3995	
R^2	0.8897		0.8878		0.8878	

Note: No values are statistically significant at $p < 0.05$; σ_b is the standard deviation of the intercept; σ is the standard deviation of the residual.

The results of these models suggest that the concentration differences between sample collection times are likely due to chance and not another unknown factor such as contamination. Therefore the time the sample was collected does not appear to influence the concentration levels of DDE or $\sum_{32}$PCBs analyzed. Placentas collected from Milwaukee, Wisconsin had lower levels of $\sum_{32}$ PCBs than those collected from Davis, California, however DDE levels were comparable among the collection sites.

G. **Quality Assurance and Quality Control**

In order to assess the quality of the methods used several quality assurance and quality control (QA/QC) measures were taken:

1. Surrogate standards were added to each sample prior to grinding and extraction to examine the extraction method performance,
2. Spiked sorbent and spiked placental tissues (tissues from a separate study) were processed to assess recovery of analytes, and
3. Procedural blanks were processed using the same techniques as samples to assess potential contamination.

Due to scheduling of the GC/MSQQQ, all of the placenta samples and QA/QC samples were run together to determine concentrations after all analytical samples were extracted.

1. **Surrogate recoveries**

The surrogate used for this project was ^{13}CB52, purchased from AccuStandard (New Haven, Connecticut). Two other surrogates were used to assess PBDE losses: FBDE69 and FBDE208. The average recovery for ^{13}CB52 was 64.62% (standard deviation 9.69) with a range of 43.02% to 84.17%. Although the overall recovery was lower than expected, the levels were within the control levels and there does not appear to be a trend in surrogate recovery over time (Figure 19). The differences between analysts were also assessed and the differences were found to be negligible (Figure 20).

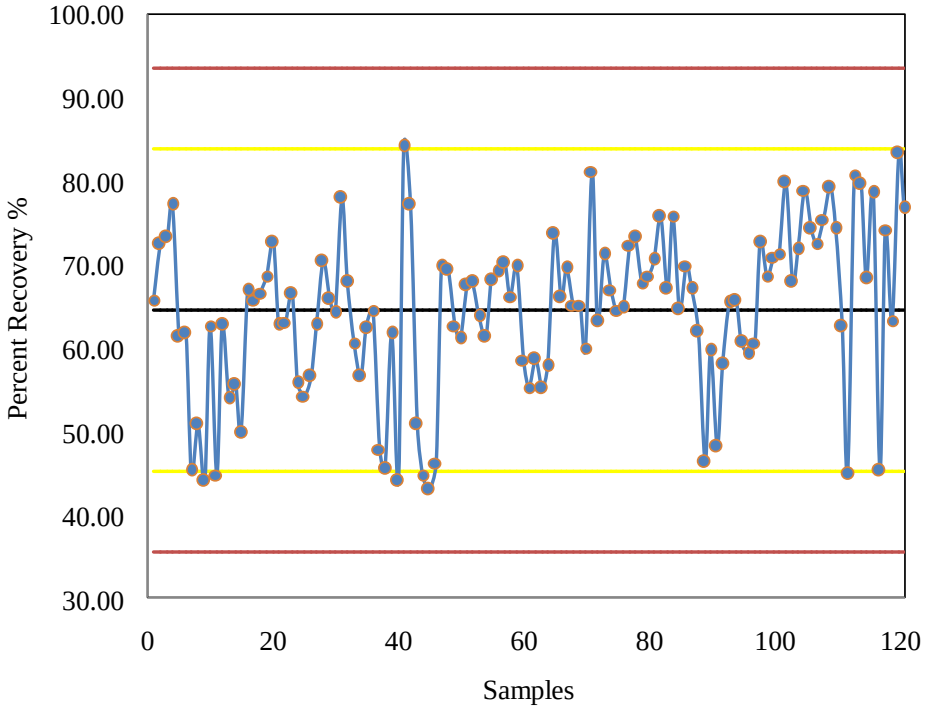


Figure 19. CB52L surrogate recovery chart over time.

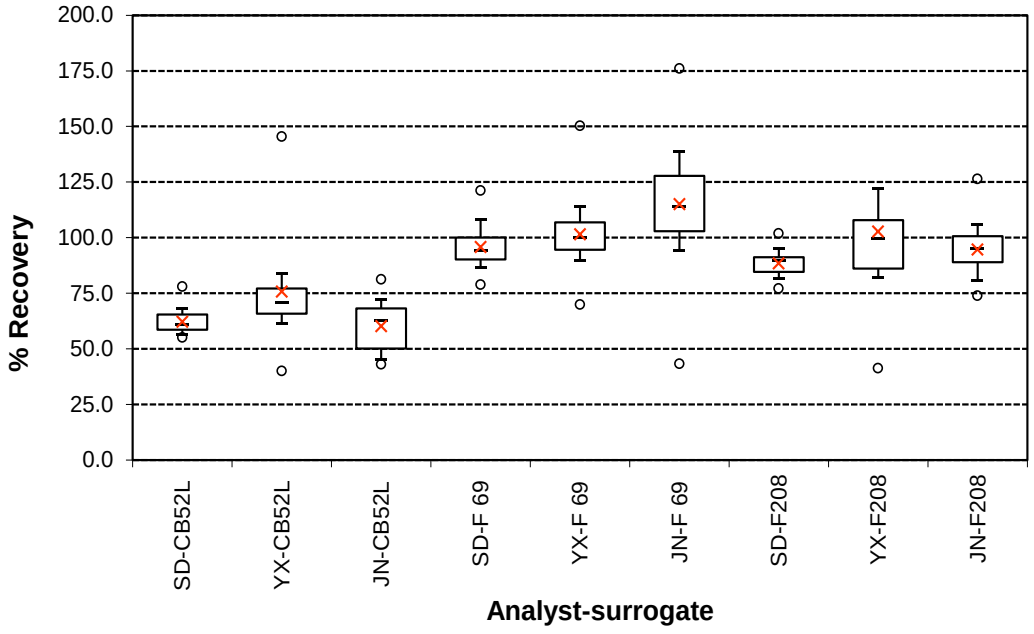


Figure 20. Surrogate recoveries by analyst.

2. **Spike recoveries**

Placenta tissue and Florisil blanks (n=2) were spiked with PCB and DDE at a 2 ng/mL concentration. Each was extracted per the described method and the recoveries are listed below (Table XXV). The recovery range for the Blank Spikes was 76% –106% (±1– 4%). The recovery range for the Matrix (placenta) Spikes was 75% –104% (±1– 6%). The recovery was significantly lower in the matrix-spiked samples compared to the blank spikes (paired t-test, $p < 0.05$). However similar tests on PBDE spikes did not find a statistically significant difference between blank spikes and matrix spikes (paired t-test, $p > 0.05$). This indicates that there is matrix interference for PCBs but not for PBDEs.

TABLE XXV
QC SPIKE RECOVERY DATA FOR SELECT PCB CONGENERS

	Blank Spiked (N=2)					Matrix Spiked (n=2)					
PCBs	**BSPK1**	**BSPK2**	**REF LEVEL**	**AVE SPK**	**% REC**	**MSPK1**	**MSPK2**	**REF LEVEL**	**Matrix**	**AVE SPK**	**% REC**
8	1.98	2.01	2.61	2.00	76.5	2.18	2.28	2.64	0.21	2.23	76.3
28	2.01	2.13	2.46	2.07	84.2	2.12	2.14	2.45	0.17	2.13	80.1
52	2.13	2.05	2.35	2.09	88.8	2.19	2.07	2.31	0.24	2.13	81.8
60	1.99	1.92	2.09	1.95	93.5	2.02	1.92	2.09	0.23	1.97	83.3
70	2.12	2.08	2.21	2.10	94.9	2.11	2.04	2.23	0.17	2.08	85.5
87	1.97	1.86	1.96	1.92	97.8	1.85	1.75	1.99	0.10	1.80	85.4
118	1.93	1.86	1.84	1.89	103.2	1.92	1.81	1.99	0.12	1.86	87.4
138	2.03	1.96	1.96	1.99	101.6	1.87	1.75	2.09	0.06	1.81	83.5
153	2.00	1.91	2.01	1.95	97.2	1.90	1.76	2.10	0.09	1.83	82.7
180	1.93	1.84	1.90	1.89	99.5	1.78	1.65	2.11	0.03	1.72	80.0
187	2.01	1.93	2.02	1.97	97.5	1.78	1.65	2.10	0.02	1.72	81.0
52L	–	–	–	–	–	1.58	1.54	1.94	0.00	1.56	80.3
DDE	–	–	–	–	–	2.81	2.62	2.12	0.91	2.71	85.2

3. **Procedural blanks and contamination study**

A total of 14 procedural blanks were processed for this project (Appendix D). The results indicate some PCB contamination: PCB 60 was found in the highest concentration in most blank samples. This was the fourth most prevalent compound in our tissue samples. Since the blanks were not quantified until all of the samples had been extracted we were unaware of the contamination issues. In order to assess where contamination was occurring and prevent it in the future, several experiments were run:

1. Glassware was evaluated to ensure that cleaning protocols were thorough.
2. The solvent evaporators, rotary evaporator and nitrogen blow, were tested for contaminate levels by evaporating volumes of pure solvent and analyzing them.
3. Procedural blanks were extracted in each analyst's hood to determine if there was contamination in the working area.
4. Sorbent preparation was evaluated by comparing the general activation method used for this project (see Materials and Methods, Sorbent Preparation section) and sorbent that was burned in a furnace at 500°C.

a. **Glassware evaluation**

The following glassware was evaluated: mortar and pestles, 1 mL volumetric flasks, and whole sets of glassware by running blanks with all burned glassware compared to simple solvent washed glassware. The glassware contributed small amounts of PCBs, but there was not a statistical difference between solvent–rinsed and burned glassware (paired t-test, $p > 0.05$). Based on these results, the glassware cleaning protocol was determined to be adequate.

b. **Solvent evaporators**

The nitrogen blow was negligible to the overall contamination measured. For the rotary evaporator test, the 50 mL of hexane or 8:2 hexane:DCM mixture were run in triplicate one after another on the single rotary evaporator. There was a significant difference between the first test and subsequent tests (paired t-test, $p < 0.05$). This indicates that the rotary evaporator should be cleaned more thoroughly between each sample. Previously the protocol was to evaporate ~20 mL of hexane between each use. Based on these results, it is suggested that at least 50 mL of hexane should be evaporated prior to use and further investigations should be performed.

c. **Hood comparisons**

A single analyst performed comparisons of each hood on the same day. Triplicates of procedural blanks were performed in each hood and analyzed to determine if there is workspace contamination. There was not a significant difference between the three hoods (ANOVA single factor test, $p > 0.05$), indicating that individual hoods are not responsible for elevated contamination levels.

d. **Florisil preparation**

In order to assess whether Florisil sorbent was contributing to the contamination three different Florisil sorbent preparations were compared for extraction:

1. Heating Florisil activated in the oven at 150°C for between 12–14 hours,
2. Burning Florisil in the furnace at 500°F for 12 hours, or
3. Solvent rinsing Florisil with acetone, DCM and hexane, vacuuming it dry and then activating in the oven at 150°C for between 12–14 hours.

There was a significant difference between PCB levels and Florisil preparation (ANOVA single factor test, $p < 0.05$; paired t–tests between each, $p < 0.05$). The solvent rinsed Florisil yielded the best results. However, an independent study for PBDE levels found the oven heated Florisil had the reduced contamination levels.

e. **Silica preparation**

To determine if silica sorbent preparation impacted PCB concentrations, the following preparation techniques were compared for the cleanup column:

1. Heating silica until activated 150°C for at least 15 hours, or
2. Burning silica in the furnace at 500°F for 12 hours.

There was not a significant difference between PCB levels and silica preparation (paired t-test, two-tailed distribution, $p > 0.05$). The furnace–burned silica had slightly lower PCB levels (0.1133 ng/mL in burned silica versus 0.1162 ng/mL activated silica). The furnace-burned silica also had lower levels of PBDEs, indicating that silica should be burned prior to use in further experiments.

V. CONCLUSIONS

The persistent organic pollutants, DDE and PCBs, are still detectible in human placental tissues many years after their use was banned in the United States. Because the placenta functions as the intermediate barrier between maternal and fetal circulatory systems, analysis of its tissue can serve as a biomarker of fetal exposure *in utero*. The aim of this research was to measure the concentrations of DDE and select PCBs in human placental tissues and to determine if the time of collection after delivery of the placenta influences the levels of these concentrations. The major findings of this research are as follows:

1. The chemical DDE is present in detectable levels in all tissue samples and is the most dominant compound by concentration when compared with individual PCB congeners in this sample series. The median DDE concentration in the tissue samples was 82.2 pg/g wet weight (wwt), with an average of 208 pg/g wwt, and a range of 9.8 to 3220 pg/g wwt.
2. The PCBs are present in all tissue samples, although the congener profile (most abundant to least abundant PCB) differs from previous placental tissue studies. The median of the $\sum_{32}$ PCBs analyzed in the tissue samples was 395 pg/g wwt, with an average of 442 pg/g wwt, and a range of 76 to 1572 pg/g wwt.
3. The PCBs with fewer chlorine molecules tend to be more abundant than those with more such as the hexachlorobiphenyls and heptachlorobiphenyls. The PCB 52 congener was the prevailing congener and PCB 126 was the least detected congener.
4. Collection time after child delivery does not significantly affect the levels of PCBs or DDE in the placenta tissue samples. Coefficients were positive,

indicating an increase over time, but these were not statistically significant and the change was small relative to the mean values.

5. There is no significant difference in DDE concentration levels among collection sites. However, $\sum_{32}$ PCBs were statistically significantly different between the Wisconsin and California samples, with samples collected at UCD having higher total PCB concentrations than those from MCW, indicating possible regional differences in exposure.

The placentas collected for this study were collected anonymously so factors that could influence POP concentrations such as maternal age, weight, and diet could not be correlated to the data presented. The main objective of this pilot study was to assess whether collection time influences PCB and DDE concentrations in the placental tissues. This is important for determining a consistent collection procedure at all hospitals participating in the NCS main study.

One limitation to this study was the lack of quantified placental lipid concentrations for each sample. The small quantity of tissue provided did not allow for further analyses. This makes the results difficult to compare with other studies that report only on a lipid mass basis. However, using the technique of lipid normalization estimation, general comparisons can be done.

Laboratory contamination is often a concern when dealing with very low concentrations, such as to the parts per trillion level as in this study. Detectible levels of PCBs were found in the procedural blanks preformed. Based on our contamination study recommendations were made to prevent similar contamination in the laboratory for future studies. These include additional pre-

cleaning of the rotary evaporator before solvent condensation and burning silica gel at 500ºC prior to use.

This research did not include the analysis of PCB metabolites such as hydroxylated PCBs. Hydroxylated PCBs are endocrine disruptors like their parent compounds (Glynn et al., 2011; Gómara et al., 2012). However these metabolites are rarely measured in placental studies. It has been established that hydroxylated PCBs cross the placental barrier and further studies are warranted to assess prenatal exposures (Gómara et al., 2012).

Additional research recommended to enhance our knowledge of prenatal exposures, health outcomes, and to improve analytical procedures include:

1. Explore reasons behind differences in PCB congener distributions among various placental studies;
2. Regional assessment and evaluation of specific PCB congeners to determine reasons why California samples have higher placental tissue concentrations;
3. Continued laboratory contamination evaluations throughout future experiments;
4. Assessment of PCB metabolites in human placental tissues; and
5. Continuation of the main NCS, with collection of additional factors such as environmental exposures and health outcomes.

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APPENDICES

APPENDIX A

Reported Values of DDE in placenta tissue

Population	**Date of Collection**	***n***	**Mean Conc.**	**Standard Deviation**	**Median Conc.**	**Units**	**Detection Method**	**Source**
California, USA	Before 1968	39	5			ug/g placenta as fat	GC/ECD	Rappolt, 1968
Lucknow, India	1978	50	50.54			ppb	GC with 3H+ detector	Saxena et al., 1980
Lucknow, India	1979–1980	9	12.4		11.5	ppb	GC/ECD	Saxena et al., 1983
Lucknow, India	1979–1980	27	18.3		11.3	ppb	GC/ECD	Saxena et al., 1983
North Carolina, USA	Before 1986	790			6.77	ppb	GC/MS	Rogan et al., 1986
Denmark	1997–2001	43	47.15	35.01		ng/g lipid	GC/MS	Shen et al., 2007
Finland	1997–2001	43	21.23	23.85		ng/g lipid	GC/MS	Shen et al., 2007
Murcia Province, Spain	1998–2000	102	17.7	16.37		ng/g wet tissue	GC/ECD	Falcón et al., 2004
Bratislava, Slovakia	Before 1999	57	0.1			ug/kg	GC/ECD	Reichrtová et al., 1999
Stará Lubovna, Slovakia	Before 1999	63	0.1			ug/kg	GC/ECD	Reichrtová et al., 1999
Brescia, Italy	2006	69	69.3	2.03*	62.5	ng/g lipid	GC/MS (EI)	Bergonzi et al., 2009
USA	Before 2007	19	58.3	734.1		pg/g lipid	GC/ECD	Brooks et al., 2007

* Geometric standard deviation

APPENDIX B

Reported Values of select PCB congeners in placenta tissue

Population	Date	Analyte(s)	*n*	Mean (Std Dev)	Median.	Units	Method	Reference
Brescia, Italy	2006	$\sum_{30}$ PCBs	70	98.7 (1.8)	92.5	ng/g lipid	GC/MS (EI)	Bergonzi et al., 2009
		PCB 28 & 52			ND			
		PCB 153			*26*			
Madrid, Spain	2003–2004	$\sum_{15}$ PCBs	17	2546 (1058)	2292	pg/g fresh weight	GC/ECD	Gómara et al., 2012
		PCB 28		169 (80)	169			
		PCB 52		638 (386)	658			
		PCB 153		309 (132)	258			
Bratislava, Slovakia	Before 1999	PCB 28	57		0.1	ug/kg	N.P.	Reichrtová et al., 1999
		PCB 52			0.1			
		PCB 153			0.2			
Stará Lubovna, Slovakia	Before 1999	PCB 28	63		ND	ug/kg		
		PCB 52			ND			
		PCB 153			0.1			
N.P.	before 1977	PCBs	19	5.0267		ppm fat basis	GC/ECD	DeKoning and Karmaus, 2000
				5027		ng/g lipid		
Germany	before 1994	PCBs	46		3.73	ppb	GC/ECD	
					248–373	ng/g lipid		
N.P.	before 1996	PCBs	25		0.95	mg/kg fat tissue	GC/ECD	
					950	ng/g lipid		
Central Taiwan	2000–2001	Dioxin–like PCBs	20	5292 (2130)	4848	pg/g lipid	GC/MS	Wang et al., 2004
		Non–ortho PCBs		67.6 (63.55)	44.05			
		Mono–ortho PCBs		5224 (2142)	4824			
		PCB 138, 153, 180		28837 (12781)	26228			
Upstate New York, USA	1995–1996	$\sum_{3}$ PCBs	5	18.2		pg/g lipid	GC/MS	Schecter et al., 1998
		PCB 77		4		pg/g lipid		
		PCB 126		10.1				
		PCB 169		4.1				

Notes: Not Provided (N.P.); Not Detected (ND); Non–ortho PCBs 81, 77, 126, 169; Mono–ortho PCBs 105, 114, 118, 123, 156, 157, 167, 189.

APPENDIX C

Data for each placenta, including all analytical samples are shown below.

Placenta ID	Analytical ID	Hour Collected	Σ_{32}PCB Concentration (pg/g wwt)	DDE Concentration (pg/g wwt)
1001	1001	0	504.75	392.28
	1002	1	471.00	329.49
	1003	2	358.76	286.26
	1004	8	875.84	259.13
	1009	48	554.33	271.72
1002	1005	0	856.35	237.73
	1006	1	498.33	225.94
	1007	2	720.82	167.87
	1008	8	571.48	157.17
1003	1010	0	340.48	73.52
	1011	1	243.74	163.17
	1012	2	525.43	224.65
	1013	8	300.94	92.97
	1020	48	449.99	104.59
1004	1103	0	394.83	71.03
	1101	1	324.63	56.33
	1104	2	274.94	82.13
	1102	8	455.33	76.35
1005	1063	0	436.48	47.21
	1062	1	332.07	22.32
	1060	2	521.84	28.10
	1059	8	375.35	23.42
	1061	48	618.38	32.77
1006	1068	0	681.65	34.32
	1067	1	415.62	30.20
	1066	2	392.12	32.58
	1065	8	473.74	70.86
	1064	48	725.15	82.45
3007	1021	0	576.74	1039.03
	1022	4	442.36	1951.21
	1023	8	902.02	1017.88
	1024	12	136.86	1768.97
3008	1025	0	350.65	102.63
	1026	4	554.68	77.11
	1027	8	597.73	140.65
	1028	12	791.62	123.83

APPENDIX C (continued)

Data for each placenta, including all analytical samples are shown below.

Placenta ID	Analytical ID	Hour Collected	Σ_{32}PCB Concentration (pg/g wwt)	DDE Concentration (pg/g wwt)
3009	1029	0	413.24	150.89
	1030	4	636.15	88.69
	1031	8	653.88	102.72
	1032	12	134.28	19.17
3010	1172	0	268.17	32.58
	1173	4	260.44	27.85
	1054	8	335.29	14.56
	1053	12	391.42	14.28
3011	1122	0	281.60	48.27
	1124	4	196.72	54.57
	1125	8	360.18	41.05
	1123	12	403.09	46.35
3012	1141	0	405.89	56.86
	1140	4	253.63	40.64
	1142	12	301.57	89.25
2013	1015	0	311.33	128.59
	1016	2	1211.33	130.38
	1017	4	859.51	164.24
	1018	12	658.18	151.81
	1019	24	429.03	48.01
2014	1033	0	832.17	1968.08
	1034	2	355.29	2401.53
	1035	4	648.35	3073.50
	1036	12	801.95	3222.53
	1037	24	972.28	1477.11
2015A	1038	0	848.37	123.85
	1040	2	345.59	79.60
	1042	4	657.86	85.41
	1044	12	613.99	99.15
	1046	24	1471.58	82.16
2015B	1039	0	412.16	100.17
	1041	2	395.81	72.37
	1043	4	1572.02	267.37
	1045	12	735.95	63.35
	1047	24	1093.96	192.76
2016	1117	0	560.16	172.00
	1121	2	543.29	164.44
	1118	4	265.44	252.33
	1119	12	568.22	187.84
	1120	24	394.63	75.06

APPENDIX C (continued)

Data for each placenta, including all analytical samples are shown below.

Placenta ID	Analytical ID	Hour Collected	Σ_{32}PCB Concentration (pg/g wwt)	DDE Concentration (pg/g wwt)
2017	1110	0	247.78	83.57
	1108	2	288.28	116.64
	1107	4	376.35	118.63
	1109	12	573.80	101.81
	1106	24	591.42	129.67
2018	1132	0	542.67	67.44
	1136	2	263.41	69.73
	1133	4	378.63	86.05
	1134	12	441.41	76.91
	1135	24	262.29	76.69
1019	1072	0	467.35	184.15
	1069	1	676.97	112.69
	1070	24	467.17	155.65
	1071	36	859.48	171.81
1020	1051	0	552.29	41.72
	1050	1	566.22	43.79
	1049	24	543.25	44.34
	1048	36	519.05	56.33
1021	1080	0	323.69	24.92
	1078	1	577.21	30.04
	1077	24	355.68	33.12
	1079	36	442.14	36.98
1022	1097	0	343.18	57.74
	1099	1	344.70	56.22
	1100	24	338.35	72.39
	1098	36	163.68	89.71
1023	1167	0	487.16	49.59
	1166	1	298.38	55.70
	1165	24	N/A	N/A
	1164	36	532.45	88.07
1024	1171	0	482.38	397.10
	1169	1	231.29	636.95
	1170	24	250.54	497.65
	1168	36	340.76	456.02
3025	1111	0	384.57	62.85
	1113	36	161.98	57.35
	1112	72	384.29	82.76
3026	1114	0	336.83	177.88
	1116	36	402.64	233.60
	1115	72	400.40	247.92

APPENDIX C (continued)

Data for each placenta, including all analytical samples are shown below.

Placenta ID	Analytical ID	Hour Collected	Σ_{32}PCB Concentration (pg/g wwt)	DDE Concentration (pg/g wwt)
3027	1163	0	343.56	93.45
	1162	36	263.15	86.03
	1161	72	416.71	141.51
3028	1151	0	370.72	75.43
	1150	36	186.73	55.60
	1149	72	400.95	92.89
3029	1160	0	206.24	36.20
	1158	36	264.16	29.72
	1159	72	438.43	27.36
3030	1138	0	185.02	33.68
	1137	36	148.38	77.99
	1139	72	229.96	57.09
2031	1156	0	507.32	68.79
	1155	48	351.58	125.06
	1157	96	182.94	22.32
2032	1152	0	576.70	120.16
	1154	48	287.61	12.50
	1153	96	347.72	139.31
2033	1143	0	336.95	50.66
	1144	48	204.04	145.21
	1145	96	221.72	136.39
2034	1127	0	254.79	140.04
	1128	48	342.03	169.97
	1126	96	323.54	221.23
2035	1129	0	373.63	102.41
	1131	48	421.34	61.74
	1130	96	399.72	84.72
2036	1147	0	76.16	168.45
	1146	48	362.27	184.77
1037	1087	0	267.11	85.00
	1088	1	331.61	78.98
	1086	72	308.19	106.35
	1085	96	308.57	73.97
1038	1055	0	174.07	9.83
	1058	1	312.78	35.21
	1057	72	252.07	30.77
	1056	96	292.87	39.73

APPENDIX C (continued)

Data for each placenta, including all analytical samples are shown below.

Placenta ID	Analytical ID	Hour Collected	Σ32PCB Concentration (pg/g wwt)	DDE Concentration (pg/g wwt)
1039	1076	0	255.92	44.48
	1075	1	449.03	46.96
	1073	72	522.08	61.56
	1074	96	356.65	66.16
1040	1083	0	N/A	N/A
	1082	1	223.66	37.37
	1084	72	430.11	48.25
	1081	96	529.01	15.19
1041	1092	0	256.20	38.11
	1091	1	313.19	37.25
	1090	72	685.52	90.25
	1089	96	233.83	32.26
1042	1095	0	175.39	46.89
	1096	1	204.52	59.19
	1093	72	514.72	79.92
	1094	96	464.15	73.95

APPENDIX D

Blank Concentration levels (ng/mL)

Analyte	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
8	0.030	0.025	0.032	0.028	0.109	0.050	0.025	0.079	0.049	0.038	0.069	0.035	0.038	0.032
28	0.054	0.051	0.056	0.057	0.114	0.040	0.022	0.103	0.033	0.024	0.039	0.028	0.029	0.039
37	0.012	0.015	0.015	0.015	0.033	0.010	0.006	0.042	0.008	0.006	0.009	0.008	0.007	0.009
44	0.022	0.023	0.027	0.029	0.055	0.041	0.021	0.145	0.032	0.024	0.037	0.029	0.024	0.027
49	0.015	0.013	0.017	0.016	0.041	0.025	0.015	0.078	0.019	0.014	0.024	0.019	0.015	0.023
52	0.037	0.035	0.040	0.043	0.085	0.062	0.030	0.199	0.049	0.035	0.058	0.043	0.035	0.043
60	0.010	0.008	0.009	0.011	0.111	0.075	0.048	0.565	0.061	0.047	0.070	0.061	0.006	0.057
66	0.011	0.010	0.013	0.011	0.055	0.022	0.013	0.115	0.017	0.012	0.019	0.017	0.031	0.019
70	0.032	0.026	0.034	0.036	0.111	0.046	0.030	0.279	0.037	0.027	0.043	0.037	0.009	0.040
74	0.010	0.008	0.010	0.011	0.039	0.015	0.010	0.073	0.011	0.008	0.013	0.011	0.010	0.013
77	0.000	0.069	0.074	0.107	0.003	0.003	0.002	0.015	0.002	0.001	0.003	0.002	0.003	0.001
82	0.000	0.007	0.008	0.012	0.008	0.011	0.007	0.081	0.007	0.005	0.009	0.007	0.016	0.005
87	0.034	0.028	0.032	0.039	0.028	0.043	0.027	0.342	0.032	0.024	0.037	0.033	0.011	0.028
99	0.028	0.019	0.021	0.025	0.048	0.029	0.019	0.211	0.023	0.017	0.027	0.024	0.049	0.022
101	0.067	0.049	0.058	0.070	0.016	0.007	0.004	0.029	0.004	0.004	0.006	0.004	0.013	0.005
105	0.022	0.016	0.018	0.034	0.021	0.021	0.015	0.205	0.012	0.010	0.014	0.012	0.001	0.011
114	0.000	0.000	0.000	0.000	0.002	0.002	0.002	0.013	0.002	0.001	0.002	0.001	0.017	0.001
118	0.052	0.041	0.050	0.074	0.056	0.053	0.037	0.559	0.037	0.030	0.042	0.039	0.001	0.034
126	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.001
128	0.000	0.000	0.000	0.000	0.003	0.007	0.007	0.086	0.004	0.004	0.006	0.005	0.003	0.004
138	0.046	0.039	0.034	0.086	0.000	0.032	0.026	0.385	0.021	0.017	0.024	0.021	0.011	0.017
153	0.044	0.037	0.032	0.067	0.021	0.035	0.026	0.407	0.025	0.020	0.030	0.025	0.001	0.021
156	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.028	0.002	0.001	0.002	0.002	0.001	0.001

APPENDIX D (continued)

Blank Concentration levels (ng/mL)

Analyte	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14
158	0.000	0.000	0.000	0.000	0.003	0.005	0.003	0.048	0.003	0.002	0.004	0.003	0.002	0.003
166	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.001	0.000	0.000
169	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.008	0.002	0.000	0.001	0.001	0.000	0.001
170	0.000	0.000	0.000	0.000	0.001	0.002	0.002	0.026	0.002	0.001	0.002	0.002	0.001	0.001
179	0.000	0.000	0.005	0.000	0.002	0.005	0.003	0.033	0.003	0.003	0.005	0.003	0.012	0.002
180	0.000	0.004	0.004	0.011	0.002	0.006	0.006	0.073	0.005	0.004	0.005	0.004	0.002	0.003
183	0.000	0.000	0.000	0.000	0.002	0.003	0.003	0.032	0.002	0.002	0.003	0.003	0.002	0.002
187	0.000	0.000	0.006	0.000	0.004	0.008	0.006	0.084	0.006	0.004	0.007	0.005	0.004	0.005
189	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.004	0.002	0.001	0.001	0.001	0.000	0.000
DDE	0.000	0.000	0.000	0.000	0.013	0.023	0.013	0.128	0.013	0.012	0.018	0.014	0.008	0.012
Σ_{32} PCB	0.523	0.522	0.594	0.782	0.974	0.664	0.418	4.346	0.509	0.388	0.612	0.486	0.353	0.470

APPENDIX E

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VITA

Name	Jessica Anne Nanes
Education	Colorado State University – Pueblo, Pueblo, Colorado, 2008 B.S. in Biological Sciences, Minor in Chemistry.
Honors	Finalist for the Thelkeld Award 2008 Who's Who Among College Students 2008 3rd Place at the Regional Tri–Beta Research Conference 2008 Beta Beta Beta National Biological Honor Society 2007 Outstanding Club Member 2007 Climate Crews Finalist 2007 Dean's List 2001–2008
Research Experience	Research Assistant: EOHS Department, University of Illinois at Chicago, 2010–Present (Research adviser: Dr. An Li). 1. Analysis of persistent environmental pollutants in human placental tissue for the National Children's Study. 2. Dechlorane plus photodegradation study. Undergraduate Research: Biology Department, Colorado State University – Pueblo, 2007(research adviser: Dr. Brian Vanden Heuval). 1. Microbial source tracking in the Fountain Creek. 2. Included water sampling, E. coli analysis, DNA extraction and analysis. 3. Sediment analysis for E. coli distribution performed.
Work Experience	Laboratory Analyst, Downers Grove Sanitary District, 2008–2010. 1. Analysis of wastewater, water, and biosolid samples. 2. Adhered to quality assurance protocol by performing required testing and generating data. 3. Obtained samples from sources on and off site using approved methods for collection.

Work Experience (continued)	Environmental Health & Safety Inspector, Colorado State University – Pueblo, 2007–2008. 1. Assistant to the EHS Director of University, conducted safety inspections, and evaluated equipment. 2. Attended regional meetings and organized scheduling and training. 3. Wrote grant proposals, edited and revised campus memos, policies and procedures. Office Assistant, Continuing Education, 2006–2007. 1. Responsible for entering students into the database and sending out course materials. 2. Edited and updated final drafts of syllabi and performed general office duties. Business Administrator, AJV Corporation, 2000–2005. 1. Responsible for training of new customers and employees. 2. Managed accounts payable/receivable and employee payroll. 3. Designed company literature and graphic related materials.
University Services	President and founder: UIC Run Club, 2011–2012. Committee Member: SPH Committee on Admissions and Recruitment Policies, University of Illinois at Chicago, 2010–2011. Ad Hoc Committee for Sustainability: Colorado State University – Pueblo, 2006–2008. Founder, president and member: Students for Environmental Awareness, Colorado State University – Pueblo, 2007–2008. Organized and implemented recycling program: organized locations and volunteers, obtained receptacles, awarded grant from Pepsi Corporation 2007–2008. Organized Educational Events: Focus the Nation, 2008. Hosted documentary showings, webcasts, and other educational events: in collaboration with Students for Environmental Awareness, 2007–2008.
Presentations	Martinez, J; and Viges, J. 2008. Sediment Removal and Escherichia coli Levels in Fountain Creek, Poster Presentation at the Regional Tri–Beta Research Conference.