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Polychlorinated Biphenyls, Dichlorodiphenyldichloroethylene, and Infant Development and Growth: An Analysis of the PIN Babies Study

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A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

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ABSTRACT

I-Jen Pan: Polychlorinated Biphenyls, Dichlorodiphenyldichloroethylene, and Infant Development and Growth: An Analysis of the PIN Babies Study
(Under the direction of Julie L. Daniels, PhD)

Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) are persistent, bioaccumulative and toxic pollutants which were broadly used in the US until the 1970s. Common exposure to PCBs, DDT, and dichlorodiphenyldichloroethylene (DDE), the most stable metabolite of DDT, may influence children's neurodevelopment and growth, but study results are not consistent. This dissertation reported the concentrations of PCBs, DDT and DDE in breast milk of lactating women in Central North Carolina in 2004-2006, and examined the associations between lactational exposure to PCBs, DDT and DDE and infant development and growth at 12 months using data from the Pregnancy, Infection and Nutrition Babies Study, 2004-2006. PCBs, DDT and DDE were measured in breast milk at the third month postpartum. Lactational exposure of these chemicals was estimated by the product of chemical concentrations and the duration of breast feeding. Infant development at 12 months was measured by the Mullen Scales of Early Learning (n=231) and the Short Form: Level I (infant) of the MacArthur-Bates Communicative Development Indices (CDI) (n=218). Serial infant growth measurements through the first 12 months of life were regularly recorded in each child's growth card by pediatric practitioners (n=206). No consistent associations were

observed between lactational exposure to PCBs, DDT and DDE through the first 12 months and the measures of infant development. However, DDE was associated with scoring below average on the gross motor scale of the Mullen among males only (adjusted OR=1.9, 95%CI=1.1, 3.3). Among infants breast fed for 6 months or longer there was no difference in weight and length through the first 6 months as the concentrations of PCBs, DDT and DDE increased. No difference was observed in weight and length at 12 months when comparing the accumulated lactational exposure through 12 months after controlling for total duration of breast feeding. In the ranges of chemical concentrations studied here, combined with the beneficial effects of the long duration of breastfeeding in this study population, lactational exposure to PCBs, DDT and DDE did not appear to impair infant neurodevelopment at 12 months, and resulted in no negative influence on infant growth in the first 12 months.

To my parents, my brother, and Jim

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LIST OF ABBREVIATIONS

AA	arachidonic acid
ALA	alpha-linolenic acid
AhR	aryl-hydrocarbon receptor
BMI	body mass index
BSID	Bayley Scales of Infant Development
CDC	Centers for Disease Control and Prevention
CDI	MacArthur-Bates Communicative Development Inventories
CI	confidence interval
CPP	Collaborative Perinatal Project
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
GC/IDHRMS	gas chromatography/isotope dilution high-resolution mass spectrometry
LA	linoleic acid
LC-PUFAs	long-chain polyunsaturated fatty acids
LEM	Lactational Exposure Metric
LOD	limit of detection
MD	mean difference
MDI	mental development index
OC	organochlorine compounds

OR	odds ratio
PBT	persistent bioaccumulative toxicants
PCB	polychlorinated biphenyls
PCDF	polychlorinated dibenzofurens
PDI	psychomotor development index
PIN	Pregnancy, Infection and Nutrition
SD	standard deviation
ω-3 FAs	omega-3 fatty acids
ω-6 FAs	omega-6 fatty acids
WHO	World Health Organization

CHAPTER I. LITERATURE REVIEW

A. Introduction

Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) are persistent organic pollutants which have been widely distributed in the environment and accumulated in the ecosystem. Research so far can not confirm the adverse effects on infant health from PCBs, DDT and DDE (the most stable metabolite of DDT) at the low background levels. Therefore, this study aims to evaluate the effects of lactational exposure to persistent organic pollutants on early growth and neurodevelopment measured at 12 months of life.

PCBs are a mixture of synthetic organic compounds which do not exist naturally in the environment. These compounds are composed of chlorinated biphenyl rings. Theoretically, 209 isomers could be produced, which are different in the degree of chlorination and chlorinated positions. PCBs are hydrophobic, lipophilic, colorless to light yellow, nonflammable, and oily liquids or crystalline solids without smell or taste. Their viscosity, hydrophobility and stability increase as the number of chlorines increases. The half-lives of the congeners range from 1 day to 70 years [1].

Because of their electrical insulating properties, mixtures of PCBs had been broadly

used as coolants and lubricants in electrical transformers, capacitors and hydraulic equipment, and also used as plasticizers in plastic and rubber products. The massive production of PCBs in industrial and commercial applications started in 1929. Following the widespread usage, their hazardous effects on ecosystem and humans were gradually observed, leading to the ban on manufacture in the U.S. in 1977 and the removal of the PCB-containing equipment by 2025 under the Stockholm Convention on Persistent Organic Pollutants.

DDT was the first widespread organochlorine pesticide. DDT compounds are lipophilic, hydrophobic, and colorless crystals without smell or taste. The active ingredient of a mixture of DDT used for insecticides was p,p'-DDT, accounting for 65-80%. The half-life of DDT is about 7 years, but dichlorodiphenyldichloroethylene (DDE), the DDT metabolite, persists longer [2].

DDT was initially produced in 1945, and was broadly used as an insecticide in agriculture and mosquito control during the 1950s and 1960s. Like PCBs, DDT was also observed to be harmful to the ecosystem and human health. The most well-known example is the eggshell thinning effects on birds. Although DDT was banned in 1972 in the United States and has been listed as one of the persistent bioaccumulative toxicants (PBT) by the US Environmental Protection Agency, it is still used for malaria vector control in other developing countries because of its high cost-effectiveness on disease control [3,4].

Although both PCBs and DDT have been banned for decades, they can still be detected in the present environment and in the blood, urine and breast milk of the U.S. population [5-7]. The potential sources of these two chemicals in the US now could be the leakage from contaminated waste sites, emissions from the pollutants built up in the environment, and continued use abroad. PCBs and DDT have also accumulated in the food chain resulting in human exposure mainly through diet, through eating contaminated meat, fish, and shellfish. After the ban on usage, occupational exposure to both chemicals and high-dose community spraying of DDT are not the main concerns in the US. For infants, they could be exposed to PCBs, DDT and DDE prenatally through placental transfer and postnatally from breast-feeding.

Along with environmental pollutants, breast-fed infants obtain several essential nutrients from breast milk. Long-chain polyunsaturated fatty acids (LC-PUFAs) are one of them, which come from the maternal diet. LC-PUFAs are carboxylic acids with a long unbranched aliphatic tail consisting of two or more double bonds between carbons. LC-PUFAs have two families – omega-3 fatty acids (ω-3 FAs) and omega-6 fatty acids (ω-6 FAs). ω-3 FAs includes alpha-linolenic acid (18:3, ALA), eicosapentaenoic acid (20:5, EPA), and docosahexaenoic acid (22:6, DHA); ω-6 FAs includes linoleic acid (18:2, LA) and arachidonic acid (20:4, AA). Since ω-3 and ω-6 FAs may act either synergistically or antagonistically on health effects, the balance between ω-3 and ω-6 FAs, evaluated as a ratio, needs be considered. Some literature has shown evidence of the importance of LC-PUFAs to the fetal and neonatal brain development [8-10], but the results of human studies about LC-PUFAs contribution to the improvement of later infant

neurodevelopment and growth are not confirmed yet [11-19].

The developing embryonic and fetal stage and the periods of rapid-growth in infancy and childhood are highly vulnerable to the effects of environmental pollutants. From cell multiplication, differentiation and migration to organogenesis and physical size change, the disruption of each process can cause irreparable damage and further influence the functioning of later life. The critical time windows of the vulnerability to various environmental exposures differ from organ to organ [20]. For neurodevelopment, brain development starts at the embryonic stage and lasts through adolescence. In the first month of pregnancy, neurulation, which induces the neural plate and forms the neural tube, begins. Interruption during this period results in neural tube defects and malformations. The subsequent processes include proliferation, migration, differentiation, synaptogenesis, apoptosis and myelination, and these processes last from the embryonic stage through the postnatal period. The immature blood-brain barrier during embryonic, fetal and infant periods makes it easier for chemicals to enter the brain. Small, fat soluble molecules can easily enter the brain and possibly produce toxic effects. Since brain maturation is not simultaneous in all areas, chemical exposures at different time windows may cause adverse effects on different behavioral domains, such as cognitive, language, sensory, motor, emotional and social development domains [21,22]. Neurotoxicants that are recognized so far include lead, methylmercury, arsenic, PCBs, pesticides (like DDT and chlorpyrifos) and so on [23]. Maternal smoking, alcohol consumption and infections during pregnancy, fetal and infant nutrient absorption, and parenting and the home environment also influence children's neurodevelopment along the way.

For physical development, the growth rate in the first year after birth is astonishingly rapid. The rate of infant weight-gain is 680 g per month in the first 5-6 months, and then weight triples by the end of the first year while body length increases by approximately 50% over the first 12 months [24]. Infant growth retardation can be due to intrauterine growth inhibition, childhood diseases, malnutrition, and disruption of hormone system caused by environmental exposures. Infant growth has been related to children's cognition, pubertal development, adult obesity, chronic diseases, and workplace success [25-32].

Both PCBs and DDT have been suspected to have health effects on children's neurodevelopment and growth. *In vivo* and *in vitro* studies have showed that PCBs may alter dopamine, estrogen and thyroid effects; animal and human studies have shown that prenatal and perinatal exposure to PCBs at environmental levels might influence cognition and behavior in infants and children [33-41]. DDT and DDE are also suspected to have estrogenic, antiestrogenic and anti-androgenic properties which contribute to their adverse effects on reproduction and development [42-46]. Although some epidemiological studies have focused on investigating the effects of PCBs, DDT and DDE on children's neurodevelopment and growth, previous studies still have not reached any firm conclusions. In addition, only two studies discussed the joint effect of PCBs and LC-PUFAs on children's visual function at 12 months and neuromotor function at preschool age [47,48], but both studies had small sample sizes and failed to find any associations.

Therefore, the goal of this dissertation is to describe the correlations between PCBs, DDT, DDE and LC-PUFAs in breast milk at the third month postpartum, and also to estimate the relationships between PCB, DDT and DDE postnatal exposure and infant development and physical growth in the first 12 months by using data from the PIN Babies Study. We hope to contribute to current knowledge of the effects of contemporary environmental exposures to PCBs, DDT and DDE on early human development.

B. Systematic Review of Epidemiological Studies

The literature search was done via PUBMED in January of 2007 by using keyword combinations of each one of the exposures and outcomes. Keywords for exposures were PCB, DDT, and DDE, and for outcomes, they were neurodevelopment, development, growth and anthropometry. Limitations were set as English language, human studies published from January 1st 1970 to December 31st 2006, involving children aged 0-18 years. Additionally, references cited in the papers but not found in PUBMED search were also reviewed manually. For dose-response comparison purposes, this review excluded studies without direct measurements of chemical concentrations in biospecimens. Since we are interested in the effects on postnatal growth, the chemical effects on birth weight, gestational age, *in utero* growth, and any other growth measurements at birth are not considered in this review.

B.1 Polychlorinated Biphenyls (PCBs) and Children's Neurodevelopment

Neurotoxicity of PCBs in humans was first observed in the Yusho mass poisoning accident in Japan in 1968, caused by ingestion of the rice oil contaminated with PCBs and polychlorinated dibenzofurens (PCDFs) [49]. Later in 1978 and 1979, a similar accident also occurred in Taiwan, called the Yucheng accident, which affected around 2,000 people [50]. Several follow-up studies of the children born to exposed women in Taiwan have been conducted. The investigators matched Yucheng children to other children who were born to women who were not exposed in this accident by age, sex, neighborhood, maternal age, and socio-economic status. Children's cognition, behavior and development were assessed by the Chinese Child Developmental Inventory, Bayley Scales for Infant Development, Stanford-Binet Test, Wechsler Intelligence Scale for Children-Revised, Raven's Coloured and Standardized Progressive Matrices, and Rutter's Child Behavior Scale A. The results showed that Yucheng children, compared to their matched controls, had poorer cognitive ability, delayed development and behavioral problems [51-57]. Concentrations of PCBs in biological samples were not examined in all Yucheng children, but a subset study reported the median of mother's serum PCBs levels at the end of pregnancy as 26.8 ng/ml [50], which is much higher than maternal concentrations during pregnancy in any subsequent cohort study in other countries.

Since the concern of PCBs' neurotoxic effects on children was raised by these two accidents, several cohort studies or secondary analyses were conducted in different countries to further investigate whether prenatal and postnatal exposures to

environmental levels of PCBs would impair children's development. To the best of our knowledge, these studies included four cohort studies in the USA, one follow-up study of the monitoring program in Canada, and one cohort study each done in 5 additional countries - Germany, Netherlands, Denmark, Spain, and Japan (table 1). Although these studies covered the periods both before and after the ban and also covered infants through adolescents, no firm conclusion could be suggested. The study results are summarized below, and the tests used for assessing children's neurodevelopment in these studies are listed in table 2.

For newborns, in the North Carolina cohort the researchers found neonatal muscle tonicity and reflexes decreased as the concentrations of PCBs in breast milk at birth increased [58], while in the Oswego cohort, researchers observed that only the decrements in habituation scores and autonomic scores at 25-48 hours after birth were associated with highly chlorinated PCBs in cord blood [59]. The Dutch study used the Prechtl Newborn Neurological Examination to assess neonatal neurological optimality. No association was found between cord blood PCBs concentrations and neurological optimality, but high exposure to planar PCBs in breast milk, collected in the 2-6 weeks after delivery, was associated with hypotonia but not with reflexes [60].

For infants (1 to 24 months), prenatal PCBs exposure was found to be associated with deficits in motor development at 6 to 24 months in the North Carolina cohort [61,62] but in the Dutch cohort it was only found at 3 months [63]. In the Dutch cohort, they also found that postnatal PCBs exposure, after controlling the trichotomised lactational

periods, was associated with deficits in motor development at 7 months but not at 3 months or 18 months. In contrast to these two studies, in the Collaborative Perinatal Project (CPP) cohort, the German cohort, the Spain cohort and the Hokkaido cohort, researchers did not find any similar associations of PCBs with infant motor development [64-67]. Infant mental development, assessed by the Bayley Scales of Infant Development (BSID), was not affected by PCBs exposure in most of the studies [61-64,66,67], except in the German cohort [65,68]. A deficit in infant novelty preference, assessed by the Fagan Test of Infant Intelligence (FTII), was found in the Michigan cohort [69] and the Oswego cohort [70], but not in the German cohort [65].

For preschool children aged 2-5 years, children's cognition, assessed by the McCarthy Scales of Children's Abilities (MSCA), was slightly associated with highly chlorinated PCBs when assessed at around 3 years in the Oswego cohort [71], but was not associated with PCBs in the North Carolina cohort when assessed at 3, 4 and 5 years [72]. In the Oswego study, a potential interaction between PCBs exposure and the size of the splenium of the corpus callosum on children's sustained attention was suggested [73]. This may imply a plausible biomechanism of the relationship between PCBs and preschool children's response inhibition. In the Michigan cohort, they found PCBs might be associated with the deficits in children's cognition, memory, and visual information processing, but the results did not show constant patterns across bio-samples or across assessment methods [74,75]. Associations between PCBs and the total score on the Kaufman Assessment Battery for Children (K-ABC) were found in two European studies, but in the Dutch cohort, it was suggested that this effect was caused by prenatal exposure [76]

whereas in the German cohort it appeared to be related to postnatal exposure [68]. No other studies observed any effects on children's motor function [48,77].

For children aged 6-12 years, in the Dutch cohort researchers found exposure to prenatal PCBs influenced children's cognition and memory at 6.5 years, and these effects were modified by maternal age and maternal verbal intelligence quotient (IQ) [78]. The Michigan studies also reported prenatal PCBs reduced children's IQ, verbal capability and freedom from distractibility at 11 years [79]. However, in the CPP cohort and the Denmark cohort, in contrast, researchers did not find any associations at 7 years [80,81]. PCBs' effects on children's motor function were not observed in this period of life, except in the Dutch cohort, with a decrement in motor scores assessed by MSCA at 6.5 years [78]. In addition, in the Dutch cohort, researchers also observed that prenatal PCBs influenced children's play behavior [82], but no replications of this were reported by others.

Environmental levels of PCBs seem to have adverse effects on children's motor functioning in neonatal and infant stages, and the adverse effects on children's cognition and neurobehaviors might persist longer to school age. However, no consistent results were reported across previous studies. It is still not clear whether the observed effects by PCBs should be attributed to prenatal exposure via the transplacental route or to postnatal exposure via breast-feeding. From animal studies and in vitro studies, PCBs have been observed to have high binding affinity for the aryl-hydrocarbon receptor (AhR), have influence on thyroid hormone homeostasis, and cause alterations of dopamine and estrogen levels, which may be the potential mechanisms responsible for the neurotoxicity

observed in exposed humans. PCBs are fat soluble molecules which can easily enter the brain and possibly produce toxic effects.

B.2 Dichlorodiphenyldichloroethylene (DDE) and Children's Neurodevelopment

DDT insecticidal function is to prolong neuronal repolarization in mosquitoes and other insects, but its metabolite, DDE, has less toxicity. Both DDT and DDE persist for a long time and are bioaccumulated in the ecosystem. In animal studies, DDT exposure during the period of the brain growth spurt can cause cholinergic disturbance and neurobehavioral alteration in adult life [83]. The neurological toxic effects of DDT and DDE on humans, except by acute poisoning of DDT, are still unclear. To our knowledge, only three studies in the United States, one in Denmark, and one in Spain have investigated the potential adverse effects of DDT or DDE on children's neurodevelopment (table 3). Among the studies in the United States, the California study is the only study in which *p,p'*-DDT could be detected in 100% of maternal blood samples, and *o,p*'-DDT could be detected in 95.8% when DDT can not be detected in most of the general population around the same time periods [5,84]. Findings of the studies have been inconsistent.

For newborns, assessed by Brazelton Neonatal Behaioral Assessment Scale, the North Carolina study observed a gradual upward association between *p,p'*- DDE and hyporeflexia [58], whereas the Oswego study found no association. It might be because the Oswego cohort was prenatally exposed to lower DDE concentration than the North

Carolina cohort.

For infants (1 to 24 months), the North Carolina study did not find any associations, and the only significantly positive association found between DDE and Bayley Mental Development Index (MDI) at 6 months of age might be a chance finding [61,62]. In the California study of Mexican farm-workers' children, DDE was only observed to be associated with a decrement of Bayley Psychomotor Development Index (PDI) at 6 months, but not associated with either PDI at 12 and 24 months or MDI at 6, 12, 24 months [85]. In the Oswego cohort, results also suggested that prenatal DDE exposure was not associated with infant novelty preference [70]. However, in the Spain cohort of 92 mother-child pairs, cord blood DDE levels was associated with the decrements in MDI, PDI, and infant's social, performance, and locomotor scores, assessed by Griffiths Mental Development Scales, at the age of 13 months. The California study, which is the only study that assessed the effects of DDT on infants, found p,p'-DDT and o,p'-DDT were negatively associated with infant's MDI at 12 and 24 months of age, but only p,p'-DDT was negatively associated with infant's PDI at aged 6 and 12 months.

For preschool children aged 2-5 years, none of the studies found associations between prenatal DDE exposure and children's neurodevelopment [72,73,86], but in the Spain study, they observed prenatal *p,p'*-DDT exposure to be associated with the deficits in children's cognition on memory and verbal functions [86]. For 7-year-olds, no clear association was observed [73,81]. One ecologic study using the data from 11 countries and 14 federal states of Germany suggested an inverse relationship between DDT

concentrations in breast milk and the general mental capacity of 15-year-old children [87], but further studies are still needed to rule out the possibility of ecological fallacy.

In summary, the effects of prenatal DDE exposure on children's neurodevelopment might be slight, but not confirmed. So far there are too few studies to make any conclusions about the relationships between prenatal DDT exposure and children's neurodevelopment. The effects of postnatal exposure from breast-feeding can not be determined, since no previous studies have directly assessed it.

B.3 Polychlorinated Biphenyls (PCBs) and Children's Growth

In the Yucho accident in Japan and the Yucheng accident in Taiwan, the studies reported that the growth of children was suppressed after being exposed to PCBs, but after several years on average they returned to normal [51,88,89]. PCBs acting as endocrine disruptors may be the plausible mechanism for the alteration in children's growth. Five cohorts in the United States, one cohort each in Germany, in the Netherlands, and in Denmark have investigated the association between maternal exposure to environmental PCBs levels and children's growth retardation (table 4). However, the results contradict each other.

Children prenatally exposed to higher PCBs levels were reported to be shorter from birth to 3 months of age in the formula-fed group in the Dutch cohort [90], and were also reported to be shorter at 18 months of age in the Faroe Islands cohort study [91]. Conversely,

the California study reported that higher prenatal PCBs exposure was associated with greater growth in height among 5-year-old girls [92]. None of the others found any differences in children's height caused by prenatal PCBs exposure.

As for body weight, the negative association with prenatal PCBs exposure was observed among girls only in the small subset of the CPP cohort in New York [93], and also in the Michigan polybrominated biphenyl (PBB) incident cohort [94]. This negative association was found without gender differentiation among children aged 18 months in the Faroe Islands cohort [91] and among 4-year-old children in the Michigan fish consumption cohort [95]. In the Dutch cohort, they reported that the growth rate estimated by weight change was negatively related to prenatal PCBs exposure, but this association was only observed in the formula-fed group from birth to 3 months [90]. Conversely, the positive association between prenatal PCBs exposure and growth in weight was found in children during 0-5 years in the North Carolina study when the analysis was restricted to white girls only [96]. None of the others reported any differences in children's weight caused by prenatal PCB exposure.

In summary, gender differentiation on the effects of prenatal PCBs exposure on children's growth was found in several studies, although the potential biomechanisms are still not clear. Because of inconsistent results, no conclusive suggestion can be made about in which age period growth is vulnerable to prenatal PCBs exposure. In addition, previous papers investigating the effects of postnatal PCBs exposure via breast-feeding are too scarce, so further research is still needed.

B.4 Dichlorodiphenyldichloroethylene (DDE) and Children's Growth

DDT and DDE, suspected as endocrine disruptors, have been found to have estrogenic and antiandrogenic properties (11, 61-63). Because children's growth depends on hormonal regulation, the prenatal and postnatal exposures to DDT and DDE may adversely affect children's physical development. There were only three cohort studies in the United States and one cohort study in Germany evaluating the relationships between prenatal or postnatal DDT or DDE exposure and children's growth (table 5). Most of the studies did not observe any associations between prenatal DDT or DDE exposure and children's growth in height or weight. However, the CPP cohort found a negative association between p,p'-DDE and children's height at 1, 4, and 7 years [97]. This negative association was also found in the German study, but only among girls [98]. Contrarily, positive association was found between prenatal p,p'-DDE and boys' height and weight at age of 0-5 years in the North Carolina study [96]. Among these studies, only in the German cohort did researchers use the concentrations detected in children's blood at 8 years to index perinatal exposure, which may induce exposure misclassification.

In summary, gender differentiation of the effects of DDE on children's growth was observed in two studies, but the results were not consistent. Only two studies have investigated the effects of prenatal DDT exposure, but neither found any associations. Although previous studies suggested that no adverse effects on children's growth were caused by prenatal DDT or DDE exposure, we are still far from any conclusions, because

there are so few studies and they have diverse study characteristics and inconsistent findings. Postnatal exposure was only evaluated in the North Carolina study by the multiplication of maternal concentration in blood and duration of breast-feeding, but no association was found.

C. Discussion

There are several differences among studies which could explain the discordant results observed in different studies. First, the exposure assessments differed among studies. Previously, researchers used three different ways to estimate children's prenatal exposure: 1) the concentration detected in maternal blood obtained at the third trimester of the index pregnancy, 2) the concentration detected in the cord blood, and 3) the estimated concentration calculated from the concentration in maternal breast milk collected at delivery. In the German cohort, the correlation between PCBs in cord blood and milk was 0.57 [68]. In the Dutch cohort, the correlations between PCBs in maternal blood and milk were 0.70-0.79, while the correlations between maternal blood and cord blood were 0.52-0.74 [99]. In the North Carolina study, the correlation of PCBs concentration between maternal serum at birth and milk at birth was 0.64, while the correlation of DDE concentrations was 0.82 [100]. However, the correlation between the concentrations of PCBs in cord blood and breast milk in the Oswego cohort was low (r=0.15) [59]. Overall, if the correlations between these three biospecimens, maternal blood at pregnancy, cord blood, and breast milk at birth, were high, all could be good surrogates for prenatal exposure, but if the correlations are merely low to medium, certain amounts

of exposure misclassification would be induced and influence the study results.

As for postnatal exposures, the total amount of PCBs or DDE consumed by infants through breast-feeding is influenced by the duration of lactation. The Faroe Island study and the Michigan study used the multiplication of total PCBs concentration in maternal milk and duration of breast-feeding to estimate the levels of postnatal exposure, while the German study treated lactational period as a covariate in the statistical models. It is more accurate to use both lactational period and the concentrations in breast milk to indicate postnatal exposure, because this estimate would be more highly correlated with infant's PCB plasma concentration [101]. It is hard to distinguish postnatal effects from prenatal effects, especially when the breast milk samples were collected at the time close to the delivery to make postnatal concentrations highly correlated with prenatal concentrations. The findings from the Rogan et al. paper suggested a decline in concentrations as an increase in the length of lactation [100], whereas the Hooper paper suggested that the concentration was not substantially reduced in the first 3 months of breast feeding [102]. Breast milk is also an excellent source of nutrition and provides several potential benefits to the infant's immunologic system, growth and neurodevelopment [103,104], so the comparisons between the formula group, which generally was assumed to have no chemical exposure, and the breast-feeding group may mask the potential adverse effects of environmental exposures in breast milk.

The other differences in exposure assessments that could contribute to the different findings in PCBs effects are different laboratory analyses, and different summary

estimates of total PCBs concentrations. In the earlier papers, investigators could only use packed column gas chromatography to detect the concentrations of total PCBs, while later investigators could use the capillary column gas chromatography method to detect the concentrations of specific congeners. The new laboratory analysis is better at quantification of the lower environmental concentrations because of their lower detection limits and higher sensitivity, and it also has the capability to detect each specific congener concentration. However, different studies may not use the same method to compose the summary estimate of total PCBs concentrations. One may choose to include all detectable congeners, whereas another may choose several peak levels of PCB congeners to estimate the total amounts, so the differing total PCBs concentrations in previous studies do not allow for direct comparisons. Besides, some findings in the previous studies were observed only when their analyses were restricted to some specific grouping, such as highly chlorinated PCBs or dioxin-like PCBs or coplanar PCBs. Using the gross concentration instead of specific target chemical concentrations may introduce noise into the exposure estimation.

Secondly, different study population characteristics, like population size, racial composition, the distribution of children's gender, parental education and economic status, may also contribute to different findings across studies. In addition, different mixtures of environmental exposures across studies would also influence the associations of interest in PCBs or DDE. For example, the Faroe Island population consumed large amounts of seafood. The Michigan cohort and the Oswego cohort consumed more freshwater fish. Parts of the Dutch cohort lived in a highly industrialized city. The California Mexican

farm-workers cohort was exposed to more agricultural pesticides. The mixtures of environmental chemicals could be methyl mercury and PCBs, dioxin and PCBs, PCDF and PCBs, or DDT/DDE and PCB. For fish eaters, they also obtained more fish protein and ω-3 fatty acids, which has been reported to be beneficial for fetal growth [105-108]. If these chemicals act synergistically or antagonisticly on infant growth or neurodevelopment, the association observed for either one of chemicals may be biased if other chemicals are not considered in the statistical analyses.

Thirdly, differences in outcome measurements may partially explain the differences in findings. For neurodevelopment, studies used different scales to test children's cognition, motor function and/or neurobehaviors. The different scales did not allow for direct comparisons among results, because different scales may measure different brain functions, may have different sensitivities and differing ability to predict children's future neurodevelopment and capacity. The neurodevelopment measurements could be either quantitative or qualitative. Different examination age, testing protocols or administrative efforts used for the same measurements may also cause the differences in the results. For infant physical growth, few studies were able to use serial measurements instead of measuring at only one time point to investigate the differences in children's growth rates. Since development is a continuous process, it would be better to have serial growth measurements and employ statistical regression models of repeated measurements.

Fourthly, inadequate confounding controls may attenuate the effect estimates of interest or decrease the precision of effect estimates. Home observation for measurement

of the environment characteristics, parental intelligence, socioeconomics status, and parental nursing have been linked to children's development, but if the distribution of these factors is not differentiated between exposure groups or not related to chemical concentrations, then it is redundant to control these factors. On the other hand, several reproductive factors, such as parity, lactational periods, number of children nursed, and maternal age, have been observed to be associated with chemical concentrations, and if these reproductive factors are also associated with children's development and/or growth, then these factors should be controlled for the statistical models. Still residual confounding effects could bias the results in either direction.

Several methodological challenges could be identified from previous studies. Firstly, the accuracy of exposure measurement is crucial. Secondly, when prenatal exposure is highly correlated with postnatal exposure, it is difficult to assess the critical windows for adverse effects of environmental chemicals on children's development and growth. Thirdly, there is not just one single chemical existing per time in the environment. If the chemicals of interest are highly correlated and have mixed effects on the outcome of interest, collinearity will be an issue when all chemicals are involved in one traditional regression model. Fourthly, low environmental exposure levels tend to have minor effects, which require larger study populations or a wider range of chemical concentrations to obtain sufficient statistical power to detect the effects. Fifthly, the simple neurobehavioral tests would have limited ability to detect subtle physiological deficits of the brain and nervous system which might be identified with more sensitive clinical assessments.

This dissertation will be focused on investigating the associations between postnatal exposure to PCBs, DDT and/or DDE through breast-feeding and infant development and growth through the first twelve months. This dissertation also will estimate whether the associations between lactational exposure to PCBs, DDT and DDE and infant development would be modified by the concentrations of LC-PUFAs in breast milk. The postnatal exposures will be directly estimated by the duration of lactation and the concentrations detected in breast milk collected at the third month postpartum by gas chromatography/isotope dilution high-resolution mass spectrometry. Neurodevelopment at age 12 months is measured by the Mullen Scales and the MacArthur Communicative Development Inventories Short Form. These two measures are able to assess different aspects of infant neurodevelopment, and the administration is relatively easy and feasible for assessing children at 12 months. Repeated growth measurements in height and weight through the first 12 months of life are recorded on the child's growth card by the pediatric practitioners, so each infant's growth pattern can be more accurately estimated. The sample size of breast-feeding mother-child pairs is larger than in most of the previous studies. All pre-, peri- and post-natal covariates were collected prospectively by questionnaires in PIN studies, so potential confounders could be appropriately controlled. However, this dissertation has limitations on distinguishing the effects of postnatal exposure from the effects caused by prenatal exposure. We also will have difficulties in controlling the effects of other environmental exposures. Nonetheless, this dissertation's results will contribute to our knowledge about the effects of lactational exposure to low background levels of PCBs, DDT and DDE on early human development.

Table 1. Reviews of studies about the effects of polychlorinated biphenyls (PCBs) on children's neurodevelopment. *

Study	Size#	Biospecimen	PCBs Conc †	Age	Test ‡	Result §
USA						
CPP Cohort (1959-1965)						
Daniels 2003 [64]	1207	Maternal blood at 3rd triemester	Median=2.7 (95%CI=1.8, 3.7) (µg/l, wet weight)	8 mo	BSID	–
Gray 2005 [80]	894	Maternal blood at 3rd triemester	Median=2.85, 95th=6.75 (µg/l, wet weight)	7 yr	WISC WRAT	–
North Carolina Cohort (1978-1982)						
Rogan 1986 [58]	(912)	Milk at birth	Median=1.74, 95th=3.64 (ppm)	<1 mo	BNBAS	Muscle tonicity ↓ Reflex ↓
Gladen 1988 [61]	(802)	Milk at birth	(similar to Rogan 1986)	6 mo 12 mo	BSID	PDI at 6 mo ↓ PDI at 12 mo ↓
Rogan 1991 [62]	(676)	Milk at birth	(similar to Rogan 1986)	18 mo 24 mo	BSID	PDI at 18 mo ↓ PDI at 24 mo ↓
Gladen 1991 [72]	(645)	Milk at birth	(similar to Rogan 1986)	3 yr 4 yr 5 yr	MSCA English, math grades	–
Michigan Cohort (1980-1981)						
Jacobson 1985 [69]	81 (67)	Cord blood Breast milk within 2 months	Cord serum (ng/ml): range: 0.2-7.9 Breast milk: (unmentioned)	7 mo	FTII	Prenatal PCBs: recognition memory ↓ preference for novelty ↓ Postnatal PCBs: –
Jacobson 1990 [95]	146 (87)	Cord blood Breast milk within 2 months	Cord serum (ng/ml): mean=2.5, SD=2.0, range: 0-12.3 Breast milk (ng/ml): mean=835.9, SD=388.4, range: 185.7-2600,	4 yr	Activity rating from EASI+BSID	Prenatal PCBs: – Postnatal PCBs: activity ↓

Jacobson 1990 [74]	146 (120)	Cord blood Breast milk within 2 months	Cord serum (ng/ml): mean=2.5, SD=2.0 Breast milk (ng/ml): mean=835.9, SD=388.4	4 yr	MSCA	Prenatal PCBs: verbal ↓ quantitative ↓ Postnatal PCBs: memory ↓
Jacobson 1992 [75]	143 (118)	Cord blood Breast milk within 2 months	Cord serum (ng/ml): mean=2.5, SD=2.0 Breast milk (ng/ml): mean=833, SD=389.5	4 yr	SMT MFFT CPT	Prenatal PCBs: # of errors on the Sternberg memory task ↑ Postnatal PCBs: RT on the visual discrimination task ↑
Jacobson 1996 [79] Jacobson 2002 [109]	139 (124)	Cord blood	Cord serum (ng/ml): mean=3, SD=2	11 yr	WISC-R WRAT WRMT-R	Prenatal PCBs: IQ ↓ verbal ↓ verbal comprehension ↓ freedom from distractibility ↓ word comprehension ↓ reading comprehension ↓ (effects modified by maternal intellectual input)
Oswego Cohort (1991-1994)						
Stewart 2000 [59]	293	Cord blood	25th=0.17, 50th=0.52, 75th=1.11 (ng/g wet)	birth	BNBAS	Highly chlorinated PCB: (at 25-48 hr after birth) Habituation scores ↓ autonomic scores ↓

Darvill 2000 [70]	230 (86)	Cord blood Breast milk b/w 1-3 months	Cord serum (ng/g wet): 25th=0.17, 50th=0.52, 75th=1.11 Breast milk (n=86) (ng/g lipid): 25th=87, 50th=153, 75th=249	6 mo 12 mo	FTII	Prenatal total PCBs: FTII scores at 6 mo ↓ FTII scores at 12 mo ↓ Prenatal highly chlorinated PCBs: FTII scores at 6 mo – FTII scores at 12 mo ↓ Postnatal total PCBs: –
Stewart 2003 [71]	212 (86)	Cord blood Breast milk b/w 1-3 months	(similar to Darvill 2000)	38 mo 54 mo	MSCA	Prenatal highly chlorinated PCBs: GCI at 38 mo ↓ GCI at 54 mo –
Stewart 2003 [73]	189 (86)	Cord blood Breast milk b/w 1-3 months	(similar to Darvill 2000)	4.5 yr 7.8 yr	CPT MRI (n=60)	Prenatal highly chlorinated PCBs: # of errors of commission ↑ (modified by the size of the splenium of the corpus callosum)
Canada						
Nunavik Monitoring Program(1993-1996)						
Despres 2005 [48]	110	Cord blood	Geometric mean=330.3, SD=267.6, range: 99.5-1725.7 (µg/kg)	4-6 yr	ATNA	–
Germany						
Dusseldorf cohort (1993-1995)						
Winneke 1998 [65]	171 (131)	Cord blood Breast milk within 1 month	Cord serum (ng/g fat): mean=218, SD=100.2 Breast milk (n=131) (ng/g fat): mean=426.5, SD=184.4	7 mo	BSID FTII	Prenatal PCBs: – Postnatal PCBs: MDI ↓ PDI – Fagan –

Walkowiak 2001 [68]	171 (126)	Cord blood Breast milk within 1 month	Cord serum (n=141) (ng/ml): median=0.39, 5th=0.11, 95th=0.83 Breast milk (n=126) (ng/g lipids): median=404, 5th=173, 95th=679	7 mo 18 mo 30 mo 42 mo	BSID K-ABC	Prenatal PCBs: – Postnatal PCBs: MDI at 7-30 mo ↓ MDI at 30 mo ↓ PDI at 7-30 mo ↓ K-ABC at 42 mo ↓
Netherlands						
Dutch cohort (1990-1992)						
Huisman 1995 [60]	418 (209)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum (n=415) (µg/l): median=2.04, 5th=1.00, 95th=3.81 Cord serum (n=373) (µg/l): median=0.38, 5th=0.18, 95th=0.86 Breast milk PCB169 (µg/kg fat): median=79.53, 5th=42.35, 95th=143.70	<1 mo	Prechtl	Prenatal PCBs: – Postnatal planar PCBs: Tonicity ↓ Reflex – neurological optimality ↓
Huisman 1995 [110]	418 (209)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum: (same as Huisman 1995) Cord serum: (same as Huisman 1995) Breast milk (ng TEQ/kg milk fat): median=33, 5th=17, 95th=61	18 mo	THNE	Prenatal PCBs: Neurological optimality ↓ Postnatal PCBs: –

Koopman-Esseboom 1996 [63]	207 (105)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum (ng/g plasma): mean=2.2, SD=1.0 Cord serum (ng/g): mean=0.5, SD=0.3 Brest milk (pg TEQ/g fat): mean=66.6, SD=24.2	3 mo 7 mo 18 mo	BSID	Maternal PCBs: PDI at 3 mo ↓ PDI at 7 mo – PDI at 18 mo – Breast milk PCBs: PDI at 3 mo – PDI at 7 mo ↓ PDI at 18 mo –
Lanting 1998 [77]	418 (209)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	(same as Huisman 1995)	42 mo	THNE	–
Patandin 1999 [76]	373 (195)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum: (same as Huisman 1995) Cord serum: (same as Huisman 1995) Breast milk (n=193) (µg/kg fat): median=405, 5th=205, 95th=723	42 mo	K-ABC RDLS	Maternal PCBs: K-ABC score ↓ Breast milk PCBs: –
Vreugdenhil 2002 [78]	376 (194)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum (µg/l): median=2.04, range: 0.59-7.35 Cord serum (µg/l): median=0.38, range: 0.08-2.08 Breast milk (n=194) (µg/kg fat): median=403.66, range: 158.35-1226.38	6.5 yr	MSCA	Maternal PCBs: GCI ↓ (modified by maternal age and VIQ) Memory ↓ (modified by maternal age and VIQ) Motor ↓ (modified by VIQ and HOME) Breast milk PCBs: –

Vreugdenhil 2002 [82]	160 (85)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum (μg/l): median=2.06, range: 0.73-5.08 Cord serum (μg/l): median=0.42, range: 0.08-1.99 Breast milk (μg/kg fat): median=390, range: 174-805	7.5 yr	PSAI	Maternal PCBs: masculinized play behavior in boys ↓ Cord serum PCBs: masculinized play behavior in boys ↓ masculinized play behavior in girls ↑ Breast PCBs: –
Denmark						
Faroe Islands Cohort (1986-1987)						
Grandjean 2001 [81]	435	Cord tissue	median=1.02, interquartile range: 0.53-1.71, max=65.2 (μg/g lipid)	7 yr	NES2 WISC-R BVMG CVLT BNT Neurophysiological and sensory test	Wet-weight PCBs: Boston naming test ↓ RT in NES2 ↑ Lipid adjusted PCBs: –
Spain						
Spain cohort (1997-1999)						
Ribas-Fito 2003 [66]	92	Cord blood	<LOD - ?	13 mo	BSID GMDS	–
Japan						
Hokkaido cohort (2002-2004)						
Nakajima 2006 [67]	134	Maternal blood at 3rd triemester	Geometric mean=11770.9, range: 3311.1-37267.2 (pg/g lipid)	6 mo	BSID-II	–

* Abbreviation used in this table: CPP, the Collaborative Perinatal Project; CI, confidence interval; SD, standard deviation; hr, hours; mo, months; yr, years; b/w, between; PDI, psychomotor development index; MDI, mental development index; #, numbers; RT, reaction time; IQ, intelligence quotient; GCI, General Cognitive Index; VIQ, verbal intelligence quotient; HOME, Home Observation for Measurement of the Environment.

† PCBs concentrations listed in this table may not be directly comparable, because different studies used different congeners to compose the total PCBs concentrations. Different studies also used different biospecimens to estimate the PCBs concentrations. In addition, sensitivities, detection limits, equipments, and recovery methods of laboratory analyses were different.

‡ ATNA, Ameiel-Tison and Gosselin Examination; BNBAS, Brazeltom Neonatal Behavioral Assessment Scale; BNT, Boston Naming Test; BSID(-II), Bayley scales of Infant Development (-second version); BVMG, Bender Visual Motor Gestalt Test; CPT, Continuous Performance

Testing; CVLT, California Verbal Learning Test; FTII, Fagan Test of Infant Intelligence; EASI, Emotionality, Activity, Sociability and Impulsivity Temperament Survey; GMDS, Griffiths Mental Development Scales; NES2, Neurobehavioral Evaluation System; MFFT, Matching Familiar Figures Test; MRI, magnetic resonance imaging; MSCA, McCarthy Scales of Children's Abilities; K-ABC, Kaufman Assessment Battery for Children; Prechtl, Prechtl Newborn Neurological Examination; PSAI, Pre-school Activities Inventory; RDLS, Reynell Development Language Scales; SMT, Sternberg Memory Test; THNE, Touwen/Hempel Neurologic Examination; WISC(-R), Wechsler Intelligence Scale for Children (-Revised); WRAT, Wide Range of Achievement Test; WRMT-R, Woodcock Reading Mastery Test-Revised.

[§] – indicates no association. ↑ indicates positive association with the increments of PCBs concentrations. ↓ indicates negative association with the increments of PCBs concentrations.

[#] Numbers in the parentheses represent the numbers of breast milk samples in each study.

Table 2. Neurodevelopment tests used in previous papers.

Test	Abbrev	Age	Assessment
Neonatal Tests			
Brazelton Neonatal Behavioral Assessment Scale	BNBAS	<3 d	Habituation Orientation Motor performance Range of state Regulation of state Autonomic regulation Reflexes
Prechtl Newborn Neurological Examination	Prechtl	10-21 d	Neurodevelopment
Children's Tests – Mental			
Bayley Scales of Infant Development	BSID BSID-II	2-30 mo 1-42 mo	Mental development index (MDI) – cognition, language and social development
Fagan Test of Infant Intelligence	FTII	3-12 mo	Novelty preference
Griffiths Mental Development Scales	GMDS	0-8 yr	Locomotor scale Personal-social scale Hearing and language scale Eye-hand coordination scale Performance scale
Reynell Developmental Language Scales	RDLS	1.5-6 yr	Language ability General mental ability
Kaufman Assessment Battery for Children	K-ABC	2.5-12.5 yr	Short term memory Visual processing Learning ability Planning ability Crystallized ability
McCarthy Scales of Children's Abilities	MSCA	2.5-8.5 yr	Verbal scale - language comprehension and use Quantitative scale - mathematical ability Perceptual-performance scale - ability to conceptualize and reason without words Memory scale - short-term recall of words, numbers, pictures, and tonal sequences Motor scale - gross and fine motor coordination General cognitive scale - overall intellectual functioning

Wechsler Intelligence Scale for Children	WISC	6-16 yr	Verbal IQ Verbal comprehension factor Freedom from distractibility factor Performance (nonverbal) IQ Perceptual organization (nonverbal) factor Processing speed factor Full scale IQ
Wide Range Achievement Test	WRAT	5-11 yr >12 yr	Learning ability – reading, spelling, arithmetic
Woodcock Reading Mastery Test-Revised	WRMT-R	>5 yr	Word comprehension Passage comprehension Reading comprehension
Boston Naming Test	BNT		Learning ability
California Verbal Learning Test	CVLT		Verbal memory ability
Sternberg Memory Test	SMT		Short-term memory search
Matching Familiar Figures Test	MFFT		Visual discrimination
Children's Tests – Motor			
Bayley Scales of Infant Development	BSID BSID-II	2-30 mo 1-42 mo	Psychomotor development index (PDI) - fine and gross motor development
Amiel-Tison and Gosselin Examination	ATNA	0-6 yr	Head circumference and growth Craniofacial examination of the cranial nerves Motor development Passive muscle tone Motor activity Deep tendon and cutaneous reflexes Primitive reflexes Postural reactions Qualitative abnormalities in gross motor function and acquired deformities
McCarthy Scales of Children's Abilities	MSCA	2.5-8.5 yr	Motor scale - gross and fine motor coordination
Bender Visual Motor Gestalt Test	BVMG	>3 yr	Visual-motor maturity
Touwen/Hempel Neurologic Examination	THNE		Motor functions – grasping, sitting, crawling, standing, and walking
Children Tests – Neurobehavioral			
Continuous Performance Testing	CPT	>4 yr	Sustained attention

Emotionality, Activity, Sociability, and Impulsivity Temperament Survey	EASI	3-18	Tendency to emotionality, activity, sociability, and impulsivity
Neurobehavioral Evaluation System	NES2		Reaction time Hand-eye coordination Continuous performance
Pre-school Activities Inventory	PSAI		Gender role behavior

* d, days; mo, months; yr, years.

Table 3. Reviews of studies about the effects of dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) on children's neurodevelopment. [*]

Study	Size [**]	Biospecimen	DDT/DDE Conc [†]	Age	Test [‡]	Result [§]
USA						
North Carolina Cohort (1978-1982)						
Rogan 1986 [58]	(912)	Milk at birth	DDE: Median=2.51 ppm, 95th=7.59 (ppm)	<1 mo	BNBAS	DDE: Reflex ↓
Gladen 1988 [61]	(802)	Milk at birth	(similar to Rogan 1986)	6 mo 12 mo	BSID	DDE: MDI at 6 mo ↑ MDI at 12 mo –
Rogan 1991 [62]	(676)	Milk at birth	(similar to Rogan 1986)	18 mo 24 mo	BSID	DDE: –
Gladen 1991 [72]	(645)	Milk at birth	(similar to Rogan 1986)	3 yr 4 yr 5 yr	MSCA English, math grades	DDE: –
Oswego Cohort (1991-1994)						
Stewart 2000 [59]	293	Cord blood	DDE: 25th=0.06, 50th=0.10, 75th=0.18 (ng/g wet)	Birth	BNBAS	DDE: –
Darvill 2000 [70]	230 (86)	Cord blood Breast milk b/w 1-3 months	Not mentioned	6 mo 12 mo	FTII	DDE: –
Stewart 2003 [73]	189 (86)	Cord blood Breast milk b/w 1-3 months	Not mentioned	4.5 yr 7.8 yr	CPT MRI (n=60)	DDE: –
California – CHAMACOS study (1999-2000)						

Eskenazi 2006 [85]	330	Maternal blood at 3rd trimester or delivery	*p,p'*-DDT: range: 1.55-33174, geometric mean (95%CI)=22.0 (18.4-26.4) (ng/g of lipid) *o,p'*-DDT: range: 0.07-1878.1 geometric mean (95%CI)=1.8 (1.5-2.1) (ng/g of lipid) *p,p'*-DDE: range: 48.80-159303.3, geometric mean (95%CI) = 1436.9 (1257.4-1642.1) (ng/g of lipid).	6 mo 12 mo 24 mo	BSID	*p,p'*-DDT: MDI at 6 mo – MDI at 12, 24 mo ↓ PDI at 6, 12 mo ↓ PDI at 24 mo – *o,p'*-DDT: MDI at 6 mo – MDI at 12, 24 mo ↓ PDI at 6, 12, 24 mo – *p,p'*-DDE: MDI at 6, 12, 24 mo – PDI at 6 mo ↓ PDI at 12, 24 mo –
Denmark						
Faroe Islands Cohort (1986-1987)						
Grandjean 2001 [81]	435	Cord tissue	p,p'-DDE: median=0.713 (µg/g lipid)	7 yr	NES2 WISC-R BVMG CVLT BNT Neurophysiological and sensory test	Collinear with total PCBs (data not shown)
Spain						
Spain cohort (1997-1999)						
Ribas-Fito 2003 [66]	92	Cord blood	p,p'-DDE: <LOD - ?	13 mo	BSID GMDS	MDI ↓ PDI ↓ Social ↓ Performance ↓ Hearing & language – Locomotor ↓ Eye-hand coordination –

Ribas-Fito 2006 [86]	475[#]	Cord blood	*p,p'*-DDT: [Ribera d'Ebre] 50th=0.05, max=1.87 (ng/ml) [Menorca] 50th=0.08, max=2.28 (ng/ml) *p,p'*-DDE: [Ribera d'Ebre] 50th=0.86, Max=7.11 (ng/ml) [Menorca] 50th=1.03, Max=19.54 (ng/ml)	4 yr	MSCA (also regrouped as verbal memory, working memory, memory span or short-term memory, and executive function by authors)	*p,p'*-DDT: GCI ↓ Memory ↓ Verbal ↓ Executive function ↓ Memory span ↓ Verbal memory ↓ *p,p'*-DDE: –

[*] Abbreviation used in this table: CI, confidence interval; mo, months; yr, years; PDI, psychomotor development index; MDI, mental development index; GCI, General Cognitive Index; CHAMACOS, the Center for the Health Assessment of Mothers and Children of Salinas.

[†] DDT or DDE concentrations listed in this table may not be directly comparable, because different studies used different biospecimens to estimate the concentrations. In addition, sensitivities, detection limits, equipments, and recovery methods of laboratory analyses might be different.

[‡] BNBAS, Brazeltom Neonatal Behavioral Assessment Scale; BNT, Boston Naming Test; BSID, Bayley scales of Infant Development; BVMG, Bender Visual Motor Gestalt Test; CPT, Continuous Performance Testing; CVLT, California Verbal Learning Test; FTII, Fagan Test of Infant Intelligence; GMDS, Griffiths Mental Development Scales; NES2, Neurobehavioral Evaluation System; MRI, magnetic resonance imaging; MSCA, McCarthy Scales of Children's Abilities; WISC-R, Wechsler Intelligence Scale for Children-Revised.

[§] – indicates no association. ↑ indicates positive association with the increments of DDT or DDE concentrations. ↓ indicates negative association with the increments of DDT or DDE concentrations.

[#] This study included two cohorts, Ribera d'Ebre cohort (n=70) and Menorca cohort (n=405).

[**] Numbers in the parentheses represent the numbers of breast milk samples in each study.

Table 4. Reviews of studies about the effects of polychlorinated biphenyls (PCBs) on children's growth. [*]

Study	Size[#]	Biospecimen	PCBs Conc [†]	Age	Outcome	Result [‡]
USA						
CPP Cohort (1959-1965)						
Ribas-Fito 2006 [97]	1712	Maternal blood at 3rd trimester	Median=2.7, interquartile range: 1.8-3.7 (µg/l, wet weight)	1 yr 4 yr 7 yr	Height Weight	–
Lamb 2006 [93]	150	Maternal blood at 3rd trimester	25th=6.8, 75th=10.4, mean=9.2, SD=3.5 (µg/l)	Birth 1 yr 4 yr 7 yr 17 yr	Height Weight	Ortho-substituted PCBs: Height ↑↓ Weight ↓ (through 17 yrs, girls only)
California – CHDS cohort (1964-1967)						
Hertz-Picciotto 2005 [92]	399	Maternal blood at 2nd or 3rd trimester	Median=616, 5th=378, 95th=1115 (ng/g lipids)	Birth 5 yr	Height Sitting height Weight Head circumference Bi-acromial distance Bi-iliac distance Chest depth Chest breadth	Height at 5 yr ↑ (girls only) Sitting height at 5 yr ↑ (girls only)
Michigan – PBB incident cohort (1976-1979)						
Blanck 2002 [94]	206	Maternal blood at enrollment	Median=5, range: ND-78 (ppb)	5-24 yr (mean =15.2 yr)	Height Weight	Height – Weight adjusted for height and age ↓ (girls only)
North Carolina Cohort (1978-1982)						
Rogan 1987 [111]	(802)	Milk at birth	(similar to Rogan 1986)	0-1 yr	Weight gain	–
Gladen 2000 [96]	(594)	Milk at birth	Median=1.7, range: 0.5-5.5 (ppm)	0-5 yr	Height Weight Stage of pubertal development	Weight adjusted for height ↑ (white girls only)

Michigan Cohort (1980-1981)						
Jacobson 1990 [95]	146 (87)	Cord blood Breast milk within 2 months	Cord serum: mean=2.5, SD=2.0, range: 0-12.3 (ng/ml) Breast milk: mean=835.9, SD=388.4, range: 185.7-2600 (ng/ml)	4 yr	Height Weight Head circumference	Prenatal PCBs: Weight ↓ Height – Head circumference – Postnatal PCBs: –
Germany						
Hesse cohort (1994-1997)						
Karmaus 2002 [98]	343	Child's blood at 8 years old	Not mentioned	Birth 1-48 mo 8 yr 9 yr 10 yr	Height Weight	–
Netherlands						
Dutch cohort (1990-1992)						
Patandin 1998 [90]	207 (105)	Maternal blood at last month of pregnancy Cord blood Breast milk in the 2-6 weeks	Maternal serum: Median=2.04, range: 0.59-7.35 (µg/l) Cord serum: Median=0.40, range: 0.08-2.08 (µg/l) Breast milk (n=105): Median=391.5, range: 173.7-1226.4 (µg/kg fat)	10 d 3 mo 7 mo 18 mo 42 mo	Length Weight Head circumference	Prenatal PCBs: (b/w birth-3 mo, in formula-fed group only) Growth rate (weight change) ↓ Length ↓ Head circumference ↓ Postnatal PCBs: –
Denmark						
Faroe Islands Cohort (1994-1995)						
Grandjean 2003 [91]	(182)	Breast milk in the first 2 weeks	Geometric mean=1.52, range: 0.07-18.5 (µg/g lipid)	18 mo 42 mo	Standing height Weight	Height at 18 mo ↓ Weight at 18 mo ↓ Height at 42 mo – Weight at 42 mo –

[*] Abbreviation used in this table: CPP, the Collaborative Perinatal Project; CHDS, the Child Health and Development Study; SD, standard deviation; d, days; mo, months; yr, years; b/w, between; PBB, polybrominated biphenyl.

[†] PCBs concentrations listed in this table may not be directly comparable, because different studies used different congeners to compose the total PCBs concentrations. Different studies also used different biospecimens to estimate the PCBs concentrations. In addition, sensitivities, detection limits, equipments, and recovery methods of laboratory analyses were different.

[‡] – indicates no association. ↑ indicates positive association with the increments of PCBs concentrations. ↓ indicates negative association with the increments of PCBs concentrations. ↑↓ indicates inconsistent associations.

[#] Numbers in the parentheses represent the numbers of breast milk samples in each study.

Table 5. Reviews of studies about the effects of dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) on children's growth. [*]

Study	Size[#]	Biospecimen	DDT/DDE Conc [†]	Age	Test	Result [‡]
USA						
CPP Cohort (1959-1965)						
Gladen 2004 [112]	304	Maternal blood at 3rd triemester	*p,p'*-DDT: Median=1.9, range: ND-12.7 (µg/g lipid) *o,p'*-DDT: Median=0.14, range: ND-1.33 (µg/g lipid) *p,p'*-DDE: Median=5.7, range: 1-25 (µg/g lipid)	Puberty	Height BMI Ratio of sitting height to height Triceps skinfold thickness Ratio of subscapular to the sum of triceps and subcapular skinfold thickness Skeletal age	– (male African Americans only)
Ribas-Fito 2006 [97]	1712	Maternal blood at 3rd triemester	*p,p'*-DDE: Median=24.4, interquartile range: 17.0-36.2 (µg/l)	1 yr 4 yr 7 yr	Height Weight	*p,p'*-DDT: – *p,p'*-DDE: Height ↓
Lamb 2006 [93]	150	Maternal blood at 3rd triemester	Not mentioned	Birth 1 yr 4 yr 7 yr 17 yr	Height Weight	–
California – CHDS cohort (1964-1967)						

Jusko 2006 [113]	399	Maternal blood at 2nd or 3rd trimester	p,p'-DDT: Mean=1.93, SD=1.25, 25th=1.11, 75th=2.30 (µg/g lipid) o,p'-DDT: Mean=0.27, SD=0.22, 25th=0.12, 75th=0.35 (µg/g lipid) p,p'-DDE: Mean=6.85, SD=4.80, 25th=3.90, 75th=8.56 (µg/g lipid)	Birth 5 yr	Height Sitting height Weight Head circumference Bi-acromial distance Bi-iliac distance Chest depth Chest breadth	–
North Carolina Cohort (1978-1982)						
Rogan 1987 [111]	(802)	Milk at birth	(similar to Rogan 1986)	0-1yr	Weight gain	–
Gladen 2000 [96]	(594)	Milk at birth	*p,p'*-DDE: Median=2.4, range: 0.3-23.8 (ppm)	0-5 yr	Height Weight Stage of pubertal development	Height ↑ (boys only) Weight adjusted for height ↑ (boys only)
Germany						
Hesse cohort (1994-1997)						
Karmaus 2002 [98]	343	Child's blood at 8 years old	p,p'-DDE: 0.04-4.02 (µg/l)	Birth 1-48 mo 8 yr 9 yr 10 yr	Height Weight	Height ↓ (girls only)

[*] Abbreviation used in this table: CPP, the Collaborative Perinatal Project; SD, standard deviation; mo, months; yr, years.

[†] DDT or DDE concentrations listed in this table may not be directly comparable, because different studies used different biospecimens to estimate the concentrations. In addition, sensitivities, detection limits, equipments, and recovery methods of laboratory analyses might be different.

[‡] – indicates no association. ↑ indicates positive association with the increments of DDT or DDE concentrations. ↓ indicates negative association with the increments of DDT or DDE concentrations.

[#] Numbers in the parentheses represent the numbers of breast milk samples in each study.

CHAPTER II. RESEARCH AIMS AND HYPOTHESIS

Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) are persistent, bioaccumulative and toxic halogenated compounds, which were broadly used in the United States until the 1970s when they were banned because of their adverse effects on human health and the ecosystem. Although both chemicals have not been used for decades, PCBs and dichlorodiphenyl dichloroethylene (DDE), a metabolite of DDT, can still be detected in the environment and in the blood, urine and breast milk of the U.S. population [5-7]. Due to the lipophilic property of PCBs, DDT and DDE, infants could be exposed to these chemicals prenatally through placental transfer or postnatally through breast-feeding.

Some epidemiological studies have shown that PCBs and DDE may influence children's neurodevelopment and growth, but the findings remain inconclusive. In vivo and in vitro studies have shown that PCBs may alter synaptic transmission and dopamine, estrogen and thyroid effects [114-121]; animal and human studies have shown that prenatal and perinatal exposure to PCBs at background levels may influence cognition and behavior in infants and children [33-41]. DDT has estrogenic properties, while DDE has antiandrogenic properties [42,44,45]. In an animal study, DDT exposure during the brain growth spurt period caused cholinergic disturbance and neurobehavioral change in adult life [83].

Since few of the previous studies were focused on the effects of infant lactational exposure and most of the previous studies were conducted during 1980s and 1990s, this dissertation proposes a secondary data analysis of the PIN Babies Study in 2004-2006 to investigate whether lactational exposures to low background levels of PCBs, DDT and DDE affect infant development and physical growth in the first 12 months of life. The specific aims are to:

1. Describe distributions of PCBs, DDT and DDE in breast milk and their correlations with long-chain polyunsaturated fatty acids in the same subsamples.

2. Estimate the associations between the concentrations of PCBs, DDT and DDE in breast milk and infant communicative development at 12 months, measured by the MacArthur-Bates Communicative Development Indices (CDI). The alternative hypothesis for this study aim is that PCBs, DDT and DDE are separately associated with infant CDI scores at 12 months.

3. Estimate the associations between the concentrations of PCBs, DDT and DDE in breast milk and infant physical, cognitive, and communicative development at 12 months, measured by the Mullen Scales of Early Learning (Mullen). The alternative hypothesis for this study aim is that PCBs, DDT and DDE are separately associated with infant Mullen scores at 12 months.

4. Estimate the associations between the concentrations of PCBs, DDT and DDE in breast milk and serial infant growth measurements through the first 12 months. The alternative hypothesis for this study aim is that PCBs, DDT and DDE are separately associated with infant weight and length through the first 12 months.

From sub-analyses, we will also be able to 1) assess the degree of effect measure modification by infant gender, and LC-PUFAs concentration in breast milk, 2) examine which pre-, peri-, and post-natal variables, such as fish consumption, maternal parity, and maternal body weight, could predict PCBs, DDT and DDE concentrations in breast milk at 3 months.

The study population was the children of the PIN Postpartum Study participants, who were recruited for participation in the PIN Babies Study. For this study, samples of breast milk were collected from breast-feeding mothers at 3 months postpartum in order to determine the concentration of PCBs, DDT and DDE in breast milk. The Mullen Scale and MacArthur-Bates CDI were used to assess the children's development at 12 months postpartum. Infant weight and height were recorded regularly on the child's growth card, which was filled out by medical care providers. The expected size of the study population for having both exposure and outcome measurements is approximately 219 mother-child pairs. We hypothesize that in the PIN Babies Study, PCBs and DDE levels in breast milk will have minor associations with the deficits on infant's cognition and also with the delay in infant's growth of the first 12 months.

CHAPTER III. METHODS

A. Study Design Overview

The Pregnancy, Infection and Nutrition (PIN) Study is a cohort study of pregnancy. Pregnant women were recruited before 20 gestational weeks from prenatal care clinics at the University of North Carolina (UNC) Hospitals from January 2001 to June 2005. The women who participated in the PIN were followed up to their delivery. During pregnancy, they completed two research clinic visits at less than 20 gestational weeks and during 24-29 gestational weeks, and completed two telephone interviews during 17-22 gestational weeks and during 27-30 gestational weeks. After delivery, women also completed one brief questionnaire while in the hospital, and were asked to continue study participation as part of the PIN Postpartum Study.

In the PIN Postpartum Study, participant women were interviewed in their homes around the third and the twelfth months postpartum; at the same time, they were asked whether they were willing to have their child participate in PIN Babies Study. Data collection was completed during home visits for women who consented. Each mother completed a computer assisted interview about her health and lifestyle and that of her child. In addition, the child's development was assessed using the Mullen Scales of Early Learning. Self-administered MacArthur-Bates Communicative Development Inventories

(CDI) and a child's growth card were left for mothers to return later. A sample of breast milk was collected from breast-feeding mothers at the third month postpartum and stored at -80°C.

Overall, the PIN Study, the PIN Postpartum Study, the PIN Babies Study collected data about prenatal and postpartum maternal and infant information on basic characteristics, diet, physical activity, psychosocial status, infant feeding habits, etc. The informed consent forms were signed at each study stage, and the participants were compensated for participation to aid retention. The Institutional Review Board of University of North Carolina at Chapel Hill has approved all the study protocols of the PIN studies. Details of these studies are available at the website: http://www.cpc.unc.edu/projects/pin/.

B. Study Population

A total of 2006 women were recruited in the PIN Study from the prenatal care clinics at the University of North Carolina (UNC) Hospitals between January 2001 and June 2005 (participation rate: 64%), of whom 1942 women's delivery information was available. In this PIN cohort, 69% of the women are white, 22% are African American, and 9% are other races. Approximately 80% of the women were between 20-34 years of age, 6% were less than 20 years and 16% were 35 or over. Most women were married (71%), and about half of them had education levels at 16 years and more. Thirty percent of the women self-reported smoking during the pregnancy. For this large cohort, 14.6%

had preterm births (n=284) and 9.9% had a low birth weight baby (n=192).

About 65% of PIN Study participants gave consent for the PIN Postpartum Study. The women having singleton births without major birth defects were further asked to have their children participate in the PIN Babies study at 3 months postpartum and 12 moths postpartum. The end of data collection of PIN Postpartum Study and PIN Babies Study was in December 2006. There were 589 infants participated in the PIN Babies Study, 496 (84%) participated at the 3 months, and 502 (85%) participated at the 12 months. Over two-thirds of the subjects, 69% (n=409) participated in both the 3- and 12-month time periods. Of the 331 women still breast feeding at 3 months, 306 (92%) were willing to provide a breast milk sample (304 were usable). The comparison of characteristics among the PIN Studies showed that a higher percentage of white women participated in the PIN Postpartum and provided breast milk in the PIN Babies Study than participated in the original PIN Study. Participant women who were still breast feeding at 3 months and willing to provide breast milk were older and had higher education levels compared to the PIN sample (table 6).

For the purpose of dissertation, to investigate the association between the levels of PCBs and DDE in breast milk and infant development and growth, the study population will be a subset of mother-child pairs in the PIN Babies study – singleton births without major birth defects with the mother breastfeeding and providing a valid breast milk sample at the third month postpartum. The expected size is approximately 219 mother-child pairs.

C. Data Collection

In the PIN Postpartum Study, the participant women were called beforehand to schedule a home visit interview around the third and twelfth months postpartum. A milk collection kit and a growth card were mailed to the mother before the scheduled visit and collected at the home visit. A confirmation call was made in a few days before the visit to ensure that both the mother and the child were generally healthy and comfortable with the visit.

A two-person research team was sent out for the home visit at the appointed time. The team had standardized training on conducting the computer-assisted interview and administering the Mullen Scales of Early Learning. At the home visit, the research staff explained the research goals and interview steps to the mother, and obtained signed consent. One interviewer, then interviewed the mother, asking questions about her health status, diet, psychosocial status, physical activity, and infant health and feeding, while the other person observed and interacted with the infant to score the Mullen Scales. After the 12-month visit interview, the child's growth card was collected, and the self-administered short-form version of the MacArthur-Bates Communicative Development Indices (CDI) was left for mothers to return by mail. The completion time of the whole 12-month home visit was around 2 hours.

C.1 Exposure Measurement

Participant women who breastfed their child at 3 or 12 months postpartum were asked to provide a breast milk sample for measuring the concentrations of chemicals in the breast milk. A milk collection kit, containing three 1.5ml tubes, a plastic pipette, and the instructions, was sent before the scheduled visit. At around 10 in the morning of the scheduled visit day, participants followed the instructions to use the pump to empty both breasts, gently mix the milk and use the plastic pipette to transfer the milk into 3 tubes. These milk samples were frozen in the refrigerator until the interviewers came, and the samples were transported on ice back to the -80°C storage freezers in the Carolina Population Center.

Breast milk samples were analyzed for nine dioxin-like PCBs, twenty-seven nondioxin-like PCBs, p,p'-DDT, o,p'-DDT, and p,p'-DDE (table 7) according to the existing methodology at the Organic Analytic Toxicology Branch of the National Center for Environmental Health at CDC under the supervision of Dr. Andreas Sjödin [122]. The measurement of selected chemicals in breast milk samples was performed using gas chromatography/isotope dilution high-resolution mass spectrometry (GC/IDHRMS) using a MAT95 instrument (ThermoFinnigan MAT; Bremen, Germany). All milk samples were pasteurized prior to analysis to eliminate risk of pathogen exposure to the experimenter. Breast milk (1g) was added to cartridges containing Hydromarix (1g) (Varian Inc, Walnut Creek, CA). To the specimens were then added ^{13}C-labeled internal standards. The specimens were then dried using pressurized nitrogen and the milk lipids

eluted with dichloromethane (10mL), using the Rapid Trace Solid-phase Extraction Workstation (Caliper Life Sciences; Hopkinton, MA) for automation. The lipid concentration was gravimetrically determined by an analytical balance AX105 Delta Range (Mettler Toledo; Columbus, OH) with an accuracy of $\pm 10^{-4}$ g. The lipid extracts were then purified on a two layered column containing silica (top layer) and silica/sulfuric acid (bottom layer), using the Solid-phase Extraction Workstation for automation. The specimens were then evaporated to 10uL and fortified with recovery standards. Each analysis batch contained sixteen unknowns, two method blanks and two quality control (QC) specimens. The specimens were then analyzed by GC-IDHRMS together with a 6-point calibration curve spanning the concentration range of 0.2-2000 μg/l. The between-assay coefficient of variation (CV) is normally less than 10%. The medians of limits of detection (LODs) are given in the table 7.

C.2 Outcome Ascertainment

The outcomes of interest are infant early development and growth in the first 12 months of life, which is a period of dramatic change in babies' cognitive development and physical growth. A baby learns to recognize sounds, focus vision, produce sounds, and interact with others by using cries, chuckles, gestures, and babbling. At the end of the first year, an infant's weight is triple and body length is increased by a half. PIN Babies study chose the Mullen Scales of Early Learning (the Mullen) and the infant short-form version of the MacArthur Communicative Development Inventories (CDIs) to detect the variation of cognition, motor skills, and language developments among the one-year-old

infants. In addition, the study collected babies' growth measurements from routine clinical checkups in the first year as a means to measure each infant's growth trajectory.

C.2.1 Cognitive and Motor Development – by the Mullen Scales of Early Learning

The Mullen Scales of Early Learning (the Mullen) were administered at the 12-month postpartum home visit by trained testers. This instrument was designed for evaluating children aged from birth to 68 months old on five dimensions of development – visual reception, fine motor, receptive language, expressive language, and gross motor. The Mullen can provide scores of verbal and nonverbal skills separately along with an overall index of developmental ability. Some of the evaluations using the Mullen involved the mother's assistance and her observation from daily care. The overall administration time for evaluating a 12-month-old infant is about 30 minutes. The Mullen has fair reliability and moderate correlations with other developmental scales [123]. It has been used in several autistic spectrum disorders studies [124-126].

C.2.2 Language Comprehension and Production – by the Infant Short-form of the MacArthur Communicative Development Inventories

Infant language comprehension and production at 12 months postpartum were measured by the MacArthur Communicative Development Inventories (CDIs). The CDIs are parent-report instruments for evaluating children's early language development (http://www.sci.sdsu.edu/cdi/cdiwelcome.htm). There are two forms – one for infants

aged 8-18 months and the other for toddlers aged 16-30 months [127]. Compared to two other traditional means, language sampling and structured testing, CDIs are less labor-intensive, have better cost-efficiency, are easier in larger studies, and have greater feasibility for assessing the children younger than two-and-a-half years old [127]. However, since the forms are completed by parents, it is limited to literate parents. In the PIN cohort, only 8% of participant women had education level less than 12 years and all could read, so this limitation won't influence the validity of our outcome measurement.

To shorten the self-administration time for the mother to increase the response rate, we used the short-form version of the infant form for infants aged between 8 and 18 months, which is an 89-word inventory for vocabulary comprehension and production instead of 396-word full form [128]. At the 12-month postpartum home visit, the interviewers left this self-administrative short-form for participant mothers to fill out and return by mail. The short-form checklist contains two columns, one labeled as "Understands" and the other labeled as "Understands and Says". These two choices are exclusive. If a baby can understand the word on the list but can't say it, the mother should check "Understands". If a baby can both understand and say the word, the mother should check "Understands and Says" rather than "Understands". The overall correlation between the short-form and the original long-form is very high (r=0.97), but an action and gesture scale is not included in the short-form because of inability to reliably simplify it in the brief format [128,129]. Meadows et al paper has showed that mothers can consistently report their infants' communicative acts, which suggests the certain reliability of parent-report instrument [130]. The infant short-version of CDIs can not be

used for identifying children at risk for later clinical language deficits, but the correlation between the early infant form and later toddler form provide the evidence for the instrument's predictive validity for language development [127].

C.2.3 Physical Growth – by the Babies' Growth Card

Mothers were provided with the babies' growth card and were asked to take the card to the child's 12-month well-child medical visit. This card, filled out by medical care providers, recorded infant weight and length at each previous routine clinical baby visit. The suggested time for visits are at 2, 4, 6, 9, and 12 months of age, but the frequency and times of visits may vary among infants by their health status. This growth card was then collected at the 12-month postpartum home interview.

C.3 Other Covariates

We're also interested in knowing the correlation between PCBs, DDT, DDE and LC-PUFAs in breast milk. Fatty acid extraction and assessment was performed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, MN). Fatty acids were extracted from 0.5 ml of breast milk mixed with 0.5 ml of 0.9% saline. 0.1 mg of 17:0 standard (diheptadecanoyl phosphatidylchloine) was added to monitor efficacy of extraction. The extraction process was conducted by a Hewlett Packard 5890 gas chromatograph equipped with a HP6890A autosampler as previously described [131]. Nine LC-PUFAs in breast milk were identified:

five ω-3 FAs – 18:3 (ALA), 20:3, 20:5 (EPA), 22:5 (docosapentaneoic acid), and 22:6 (DHA); four ω-6 FAs - 18:2 (LA), 18:3 (gamma-LA), 20:3 (dihomo-gamma-LA), and 20:4 (AA).

Other covariates of interest are potential confounders and risk factors (figure 1). Potential confounders are the factors which are associated with both lactational PCBs, DDT and DDE and infant development or growth, but which should not be an effect of either exposure or outcome. These factors are race/ethnicity, maternal age, socioeconomic status, duration of breastfeeding, parity and additionally maternal BMI for the investigation of infant growth. Risk factors are the factors related to outcome, but not apparently associated with exposure. These include maternal smoking, alcohol consumption and drug use during pregnancy, infant gender, and infant age. For infant growth association, maternal diabetes, paternal weight and height, and in utero growth retardation are additionally included. All these data are collected through interviews, self-adminitered questionnaires and the prenatal medical records in PIN, PIN Postpartum, and PIN Babies studies.

D. Data Analyses

The data from the PIN, PIN Postpartum, and PIN Babies studies will be merged using subject identification numbers by the programmer at the Carolina Population Center. Data will be further checked and cleaned for plausible range and consistency across studies. The conflicts among different sub-files may indicate the errors of data linkage or

key-in. Before the analyses for specific aims, frequency distribution or descriptive statistics of each variable of interest will be assessed to detect potential outliers and missing data. Unrealistic answers or numbers for each variable will be flagged for verification.

D.1 Specific Aim 1 – Describe Distributions of PCBs, DDT and DDE in Contemporary Breast Milk and Their Correlations with Long-Chain Polyunsaturated Fatty Acids in a Subsample

Descriptive analyses of the concentrations of PCBs, DDT, DDE, and LC-PUFAs will be done via univariate analyses. The minimum, maximum, median, mean, standard error, number of missing, skewness and graphical distribution of each chemical will be described. The correlations between PCBs, DDT and DDE and LC-PUFAs will be assessed in the sub-sample of PIN Babies Study participants who have observations of both environmental pollutants and LC-PUFAs in breast milk. The sample size is approximately 175 participant mothers. The demographic distribution of mothers with LC-PUFAs data and mothers without LC-PUFAs data will be compared.

D.2 Specific Aim 2 – Estimate the Associations Between PCBs, DDT and DDE in Breast Milk and Infant Development at 12 Months

Initial exploratory analyses for the concentrations of PCBs, DDT and DDE in breast milk, developmental scores of the Mullen and the CDI, and the potential confounders will

be done via univariate analyses. For continuous variables, the minimum, maximum, median, mean, standard error, number of missing, skewness and graphical distribution will be described; for categorical variables, sample size, frequency and number of missing will be given. Categories with sparse data will be combined to reduce numbers of strata to increase sample size of the stratum, if the effect estimates are homogeneous across strata. Mother-child pairs with unverifiable erroneous numbers or missing variables will be excluded from the data analyses, if the number of missing is less than 10% and not related to their exposure or outcome status, i.e. under the assumption of missing at random (MAR).

Concentrations of PCBs, DDT and DDE are the main exposures of interest. Nine dioxin-like PCBs, twenty-seven nondioxin-like PCBs, p,p'-DDT, o,p'-DDT, and p,p'-DDE were measured in maternal breast milk at 3 months postpartum (table 8). From linear correlation coefficients between the most prevalent PCB congeners, which are detectable in more than 90% of samples, we find that tri-*ortho*-substituted PCBs are not highly correlated with mono-ortho-substituted PCBs ($r<0.6$) and some of the di-ortho-substituted congeners are not highly correlated with some of the mono-*ortho*-substituted congeners either (table 9). Therefore, we may need to treat each PCB congener as an exposure variable in separate models to explore the potential difference of the effects. We will also group PCB congeners based on the degree of *ortho*-substitution in 3 groups – mono-*ortho*-substituted PCBs, di-*ortho*-substituted PCBs and tri-*ortho*-substituted PCBs, and also use the sum of total PCB congeners to provide one composite exposure value (ΣPCBs). For the samples measured as under the limits of

detection (LOD), three manipulations are used: 1) <LOD is imputed as 0; 2) <LOD is imputed as $LOD/\sqrt{2}$; and 3) <LOD is imputed as $median(LOD)/\sqrt{2}$. Since there is no standard method to calculate ΣPCBs, we decided that only the PCB congeners which have less than 30% of the samples with concentrations under LOD, will be counted in ΣPCBs in order to reduce the exposure misclassification resulting from data imputation. To treat exposure as a continuous variable, natural-log transformation may be needed because of the right-skewness of the distribution. Since the exposure-response trend may not be strictly monotonic, we will further use quartiles of the individual chemical distribution of this study cohort to categorize the study population. The multiplication of concentrations of chemicals in breast milk and the duration of mostly breast-feeding will be calculated to represent the accumulated lactational exposure under the assumptions that the amount of milk fat is similar among mothers and infants consumed the same amount of milk daily.

The Mullen will provide scores of five dimensions of development, visual reception, fine motor, receptive language, expressive language, and gross motor, along with a composite score of early learning. Standard T scores for each scale, with mean=50 and standard deviation (SD)=10, and standardized composite score, with mean=100 and SD=15, will be provided based on the US normative sample. Standard scores will be used when continuous outcomes are of interest, and the percentile ranking and descriptive categories will be used to provide proper categorization of the study population when the risk association is of interest. The lowest 15^{th} percentile or 1 SD below age-adjusted mean will be used as a cut point for low scores.

The short form of the CDI at 12 months provides the scores of infant's language comprehension and production. Raw scores can further be standardized for boys and girls separately or both sexes combined. These percentile scores can be treated as a continuous outcome in the statistical models. In addition, for risk associations of interest, the infants with scores of one SD and greater below the mean will be categorized as a group with lower levels of language development.

The multivariable generalized linear models with identity link function with Gaussian (normal) distribution or log link function with Bernoulli (binomial) will be used to assess the associations between lactational exposure to PCBs, DDT, and DDE and infant development of cognitive skills and language at the 12 months of life. Effect measure modification (EMM) by race/ethnicity and infant gender will be assessed via stratification analysis of the full model. If the p-value of the homogeneity test is less than 0.10, the interaction terms of main exposure and effect modifier will be included in the models. The models will also contain a priori confounders and risk factors based on substantive knowledge – race/ethnicity, maternal age, socioeconomic status, duration of breastfeeding, parity, maternal smoking, alcohol consumption, infant gender, and infant age. The effect estimates with 95% confidence intervals will be reported. All the statistical analyses will be conducted by PC-SAS computer program (version 9.1).

D.3 Specific Aim 3 – Estimate the Associations Between PCBs, DDT and DDE in Breast Milk and Infant Growth through the First 12 Months of Life.

The initial descriptive analyses and defining exposure variables will be the same as the analyses describing in specific Aim 1. Infant serial growth measurements – weight and length – recorded on the babies' growth card through the first 12 months of life will be the outcomes of interest for our second specific aim. The visit time for regular medical checkups are suggested at 2, 4, 6, 9, and 12 months of age, but the number and the time of visits could vary among infants. According to 2000 CDC growth charts [132], infants' standardized sex-specific percentiles for weight-for-age, length-for-age and weight-for-length will be further determined. However, very-low-birth-weight infants, whose birth weight <1,500g, will be omitted, since they have different growth patterns and are excluded from the nationwide reference data. Infants who are ranked as $<10^{th}$ percentile will be categorized as having stunted growth.

To assess the relationships of lactational exposure to PCBs, DDT and DDE and infant growth, we will use the generalized estimating equations (GEE), which handle repeated measurements of outcomes over time and unequal number and time of visits of study subjects [133,134]. Residual plots will be used to assess the homoscedasticity assumption for generalized linear models. If the graphs show heteroscedasticity, mixed models will be used instead of GEE. Identity link function with Gaussian (normal) distribution will be used when weight and length are treated as continuous variables; log link function with Bernoulli (binomial) distribution will be used when the risk ratio of

high risk of slow growth are interested. The working correlation matrix will be defined as unstructured first to examine the correlation structure of repeated measurements. Infant's age will be treated as a time-dependent predictor. A priori covariates contain race/ethnicity, socioeconomic status, maternal age at pregnancy, maternal smoking, alcohol consumption and drug use during pregnancy, maternal diabetes, maternal postpartum BMI, duration of breastfeeding, parity, infant gender, paternal weight and height, and in utero growth retardation. The interaction terms of chemical concentrations with infant age, chemical concentrations with infant gender, and chemical concentrations with in utero growth retardation will be considered. The covariates will be eliminated from the full model if they are not predictors of the outcome ($p>0.10$) and do not confound the main effect estimates of interest by 10% (10% change-in-estimate criterion) [135]. Test for the trend of the associations will be considered through analyses. The effect estimates with 95% confidence intervals will be reported. All the statistical analyses will be conducted by PC-SAS computer program (version 9.1).

D.4 Statistical Power

The expected size of the study population for having both exposure and outcome measurements is approximately 219 mother-child pairs. To simplify the calculations of statistical power, the statistical analyses for specific aim 2 and specific aim 3 are grouped in three situations – linear regression models, log-linear regression models and GEEs. All the hypothesis tests are two-sided and with type 1 error (α) equal to 0.05.

For simple linear regression models, for example, assessing the relationships between the concentrations of PCBs, DDT, and DDE in breast milk and the scores on the infant neurodevelopment tests, the statistical power is calculated by the nQuery Advisor® software given several assumed values (α=0.05, n=219):

If the correlation coefficient (ρ) = 0.05, the power will be 11%.

If the correlation coefficient (ρ) = 0.10, the power will be 31%.

If the correlation coefficient (ρ) = 0.15, the power will be 60%.

If the correlation coefficient (ρ) = 0.20, the power will be 85%.

For log-linear regression models, for example, assessing the risk ratio of having deficits in neurodevelopment at the twelfth month between exposed and non-exposed groups, the statistical power is calculated by episheet.xls, developed by Ken Rothman, given several assumed values (α=0.05, n=219, unexposed/exposed ratio=3, 15% of unexposed group categorized as having low scores on the tests):

If the risk ratio (RR) = 1.2, the power will be 8%.

If the risk ratio (RR) = 1.5, the power will be 26%.

If the risk ratio (RR) = 2.0, the power will be 68%.

If the risk ratio (RR) = 3.0, the power will be 99%.

To simplify the power calculations for GEEs, we have focused on calculating the sample size for testing the hypothesis that the infant growth rates of change through the first 12 months, as measured by weight, are the same for two equal-sized exposure groups. We assume: 1) the correlations (ρ) among repeated growth measurements are consistent

through the time period, i.e. exchangeable correlation matrix; 2) every child has an equal number (n) and time of visits, i.e. 5 visits at 2, 4, 6, 9, and 12 months of age. Given type I error (α) =0.05, power($1-\beta$)=80%, and assumptive measurement variation (σ^2)=100, we use the formula below to calculate that if the correlation between each measurement is 0.2, we will need 79 subjects per exposed group to detect the difference in infant weight change rate of 0.5 kg/month. When the correlation between each measurement (ρ) increases and measurement variation (σ^2) decreases, the sample size to detect the same effect will decrease. Since we may have at least a total of 200 subjects for this study aim, this study should be able to reach an acceptable statistical power.

$$N = \frac{2\left(Z_{\alpha/2} + Z_{\beta}\right)^2 \sigma^2 (1-\rho)}{n s_x^2 d^2} \qquad 136$$

Table 6. Characteristics of the participant women in this study, in the PIN Postpartum Study and in the PIN 3 Cohort.

	OCs+FAs (n=175)		Milk Samples (n=304)		PIN Postpartum (n=688)		PIN3 Cohort (n=2006)	
Characteristics	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Race								
White	152	87	262	86	526	76	1382	69
Black	12	7	18	6	102	15	434	22
Other	11	6	24	8	60	9	189	9
Missing							1	
Age								
<25	18	10	32	11	132	19	479	24
25-29	51	29	88	29	195	28	557	28
30-34	76	43	130	43	250	36	657	33
≥35	30	17	54	18	111	16	313	16
Education								
< High school	4	2	7	2	36	5	166	8
High school	6	3	10	3	82	12	328	16
≥ College degree	165	94	287	94	570	83	1503	75
Missing							9	
Parity								
0	95	54	160	53	334	49	901	45
≥1	80	46	144	47	354	51	1098	55
Missing							7	
Pre-pregnancy BMI								
<19.8	30	17	52	17	97	14	266	14
19.8-26.0	111	63	189	62	371	54	930	49
26.0-29.0	12	7	27	9	82	12	215	11
>29.0	22	13	36	12	136	20	487	26
Missing						2	108	

Table 7. List of chemicals of interest measured in breast milk samples and the median of lipid adjusted limit of detection (LOD) (ng/g lipid).

Chemicals	Name	Median(LOD)
PCBs		
mono-*ortho*-substituted PCBs		
PCB 28	2,4,4'-trichlorobiphenyl	2.50
PCB 66	2,3',4,4'-tetrachlorobiphenyl	0.30
PCB 74	2,4,4',5-tetrachlorobiphenyl	0.30
PCB 105	2,3,3',4,4'-pentachlorobiphenyl	0.30
PCB 118	2,3',4,4',5-pentachlorobiphenyl	0.30
PCB 156	2,3,3',4,4',5-hexachlorobiphenyl	0.30
PCB 157	2,3,3',4,4',5'-hexachlorobiphenyl	0.30
PCB 167	2,3',4,4',5,5'-hexachlorobiphenyl	0.30
PCB 189	2,3,3',4,4',5,5'-heptachlorobiphenyl	0.30
di-*ortho*-substituted PCBs		
PCB 44	2,2',3,5'-tetrachlorobiphenyl	0.30
PCB 49	2,2',4,5'-tetrachlorobiphenyl	0.30
PCB 52	2,2',5,5'-tetrachlorobiphenyl	0.30
PCB 87	2,2',3,4,5'-pentachlorobiphenyl	0.30
PCB 99	2,2',4,4',5-pentachlorobiphenyl	0.30
PCB 101	2,2',4,5,5'-pentachlorobiphenyl	0.30
PCB 110	2,3,3',4',6-pentachlorobiphenyl	0.30
PCB 128	2,2',3,3',4,4'-hexachlorobiphenyl	0.30
PCB 138	2,2',3,4,4',5'-hexachlorobiphenyl	0.30
PCB 146	2,2',3,4',5,5'-hexachlorobiphenyl	0.30
PCB 153	2,2',4,4',5,5'-hexachlorobiphenyl	0.30
PCB 158	2,3,3',4,4',6-hexachlorobiphenyl	0.30
PCB 170	2,2',3,3',4,4',5-heptachlorobiphenyl	0.30
PCB 172	2,2',3,3',4,5,5'-heptachlorobiphenyl	0.30
PCB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl	0.30
PCB 194	2,2',3,3',4,4',5,5'-octachlorobiphenyl	0.30
tri-*ortho*-substituted PCBs		
PCB 149	2,2',3,4',5',6-hexachlorobiphenyl	0.30
PCB 151	2,2',3,5,5',6-hexachlorobiphenyl	0.30
PCB 177	2,2',3,3',4',5,6-heptachlorobiphenyl	0.30
PCB 178	2,2',3,3',5,5',6-heptachlorobiphenyl	0.30
PCB 183	2,2',3,4,4',5',6-heptachlorobiphenyl	0.30
PCB 187	2,2',3,4',5,5',6-heptachlorobiphenyl	0.30
PCB 195	2,2',3,3',4,4',5,6-octachlorobiphenyl	0.30
PCB 196	2,2',3,3',4,4',5',6-octachlorobiphenyl	0.30
PCB 199	2,2',3,3',4,5,5',6'-octachlorobiphenyl	0.30
PCB 203	2,2',3,4,4',5,5',6-octachlorobiphenyl	0.30
PCB 206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	0.30
DDT and DDE		
p,p'-DDT	2,2-bis(4-chlorophenyl-1,1,1-trichloroethan	0.30
o,p'-DDT	2-(4-chlorophenyl)-2-(2-chlorophenyl)- 1,1,1-trichloroethan	0.30
p,p'-DDE	2,2-bis(4-chlorophenyl)-1,1-dichloroethene	0.30

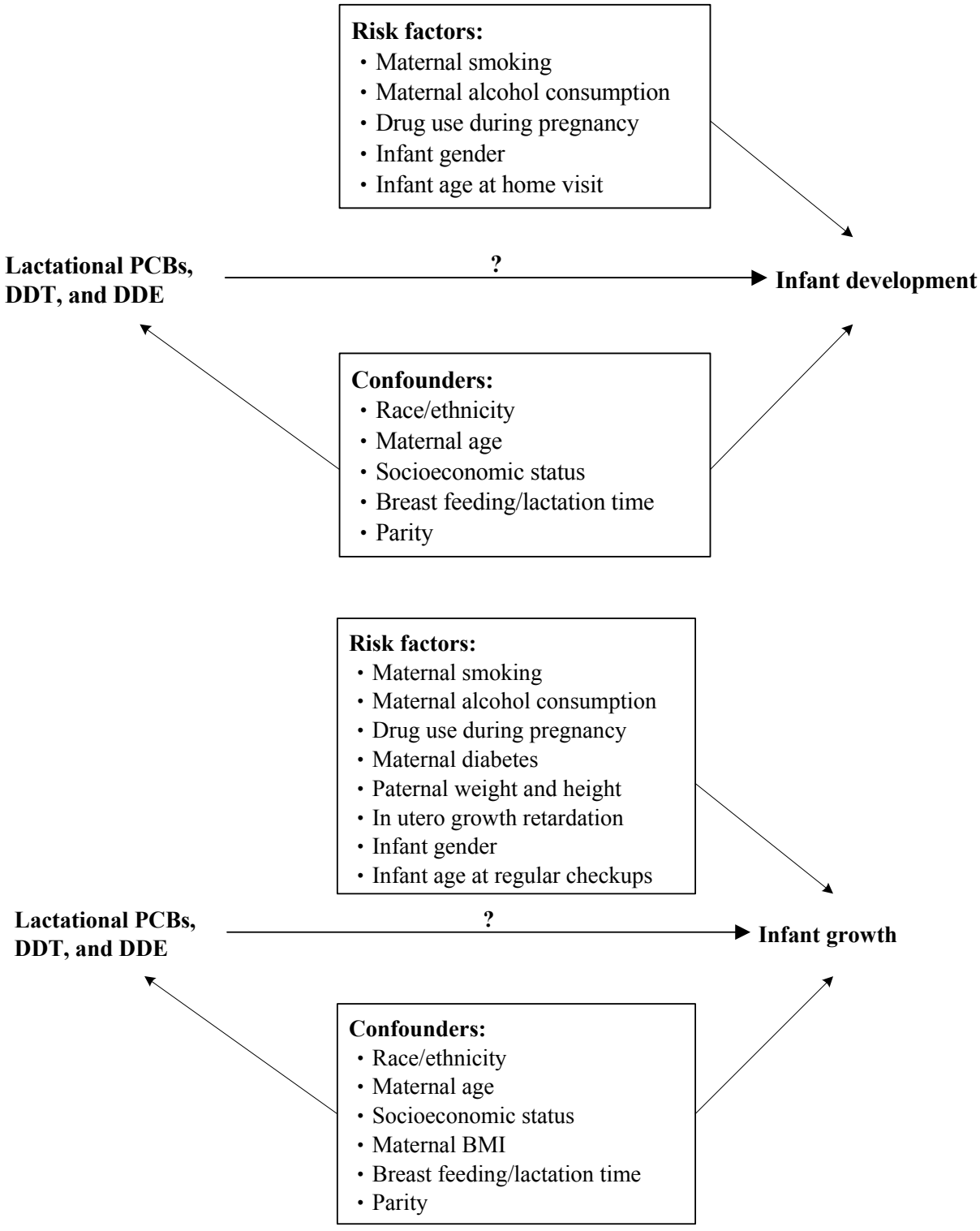

Figure 1. Conceptual diagram of potential covariates of the association between PCBs, DDT and DDE in breast milk and infant development and growth. An arrow indicates the causal effect.

Table 8. Lipid-adjusted chemical concentrations (ng/g lipid) in maternal breast milk at 3 months postpartum (n=304).

Chemicals	D (%)*	Minimum	25th	Median	75th	Maximum	(a)Mean (SD)†	(b)Mean (SD)†	(c)Mean (SD)†
PCB28	106 (34.9)	<LOD	<LOD	<LOD	2.3	22.4	1.23 (2.28)	3.1 (2.49)	2.39 (1.75)
PCB44	34 (11.2)	<LOD	<LOD	<LOD	<LOD	6.4	0.1 (0.52)	0.37 (0.52)	0.29 (0.48)
PCB49	29 (9.5)	<LOD	<LOD	<LOD	<LOD	4.3	0.05 (0.29)	0.33 (0.35)	0.24 (0.26)
PCB52	187 (61.5)	<LOD	<LOD	0.3	0.6	5.9	0.45 (0.64)	0.59 (0.60)	0.53 (0.59)
PCB66	264 (86.8)	<LOD	0.55	1	1.4	7	1.08 (0.88)	1.15 (0.82)	1.1 (0.85)
PCB74	301 (99.0)	<LOD	3.35	5	6.9	24.7	5.68 (3.63)	5.69 (3.63)	5.68 (3.63)
PCB87	44 (14.5)	<LOD	<LOD	<LOD	<LOD	6.6	0.11 (0.44)	0.38 (0.46)	0.29 (0.41)
PCB99	301 (99.0)	<LOD	2.6	4	5.8	33.3	4.75 (3.66)	4.75 (3.65)	4.75 (3.66)
PCB101	154 (50.7)	<LOD	<LOD	0.2	0.5	8	0.32 (0.57)	0.51 (0.54)	0.42 (0.52)
PCB105	280 (92.1)	<LOD	0.9	1.4	2	11.7	1.69 (1.54)	1.74 (1.50)	1.7 (1.52)
PCB110	18 (5.9)	<LOD	<LOD	<LOD	<LOD	7.6	0.05 (0.45)	0.34 (0.49)	0.25 (0.43)
PCB118	303 (99.7)	<LOD	4.1	5.95	9.35	52.6	7.65 (6.07)	7.65 (6.07)	7.65 (6.07)
PCB128	11 (3.6)	<LOD	<LOD	<LOD	<LOD	6.3	0.04 (0.37)	0.33 (0.43)	0.24 (0.35)
PCB138_158	303 (99.7)	<LOD	9.7	15.15	24.1	144	18.93 (14.83)	18.93 (14.83)	18.93 (14.83)
PCB146	293 (96.4)	<LOD	1.1	1.9	3.1	11.9	2.42 (1.99)	2.45 (1.97)	2.43 (1.99)
PCB149	15 (4.9)	<LOD	<LOD	<LOD	<LOD	6.1	0.05 (0.47)	0.34 (0.51)	0.25 (0.45)
PCB151	24 (7.9)	<LOD	<LOD	<LOD	<LOD	6.7	0.1 (0.66)	0.38 (0.67)	0.29 (0.64)
PCB153	304 (100.0)	2.2	10.5	16.55	26.6	199	21.58 (18.64)	21.58 (18.64)	21.58 (18.64)
PCB156	274 (90.1)	<LOD	1.2	2.15	3.65	19.7	2.87 (2.75)	2.93 (2.69)	2.89 (2.73)
PCB157	177 (58.2)	<LOD	<LOD	0.4	0.8	7.8	0.56 (0.79)	0.74 (0.72)	0.65 (0.73)
PCB167	155 (51.0)	<LOD	<LOD	0.2	0.7	7.1	0.47 (0.72)	0.66 (0.65)	0.57 (0.66)
PCB170	300 (98.7)	<LOD	2.4	3.5	5.95	54.5	4.83 (4.61)	4.84 (4.60)	4.83 (4.61)
PCB172	161 (53.0)	<LOD	<LOD	0.3	0.7	6.6	0.47 (0.71)	0.66 (0.65)	0.57 (0.65)
PCB177	215 (70.7)	<LOD	<LOD	0.6	1	8.9	0.74 (0.95)	0.88 (0.88)	0.8 (0.9)
PCB178	218 (71.7)	<LOD	<LOD	0.6	1.2	10.4	0.83 (1.02)	0.97 (0.95)	0.88 (0.97)
PCB180	303 (99.7)	<LOD	5.15	8.2	13.75	128	11.13 (10.94)	11.13 (10.94)	11.13 (10.94)
PCB183	253 (83.2)	<LOD	0.6	1.1	1.6	16.2	1.28 (1.52)	1.38 (1.46)	1.31 (1.49)
PCB187	289 (95.1)	<LOD	1.7	2.9	4.7	55.3	3.82 (4.25)	3.85 (4.23)	3.83 (4.24)
PCB189	41 (13.5)	<LOD	<LOD	<LOD	<LOD	7.4	0.09 (0.46)	0.36 (0.49)	0.27 (0.43)
PCB194	257 (84.5)	<LOD	0.7	1.2	2.1	10.4	1.57 (1.54)	1.65 (1.47)	1.61 (1.5)
PCB195	129 (42.4)	<LOD	<LOD	<LOD	0.5	22.1	0.36 (1.37)	0.58 (1.35)	0.48 (1.35)
PCB196_203	265 (87.2)	<LOD	0.8	1.4	2.1	15.9	1.68 (1.61)	1.75 (1.55)	1.7 (1.59)
PCB199	260 (85.5)	<LOD	0.65	1.3	2	9.5	1.64 (1.56)	1.72 (1.50)	1.67 (1.53)
PCB206	190 (62.5)	<LOD	<LOD	0.4	0.6	7.3	0.49 (0.77)	0.65 (0.72)	0.56 (0.72)
mono-*ortho*-PCBs ‡	--	<LOD	10.9	15.45	23.6	100.3	18.96 (13.32)	19.17 (13.21)	19.03 (13.27)
di-*ortho*-PCBs ‡	--	6	33.8	51.7	81.5	567.5	65.26 (53.11)	65.39 (53.06)	65.31 (53.09)

tri-*ortho*-PCBs ‡	--	<LOD	4.15	7.75	12.9	100.7	9.97 (9.91)	10.54 (9.65)	10.2 (9.76)
ΣPCBs ‡	--	6	48.1	77.1	119	708.1	94.21 (72.05)	95.11 (71.67)	94.55 (71.85)
o,p'-DDT	184 (60.5)	<LOD	<LOD	0.5	0.8	8.8	0.57 (0.86)	0.73 (0.80)	0.65 (0.81)
p,p'-DDT	292 (96.1)	<LOD	3.2	4.7	6.9	80.4	6.25 (7.61)	6.28 (7.59)	6.25 (7.6)
p,p'-DDE	304 (100.0)	1.4	85.85	120.5	187	2140	177.44 (222.8)	177.44 (222.80)	177.44 (222.8)

* D (%), number and percent of samples with detectable chemicals.

† (a) <LOD is imputed as 0 to calculate the mean and SD; (b) <LOD is imputed as $LOD/\sqrt{2}$ to calculate the mean and SD; (c) <LOD is imputed as $median(LOD)/\sqrt{2}$.

‡ Mono-*ortho*-PCBs is the sum of the mono-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB66, 74, 105, 118 and 156; di-*ortho*-PCBs is the sum of the di-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB99, 138_158, 146, 153, 170, 180 and 194; tri-*ortho*-PCBs is the sum of the tri-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB 177, 178, 183, 187, 196_203 and 199; ΣPCBs is the sum of the PCB congeners which are detectable in more than 70% samples, i.e. PCB66, 74, 99, 105, 118, 138_158, 146, 153, 156, 170, 177, 178, 180, 183, 187, 194, 196_203 and 199.

Table 9. Linear correlation coefficients between the lipid-adjusted concentrations (ng/g lipid) of the most prevalent PCB congeners in maternal breast milk at 3 months postpartum.

Congener *	74	105	118	156	99	138_158	146	153	170	180	187	Mono- †	Di- †	Tri- †	ΣPCBs †
74	1	0.73776	0.83683	0.71801	0.79582	0.76973	0.72256	0.69874	0.59574	0.57976	0.41219	0.92199	0.72855	0.49353	0.77603
		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
105	0.73776	1	0.93506	0.62813	0.78279	0.699	0.65322	0.58621	0.4686	0.42282	0.33193	0.90851	0.61864	0.42147	0.6825
	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
118	0.83683	0.93506	1	0.69372	0.85265	0.79607	0.73082	0.69558	0.54623	0.52098	0.39327	0.96611	0.71951	0.44966	0.77154
	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
156	0.71801	0.62813	0.69372	1	0.64585	0.86863	0.83486	0.82063	0.80161	0.75336	0.47274	0.81104	0.85284	0.60302	0.86225
	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
99	0.79582	0.78279	0.85265	0.64585	1	0.81852	0.68317	0.68501	0.50638	0.47006	0.3508	0.85206	0.71462	0.41075	0.74152
	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
138_158	0.76973	0.699	0.79607	0.86863	0.81852	1	0.89872	0.96366	0.85812	0.84158	0.641	0.85183	0.97507	0.71158	0.97482
	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
146	0.72256	0.65322	0.73082	0.83486	0.68317	0.89872	1	0.88802	0.76477	0.76638	0.74895	0.80241	0.89163	0.80263	0.91642
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
153	0.69874	0.58621	0.69558	0.82063	0.68501	0.96366	0.88802	1	0.9125	0.92369	0.75517	0.76095	0.99145	0.80466	0.98277
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
170	0.59574	0.4686	0.54623	0.80161	0.50638	0.85812	0.76477	0.9125	1	0.98242	0.60497	0.64584	0.93777	0.72947	0.91159
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
180	0.57976	0.42282	0.52098	0.75336	0.47006	0.84158	0.76638	0.92369	0.98242	1	0.67999	0.61277	0.93684	0.77783	0.91136
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
187	0.41219	0.33193	0.39327	0.47274	0.3508	0.641	0.74895	0.75517	0.60497	0.67999	1	0.44185	0.70684	0.95458	0.73386
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001
Mono- †	0.92199	0.90851	0.96611	0.81104	0.85206	0.85183	0.80241	0.76095	0.64584	0.61277	0.44185	1	0.79207	0.53208	0.84263
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001
Di- †	0.72855	0.61864	0.71951	0.85284	0.71462	0.97507	0.89163	0.99145	0.93777	0.93684	0.70684	0.79207	1	0.78639	0.99234
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001

Tri- †	0.49353	0.42147	0.44966	0.60302	0.41075	0.71158	0.80263	0.80466	0.72947	0.77783	0.95458	0.53208	0.78639	1	0.81552
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001
ΣPCBs †	0.77603	0.6825	0.77154	0.86225	0.74152	0.97482	0.91642	0.98277	0.91159	0.91136	0.73386	0.84263	0.99234	0.81552	1
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

* First row represents Pearson correlation coefficient (r); second row represents p-value for null hypothesis: r=0.

† Mono-*ortho*-PCBs is the sum of the mono-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB66, 74, 105, 118 and 156; di-*ortho*-PCBs is the sum of the di-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB99, 138_158, 146, 153, 170, 180 and 194; tri-*ortho*-PCBs is the sum of the tri-*ortho*-subsituted PCBs concentrations which are detectable in more than 70% of the samples, which are PCB 177, 178, 183, 187, 196_203 and 199; ΣPCBs is the sum of the PCB congeners which are detectable in more than 70% samples, i.e. PCB66, 74, 99, 105, 118, 138_158, 146, 153, 156, 170, 177, 178, 180, 183, 187, 194, 196_203 and 199.

CHAPTER IV. RESULTS

A. Manuscript 1: Organochlorine Compounds and Fatty Acids in Breast Milk in Central North Carolina 2004-2006

A.1 Introduction

Polychlorinated biphenyls (PCB) and organochlorine pesticides, such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), chlordane, lindane and mirex, were widely used from the 1920s. When they were found to be widespread, toxic environmental contaminants with effects on ecosystems, wildlife and potentially human beings, they were banned for most usage in the United States. These organochlorine compounds (OCs) have long half-lives and are lipophilic, and thus they bioaccumulate in the environment through the food chain. Although most uses are restricted, people are still exposed to these chemicals and their metabolites through diet, most commonly by eating contaminated meat and seafood. Infants are exposed mainly through breast milk consumption. Infants are more vulnerable than adults to the potential adverse effects of these environmental pollutants.

Despite the presence of environmental pollutants, breast feeding is still encouraged because it benefits infants. Among other things, breast milk contains long chain fatty

acids (FAs), which are used by the child to make central nervous system tissue. The concentrations of both fatty acids and organochlorines in breast milk are determined by maternal diet, and fish (or marine mammals) in particular can be relatively high in both. A study of Faroe Islanders who consumed fish and pilot whale showed that total PCBs (defined as two times of the summation of PCB138, 153 and 180) were positively correlated with the relative concentrations of eicosapentaenoic acid (EPA) (r=0.42) and docosahexaenoic acid (DHA) (r=0.24) in maternal serum at 34 gestational weeks and negatively correlated with arachidonic acid (AA) in cord serum (r=-0.32) [137]. The Inuit cohort of pregnant women in 1995-2001 showed PCB153 was positively correlated with DHA (r=0.27) in cord blood [138]. None of the previous studies examined the potential correlations between OCs and FAs in breast milk of populations exposed to background levels.

Since most of the previous studies were conducted during the 1980s and 1990s, there are limited data published on the levels of OCs in breast milk of American women in the 21st century [7]. The objectives of this paper are to report the levels of OCs and FAs in the breast milk of lactating women in central North Carolina from 2004 to 2006, to estimate the correlations within and between OCs and FAs, and to identify the factors determining the variability of the chemical concentrations.

A.2 Methods

Study Design and Population

The Pregnancy, Infection and Nutrition (PIN) Study is a cohort study that recruited pregnant women before 20 gestational weeks with a singleton birth who were receiving prenatal care at the University of North Carolina at Chapel Hill hospital from 2001 to 2005. Mothers completed several self-administered questionnaires and two phone interviews during pregnancy and one brief questionnaire after delivery at the hospital to provide details about their health and lifestyle during pregnancy. After delivery, women were asked to continue participation as part of the PIN Postpartum Study. Participant mothers were interviewed in their homes around 3 months postpartum about their health and lifestyle and the health of their child. The Block Food Frequency Questionnaire was self-administered and used to assess maternal diet during the first 3 months postpartum. Between 2004-2006, participant women were also asked whether they were willing to have their child participate in the PIN Babies Study. Women who were still breastfeeding at the 3-month home visit were asked to provide a breast milk sample.

A milk collection kit, containing three 1.5ml tubes, a plastic pipette, and the instructions, was sent before the scheduled visit. At around 10 o'clock in the morning of the scheduled visit day, participants were to follow the written instructions to pump both breasts, gently mix the milk extracted, and use the plastic pipette to transfer the milk into 3 tubes and store them in the freezer until the interviewers arrived. Samples were then transported on ice to the -80 ºC storage freezers. The appropriate informed consent forms were signed at each stage of the study, and the participants were compensated for participation. The Institutional Review Board of University of North Carolina at Chapel

Hill approved all the study protocols of the PIN studies.

Exposure Measurement

Breast milk samples were analyzed for thirty-five PCB congeners, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, HCB, β-hexachlorocyclohexane (HCH), γ-HCH, oxychlordane, *trans*-nonachlor and mirex according to the existing methodology at the Organic Analytic Toxicology Branch of the National Center for Environmental Health at the Centers for Disease Control and Prevention [122]. The measurement of selected chemicals in breast milk samples was performed using gas chromatography/isotope dilution high-resolution mass spectrometry (GC/IDHRMS) using a MAT95 instrument (ThermoFinnigan MAT; Bremen, Germany). All milk samples were pasteurized prior to analysis to eliminate risk of pathogen exposure to the experimenter. Breast milk (1g) was added to cartridges containing Hydromarix (1g) (Varian Inc, Walnut Creek, CA). To the specimens were then added ^{13}C-labeled internal standards. The specimens were then dried using pressurized nitrogen and the milk lipids eluted with dichloromethane (10mL), using the Rapid Trace Solid-phase Extraction Workstation (Caliper Life Sciences; Hopkinton, MA) for automation. The lipid concentration was gravimetrically determined by an analytical balance AX105 Delta Range (Mettler Toledo; Columbus, OH) with an accuracy of $\pm 10^{-4}$ g. The lipid extracts were then purified on a two layered column containing silica (top layer) and silica/sulfuric acid (bottom layer), using the Solid-phase Extraction Workstation for automation. The specimens were then evaporated to 10uL and fortified with recovery standards. Each analysis batch contained sixteen unknowns, two method

blanks and two quality control (QC) specimens. The specimens were then analyzed by GC-IDHRMS together with a 6-point calibration curve spanning the concentration range of 0.2-2000 μg/l. The between-assay coefficient of variation (CV) was normally less than 10%. Only chemicals that were detected in more than 70% of the samples were reported in this paper. The concentrations were lipid adjusted. Total PCBs was calculated as the sum of the eighteen PCB congeners that were detectable in more than 70% samples: PCB66, PCB74, PCB99, PCB105, PCB118, PCB138_158, PCB146, PCB153, PCB156, PCB170, PCB177, PCB178, PCB180, PCB183, PCB187, PCB194, PCB196_203 and PCB199. The samples with concentrations lower than the limits of detection (LOD) were imputed to the median of the method LOD of each measurement divided by a square root of 2.

For 175 milk samples collected in the first 18 months of the study, long chain polyunsaturated fatty acids were measured. The FAs extraction and assessment were performed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, MN). FAs were extracted from 0.5 ml of breast milk mixed with 0.5 ml of 0.9% saline. 0.1 mg of 17:0 standard (diheptadecanoyl phosphatidylcholine) was added to monitor efficacy of extraction. Milk total lipids were determined gravimetrically after extraction with a mixture of chloroform:methanol (2:1, v/v) [139]. FA methyl esters from milk lipid extracts were prepared with 1.5 ml of 14% boron trifluoride in methanol at 80 ºC for 90 minutes, and then extracted with petroleum ether [140].The final product was dissolved in heptane and injected onto a capillary Varian CP7420 100m column. The extraction process was

conducted by a Hewlett Packard 5890 gas chromatograph equipped with a HP6890A autosampler as previously described [131]. Programmed temperature started with 190 ºC for 25 minutes and then 2ºC/min increments up to 240 ºC, and finally was held at 240 ºC for 5 minutes. Adequate separation of fatty acid methylesters was obtained over 50 minutes. Relative concentration of each FA was expressed as percent of total fat. Five ω-3 FAs and four ω-6 FAs in breast milk were identified: 18:3n-3 (alpha-linolenic acid, ALA), 20:3n-3, 20:5n-3 (EPA), 22:5n-3 (docosapentaneoic acid), 22:6n-3 (DHA), 18:2n-6 (linolenic acid, LA), 18:3n-6 (gamma-LA), 20:2n-6, 20:3n-6 (dihomo-gamma-LA), and 20:4n-6 (AA). The ratio of ω-6 FAs to ω-3 FAs was calculated by the concentration of total ω-6 FAs divided by the concentration of total ω-3 FAs.

Statistical Analysis

Initial analyses describing the concentrations of OCs and FAs in breast milk were done via univariate analyses. The number of missing values and minimum, maximum, quartiles, and skewness of the concentration distributions were examined. Pearson correlation coefficients were used to estimate the linear correlations among OCs, among FAs and between OCs and FAs.

Multivariable linear regression models were used to examine which characteristics of the mother were associated with the chemical concentrations in breast milk at 3 months postpartum. The OC concentrations were right skewed and so were log transformed before analysis. The factors of interest included maternal age at the start of

pregnancy (years), parity (0 vs. ≥1), maternal race (nonwhite vs. white), maternal education (≤12 years, 13-15 years vs. ≥16 years), pre-pregnancy body mass index (BMI) (kg/m^2), weight gain during pregnancy (kg), % body fat at 3 months postpartum, maternal smoking in the first three months postpartum (yes vs. no), income as a percentage of the poverty level during pregnancy (100%), and maternal postpartum diet in the first 3 months estimated by daily grams of animal protein and the proportion of daily fish grams to animal protein grams. If maternal postpartum diet data were missing but maternal diet at pregnancy was available, then single imputation was used based on the equations constructed from the women who had diet information available at both durations.

For the multivariable linear regression models of FAs, the factors of interest additionally included the natural log of OCs which were linearly correlated in order to examine the associations between OCs and FAs.

Observations with missing values for OC concentrations and maternal characteristics were dropped from the models (5 missing poverty level during pregnancy, 13 missing maternal diet, 1 missing PCBs, 1 missing *p,p'*-DDT, 29 missing HCB and 2 missing oxychlordane). The statistical analyses were conducted by PC-SAS (version 9.1).

A.3 Results

A total of 585 infants without major birth defects were eligible to participate in the PIN Babies Study, and among them 496 infants participated at 3 months postpartum.

Three hundred and thirty-one women in the PIN Babies Study were still breast-feeding at the time of the three month postpartum visit and 306 (92%) were willing to provide a breast milk sample. There were only 2 of 306 milk samples unusable.

The characteristics of the 304 participant women included in this study are listed in Table 10. They were mainly white, aged 25 years or older and had more than 12 years of education. More than half of the women had a normal pre-pregnancy BMI (19.8-26.0 kg/m^2). Most of the women (95%) did not smoke postpartum. One hundred and seventy-five out of 304 women had data on both OCs and FAs. The characteristics of this sub-group were not different from the larger study population.

Table 11 presents lipid-adjusted concentrations of the OCs that were detected in more than 70% of breast milk samples. The dominant congener of PCBs was PCB153, followed by PCB138_158 and PCB180. The range of PCB153 concentration was from 2.20 to 199.00 ng/g lipid with a median of 16.55 ng/g lipid, and for total PCBs the range was from 9.15 to 708.10 ng/g lipid with a median of 77.30 ng/g lipid. The correlation between PCB153 and total PCBs was high (r=0.98). *P,p'*-DDE was detected in all the 304 samples and its concentration was much higher than *p,p'*-DDT. The range of *p,p'*-DDE was from 1.40 to 2140.00 ng/g lipid with a median of 120.50 ng/g lipid. The concentration of mirex compared to other organochlorine pesticides was much lower. Total PCBs in breast milk was positively correlated with other OCs except for β-HCH (table 12). *P,p'*-DDE were positively correlated with HCB and β-HCH but not other organochlorine pesticides. Generally the magnitude of the correlations was low to

moderate (Pearson correlation coefficient (r)<0.6), except that the chlordane-related compounds, oxychlordane and *trans*-nonachlor, were highly correlated (r=0.88, p<0.001).

The 175 breast milk samples contained an average of 0.08% (standard deviation (SD)=0.05%) EPA, 0.20% (SD=0.14%) DHA and 0.50% (SD=0.10%) AA (table 13). The range of the ratio of ω-6 FAs to ω-3 FAs was from 5.6 to 22.8 with a median of 10.40. EPA and DHA were highly correlated (r=0.71, p<0.001), but AA was correlated with neither EPA (r=0.14, p>0.05) nor with DHA (r=0.10, p>0.05). Only weak correlations were found between OCs and ω-3 and ω-6 FAs in breast milk: between the natural log of total PCBs and DHA (r=0.15), between the natural log of PCBs and AA (r=0.20), and between the natural log of mirex and AA (r=0.28) (table 14).

In the multivariable linear regression models for the natural log of OCs concentrations, we found primiparous women had higher OCs concentrations than multiparous women with a difference in natural log of concentrations from 0.22 for mirex to 0.61 for *trans*-Nonachlor (table 15). Because a monotonic association between maternal age and the natural log of OCs concentrations was observed, maternal age was treated as a continuous variable in the multivariate linear regression models. In our study, the natural log of OCs in breast milk increased 0.11 to 0.56 per 5-year increase in maternal age except for HCB. Non-white women compared to white women had higher concentrations of *p,p'*-DDT, *p,p'*-DDE and β-HCH, but had lower concentrations of *trans*-Nonachlor in breast milk. Women with education less than or equal to 12 years compared to women with education equal to or more than 16 years had higher

concentrations of *p,p'*-DDE, HCB, β-HCH and *trans*-nonachlor. Concentrations of PCBs, *p,p'*-DDE, HCB and β-HCH slightly increased with each 100% of poverty level increase. Pre-pregnancy BMI, weight gain during pregnancy, % body fat at 3 months postpartum and postpartum smoking were not associated with the concentrations of any OCs in breast milk, although Mirex concentration slightly decreased when % body fat at 3 months postpartum increased. Maternal diet in the first 3 months postpartum was not associated with OCs concentrations in breast milk, except that β-HCH slightly increased as an increase in the proportion of daily fish intake to total daily animal protein intake (β=0.02, p=0.01), and the association between *p,p'*-DDT and proportion of fish intake was borderline (β=0.01, p=0.06).

In the multivariable linear regression models for FAs concentrations, we found most of the factors of interest were not associated with FAs concentrations in breast milk, but an increase in proportion of daily fish intake was associated with EPA, DHA, total ω-3 FAs and AA (table 16). Weight gain during pregnancy was positively associated with the concentration of AA in breast milk, but magnitude of the change was small. Women who smoked postpartum had higher DHA concentrations than non-smokers, but the confidence interval of the estimate was wide. The positive associations between total PCBs and DHA, PCBs and AA, and mirex and AA were still observed after controlling for other factors in the multivariate models, but the association between PCBs and DHA and between PCBs and AA did not achieve statistical significance.

A.4 Discussion

This paper presents the contemporary concentrations of OCs and ω-3 and ω-6 FAs in breast milk at 3 months postpartum, which indicates the potential for infant exposure to these chemicals and fatty acids. ΣPCBs concentration in our study were relatively low when compared to PCBs concentrations published in the previous studies which examined the hazardous effects on children's development [65,71,77,79,91,100]. Longnecker et al compared PCB153 concentrations from different studies standardized to the same base. Across the studies, the range of PCB153 median concentrations were from 30 to 450 ng/g lipid in maternal serum [141]. The median ratio of PCBs concentration in breast milk at birth to concentration in serum at pregnancy was suggested to be 1.34 in the Longnecker et al paper [141]. The ratio of median concentration in milk at birth to concentration in milk at 3 months postpartum was 1.21 suggested from the findings of Rogan et al paper [100]. According to these two values, the medium PCB153 concentration of our study population would approximate to 15 ng/g serum lipid (16.55 x 1.21 / 1.34 ~ 15), which is much lower than the lowest concentration (Massachusetts study [142], 1993-1998, median=30 ng/g lipid in serum) in the Longnecker paper [141].

DDT and DDE concentrations were also lower in this study compared to others. The average concentration of *p,p'*-DDT in our study (mean= 6.25, SD= 7.60 ng/g lipid) was lower than the extrapolated DDT concentration obtained from the regression line suggested by Smith et al (DDT_{2005}=exp(8.497 - 0.179 (2005-1975) = 22.81 ng/g in milk fat) [143]. The *p,p'*-DDE concentration in a previous North Carolina study in 1978-1982

(median concentration in milk at 3 months=2.07 ppm, i.e. 2070 ng/g) was more than one order of magnitude of that observed in our study [100].

The correlations among OCs observed in our study were low to moderate, and different from those of the Quebec Inuit population, where the correlations among OCs in breast milk were medium to high (r=0.45-0.95) [138]. This difference may be due to different diet habits of the Inuit population who consumed mainly marine food and had higher chances of being exposed to OCs. In contrast, maternal fish consumption and daily animal protein intake in the first 3 months postpartum were generally not associated with the OCs concentrations in breast milk at 3 months postpartum in our study, which indicates that the OCs in this population were possibly accumulated throughout the lifetime rather than through short-term food consumption.

Our study supports previous reports that maternal age and parity predict OCs concentrations in breast milk [7,144]. The persistent and lipophilic nature of OCs contributes to the body's accumulation with age and elimination by pregnancy and lactation. Since 88% of 304 women in our study were exclusively or mostly breast-feeding in the first 3 months, this study had little variability in duration of lactation thus we were unable to examine its association with OCs concentrations in breast milk. Racial differences of the concentrations was observed for *p,p'*-DDT, *p,p'*-DDE, β-HCH and *trans*-Nonachlor, although only 14% of our study was non-white women. Three factors – pre-pregnancy BMI, weight gain during pregnancy and % body fat at 3 months, which indicated the body weight status during pregnancy and after pregnancy, generally failed to predict OCs

concentration in breast milk at the third month postpartum. This finding was similar to other studies that found little or no correlation between organochlorine pesticides and BMI or body weight changes [7,144]. Although relatively low maternal education was related to high concentrations of *p,p'*-DDE, HCB and β-HCH in this population, the concentrations of these chemicals were slightly decreased when the household was relatively poor during pregnancy. The paradoxical contradiction between these two variables, which were both used to be a proxy of socioeconomic status of the women, may be because these two variables represent socioeconomic status at two different time (long-term vs. at pregnancy) or because of the instable findings from the small sample size of education levels ≤ 12 years (n=17, 6%).

AA, and EPA and DHA can be formed in the human body from desaturation and elongation of LA and ALA, respectively; they also can be obtained from dietary intake of animal tissues [145], which was supported by our findings that an increase in the proportion of daily fish intake to total animal protein intake was associated with AA, EPA and DHA measured in breast milk. Worldwide mean±SD concentration of DHA in breast milk was 0.32±0.22% with range from 0.06% to 1.4%, and for AA, mean±SD concentration was 0.47±0.13% with range from 0.24% to 1.0% [146]. The breast milk of our study population, compared to worldwide average, contained similar amounts of AA (0.50±0.10%) but smaller amounts of DHA (0.20±0.14%). In the worldwide meta-analysis study, they also found positive but small correlation between DHA and AA in breast milk (r=0.25), which was not supported by our study.

Overall, no strong linear correlation was observed between the natural log of concentrations of OCs and concentrations of ω-3 and ω-6 FAs in breast milk. Without natural log transformation of OCs, PCBs and mirex were still positively but weakly correlated with AA ($r=0.15$, $p<0.05$ and $r=0.25$, $p<0.001$, respectively). After adjustments for other factors, a two-fold increase in concentrations of mirex increased AA levels by 0.002 (% of total fat), and the positive association between PCBs and AA disappeared. Our results differed from the Faroe Island cohort study, which found a negative association between maternal serum PCBs concentration and umbilical cord serum AA concentration [137]. The differences in the observed relationships between OCs and FAs between the studies may reflect different diet habits and variation in the specimen type. *In vivo* and *in vitro* studies have shown that PCBs can induce the release of AA [147-150]. This may suggest a potential biomechanism responsible for the positive correlation between PCBs and AA. However, the biomechanism for the slight positive association between mirex and AA was not clear. It was also not clear whether OCs would influence the elongation and desaturation of FAs.

This paper provides concentrations of OCs and FAs in breast milk of lactating women in Central North Carolina from 2004 to 2006, and supports the decline in the concentrations of OCs in the US population [5] and as seen in the Canadian population [151,152]. Since maternal age, maternal education level and maternal race were found to explain some variation in the concentrations of OCs in breast milk, the direct comparison of our study results to others should take into account that the population of this study was largely white lactating women greater than 25 years of age and with more than 12

years of education. Although infant lactational exposure to OCs has decreased, it remains unclear whether infant development is unaffected by these low background levels. The amounts of fatty acids in breast milk may influence the health and development of infants [153-156]. Having information on both OCs and FAs in the same population allows us to examine the correlations between environmental pollutants and nutrients in breast milk. Many studies only have information on one type of chemicals, beneficial or harmful, in breast milk. Our results helped to fill in this gap of knowledge and also provided additional information for the risk and benefit assessment of breast-feeding.

Table 10. Characteristics of participant women. *

Characteristics	Milk Samples (n=304) No.	%	OCs+FAs † (n=175) No.	%
Race				
White	262	86.2	152	86.9
Black	18	5.9	12	6.9
Other	24	7.9	11	6.3
Age (years)				
<25	32	10.5	18	10.3
25-29	88	29.0	51	29.1
30-34	130	42.8	76	43.4
≥35	54	17.8	30	17.1
Mean±SD	30.7±4.9		30.7±4.8	
Education (years)				
≤12	17	5.6	10	5.7
13-15	37	12.2	20	11.4
≥16	250	82.2	145	82.9
Parity				
0	160	52.6	95	54.3
≥1	144	47.4	80	45.7
Pre-pregnancy BMI (kg/m^2)				
<19.8	52	17.1	30	17.1
19.8-26.0	189	62.2	111	63.4
26.0-29.0	27	8.9	12	6.9
>29.0	36	11.8	22	12.6
Smoking postpartum				
Yes	14	4.6	9	5.1
No	290	95.4	166	94.9
Percent of 2001 poverty level during pregnancy				
Mean±SD	490±199		499±194	
Missing	5		2	

* Abbreviation: OCs, organochlorine compounds; FAs, fatty acids; BMI, body mass index (kg/m^2); SD, standard deviation.
† OCs+FAs represents the sub-group that has both OCs and FAs concentrations available.

Table 11. Lipid-adjusted chemical concentrations (ng/g lipid) in maternal breast milk at 3 months postpartum (n=304).

Chemicals	D (%)*	Minimum	25th	Median	75th	Maximum
PCB66	264 (86.8)	<LOD	0.55	1.00	1.40	7.00
PCB74	301 (99.0)	<LOD	3.35	5.00	6.90	24.70
PCB99	301 (99.0)	<LOD	2.60	4.00	5.80	33.30
PCB105	280 (92.1)	<LOD	0.90	1.40	2.00	11.70
PCB118	303 (99.7)	<LOD	4.10	5.95	9.35	52.60
PCB138_158	303 (99.7)	<LOD	9.70	15.15	24.10	144.00
PCB146	293 (96.4)	<LOD	1.10	1.90	3.10	11.90
PCB153	304 (100.0)	2.20	10.50	16.55	26.60	199.00
PCB156	274 (90.1)	<LOD	1.20	2.15	3.65	19.70
PCB170	300 (98.7)	<LOD	2.40	3.5	5.95	54.50
PCB177	215 (70.7)	<LOD	<LOD	0.60	1.00	8.90
PCB178	218 (71.7)	<LOD	<LOD	0.60	1.20	10.40
PCB180	303 (99.7)	<LOD	5.15	8.20	13.75	128.00
PCB183	253 (83.2)	<LOD	0.60	1.10	1.60	16.20
PCB187	289 (95.1)	<LOD	1.70	2.90	4.70	55.30
PCB194	257 (84.5)	<LOD	0.70	1.20	2.10	10.40
PCB196_203	265 (87.2)	<LOD	0.80	1.40	2.10	15.90
PCB199	260 (85.5)	<LOD	0.65	1.30	2.00	9.50
ΣPCBs †	--	9.15	48.51	77.30	119.00	708.10
p,p'-DDT	292 (96.1)	<LOD	3.20	4.70	6.90	80.40
p,p'-DDE	304 (100.0)	1.40	85.85	120.50	187.00	2140.00
HCB	265 (87.2)	<LOD	7.50	10.50	13.50	106.00
β-HCH	290 (95.4)	<LOD	3.00	4.60	6.90	1580.00
Oxychlordane	293 (96.4)	<LOD	10.10	14.35	22.70	59.00
trans-Nonachlor	299 (98.4)	<LOD	12.40	19.45	31.00	91.10
Mirex	238 (78.3)	<LOD	0.30	0.70	1.30	17.60

* D (%), number and percent of samples with detectable concentrations.
† ΣPCBs is the sum of the PCB congeners which are detectable in more than 70% samples, in which <LOD is treated as median(LOD) divided by a square root of 2.

Table 12. Linear correlation coefficients* between the lipid-adjusted concentrations (ng/g lipid) of PCBs and organochlorine pesticides in maternal breast milk at 3 months postpartum (n=304).

	ΣPCBs	*p,p'*-DDT	*p,p'*-DDE	HCB	β-HCH	Oxychlordane	*trans*-Nonachlor	Mirex
ΣPCBs	1	0.17 **	0.21 ***	0.48 ***	0.04	0.37 ***	0.28 ***	0.24 ***
p,p'-DDT	0.17 **	1	0.57 ***	0.16 **	0.59 ***	0.12 *	0.09	0.06
p,p'-DDE	0.21 ***	0.57 ***	1	0.21 ***	0.55 ***	0.08	0.05	0.02
HCB	0.48 ***	0.16 **	0.21 ***	1	0.09	0.25 ***	0.17 **	0.14 *
β-HCH	0.04	0.59 ***	0.55 ***	0.09	1	-0.02	-0.05	-0.04
Oxychlordane	0.37 ***	0.12 *	0.08	0.25 ***	-0.02	1	0.88 ***	0.40 ***
trans-Nonachlor	0.28 ***	0.09	0.05	0.17 **	-0.05	0.88 ***	1	0.39 ***
Mirex	0.24 ***	0.06	0.02	0.14 *	-0.04	0.40 ***	0.39 ***	1

* Values shown are Pearson correlation coefficient (r); *, p<0.05; **, p<0.01, ***, p<0.001.

Table 13. Polyunsaturated fatty acids relative concentrations (% of total fat) in maternal breast milk at 3 months postpartum (n=175).

Fatty acids		Minimum	25th	Median	75th	Maximum	Mean (SD)
Eicosapentaenoic acid (EPA)	20:5n-3	0.03	0.05	0.07	0.09	0.31	0.08 (0.05)
Docosahexaenoic acid (DHA)	22:6n-3	0.07	0.12	0.16	0.23	0.92	0.20 (0.14)
Total ω-3 fatty acids *		0.79	1.27	1.62	1.97	2.95	1.66 (0.51)
Arachidonic acid (AA)	20:4n-6	0.27	0.43	0.49	0.55	0.80	0.50 (0.10)
Total ω-6 fatty acids *		8.80	15.08	16.86	19.04	27.39	16.98 (3.38)

* The relative concentration of total ω-3 fatty acids is the sum of the relative concentrations of fatty acids 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3. The relative concentration of total ω-6 fatty acids is the sum of the fatty acids 18:2n-6, 18:3n-6, 20:2n-6, 20:3n-6 and 20:4n-6.

Table 14. Linear correlation coefficients* between the natural log of the concentrations of organochlorine compounds and polyunsaturated fatty acids in maternal breast milk at 3 months postpartum (n=175).

Fatty acids	Ln(ΣPCBs)	Ln(*p,p'*-DDT)	Ln(*p,p'*-DDE)	Ln(HCB)	Ln(β-HCH)	Ln(Oxychlordane)	Ln(*trans*-Nonachlor)	Ln(Mirex)
EPA	0.06	0.06	0.002	0.09	0.03	0.07	0.04	0.05
DHA	0.15 *	0.11	0.05	0.11	0.09	0.09	0.06	0.09
Total ω-3	0.04	0.01	0.06	0.01	0.02	0.01	0.06	0.08
AA	0.20 **	0.03	-0.01	0.01	-0.04	0.06	0.11	0.28 ***
Total ω-6	-0.12	-0.04	0.08	-0.12	0.02	-0.05	-0.06	-0.02

* Values shown are Pearson correlation coefficient (r); *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$.

Table 15. β coefficients and 95% confidence intervals of the factors of interest in multivariable linear regression models for the natural log of PCBs and organochlorine pesticides. *

OCs	Parameter	β	LCL	UCL	p-value
ΣPCBs	Maternal age (per 5 years)	0.2639	0.1928	0.3351	<.0001
	Nulliparous (ref. multiparous)	0.3809	0.2509	0.5108	<.0001
	None-white (ref. white)	-0.0586	-0.2437	0.1265	0.54
	Maternal education (ref. ≥16 years)				
	≤12 years	0.0583	-0.2463	0.3629	0.71
	13-15 years	-0.0461	-0.2626	0.1704	0.68
	Pre-pregnancy BMI (kg/m^2)	-0.0018	-0.0205	0.0169	0.85
	Weight gain during pregnancy (kg)	-0.0039	-0.0156	0.0078	0.51
	% body fat at 3 months	-0.0060	-0.0166	0.0047	0.27
	Postpartum smoking (ref. non-smoking)	0.0780	-0.2116	0.3676	0.60
	Poverty level during pregnancy (per 100%)	0.0524	0.0137	0.0911	0.01
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0013	-0.0049	0.0075	0.68
	Total animal protein daily intake (g)	0.0003	-0.0002	0.0008	0.26
***p,p'*-DDT**	Maternal age (per 5 years)	0.1519	0.0415	0.2623	0.01
	Nulliparous (ref. multiparous)	0.4060	0.2047	0.6072	<.0001
	None-white (ref. white)	0.4525	0.1654	0.7397	0.002
	Maternal education (ref. ≥16 years)				
	≤12 years	0.1022	-0.3709	0.5753	0.67
	13-15 years	0.0242	-0.3121	0.3604	0.89
	Pre-pregnancy BMI (kg/m^2)	0.0196	-0.0094	0.0487	0.19
	Weight gain during pregnancy (kg)	-0.0121	-0.0302	0.0061	0.19
	% body fat at 3 months	-0.0040	-0.0206	0.0126	0.64
	Postpartum smoking (ref. non-smoking)	-0.3008	-0.7500	0.1485	0.19
	Poverty level during pregnancy (per 100%)	0.0243	-0.0362	0.0848	0.43
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0094	-0.0002	0.0190	0.06
	Total animal protein daily intake (g)	0.0005	-0.0003	0.0013	0.21
***p,p'*-DDE**	Maternal age (per 5 years)	0.1141	0.0293	0.1990	0.01
	Nulliparous (ref. multiparous)	0.3008	0.1460	0.4556	0.0001
	None-white (ref. white)	0.5700	0.3492	0.7908	<.0001
	Maternal education (ref. ≥16 years)				
	≤12 years	0.4675	0.1041	0.8309	0.01
	13-15 years	-0.0076	-0.2658	0.2506	0.95
	Pre-pregnancy BMI (kg/m^2)	-0.0059	-0.0283	0.0164	0.60
	Weight gain during pregnancy (kg)	-0.0105	-0.0244	0.0034	0.14
	% body fat at 3 months	-0.0025	-0.0153	0.0102	0.70
	Postpartum smoking (ref. non-smoking)	-0.0820	-0.4276	0.2636	0.64
	Poverty level during pregnancy (per 100%)	0.0615	0.0153	0.1076	0.01
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0024	-0.0050	0.0098	0.52
	Total animal protein daily intake (g)	-0.0001	-0.0008	0.0005	0.69

HCB	Maternal age (per 5 years)	0.0104	-0.0691	0.0899	0.80
	Nulliparous (ref. multiparous)	0.3308	0.1830	0.4786	<.0001
	None-white (ref. white)	-0.1790	-0.3879	0.0299	0.09
	Maternal education (ref. ≥16 years)				
	≤12 years	0.3582	-0.0062	0.7227	0.05
	13-15 years	0.0580	-0.1917	0.3076	0.65
	Pre-pregnancy BMI (kg/m^2)	0.0106	-0.0101	0.0313	0.32
	Weight gain during pregnancy (kg)	0.0015	-0.0116	0.0147	0.82
	% body fat at 3 months	-0.0003	-0.0121	0.0116	0.96
	Postpartum smoking (ref. non-smoking)	-0.2069	-0.5373	0.1235	0.22
	Poverty level during pregnancy (per 100%)	0.0517	0.0069	0.0965	0.02
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0032	-0.0039	0.0102	0.38
	Total animal protein daily intake (g)	0.0005	-0.0001	0.0011	0.12
β-HCH	Maternal age (per 5 years)	0.1518	0.0130	0.2906	0.03
	Nulliparous (ref. multiparous)	0.5720	0.3187	0.8253	<.0001
	None-white (ref. white)	0.7992	0.4379	1.1606	<.0001
	Maternal education (ref. ≥16 years)				
	≤12 years	0.5838	-0.0109	1.1785	0.05
	13-15 years	0.0764	-0.3462	0.4990	0.72
	Pre-pregnancy BMI (kg/m^2)	-0.0096	-0.0461	0.0269	0.61
	Weight gain during pregnancy (kg)	-0.0105	-0.0333	0.0122	0.36
	% body fat at 3 months	-0.0009	-0.0217	0.0199	0.93
	Postpartum smoking (ref. non-smoking)	-0.2181	-0.7836	0.3474	0.45
	Poverty level during pregnancy (per 100%)	0.1351	0.0595	0.2107	0.001
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0153	0.0032	0.0275	0.01
	Total animal protein daily intake (g)	0.0009	-0.0001	0.0019	0.09
Oxychlordane	Maternal age (per 5 years)	0.1478	0.0182	0.2775	0.03
	Nulliparous (ref. multiparous)	0.5111	0.2743	0.7479	<.0001
	None-white (ref. white)	-0.1418	-0.4795	0.1959	0.41
	Maternal education (ref. ≥16 years)				
	≤12 years	0.0908	-0.4750	0.6565	0.75
	13-15 years	-0.0235	-0.4176	0.3706	0.91
	Pre-pregnancy BMI (kg/m^2)	0.0009	-0.0332	0.0350	0.96
	Weight gain during pregnancy (kg)	-0.0027	-0.0240	0.0185	0.80
	% body fat at 3 months	0.0001	-0.0193	0.0196	0.99
	Postpartum smoking (ref. non-smoking)	0.3130	-0.2146	0.8406	0.25
	Poverty level during pregnancy (per 100%)	0.0411	-0.0295	0.1117	0.25
	Proportion of fish daily intake to total animal protein daily intake (%)	-0.0004	-0.0117	0.0110	0.95
	Total animal protein daily intake (g)	0.0001	-0.0009	0.0011	0.83
***trans*-Nonachlor**	Maternal age (per 5 years)	0.2186	0.0982	0.3390	0.0004
	Nulliparous (ref. multiparous)	0.6083	0.3887	0.8280	<.0001
	None-white (ref. white)	-0.3418	-0.6551	-0.0284	0.03

	Maternal education (ref. ≥16 years)				
	≤12 years	0.6495	0.1338	1.1651	0.01
	13-15 years	0.1701	-0.1964	0.5365	0.36
	Pre-pregnancy BMI (kg/m^2)	-0.0276	-0.0593	0.0041	0.09
	Weight gain during pregnancy (kg)	-0.0098	-0.0295	0.0100	0.33
	% body fat at 3 months	0.0063	-0.0118	0.0244	0.49
	Postpartum smoking (ref. non-smoking)	0.3399	-0.1505	0.8303	0.17
	Poverty level during pregnancy (per 100%)	0.0130	-0.0526	0.0785	0.70
	Proportion of fish daily intake to total animal protein daily intake (%)	-0.0036	-0.0141	0.0069	0.50
	Total animal protein daily intake (g)	0.0006	-0.0003	0.0015	0.19
Mirex	Maternal age (per 5 years)	0.5639	0.4573	0.6705	<.0001
	Nulliparous (ref. multiparous)	0.2215	0.0270	0.4160	0.03
	None-white (ref. white)	0.0534	-0.2240	0.3308	0.71
	Maternal education (ref. ≥16 years)				
	≤12 years	0.2396	-0.2169	0.6961	0.30
	13-15 years	0.0229	-0.3015	0.3473	0.89
	Pre-pregnancy BMI (kg/m^2)	-0.0058	-0.0339	0.0222	0.68
	Weight gain during pregnancy (kg)	-0.0079	-0.0253	0.0096	0.38
	% body fat at 3 months	-0.0186	-0.0346	-0.0026	0.02
	Postpartum smoking (ref. non-smoking)	0.1714	-0.2628	0.6055	0.44
	Poverty level during pregnancy (per 100%)	-0.0307	-0.0887	0.0274	0.30
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0009	-0.0084	0.0102	0.85
	Total animal protein daily intake (g)	0.0003	-0.0004	0.0011	0.40

* Abbreviation: LCL, lower confidence limit; UCL, upper confidence limit; ref., reference group; BMI, body mass index.

Table 16. β coefficients and 95% confidence intervals of the factors of interest in multivariable linear regression models for ω-3 and ω-6 fatty acids. *

FAs	Parameter	β	LCL	UCL	p-value
EPA	Maternal age (per 5 years)	-0.0054	-0.0147	0.0039	0.26
	Nulliparous (ref. multiparous)	0.0033	-0.0130	0.0196	0.69
	None-white (ref. white)	-0.0268	-0.0527	-0.0009	0.04
	Maternal education (ref. ≥16 years)				
	≤12 years	-0.0305	-0.0711	0.0101	0.14
	13-15 years	-0.0078	-0.0347	0.0190	0.57
	Pre-pregnancy BMI (kg/m^2)	0.0011	-0.0012	0.0033	0.35
	Weight gain during pregnancy (kg)	-0.0007	-0.0021	0.0008	0.39
	% body fat at 3 months	-0.0004	-0.0016	0.0008	0.51
	Postpartum smoking (ref. non-smoking)	-0.0005	-0.0347	0.0338	0.98
	Poverty level during pregnancy (per 100%)	-0.0002	-0.0053	0.0048	0.93
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0009	0.0001	0.0017	0.02
	Total animal protein daily intake (g)	0.0000	-0.0001	0.0000	0.34
DHA	Maternal age (per 5 years)	-0.0105	-0.0380	0.0170	0.45
	Nulliparous (ref. multiparous)	0.0008	-0.0439	0.0456	0.97
	None-white (ref. white)	-0.0286	-0.0964	0.0392	0.41
	Maternal education (ref. ≥16 years)				
	≤12 years	-0.0598	-0.1665	0.0469	0.27
	13-15 years	-0.0221	-0.0923	0.0482	0.54
	Pre-pregnancy BMI (kg/m^2)	-0.0012	-0.0070	0.0047	0.70
	Weight gain during pregnancy (kg)	0.0003	-0.0036	0.0042	0.88
	% body fat at 3 months	-0.0012	-0.0043	0.0019	0.44
	Postpartum smoking (ref. non-smoking)	0.0974	0.0080	0.1868	0.03
	Poverty level during pregnancy (per 100%)	-0.0001	-0.0135	0.0133	0.99
	ln(ΣPCBs)	0.0283	-0.0124	0.0691	0.17
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0048	0.0028	0.0069	<.0001
	Total animal protein daily intake (g)	0.0000	-0.0001	0.0002	0.63
Total ω-3	Maternal age (per 5 years)	-0.0292	-0.1197	0.0614	0.53
	Nulliparous (ref. multiparous)	-0.0632	-0.2218	0.0954	0.43
	None-white (ref. white)	-0.0769	-0.3286	0.1748	0.55
	Maternal education (ref. ≥16 years)				
	≤12 years	0.0460	-0.3484	0.4403	0.82
	13-15 years	0.0193	-0.2414	0.2800	0.88
	Pre-pregnancy BMI (kg/m^2)	-0.0029	-0.0245	0.0188	0.80
	Weight gain during pregnancy (kg)	-0.0096	-0.0240	0.0048	0.19
	% body fat at 3 months	-0.0059	-0.0174	0.0055	0.31
	Postpartum smoking (ref. non-smoking)	-0.1780	-0.5108	0.1548	0.29
	Poverty level during pregnancy (per 100%)	0.0342	-0.0149	0.0833	0.17
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0141	0.0065	0.0217	0.0003
	Total animal protein daily intake (g)	-0.0003	-0.0011	0.0004	0.36
AA	Maternal age (per 5 years)	-0.0098	-0.0313	0.0117	0.37
	Nulliparous (ref. multiparous)	0.0064	-0.0262	0.0390	0.70
	None-white (ref. white)	0.0046	-0.0448	0.0541	0.85

	Maternal education (ref. ≥16 years)				
	≤12 years	0.0295	-0.0483	0.1073	0.46
	13-15 years	0.0015	-0.0497	0.0527	0.95
	Pre-pregnancy BMI (kg/m^2)	0.0006	-0.0037	0.0048	0.80
	Weight gain during pregnancy (kg)	0.0036	0.0008	0.0065	0.01
	% body fat at 3 months	-0.0011	-0.0034	0.0012	0.34
	Postpartum smoking (ref. non-smoking)	0.0158	-0.0496	0.0812	0.64
	Poverty level during pregnancy (per 100%)	0.0003	-0.0096	0.0102	0.95
	ln(ΣPCBs)	0.0230	-0.0086	0.0546	0.15
	ln(Mirex)	0.0329	0.0116	0.0543	0.003
	Proportion of fish daily intake to total animal protein daily intake (%)	-0.0017	-0.0032	-0.0003	0.02
	Total animal protein daily intake (g)	0.0000	-0.0001	0.0002	0.60
Total ω-6	Maternal age (per 5 years)	-0.0702	-0.6823	0.5418	0.82
	Nulliparous (ref. multiparous)	-0.7354	-1.8077	0.3369	0.18
	None-white (ref. white)	0.8316	-0.8700	2.5331	0.34
	Maternal education (ref. ≥16 years)				
	≤12 years	1.4799	-1.1862	4.1459	0.28
	13-15 years	1.0490	-0.7135	2.8116	0.24
	Pre-pregnancy BMI (kg/m^2)	0.0110	-0.1352	0.1572	0.88
	Weight gain during pregnancy (kg)	-0.0039	-0.1012	0.0933	0.94
	% body fat at 3 months	-0.0381	-0.1155	0.0393	0.33
	Postpartum smoking (ref. non-smoking)	-1.7835	-4.0333	0.4662	0.12
	Poverty level during pregnancy (per 100%)	0.0787	-0.2534	0.4107	0.64
	Proportion of fish daily intake to total animal protein daily intake (%)	0.0304	-0.0210	0.0817	0.25
	Total animal protein daily intake (g)	-0.0014	-0.0063	0.0034	0.56

* Abbreviation: LCL, lower confidence limit; UCL, upper confidence limit; ref., reference group; BMI, body mass index.

B. Manuscript 2: Lactational Exposure to Polychlorinated Biphenyls, Dichlorodiphenyltrichloroethane, and Dichlorodiphenyldichloroethylene and Infant Neurodevelopment: An Analysis of the Pregnancy, Infections, and Nutrition Babies Study

B.1 Introduction

Polychlorinated biphenyls (PCBs) are mixtures of synthetic organic compounds that were widely used as insulators, coolants and lubricants in electrical transformers, capacitors and hydraulic equipment, and as plasticizers in plastic and rubber products. Production of PCBs for industrial and commercial applications in the United States started in 1929 [157]. Dichlorodiphenyltrichloroethane (DDT) was produced in the United States beginning in 1940. DDT was the first organochlorine pesticide in widespread use, and was used extensively as an insecticide in agriculture and mosquito control during the 1950s and 1960s [158]. The active ingredient in the commercial DDT sold as an insecticide was *p,p'*-DDT, accounting for 65-80% [4,159]. Dichlorodiphenyldichloroethylene (DDE) is the most stable metabolite of DDT [160].

PCBs and DDT are both halogenated compounds that were banned in the United States in 1977 and 1972, respectively, because they persist in the environment and bioaccumulate, adversely affecting the ecosystem and possibly human health [158]. Although production and use of these two organic pollutants have been banned for decades, they can still be detected in the environment and in the blood and breast milk of

the US population [5-7]. The main exposure route for humans is their diet, most commonly by eating contaminated meat, fish, and shellfish. For infants, the main exposure route is breast feeding.

Infancy is a highly vulnerable period of exposure to these persistent environmental pollutants. Postnatally, synaptogenesis occurs rapidly over the first two years [161]. Gilmore et al found that the neonate's cortical gray matter of regions for visual and motor function grows rapidly in the first month, and total brain size increases 100% in the first year [162]. Extremely rapid growth in specific areas may increase the vulnerability of the postnatal brain to environmental pollutants during the first year of life. Since brain maturation is not simultaneous in all areas, chemical exposures at different times may cause adverse effects on different developmental domains [21,22].

Epidemiological studies have investigated the associations between exposure to PCBs, DDT and DDE and infant neurodevelopment for decades, but the findings have been conflicting and remain inconclusive. Most of the previous studies were conducted during the 1980s and 1990s and focused on infant prenatal exposure to PCBs and DDE through placental transfer from the mother. Little is known about the effects of infants' lactational exposure to the low background levels of persistent organic pollutants in this century. Assessing these effects is also complicated by the co-existing beneficial attributes of breastfeeding, both social and nutritional. Long-chain polyunsaturated fatty acids are essential to fetal and neonatal brain development [8-10], yet the richest dietary sources of them for mothers, such as fish, may also be sources for the contaminant

chemicals. The potential for any harmful effects from persistent organic pollutants in breast milk to be modified by long-chain polyunsaturated fatty acids warrants consideration. Thus, this study was designed to examine associations between the exposure to environmental levels of PCBs, *p,p'*-DDT and *p,p'*-DDE through breast-feeding and infant neurodevelopment at 12 months of age in central North Carolina in 2004-2006.

B.2 Methods

Study Population

Subjects were participants of the Pregnancy, Infection and Nutrition (PIN) Babies Study. The study follows children born to women who participated in the PIN3 and PIN Postpartum Studies [163]. The PIN3 Study enrolled pregnant women from 2001 to 2005 receiving prenatal care at the University of North Carolina Hospitals before 20 weeks gestation and the PIN Postpartum Study followed a subset of them through the first year postpartum. Mothers completed several self-administered questionnaires and two phone interviews during pregnancy and one brief questionnaire after hospital delivery at that provided details about their health and lifestyle during pregnancy. After delivery, women were invited to continue participation by allowing two in-home interviews at 3 and 12 months postpartum. Details of these studies are available at the website: http://www.cpc.unc.edu/projects/pin/.

Beginning in January 2004, participants in the PIN Postpartum Study were invited to enroll their infants in the PIN Babies Study. This study added developmental assessment of the child at 3 and 12 months of age. All children were singletons, without major birth defects. The study protocols of the PIN, PIN Postpartum, and PIN Babies studies have been approved by the Institutional Review Board of the University of North Carolina at Chapel Hill.

Exposure Measurement

Participant women who were still breast-feeding at the time of the three-month postpartum home visit were asked to provide a breast milk sample; 88% of the total 304 women who provided samples were exclusively or mostly breastfeeding at 3 months. A milk collection kit containing three 1.5ml tubes, a plastic pipette and instructions was sent before the scheduled visit. At around 10 o'clock in the morning of the scheduled visit day, participants were to follow the written instructions to pump both breasts, gently mix the milk extracted, and use the plastic pipette to transfer the milk into 3 tubes and store them in the freezer until the interviewers arrived. Samples were then transported on ice to the -80 ºC storage freezers. Breast milk was collected from women who participated between 2004 and 2006.

Breast milk samples were analyzed for *p,p'*-DDT, *p,p'*-DDE and thirty-five PCB congeners according to the existing methods at the Organic Analytic Toxicology Branch of the National Center for Environmental Health at the Centers for Disease Control and

Prevention [122]. The measurement of selected chemicals in breast milk samples was performed using gas chromatography/isotope dilution high-resolution mass spectrometry (GC/IDHRMS) using a MAT95 instrument (ThermoFinnigan MAT; Bremen, Germany). The lipid concentration was gravimetrically determined by an analytical balance AX105 Delta Range (Mettler Toledo; Columbus, OH) with an accuracy of $\pm 10^{-4}$ g. Each analysis batch contained sixteen unknowns, two method blanks and two quality control specimens. The between-assay coefficient of variation was normally less than 10%. Concentrations reported here were all lipid adjusted. PCB153 and *p,p'*-DDE were detected in all 304 samples, and *p,p'*-DDT was detected in 292 samples (96%). Total PCBs was calculated as the sum of eighteen detectable PCB congeners in more than 70% samples, that is PCB66, PCB74, PCB99, PCB105, PCB118, PCB138_158, PCB146, PCB153, PCB156, PCB170, PCB177, PCB178, PCB180, PCB183, PCB187, PCB194, PCB196_203 and PCB199. Concentrations lower than the limits of detection (LOD) were imputed to the median of the method LOD of each measurement divided by a square root of 2 [164].

In addition to the analyses for DDT, DDE and PCBs, long chain polyunsaturated fatty acids were measured in the first 175 of the 304 total breast milk samples. The fatty acid extraction and assessment was performed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, MN). Relative concentrations of docosahexaenoic acid (DHA) and arachidonic acid (AA) were expressed as percent of total fat.

Lactational Exposure Determination

We quantified the duration of breast feeding in order to estimate the infant's lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE through the first 12 months of life. Infant feeding status was reported during the maternal home interview at 3 and 12 months postpartum. Women were asked to recall their child's feeding practices for each month, indicating the frequency that they breast fed, fed infant formula, and fed other types of food. *Exclusively breast-feeding* was defined as breast feeding with no other food or liquid; *mostly breast-feeding* was defined as breast feeding and feeding of other supplements equal to or less than one time per day; and *breast-feeding with supplements* was defined as breast feeding with feeding any other liquids or solids >1 time per day. The number of months representing each type of feeding was summed and used in a Lactational Exposure Metric (LEM).

The LEM was developed to represent the infant's exposure to each chemical in the first 12 months as follows:

$$LEM = C \times (D_1+D_2) + ½ \times C \times D_3,$$

in which C denotes chemical concentration in breast milk at the third month postpartum (ng/g lipid), D_1 is duration of exclusively breast-feeding (months), D_2 is duration of mostly breast-feeding (months), and D_3 is duration of breast-feeding with other supplements (months). The unit of this estimate is concentration-months, i.e. ng/g-months. Using this metric, breast milk concentration for each chemical was assumed to be constant through the lactation period, and breast milk consumption was assumed to be decreased by half during the period when breast milk was supplemented with formula,

other liquids or solids.

Developmental Assessment

Infant cognition was measured at twelve months by the Mullen Scales of Early Learning: AGS Edition (Mullen, 1995). The Mullen was administered in the home by trained study staff members. This standardized assessment instrument was designed to evaluate cognitive and motor functioning of children from birth to 68 months of age in five domains of development – receptive language, expressive language, visual reception, fine motor, and gross motor [165].

Raw scores for each of the five scales are used to derive T scores that take into account the infant's age. Mullen T scores have a mean of 50 and a standard deviation (SD) of 10. The T scores of receptive language, expressive language, fine motor and visual reception scales are added together to form the *Cognitive T Score Sum*, which is used to derive the *Early Learning Composite* standardized score (mean=100, SD=15). This composite score provides an overall estimate of the infant's developmental level. Standardized scores below one standard deviation below the mean (i.e. <40 for T scores on the five individual scales and scores <85 for the *Early Learning Composite*) are categorized as *below average* for the corresponding scale or overall index.

Infant language comprehension and production were also measured at 12 months via parental report, using the Short Form: Level I (for infants) of the MacArthur-Bates

Communicative Development Inventories (CDI) (Fenson, Pethic, Renda, Cox, Dale & Reznick 2000; see also http://www.sci.sdsu.edu/cdi/cdiwelcome.htm). The Infant Short Form (for 8- to 18-month-olds) was used to reduce self-administration time and increase the response rate; its scores are highly correlated with the original 396-word full form (r=0.97) [128,129]. The instrument prompts parents to endorse whether their child "understands" or both "understands and says" 89 specific words. The raw score for vocabulary comprehension is the sum of the words the child "understands" and "understands and says". The raw score for infant vocabulary production is the number of words endorsed as "understands and says". At the 12-month postpartum home visit, mothers were asked to complete the form and return it in a self-addressed stamped envelope. Infants' raw scores for vocabulary comprehension were dichotomized as below the 15th percentile (below average), comparable to the cutpoints used for the Mullen, vs. scores at or above the 15th percentile (average and above average). Production scores were generally low, as would be expected at this age, and did not have sufficient variability to warrant analysis.

Statistical Analysis

All the analyses were conducted using PC-SAS (version 9.1). The associations between lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE and the T scores for each of the Mullen scales were estimated using multivariable linear regression models. LEM of PCB153, PCBs, *p,p'*-DDT and *p,p'*-DDE were natural log transformed and modeled separately. Robust regression with Least Trimmed Squares estimation for multiple linear

regression models was used to examine the influence by the outliers of chemical concentrations on beta coefficients [166].

Multivariable logistic regression models were used to estimate the associations between each chemical and the odds of scoring *below average* on the Mullen scales and below the 15th percentile scores for vocabulary comprehension of the CDI. LEM of PCB153, PCBs, *p,p'*-DDT and *p,p'*-DDE were natural log transformed and modeled separately.

To control the positive confounding effects from breast feeding, the residuals from the models of breastfeeding and Mullen scores were first calculated using simple linear regression, and then were included in the multiple linear regression models as dependent variables to estimate the effects of the LEM. Because we were unable to pre-adjust the confounding effects from breast feeding for logistic regression models, total months of breast feeding was included in the models as a continuous variable.

Other covariates that were associated with both lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE and infant neurodevelopment, but were not an effect of either exposure or outcome, were evaluated as potential confounders. These covariates included maternal age at the start of pregnancy (years), parity (0 vs. ≥1), maternal race (nonwhite vs. white), maternal education (≤12 years, 13-15 years vs. ≥16 years), income as a percentage of the poverty level during pregnancy (100%), and infant gender (male vs. female). Mother-child pairs missing values for variables in the model were excluded

because only 6% were missing any data and missingness did not appear to be related to exposure or outcome status.

The potential for infant gender to modify the measured effects was assessed via interaction terms between gender and the natural log of LEM of PCBs, *p,p'*-DDT and *p,p'*-DDE in the multiple logistic regression models. The potential for the fatty acids in breast milk to modify the measured effects was assessed by dichotomizing the relative concentrations of DHA and AA as <25th percentile vs. ≥25th percentile and creating interaction terms between the natural logs of LEM of PCBs, *p,p'*-DDT and *p,p'*-DDE and DHA and AA. The interaction terms were added in the multiple logistic regression models and assessed by the likelihood ratio test. If interaction was indicated ($p<0.1$), we calculated stratum-specific odds ratios, 95% confidence intervals, and confidence limit ratios, i.e. upper confidence limit divided by lower confidence limit. Because of the smaller sample size of mother-child pairs with known fatty acids concentrations (n=175), the covariates included in the multiple logistic regression models were reduced to major factors, which were total months of breast-feeding, maternal age at the start of pregnancy, and infant gender.

B.3 Results

Three hundred and four participants provided samples of breast milk at three months postpartum (mean±SD = 3.5±0.6 months). Two hundred and sixty-four of the 304 women continued their participation at the 12-month postpartum home visit and provided

complete infant feeding information. Among these 264 women, 231 consented to allow their child to be evaluated by the Mullen and 218 women returned the parent report Short Form of the CDI. The study sample included more male than female infants, and most participant women were white, aged 25 years or older and had more than 12 years of education (Table 1). Most of the women breast fed for 6 months or longer, and were not smoking in the 12 months postpartum.

In this study population, female infants had higher scores than male infants on the receptive language, expressive language, and fine motor scales of the Mullen and on the Early Learning Composite. Infants who were breast fed longer than 9 months compared to 9 months or less had higher scores on expressive language, fine motor and the Early Learning Composite scales. Non-white infants and infants whose mothers had 12 or fewer years of education had higher scores on the gross motor scale.

In the multivariable linear regression models, a two-fold increase in the LEM of PCB153, total PCBs, *p,p'*-DDT and *p,p'*-DDE did not change any of the Mullen Scale T Scores or the Early Learning Composite standardized score by more than 2 points (Table 2). All confidence intervals included zero, indicating no difference. Zero to 0.9% outliers were identified in the robust regression models, but none affected the effect estimates.

In the multivariable logistic regression models, a two-fold increase in LEM of PCB153 tended to increase the odds of scoring *below average* on the receptive language scale (OR=1.3, 95%CI=0.8, 2.0), though the association is not statistically significant

(Table 3). A two-fold increase in LEM of *p,p'*-DDT and *p,p'*-DDE increased the odds of scoring *below average* on the gross motor scale (DDT: OR=1.4, 95%CI=0.9, 2.0; DDE: OR=1.4, 95%CI=0.9, 2.2), the fine motor scale (DDT: OR=1.4, 95%CI=1.0, 2.0; DDE: OR=1.3, 95%CI=0.9, 2.1), and the Early Learning Composite score (DDT: OR=1.4, 95%CI=0.9, 2.2; DDE: OR=1.4, 95%CI=0.8, 2.2), although all confidence intervals included 1.

Using the norms appropriate for gender and age, 19% of the 218 infants scored below the 15th percentile for word comprehension. The infants who were breast fed longer than 9 months were less likely to score below the 15th percentile for comprehension. None of other factors showed an association with comprehension. After adjusting for duration of breast feeding and other covariates in the multivariable logistic regression models, two-fold increases in LEMs of PCB153, total PCBs, *p,p'*-DDT and *p,p'*-DDE were not associated with CDI scores (Table 4).

Infant gender did not modify the observed effects in most models (p-values of the interaction terms ≥0.1). However, male infants who had a two-fold increase in LEM of *p,p'*-DDE were more likely to score *below average* on the gross motor scale (adjusted OR=1.9, 95%CI=1.1, 3.3); this association was not observed among female infants (adjusted OR=0.7, 95%CI=0.3, 1.5) (p-value of the likelihood ratio test=0.02).

The 25th percentile of DHA and AA concentrations were 0.12% and 0.43% of total fat, respectively. The effects of exposure to PCB153, PCBs, *p,p'*-DDT and *p,p'*-DDE

were generally similar above and below the 25th percentile of each fatty acid, although the effects of DDE on the odds of scoring *below average* on the Mullen fine motor scale was higher when DHA level was <25th percentile (OR=1.9, 95%CI=0.8, 4.8) compared to the level ≥25th percentile (OR=0.7, 95%CI=0.4, 1.4). Because data were sparse, the stratum-specific estimates were unstable; consequently, this study was unable to confirm or interpret potential differential effects.

B.4 Discussion

In this study, lactational exposures to PCBs, *p,p'*-DDT and *p,p'*-DDE were generally not associated with infant neurodevelopment at age of 12 months, as assessed by the Mullen and the Short Form of the CDI. However, we found that lactational exposure to *p,p'*-DDE through the first 12 months was associated with scoring *below average* on the gross motor scale among males but not females. The neurological toxic effects of DDE on male infants in these data were not precise; however, as a known antiandrogen, *p,p'*-DDE can inhibit the biologic effects of androgens, which might differentially affect the development of males. The effect of DDE on the gross motor modified by infant gender hasn't been reported before. Our results suggest that the potential for this interaction should be examined in other large datasets with available data.

Most prior epidemiological studies used the Bayley Scales of Infant Development (BSID) which included only two scales (mental and psychomotor). We used the Mullen to assess neurodevelopment because the five subscales may help distinguish among finer

domains of development. The Mullen Early Learning Composite score has been found to be highly correlated with the mental development index (MDI) of the BSID (r=0.70), and the Mullen gross motor score has also been found to be highly correlated with the psychomotor development index (PDI) of the BSID (r=0.76) [165], which may provide the basis for comparisons of our study results with those of previous studies. However, it is important to note that both the Mullen and the Bayley Scales are limited in the ability to detect subtle physiological deficits of the brain and nervous system which might be identified with more sensitive clinical assessments.

In a study of Mexican farm-workers' children in California, 1999-2000, prenatal *p,p'*-DDE was found to be associated with infant PDI at 6 months but not at a later age [85]. A study of a cohort of 92 mother-child pairs in Spain found that an increase in cord blood DDE levels was associated with decrements in MDI and PDI, and was also associated with lower scores on the personal-social scale, locomotor scale and performance scale assessed by the Griffiths Mental Development Scales at 13 months [66]. In a cohort study in Mexico, 2001-2005, they only found an association between maternal DDE serum level in the first trimester of pregnancy and a reduction in infant PDI throughout the first year [167]. However, neither study investigated postnatal exposure or reported effect measure modification by infant gender. Three other studies did not observe associations between DDE and infant neurodevelopment [61,62,70].

Prenatal exposure to PCBs was associated with lower scores on the PDI at 6 to 24 months in a North Carolina cohort between 1978-1982 [61,62]. A Dutch study (1990-1992)

reported delays in motor development at 3 months, but not at older ages, for prenatal exposure [63]. In addition, in the Dutch cohort, infants with postnatal exposure to PCBs scored lower on the PDI at 7 months, after controlling for lactational periods [63]. In contrast to these two studies, the Collaborative Perinatal Project cohort (in the US), and cohorts in Germany, Spain and Japan did not find any associations between infant motor development and either prenatal PCBs [64-67] or postnatal PCBs [65]. None of the studies found associations using the BSID mental development index [61-64,66,67], although the German study found that PCBs in breast milk related to a decrease in infant MDI [65,68]. The Michigan cohort [69] and the Oswego cohort [70], both of which used the Fagan Test of Infant Intelligence to assess infant cognition, found a deficit in infant novelty preference associated with prenatal PCBs exposure.

This study differed from other studies focused on infants' lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE by the method of exposure assignment. Rather than using only the chemical concentration at the time of measurement, we used the LEM to estimate each infant's total exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE through breast feeding during the first year of life. This metric accounted for both the chemical concentration in breast milk and the duration of breast feeding. According to the Hooper et al study [102], PCB153 concentration in breast milk decreased 4% over the first 6 months of breast feeding; after 6 months the change in PCB153 concentration did not change more than 1% per month. Among 83 women in our study who had concentrations available in both 3-month and 12-month breast milk samples, there was no statistical difference in the percentage change of the concentrations of PCB153, PCBs, *p,p'*-DDT

and *p,p'*-DDE. These findings support our assumption of a relatively constant concentration throughout the lactation period of interest here. Additionally, under the assumptions that infants in the same feeding status group consume similar breast milk volume and that the each mother's milk had similar fat composition, the LEM should be highly correlated with the absolute value of the accumulated dose of the chemicals throughout the first 12 months of life. The LEM is therefore appropriate for estimating the relative effects of chemical exposure.

In contrast to the potential detrimental effects of these persistent organic pollutants in breast milk, breastfeeding could also convey beneficial effects through enhanced nutrition or through social enrichment. Our study observed that infants who were breast fed longer scored higher than infants breast fed for a shorter period on the expressive language and fine motor scales and the Early Learning Composite of the Mullen; this finding of a beneficial effect of breast feeding on infant cognitive development is in agreement with that found in previous studies [104]. Because most of the women in this study did breast feed for 6 months or longer, to further diminish this confounding effect by long-term breast feeding, we carefully controlled for breast feeding and the potential confounding effects from long-term breast feeding are reduced in the analyses.

Beneficial fatty acids and possibly harmful environmental pollutants unavoidably coexist in breast milk, mainly from maternal dietary sources. It is difficult to know how the beneficial and harmful constituents of breast milk interact to affect development. While we evaluated the interaction between the pollutants PCBs, DDT and DDE, and

fatty acids DHA and AA in breast milk on infant neurodevelopment, only one interaction term appeared statistically significant at an *a priori* significant level of 0.1 in the multiple logistic regression models. However, the sample size of this study was too small to provide precise effect estimates. Other studies of larger sample sizes are needed to examine these relationships.

Animal studies have shown that different degrees of chlorination of PCB congeners may cause different neurochemical effects [40], but based on the number of chlorine substitutions in the *ortho* positions (2, 2', 6, and 6'), our study did not find an association between lactational exposure to different *ortho*-chlorinated PCBs groupings with any measurements of infant neurodevelopment.

The Mullen scores at 12 months in this study were calculated without the conventional age adjustment for prematurity (infants born earlier than 37 gestational weeks) that is common in clinical practice. In this sample, conventional age adjustment led to artificially-elevated T scores for the 18 preterm infants. These scores were significantly higher than those of the 200 full term infants on the visual reception and fine motor scales and the Early Learning Composite, after controlling for infant gender, parity, maternal race, maternal age, maternal education, poverty level, and duration of breast feeding. Therefore, we determined that it was better to use actual chronological age in calculating the Mullen scores. The norming sample for the CDI Short Form had excluded babies born at 34 weeks or younger. We conducted a sensitivity analysis by excluding the 3 babies in our sample who were born at 34 weeks and 1 baby at 33 weeks from the

models, and the results were not changed. Therefore, we retained those infants in our analyses.

One of the study limitations is that we were unable to distinguish the effects of lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE from the effects of prenatal exposure. PCBs and DDE concentration in breast milk have been shown to be highly correlated with the concentrations in cord plasma [100,101], so infants exposed to high chemical concentrations postnatally in breast milk were also likely to be exposed to high chemical concentrations prenatally. The other limitation is that most of our study population breast fed 6 months or longer, so we had limited sample size to differentiate the effects of short-term high-concentration exposure to PCBs, DDT and DDE from the effects of long-term low-concentration exposure.

Overall, our study did not find lactational exposure to PCBs, DDT and DDE to be associated with infant neurodevelopment at 12 months. This may be attributed to the combination of low chemical concentrations in breast milk and the beneficial effects of the long duration of breastfeeding in this study population. Larger studies are needed to assess the potential for gender to modify the association between lactational exposure to *p,p'*-DDE and infant gross motor development, and to assess the potential effect measure modification on the associations between environmental pollutants in breast milk and infant neurodevelopment by the concentrations of beneficial long chain polyunsaturated fatty acids in breast milk. This study contributes to the knowledge of potential effects of accumulated exposure to low levels of PCBs, DDT and DDE through lactation in the first

year of life on infant neurodevelopment, taking into account interactions with fatty acids.

B.5 Acknowledgement

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Table 17. Characteristics of participant mothers who provided breast milk at 3 months in the PIN Babies study and the mother-child pairs included in this study. *

Participant characteristics	Milk Samples (n=304) No.	%	Mullen Subgroup (n=231) No.	%	CDI Subgroup (n=218) No.	%
Maternal chracteristics						
Race						
White	262	86	204	88	194	89
Black	18	6	12	5	9	4
Other	24	8	15	7	15	7
Age (years)						
<25	32	11	24	10	20	9
25-29	88	29	63	27	61	28
30-34	130	43	99	43	94	43
≥35	54	18	45	20	43	20
Mean±SD	31±5		31±5		31±5	
Education (years)						
≤12	17	6	11	5	10	5
13-15	37	12	26	11	23	11
≥16	250	82	194	84	185	85
Mean±SD	17±2		17±2		17±2	
Parity						
0	160	53	118	51	113	52
≥1	144	47	113	49	105	48
Percent of 2001 poverty level during pregnancy						
Mean±SD	490±199		493±197		513±190	
Missing	5		4		4	
Postpartum smoking						
Yes	13	5	9	4	10	5
No	251	95	222	96	208	95
Missing	40		0		0	
Median (range) of chemical concentrations in breast milk (ng/g lipid) ‡						
PCB153	17 (2-199)		18 (2-199)		17 (2-199)	
ΣPCBs †	77 (9-708)		79 (12-708)		79 (12-708)	
p,p'-DDT	5 (<LOD-80)		5 (<LOD-80)		5 (<LOD-80)	
p,p'-DDE	121 (1-2140)		117 (15-2140)		117 (15-2140)	
Infant characteristics						
Median (range) of LEM (ng/g lipid - months) ‡						
PCB153	119 (12-1194)		118 (12-1194)		119 (12-1194)	
ΣPCBs †	546 (64-4249)		550 (64-4249)		549 (64-4249)	
p,p'-DDT	33 (1-523)		33 (1-523)		33 (1-523)	
p,p'-DDE	871 (134-19260)		867 (134-19260)		864 (134-19260)	

Duration of breast feeding (months)						
0-<6	11	4	10	4	11	5
6-9	66	25	60	26	54	25
>9-12	187	71	161	70	153	70
Missing	40		0		0	
Mean±SD	10±2		10±2		10±2	
Gender						
Male	163	54	122	53	120	55
Female	141	46	109	47	98	45
Preterm birth						
Yes	22	7	18	8	18	8
No	282	93	213	92	200	92
Age at developmental assessment (months)						
Mean±SD			12±1		13±1	
Mullen Scales of Early Learning (Mean±SD) ‡						
Receptive Language			46±8			
Expressive Language			54±9			
Visual Reception			51±11			
Gross Motor			50±12			
Fine Motor			51±11			
Composite Standard Score			101±14			
MacArthur CDI						
Words comprehended						
Mean±SD					29±20	
Comprehension						
<15th					42	19
15th-85th					149	68
>85th					27	12

* Abbreviation: CDI, the MacArthur Communicative Development Inventories; SD, standard deviation; <LOD, lower than detection limit; PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; LEM, Lactational Exposure Metric.

† ΣPCBs is the sum of the concentrations of PCB66, PCB74, PCB99, PCB105, PCB118, PCB138_158, PCB146, PCB153, PCB156, PCB170, PCB177, PCB178, PCB180, PCB183, PCB187, PCB194, PCB196_203 and PCB199, in which <LOD is treated as median(LOD) divided by a square root of 2.

‡ There was 1 subject missing ΣPCBs concentration and the LEM of ΣPCBs and 1 subject missing *p,p'*-DDT concentration and the LEM of *p,p'*-DDT. An additonal 40 subjects in the milk sample group were missing LEM because of missing duration of breast feeding through 12 months. These Mullen scores were missing: 2 for receptive language, 2 for expressive language, 6 for visual reception, 7 for fine motor, 2 for gross motor, and 8 for the Early Learning Composite.

Table 18. Adjusted mean difference in the scores of the Mullen Scales of Early Learning associated with a two-fold increase in lactational exposure metrics. [*, †]

	Receptive language		Expressive language		Visual reception		Gross motor		Fine motor		Early Learning Composite	
	MD	95% CI	MD	95% CI	MD	95% CI	MD	95% CI	MD	95% CI	MD	95% CI
PCB153	0.0	-1.2, 1.2	0.7	-0.6, 2.1	0.5	-1.2, 2.2	-0.0	-1.9, 1.8	1.1	-0.5, 3.0	1.4	-0.6, 3.4
ΣPCBs	0.1	-1.2, 1.3	0.8	-0.6, 2.2	0.3	-1.6, 2.1	-0.3	-2.3, 1.7	1.2	-0.5, 3.0	1.4	-0.7, 3.5
***p,p'*-DDT**	0.1	-0.8, 1.0	-0.1	-1.1, 1.0	-0.1	-1.4, 1.2	-1.3	-2.7, 0.1	0.3	-0.9, 1.5	-0.2	-1.7, 1.3
***p,p'*-DDE**	-0.2	-1.3, 1.0	-0.5	-1.8, 0.8	0.3	-1.4, 2.0	-1.6	-3.4, 0.2	-0.2	-1.8, 1.4	-0.3	-2.2, 1.7

[*] Abbreviation: MD, mean difference; CI, confidence interval; PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene.

[†] Adjusted for maternal age at the start of pregnancy (years), parity (0 vs. ≥1), maternal race (nonwhite vs. white), maternal education (≤12 years, 13-15 years vs. ≥16 years), poverty level during pregnancy and infant gender (male vs. female).

Table 19. Adjusted odds ratio of being scored below average in the Mullen Scales of Early Learning for a two-fold increase in lactational exposure metrics. [*, †]

	Receptive language		Expressive language		Visual reception		Gross motor		Fine motor		Early Learning Composite	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
PCB153	1.3	0.8, 2.0	0.8	0.4, 1.6	0.9	0.6, 1.3	1.0	0.7, 1.5	0.9	0.6, 1.3	0.8	0.5, 1.4
ΣPCBs	1.3	0.8, 2.0	0.9	0.4, 1.8	0.9	0.6, 1.4	1.1	0.7, 1.6	0.8	0.5, 1.3	0.8	0.5, 1.5
p,p'-DDT	0.8	0.6, 1.1	1.0	0.6, 1.7	1.0	0.7, 1.3	1.4	0.9, 2.0	1.4	1.0, 2.0	1.4	0.9, 2.2
p,p'-DDE	1.1	0.7, 1.6	1.3	0.7, 2.5	0.9	0.6, 1.4	1.4	0.9, 2.2	1.3	0.9, 2.1	1.4	0.8, 2.2

[*] Abbreviation: OR, odds ratio; CI, confidence interval; PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene.

[†] Adjusted for duration of breast feeding (months), maternal age at the start of pregnancy (years), parity (0 vs. ≥1), maternal race (nonwhite vs. white), maternal education (≤12 years, 13-15 years vs. ≥16 years), poverty level during pregnancy and infant gender (male vs. female).

Table 20. Adjusted odds ratio for the infant Short Form of the MacArthur Communicative Development Inventories associated with a two-fold increase in lactational exposure metrics. [*, †]

	Comprehension <15th percentile	
	OR	95% CI
PCB153	1.0	0.7, 1.6
ΣPCBs	1.0	0.6, 1.6
***p,p'*-DDT**	1.0	0.8, 1.4
***p,p'*-DDE**	1.0	0.7, 1.5

[*] Abbreviation: OR, odds ratio; CI, confidence interval; PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene.

[†] Adjusted for duration of breast feeding (months), maternal age at the start of pregnancy (years), parity (0 vs. ≥1), maternal race (nonwhite vs. white), maternal education (≤12 years, 13-15 years vs. ≥16 years), poverty level during pregnancy and infant gender (male vs. female).

C. Manuscript 3: Lactational Exposure to Polychlorinated Biphenyls, Dichlorodiphenyltrichloroethane, and Dichlorodiphenyldichloroethylene and Infant Growth: An Analysis of the Pregnancy, Infections, and Nutrition Babies Study

C.1 Introduction

The first 12 months of life is a period of dramatic change in babies' physical growth. Infants gain weight at a rate of about 680 g per month during the first 5 to 6 months [24]. Weight triples by the end of the first year, and body length increases by approximately 50% [24]. Growth in infancy has been related to long-term outcomes such as children's cognition, pubertal development, adult obesity, chronic diseases, and workplace success [25-32]. Infant growth retardation can be due to intrauterine growth inhibition, early childhood diseases, malnutrition, or disruption of hormone systems, potentially caused by environmental pollutants.

Polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its most stable metabolite, *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), are persistent organic compounds in the environment, which have been recognized as endocrine disruptors [168]. In vivo and in vitro studies have shown that PCBs may alter dopamine, estrogen and thyroid effects [33,35,38,41]. DDT has been suspected to have estrogenic effects, and *p,p'*-DDE is known as an antiandrogen which inhibits the biologic effects of androgens [42-46]. The potential for these compounds to interfere with the endocrine system

could adversely affect infant development and growth.

The main route of exposure to PCBs, DDT and DDE for infants is through breast feeding. As the duration of lactation increases, the infant's accumulated dose of these long half-life chemicals increases. Exclusive breastfeeding up to 6 months postpartum is recommended by the World Health Organization (WHO) because breast milk provides essential nutrients that an infant needs for growth and development [169]. Assessing the adverse effects of chemical exposure through breastfeeding is thus complicated by the co-existing beneficial attributes.

Breast-fed infants compared to formula-fed infants generally gain less weight in the first twelve months without much difference in length [170,171]. Breast feeding duration could affect the infant's growth curve as well as the accumulated lactational exposure. We estimated the relationships between lactational exposure to PCBs, DDT and DDE and infant growth in the first 12 months using two methods. We first restricted our study to the infants who were breast fed for 6 months or longer to make breast feeding duration in the first 6 months uniform across the sample. This allowed us to assess the effects of chemical exposure on growth in the first 6 months independently from effects of breastfeeding. We also examined exposure to the accumulated chemicals over the first 12 months of life, incorporating duration of breastfeeding into an exposure metric.

C.2 Methods

Study Population

The Pregnancy, Infection and Nutrition (PIN) Babies Study from January 2004 to the end of 2006 followed the infants of the PIN and PIN Postpartum Study participants. These studies enrolled pregnant women receiving prenatal care at the University of North Carolina Hospitals before 20 weeks gestation and followed them through the first year postpartum. Mothers completed several self-administered questionnaires and two phone interviews during pregnancy, one brief questionnaire after delivery at hospital, and two in-home interviews at 3 and 12 months postpartum to provide details about their health and lifestyle during and after pregnancy. Infants in the PIN Postpartum and PIN Babies studies were singletons without major birth defects. Details of these studies are available at the website: http://www.cpc.unc.edu/projects/pin/. The study protocols of the PIN, PIN Postpartum, and PIN Babies studies have been approved by the Institutional Review Board of the University of North Carolina at Chapel Hill.

Three hundred and four of the participant women in the PIN Postpartum Study were still lactating at 3 months postpartum and willing to provide a valid breast milk sample for the postpartum in-home interview. They followed the written instructions to pump both breasts at around 10 o'clock in the morning of the scheduled interview. They gently mixed the milk extracted, used the plastic pipette to transfer the milk into three 1.5ml tubes, and stored the milk in the freezer until the interviewers arrived. Samples were then transported on ice to the -80 ºC storage freezers.

Two hundred and sixty-four women continued participation in the postpartum 12-month in-home interview. Infant feeding status for each preceding month was reported at these two home interviews. Two hundred and fifty-three women were accordingly identified as breast feeding for 6 months or longer in the first year.

Growth Measurement

Mothers were provided with an infant growth card to take to their child's 12 month pediatric well-child visit. The children's medical care providers completed the growth card by recording the infant's weight and length at each routine clinical visit from birth through 12 months. The schedule for visits recommended by the American Academy of Pediatrics is at 1, 2, 4, 6, 9, and 12 months of age, but actual dates of visits were recorded because the frequency and times of visits varied among infants. The growth card was then collected at the 12-month postpartum home visit.

The weights and lengths of infants were compared to the nationally representative data, the 2000 Centers for Disease Control and Prevention (CDC) growth charts, to determine their age- and gender-standardized z-scores of weights and lengths and their age- and gender-specific percentiles of weights, lengths and weight-for-length [132]. Growth measurements were considered as biologically implausible if the z-scores were in the fixed exclusion range suggested by the World Health Organization [172]. Infants who were less than the 5th percentile of length-for-age were classified as having stunted growth. Underweight was the infant's percentile of weight-for-length less than the 5th percentile,

and overweight was the infant's percentile of weight-for-length equal to or greater than the 95th percentile. The 2000 CDC growth charts were based on a mix of both breast-fed and formula-fed infants, in which only 33% was breast-fed for 3 months or longer. It is likely that the weight of the 206 infants in this sample, in general, will be on the "light" side on the 2000 charts because the entire sample breast fed for 6 months or longer.

Exposure Measurement

Breast milk samples were analyzed for *p,p'*-DDT, *p,p'*-DDE and thirty-five PCB congeners according to the existing methodology at the Organic Analytic Toxicology Branch of the National Center for Environmental Health at the CDC [122]. The measurement of selected chemicals in breast milk samples was performed using gas chromatography/isotope dilution high-resolution mass spectrometry (GC/IDHRMS) using a MAT95 instrument (ThermoFinnigan MAT; Bremen, Germany). The lipid concentration was gravimetrically determined by an analytical balance AX105 Delta Range (Mettler Toledo; Columbus, OH) with an accuracy of $\pm 10^{-4}$ g. Each analysis batch contained sixteen unknowns, two method blanks and two quality control specimens. The between-assay coefficient of variation was normally less than 10%. Concentrations reported here were all lipid adjusted. PCB153 and *p,p'*-DDE were detected in all 304 samples, and *p,p'*-DDT was detected in 292 samples (96%). Total PCBs was calculated as the sum of eighteen detectable PCB congeners in more than 70% samples, that is PCB66, PCB74, PCB99, PCB105, PCB118, PCB138_158, PCB146, PCB153, PCB156, PCB170, PCB177, PCB178, PCB180, PCB183, PCB187, PCB194, PCB196_203 and PCB199.

Concentrations lower than the limits of detection (LOD) were imputed to the median of the method LOD of each measurement divided by a square root of 2 [164].

Lactational Exposure Metric

We quantified the duration of breast feeding in order to estimate the infant's the lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE through the first 12 months of life. At the 3- and 12-month interviews, women were asked to recall their child's feeding practices for each of the preceding months, indicating the frequency that they breast fed, fed infant formula, and fed other types of food. For each month, feeding status was classified. *Exclusively breast-feeding* was defined as breast feeding with no other food or liquid; *mostly breast-feeding* was defined as breast feeding with feeding of other supplements equal to or less than one time per day; and *breast-feeding with supplements* was defined as breast feeding with feeding any other liquids or solids >1 time per day. The number of months representing each type of feeding was summed and used in a Lactational Exposure Metric (LEM).

The LEM was developed as follows to represent the infant's cumulative exposure to each chemical at 12 months:

$$LEM = C \times (D_1+D_2) + ½ \times C \times D_3,$$

in which C denotes chemical concentration in breast milk (ng/g lipid), D_1 is duration of exclusively breast-feeding (months), D_2 is duration of mostly breast-feeding (months), and D_3 is duration of breast-feeding with other supplements (months). The unit of this

estimate is concentration-months, i.e. ng/g-months. Using this metric, breast milk concentration for each chemical was assumed to be constant through the lactation period, and breast milk consumption was assumed to be decreased by half during the period when breastmilk was supplemented with formula, other liquids or solids.

Statistical Analysis

To estimate the relationships between lactational exposure to PCBs, DDT and DDE and infant growth in the first 12 month, two statistical analyses were conducted using PC-SAS (version 9.1). In the first analysis, linear mixed effects models (SAS PROC MIXED) were used to assess the relationships between PCB153, PCBs, DDT and DDE concentration in breast milk and longitudinal infant weight (grams) and length (centimeters) measurements in the first 6 months [173]. This analysis was restricted to infants who were breast fed for 6 months or longer, in order to distinguish the effects of the chemicals from effects of the duration of breast feeding. The concentrations of PCB153, total PCBs, DDT and DDE in breast milk at 3 months postpartum were included in the separate mixed models as time-independent variables. Infant age at the pediatric well-child visits was a time dependent variable, and centered at 90 days. A linear term, a quadratic term and a cubic term of infant age at the visit were included in the model to allow a curvilinear growth pattern. Models also included a random intercept and a random slope for infant age to allow infants to have their own growth patterns. The interaction terms between infant age and gender, between infant age and preterm status, between infant age and maternal race, and between infant gender and the concentrations

of chemicals were added in the models and assessed by likelihood ratio tests using maximum likelihood. The *a priori* α-level for interaction tests was 0.1. Influence statistics were assessed to examine how observations impacted the effect estimators [174]. The parameters in the final models were estimated using restricted maximum likelihood. Other covariates in the final model were potential confounders and the important factors of the outcomes: infant gender (male vs. female), preterm birth (yes vs. no), birth weight (grams), maternal race (nonwhite vs. white), education (<16 years vs. ≥16 years), income as a percentage of the poverty level during pregnancy (100%), parity (0 vs. ≥1), and maternal pre-pregnancy body mass index (kg/m^2).

In the second analysis, multivariable linear regression models were used to assess the relationships between lactational exposure to PCB153, PCBs, DDT and DDE through 12 months, which was estimated by LEM, and the z-scores of infant weight and length at 12 months. Infants who had a growth measurement in the interval 12±1.5 months were included in this analysis. If there were multiple measurements within the range, we used the one measured at the age closest to 12 months. To control the confounding effects from breast feeding, the residuals were first calculated separately from the models of breastfeeding and z-scores of weight and z-scores of length using simple linear regression, and then were included in the multiple linear regression models as dependent variables to estimate the effects of the LEM. Other covariates included in the linear regression models were z-score of birth weight, preterm birth (yes vs. no), maternal race (nonwhite vs. white), education (<16 years vs. ≥16 years), income as a percentage of the poverty level during pregnancy (100%), parity (0 vs. ≥1) and maternal pre-pregnancy body mass index

(kg/m^2). Gender and LEM interaction was included in the models and assessed by the likelihood ratio test (a priori α-level=0.10). Robust regression with Least Trimmed Squares (LTS) estimation for multiple linear regression models was used to examine the influence by the outliers of chemical concentrations on beta coefficients [166].

C.3 Results

Among 253 women who breast fed for 6 months or longer, 206 women returned the growth card. The study population was primarily white women with 16 or more years of education who were about 30 years of age at the start of pregnancy (table 21). Comparison of the 206 mother-child pairs in the growth card subgroup with the original 304 participants showed that those who breast fed for 6 months or longer and also participated in the follow-up studies were more highly educated and more likely to be white women. However, this subgroup had similar distributions of chemical concentrations and LEMs to those of the original population. In the subgroup of 206 mother-child pairs, one infant had no records of clinical visits in the first 6 months and was not included in the mixed models; 8 infants had no clinical visits around 12 months and were not included in the linear regression models.

Children averaged 3.7 (range 1 to 7) well-child visits in the first 6 months. A total of 750 visits for 205 infants were included in the analysis. After controlling for other covariates in the mixed models, no difference were observed in growth curves of infant weight and length through 6 months as an increase of 10 ng/g-lipid in the concentrations

of PCB153, total PCBs, DDT and DDE (table 22). Although the p-values for the parameter estimates of PCB153 and total PCBs in the models for body weight were at borderline (p=0.06), these borderline effects disappeared when excluding chemical concentration outliers (>mean+3 standard deviation (SD)). Several chemical concentration outliers also appeared to be influential on other parameter estimates. However, excluding these outliers (2 for analysis of PCB153, 1 for total PCBs, and 4 for DDT and DDE) only changed the effect estimates by 2.8 to 48.6 grams on weight and by 0.06 centimeters or less on length, and the confidence intervals of the associations became wider and still remained inclusive of zero.

The interaction terms between infant age and gender, infant age and preterm status, and infant age and maternal race contributed to the fit of the models, which indicated the growth curves of weight in the first 6 months varied by gender, preterm status and maternal race; however, in the models of lengths, the growth curves did not vary by maternal race. The likelihood ratio tests for the interaction between the chemical concentrations and gender were greater than 0.1, so the effects of chemicals were not modified by infant gender.

At twelve months, the mean weight of 198 infants who had medical visits at 12 months was 9570 grams (standard deviation (SD)=1126) and the mean z-score of weight was -0.50 (SD=1.03). The mean length was 75.7 centimeters (SD=2.7) and the mean z-score of length was 0.17 (SD=0.88). Twenty-one of 198 infants (10.6%) were classified as underweight, 9 infants (4.6%) were overweight, and 4 infants (2.0%) were stunted

using the 2000 CDC growth charts. As the duration of breast feeding increased by a month, the z-scores of weight at 12 months decreased by 0.13 ($p<0.001$) and the z-score of length at 12 months decreased by 0.06 ($p=0.04$). Table 23 shows that after covariate adjustment, no differences in z-scores of weight or length were observed with increases in LEM of PCB153, PCBs, DDT and DDE. No gender modification was observed on the effect measurements of LEM. Robust regression identified 1 to 2 outliers (0.5 to 1 %) in the multivariable linear regression models, but none changed the effect estimates.

C.4 Discussion

Our results suggest that among infants breast fed for 6 months or longer there was no difference in weight and length through the first 6 months as PCBs, DDT and DDE concentration in breast milk increased. No difference was observed in weight and length at 12 months when comparing the accumulated lactational exposure through 12 months after controlling for their total duration of breast feeding. No sex-specific association was observed.

The associations between PCBs, DDT and DDE and children's growth have not been consistent throughout the literature, and nor has the effect modification of gender. Inconsistent results may be due to different exposure periods assessed (prenatal, lactational or childhood), different chemical concentrations in the different study populations and different mixtures of environmental chemicals across studies, or inadequate confounding control for duration of breast feeding. Our study measured

chemical concentrations in breast milk at 3 months postpartum to examine the effects of infants' lactational exposure. Our study population was exposed to low background levels reflected by the relatively lower chemical concentrations compared to other previous studies. Because breast feeding itself can affect growth patterns, we restricted the analyses to those breast fed for 6 months or longer. This restriction should have minimized the potential for confounding by the duration of breast feeding per se.

Despite different methods of exposure estimation and different time windows of outcome measurements, this study supported previous findings of no associations between children's growth and postnatal exposure to PCBs [90,95,98]. Our results contrast those from the Faroe Islands cohort study, which suggested that an increase in lactational exposure, estimated by the product terms of chemical concentrations and the duration of exclusive breast feeding, decreased body height and weight at 18 months [91]. As for prenatal exposure to PCBs in previous studies, children with higher prenatal PCBs were reported to be shorter from birth to 3 months of age in a formula-fed group in the Dutch cohort [90], but was associated with greater height among 5-year-old girls in the California study [92]. A negative association with body weight was observed only among girls in the small subset of the Collaborative Perinatal Project (CPP) cohort in New York [93] and in a Michigan cohort of polybrominated biphenyl accident victims [94]. This negative association was found without gender differentiation among 4-year-old children in the Michigan fish consumption cohort [95], and from birth to 3 months in a formula-fed group in the Dutch cohort [90]. However, there were two other studies that reported no difference was observed in children's growth when prenatal exposure to PCBs increased [97,111].

No prior studies have investigated lactational exposure to DDT and DDE in relation to infant growth. In several studies regarding prenatal exposure to DDT and DDE, no difference was observed in children's growth [93,111,113]. However, the CPP cohort found a negative association between prenatal *p,p'*-DDE and children's height at 1, 4, and 7 years [97]. This negative association was also found only among girls in the German study by using the concentrations detected in child's blood at 8 years old to index perinatal exposure [98].

Compared to the use of a single chemical concentration at the time of measurement, the lactational exposure metrics used in this study can more accurately reflect an infant's accumulated lactational exposure through the first 12 months. According to the Hooper et al study [102], PCB153 concentration in breast milk decreased 4% over the first 6 months of breast feeding; after 6 months the change in PCB153 concentration did not change more than 1% per month. These findings support our assumption of a relatively constant concentration throughout the lactation period of interest. Additionally, under the two assumptions that infants in the same feeding status group consumed similar amounts of breast milk and that the mothers had similar fat composition in their breast milk, the LEM should be highly correlated with the absolute value of the accumulated dose of the chemicals. The LEM is therefore appropriate for estimating the relative effects of chemical exposure.

For the investigation on the growth trajectory in the first 6 months, we used the

concentration of chemicals in breast milk by itself in the mixed models. This measurement should be highly correlated with the accumulated exposure through the first 6 months, because all the infants in this study were at least breast fed for 6 months and 90% of the infants were exclusively and mostly breast fed for 4 months or longer. This selection criterion provided comparability among the infants, and also provided an unambiguous measure of the chemical effect independent of the duration of breast feeding. We also used the LEM at each pediatric well-child visit as a time-dependent variable in the mixed models, and the findings confirmed the results of no association.

The logarithmic transformation of the concentrations of PCB153, total PCBs, DDT and DDE was considered for the general linear mixed models, but it did not substantially improve the normality of residuals or reduce leverage points. We decided to use a linear term of the concentrations in the models for simplicity and ease of interpretation.

The babies' growth cards provided a low cost collection of serial growth measurements recorded from the pediatric medical record to construct each infant's growth pattern. Although it was not feasible to standardize the measurement procedures and equipment used in the physical examination at the various pediatric clinics, there is no reason to suspect that any potential measurement error was associated with infants' true growth or lactational exposure to chemicals.

Several limitations of this study should be noted. This study population was primarily well educated white mothers, which may constrain the generalization of the

findings if heterogeneous effects by race and socioeconomic status were believed to be true. There were no direct measurements of prenatal exposure to PCBs, DDT and DDE in this study, thus the effects of lactational exposure could not be differentiated from the effects of prenatal exposure. However, we adjusted for infant birth weight in the mixed models and adjusted the z-scores of birth weight in the multiple linear regression models to control for the potential adverse effects of prenatal exposure on intrauterine growth.

Women have been encouraged to breast feed for at least 6 months by the WHO and the American Academy of Pediatrics. Although longer durations of breast feeding may involve increased exposure to potentially hazardous chemicals, our study indicates that breast feeding for 6 months or longer, with lactational exposure to PCBs, DDT and DDE in the ranges of low background level concentrations studied here, results in no negative influence on infant growth in the first 12 months.

Table 21. Characteristics and chemical concentrations in breast milk of participant mothers who provided breast milk at 3 months in the PIN Babies study and characteristics and chemical concentrations of the mother-child pairs included in this study. *

	Milk Samples (n=304)		Growth Card Subgroup (n=206)	
	No.	%	No.	%
Maternal characteristics				
Race				
White	262	86	185	90
Non-white	42	14	21	10
Education (years)				
<16	54	18	28	14
≥16	250	82	178	86
Parity				
0	160	53	106	52
≥1	144	47	100	48
Age (years)				
Mean±SD	31±5		31±5	
Percent of 2001 poverty level during pregnancy (%)				
Mean±SD	490±199		508±188	
Missing	5		4	
Pre-pregnancy body mass index (kg/m^2)				
Mean±SD	23.8±4.9		23.5±4.8	
Median (range) of chemical concentrations in breast milk (ng/g lipid) ‡				
PCB153	17 (2-199)		18 (2-199)	
ΣPCBs †	77 (9-708)		81 (12-708)	
p,p'-DDT	5 (<LOD-80)		5 (<LOD-36)	
p,p'-DDE	121 (1-2140)		114 (15-2140)	
Infant characteristics				
Baby gender				
Male	163	54	109	53
Female	141	46	97	47
Preterm birth				
Yes	22	7	15	7
No	282	93	191	93
Birth weight (g)				
Mean±SD	3426±729		3358±468	
Duration of breast feeding (months)				
Mean±SD	10±2		11±2	
Missing	40		0	

Months of exclusive breastfeeding		
Mean±SD	4±2	5±2
Missing	40	0
Median (range) of LEM at 12 months (ng/g lipid - months) ‡		
PCB153	119 (12-1194)	126 (19-1194)
ΣPCBs †	546 (64-4249)	574 (100-4249)
p,p'-DDT	33 (1-523)	35 (1-326)
p,p'-DDE	871 (134-19260)	887 (134-19260)

* Abbreviation: SD, standard deviation; <LOD, lower than detection limit; PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene.
† ΣPCBs is the sum of the concentrations of PCB66, PCB74, PCB99, PCB105, PCB118, PCB138_158, PCB146, PCB153, PCB156, PCB170, PCB177, PCB178, PCB180, PCB183, PCB187, PCB194, PCB196_203 and PCB199, in which <LOD is treated as median(LOD) divided by a square root of 2.
‡ There were 1 subject missing ΣPCBs concentration and the LEM of ΣPCBs and 1 subject missing *p,p'*-DDT concentration and the LEM of *p,p'*-DDT. Additonal 40 subjects in the milk sample group missed LEM because of missing duration of breast feeding through 12 months.

Table 22. Fixed effect estimates of a 10 ng/g lipid increase in the concentrations of PCB153, PCBs, DDT and DDE in breast milk at 3 months postpartum on infant weight and length in the first 6 months. *

Chemical concentration	Weight (g)		Length (cm)	
(per 10 ng/g lipid)	β †	95% CI	β †	95% CI
PCB153	-22.8	-46.1, 0.6	-0.04	-0.18, 0.10
ΣPCBs	-6.1	-12.5, 0.3	-0.02	-0.05, 0.02
***p,p'*-DDT**	21.0	-89.7, 131.6	0.24	-0.39, 0.88
***p,p'*-DDE**	0.6	-1.7, 2.8	0.01	-0.005, 0.02

* Abbreviation: PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; CI, confidence interval.

† Adjusted for infant gender (male vs. female), preterm birth (yes vs. no), birth weight (grams), maternal race (nonwhite vs. white), education (<16 years vs. ≥16 years), income as a percentage of the poverty level during pregnancy (100%), parity (0 vs. ≥1), and maternal pre-pregnancy body mass index (kg/m^2).

Table 23. Mean difference in age- and gender-standardized weight and length (z-scores) at 12 months per 10 ng/g-month increase in lactational exposure metrics of PCB153, PCBs, DDT and DDE. *

LEM	Weight		Length	
(per 10 ng/g-month)	β †	95% CI	β †	95% CI
PCB153	0.00	-0.01, 0.01	0.00	-0.01, 0.01
ΣPCBs	0.00	-0.003, 0.003	0.00	-0.002, 0.003
***p,p'*-DDT**	-0.01	-0.04, 0.03	-0.002	-0.03, 0.03
***p,p'*-DDE**	0.00	-0.0002, 0.001	0.0004	-0.0002, 0.001

* Abbreviation: PCB, polychlorinated biphenyls; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; CI, confidence interval.

† Adjusted for z-score of birth weight, preterm birth (yes vs. no), maternal race (nonwhite vs. white), education (<16 years vs. ≥16 years), income as a percentage of the poverty level during pregnancy (100%), parity (0 vs. ≥1) and maternal pre-pregnancy body mass index (kg/m^2)

CHAPTER V. DISCUSSION

A. Summary of Findings

The Pregnancy, Infection and Nutrition (PIN) Babies Study from January 2004 to the end of 2006 followed up the infants of the participant women of the PIN and PIN Postpartum Studies. Three hundred and four of the participant women in the PIN Babies Study were still lactating at 3 months postpartum and willing to provide a valid breast milk sample. The range of PCB153 concentration in breast milk of this study population was 2.2–199.0 ng/g lipid (median: 16.6 ng/g lipid); for total PCBs the range was 9.2–708.1 ng/g lipid (median: 77.3 ng/g lipid); for *p,p'*-DDT the range was <LOD–80.4 ng/g lipid (median: 4.7 ng/g lipid); and for *p,p'*-DDE the range was 1.4-2140.0 ng/g lipid (median: 120.50 ng/g lipid). Compared to other previous studies which examined the potential hazardous effects on children's development [141], PCB153 median concentration in this study was the lowest. Compared to *p,p'*-DDE concentration in a previous North Carolina study in 1978-1982 (median concentration in milk at 3 months=2.07 ppm, i.e. 2070 ng/g) [100] this study DDE concentration was very much lower.

The correlation between the concentrations of PCBs and DDE in breast milk at 3 months postpartum was low (r=0.21). PCBs was observed to be positively correlated with the concentrations of DHA (r=0.15) and AA (r=0.20) in breast milk at 3 months

postpartum, but the associations disappeared when adjusted for other covariates. No correlations were observed between the concentrations of *p,p'*-DDT and *p,p'*-DDE and the concentrations of DHA, EPA and AA in breast milk.

Findings of this dissertation do not suggest adverse effects of lactational exposure to PCBs, DDT and DDE on infant neurodevelopment at 12 months, although lactational exposure to *p,p'*-DDE through the first 12 months appeared associated with scoring *below average* on the gross motor scale among males but not females. The interaction between PCB153 and EPA in the model of scoring *below average* on the visual reception scale, and the interaction between *p,p'*-DDE and DHA in the model of scoring *below average* on the fine motor scale appeared statistically significant at an a priori significant level of 0.1 in the multiple logistic regression models. However, the sample size of this study was too small to provide precise effect estimates. Other studies of larger sample sizes are needed to examine these relationships.

Findings of the linear mixed effects models among the infants who were breast fed for 6 months or longer suggested no difference in infants' growth patterns in the first 6 months when concentrations of PCBs, DDT and DDE in breast milk increased. Cumulative lactational exposure to PCBs, DDT and DDE through 12 months was not associated with infant weight and length at 12 months after pre-adjustment for the duration of breast feeding. No sex-specific association was observed.

B. Strengths and Limitations

B.1 Strengths

One of the main strengths of this dissertation is an appropriate estimation of lactational exposure of infants by using LEM. This metric, accounting for both the chemical concentration in breast milk and the duration of breast feeding, reflected an infant's accumulated lactational exposure through the first 12 months of life better than the use of a single chemical concentration at the time of measurement. Because infant feeding status and frequencies of breast feeding, feeding infant formula and feeding other type of food at each month were reported at the maternal home interview at 3 and 12 months postpartum, the durations of exclusively breast-feeding, mostly breast-feeding and breast-feeding with supplements were able to be accordingly defined. This exposure measurement is also better than using indirect exposure surrogates such as the amounts of maternal fish consumption and the duration of lactation only.

Assessing the adverse effects of chemical exposure through breastfeeding is complicated by the co-existing beneficial attributes of breastfeeding. This study adequately controlled this confounding effect by pre-adjusting the duration of breast feeding in the separate linear regression models or by sample selection of infants so they had a uniform duration of breast feeding in the study period.

This dissertation has direct measurements of environmental pollutants – PCBs, DDT

and DDE and nutrients – LC-PUFAs in breast milk, so the correlations between PCBs, DDT, DDE and LC-PUFAs in breast milk could be examined and the modification by LC-PUFAs on the effects of lactational exposure to PCBs, DDT and DDE on infant neurodevelopment could be assessed.

The Mullen Scales of Early Learning has not been used in previous related papers. This developmental assessment, compared to the commonly used Bayley Scales, may help distinguish effects on more refined domains of development. The administration time for evaluating an infant at 12 months old is about 30 minutes, which is feasible to be conducted at a home interview. The Mullen Early Learning Composite score has been found to be highly correlated with the mental development index of the Bayley Scales (r=0.70), and the Mullen gross motor score has also been found to be highly correlated with the psychomotor development index of the Bayley Scales (r=0.76) [165], which may provide the basis for comparisons of study results. The parent-report approach of the infant Short Form of the MacArthur-Bates Communicative Development Inventories requires no child cooperation and therefore can be used to evaluate the language development of the infant who is often unwilling to interact with strangers or cooperate during testing. Meadows et al have reported that mothers can consistently report their infants' communicative acts [130], supporting the use of a self-administered instrument in research studies.

Infant growth through age 12 months is measured regularly at the pediatric well-child visits. The babies' growth cards provided a cost-effective collection of these

serial growth measurements to construct each infant's growth pattern. Although it was not feasible to standardize the measurement procedures and equipment used in the physical examination at the various pediatric clinics, there is no reason to suspect that any potential measurement error was associated with infants' true growth or lactational exposure to chemicals.

All pre-, peri-, and post-natal covariates in the PIN studies were collected prospectively, so recall of the relative information is likely accurate. Because of the availability and accuracy of the covariate information, this dissertation can properly control potential confounders. However, as in any observational study, residual confounding effects may still exist.

B.2 Limitations

The main study limitation is that we were unable to distinguish the effects of lactational exposure to PCBs, *p,p'*-DDT and *p,p'*-DDE from the effects of prenatal exposure. PCBs and DDE concentration in breast milk have been shown to be highly correlated with the concentrations in cord plasma [100,101], so infants exposed to high chemical concentrations postnatally in breast milk were also likely to be exposed to high chemical concentrations prenatally.

Another limitation is that most of our study population breast fed more than 6 months, so we had limited variability in duration of breast feeding and also limited

sample size to differentiate the effects of short-term high-concentration exposure to PCBs, DDT and DDE from the effects of long-term low-concentration exposure when using LEM (concentrations-months) as an index of lactational exposure for analysis.

Although the sample size of lactating mother-child pairs in this dissertation is larger than in some of the previous studies, this study still has limited power to detect a small magnitude of the association. Because only 175 mothers had measurements for the concentrations of LC-PUFAs in breast milk and only 14 of 304 participants were non-white women, the stratification analyses by LC-PUFAs and maternal race was limited.

Finally, this study population was primarily well educated white mothers who had their pregnancy at around 30 years old, which may constrain the generalization of the findings in this dissertation if heterogeneous effects by maternal race, age and socioeconomic status were believed to be true.

C. Conclusions

Women have been encouraged to breast feed for at least 6 months by the WHO and the American Academy of Pediatrics. Although longer durations of breast feeding may involve increased exposure to potentially hazardous chemicals, our study indicates that lactational exposure to PCBs, DDT and DDE was generally not found to be associated with infant neurodevelopment at 12 months, and breast feeding for 6 months or longer,

with lactational exposure to PCBs, DDT and DDE in the ranges of concentrations studied here, results in no negative influence on infant growth in the first 12 months.

Because previous studies had higher chemical exposure and some of the studies investigated populations who consumed large amounts of fish or seafood, this dissertation, in which the study population was lactating women and their infants in Central North Carolina in 2004-2006, who were generally exposed to low background levels of chemicals, is informative for future risk assessment of infants exposed to low background levels of PCBs, DDT and DDE through lactation.

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