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Regina Marie Simeone Date

Pre- and Postnatal Exposure to Polychlorinated Biphenyls and Cognitive Development in Early Childhood

By

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Master of Public Health

Epidemiology

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B.A., Swarthmore College, 2006
M.S., University of Georgia, 2010

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Abstract

Pre- and Postnatal Exposure to Polychlorinated Biphenyls and Cognitive Development in Early Childhood

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Prior cohort studies suggest that exposure to polychlorinated biphenyls (PCBs) may hinder cognitive development. The aim of this study was to determine if perinatal exposure to PCBs affects behavior and intelligence in children at age 45 months. We sampled 438 mother-child pairs from a birth cohort conducted in a highly exposed district in the Slovak Republic. Mothers in our population had a median PCB 153 serum level of 1.73 ng/ml. Prenatal exposure was estimated as maternal PCB 153, the most abundant congener, and PCB 118, the most abundant mono-ortho dioxin-like congener. Children's body burdens of PCB 153 and 118 were measured at 16- and 45-months. At 45-months, mothers completed the Child Behavior Checklist ages 1 ½ - 5 (CBCL), which is used to identify behavioral problems. Children completed five subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III), which is an intelligence test. Negative binomial and multiple linear regression analyses were used to measure the associations between PCB exposure and CBCL syndrome score or WPPSI-III subtest score, respectively. After adjusting for potential confounders, increased anxious/depressed behavior problems were reported more frequently among children more highly exposed to PCB 153 and 118 (exponentiated beta coefficients representing a change in score for a 1-unit increase in PCB; PCB 153: 1.04, 95% confidence interval (CI): 1.0, 1.1; PCB 118: 1.4, 95% CI: 1.1, 1.9). Other associations between 16- or 45-month PCB exposure and cognitive outcomes were null. Maternal exposure to PCB 153 and 118 was not associated with any of the 14 outcomes. Perinatal exposure in a highly exposed population did not appear to substantially affect cognitive development at 45-months; however, internalizing behaviors, as found on the anxious/depressed syndrome scale, may be more affected by exposure than intelligence and other behaviors.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Polychlorinated biphenyls (PCBs) are a class of manmade chemicals that persist in the environment and were widely produced between their introduction in 1929 until the late 1970s (1). These chemicals were used in various industrial and consumer products, including heat transfer agents, electric capacitors, plasticizers, lubricants, flame retardants, and carbonless paper (2, 3). In 1977, the United States banned production of PCBs due to recognition of their persistence in the environment and concern regarding potential health effects (3). PCBs are stable and lipophilic chemicals, and once released into the environment, tend to remain in soil, water, and animal fat for long periods of time (2-4). Moreover, due to their lipophilic nature, body burdens of PCBs accumulate up the food chain and are found in highest concentrations in mammals with high-fat diets, such as humans (5).

Most human exposure to PCBs occurs through consumption of contaminated food, usually fish or other fatty meats, or through environmental exposure due to improper disposal of PCB waste (2-4). Although declining, individuals born after the cessation of PCB production have detectable levels of PCBs in their bodies (6). Additional exposure routes occur prenatally or immediately after birth, as PCBs are able to cross the placental barrier and are excreted through breast milk. Perinatal exposure to PCBs is worrisome due to potential adverse health effects.

The chemical structure of PCBs is similar to that of chemicals with known harmful health effects. Polychlorinated biphenyls consist of two benzene rings and one to ten chlorine atoms. Different numbers and positions of the chlorine atoms allow for 209

different congeners of PCBs, although only a subset were used commercially (2, 7). The more highly chlorinated the benzene rings, the more persistent the compound, and the more readily it is absorbed into the environment (7). In addition to differences in chlorination, PCBs may be dioxin-like mono-ortho or non-dioxin-like di-ortho in structure. Dioxin-like PCBs are similar in structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxins (TCDDs) (5). Dioxins are persistent environmental toxins known to have adverse effects on the immune system, the reproductive system, the endocrine system, and development; they are also known carcinogens (8). Non-dioxin-like PCBs have their own toxic health effects that include, but are not limited to, effects on the endocrine system and potential immunotoxicity (9). Due to these adverse health effects, human exposure to PCBs has become an area of concern; this concern was validated after the occurrence of two PCB mass-poisoning events that ultimately led to the banning of the chemicals.

In 1968 in Western Japan, numerous individuals presented with an unusual syndrome, whose symptoms included chloracne, hyperpigmentation of the skin and nails, loss of hair, fatigue, and nausea, among others (10). The cause of this syndrome was found to be rice oil contaminated with heat-degraded Kanechlor 400, a PCB, and the syndrome was named *Yusho*, or "oil disease" (10). It was estimated that over 15,000 people consumed the contaminated rice oil, although only about 2,000 individuals were diagnosed (10, 11). A similar incident occurred in 1978 in Taiwan. More than 1,800 cases had been identified by 1980 and presented with similar symptoms as those in Japan; the outbreak was also traced to contaminated rice oil and was called *Yu-cheng* (11). Children born to mothers affected by *Yusho* or *Yu-cheng* disease experienced developmental delays and cognitive deficiencies due to PCB poisoning.

In a pair-matched cohort study of the *Yu-cheng* cohort, 117 children aged 4-7 born to mothers exposed to contaminated rice oil were examined (12). The authors matched exposed children on neighborhood, age, sex, maternal age, parental education, and parental occupation to unexposed children of families, neighbors, and friends recommended by *Yu-cheng* parents. Older siblings of exposed children were also studied since they were born prior to the oil contamination. PCB levels of *Yu-cheng* Mothers averaged 49.3 ppb; control mothers did not have PCB levels measured, but control PCB levels were estimated from local blood-bank specimens, which averaged 9.8 ppb. The authors assessed cognitive development using Chinese versions of the Stanford-Binet test and the Wechsler Intelligence Scale for Children. They found a consistent and significant deficit in IQ scores of approximately five IQ points between children prenatally exposed to PCB compared to controls. Moreover, this association was consistent regardless of whether children were born immediately after maternal exposure or up to six years after maternal exposure, suggesting that the effects of PCB exposure in the mother were long lasting and detrimental.

A similar study by Lai et al. examined the same cohort of exposed children for behavioral deficits (13). Of the 115 matched pairs examined in the previous study, 112 participated in the Lai et al. study. Three measurements of behavior and IQ were obtained: the Achenbach Child Behavior Checklist (CBCL), the Rutter Child Behavior Scale, and the Wechsler Intelligence Scale for Children. Higher scores on the CBCL and Rutter Child Behavior Scale indicate increased behavioral problems; higher scores on the Wechsler Intelligence Scale indicate greater intelligence. The authors used mixed-models to examine each outcome and adjusted for age at testing, sex, and birth year. Exposed

children had a higher mean CBCL score (β=2.77, SE=0.88), a higher Rutter Child Behavior Score (β=6.03, SE=1.28), and a lower IQ score (β=-3.32, SE=1.69) compared to non-exposed children. These findings were consistent across age, suggesting again that maternal exposure had lasting effects on the pre-natal exposure of her children.

Cognitive Testing

Cognitive development is measured in a variety of ways. A commonly used tool in the measurement of infant neurodevelopment is the Bayley Scales of Infant Development assessment (BSID), which is appropriate for use in infants ages one to 42 months (14). The BSID is administered by a psychologist and is comprised of two subscales, Mental and Psychomotor, which are used to determine cognitive and motor development (14, 15).

The Child Behavior Checklist (CBCL) is another commonly used clinical assessment tool for children ages two to 18 years. For younger children, the CBCL ages 1 ½ - 5 years is used. The assessment is usually completed by a parent. Seven areas of behavior are measured: Anxious/Depressed, Withdrawn, Emotionally Reactive, Sleep Problems, Somatic Complaints, Aggressive Behavior, and Attention Problems. Anxious/Depressed, Withdrawn, Somatic Complaints, and Emotionally Reactive scores comprise an Internalizing behavior score and Aggressive Behavior and Attention Problem scores comprise an Externalizing behavior score (16, 17). Increased scores on the CBCL indicate higher reports of behavioral problems.

The McCarthy Scales of Infant Ability are comprised of six scales and are conducted on preschool-aged children. The six McCarthy Scales are verbal, perceptual-performance, quantitative, general cognitive, memory, and motor (15). The Fagan Test of

Infant Development is designed for infants up to age 52 weeks and measures the amount of time an infant spends examining a novel stimulus (a picture) (18). This test is considered predictive of later verbal IQ in young children (19). Commonly used tests to measure infant intelligence are the Stanford-Binet and Wechsler Intelligence Scale for Children. The variety of tests performed at different ages in childhood can make comparisons between studies difficult; moreover, tests performed in infancy may not be predictive of later cognitive abilities.

Probable Risk and Protective Factors

Exposures of the mother can have profound effects on the development of the fetal brain and these effects can last into adulthood. The developing brain is vulnerable to a number of harmful exposures both pre- and postnatally (20). Known environmental exposures that affect brain development include lead and arsenic (21). Prenatal exposure to lead is associated with reduced IQ and poorer behavioral scores compared to children with low or no prenatal exposure to lead (21, 22). Maternal exposure to infectious diseases, poor maternal nutrition, iodine deficiency, thyroxine deficiency, and iron deficiency, can impact all fetal neurodevelopment (21, 23).

The environment of a child after birth has a significant impact on his or her cognitive development. Breastfeeding is associated with better performance on the Bayley Scales of Infant Development between one and five years of age; increased duration of breastfeeding also has a positive association with development (15, 24, 25). This association may be due to maternal stimulation of and interaction with the child during breastfeeding, a potential biological mechanism, or even confounding with other environmental or biological factors. Additionally, maternal and paternal intelligence and

education, socioeconomic factors, race and ethnicity, and the home environment have significant effects on childhood development (26, 27). The HOME score is used to assess the home environment and how it may affect development (28). It evaluates parental verbal and emotional abilities, as well as parental responsiveness, presence of toys or other stimulating activities, and daily experience of the child (28). This assessment is often used in analyses of cognitive development because the home environment may be an important influence or confounder related to child development.

PCBs could affect the developing brain through several pathways. First, the presence of PCBs during fetal development could interfere with thyroid hormones during neurodevelopment (14, 29). Thyroid hormones are crucial during development of the brain and PCBs could disrupt natural developmental processes because they are similar in structure to naturally occurring thyroid hormones (29). Second, some PCBs have anti-estrogenic behaviors and may interfere with sex steroids and hormones during development (14). In animal studies, *in utero* exposure to anti-estrogenic PCBs caused altered brain development and behavior (30); *in utero* exposure to PCBs in humans could also affect sex hormones and thus brain structure (14). Third, dioxin-like PCBs are known to bind to aryl hydrocarbon (Ah) receptors; the Ah pathway is related to thymic atrophy and could affect brain development (14). Exposure to PCBs during development could subtly alter brain structure and function prior to birth. Various human populations have been exposed to a range of PCB contamination; these populations have been used to study the effects of PCB exposure on developing children.

Cohort Studies of the Effects of PCB exposure

Michigan Cohort

Several studies have been conducted in the United States to examine the effects of background and increased levels of pre- and postnatal exposure to of PCBs. Researchers established a cohort in the Grand Rapids, Michigan area because of high levels of PCBs in the local population due to diets high in fatty fish. PCBs bioaccumulate up the food chain and bodies of water are often highly contaminated from prior PCB runoff. Fatty fish tend to have high levels of PCBs. Individuals with a diet high in these fish are subsequently exposed to higher levels of PCBs than individuals who do not share the same diet (19). Women were recruited into the cohort one to two days after delivery between 1980 and 1981 and came from four Michigan hospitals (19, 31-34).

Fein et al. examined the effects of increased PCB levels on neonatal outcomes of birth weight, crown-heel length, and head circumference of 242 infants in the Grand Rapids, Michigan cohort (31). Researchers used packed gas chromatography to measure PCB exposure in umbilical cord and maternal serum. Maternal report of fish consumption was used as a proxy for dietary PCB exposure. Analysis of Covariance was used to detect differences in neonatal outcomes and PCB exposure. The authors compared women with greater than or equal to 3 ng/mL PCB in their cord blood to women with less than 3 ng/mL PCB in their cord blood. After adjusting for maternal pre-pregnancy weight, type of delivery, alcohol and caffeine consumption during pregnancy, and use of cold remedies during pregnancies, increased levels of prenatal PCB exposure were associated with decreased birth weight (high PCB: 3.41±0.54 kg; low PCB: 3.57±0.54 kg; $p < 0.05$) and decreased head circumference (high PCB: 34.63±1.19; low PCB: 35.28±1.18 cm; $p <$

0.001). This study suggested that prenatal PCB exposure may negatively affect neonatal outcomes but did not examine the effects of PCBs on cognitive function (Table 1.1).

Jacobson, S. et al. examined the association between increased PCB exposure and performance on the Fagan Test of Infant Development in a cohort of 123 seven-month old infants drawn from the original Michigan cohort (19). The authors measured maternal PCB exposure in three ways: self-report of amount of contaminated fish consumption, levels of PCB in umbilical cord serum, and levels of PCB in breast milk samples. The cord blood samples were considered proxies of prenatal exposure. The authors conducted multiple linear regression analysis to determine the association between exposure to PCB and performance on the Fagan Test. Results indicated that fish consumption and cord blood PCB levels were predictive of performance on the Fagan Test. Controlling for maternal age, socioeconomic status, and parity, increased levels of PCB in the umbilical cord blood predicted reduced interest in the novel stimuli (β=-0.39, $p < 0.005$). Reported fish consumption was also predictive of reduced interest in novel stimuli, with increased fish consumption associated with poorer performance (β=-0.23, $p < 0.05$). PCB levels in breast milk were not associated with performance on the Fagan Test (Table 1.1).

Another study of the Michigan cohort examined the effects of PCB exposure and cognition in a sample of 236 children at age four using the McCarthy Scales of Children's Abilities (35). The authors used cord blood serum and reported fish consumption as proxies of prenatal PCB exposure; they used breast milk serum samples to determine postnatal PCB exposure. Increased umbilical cord PCB was negatively associated with the verbal and numerical memory subtests of the McCarthy Scales (β=-0.22, p=0.02 and β=-0.24, p=0.01, respectively). The authors observed a negative

association between increased PCB exposure through breast milk and performance on the memory scale (β=-0.27, p=0.01); however, they observed a positive association between increased duration of breast feeding, regardless of PCB level, and performance on the verbal and numerical memory subtests (β=0.23, p=0.05 and β=0.25, p=0.04, respectively). The authors concluded that prenatal exposure was a more important predictor of performance than postnatal exposure.

The Michigan cohort was examined again when the children were 11 years old; of the original sample, 212 were included in the 11-year assessment (32). The authors used multiple linear regression analyses to examine prenatal exposure and its association with children's performance on the Wechsler Intelligence Scales for Children and other achievement tests (32). At 11 years, prenatal PCB exposure was negatively associated with Full-Scale IQ (β=-0.17, p=0.02), Verbal IQ (β=-0.16, p=0.02), verbal comprehension (β=-0.16, p=0.02), and Freedom from Distractibility (β=-0.17, p=0.02). Additionally, children in the highest exposure group performed poorly on word and reading comprehension (β=-0.17, p=0.01 and β=-0.13, p=0.06, respectively).

Prenatal exposure to PCBs and its effect on cognitive development may be modified by breastfeeding. Researchers examined this possibility in the Michigan cohort when children were four and eleven years old (33). They estimated prenatal exposure as the normalized average of PCBs in maternal serum, cord blood serum, and maternal milk serum. Previous cognitive measurements using the McCarthy Scales (four-years, sample size=181) and the Wechsler Intelligence Scales (11-years, sample size=178) were used in the analysis. At four-years of age, children breastfed for less than six weeks exhibited negative associations with various components of the McCarthy Scales: general cognitive

index (β=-0.29, $p<0.05$), verbal (β=-0.45, $p<0.01$), quantitative (β=-0.36, $p<0.05$), and memory (β=-0.45, $p<0.01$). For children breastfed for at least six weeks, the associations were weakened and non-significant (Table 1.1).

At 11-years of age, a positive interaction was observed between prenatal PCB exposure and breastfeeding for multiple linear regressions of verbal comprehension (PCB β=-0.16, PCB and breastfeeding interaction β=0.23, $p<0.05$) and word comprehension (PCB β=-0.17, PCB and breastfeeding interaction β=0.19, $p<0.05$). This can be interpreted as the estimated effect of high PCB exposure compared to low PCB exposure was decreased among children who were breastfed for at least six weeks, compared to those breastfed for less than six weeks. For example, a one-unit change in PCB exposure among children breastfed at least six weeks would result in a 0.07 point decrease in verbal comprehension, while the same exposure would result in a 0.16 point decrease in children breastfed for less than six weeks. When examined by breastfeeding status, children breastfed for less than six weeks showed negative associations between PCB exposure and full scale IQ (β=-0.32, $p<0.01$), verbal comprehension (β=-0.38, $p<0.001$), freedom from distractibility (β=-0.36, $p<0.05$), word comprehension (β=-0.36, $p<0.05$), and arithmetic (β=-0.25, $p<0.05$). Children breastfed for at least six weeks showed no significant negative associations between PCB exposure and IQ or achievement but did show positive associations between mental rotation (an assessment of processing speed) and PCB exposure (Table 1.1).

From the Michigan cohort, results from studies up to age 11 years support the hypothesis that prenatal PCB exposure could have adverse effects on cognitive

development that last into childhood. Breastfeeding, however, emerged as a potential effect modifier for the detrimental effects of PCB exposure.

North Carolina Cohort

Between 1978 and 1983, researchers from North Carolina developed a birth cohort in order to examine morbidity due to PCB and dichlorodiphenyl exposure (36). Researchers obtained data on 912 newborn infants whose mothers were recruited from three medical centers in North Carolina. The authors measured PCB exposure in maternal and cord blood serum samples and maternal breastmilk serum samples.

At six and twelve months of age, the authors used Bayley Scales of Infant Development to assess the relationship between PCB exposure and cognitive development in a sample of 706 members of the original cohort (37). Using multiple linear regression, the authors found a significant, negative association between increased cord blood PCB levels and the psychomotor developmental index at six months (β=-0.96, SE=0.46) and 12 months (β=-1.34, SE=0.61). No association was observed between PCB exposure and the Mental developmental index at six months (β=-0.12, SE=0.44). The authors observed a weak association at 12 months (β=-0.54, SE=0.54). Similarly, no association was found between postnatal exposure measured through breast milk PCB levels and the Mental or Psychomotor developmental indices (MDI and PDI, respectively) (Table 1.1).

Researchers used The McCarthy Scales of Children's abilities and children's academic report cards to assess the relationship between PCB exposure and cognitive development between ages three and ten years in the North Carolina cohort (38). At three-years of age, no relation was found between postnatal PCB levels and performance

on the McCarthy Scales (data not provided). The authors used Analysis of Covariance to examine the relationship between pre- and postnatal PCB levels and McCarthy scores and third-grade report cards (age ten-years). Report card grades were given the following scores: A=4, B=3, C=2, and D or F=1. There was no significant difference between mean English scores among children with the lowest (≤0.9 ppm) and highest (>4 ppm) prenatal PCB exposure (mean $(SE)_{\text{low exposure}}$= 3.54 (0.23); mean $(SE)_{\text{high exposure}}$=3.66 (0.24)). Similarly, no significant difference existed in Mathematics scores (mean $(SE)_{\text{low exposure}}$= 3.69 (0.30); mean $(SE)_{\text{high exposure}}$=3.56 (0.32)) .

The North Carolina and Michigan cohorts found seemingly conflicting results. More negative associations were detected between PCB exposure and cognitive development in the Michigan cohort through age eleven years compared to the North Carolina cohort through age ten years. One possible explanation for these differences is the amount of PCB exposure in the cohorts. While the reported PCB levels at the time of publication were similar, the gas chromatography techniques measuring PCB levels differed in how the levels were quantified and accuracy of the measurement (39). Longnecker et al. recomputed the PCB 153 levels from each study accounting for assessment techniques to make them comparable (40). Based on these computations, the Michigan cohort had a higher median level of PCB 153 exposure compared to the North Carolina cohort (120 ng/g lipid and 100 ng/g lipid, respectively) (40). The conflicting results of these studies encouraged the development of additional cohorts, both in the United States and internationally.

Oswego, New York Cohort

Following the North Carolina and Michigan cohorts, additional studies were conducted using technology that could distinguish between the various PCB congeners.

Darvill et al. examined the association between prenatal PCB exposure and infant performance on the Fagan Test of Infant Intelligence at six and twelve months (41). At six months, 230 infants participated; at twelve months, 219 of the original 230 participated. Researchers recruited participants between 1991 and 1994 from the Lake Ontario area in Oswego, New York, another population where diets are high in fatty fish. PCB exposure was evaluated using cord blood serum levels and maternal breast milk. Prenatal PCB exposure was found to be negatively associated with performance on the Fagan Test at six but not twelve months. The authors did not observe an association with postnatal exposure (Table 1.1). Although this association is similar to what was observed in the Michigan cohort, the effect estimate was smaller. This may be due to differences in the populations studied or differences in types of PCB exposure. Longnecker et al. estimated that exposure in the Michigan cohort was three-times that of exposure in Oswego (median PCB 153 was 40 ng/g lipid) (40).

Stewart et al. examined the association between prenatal exposure to highly chlorinated PCBs and infant performance at 38 and 54 months using the McCarthy Scales of Infant Ability (42). A total of 194 children completed the assessment at 38 months and 197 completed the assessment at 54 months. A significant negative trend was found between increasing levels of PCB exposure and the children's scores on the McCarthy general cognitive index at 38 months (F-test=4.86, $p<0.05$). In contrast, the authors did not observe an association between prenatal PCB exposure and performance on the McCarthy general cognitive index at 54 months (F-test=0.59, $p>0.05$). From these results, the authors suggested that the adverse effects of PCB exposure may be transient.

Stewart et al. evaluated 156 members of the Oswego cohort at nine years of age to determine if prenatal PCB exposure was associated with IQ (43). They used the Wechsler Intelligence Scale for Children to calculate Full Scale IQ, Verbal IQ, Freedom from Distractibility, and Verbal Comprehension Index. At nine years, a significant negative linear association was found between increasing prenatal PCB exposure and Full Scale IQ (β=-0.167, p=0.021), Verbal IQ (β=-0.213, p=0.003), and Freedom from Distractibility (β=-0.235, p=0.004). The authors did not find an association between PCB exposure and performance on the Verbal Comprehension Index (β=-0.025, p=0.682). Despite their previous study, in which researchers observed no association between cognitive ability and PCB exposure at 54 months, the current study suggested that prenatal PCB exposure may have long-term effects on IQ into childhood.

Inuit Cohort

Because of high marine diets, Inuit populations are potentially exposed to high levels of PCBs and methyl mercury. Between 1995 and 2001, researchers at local clinics invited pregnant women to participate in the Cord Blood Monitoring Program in Nunavik, Canada (44). Umbilical cord blood samples were used to measure prenatal PCB exposure in 110 mother-infant pairs; PCB exposure was further broken down by congener. Median PCB 153 level was estimated to be 100 ng/g lipid (40). Children age four to six years performed tasks designed to test neuromotor and gross motor functions, including posture, reflexes, and various walking tasks. Multiple linear regression analyses examined the associations between prenatal PCB exposure and motor function. After adjusting for confounding, no association was found between prenatal PCB exposure and neurological ability or gross motor function (Data not shown).

A second study of children in the Nunavik cohort was conducted to examine the relationship between prenatal PCB exposure and visual evoked potentials (VEPs) (45). VEPs test neurological development of the visual system. Changes in amplitude and latency patterns of VEPs can indicate whether environmental exposures have damaged visual processing and may be more sensitive to subtle changes in neurodevelopment than traditional cognitive testing (45). After adjusting for confounding, increased levels of PCB congener 153 (PCB 153) were associated with increased VEP latency of response ($\beta=2.50$, $p<0.001$) and decreased VEP amplitude of response ($\beta=-3.74$, $p<0.05$).

The relationship between prenatal PCB and methyl mercury exposure with event related potentials (ERPs) were examined in the Nunavik children at 11-years of age (46). ERPs are an electrophysical assessment used to measure deficits in cognitive and attention processing (46). The oddball detection task measures how an individual reacts to randomly occurring target objects placed within a series of standard stimuli (46). The only significant association between prenatal PCB exposure and the oddball task occurred among children breastfed for less than three months. Prenatal PCB exposure to congener 153 was negatively associated with the P3b brain wave ($\beta=-0.32$, $p<0.05$). The P3b wave is associated with working memory processing and information categorization (46). The results indicate that children prenatally exposed to PCB 153 and breastfed for less than three months had poorer working memory; these results are consistent with the breastfeeding association found in the Michigan cohort (33).

Faroe Islands Cohort

Several studies of PCB exposure have also been completed in Europe. Inhabitants of the Faroe Islands have a diet high in seafood and have PCB levels higher that what is considered normal background level (47). Median PCB 153 levels in the Faroe Island

cohort are estimated to be 450 ng/g lipid (40). Between 1986 and 1987, researchers collected umbilical cord blood specimens to examine mercury and neurobehavior; when they discovered high PCB levels, additional analyses were completed to examine the effects of PCB exposure and neurodevelopment. A total of 435 children completed neuropsychological tests at age seven-years. These tests included the Neurobehavioral Evaluation System (Finger Tapping Test, Hand-Eye Coordination Test, and Continuous Performance Test) and the Wechsler Intelligence Scale for Children, among others (47). Multiple linear regression analyses were used to evaluate the association between lipid-adjusted prenatal PCB exposure (the sum of PCB congeners 118, 138, 153, 170, and 180) on these neuropsychological outcomes; an interaction between mercury exposure and PCB exposure was included in all models. After adjustment for potential confounders, no significant associations between prenatal PCB exposure and neurodevelopment at seven-years were indicated (Table 1.1).

Dutch Cohort

A Dutch cohort studied the effects of background levels of PCBs and dioxins on the development of children in the Netherlands. Participants came from two cities: Rotterdam, an industrialized region, and Groningen, a semi-urban region (48). Pregnant women were recruited between 1990 and 1992; maternal and umbilical cord plasma samples were collected. Patandin et al. examined the association between prenatal exposure to PCBs and children's cognitive abilities at 42 months in a cohort of 395 children (48). PCB congeners 118, 138, 153, and 180 were measured in umbilical cord blood; median PCB 153 levels in the Dutch cohort were estimated to be similar to those in Michigan and North Carolina (approximately 100 ng/g lipid) (40). The Kaufman

Assessment Battery for Children measured cognitive ability; this assessment includes a sequential processing scale and a simultaneous processing scale (48). Multiple linear regression analyses were used to evaluate the relationship between PCB exposure and cognitive ability. After adjustment for potential confounders, a significant negative association was found between increased prenatal PCB and overall cognitive ability (β=-4.56, SE=1.62), sequential processing ability (β=-4.16, SE=1.79), and simultaneous processing ability (β=-3.82, SE=1.60). Verbal comprehension was weakly associated with prenatal PCB exposure (β=-3.36, SE=1.91). When examined by breast feeding status, no significant PCB association was observed among infants that were solely breastfed. Conversely, researchers observed a stronger negative association between prenatal PCB exposure and cognitive ability in formula fed infants compared to the overall cohort (48) (Table 1.1). These results support those from the Michigan cohort that indicate that breastfeeding may be an effect modifier of the association between PCB exposure and cognitive development (33).

Vreugdenhil et al. completed a second study of the Dutch cohort using 418 of the original children at approximately six-and-a-half-years of age (26). Researchers used McCarthy Scales of Children's Abilities to measure cognitive development. Multiple linear regression analysis assessed the association between prenatal exposure to PCB and performance on the McCarthy Scales. After adjusting for confounders, prenatal PCB was not associated with performance on the general cognitive index (β=-0.14, SE=1.58), memory scores (β=-0.36, SE=1.02), or motor scores (β=-2.46, SE=1.45). However, significant interaction was found between PCB exposure and parental IQ and PCB exposure and the home environment; this effect was stronger when parental IQ was lower

or children lived in a poorer home environment (Table 1.1). Statistical interaction was not present between the performance of formula fed compared to breastfed children, which was observed in the earlier study (General Cognitive Index, $\beta_{PCB*formula\ fed}$=-0.29, SE=2.79; memory scores, $\beta_{PCB*formula\ fed}$ =-0.24, SE=1.81; motor scores, $\beta_{PCB*formula\ fed}$ =-2.64, SE=2.56). The authors suggest that parental and home environment may compensate for negative effects of PCB exposure. Alternatively, residual confounding may cause the appearance (or lack thereof) of effect measure modification.

German Cohort

Another European cohort was established in Düsseldorf, Germany. The original cohort consisted of 171 mother-infant pairs recruited between 1993 and 1995 from three local hospitals; PCBs measured in cord blood and maternal milk samples established prenatal PCB exposure (congeners 138, 153, and 180); PCBs measured in children's blood samples taken at 42 months estimated postnatal exposure (28). Longnecker et al. estimated that the median PCB 153 exposure for this cohort was approximately 140 ng/g lipid (Table 1.1) (40).

The authors tested infants at seven months using the Fagan Test of Infant Intelligence and the Bayley Scales of Infant Development; at 18 months and 30 months using the Bayley Scales of Infant Development; and at 42 months using the Kaufman Assessment Battery for Children. Authors also considered the HOME score and its influence on cognitive development. Multiple linear regression analyses examined the relationship between PCB exposure and development. At seven months, there were no significant associations between prenatal PCB exposure measured through cord blood and either performance on Bayley scores or the Fagan Test (Bayley MDI: β=-0.06,

SE=0.38; Bayley PDI: β=0.009, SE=0.63; Fagan: β=0.93, SE=1.13) (49). PCB measured through maternal milk yielded a significant association with the Bayley MDI scores (β=-0.69, SE=0.41), but not the Bayley PDI scores (β=-0.71, SE=0.63) or Fagan Test (β=-0.20, SE=1.19) (49).

At age 30 months, increased prenatal PCB exposure was associated with decreased performance on the Mental Development Index of the Bayley Scales (β=-4.98, one-sided p=0.035); conversely, the HOME score was positively associated the Mental and Psychomotor development indices at 18 and 30 months (18-month Bayley MDI: β=0.65, one-sided p=0.07; 18-month Bayley PDI: β=-4.78, one-sided p=0.045; 30-month Bayley MDI: β=1.77, one-sided p=0.0002; 30-month Bayley PDI=1.82, one-sided p=0.0002) (28). At 42 months, increased prenatal PCB exposure, measured through maternal milk, was negatively associated with performance on the Kaufman Assessment (β=-4.30, one-sided p=0.028). The authors also evaluated postnatal PCB exposure. Postnatal PCB exposure was measured in child serum levels at 42 months and was adjusted for prenatal PCB exposure. In this cohort, postnatal exposure was negatively associated with performance on the Kaufman Assessment (t=-2.64, one-sided p=0.025); this finding differs from results of other cohort studies that have found no relationship between postnatal PCB exposure and subsequent cognitive development.

Researchers examined children from the German cohort again at six years of age using the Kaufman Assessment (50). This allowed for comparison of results at two ages using the same testing battery. While a significant negative association was found between prenatal PCB and performance on the Kaufman Assessment at 42 months, after adjustment for confounders, no significant association was found between prenatal PCB

exposure and performance at six years (prenatal exposure through maternal milk: β=-1.50, one-sided p=0.23; postnatal exposure through child serum at 42 months: β=-1.18, one-sided p=0.18) (50). The estimated association decreased by approximately 65% when evaluated at six years age. HOME score was more strongly associated with performance at six years than it was at 42 months (β=1.26, one-sided p=0.0001) (50). Lack of negative associations could indicate that effects of prenatal PCB exposure may not be permanent with adequate home environment or other external influences. Alternatively, insufficient control of confounding, or even chance, could explain lack of significant results. Additionally, by 72 months, attrition reduced the cohort to 70 observations. This attrition could have reduced the power of the analysis or the magnitude of the observed effect.

Summary

In summary, the evidence of how perinatal PCB exposure might affect cognitive development is mixed. The historical *Yu-cheng* and *Yusho* cohorts are unique in that mothers were exposed to heat-degraded PCBs, meaning that they were simultaneously exposed to other potential teratogens (51). Moreover, no sound exposure assessment is available to estimate accurately the true levels of prenatal PCB exposure at that time (51). Despite these limitations, evidence from the *Yusho* and *Yu-cheng* incidents suggest that high levels of PCB exposure, or that heat degraded PCBs, can have severe cognitive and physical effects on prenatally exposed children. The Michigan cohort found negative associations with infant cognitive development at seven months using the Fagan test (19); cognitive deficits persisted at four and eleven years of age (32, 35). Furthermore, the effects of prenatal PCB exposure appeared to be modified by breastfeeding (33). In

contrast, the North Carolina cohort did not observe detrimental effects of prenatal PCB on cognitive development exposure persisting into early childhood (38).

More recent studies, which have better exposure assessment techniques, also reveal conflicting evidence. Results from a Dutch cohort found negative associations between prenatal PCB exposure and performance on the Kaufman Battery of cognitive tests at 42 months, with the effects potentially persisting until six-and-a-half-years (26, 48). The German cohort suggests that prenatal PCB exposure may be negatively associated with both Mental and Psychomotor scores on the Bayley Scales at 30 months, as well as the Kaufman Battery at 42 months (28); these associations, however, all but disappear at six years of age (50). The Oswego cohort, which had lower levels of PCB exposure than the Dutch, German, Michigan, and North Carolina cohorts, reported negative associations between PCB exposure and performance on the McCarthy Scales of Infant Ability at 38 but not 54 months (42). The PCB association, however, persisted until approximately nine years of age, when a negative linear association was found between PCB exposure and nine-year-old IQ (43).

Differences in results may be partially explained by the different cognitive assessments used in the various cohorts. Additionally, exposure routes of PCB varied. Some cohorts (Michigan, Oswego, Faroe Islands, and Nunavik) were primarily exposed through a seafood diet, while others (North Carolina, Dutch, German) were primarily exposed through background levels of PCB contamination in the environment. Exposure assessment also differed according to study. Differences in controlling for confounding and statistical analysis are potential reasons for differences in results. Additionally, PCB associations may be affected by differences in assessment methods and cultural variations

between the study populations. The body of evidence suggests, however, that some negative cognitive effects due to increased prenatal, and perhaps postnatal, PCB exposure are to be expected. Whether these effects will persist into childhood, or even adulthood, remains to be better understood.

Present Study: Slovakia cohort

While most western countries halted the production of PCBs in the mid- to late 1970s, PCBs were produced in Czechoslovakia between 1959 and 1984. The Chemko chemical plant, located in the Michalovce district of Eastern Slovakia produced more than 20,000 tons of PCBs in that time (52). Waste from the plant was discarded into the nearby Laborec River, resulting in widespread contamination of the air, water, soil, and wildlife (52, 53). In 1994, Kocan et al. examined PCB levels in the blood and adipose tissue of residents from several areas of Slovakia, as well as other industrialized countries (54). Residents of the Michalovce districts had levels of PCB in their adipose tissue that were approximately three times higher than residents in other areas of Slovakia. Michalovce residents had PCB levels several times higher than residents of other industrialized countries, including Japan, the United Kingdom, Poland, and the Netherlands.

In 2001, PCB levels in the Michalovce district were compared to levels in the Stopkov/Svidnik district, which is approximately 60 km away from Michalovce (53). Kocan et al. found that air samples, soil samples, water sediment samples, water surface samples, and game-animal samples from the Michalovce district consistently had higher levels of PCB than comparable samples from the Stropkov/Svidnik district (53).

A recent cross-sectional study examined levels of PCBs and their metabolites in individuals from the Michalovce and Svidnik districts. Hovander et al. estimated that median PCB 153 concentration among males and females in Michalovce was 570 ng/g lipid compared to 240 ng/g lipid in Svidnik (52). Exposure in the Michalovce population appears to be significantly higher than PCB exposure in other populations.

Between 2002 and 2004, pregnant women over age 18 in the districts of Michalovce and Svidnik were prospectively recruited to participate in a birth cohort study to examine the effects of PCB exposure on development (55). Mothers were screened as they entered the hospital for delivery; as each district had only one hospital, this method likely captured most deliveries during the study period. Mothers were excluded if they were less than 18 years of age, had lived in their respective districts for less than 5 years, had more than four previous births, had a difficult illness during pregnancy, or a complication during pregnancy. Additionally, children were excluded if they were born with serious birth defects (56). Using maternal serum, researchers estimated median PCB 153 exposure among Michalovce women to be 160 ng/g lipid and among Svidnik women to be 100 ng/g lipid (57). Studies already conducted in the population indicate that PCB exposure is associated with potential adverse health effects.

Park et al. evaluated the association between prenatal exposure to PCBs and neurodevelopment among 760 16-month-old infants in Michalovce and Svidnik (14). At 16 months, the quality of the home environment and children's neurodevelopment was assessed. The authors evaluated neurodevelopment using the Mental and Psychomotor Developmental indices of the Bayley Scales of Infant Development. Congener-specific PCB effects and exposure were evaluated by chemical structure as well as estrogenic

activity (14). Dioxin-like PCBs included PCB congeners 118 and 156; non-dioxin-like PCBs included PCB congeners 138, 153, 170, and 180; anti-estrogenic PCB included PCB congeners 138, 156, 170, and 180. Each of these subsets of congeners could potentially impact neurodevelopment through different biological mechanisms. No significant association was found between performance on the Mental or Psychomotor indices with non-dioxin-like or anti-estrogenic PCBs (Table 1.1). The effect estimates were small in magnitude, indicating small changes in Bayley scores for one-unit increases in PCB congeners. The authors observed a negative association between increased levels of dioxin-like PCB congeners and scores on the Mental and Psychomotor indices (β=-1.60, SE=0.45; β=-1.26, SE=0.53, respectively). These estimates for dioxin-like congeners were two- to four-times those for the major and anti-estrogenic congeners. This relationship held for PCB congeners estimated from maternal and cord serum (14).

It is unknown whether the association between prenatal PCB exposure and cognitive development will persist past 16-months. Evidence from other cohort studies suggests that effects of prenatal PCB exposure may continue into early childhood, but this evidence is unconvincing. In order evaluate the potential association between perinatal exposure to PCBs and cognitive development, the Child Behavior Checklist and several subtests of the Wechsler Preschool and Primary Scale of Intelligence were administered at age 45 months to children from the Michalovce population.

Prenatal PCB exposure could have lasting effects into childhood and even adulthood. Postnatal PCB exposure may impact brain development after birth. Currently, it is unknown whether individuals exposed to high levels of perinatal PCBs experience effects of exposure into childhood. Examining behavioral and intelligence outcomes at 45

months will give an indication of whether previously reported deficits in neurodevelopment of infants continue into early childhood.

The current study differs from previous studies in several ways. Exposure assessment ensured that precise measures of prenatal PCB levels were obtained for each child in the population. Multiple PCB congeners were measured and recorded to allow for an accurate estimate of prenatal exposure. Breast milk and fish consumption were not used as proxies for prenatal exposure. The population being studied was drawn from the members of the original cohort with the highest potential PCB exposure. Cognitive development was assessed in two ways. The CBCL assessment was used to identify children with abnormal reported behavioral problems, while the Wechsler subtests assessed cognitive and reasoning abilities. Examining different outcomes will indicate whether PCB exposure is associated with behavioral deficits, cognitive deficits, or both.

A thorough understanding of how PCB exposure affects development into childhood and adulthood is crucial to discerning how and why populations with high exposure to PCB may differ in their cognitive and social potential. Most individuals currently have had some perinatal exposure to PCBs; current body burdens are transferred transplacentally during pregnancy and after birth through breastfeeding. The neurodevelopmental deficiencies found in children perinatally exposed to PCBs could be predictive of later problems in childhood and adulthood; the long latency of PCBs in human populations suggests that these effects could persist for years to come. Studying cognitive development at 45-months provides an important step in determining how PCB exposure continues to affect childhood development and, potentially, what steps might be taken to alleviate this public health concern.

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Table 1.1: Previous Literature of perinatal PCB exposure and cognitive development.

Study Population	First Author	Year	Age	Breastfed	N	Exposure Measure	PCB Categories	Median PCB-153 (ng/g Lipid)[1]	Outcome	Result	
										<3 ng/ML	≥3 ng/ML
									Birthweight (kg)	3.6±0.54	3.4±0.54
						Prenatal PCB			Head circumference (cm)	35.3±1.2	34.6±1.2
						Fish consumption			Gestational age (wk)	41.0±3.0	39.8±3.1
						Umbilical cord serum	<3 ng/ML		Neuromuscular maturity	20.0±2.4	19.0±2.4
Michigan	Fein, G.	1984	Newborn		242	Maternal serum	≥3 ng/ML	120	Physical maturity	17.0±2.1	16.9±2.2
						Prenatal PCB					
						Fish consumption				Fish consumption	β=-0.23, p<0.05
						Umbilical cord serum				Cord serum	β=-0.39, p<0.01
	Jacobson, S.	1985	7 months		123	Breast milk (postnatal)	Continuous	120	Fagan's test of visual recognition memory	Breast milk	F(3,80)=1.18
	Jacobson, J.	1990	4 years		236	Umbilical cord serum	Continuous	120	McCarthy Scales of Children's Abilities		
						(Prenatal)			Verbal	β=-0.18	p=0.05
									Perceptual Performance	β=-0.03	p=0.73
									Quantitative	β=-0.17	p=0.07
									Memory	β=-0.22	p=0.02
									Motor	β=0.03	p=0.74
									General Cognitive Index	β=0.08	p=0.22
									Verbal Memory	β=-0.22	p=0.02
									Numerical Memory	β=-0.24	p=0.01
						Time breastfed*Breast milk					
						(postnatal)	Continuous		Quantitative	β=-0.20	p=0.07
									Memory	β=-0.27	p=0.01
									Verbal Memory	β=-0.22	p=0.05
									Numerical Memory	β=-0.21	p=0.05
						Child serum (postnatal)	Continuous		No significant association (data not shown)		
	Jacobson, J.	2002	4 Years	Overall	181	Umbilical cord serum	Continuous	120	McCarthy Scales of Children's Abilities		
						(Prenatal)			Verbal	β=-0.12	p<0.05
									Perceptual Performance	β=-0.01	Not significant
									Quantitative	β=-0.20	p<0.01
									Memory	β=-0.18	p<0.05
									Motor	β=0.07	Not significant
									General Cognitive Index	β=-0.13	p<0.05

Table 1.1, Continued

Study Population	First Author	Year	Age	Breastfed	N	Exposure Measure	PCB Categories	Median PCB-153 (ng/g Lipid)[1]	Outcome		Result
		2002	4 Years	< 6 weeks	56	Umbilical cord serum (Prenatal)	Continuous	120	McCarthy Scales of Children's Abilities		
									Verbal	β=-0.45	p<0.01
									Perceptual Performance	β=0.04	Not significant
									Quantitative	β=-0.36	p<0.05
									Memory	β=-0.45	p<0.01
									Motor	β=0.14	Not significant
									General Cognitive Index	β=-0.29	p<0.05
				≥ 6 weeks	122						
									Verbal	β=0.09	Not significant
									Perceptual Performance	β=0.06	Not significant
									Quantitative	β=0.03	Not significant
									Memory	β=0.03	Not significant
									Motor	β=0.10	Not significant
									General Cognitive Index	β=0.09	Not significant
		2002	11 Years	Overall	178	Umbilical cord serum (Prenatal)	Continuous	120	Wechsler IQ scale		
									Full-scale IQ	β=-0.17	p<0.05
									Verbal comprehension	β=-0.16	p<0.05
									Freedom from distractibility	β=-0.17	p<0.05
									Woodcock Reading Mastery		
									Word comprehension	β=-0.17	Not significant
									Wide Range Achievement		
									Arithmetic	β=-0.04	Not significant
		2002	11 Years	< 6 weeks	56			120	Wechsler IQ scale		
									Full-scale IQ	β=-0.32	p<0.01
									Verbal comprehension	β=-0.38	p<0.001
									Freedom from distractibility	β=-0.36	p<0.05
									Woodcock Reading Mastery		
									Word comprehension	β=-0.36	p<0.01
									Wide Range Achievement		
									Arithmetic	β=-0.25	p<0.05

Table 1.1, Continued

Study Population	First Author	Year	Age	Breastfed	N	Exposure Measure	PCB Categories	Median PCB-153 (ng/g Lipid)[1]	Outcome	Result	
				≥ 6 weeks	122			120	Full-scale IQ	β=-0.06	Not significant
									Verbal comprehension	β=-0.00	Not significant
									Freedom from distractibility	β=-0.08	Not significant
									Woodcock Reading Mastery		
									Word comprehension	β=-0.02	Not significant
									Wide Range Achievement		
									Arithmetic	β=0.08	Not significant
North Carolina	Gladen	1988	6 Months		706	Breastmilk (prenatal)	Continuous	100	Bayley MDI	β=0.12	p=0.78, SE=0.44
									Bayley PDI	β=-0.96	p=0.04, SE=0.46
						Time breastfed*Breastmilk (postnatal)	Continuous	100	Bayley MDI	β=-0.18	p=0.36, SE=0.20
									Bayley PDI	β=-0.27	p=0.17, SE=0.20
			12 Months		706	Breastmilk (prenatal)	Continuous	120	Bayley MDI	β=-0.54	p=0.32, SE=0.54
									Bayley PDI	β=-1.34	p=0.03, SE=0.61
						Time breastfed*Breastmilk (postnatal)	Continuous	120	Bayley MDI	β=-0.06	p=0.70, SE=0.16
									Bayley PDI	β=-0.27	p=0.13, SE=0.18
Oswego	Darvill	2000	6 Months		230	Umbilical cord serum (prenatal)	Not dectable >0.18 ng/g >0.52 ng/g >1.10 ng/g	40	Fagan test of infant development Linear trend analysis	F(2, 214)=4.87	p=0.014
						Breastmilk (postnatal)	Continuous	40	Fagan test of infant development	β=0.065	p=0.707
			12 Months		219	Umbilical cord serum (prenatal)	Not dectable >0.18 ng/g >0.52 ng/g >1.10 ng/g	40	Fagan test of infant development Linear trend analysis	F(2, 207)=2.04	p=0.75
						Breastmilk (postnatal)	Continuous	40	Fagan test of infant development	β=-0.075	p=0.304

Table 1.1, Continued

Study Population	First Author	Year	Age	Breastfed	N	Exposure Measure	PCB Categories	Median PCB-153 (ng/g Lipid)[1]	Outcome		Result
Faroe Islands	Grandjean	2001	7 Years		435	Umbilical cord serum (prenatal)	Continuous	400	Finger tapping (preferred hand)	β=-0.76	p=0.30
									Hand-eye coordination (errors)	β=-0.036	p=0.26
									NES2 Continuous Performance Test		
									Missed responses	β=0.03	p=0.74
									Reaction time	β=7.2	p=0.41
									Wechsler Intelligence Scale for Children		
									Digit spans	β=0.07	p=0.68
									Similarities	β=0.62	p=0.18
									Block designs	β=0.09	p=0.52
									Bender Visual Motor Gestalt Test		
									Errors on copying	β=0.25	p=0.71
									Reproduction	β=0.05	p=0.79
									Boston naming test		
									No cues	β=-0.50	p=0.41
									With cues	β=-0.74	p=0.22
									California Verbal Learning Test (Children)		
									Learning	β=1.38	p=0.18
									Short-term reproduction	β=0.22	p=0.50
									Long-term reproduction	β=-0.13	p=0.73
									Recognition	β=-0.07	p=0.77
Dutch	Patandin	1999	42 Months	Breastfed	195	Maternal Plasma	Continuous	100	Overall Cognitive Scale	β=-2.20	SE=2.14
					195		(lipid adjusted)		Sequential Processing Scale	β=-1.49	SE=2.46
					198				Simultaneous Processing Ability	β=-2.45	SE=2.18
					100				Verbal Comprehension Scale	β=-0.20	SE=2.74
				Formula fed	178	Maternal Plasma	Continuous	100	Overall Cognitive Scale	β=-8.69	SE=2.49
					178				Sequential Processing Scale	β=-8.34	SE=2.68
					186				Simultaneous Processing Ability	β=-6.54	SE=2.39
					90				Verbal Comprehension Scale	β=-6.13	SE=2.79

Table 1.1, Continued

Study Population	First Author	Year	Age	Breastfed	N	Exposure Measure	PCB Categories	Median PCB-153 (ng/g Lipid)[1]	Outcome	Result	
	Vreugdenhil	2002	6.5 Years		418	Maternal Plasma	Continuous	100	General Cognitive Index	β=-26.06	SE=10.37
									Interaction term: PCB*Parental IQ	β=0.22	SE=0.09
									General Cognitive Index	β=-26.92	SE=22.79
									Interaction term: PCB*HOME score	β=0.60	SE=0.48
									Memory	β=-16.49	SE=6.72
									Interaction term: PCB*Parental IQ	β=0.13	SE=0.06
									Memory	β=-12.99	SE=14.75
									Interaction term: PCB*HOME score	β=0.26	SE=0.31
									Motor	β=-21.24	SE=9.58
									Interaction term: PCB*Parental IQ	β=0.16	SE=0.08
									Motor	β=-46.65	SE=20.92
									Interaction term: PCB*HOME score	β=0.92	SE=0.44
German	Walkowiak	2001	7 Months		110	Breast Milk (prenatal)	Continuous	140	Bayley MDI	β=-3.61	p=0.10
									Bayley PDI	β=03.13	p=0.12
			18 Months		112				Bayley MDI	β=-4.11	p=0.06
									Bayley PDI	β=-4.78	p=0.045
			42 Months		87	Breast Milk (prenatal)			Kaufman Achievement Battery	β=-4.30	p=0.0006
						4 -Month child's serum (postnatal)			Kaufman Achievement Battery	t=2.01	p=0.025
Slovak	Park	2010	16-months		760	Maternal Serum	Continuous, Major PCBs	170	Bayley MDI	β=-0.39	SE=0.62
									Bayley PDI	β=-0.59	SE=0.73
							Continuous, Dioxin-like PCBs		Bayley MDI	β=-1.60	SE=0.45
									Bayley PDI	β=-1.26	SE=0.53
							Continuous, Anti-estrogenic PCBs		Bayley MDI	β=-0.47	SE=0.61
									Bayley PDI	β=-0.58	SE=0.72

[1] Estimated by Longnecker er al., 2003; Slovak PCB 153 levels estimated from current study

CHAPTER 2: A COHORT STUDY OF PERINATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS AND COGNITIVE DEVELOPMENT AT 45 MONTHS

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ABSTRACT: The aim of this study was to determine if perinatal exposure to PCBs negatively affects behavior and intelligence in children at age 45 months. We sampled 438 mother-child pairs from a birth cohort conducted in a highly exposed district in the Slovak Republic. Prenatal exposure was estimated as maternal PCB 153, the most abundant congener, and PCB 118, the most abundant mono-ortho dioxin-like congener. Children's body burdens of PCB 153 and 118 were measured at 16 and 45 months. At 45 months, mothers completed the Child Behavior Checklist ages 1 ½-5 (CBCL) and children completed five subtests of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III). Negative binomial and multiple linear regression analyses measured the associations between PCB exposure and CBCL syndrome score or WPPSI-III subtest score, respectively. After adjusting for potential confounders, increased CBCL anxious/depressed syndrome scores were observed among children more highly exposed to 16-month PCB 153 and 118 (exponentiated beta coefficients representing a change in score for a 1-unit increase in PCB; PCB 153: 1.04, 95% confidence interval (CI): 1.0, 1.1; PCB 118: 1.4, 95% CI: 1.1, 1.9). Other associations between 16- or 45-month PCB exposure and cognitive outcomes were null. Maternal exposure to PCB 153 and 118 was not associated with any of the 14 outcomes. Perinatal exposure in a highly exposed population did not substantially affect cognitive development at 45-months; it is possible that anxious and depressed behaviors may be more affected by PCB exposure than intelligence or other behaviors.

Introduction

Polychlorinated biphenyls (PCBs) are an environmentally persistent class of manmade chemicals widely produced from their introduction in 1929 until the late 1970s (1). PCBs were used in various industrial and consumer products, including heat transfer agents, electric capacitors, plasticizers, lubricants, flame retardants, and carbonless paper (2, 3). Although the United States banned their production in the late 1970s (3), their lipophilic nature causes PCBs to bioaccumulate up the food chain (2-4). As a result, most individuals have detectable levels of PCBs in their bodies.

Generally, human exposure to PCBs occurs through consumption of contaminated food or through environmental exposure due to improper disposal of PCB waste (2-4). Additional exposure routes occur prenatally or immediately after birth, as PCBs are able to cross the placental barrier and are excreted through breast milk. Perinatal exposure to PCBs is worrisome due to potential adverse health effects.

Numerous studies have shown that PCBs have toxic effects in the reproductive system, the endocrine system, and the immune system (5-8). Moreover, prenatal exposure to PCBs may have detrimental effects on the developing brain, and several epidemiologic studies have indicated that increased prenatal PCB exposure is associated with decreased cognitive ability (9-17). Cognitive development is a complicated process and is affected by numerous pre- and postnatal exposures.

Studies examining the effects of prenatal PCBs on cognitive development into childhood have inconsistent results. Results from a cohort study of Michigan infants transplacentally exposed to PCBs suggest a negative association exists between prenatal PCB exposure and cognitive development at age seven months using the Fagan test of

infant development (9); cognitive deficits persisted at ages four and eleven years (10, 18). In contrast, using the McCarthy Scales of Children's abilities, results from a cohort study of North Carolina infants also transplacentally exposed suggest that detrimental effects of prenatal PCBs do not persist into early childhood (19). More recent studies also reveal conflicting results. In a cohort of Dutch children, performance on the Kaufman Battery of cognitive tests at 42-months was negatively associated with increased prenatal PCB exposure, with the association potentially persisting until six-and-a-half years (16, 20). Results from a cohort study of German children suggest that prenatal PCB exposure may be negatively associated with both Mental and Psychomotor Indices on the Bayley Scales of Infant Development (BSID) at 30 months, as well as the Kaufman Battery at 42 months (21); however, these effects all but disappear at six years of age (22).

Evidence of adverse effects of postnatal PCB exposure is limited. While children from the Michigan cohort with higher postnatal exposure through breast milk performed more poorly on the McCarthy Scales of Children's Abilities at four years compared to those with lower postnatal exposure, the same association was not observed in the same cohort using children's total body burden at four years (10). No association was observed between postnatal PCB exposure through breast milk and performance on either the Mental or Psychomotor BSID at six or twelve months in children from the North Carolina cohort (11). Similarly, postnatal exposure in children in a cohort from Oswego, New York was not associated at six or twelve months with performance on the Fagan Test of Infant Development (12). Postnatal exposure measured through breast milk exposure and total body burden in children from the German cohort, however, was negatively associated with performance on the Kaufman Battery (21).

In Slovakia, PCBs were produced by the Chemko plant in the eastern district of Michalovce until 1984 (23). Disposal of waste into the nearby Laborec River resulted in widespread environmental contamination and numerous potential exposure pathways (23, 24). A study of 16-month-old infants in Michalovce and the nearby Svidnik, an area with lower PCB exposure, observed negative associations between increased prenatal PCB levels, specifically with the combined exposure of dioxin-like mono-ortho congeners 118 and 156, and performance on the BSID (25). The children were evaluated again at 45 months using the Child Behavior Checklist for ages 1 ½-5 years (CBCL) and a subset of tests from the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III). The present study investigated the associations between behavioral problems and intelligence deficiencies and prenatal PCB exposures, as measured in maternal serum, and postnatal PCB exposure, as measured in child serum at 16 and 45 months.

Methods

Study Population

Between 2002 and 2004, 1,134 mother-infant pairs were prospectively recruited from two districts in Slovakia: 811 from Michalovce, an area highly contaminated by PCBs, and 323 from Svidnik, an area less highly contaminated and located approximately 70 km northwest of Michalovce (25). Mothers were screened as they entered the hospital for delivery; as each district had only one hospital, this method likely captured most deliveries during the study period. Mothers were excluded if they were less than 18 years of age, had lived in their respective districts for less than 5 years, had more than four previous births, had a major illness, such as cancer, during pregnancy, or had a

complication during pregnancy. Children were also excluded if they were born with serious birth defects (26).

When the child was approximately 45 months of age, mothers from the Michalovce district were contacted to schedule a follow-up visit. At 45 months, 438 mother-child pairs participated in the study (54% of the original Michalovce sample). The follow-up visit was conducted at the local hospital pediatric department. At the follow-up, mothers completed the CBCL (27). An experienced psychologist administered five subtests of the WPPSI-III to the participating children (28). Both the CBCL and WPPSI-III subtests were translated into Slovak from English by a specialist-translator; unique psychological expressions were discussed with the lead researcher in Michalovce. The same psychologist conducted all tests.

This study was approved by the Institutional Review Board at the University of California, Davis and the Slovak Medical University. Detailed descriptions of population selection, consent protocols, and data and specimen collection are described in previous papers (7, 25, 26, 29, 30).

PCB/Lipid Measurement

After consent was provided, two 9-mL vacutainer tubes were used to collect a maternal blood specimen at delivery. Because PCBs are able to cross the placenta and are resistant to excretion from the blood, maternal PCB levels at birth represent the prenatal exposure of the child (25, 26, 29). At 16 and 45 months, 9 mL of blood were collected from children to estimate their body burdens. Samples were transported to the Slovak Medical University in Bratislava and were analyzed at the Department of Toxic Organic Pollutants.

A total of 15 PCB congeners were measured in maternal and child serum: International Union of Pure and Applied Chemistry (IUPAC) numbers 28, 52, 101, 105, 114, 118, 123^{+149}, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180, and 189. Congeners with samples below the limit of detection (LOD) greater than 20% of the time were excluded from the analysis (7, 25). The LOD for each congener at each time point was determined using the mean of background noise plus three standard deviations from five blank reagent samples. Congeners with concentrations below the LOD were set equal to the LOD divided by square root of two (31). Total maternal PCBs were calculated as the sum of six congeners with at least 80% of the samples above the LOD: IUPAC numbers 118, 138^{+163}, 153, 156^{+171}, 170, and 180. Total child PCBs were not calculated, because a one unit change in total PCBs would differ in maternal serum, 16-month serum, and 45-month serum due to of differences in the LODs. PCB congener 153, the most abundant congener, was used as a proxy for total PCBs. Additionally, PCB 118, the most abundant dioxin-like mono-ortho congener, was examined. Total maternal serum lipids, and total children's 16- and 45-month serum lipids were measured and were calculated using the enzymatic summation formula of Akins and Takayama (7, 25, 32, 33). Laboratory methods are described in detail elsewhere (7, 25, 26, 30). Prenatal PCB exposure was estimated based on maternal PCBs 153 and 118 at time of birth. Postnatal PCB exposure was estimated based on 16 and 45 month PCB congener 153; dioxin-like postnatal exposure was estimated based on 16 and 45 month PCB congener 118.

Cognitive Assessment

The CBCL assessed behavior problems at 45 months. The CBCL is completed by a parent and is comprised of 100 items, in which the parent is asked to consider his or her child's behavior in the last two months. For each item, the parent chooses zero if the

behavior is not true, one if the behavior is somewhat or sometimes true, or two if the behavior is very true or often true (27, 34). Syndrome scores are computed by summing select subsets of scores from the 100 items, providing a raw score for each syndrome. In total, seven syndrome scores are calculated: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behavior. Higher scores indicate increased behavioral problems. Additionally, externalizing behavior and internalizing behavior scores are obtained by summing the attention problems and aggressive behavior syndrome scores, and the emotionally reactive, anxious/depressed, somatic complaints, and withdrawn syndrome scores, respectively (34, 35).

The WPPSI-III is made up of several subtests designed for children aged two years, six months to seven years, three months (28). Five subtests were administered: information, which asks children to provides answers to questions in the form of pictures or verbal responses; receptive vocabulary, which asks children to select the correct picture out of a group of four pictures; block design, which asks children to recreate a shape using colored blocks; picture naming, which asks children to name pictures from a book; and object assembly, which asks children to put together a puzzle. These subtests were selected in order to avoid issues with translation.

Covariate Assessment

Maternal information was collected through a questionnaire completed shortly after birth. Mothers provided information regarding smoking habits, socioeconomic status, years of education completed, illness during pregnancy, past pregnancies, and other lifestyle factors (7). While in the hospital, mothers completed the Raven's Progressive Matrices, a non-verbal IQ test (36). When the child was 16 months old, a

follow-up visit was scheduled. The Home Observation for Measurement of the Environment (HOME) was completed at this visit, as well as the Mental and Psychomotor Indices of the BSID. The HOME assessment is designed to evaluate the quantity and quality of stimulation provided to a child in his or her home environment (37).

Data Analysis

In order to account for the variation present in the population, and because the CBCL is not standardized to the Slovak population, raw CBCL scores were used (34). Raw WPPSI-III scores were used because a standardized score could not be obtained using the five subtests completed. The crude association between pre- and postnatal PCBs and each of the seven syndrome CBCL scores, the externalizing and internalizing CBCL scores, and the five WPPSI-III subtests were calculated. Potential confounders were initially identified using directed acyclic graphs (DAGs) and literature review. The following covariates were considered as potential confounders: child's sex, maternal age, ethnicity (Romani vs. Slovak/other Eastern European), education, maternal smoking during pregnancy, maternal alcohol consumption during pregnancy, birth weight, duration of breastfeeding (less than six months vs. more than six months), maternal Raven score, HOME score, and parity. Potential confounders associated with WPPSI-III or CBCL scores in the DAGs and in bivariate analyses ($p<0.2$) were considered in model selection. The final models for each cognitive outcome included all covariates whose removal changed the effect estimates for PCB exposure by greater than 10% when compared to a model adjusted for all potential confounders. In addition, maternal Raven

score and HOME score were kept in all models based on DAG analysis and literature review. Prenatal and postnatal PCB exposure was evaluated in separate models.

The CBCL scores were highly skewed right, with large clusters of children having scores of zero for each syndrome (range from 9.1% to 30.4% zeros). Data transformation did not yield normal conditional distributions. Therefore, negative binomial regression models were fit to analyze the association between PCBs and CBCL syndrome scores (38, 39). We theorized that the causal relationships between each externalizing behavior syndrome and PCB exposure would be similar, as would the causal relationships between each internalizing behavior syndrome and PCB exposure. Multiple linear regression models were fit to analyze the association between PCBs and WPPSI-III scores. We theorized that the causal relationships between each WPPSI-III subtest score and PCB exposure would be similar. For those sets of CBCL syndrome scores and WPPSI-III subtests theorized to have similar causal relationships, the same covariates were included in the regression models. All analyses were conducted in the SAS statistical package (Version 9.2, SAS Institute, Cary, NC).

Results

Table 2.1 presents the median and interquartile range (IQR) of population characteristics for the 438 mother-child pairs participating at 45 months by maternal PCB 153, 16-month child PCB 153, and 45-month child PCB 153. Overall, maternal and child characteristics were similar. Mothers older than 30 years were more likely to have higher levels of PCB 153 (compared to mothers less than 20 years, $p < 0.0001$) and children of mothers over 30 years were also more likely to have higher levels of PCB 153 (16-months, $p=0.01$; 45-months, $p=0.016$). Mothers of Slovak or other Eastern European

descent had higher levels of PCB 153 compared to mothers of Romani descent. While there was no difference in maternal PCB 153 among mothers who breastfed for more than six months compared to mothers who breastfed for less than six months, children of mothers who breastfed for more than six months had substantially higher levels of PCB 153 at 16 and 45 months ($p < 0.0001$).

Table 2.2 presents descriptive statistics for the 438 mother-child pairs participating at 45 months by externalizing CBCL scores, internalizing CBCL scores, and block design WPPSI-III scores. The CBCL scores were not meaningfully different by maternal age, but the children of mothers over 30 had substantially higher block design scores compared to children of mothers less than 20 ($p < 0.0001$). Children of mothers of Slovak or other Eastern European descent had much higher CBCL scores compared to children of Romani descent ($p < 0.0001$). Mothers who did not smoke during pregnancy reported increased externalizing and internalizing behavioral problems in their children compared to mothers who smoked during pregnancy ($p=0.0209$ and $p=0.0176$, respectively). Children of mothers who did not smoke performed better on the block design WPPSI-III subtest than did children of mothers who smoked ($p < 0.0001$). Increased birth weight was associated with increased externalizing CBCL scores and better performance on the block design WPPSI-III subtest ($p=0.0298$ and $p=0.0001$, respectively). Breastfeeding for at least six months was moderately associated with lower internalizing CBCL scores ($p=0.0234$). Increased parity was associated with decreased externalizing and internalizing CBCL scores ($p < 0.0001$) and decreased block design WPPSI-III scores ($p=0.0058$). Increased maternal Raven score and HOME score were associated with increased externalizing and internalizing CBCL scores (Raven score: $p <$

0.0001; HOME score: $p < 0.0001$ and $p=0.0021$, respectively), as well as increased block design WPPSI-III scores ($p < 0.0001$).

Distributions of total maternal PCBs, maternal, 16-month, and 45-month PCB 153, and maternal, 16-month, and 45-month PCB 118 are presented in Table 2.3. Total maternal PCBs were highly correlated with maternal PCB 153 ($r=0.99$). Maternal PCB 153 was moderately correlated with 16-month PCB 153 ($r=0.58$) and 45-month PCB 153 ($r=0.53$). The 16-month PCB 153 levels were highly correlated with 45-month PCB 153 levels ($r=0.84$).

Table 2.4 presents the adjusted negative binomial coefficients of regression for the associations between PCB 153 and 118 and CBCL syndrome scores. Negative binomial regression is performed on the natural-log-scale and coefficients should be exponentiated for interpretation. For example, when 16-month PCB 118 increases by one ng/ml, scores on the attention problems syndrome scale increase by approximately 14%. Because congener 118 is present in low concentrations, one standard deviation of 16-month PCB 118 (0.28 ng/ml) is less than a one-unit change and is associated with only a 3.8% increase in score on the attention problems syndrome scale. Sixteen-month congener levels of PCB 153 and 118 were most strongly associated with increased scores on the anxious/depressed syndrome scale (a one ng/ml increase results in a 4% and 42% increase in score, respectively; $p=0.0412$ and $p=0.0142$, respectively; Table 2.4). While the direction and magnitude of the association with anxious/depressed scores was similar for the 45-month congeners, the association did not remain statistically significant. Generally, 16- and 45-month exposures to PCB did not appear to be meaningfully associated with CBCL performance; the direction of the association, however, was

generally consistent from 16 to 45 months. Additionally, exposure to PCB 118 had measures of association that were greater in magnitude than those found with exposure to PCB 153. Exposure to maternal PCB 153 or 118 did not have consistent or meaningful associations with CBCL performance.

Table 2.5 presents the estimated adjusted coefficients of regression for the association between PCB 153 and performance on the WPPSI-III subtests and PCB 118 and performance on the WPPSI-III subtests. The interpretation of each of these coefficients is that, for example, when 16-month PCB 153 concentrations increase by one ng/ml, scores on the block design subtests are reduced by approximately 0.10 points, while scores on the information processing subtests increase by approximately 0.07 points. Likewise, a one standard deviation increase in 16-month PCB 153 (2.23 ng/ml) is associated with a 0.22 point decrease in block design scores and a 0.15 point increase in information processing scores. Overall, we did not observe a meaningful association between maternal, 16-month, and 45-month exposure to PCB 153 and performance on the WPPSI-III subtests. The picture naming subtests had the strongest associations with 16-month and 45-month exposure to PCB 153, but the results are not significant at α=0.05, and the confidence intervals are imprecise. Similarly, there does not appear to be a meaningful association between maternal, 16-month, and 45-month exposure to PCB 118 and performance on the WPPSI-III subtests. In general, estimated coefficients for PCB 118 are larger in magnitude than those of PCB 153, but the estimates remain small and imprecise, and the direction of association is not consistent.

Discussion

The present study examined whether higher levels of prenatal exposure and early childhood exposure to PCB were associated with poor performance on the WPPSI-III and CBCL. We did not observe evidence of a meaningful association between prenatal PCB 153 and 118 exposure and children's behavior or intelligence measures at 45 months of age. Null findings were present across different behavior syndromes and intelligence tests. Additionally, there was no specific direction of association and no patterns emerged with regard to specific CBCL syndrome scores or WPPSI-III subtests. We observed null results between all WPPSI-III subtests and 16-month or 45-month PCB 153 and 118 exposures. The majority of the CBCL syndromes scores were not associated with 16- or 45-month exposure to PCB 153 or 118; however, increased syndrome scores on the anxious/depressed scale were associated with 16-month exposure to PCB 153 and 118.

Previous epidemiologic studies suggest that prenatal exposure to PCBs negatively affects cognitive development into early childhood. In contrast to our results, a Dutch cohort study reported that prenatal exposure to PCBs was associated with substantially lower scores on cognitive, sequential, and simultaneous processing scales of the Kaufman Battery in children assessed at 42 months (16). The same children were studied at six-and-a-half years using the McCarthy Scales of Children's abilities (20). Again, children highly exposed prenatally performed poorly on the McCarthy scales, although superior home environments and parental intelligence modified this effect. Likewise, among children from a German cohort, prenatal PCB exposure was associated with reduced performance on the Psychomotor and Mental scales of the BSID at 18 and 30 months, and with reduced performance on the Kaufman Achievement Battery for Children at 42 months (21).

The majority of epidemiologic studies have not observed an association between postnatal exposure PCBs and cognitive development. Children with higher postnatal exposure through breastfeeding in a Michigan cohort had poorer performance on the McCarthy Memory scale, but not on other McCarthy scales (10). Children with high postnatal exposure in the German cohort had reduced performance on the Kaufman Assessment Battery at 42-months of age (21). Our study detected a potential association between 16-month PCB body burdens and increased anxious/depressed behavioral problems at 45 months of age.

There are differences between the present study and previous studies of the effects of PCB exposure and cognitive development. Earlier studies, such as with Michigan cohort, used different PCB detection methods and were not able to distinguish PCB congeners (40). The Dutch and German studies used total lipid-adjusted maternal PCB as a measure of prenatal exposure, as opposed to measures of individual congeners. We did analyses for total maternal PCBs but results were similar to those of maternal PCB 153 and are not shown. Lipid adjustment also differed between the present study and the Dutch and German studies; if serum lipids are not actually a confounder, adjusting PCBs for lipids, as opposed to controlling for lipids in the model, could introduce bias into the results. Differences in the cognitive assessments used could also explain the contradictory results of the previous cohort studies compared to the present study.

In a previous study of infants in the Slovak cohort, negative associations were observed between prenatal exposure and performance on the Mental and Psychomotor Developmental Indices (MDI and PDI) of the BSID at 16-months of age. This association held using maternal and umbilical cord serum as an estimate of exposure (25). However,

the negative association was specific to dioxin-like mono-ortho PCBs. Bayley scales from the 16-month assessment were moderately correlated with WPPSI-III scores (Pearson correlations with MDI scores ranged from 0.29-0.59 and with PDI scores ranged from 0.23-0.51) and weakly correlated with CBCL scores (Pearson correlations with MDI scores ranged from 0.03-0.19 and with PDI scores ranged from 0.01-0.15). In the current study, associations with dioxin-like mono-ortho PCB 118 were greater in magnitude than those with PCB 153, but only the association between reported performance on the anxious/depressed syndrome scale and 16-month PCB exposure was significant at the 0.05 level.

There are several possible explanations for the inconsistent results found in the present study compared to the 16-month evaluation. First, performance on the Bayley scales is not generally predictive of performance on later cognitive assessments (41). Second, the reduction in sample size from the 16-month to the 45-month evaluation could have reduced the ability of the current study to detect small differences in CBCL and WPPSI-III outcomes. Third, the CBCL and WPPSI-III measures have not been standardized in the Slovak population. Cultural differences or translation discrepancies may weaken the ability of these evaluations to detect problems with cognitive development in this population. Fourth, it is possible that other factors have minimized the effects of PCB exposure. Previous literature has suggested that higher HOME scores are more predictive of performance on cognitive scales than prenatal PCB exposure (22). Subtle differences in CBCL and WPPSI-III scores could be compensated for by other social and demographic factors. Finally, we cannot ignore the possibility that perinatal

exposure to PCBs does not have lasting effects into childhood; while early cognitive development may be affected by PCB exposure, this effect could be temporary.

The original cohort suffered from high refusal rate (47%); furthermore, high attrition at the 45-month evaluation is a potential limitation. Only individuals from Michalovce were invited to participate in the 45-month evaluation. At 16 months, 559 mother-infant pairs from Michalovce participated in the Bayley Scales of Infant Development Evaluation (25). At 45-months, 438 mother-child pairs from Michalovce remained (78% of the 16-month sample). If loss to follow-up was related to PCB exposure and the CBCL and WPPSI-III outcomes, this could bias the results of the study. However, the difference between the distributions of total maternal PCBs for those in the 16-month and 45-month evaluations were small (p=0.6609).

There were some socioeconomic and demographic differences between the Michalovce population in the 16- and 45-month evaluations (data not shown). More mother-child pairs of Romani descent were lost to follow-up compared to mother-child pairs of Slovak/Eastern European descent (33 and 16%, respectively). Twenty-three percent of mothers with basic schooling, 29% of mothers with some high school, 19% of mothers with a high school diploma, and 6% of mothers with at least a college education were lost to follow-up from 16 to 45 months. More mothers reported smoking during pregnancy in the 45-month evaluation compared to the 16-month evaluation (16.3 and 13.7%, respectively). Fewer mothers reported alcohol consumption during pregnancy in the 45-month evaluation compared to the 16-month evaluation (13.5 and 16.9%, respectively). There was, however, no difference in the mean values of Bayley MDI and PDI scores between the 16- and 45-month populations (p=0.863, 0.861, respectively).

The present study had several strengths. Precise measures of maternal and child PCB body burdens were obtained for each mother-child pair in the sample. Multiple PCB congeners were measured and recorded to allow for an estimate of prenatal exposure that did not rely on proxies of exposure, such as reported fish consumption or duration of breast feeding. Furthermore, specific PCB congener levels were obtained, allowing for analysis by type of PCB congener, rather than a summary PCB measure. Despite attrition from 16 to 45 months, the final sample in this study was still larger than many samples in previous studies of PCB and cognitive development. Finally, cognitive development was assessed in two different ways. The CBCL assessment identified children with abnormal reported behavioral problems, while the WPPSI-III subtests assessed cognitive and reasoning abilities. This allowed for examination of different types of cognitive outcomes.

The present study indicates that prenatal PCB exposure likely does not have an association with behavioral problems and IQ performance at 45 months in a Slovak population with high exposure to PCBs. Postnatal levels of PCB at 16-months, however, may be weakly associated with increased behavioral problems. Given that the results are generally null, they may suggest that possible early neurodevelopmental deficits in children exposed prenatally and postnatally to PCBs may diminish as the children mature.

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TABLES

Table 2.1. Median (P25, P75) population characteristics of a cohort of mother-child pairs from Michalovce, Slovakia by maternal serum polychlorinated biphenyl (PCB) congener 153 (ng/ml), child 16-month serum PCB congener 153 (ng/ml), and child 45-month serum PCB congener 153 (ng/ml).

Characteristic	N[1] (n=438)	%	Maternal PCB 153 (ng/ml) Median (P25, P75)	N[1] (n=396)	%	16-month PCB 153 (ng/ml) Median (P25, P75)	45-month PCB 153 (ng/ml) Median (P25, P75)
Maternal age at delivery							
20 or younger	30	6.9	1.0 (0.8, 2.0)	28	7.1	0.6 (0.2, 1.4)	0.7 (0.3, 1.5)
21 -29	310	70.8	1.6 (1.2,2.7)	278	70.2	0.7 (0.2, 1.6)	0.6 (0.2, 1.3)
30 or older	98	22.4	2.4 (1.7, 4.0)	90	22.7	1.3 (0.6, 2.4)	1.0 (0.4, 1.8)
Romani ethnicity							
Slovak or other Eastern European	364	83.1	1.8 (1.2, 2.9)	332	83.8	0.7 (0.2, 1.8)	0.7 (0.3, 1.5)
Romani	74	16.9	1.3 (0.9, 2.0)	64	16.2	1.1 (0.5, 2.1)	0.9 (0.5, 1.6)
Maternal education							
Basic Schooling	92	21.4	1.4 (0.8, 2.1)	80	20.6	0.9 (0.2, 1.7)	0.8 (0.4, 1.5)
Some High School	108	25.1	2.0 (1.2, 3.0)	97	25.0	0.6 (0.2, 1.5)	0.6 (0.2, 1.1)
High School Graduate	206	47.9	1.8 (1.3, 2.8)	191	49.2	0.8 (0.3, 1.9)	0.7 (0.3, 1.7)
College or Higher	24	5.6	2.0 (1.3, 2.8)	20	5.2	1.6 (0.7, 2.4)	1.0 (0.6, 1.9)
Missing	8			8			
Maternal smoking during pregnancy							
Yes	70	16.5	1.7 (1.2, 2.7)	63	16.4	0.6 (0.2, 1.6)	0.6 (0.2, 1.5)
No	354	83.5	1.9 (1.1, 3.0)	322	83.6	0.8 (0.3, 1.9)	0.7 (0.3, 1.5)
Missing	14			11			
Maternal alcohol consumption during pregnancy							
Yes	56	13.5	1.8 (1.1, 2.7)	49	13.0	0.7 (0.3, 1.4)	0.5 (0.3, 1.0)
No	360	86.5	1.7 (1.2, 2.8)	327	87.0	0.8 (0.3, 1.9)	0.7 (0.3, 1.6)
Missing	22			20			
Birth Weight							
Less than 2500 g	20	4.6	1.5 (1.1, 2.5)	18	4.6	0.4 (0.2, 1.2)	0.6 (0.2, 1.0)
2500-3499 g	238	54.6	1.7 (1.2, 2.7)	214	54.3	0.8 (0.3, 1.9)	0.7 (0.3, 1.5)
Greater than or equal to 3500 g	178	40.8	1.7 (1.2, 3.0)	162	41.1	0.8 (0.3, 1.9)	0.7 (0.3, 1.6)
Missing	2			2			

Table 2.1, Continued

Characteristic	N[1] (n=438)	%	Maternal PCB 153 (ng/ml) Median (P25, P75)	N[1] (n=396)	%	16-month PCB 153 (ng/ml) Median (P25, P75)	45-month PCB 153 (ng/ml) Median (P25, P75)
Breastfeeding							
Less than 6 months	209	52.0	1.8 (1.2, 2.8)	201	51.9	0.3 (0.2, 0.6)	0.3 (0.2, 0.6)
At least 6 months	193	48.0	1.7 (1.1, 2.7)	186	48.1	1.7 (1.0, 2.8)	1.4 (0.8, 2.3)
Missing	36			9			
Marital status							
Married, living with partner	401	94.4	1.7 (1.2, 2.8)	364	94.6	0.8 (0.3, 1.9)	0.7 (0.3, 1.5)
Not married or living with partner	24	5.7	1.5 (1.1, 2.3)	21	5.5	0.5 (0.2, 1.4)	0.5 (0.2, 0.9)
Missing	13			11			
Child's gender							
Male	213	48.6	1.7 (1.1, 2.7)	194	49.0	0.7 (0.2, 1.8)	0.7 (0.3, 1.7)
Female	225	51.4	1.7 (1.2, 2.9)	202	51.0	0.9 (0.3, 1.9)	0.7 (0.3, 1.4)
Parity							
0	169	38.6	1.7 (1.2, 2.7)	151	38.1	0.6 (0.2, 1.9)	0.6 (0.2, 1.3)
1	159	36.3	1.8 (1.2, 2.9)	143	36.1	0.9 (0.4, 1.8)	0.7 (0.4, 1.7)
≥ 2	110	25.1	1.8 (1.2, 3.1)	102	25.8	0.8 (0.3, 1.9)	0.7 (03, 1.5)
Maternal raven score							
Beta	437		β = 0.006	395		β = -0.016	β = -0.008
Missing	1			1			
HOME score							
Beta	399		β = 0.021	384		β = 0.005	β = 0.019
Missing	39			12			

[1] Maternal PCB 153 sample consists of all 438 mother/child pairs participating in the cognitive assessment at 45 months; the 16- and 45-month PCB samples consist only of those mother/child pairs with both 16- and 45-month child serum levels.

Table 2.2. Population characteristics of a cohort of mother-child pairs from Michalovce, Slovakia and median (P25, P75) values by children's CBCL[1] Externalizing Scores, CBCL Internalizing Scores, and WPPSI-III[1] Block Design scores at 45 months of age.

Characteristic	N (n=438)	%	CBCL Externalizing Score Median (P25, P75)	CBCL Internalizing Score Median (P25, P75)	WPPSI Block Design Score Median (P25, P75)
Maternal age at delivery					
20 or younger	30	6.9	5.0 (1, 12)	4.5 (2, 9)	8.0 (6, 10)
21 -29	310	70.8	8.0 (4, 14)	8.0 (4, 13)	12.0 (8, 14)
30 or older	98	22.4	7.0 (4, 14)	8.5 (5, 14)	12.0 (10, 14)
Romani ethnicity					
Slovak or other Eastern European	364	83.1	9.0 (5, 14)	9.0 (5, 14)	12.0 (10, 16)
Romani	74	16.9	3.0 (1, 6)	2.0 (1,8)	8.0 (4, 10)
Maternal education					
Basic Schooling	92	21.4	3.5 (1, 7)	3.0 (1, 8)	8.0 (4, 10)
Some High School	108	25.1	9.0 (4, 14.5)	10.0 (5, 15)	10.0 (10, 14)
High School Graduate	206	47.9	9.0 (5, 14)	9.0 (5, 13)	12.0 (12, 16)
College or Higher	24	5.6	8.0 (6, 15.5)	8.0 (5.5, 17)	15.0 (11, 18)
Missing	8				
Maternal smoking during pregnancy					
Yes	70	16.5	4.0 (1, 11)	5.5 (2, 11)	9.0 (6, 10)
No	354	83.5	8.0 (4, 14)	9.0 (5, 13)	12.0 (10, 14)
Missing	14				
Maternal alcohol consumption during pregnancy					
Yes	56	13.5	10.0 (3.5, 16.5)	9.5 (5.5, 15)	12.0 (9, 16)
No	360	86.5	7.0 (4, 13)	8.0 (4, 12.5)	10.0 (8, 14)
Missing	22				
Birth Weight					
Less than 2500 g	20	4.6	6.0 (1, 12)	4.5 (2, 13.5)	8.0 (6, 13)
2500-3499 g	238	54.6	7.0 (3, 13)	8.0 (4, 12)	10.0 (8, 14)
Greater than or equal to 3500 g	178	40.8	9.0 (5, 14)	9.0 (5, 13)	12.0 (10, 16)
Missing	2				

Characteristic	N (n=438)	%	CBCL Externalizing Score Median (P25, P75)	CBCL Internalizing Score Median (P25, P75)	WPPSI Block Design Score Median (P25, P75)
Breastfeeding					
Less than 6 months	209	52.0	8.0 (4, 14)	9.0 (5, 14)	10.0 (8, 14)
At least 6 months	193	48.0	7.0 (3, 13)	7.0 (3, 12)	12.0 (8, 14)
Missing	36				
Marital Status					
Married, living with partner	401	94.4	8.0 (4, 14)	8.0 (4, 13)	12.0 (8, 14)
Not married or living with partner	24	5.7	5.0 (3, 14.5)	7.5 (4, 13)	10.0 (5, 13)
Missing	13				
Child's gender					
Male	213	48.6	8.0 (4, 14)	8.0 (3, 12)	10.0 (8, 14)
Female	225	51.4	7.0 (3, 13)	9.0 (5, 14)	12.0 (10, 14)
Parity					
0	169	38.6	9.0 (5, 14)	9.0 (5, 14)	12.0 (10, 16)
1	159	36.3	8.0 (4, 14)	8.0 (4, 13)	10.0 (8, 14)
≥ 2	110	25.1	5.0 (2, 11)	5.5 (2, 11)	10.0 (8, 14)
Maternal raven score					
Beta	437		$\beta = 0.144$	$\beta = 0.146$	$\beta = 0.155$
Missing	1				
HOME score					
Beta	399		$\beta = 0.291$	$\beta = 0.238$	$\beta = 0.392$
Missing	39				

[1] Child Behavior Checklist and Wechsler Preschool and Primary Scale of Intelligence-III. Each test was completed when the child was approximately 45 months of age.

Table 2.3. Distribution of total maternal polychlorinated biphenyls (PCBs)[1], maternal, 16-month, and 45-month PCB 153, and maternal, 16-month, and 45-month PCB 118 for a cohort of highly exposed mother-child pairs in Michalovce, Slovakia. Lipid adjusted wet weights are provided for comparison.

		Wet weight PCBs (ng/ml)						Lipid adjusted (ng/g lipid)					
PCB[2]	N[3]	Mean	Min	P25	P50	P75	Max	Mean	Min	P25	P50	P75	Max
Total maternal PCB	438	7.437	1.330	3.643	5.386	8.899	49.977	710.930	138.596	372.182	546.269	798.303	4511.561
Maternal PCB 153	396	2.365	0.434	1.175	1.727	2.787	14.270	224.558	46.451	120.756	173.639	257.109	1273.330
Maternal PCB 118	395	0.174	0.006	0.060	0.121	0.213	1.946	16.197	0.604	6.256	11.258	19.770	177.267
16 month PCB 153	396	1.512	0.002	0.251	0.784	1.841	16.212	264.113	0.453	42.247	139.076	335.013	3503.608
16 month PCB 118	395	0.126	0.001	0.012	0.052	0.127	3.275	22.027	0.162	2.154	9.387	22.646	632.605
45 month PCB 153	396	1.258	0.016	0.272	0.675	1.504	21.440	217.424	3.202	49.448	120.318	268.808	2749.419
45 month PCB 118	395	0.087	0.001	0.016	0.037	0.095	1.757	15.147	0.179	2.903	6.521	15.640	292.675

[1] Total maternal PCB was calculated as the sum of PCB congeners 118, 138^{+163}, 153, 156^{+171}, 170, and 180.

[2] Values represent PCB concentrations following imputation of PCBs below the limit of detection.

[3] Total maternal PCB included all women who had PCB levels measured at birth and who completed the 45-month evaluation; Maternal PCB 153 and 118 were limited to include only those women whose children were evaluation at 16- and 45-months.

Table 2.4. Adjusted negative binomial regression parameter estimates for the association between maternal, child 16-month, and child 45-month PCBs 153 and 118 with 45-month CBCL syndrome scales in a cohort of Slovak children highly exposed to PCBs.

	Maternal PCB 153[1]				16-Month PCB 153[2]				45-Month PCB 153[2]			
Subtest[3]	β	exp(β)	95% CI exp(β)		β	exp(β)	95% CI exp(β)		β	exp(β)	95% CI exp(β)	
Attention Problems	-0.003	0.997	0.957	1.039	0.000	1.000	0.960	1.042	0.008	1.008	0.963	1.055
Aggressive Behavior	-0.001	0.999	0.957	1.043	-0.004	0.996	0.956	1.037	0.012	1.012	0.963	1.063
Sleep Problems	-0.010	0.990	0.936	1.048	0.025	1.025	0.973	1.080	0.021	1.022	0.961	1.086
Emotionally Reactive	-0.019	0.981	0.926	1.040	-0.001	0.999	0.943	1.058	0.008	1.008	0.941	1.080
Anxious Depressed	0.007	1.007	0.966	1.049	0.040	1.041	1.002	1.082	0.021	1.022	0.975	1.071
Somatic Complaints	-0.028	0.972	0.930	1.017	-0.014	0.986	0.942	1.033	-0.018	0.982	0.931	1.036
Withdrawn	-0.031	0.969	0.922	1.019	0.004	1.004	0.957	1.052	-0.005	0.995	0.941	1.052
Externalizing	-0.002	0.998	0.958	1.039	-0.004	0.996	0.959	1.035	0.011	1.011	0.964	1.061
Internalizing	-0.013	0.987	0.950	1.025	0.015	1.015	0.979	1.053	0.006	1.006	0.961	1.053
	Maternal PCB-118[1]				16-Month PCB 118[2]				45-Month PCB 118[2]			
Attention Problems	0.028	1.028	0.675	1.566	0.135	1.144	0.846	1.547	0.272	1.313	0.766	2.252
Aggressive Behavior	0.003	1.003	0.648	1.552	0.085	1.088	0.805	1.472	0.284	1.329	0.760	2.324
Sleep Problems	-0.180	0.835	0.452	1.541	0.226	1.253	0.847	1.853	0.203	1.226	0.587	2.558
Emotionally Reactive	-0.136	0.873	0.478	1.594	0.236	1.267	0.846	1.895	0.498	1.646	0.806	3.361
Anxious Depressed	0.040	1.041	0.684	1.585	0.349	1.418	1.073	1.875	0.365	1.440	0.856	2.423
Somatic Complaints	-0.395	0.674	0.411	1.104	0.046	1.047	0.747	1.469	0.001	1.001	0.537	1.867
Withdrawn	-0.406	0.666	0.390	1.137	0.018	1.018	0.703	1.474	-0.010	0.990	0.508	1.928
Externalizing	0.009	1.009	0.668	1.523	0.095	1.099	0.827	1.462	0.270	1.311	0.772	2.224
Internalizing	-0.147	0.863	0.585	1.275	0.205	1.228	0.933	1.616	0.254	1.289	0.791	2.103

[1] Attention problems, Aggressive Behavior, Sleep problems, and Externalizing scores adjusted for total maternal serum lipids, maternal Raven score, HOME score, parity, child's sex, and Romani ethnicity. Emotionally reactive, Anxious/Depressed, Somatic complaints, Withdrawn, and Internalizing scores adjusted for total maternal serum lipids, maternal Raven score, HOME score, child's sex, and Romani ethnicity.

[2] Attention problems, Aggressive Behavior, Sleep problems, and Externalizing scores adjusted for total 16- or 45-month serum lipids, breastfed for less than 6 months, maternal Raven score, HOME score, child's sex, parity, and Romani ethnicity. Emotionally reactive, Anxious/Depressed, Somatic complaints, Withdrawn, and Internalizing scores adjusted for total 16- or 45-month serum lipids, breastfed for less than 6 months, maternal Raven score, HOME score, child's sex, and Romani ethnicity.

[3] After adjustment, sample sizes for maternal PCB 153 and 118 were 383 and 382 children, respectively; sample size for 16- and 45-month PCB 153 was 374 children and samples size for 16- and 45-month PCB 118 was 373 children.

Table 2.5. Adjusted multiple linear regression parameter estimates for the association between maternal, child 16-month, and child 45-month PCBs 153 and 118 with WPPSI-III subtests at 45 months in a cohort of Slovak children highly exposed to PCBs.

	Maternal PCB 153[1]			16-Month PCB 153[2]			45-Month PCB 153[2]		
Subtest	N	β	95% CI	N	β	95% CI	β	95% CI	
Block Design	383	-0.08	-0.27 0.12	374	-0.10	-0.28 0.08	-0.02	-0.24	0.19
Information Processing	383	-0.02	-0.23 0.18	374	0.07	-0.12 0.26	0.10	-0.13	0.33
Object Assembly	383	0.02	-0.26 0.30	374	0.03	-0.24 0.29	-0.03	-0.34	0.29
Picture Naming	382	0.09	-0.13 0.32	373	0.21	0.00 0.42	0.23	-0.02	0.48
Receptive Vocabulary	382	0.09	-0.18 0.36	373	0.08	-0.18 0.34	0.04	-0.27	0.34
	Maternal PCB 118[1]			16-Month PCB 118[2]			45-Month PCB 118[2]		
Block Design	382	-0.84	-2.74 1.07	373	-0.69	-2.06 0.69	-0.65	-3.14	1.83
Information Processing	382	-0.03	-2.03 1.96	373	0.43	-1.03 1.88	0.55	-2.10	3.20
Object Assembly	382	1.31	-1.39 4.02	373	0.17	-1.83 2.17	-0.23	-3.85	3.40
Picture Naming	381	0.64	-1.56 2.84	372	1.25	-0.36 2.86	1.57	-1.36	4.50
Receptive Vocabulary	381	-0.24	-2.92 2.44	372	0.15	-1.81 2.12	-0.84	-4.41	2.73

[1] Adjusted for total maternal serum lipids, maternal age at delivery, maternal Raven score, HOME score, child's sex, and Romani ethnicity.

[2] Adjusted for total 16- or 45-month serum lipids, breastfed for less than 6 months, maternal Raven score, HOME score, child's sex, and Romani ethnicity.

APPENDIX A. IRB LETTER OF EXEMPTION

EMORY UNIVERSITY

Institutional Review Board

October 28, 2011

RE: Determination: No IRB Review Required
Title: Pre- and postnatal exposure to polychlorinated biphenyls and cognitive development in early childhood
PI: Regina Simeone

Dear Ms. Simeone:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition(s) of "research" involving "human subjects" or the definition of "clinical investigation" as set forth in Emory policies and procedures and federal rules, if applicable. Specifically, in this project, you will be analyzing de identified data.

This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Andrea Goosen, MPH, CIP
Research Protocol Analyst
This letter has been digitally signed