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The association of prenatal exposure to polychlorinated biphenyls (PCBs) and thyroid function among children at 72 months in Slovakia

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By

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Bachelor of Science
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Abstract

The association of prenatal exposure to polychlorinated biphenyls (PCBs) and thyroid function among children at 72 months in Slovakia

By Karrie Finn

Polychlorinated biphenyls (PCBs) are purported to be endocrine disruptors with notable molecular similarities in structure to thyroid hormones. In prior research, prenatal exposure to PCBs has been reported to affect markers of postnatal thyroid function in infants, especially levels of thyroxine (T4) and thyroid-stimulating hormone (TSH), but no study has investigated the long-term effect of prenatal PCB exposure on thyroid function in children. The aim of this study was to assess whether prenatal exposure to PCBs affects the levels of triiodothyronine (T3), T4, and TSH in children aged 72 months using a cohort of mother-child pairs from Slovakia (n=256). Prenatal PCB exposure was assessed using the concentration of PCB congener 153 in maternal blood serum collected shortly after giving birth. Total T3, total T4, free T3, free T4, and TSH concentrations were measured in the serum of the children at a follow-up visit conducted 72 months after delivery. Multiple linear regression analyses were fit to evaluate the associations between log maternal PCB levels (ng/ml) and log child thyroid hormone levels. After adjusting for potential confounders, no association was found between prenatal PCB exposure and any of the thyroid hormones in 72-month old children (beta and 95% confidence interval: T4 (nmol/L)=0.008, -0.019-0035; TSH (mIU/L)=-0.017, -0.110-0.075). Although prior studies have reported an association between prenatal PCB exposure and neonatal thyroid function, the null findings of the present study suggest that prenatal PCB exposure might not have a long-term effect on child thyroid hormone function.

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CHAPTER 1 LITERATURE REVIEW

Introduction

Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals that were introduced in 1929 and used as common components in hundreds of commercial and industrial materials [1]. Widely valued for being non-flammable, non-corrosive, and of variable viscosity, PCBs were once found as plasticizers in dyes, paints, and pigments; as dielectrics in electrical transformers or capacitors; and as cable insulation, among a great number of common household and manufacturing products [2]. Historically, disposal practices of PCBs were not well-regulated, allowing for the contaminants to seep into the environment for years [3]. PCBs have integrated into the food chain after years of bioaccumulation, so now people are most commonly exposed through the ingestion of fish swimming in contaminated waters or by PCB leakage of heating coils into food for animal and human consumption [2]. Regulations and bans of PCBs began in the United States and most industrialized countries by the late 1970s, because they were suspected and later demonstrated to be carcinogenic as well as toxic to the immune system, reproductive system, nervous system, and endocrine system [2]. Though the manufacture of PCBs has been officially banned for over 30 years in the United States, PCB contamination and persistence in the environment continues to present a challenge to current attempts to regulate exposure.

PCB exposure in utero is of particular concern to the field of public health, as multiple studies have indicated that PCBs can cross the placental barrier, thereby exposing children prenatally [4-7]. Further, several studies have reported higher prenatal PCB levels to be associated with adverse health outcomes in children, including delayed neurological and cognitive development [3, 8-13]. Recent studies have investigated the association between

prenatal PCB exposure and infant thyroid function because PCBs, being similar in both structure and molecular property to the 2 main thyroid hormones, are thought to be potential endocrine disruptors [14]. Several studies reported strong associations between prenatal PCB exposure and thyroid hormones levels, but the directions of effect were inconsistent [15-19]. Further, most prior studies measured thyroid function only a few days after birth, so little is known about the potential long-term effects of prenatal PCB exposure on child thyroid hormones.

Thyroid Function

The thyroid gland belongs to the endocrine system and plays a critical role in the metabolism, growth, and development of the human body. It is responsible for producing and releasing a delicate balance of hormones into the blood that regulate many functions occurring as the body matures. Two main hormones produced by the thyroid gland are triiodothyronine (T3) and thyroxine (T4) [20]. Both hormones are responsible for increasing the basal metabolic rate, which is the amount of energy a certain mass of cells consume per unit time. The thyroid uses T3 and T4 to regulate functions including body temperature, heart rate, digestion, neurological development, growth in children, and alertness [20].

When the thyroid gland is not producing the appropriate amount of hormones to properly regulate the body, the consequences can be serious. Both over-activity and under-activity of the thyroid during the critical period of development can have a range of effects from behavioral problems to minimal brain dysfunction to gross mental retardation [14]. Generally, hyperthyroidism occurs when too much hormone is produced, leading to overactive cellular function. Typical symptoms of hyperthyroidism include irritability, tremor and tachycardia, increased bowel frequency, and in extreme cases, heart failure and periodic paralysis. On the

other end of the spectrum, hypothyroidism occurs when too little thyroid hormone is produced. Typical symptoms include slowed metabolism, fatigue, constipation, sensitivity to cold, poor concentration, hair loss, and in extreme cases, cerebellar ataxia and deafness [20].

The severity of problems related to thyroid function is dependent on when and to what extent the malfunctioning occurs [14]. While the thyroid plays a constant role in life-course homeostasis, it bears a great responsibility during the critical period of development in humans, beginning *in utero* and continuing through 2 years of age [14]. Thyroid hormones are responsible for the normal neurological development and brain maturation that occurs in this timeframe [14]. Should the production of thyroid hormones be disturbed during this critical period, then neurological development might undergo serious and irreversible damage.

The thyroid is particularly susceptible to certain environmental contaminants that alter its function by inhibiting or mimicking the thyroid hormones. PCBs are notably similar in structure and molecular property to T3 and T4. A PCB molecule is comprised of two halogenated phenol rings, which are capable of binding to the same major transport proteins like thyroxine-binding globulin and transthyretin as well as the thyroid hormone receptor, which are all biologically intended for T3 and T4 [14]. Among those exposed to high levels of PCBs, the thyroid might detect less availability of thyroid hormone receptors and, unable to distinguish between PCBs and its own hormones, reduce its production of T3 and T4. Thyroid stimulating hormone is produced in the pituitary gland to stimulate thyroidal secretion and the synthesis of these hormones [21]. In response to hormone imbalance, TSH levels increase to activate the thyroid as the body seeks to return hormone levels to normal. Thus, PCBs present in the body might compete for thyroid hormone receptor proteins and transport proteins as well as influence the overall levels of T3, T4, and TSH.

Thyroid function is, therefore, often measured by the levels of T3, T4, and TSH detected in blood serum. T3 and T4 hormone levels are generally measured as either total T3 and T4 concentrations, which would include both bound and unbound molecules, or as only the unbound free T3 and T4. The majority of T3 and T4 hormones circulate in the blood as complexes bound to globulins or albumin. Unbound thyroid hormones, on the other hand, are biologically active and their binding is responsible for changes in metabolism [20]. The range for normal values of free T3 (FT3) is 4.0 to 8.1 pmol/L and for free T4 (FT4) is 11 to 23 pmol/L [20]. FT3 levels are highly correlated with total T3 levels (r=0.6-0.7), as are FT4 levels with total T4 (r=0.8) [22]. Normal values of TSH in the blood range from 0.5 to 4.0 mIU/L [20]. Abnormally high levels of TSH are clinically indicative of an underactive thyroid and are often associated with lower levels of T3 and T4. Likewise low levels of TSH are indicative of an overactive thyroid and associated with higher levels of T3 and T4.

Prenatal PCB Exposure and Endocrine Outcomes in Neonates

Some studies have investigated different manifestations of endocrine system disruption associated with prenatal PCB exposure. Among these, prenatal PCB exposure and infant thymus size was analyzed in infant-mother pairs from eastern Slovakia [13]. Prenatal PCB exposure was estimated based on maternal levels shortly after delivery. Infant thymus index, the product of the maximal transverse diameter and the largest sagittal area measured 3 or 4 days after birth, was used to estimate thymus volume. The authors found that, for an increase in PCB across the interquartile range (280-700 ng/g), the thymic index decreased by 3.6%. A decrease in size of this magnitude could have clinically significant consequences for functioning of the immune system.

Other studies focused on PCB exposure and thyroid hormone levels in neonates using umbilical cord blood at delivery or blood drawn from the newborn shortly after birth. A few studies reported that prenatal PCB levels were positively correlated with FT3 and with TSH in newborns [15, 17, 19]. However, other studies found that higher prenatal PCB levels were associated with decreased FT3 and TSH levels in regression analyses [16, 18]. Two studies reported that increasing prenatal PCB levels were moderately to strongly associated with decreasing neonatal T4 and FT4 levels [16, 17], but several other studies reported only weak or null results [23-28].

Cohort Studies of PCB Exposure and Infant Thyroid Hormone Levels

Inuit Cohort

Many researches have investigated the effect of prenatal PCB exposure on the postnatal thyroid development of newborns. However, relatively few have evaluated the effects of PCB exposure on thyroid status for infants who are several months of age. Among these few cohorts is a study of Inuit mothers and children along the Hudson Bay coast of Nunavik, Quebec [29]. The Inuit population of Nunavik is heavily exposed to organochlorine compounds (OCs) like PCBs and hexachlorobenzene (HCB) through their dietary consumption of sea mammals. Dallaire et al. evaluated the association between background exposure to PCBs, PCB-OHs, and HCBs among pregnant women in Nunavik and thyroid status of their infants within the first year of life [29]. From November 1995 to March 2001, all pregnant women from the three largest communities in Nunavik were invited to participate in the Inuit Cohort Study after their first prenatal visit with a midwife or nurse.

Blood samples collected from the mothers at delivery (n=120), from the umbilical cord shortly after birth (n=95), and from the infants 7 months postpartum (n=130) were used to measure the selected OCs and thyroid hormones. OC levels were measured using gas chromatography and thyroid hormone levels were determined using radioimmunoassay methods. PCB and hexocholorobenzene (HCB) were adjusted for plasma lipid concentrations. After log-transforming all exposure and thyroid values, simple and multiple linear regression models were fit to evaluate the relationships between contaminant exposures and thyroid function. Models were adjusted for the primary caregiver's socioeconomic status, alcohol and cigarette consumption during pregnancy, maternal fish consumption during pregnancy, sex, breast-feeding status, gestational age, and Selenium level in cord blood.

Maternal PCB had a moderate inverse association with thyroxine-binding globulin (TBG) from umbilical cord blood (standardized $\beta = -0.25$; $p=0.01$). Likewise, higher cord blood PCB levels were associated with lower cord blood TBG levels (standardized $\beta= -0.26$; $p=0.01$). No other associations between environmental contaminants in maternal or cord plasma and thyroid hormone levels in cord plasma appeared to be meaningful. In a cross-sectional analysis of infant PCB and thyroid hormone concentrations in plasma collected at 7 months, increasing PCB levels were moderately associated with decreasing in TSH levels (standardized $\beta= -0.23$; $p>0.05$). All other contaminant-thyroid relationships from plasma collected 7 months postpartum appeared to be null. Based on this study's findings, OCs do not appear to be strongly associated with thyroid status among Inuit women and their offspring. However, this study only reported cross-sectional results on PCB and thyroid hormone levels and did not include associations between maternal or cord PCB levels at delivery and infant thyroid hormone levels at 7 months. Therefore, no conclusions were drawn regarding the long-term effects of PCB exposure.

Rotterdam Cohort

Between June 1990 and February 1992, women living in Rotterdam, The Netherlands, and the surrounding area were recruited to participate in a study to evaluate the effects of polychlorinated dibenzodioxin (PCDD), polychlorinated dibenzofuran (PCDF), and PCB on the thyroid hormone status of pregnant women and their children as part of the larger Dutch PCB/Dioxin Study [19]. All women-infant pairs were Caucasian and infants had to be breast fed for at least 6 weeks. Of 105 healthy mother-infant pairs recruited, 78 pairs fulfilled the criteria and were included in the analysis. Unlike the Nunavik study, this study was designed to assess *in utero* PCB exposure and exposure from breast milk.

PCB exposure was measured from maternal blood samples in the last month (36^{th} to 40^{th} week) of pregnancy, from an umbilical cord blood sample, and from a 24-hour representative sample of breast milk collected 2 weeks after delivery. PCB-congener levels in plasma were measured by gas chromatography with electron capture detection. Human milk samples were analyzed using GC-high-resolution mass spectrometry for PCBDD and PCDF levels as well as some planar PCB congeners. The toxic equivalent factor (TEF) approach was used to express the toxic potency of dioxins and PCBs in the breast milk samples, in which PCB congeners were given a value representative of their toxic potency in comparison to the most toxic dioxin congener 2,3,7,8,-tetrachlorodibezon-p-dioxin with a value of 1. The toxic equivalent (TEQ) value of each congener was calculated by multiplying the congener concentration by its associated TEF.

The thyroid hormones, TT4, TT3, FT4 and TSH, were measured in maternal plasma during the last month of pregnancy (n=78), in umbilical cord plasma (n=75), in maternal (n=77) and infant plasma (n=78) 9 to 14 days after birth, and in infant plasma 3 months after birth

(n=78). The hormone levels were determined by chemiluminescence immunoassay. Spearman rank correlation coefficients were evaluated between the thyroid hormone levels and individual PCB congener levels, dioxin congener levels, dioxin TEQ, PCB TEQ and total PCB-dioxin TEQ levels.

Higher TSH in infants 2 weeks and 3 months after delivery was moderately correlated with higher dioxin TEQ (2 weeks: r=0.38; 3 months: r=0.41), higher planar-PCB TEQ (2 weeks: r=0.37; 3 months: r=0.31), and higher total PCB-dioxin TEQ (2 weeks: r=0.40; 3 months: r=0.39*)* in breast milk. Higher nonplanar-PCB TEQ in breast milk was reported to be moderately correlated with higher infant TSH at 2 weeks (r=0.38), but the correlation with TSH at 3 months was not reported. Higher maternal and cord plasma PCBs were also reported to be significantly correlated with infant TSH levels at 2 weeks, but the correlation coefficients were not provided. Among children who breastfed, those with PCB-dioxin TEQ levels above the median (30.75 pg TEQ/g fat) had lower average plasma FT4 levels in the 2nd week after birth compared to those with TEQ levels less than or equal to the median (FT4 levels: 24.3 ± 3.4 pmol/L and 23.1 ± 3.4 pmol/L, respectively), but neither group presented an average level of FT4 that would cause clinical concern. Overall, this study reported that higher dioxin and PCB concentrations in maternal plasma, in cord plasma, and in breast milk collected at 2 weeks postpartum were moderately correlated with higher TSH levels 2 weeks and 3 months after birth.

Present Study: Slovakia Cohort

The Michalovce district of Slovakia was the site of an industrial plant called Chemko Inc. that produced PCBs between 1959 and 1985, and residents of Michalovce were consequently heavily exposed to improperly discharged PCB waste [30]. To investigate the impact of such

high environmental contamination on human health, researchers recruited 1,200 women who gave birth between 2002 and 2003 at the only maternity hospital in Michalovce or the only hospital in Svidnik, a comparison district where residents were not directly exposed to the plant's PCB waste [30]. Women were considered eligible to participate in the cohort if they were at least 18 years old at the time of delivery; never diagnosed with cancer, psychosis, renal disease, or other serious illness during pregnancy; had resided in the same district for at least five years; and had given birth less than 4 times.

Women who consented at the time of delivery were asked to provide a blood sample and complete a questionnaire regarding their pregnancies. Cord blood, results of the newborn examination, and placental tissue were collected from the child at the time of birth. Follow-up visits were conducted at six months of age, during which a blood sample was collected from the child and an updated questionnaire was administered to the mother. A second follow-up was conducted when the child was sixteen months of age, during which another child blood specimen was collected and a food frequency questionnaire regarding the child's eating habits was administered. At age 45 and 72 months, the final follow-up visits were conducted during which a blood sample from the child was collected and thyroid markers including T3, T4 and TSH were measured.

This study offers the unique opportunity to analyze the effects of prenatal PCB exposure on the long-term development of children by including follow-up visits up to 6 years after delivery, whereas the majority of preceding studies limited their measurements and biological sample collections to no more than a few days after birth. With 275 observations for thyroid hormone levels at 72 months, this study provides a unique perspective on the long-term effects of

prenatal PCB exposure on childhood thyroid development and contributes data from one of the largest samples to date.

Summary

PCB exposure presents a continuous potential threat to human health due to its highly toxic properties and widespread presence in the environment, despite having been banned for decades. In addition to notable molecular similarities between PCBs and thyroid hormones [14], the possibility of endocrine disruption by PCBs seems more plausible with growing evidence of associations between PCB exposure and changes in the endocrine system like decreasing thymus size or altered thyroid hormone production in prenatally exposed infants [13, 15-19]. However, less is known about the long-term impact on childhood thyroid development because most studies interested in the association of prenatal PCB exposure and thyroid development have been limited to cross-sectional analyses, using infant thyroid hormones collected shortly after birth. The thyroid is responsible for the biological growth and maturation, so hormone disruption can result in a range of serious and permanent consequences. It is therefore important to understand to what extent this common contaminant might impact thyroid function and childhood development.

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CHAPTER 2: THE ASSOCIATION OF PRENATAL EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBS) AND THYROID FUNCTION AMONG CHILDREN AT 72 MONTHS IN SLOVAKIA

Introduction

Polychlorinated biphenyls (PCBs) are a group of synthetic organic chemicals that were introduced in 1929 and used as common components in hundreds of commercial and industrial materials [1]. Widely valued for being non-flammable, non-corrosive, and of variable viscosity, PCBs were once found as plasticizers in dyes, paints and pigments, as dielectrics in electrical transformers or capacitors, and as cable insulation among a great number of common household and manufacturing products [2]. Disposal practices of PCBs were not well-regulated, allowing the contaminants to seep into the environment for years and collect in natural bodies of water [3]. Consequently, PCB exposure has contaminated the food chain from fish swimming in contaminated waters as well as PCB seepage from heating coils used in food processing [2]. Regulations and bans of PCBs began in the United States and most industrialized countries by the late 1970s because they were suspected and later demonstrated to be carcinogenic as well as toxic to the immune system, reproductive system, and nervous system [2].

Attention in research has been drawn to the possible susceptibility of the endocrine system to potential PCB interference. PCBs are thought to be endocrine disruptors because they resemble in structure and molecular property the two main hormones of the thyroid gland, triiodothyronine (T3) and thyroxine (T4). These hormones are responsible for the metabolism, growth, and development of the human body [4, 5]. Prenatal exposure to PCBs is of particular concern because multiple studies have indicated that PCBs can cross the placental barrier, thereby exposing children in the womb [6-10]. Thus, prenatal PCB exposure could potentially alter long-term hormone production if the thyroid is affected during the critical period of thyroid development that begins *in utero* and lasts through 2 years of age [5].

Preliminary research in animals suggested that maternal PCB exposure during pregnancy was strongly associated with altered thyroid hormone levels in offspring [7, 11-13]. However, some studies reported that T4, FT4, and T3 decreased with increasing dose of PCB [7, 12, 13] while others reported T4 and FT4 increased [11, 13].

Similar to the animal studies, several cross-sectional studies in humans suggest moderate to strong associations between perinatal PCB exposure and thyroid hormones [14-18], but the directions of the associations were inconsistent. Specifically, PCB levels measured in either umbilical cord blood or maternal blood and milk were positively correlated with FT3 and TSH in newborns in a few studies [14, 15, 18]. Other studies that performed regression analyses reported an association between higher cord blood PCB levels and decreased FT3 and TSH levels [15, 16]. Two of these also suggested that increasing PCB levels in cord blood were associated with decreasing neonatal T4 and FT4 levels [15, 18]. However, several other publications reported null associations between perinatal PCB levels and thyroid hormones [19-24].

Studies examining the potential long-term effects of prenatal PCB exposure on child thyroid outcomes are also inconsistent. A study of Inuit mothers and children along the Hudson Bay coast of Nunavik, Quebec reported that higher infant PCB levels were associated with lower TSH levels at 7 months of age (standardized $\beta = -0.23$; $p > 0.05$), but all other 7-month PCB-thyroid relations were null [25]. In contrast, a study from the Netherlands reported that higher infant exposure to PCBs, measured in both maternal plasma collected the last month of pregnancy and breast milk collected 2 weeks after birth, was weakly correlated with higher infant TSH levels 2 weeks and 3 months after birth [17]. No published study examines thyroid hormone levels beyond 2 years of age, which marks the end of the critical period of development

[5]. However, the potential long-term effects of prenatal PCB exposure on child thyroid function might be better assessed once the child has reached a more stable stage of development.

The present study considers a cohort of mother-child pairs from 2 districts in the Slovak Republic. The Michalovce district was the site of an industrial plant called Chemko Inc. that produced PCBs between 1959 and 1984. The residents of Michalovce were exposed to improperly discharged PCB waste from Chemko's operation [26]. Women giving birth in hospitals from either Michalovce or the comparison district of Svidnik, which was not directly exposed to the plant's PCB waste, were recruited to investigate the effect of such high environmental contamination on human health [26]. This study investigates the association between prenatal PCB exposure, measured in maternal serum at birth, and thyroid hormone levels in the serum of children collected at a follow-up visit 72 months after birth.

Methods

Study Population

The original study population consists of 1,134 women who gave birth between 2002 and 2004 at either a hospital in Michalovce or in Svidnik, Slovakia [27]. Each district has only one hospital and most women in Michalovce or Svidnik use their respective hospital for delivery [26]. Michalovce was selected as a region of high PCB exposure, because its residents were directly exposed to PCB contamination dumped from a chemical manufacturing plant into the local environment. Svidnik was selected as the comparison region with lower PCB exposure because its residents share similar characteristics to those in Michalovce, but they were not exposed to the same PCB manufacturing waste [27]. Women from both regions were eligible to participate in the original study if they were at least 18 years old at the time of delivery; never

diagnosed with cancer, psychosis, renal disease, or another serious illness during pregnancy; had resided in the same district for at least five years; and had given birth fewer than 4 times [26].

Women who consented at the hospital provided a blood sample and completed a questionnaire regarding their pregnancy, medical history and lifestyle factors. Of those who gave a blood specimen, 1,076 women had PCB measurements available. Women were re-contacted for a follow-up visit at the pediatric department of their local hospital when their child was about 72 months of age. Four hundred and forty-five women agreed to participate and completed an updated questionnaire, and a blood draw was performed on their children. The subset of 256 maternal-child pairs who had both maternal PCB 153 measurements and child thyroid hormone measurements at 72 months were included in this analysis.

This study was approved by the Institutional Review Board at Emory University, the University of California, Davis, and the Slovak Medical University. Comprehensive descriptions of participant selection, consent protocols, and data and specimen collection can be found in previous publications [8, 26-31].

PCB Measurement

Several studies have suggested that PCBs are capable of crossing the placental barrier [6-10], so maternal PCB levels at the time of the birth were used as a proxy for the prenatal exposure of the child. Samples were stored at -18 degrees Celsius and transported to the Slovak Medical University in Bratislava where they were analyzed at the Department of Toxic Organic Pollutants using high-resolution gas chromatography with electron capture detection (HP 5890; Hewlett-Packard, Palo Alto, CA, USA) [27].

Fifteen PCB congeners were measured in maternal 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. PCB congeners below the limit of detection (which was calculated as the mean of background noise plus three standard deviations from five blank reagent samples) were set as a value equal to the limit of detection divided by square root of two for analysis [31]. PCB congener 153, the predominant PCB congener, was highly correlated (r=0.99) with total PCB exposure in maternal serum and was used as a proxy for prenatal PCB exposure.

Thyroid Hormone Assessment

Analyses of free triiodothyronine (FT3), free thyroxin (FT4), total triiodothyronine (T3), total thyroxine (T4) and thyroid-stimulating hormone (TSH) in serum from children at 72 months of age were performed using the electrochemiluminiscent with the Elecsys 1010 apparatus by Boehringer, Mannehim. Measurements were conducted at the Institute of Experimental Endocrinology.

Covariate Assessment

Mothers reported their age, ethnicity, level of education, smoking habits, and pregnancy history via questionnaires completed during the 5-day hospital stay after delivery. A woman was considered to be of Romani ethnicity if either of her parents were Romani, if Romani language was spoken at home or if she intended to raise her child with the Romani language [31]. Child gender and birth weight were abstracted from standard newborn records. Low birth weight was defined as weighing less than 2500 grams at birth.

Data Analysis

All statistical analyses were performed using the SAS statistical package (Version 9.4, SAS Institute, Cary, NC). Initial descriptive analyses were performed for both the thyroid hormone and PCB variables. To assess confounding, relationships among the covariates,

maternal PCB 153 levels, and infant thyroid hormones were evaluated using a direct acyclic graph and a series of Kruskal-Wallis tests to assess the strength of each association.

Both thyroid hormone and PCB parameters were log-transformed to improve normality for the multiple linear regression models. The models included covariates that were thought to be confounders based on a directed acyclic graph, a strong association with either maternal PCB 153 or the thyroid hormones, or the result of an all-subsets model assessment using a 10% change in the estimated parameter as the inclusion criteria. One influential outlier was identified visually, and regression analyses were performed both including and excluding the single observation.

Results

Table 1 presents the descriptive characteristics of the study cohort and the median concentration of maternal PCB 153 by characteristic for the 256 mother-child pairs that participated in the 72 month follow-up visit. Most women in the cohort were between the ages of 20 and 29 years. Only 20.3% of the participants were of Romani descent and only 24.6% reported smoking during pregnancy. Seven infants weighed less than 2500g at birth, making up less than 3% of the study population. A strong positive association was observed between PCB 153 and maternal age as mothers belonging to older age group were more likely to have higher median PCB 153 levels than those of younger age groups. Increasing parity was also slightly associated with increasing concentrations of PCB 153. Otherwise, median PCB 153 levels did not meaningfully differ across the strata for ethnicity, maternal education, child's gender, and birth weight.

Median child thyroid hormone levels were also compared across the strata of the study population characteristics (Table 2). A slight increase in median FT3 levels was observed for children of Romani descent compared with those of Eastern European descent. FT4 levels generally decreased with increasing in maternal age, except for in children with mothers in the oldest age group. TSH generally increased with maternal age. T3, T4, and TSH levels did not differ by any of the measured characteristics.

Table 3 presents the multiple linear regression beta coefficients for the associations between log-transformed maternal PCB 153 and log-transformed child thyroid hormones levels taken at the 72 month follow-up visit. All regression analyses adjusted for total lipid concentration in maternal serum, ethnicity, maternal age, maternal education, parity, and maternal smoking during pregnancy. A one standard deviation increase in log maternal PCB 153 (0.73 ng/ml), was associated with an increase of 0.007 pmol/L in log FT4 and of 0.006 pmol/L in log T4, but a decrease of 0.006 nmol/L in log T3 and of 0.012 mIU/L in log TSH. There was no association with log FT3. None of the observed associations between maternal PCB 153 and child thyroid hormones were found to be statistically significant at $\alpha=0.05$.

Discussion

The purpose of this study was to determine whether prenatal PCB exposure is associated with altered child thyroid function. PCBs are capable of passing through the placental barrier [6-10], are similar in physical structure and chemical properties to hormones produced by the thyroid [5], and are possibly associated with changes in thyroid hormone production from prenatal exposure according to prior animal and infant studies, though the directions of effect were inconsistent [8, 11-18]. Only two published studies analyzed thyroid hormone levels at

least one month past birth: the cross-sectional Nunavik cohort that found an inverse association between PCB and TSH at 7 months, and the Netherlands cohort that found a positive correlation between PCB exposure in maternal blood and breast milk and TSH levels at 2 weeks and 3 months after birth [17, 25]. Neither of the studies considered thyroid outcomes at a later age when the child is past the critical period of development and thyroid function is potentially more stable. While some previous studies did find an association between prenatal PCB exposure and infant thyroid hormone levels [14-18], the findings of the present study are consistent with the null results reported for several studies assessing the relationship between maternal PCB exposure and thyroid hormones levels in serum from umbilical cord blood [19-24].

Of 1,134 mother-child pairs initially recruited and consented to participate, only 256 (23%) had both PCB and thyroid hormone measurements available for this study's analysis indicating that a significant percentage of the participants were lost to follow up. Such a large loss might have introduced selection bias if participation was related to prenatal PCB exposure and any of the child thyroid outcomes. However, the distribution of population characteristics for mother-child pairs lost to follow up appeared to be similar to those included in the analyses, suggesting that there might not be a difference between the two groups.

Prior studies have considered breastfeeding as a confounder. Breastfeeding was not controlled for in this study's analyses because it was hypothesized to be on the causal path from maternal PCB levels during pregnancy to infant thyroid hormone levels. However, PCBs are easily transmitted from mother to child by maternal breast milk as a postnatal source of PCB exposure [25, 32]. While this study intended to evaluate the association between prenatal PCB exposure and thyroid outcomes, it is challenging to disentangle the effect of prenatal exposure from postnatal exposure through breast milk. Further, although information on duration of

breastfeeding was collected for this study, data on PCB levels in breast milk were not available. Another potential source of error is the possibility that maternal serum PCB measurements did not accurately reflect the true prenatal PCB exposure of the child. The conclusions of this study rely on the assumption that maternal serum is an appropriate proxy, which is supported by evidence in the literature that PCBs in maternal serum are strongly and positively correlated with cord blood, the other common proxy for prenatal exposure [9].

With 256 observations, the present study is the largest to date investigating prenatal PCB exposure and child thyroid hormone outcomes, and unlike prior research, data were collected and analyzed for all five thyroid hormone outcomes (FT3, FT4, T3, T4 and TSH). Further, the longitudinal design of this study offers a unique perspective on the long-term effects of prenatal PCB exposure by examining child thyroid hormones levels 72 months after birth in contrast to most prior studies, which were cross-sectional and were therefore unable to address the effects of prenatal PCB exposure over time.

This study suggests that prenatal PCB exposure does not alter postnatal thyroid hormone production at 72 months of age. If there is an effect of prenatal PCB exposure on the thyroid in early development shortly after birth, it is possible that the endocrine system is able to return to homeostasis without lasting consequences at later developmental ages. Prior research reported that higher prenatal PCB exposure was associated with decreased neonatal thymus development in this cohort [29], but thyroid function does not appear to be affected at the age of 72 months.

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TABLES

Table 1. Characteristics of Mother-Child Pairs and Median (P25,P75) for Polychlorinated Bromine (PCB) congener 153 (ng/ml) in Maternal Blood Serum in Eastern Slovakia Cohort, 2002-2004

	Cohort (n=256[1])		PCB 153 (ng/ml)		
	N	**%**	**Median**	**(P25,P75)**	***p* value[2]**
Ethnicity					<0.0001
Eastern European	204	79.69	1.63	(1.08,2.54)	
Romani	52	20.31	1.63	(1.04,2.91)	
Maternal Age					
<20	19	7.42	0.80	(0.73,1.61)	
20-24	86	33.59	1.34	(0.97,2.18)	<0.0001
25-29	92	35.94	1.75	(1.18,2.72)	
30-34	43	16.80	1.99	(1.55, 3.87)	
35-39	12	4.69	2.30	(1.53, 4.35)	
40+	4	1.56	4.56	(1.93, 7.53)	
Maternal Education					0.6158
Basic Schooling	53	21.20	1.49	(0.96, 2.40)	
Some High School	67	26.80	1.69	(1.09, 2.62)	
High School Graduate	105	42.00	1.66	(1.10, 2.55)	
College or Higher	25	10.00	1.47	(1.02, 2.08)	
Missing	6				
Parity					0.0705
0	83	32.55	1.60	(1.05, 2.30)	
1	110	43.14	1.61	(1.10, 2.79)	
2	43	16.86	1.68	(0.97, 3.28)	
3-4	19	7.45	1.74	(0.87, 3.27)	
Missing	1				
Maternal smoking during pregnancy					0.6088
No	184	75.41	1.65	(1.11, 2.56)	
Yes	60	24.59	1.39	(0.94, 3.06)	
Missing	12				
Sex of Child					0.1436
Male	142	55.69	1.68	(1.09, 2.60)	
Female	113	44.31	1.59	(1.06, 2.68)	
Missing	1				
Birth Weight					0.9402
Less than 2500g	7	2.73	1.63	(1.05, 3.45)	
Greater than or equal to 2500g	249	97.27	1.64	(1.09, 2.60)	

[1]Includes only maternal-child pairs that have maternal PCB 153 measurements and at least one of the following child hormone measurements at the 72 month follow-up: free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (T3), total thyroxine (T4) or thyroid stimulating hormone (TSH)

[2]Calculated by the Kruskal-Wallis test

TABLES

Table 2. Median (P25, P75) Child Thyroid Hormone Levels at 72 Month Follow-Up Visit by Each Characteristic of the Study Cohort (n=256[1])

	FT3 (pmol/L)			FT4 (pmol/L)			T3 (nmol/L)			T4 (nmol/L)			TSH (mIU/L)		
	Median	**(P25,P75)**	***p* value[2]**	**Median**	**(P25,P75)**	***p* value[2]**	**Median**	**(P25,P75)**	***p* value[2]**	**Median**	**(P25,P75)**	***p* value[2]**	**Median**	**(P25,P75)**	***p* value[2]**
Ethnicity			0.0213			0.0002			0.4163			0.6738			0.5261
Eastern European	6.05	(5.63, 6.51)		18.07	(16.88, 19.72)		2.79	(2.55, 3.01)		152.36	(142.80, 182.70)		2.75	(1.90, 3.69)	
Romani	6.39	(5.80, 6.99)		18.33	(17.10, 19.41)		2.81	(2.47, 3.15)		151.63	(143.72, 176.15)		2.50	(2.18, 3.78)	
Maternal Age			0.5715			0.0074			0.4652			0.0863			0.3419
<20	6.07	(5.66, 6.97)		18.79	(17.46, 19.61)		2.71	(2.49, 3.09)		150.70	(141.44, 177.40)		2.53	(2.02, 3.21)	
20-24	6.04	(5.64, 6.56)		18.38	(17.17, 20.03)		2.81	(2.52, 3.05)		153.64	(145.12, 181.60)		2.71	(1.93, 3.74)	
25-29	6.11	(5.70, 6.53)		18.10	(16.76, 19.66)		2.80	(2.55, 3.04)		150.32	(142.36, 180.15)		2.45	(1.77, 3.78)	
30-34	5.92	(5.48, 6.48)		17.54	(16.41, 18.62)		2.72	(2.49, 2.96)		148.56	(38.64, 183.30)		3.14	(2.25, 3.72)	
35-39	6.16	(5.49, 6.45)		17.61	(16.69, 18.90)		2.79	(2.59, 3.01)		185.50	(165.41, 187.75)		3.30	(2.11, 5.17)	
40+	6.48	(6.26, 6.67)		20.27	(18.78, 21.63)		3.03	(2.83, 3.12)		152.28	(147.36, 171.87)		3.25	(2.14, 4.06)	
Maternal Education			0.3876			0.4951			0.8422			0.6356			0.4630
Basic Schooling	6.28	(5.51, 6.86)		18.39	(17.10, 20.00)		2.81	(2.49, 3.14)		153.68	(143.76, 176.10)		2.53	(2.25, 3.61)	
Some High School	5.91	(5.60, 6.46)		17.94	(16.81, 19.45)		2.75	(2.50, 2.99)		152.30	(140.72, 180.70)		2.87	(1.96, 3.95)	
High School Graduate	6.16	(5.70, 6.55)		18.23	(17.00, 19.90)		2.79	(2.57, 3.02)		152.40	(143.36, 184.10)		2.72	(1.94, 3.56)	
College or Higher	5.94	(5.70, 6.53)		18.26	(16.72, 19.12)		2.82	(2.67, 3.04)		150.48	(142.08, 178.50)		2.17	(1.62, 3.53)	
Parity			0.4311			0.9077			0.5078			0.7068			0.2198
0	6.14	(5.66, 6.67)		18.14	(17.29, 19.40)		2.79	(2.51, 3.09)		149.04	(142.08, 177.40)		2.77	(2.05 ,3.70)	
1	5.99	(5.61, 6.42)		18.16	(16.94, 19.65)		2.78	(2.54, 3.03)		154.20	(144.32, 182.20)		2.54	(1.84, 3.52)	
2	6.19	(5.66, 6.62)		18.24	(16.41, 19.61)		2.81	(2.60, 3.02)		150.00	(142.48, 185.00)		2.94	(2.17, 4.44)	
3-4	6.49	(5.70, 7.12)		19.12	(16.60, 20.80)		2.83	(2.57, 3.15)		148.80	(140.40, 184.60)		2.57	(1.73, 4.08)	
Maternal Smoking During Pregnancy			0.6705			0.6664			0.9473			0.6456			0.1335
No	6.07	(5.62, 6.60)		18.21	(16.96, 19.57)		2.80	(2.55, 3.06)		152.48	(143.56, 183.70)		2.69	(1.92, 3.73)	
Yes	6.25	(5.77, 6.53)		18.51	(17.05, 20.12)		2.82	(2.85, 3.03)		153.16	(142.48, 182.65)		2.49	(1.98, 3.25)	
Sex of Child			0.3459			0.1224			0.8434			0.0180			0.7064
Male	6.05	(5.55, 6.55)		18.18	(16.86, 19.53)		2.78	(2.50, 3.04)		151.60	(142.88, 183.20)		2.78	(2.02, 3.61)	
Female	6.09	(5.72, 6.51)		18.21	(17.10, 19.90)		2.80	(2.55, 3.05)		152.40	(143.68, 181.70)		2.51	(1.92, 3.93)	
Birth Weight			0.2766			0.2373			0.1171			0.5157			0.0582
Less than 2500g	6.30	(5.99, 6.62)		19.05	(17.10, 22.45)		3.02	(2.71, 3.28)		155.60	(145.44, 189.50)		4.51	(2.94, 5.15)	
Greater than or equal to 2500g	6.07	(5.63, 6.53)		18.21	(16.89, 19.65)		2.79	(2.52, 3.04)		152.32	(142.88, 181.70)		2.61	(1.94, 3.61)	

[1]Includes only maternal-child pairs that have maternal PCB 153 measurements and at least one of the following child hormone measurements at the 72 month follow-up: free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (T3), total thyroxine (T4) or thyroid stimulating hormone (TSH)

[2]Calculated by the Kruskal-Wallis test

TABLES

Table 3. Adjusted Multiple Linear Regression Models of Log-Transformed Thyroid Hormones in Children at 72 months with Log-Transformed Maternal PCB 153 (ng/ml)

Thyroid Parameter	β[1]	95% CI[2]		*p* value
FT3 (pmol/L)[3]	0.000	-0.024	0.024	0.9876
FT4 (pmol/L)[4]	0.009	-0.015	0.033	0.4592
T3 (nmol/L)[5]	-0.008	-0.037	0.020	0.5685
T4 (nmol/L)	0.008	-0.019	0.035	0.5619
TSH (mIU/L)	-0.017	-0.110	0.075	0.7088

[1] Adjusted for total lipid concentration, ethnicity, maternal age, maternal education, parity, and maternal smoking during pregnancy. Excluded influential outlier for FT3, FT4, and T3.
[2] 95% Confidence Interval
[3] With outlier included: β=0.000 95%CI (-0.027, 0.028), p=0.9751
[4] With outlier included: β= 0.009 95%CI (-0.027, 0.044), p=0.6282
[5] With outlier included: β= -0.007 95%CI (-0.069, 0.055), p=0.8172