EARLY CHILDHOOD DEVELOPMENT AND PCB EXPOSURE IN SLOVAKIA

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Introduction

Children represent a most vulnerable population, with respect to exposure to environmental pollutants. Their first contact with pollutants occurs prenatally via transplacental transfer and then continues through breastfeeding after birth. Polychlorinated biphenyls (PCBs) are ubiquitous, persistent, bio-accumulative environmental pollutants exerting neurodevelopmental and immunotoxic effects in experimental animals treated prenatally. Associations with neurobehavioral deficits were found in some but not all epidemiologic studies of environmental exposures,[1,2] with data suggesting that exposures during the prenatal period may be the most sensitive.[3] Additionally, the risk of intrauterine growth retardation increases with higher environmental PCB exposures, and several reports suggest a more potent effect in male babies.[4,5]

Our international collaborative project "Early childhood development and PCB exposure in Slovakia" was launched in 2001. It is focused on the distribution of PCBs and their metabolites in biological samples of woman/baby pairs and subsequent health effects in infants and children – specifically immune markers at birth and early childhood, intrauterine and early childhood growth, and early neurobehavioral development. The study is being conducted in the Slovak Republic, where a chemical plant that manufactured PCBs until 1985 improperly discharged large quantities of contaminated waste into the surrounding area.

Materials and Methods

The study uses a cohort design, with immunologic, endocrine, and developmental, growth, and neurobehavioral outcomes ascertained prospectively. During 2001-2003, 1134 births were enrolled in two selected districts of eastern Slovakia, one with high PCB contamination (Michalovce), and the other with somewhat lower, but not negligible levels in the environment (Svidnik/Stropkov). Infants were followed up at 6 and 16 months of age at the local hospitals. Several types of biological samples were collected during the study for laboratory analyses - specimens of maternal and cord blood and placental tissue were collected at delivery, breastmilk at 4 days after delivery and infant's blood at 6- and 16-month visits.

Analyses of fifteen PCB congeners (28, 52, 101, 105, 114, 118, 123^{+149}, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180, and 189) and six pesticides (α-, β-, γ-HCH, HCB, DDT and DDE) were performed for all maternal serum samples, and in cord and infant's sera for a subsample of the cohort, using HRGC (high-resolution gas chromatography) with electron capture detection.[6] Lipids were determined by an enzymatic method according to Akins et al.[7]

PCB-metabolites - hydroxylated polychlorinated biphenyls (OH-PCBs) and methyl sulfone polychlorinated biphenyls (Me-SO_2-PCBs) - were measured in a subsample of collected maternal serum. Methods have been described elsewhere.[8,9] Analyses of the derivatives of the OH-PCBs and the MeSO_2-PCBs were performed using a Hewlett Packard Model 6890 gas chromatograph/electron-capture detector.

Besides organochlorine compounds, concentrations of toxic metals, namely lead and mercury, representing possible confounders for neurodevelopmental outcomes, were measured in the samples of maternal blood.

Interviews were conducted with the mother at the time of delivery, and again when the infant reached 6 months and 16 months of age. The first questionnaire was focused on information about the recent and previous pregnancies, sociodemographic and household characteristics, diet, lifestyle (incl. smoking and alcohol consumption), and on factors that could influence the growth and development of the child or PCB exposures. Dietary information was collected via a food frequency questionnaire with the aim to determine the sources of PCBs in the woman's diet during pregnancy, and to assess the general adequacy of her diet. The 6- and 16-month questionnaires asked about the home environment, exposure to smoking, the child's breastfeeding and eating habits, information about caregivers of the child, the daycare history of the child, respiratory symptoms, medications, developmental milestones, etc. Food frequency questionnaires were used for the children's diet as well. Data from the maternal medical charts after delivery and later from pediatric records (focused on child's development and morbidity) were abstracted. Tooth eruption was examined at birth, and at 6- and 16- month-visits.

On the second day after birth, newborn hearing screening was performed by a nurse using automated auditory brainstem response (AABR), while the child was sleeping. Infants who did not pass the AABR screening were re-screened and if a pass was again not obtained, the infant was referred to a hearing clinic for diagnostic testing by an audiologist.

As a marker of immune health of a child, an ultrasound of the thymus was done at birth, and at 6 and 16 months, according to the procedures described by Hasselbach et al.[10,11] The thymic index calculated from the ultrasound was used as an indicator of thymic volume. At birth, 6 and 16 months, cell-mediated immunity was examined in a subset of children (phenotypic analysis of peripheral blood lymphocytes). Three color analysis – using detectors set for FITC (530 nm), PE(R-PE - 580 nm) and PerCP(670 nm) detectors was performed using CellQuest(BD) and SimulSET Software (BD). At 6 months, functional immunity of the children was measured by challenge tests to assay post-vaccination antibody titers against diphtheria and tetanus by tanned cell hemagglutination. At 16 months, IgG, IgM, IgA, and IgE were measured in a subset of infants using immunoturbic method and IgE was analyzed by commercial ELISA kit (both SEVAPHARMA, Prague).

Thyroid function was assessed in a subset of maternal and cord blood sera samples and at 6- and 16-months.

Results and discussion

A total of 1134 mother/child pairs participated in the project, representing both study regions - Michalovce (N=811) and Svidnik (N=323). From this total, 1057 had sufficiently complete records. Characteristics of the study population are presented in the Table 1.

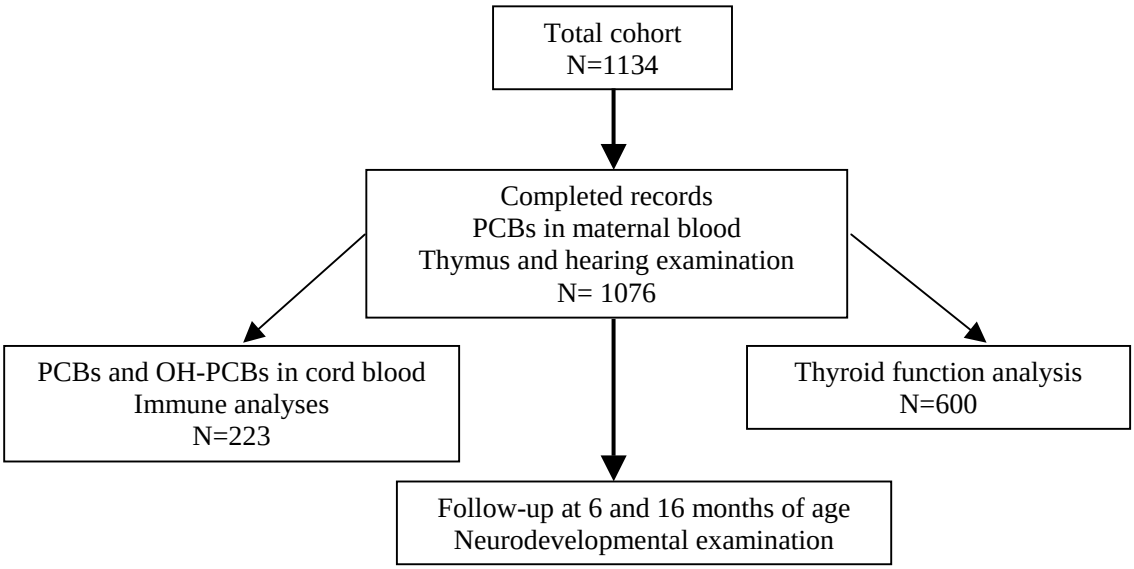
Maternal age ranged from 18 to 43 years (mean 25.7 years). Average years of education were 11.5 years, 53 % of women had high school education with graduation, or higher level of education. Out of 1057 women, 79 % were Caucasian and 21 % belonged to Romani ethnicity, 46 % women were primiparas and 36 % of women smoked prior to pregnancy. The project design concerning selection of samples for laboratory analyses is shown in Figure 1.

The mean PCB concentration was 6.53 ng/ml (SD ± 8.54 ng/ml; median 4.48 ng/ml) in maternal blood and 1.38 ng/ml (SD ± 1.63 ng/ml, median 0.87 ng/ml) in cord blood. Mean birth weight was 3325 g (SD ± 497 g; median 3350 g). The association between total maternal serum PCB levels and birth weight was not statistically significant. However, newborn boys born to Romani mothers had lower birth weight than the non-Romani boys, an effect which was larger at high PCB levels (90^{th} percentile) vs. low PCB levels (10^{th} percentiles). These differences were not found for girls. These results suggest that higher levels of PCBs in maternal blood sera have a larger growth inhibiting effect on Romani boys than girls, and the effect may be magnified by other social factors.

Table 1. Characteristics of the study population (N=1057)

		N	%
District	**Michalovce**	743	70.3
	Svidnik	314	29.7
Maternal age [years]	**18-20**	156	14.8
	21-30	728	68.9
	31 +	173	16.4
Maternal education	**University**	79	7.5
	High school with grad.	482	45.6
	Lower education	496	46.9
Ethnicity	**Caucasian**	831	78.6
	Romani	226	21.4
Smoking	**Yes**	381	36.0
	No	676	64.0
Child's gender	**Female**	512	48.4
	Male	545	51.6

Figure 1. Project design



Newborn hearing examination revealed only 3 newborns that did not pass the AABR examination; two newborns had already erupted teeth.

Thymus index was calculated in 987 children. Prenatal PCB exposure was associated with a smaller thymic index in our study (beta = -0.039, $p<0.04$). District of residence also predicted thymic index. Male sex, later gestational age,

larger birth weight z-score, and Roma ethnicity were associated with a larger thymic index while maternal smoking, alcohol consumption and respiratory disease were associated with a lower thymic index. This study provides the first evidence to date that higher PCBs exposures in infants are associated with a smaller thymus index, which may imply impaired thymus development.

The concentrations of OH-PCB metabolites were twice as high in the maternal blood sera from the Michalovce region as compared to Svidnik ($p < 0.001$). 4-OH-CB187 was a primary metabolite, followed by 4-OH-CB146. The median ratio of the sum of OH-PCB to the sum of their parent PCB congeners was 0.10. Mothers residing in eastern Slovakia are still highly exposed to PCBs, and their body burdens of these pollutants and OH-PCB metabolites may pose a risk for adverse effects on health for themselves and their children.

In future studies, we hope to follow these infants as they go through childhood and adolescence.

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