Neurobehavioral observation and hearing impairment in children at school age in eastern Slovakia

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Introduction

Neurotoxicity of PCBs has been reported in humans and confirmed in animal studies[1,2,3].

It was shown that PCBs can alter a number of developmental physiological processes in which the thyroid plays an essential role[4,5]. In children, the prenatal exposure to PCBs was associated with reduced birth weight and poor recognition memory. In children with longer duration of breast feeding implying higher PCB exposure, altered behavior, lengthening of psychomotor activities, worse attention, and worse memory performance were found [6,7].

The so far published data on the association between PCBs exposure and hearing were based mainly on animal observations. Low-frequency auditory impairments have been documented in PCB exposed rats, including elevated behavioral auditory thresholds, decreased amplitude and prolonged latency auditory evoked brain stem responses [8]. Two papers were related to humans only. The first one reported PCB-associated increased thresholds at two out of eight frequencies on audiometry, but only on the left side, and no deficits on evoked potentials or contrast sensitivity in 7-year-old children prenatally exposed to seafood neurotoxicants [9]. The other paper was focused on hearing impairments in boys of fish-eating mothers, but no individual PCB exposure data were available [10].

The aim of this study was to evaluate the associations between exposure to PCBs and health outcomes assessed, as performance in neurobehavioral tests, thyroid hormones production and hearing status. Selected confounder factors such as heavy metals and health/social background of development in children were also taken into consideration.

Methods and Materials

Study Group. **Children aged 8-9 years born and living in the region of eastern Slovakia, polluted by the Chemko factory producing PCBs in the past, were recruited as shown in Table 1.**

Sites of schools, distance and direction from the PCB plant		Number of children	PCBs serum concentrations		
		n	Mean	Median	SD
Michalovce	15 km SSE	135	511.2	404.6	467.3
Strážske	0	59	916.2	684.3	989.3
Nacina Ves	6 km SSE	22	1692.8	1423.9	1146.3
Svidník	53 NNW	121	318.6	209.1	330.4
Stropkov	41 km NW	50	364.1	248.1	365.7
Giraltovce	37 km NW	44	257.5	204.6	265.5
Total		431	529.9	321.0	655.8

Mothers of all children have been living permanently in the area for at least 5 years before their children were born.

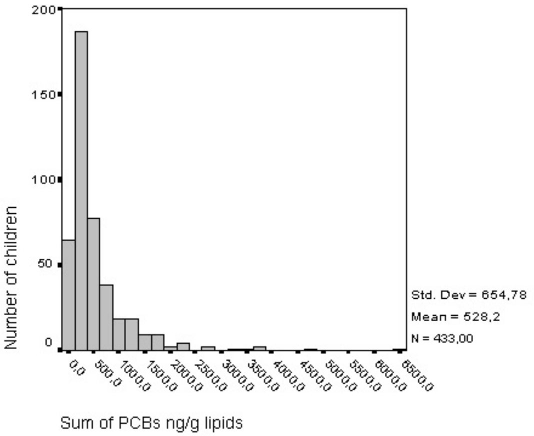
Methods: **In peripheral blood/serum, concentrations PCBs [ng/g serum lipids], TSH, T3 and T4, Pb, Hg and Mn were determined. Furthermore, the thyroid volume, basic anthropometric parameters and hearing status (tympanometry, pure tone audiometry, brainstem evoked responses, transient evoked oto-acoustic emissions) were assessed. Neurobehavioral examination consisted of the examination of (1) sensomotor and attention functions: Simple reaction time (SRT), Vienna colour discrimination test, Tapping (preferred and non-preferred hand), memory and (2) complex mental processes: Benton recognition test, Digit span (forward, backward), Digit symbols (DS), Cube-hand coordination and Raven nonverbal intelligence test (colored form for children and the non colored version for mothers). The "Health and social background questionnaire" and "The scales of child's behavior at home" were completed by the teachers and parents, respectively. Statistical analyses were performed using SPSS statistical software. The study had a cross-sectional design and therefore only associations between parameters and not causal relationships could be deduced. The main aim of this part was to look for associations between neurobehavioral performance (dependent variable) and a set of predictors (independent variables). Independent variables were chosen as follows:**

PCBs serum concentrations, concentrations of heavy metals (Pb, Hg, Mn), thyroid hormones (T_3, FT_4, TSH), and questionnaire and scale data. For testing of the validity of these associations, the multifactor analysis of variance or multiple linear regression analysis were used.

Results and Discussion

Association between neurobehavioral performance and PCB exposure**. Since the serum concentrations of PCB sums in children were not normally distributed and were skewed towards higher levels (Figure 1), in some instances the PCB serum concentrations were expressed in percentiles for statistical analysis.**

Figure 1: Frequency diagram of PCBs level in children.



Simple reaction time. Table 2 summarizes data on the significant associations between simple reaction time, a basic parameter for evaluation of sensomotor functions, and independent variables. The simple reaction time in the cohort of our children living in an area polluted by PCBs was the function of PCB serum concentrations, their gender and birth weight. Other independent variables did not reach the level of significance at $\alpha = 0.05$. The performance of children with PCB serum concentrations in the 4^{th} quartile, with lower birth weight (<3500 g) and in girls was found to be worse in the test of simple reaction time.

Table 2. Associations among simple reaction time and PCB serum concentrations, gender of children and birth weight. Results of multifactor analysis of variance.

	Mean Square	F	P value
Corrected	517021.7	12.3	<0.001
Quartiles of PCB serum concentrations	589770.7	14.0	<0.001
Gender	422991.7	10.0	0.002
Birth weight	412492.0	9.78	0.002

Vienna color discrimination test, **total number of hits evaluated**. Vienna color discrimination test is targeted at sensomotor functions as well, but if compared with simple reaction time, it involves also a discrimination process. In this version, the total number of hits was evaluated. Table 3 summarizes data on association between results of Vienna color discrimination test and significantly associated independent variables: PCB serum concentrations and age of children. The performance of children with higher blood sera PCBs levels was decreased in Vienna discrimination test (number of hits). It was also found in this test that significantly higher number of hits was attained by older children.

Table 3. Association among Vienna discrimination test (sum of hits) and PCB serum concentrations and age of children (days). Results of multifactor analysis of variance.

	Mean Square	F	p value
Corrected	3265.5	25.0	<0.001
PCB serum concentration	544.2	4.2	< 0.042
Age of children	4514.3	34.5	< 0.001

Vienna discrimination test, number of correct hits evaluated. In this version of the Vienna discrimination test, contrary to the previous one, only the number of correct hits was evaluated. Table 4 summarizes data on the association between Vienna color discrimination test results and significantly associated independent variables: groups of children with following ranges of PCB serum concentration: <200; 200-600; >600, education of the mother, gender of children and age of children. Children with higher PCBs levels and also children with mothers with elementary school education had worse performance in Vienna discrimination test. Girls performed a higher number of correct hits than boys. A significantly better performance in this test in older children was found.

Table 4. Association between the results of Vienna discrimination test (sum of correct hits) and of PCB serum concentrations, education of mother, gender and age of children. Results of multifactor analysis of variance.

	Mean Square	F	p value
Corrected	348049.0	2385.0	<0.001
Ranges of PCB serum concentration <200; 200-600; >600[ng/g serum lipids]	1024.2	7.0	0.001
Education of mother	2170.4	14.9	< 0.001
Gender of children	730.5	5.1	0.03
Age of children	12651.4	86.7	< 0.001

Benton Visual Retention Test. This test was designed to assess the visual perception, visual memory, and visuoconstructive abilities. Table 5 summarizes data on the associations between results of Benton visual retention test and significantly associated independent variables: FT_4 and PCB serum concentrations. Other independent variables did not reach level of significance at $\alpha = 0.05$. It can be seen from Table 5 that children with higher PCBs levels and higher FT_4 serum levels had worse performance in Benton visual retention test.

Table 5. Associations between the results of Benton memory test and FT_4 and PCB serum concentrations. Results of multiple linear regression analysis.

	ß	T	p-value
FT_4	-0.157	-3.259	0.001
PCB serum concentration	-0.143	-2.958	0.003

Scale of behavior at home and at school. Scale of behavior is a very important indicator of general behavior of the child at home and at school. It is targeted at the identification of subtle changes in behavior and possible associations with environmental exposures. Manifestation of hyperactive behavior of children at home was positively correlated with both, PCB serum concentrations and blood Pb levels.

Table 6. Associations between the results of Scale of behavior at home and Pb blood concentration [µmol/L] and PCB serum concentrations. Results of multifactor analysis of variance.

	Mean Square	F	p value
Corrected	45456.4	397.8	<0.001
Pb_{208}	613.3	5.4	0.021
PCB serum concentration	6510.9	53.8	<0.001

Table 7 shows the association among the results of Scale of behavior at school, PCB serum concentrations and gender and age of children. Younger boys showed more hyperactive behavior at school; and in these children, PCB serum levels were positively associated with hyperactive behavior.

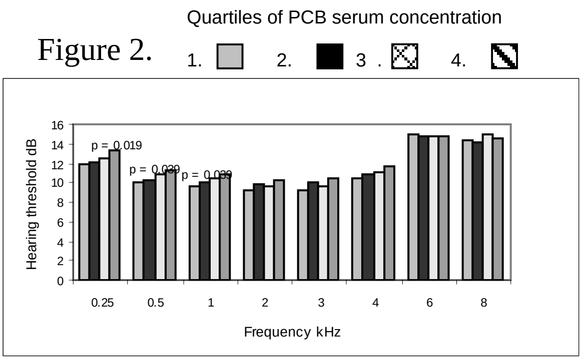
Table 7. Associations between the results of Scale of behavior at school and PCB serum
concentrations and gender and age of children. Results of multifactor analysis of variance.

	Mean Square	F	p value
Corrected	5188.7	142.8	0.001
PCB serum concentrations	106.5	2.9	0.008
Age of children	215.0	6.0	<0.001
Gender of children	860.4	23.7	<0.001

Environmental exposure to PCBs and other noxious chemical substances in the prenatal and perinatal time indicated the strong effect on neuropsychological outcomes in school period of children[,9,11,12]. In agreement with findings of other authors[9,11], we found in a population of children environmentally exposed to PCBs a significant association between neurobehavioral performance and PCBs levels and other potential predictors of environmental, social and hormonal nature. Relevant to our data is a report[1] on alteration of neurobehavioural performance in 4-year-old children environmentally exposed to low-doses of PCBs in which authors emphasized that prenatal exposure (assessed by umbilical sera PCB levels) predicted poor short–term memory function in both verbal and quantitative tests.

Association between hearing functions and PCB exposure. **Pure tone audiometry**. The results of the examination of children by pure tone audiometry are presented in Figure 2. The data are averages for the left and right ear. It can be seen that the increases of the hearing thresholds at the low frequencies were significantly associated with the PCB serum concentrations expressed as quartiles. For statistical treatment was used one way analysis of variance. This finding is in agreement with the data obtained in experimental animals exposed to PCB[8].

Figure 2.



Transient evoked otoacoustric emissions. The source of data was the examination protocol ILO 88 Otodynamics. The frequency diagram of the average sound pressure level of the transient otoacoustic emissions (mean response) for the mean of the left and right ear is shown in Figure 3. The indicated bimodal distribution of the emissions was not confirmed by statistical methods and distribution is normal. Figure 4 shows the same data distributed according to the serum PCB concentrations expressed as quartiles. It can be seen that a decreasing emission level is associated with an increase of PCB serum levels. This association was found also by multiple linear regression analysis, the results of which are shown in Table 8.

Figure 3. The frequency diagram of the average sound pressure level of the transient otoacoustic emissions (mean response) for the mean of the left and right ear.

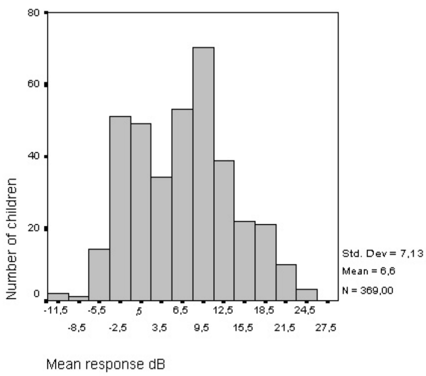


Figure 4. Average sound pressure level of the transient otoacoustic emissions (mean response) for the mean of the left and right ear according to quartiles of PCB serum level.

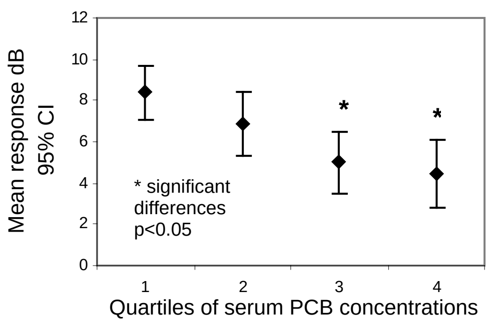


Table 8. Association between sound pressure level of transient evoked otoacoustic emissions and education of the mother and PCB serum concentration.

	β	t	p value
Constant		7.1	<0.001
Education of mother	-0.163	-3.0	0.003
PCB serum concentration	0.162	-3.0	0.003

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