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Serum PCB concentrations and cochlear function in 12-year-old children

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Abstract

Experimental evidence from animals indicates that exposure to polychlorinated biphenyls (PCBs) causes deterioration of the outer hair cells (OHCs) of the cochlea. To test this hypothesis in humans, we measured serum PCB concentrations in 574 12-year-old children residing in three districts in the Slovak Republic using high-resolution gas chromatography with micro-electron capture detection. As a marker of cochlear status, we measured transient evoked (TE) and distortion product (DP) otoacoustic emissions (OAEs), and assessed the cross-sectional association between serum PCBs and OAEs. Median total PCB concentrations were 352.8, 150.5, and 134.9 ng/g lipid in Michalovce, Svidnik, and Bratislava, respectively. In multivariate regression models where otoacoustic measures were modeled as a function of log (base 10) PCB concentrations with adjustment for gender, age, and site of examination, dioxin-like PCBs, non-dioxin-like PCBs and a PCB grouping targeting upregulation of hepatic uridine 5′-diphospho-glucuronosyltransferase were significantly associated with lower TEOAE powers at 1000 and 1500 Hz. At 1500 Hz, we observed a strong association with sum of PCBs and DL-PCBs, in the left ear only. The DPOAEs at 1000 Hz were associated with all 4 PCB groupings. The results of this study show that PCBs may affect the OHCs of the cochlea, a result consistent with findings from animal studies published to date.

Introduction

Man-made polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental pollutants and their role in developmental toxicity is of great concern. PCBs have been classified as developmental neurotoxicants (1–4), and data indicate that developmental exposure to PCBs can result in auditory impairment. For instance, prenatal exposure to Aroclor 1254 has been associated with hearing deficits when rats were tested by pure tone audiometry at the lowest frequency (1 kHz) (5), and brainstem auditory evoked responses in rats suggest

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Brief
Associations between PCB concentrations and otoacoustic measures assessed at 12 years of age were observed in children living in the Slovak Republic.

a cochlear and/or auditory nerve site of Aroclor 1254 action (6). Surface preparations of the organ of Corti in animals exposed to Aroclor 1254 have revealed a mild to moderate loss of OHCs in the upper-middle and apical turns, linking the loss of low-frequency hearing to a loss of OHCs (7). In adult rats exposed early in development to PCBs, performance on the distortion product otoacoustic emissions (DPOAEs) test was reduced (8). Auditory impairment was also observed in a study which dosed rats with a PCB mixture formulated to model the congener profile found in fish consumed by a human population in northeastern Wisconsin (9).

In humans, a study of 7-year-old children prenatally exposed to seafood neurotoxicants (10) showed increased hearing thresholds in the left ears (for 2 out of 8 frequencies) in relation to prenatal PCB exposures. In another study which included boys of fish-eating mothers, the more exposed east coast boys did not differ from the regional reference population with respect to hearing ability (11). Finally, PCB concentrations in maternal serum were examined in relation to audiometrically-determined hearing thresholds among offspring at 8 years of age. Maternal serum PCB concentrations were found to be unrelated to the adjusted odds of sensorineural hearing loss (12). In our previous study from the same cohort of children, serum PCB concentrations of children aged 8–9-years were associated with an increase in hearing thresholds at low frequencies, while a negative relationship between serum PCBs and the amplitude of transient evoked otoacoustic emissions (TEOAEs) response was observed in the uppermost tertile of serum PCBs (13). The current study extends the age of auditory assessment into later childhood/early adolescence, and includes an evaluation of DPOAEs, which add additional information about cochlear status.

Materials and Methods

Study sample

Children were recruited from three districts of Slovakia with varying degrees of environmental PCB contamination: Michalovce, an area with a high level of environmental PCB contamination (14,15); Svidnik, an area with moderate PCB contamination, and Bratislava, an urban center with generally low levels of environmental PCB contamination. From these districts, we sampled children from five schools in Michalovce, four in Svidnik, and seven in Bratislava. To be eligible for our study, children must have been 12 years of age at the time of sampling, and born to mothers who had been living in the same residence for at least 5 years before their child’s birth. Approximately 1100 families were approached, and 728 gave written informed consent for their participation in the study. Of these 728 families that provided written consent, a hearing examination and PCB determinations were completed for 574. The reasons for incomplete data were most often the result of insufficient blood volume for PCB determination or refusal of blood draw. At the analysis stage, we also excluded 54 children with middle ear pathology, a family history of hereditary hearing loss, active or recent otologic disease, a history of ear surgery, past exposure to ototoxic drugs, and those children who had an abnormal tympanogram (described below). Finally, five serum samples were damaged during analysis and 95 families missed appoints for the hearing examination. The study was approved by the Ethics Committee of the Slovak Medical University.

Otologic and audiological assessments

Before conducing the specific audiological assessments (tympanometry and OAEs) children were given an otoscopic examination to ensure that the ear was healthy and that the ear canal was free of obstructions. Hearing thresholds were tested by pure-tone audiometry in order to exclude children with serious hearing loss, which would prevent the evaluation of OAEs. Audiological assessments were performed at the Military Aviation Hospital in Košice, Slovakia for children living in the Michalovce and Svidnik regions, and at the Children’s

Faculty Hospital in Bratislava for those from the Bratislava region. Assessments were conducted in a sound-proof room at both hospitals.

Tympanometry

After examination of the outer and middle ear, tympanograms were assessed by a Siemens SD-30 tympanometer (Munich, Germany) in Košice and by an INTERACOUSTICS Impedance audiometer AZ 26 tympanometer (Yarmouth Maine, USA) in Bratislava. If the tympanogram was classified as a “B” or “C” according to Jerger’s classification (16), children were excluded from the study.

OAEs

OAEs are sounds of cochlear origin, which can be recorded by a microphone fitted into the ear canal. They arise in the ear canal when the tympanum receives vibrations transmitted backwards through the middle ear from the cochlea and are caused by the motion of the cochlea’s sensory hair cells as they energetically respond to auditory stimulation. These vibrations occur as a by-product of a unique and vulnerable cochlear mechanism which has become known as the “cochlear amplifier” and which contributes greatly to the sensitivity and discrimination of hearing (17).

TEOAEs represent the sum of the pulse responses of outer hair cells (OHCs) along the cochlea whereas DPOAEs represent cubic distortion product generated by the cochlea when stimulated simultaneously by two tones, f_1 and f_2. Thus TEOAEs and DPOAEs furnish complementary information. In humans the $2f_1 – f_2$ distortion component yields the highest amplitude (18). At both sites (Košice and Bratislava), OAEs were recorded by the same Echoport ILO 292 USB-I Otodynamics Ltd (Hatfield, Herts, United Kingdom) connected to a personal computer equipped with ILO V6 software. The “Quickscreen” version of software was employed. First, a standard ILO probe with a disposable tip was applied, and ear canal response was used to check fitting conditions of the probe. The stimulus intensity was set at 84 ± 3 dB peak equivalent. To ensure reliability across time, calibration of the probe was done on a weekly basis. For TEOAEs, a nonlinear method of recording was used, and the noise rejection level at the probe tip was set to 47 dB. During data collection, TE stimuli were delivered to the ear and OAE response data collected. Multiple stimulus presentations and response averaging are required to extract TEOAE responses from background noise. The response was averaged from 260 stimulus repetitions and an OAE waveform was displayed in the time domain.

The OAE response (total power in dB sound pressure level (SPL)) was transformed by Fourier transformation which converts the time-varying soundwave patterns into frequency spectra. The OAE (power dB SPL) and noise energy were thus displayed in a frequency spectrum as a half-octave histogram. For a more detailed acoustical analysis, we used half octave bands centered at 1000, 1500, 2000, 3000 and 4000 Hz. The signal-to-noise ratio (SNR) in dB units was calculated as the difference between the OAE response (dB SPL) and the noise level (dB SPL). DPOAEs were measured in response to pairs of primary tones ($f_2>f_1$), with f_2 set at default frequencies. The f_2/f_1 ratio was 1.22 for each primary pair. The f_1 level was 65 dB and f_2 55 dB. A signal analyzer divided the ear canal signal into its discrete frequency components so that DPOAEs at the $2f_1—f_2$ frequency were extracted as amplitude spectra. DPOAE findings were presented as the Average of DP 1/2 half octave amplitudes and as DP half-octave octave power in dB SPL units for 1000, 2000 and 4000 Hz. OAE recordings with reproducibility ≤80 % and the SNR ≤3 dB were excluded. The known nonpathologic factors influencing measurement of OAEs (19) were kept under control.

A different nurse from the two hospitals carried out the OAE measurement. The inter-examiner variation was evaluated in 18 subjects for each OAE parameter using a T-test. The mean difference between the two examinations was −0.208 dB SPL, 95% CI (−0.52, 0.104) for total TEOAE power and 0.216 dB SPL, 95% CI (−0.226, 0.658) for Average DPOAE power.

Collection and analysis of blood samples

To assess PCB concentrations, approximately 13 ml of blood was drawn from children in a fasting state. Samples were centrifuged to isolate serum, and approximately 5–6 ml of serum was extracted. Serum samples were kept frozen at −18 °C until analysis. Solid-phase extraction followed by a clean-up procedure and high-resolution gas chromatography with micro-electron capture detection was used for the analyses of PCBs (20,21). Fifteen PCB congeners (PCB-28, 52, 101, 105, 114, 118, 123^{+149}, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180, 189, IUPAC Nos.) were analyzed. Serum sample spiked with an extraction standard (PCB-174) was mixed with an equivalent amount of water - 1-propanol (85:15, v/v) mixture and sonicated. A solid phase extraction column (1g/6ml Extract-Clean High Capacity C_{18} endcapped, Alltech Associates, Inc., Belgium) conditioned with 5 ml of methanol was used. The analytes were eluted with n-hexane - DCM (1:1, v/v). The residues diluted in n-hexane were purified on a florisil-silica gel column that was treated with sulphuric acid. The analytes were eluted with 10-% DCM - n-hexane and concentrated. The cleaned-up extract was diluted with syringe standard (PCB-103) and analyzed by a Gas Chromatograph 6890N (Agilent Technologies, DB-5 capillary column 60 m × 0.25 mm ID × 0.25 μm film thickness, J&W Scientific, USA) equipped with an electron capture detector.

External standard calibration was used for quantification of PCBs. The five calibration levels of PCBs ranged from 0.5 to 200 ng/ml. Recovery was checked using PCB-174 added to each sample. PCB-103 served as a syringe standard to correct volume of samples analyzed. An analytical batch consisted of 10 serum specimens, one solvent blank, and one in-house reference sample (spiked porcine serum). The reported concentrations were blank corrected and adjusted by recovery rates. The limits of detection (LOD) were set to three times the baseline noise. Control charts were plotted for daily standard solution responses and QC sample analysis as a basis for checking the precision and reliability of the analytical process. The laboratory has periodically participated in the *External Quality Assessment Scheme Intercomparison Programme* for environmental medical toxicological analysis (PCBs in ram serum, Erlangen, Germany). Serum lipids were determined by enzymatic summation method (22).

Categorization of exposure variables

A "sum" variable was created which was the arithmetic sum of PCB congeners (28, 52, 101, 105, 114, 118, 123^{+149}, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180, 189, IUPAC Nos.). Besides using the sum of PCBs as an independent variable, PCBs were categorized into three groups based on their chemical structures, the dioxin-like (DL-PCBs), non-dioxin-like PCBs (NDL-PCBs) (23,24) and possible PCB inducers of the microsomal enzyme uridinediphosphate glucuronosyltransferase (UGT) (THY-PCBs) (25). PCB congeners 118 and 156 were regarded as DL-PCBs (26). For NDL-PCBs 138,153, 170, and 180 were included (27) and for THY-PCBs 52, 99, 101, 118, 153, 156, 157, 167, 180, 183, 187, 189, 194, and 199 (25).

Statistical analysis

For individual PCB measurements with concentrations below the limit of detection (LOD), we imputed by taking the LOD value divided by the square root of 2 if PCB congeners had fewer than 20% of samples below the LOD; Otherwise the LOD values were divided by 2. In crude analyses, the positively skewed PCB serum concentration distribution was categorized into tertiles. Regarding side and gender differences in ear functions (28–35), the auditory outcomes were analyzed separately for left and right ears. For multivariate analysis, a linear model was applied for each ear side adjusted for gender, age (days) and examination site with OAEs being the dependent variable. In these models PCB concentrations were base 10 log transformed. All statistical analyses were performed using the SPSS for Windows statistical package (version 14.0; SPSS Inc., Chicago, IL, USA).

Results

The concentration of the sum of PCBs in serum of children is shown in Table 1.

When children from all regions were split into tertiles with regard to sum of PCB serum concentration (ng/g serum lipids), the boundaries of tertiles were 129 and 262.7 and the following data were obtained (mean ± SD; median): 1. tertile 85.2±26.1; 86.2, 2. tertile 185.8 ±39.6; 183.9 and 3. tertile 701.2±670.4; 485.0. Table 2 shows the median total TEOAE and DPOAE powers in ears of children grouped into tertiles with regard to sum of PCB serum concentration and the median values of the TEOAE and DPOAE powers at the individual frequencies. Multiple linear regression analysis was used to test the association between exposure variables and auditory outcomes (Table 2).

The summary of estimated coefficients, their standard errors, and p-values for exposure variables in multiple linear regression models predicting TEOAEs and DPOAEs after adjusting for gender, age and site of examination are shown in Tables 3 and 4.

Shown are only data for covariates for which $p \leq 0.05$. There were no associations between the TEOAE total power and any of the PCB groupings, however there were strong associations on both ear sides for all four PCB groupings with TEOAE power at 1000 Hz when adjusting for age and gender. The associations remained significant but less pronounced after adjusting also for site of examination for the left ears. For the right ears they were present only for the DL-PCB grouping. At 1500 Hz was seen strong association with sum of PCBs and DL-PCBs on the left side. There were no associations observed at higher frequencies. Age was not associated with TEOAE power and gender predicted TEOAE, but did not interact with exposure to PCB.

Table 4 summarizes the associations between PCB exposures and DPOAEs. Age and gender did not predict any DPOAE outcomes. In the left ears after adjustment for gender and age there was an association between a decrease of DPOAE powers at 1000 and 2000 Hz and exposure to all PCB groupings. After adjusting also for site of examination significant associations remained for 1000 Hz for all 4 groupings. In the right ears there was a similar pattern.

Discussion

We have observed associations between environmental exposure to various subsets of PCB congeners and deficits in cochlear functions in children. In the current study, multiple regression analysis with adjustment for age, gender and examination site did not show any association between PCB exposure and total TEOAE power, however the TEOAE powers at low frequencies were predicted by PCB concentrations. The gender did not interact with association between PCB exposure and TEOAE powers. It is remarkable that an association was observed in the right ears at 1000 Hz only with the DL-PCBs.

The DPOAEs were found to be also strongly associated with PCB exposure. Again, the low frequencies appear to be the most affected. Decreased amplitudes of DPOAEs were seen in girls at all frequencies, but not in boys, similar to gender differences in rats (8,9). Surprisingly, associations at higher DPOAE frequencies were observed, contrary to the TEOAE pattern and observation in rats (8,9). The left ears showed greater deficits compared to the right ones, also seen in noise induced hearing loss (36–38).

The mechanism of the effect of PCBs on hearing in man is unknown (39). In rats, with markedly higher PCB exposures compared to our adolescents, ototoxicity involves early postnatal exposure to PCBs via lactation, an upregulation of hepatic UGTs, and subsequent hypothyroxinemia during a critical period of cochlear development, with the ultimate neurotoxic consequence of hearing loss (39). In man cochlear development is completed prenatally, but PCB exposure is greatest during breast-feeding. Thus hypothyroxinemia as a result of a possible upregulation of UGTs does not seem to play an important role in humans. With regard to the discrepancy between sensitive window and peaking PCB level in humans, other mechanisms of effect have to be sought. The association between the auditory outcomes and THY-PCBs was similar with sum of PCBs and NDL-PCBs. Moreover in untreated congenital hypothyroid newborns no correlation was found between outer hair cell dysfunction and hypothyroidism (40).

In the current study there were no striking differences between associations of the auditory outcomes with any of the PCB grouping tested. The concentrations of the individual congeners in body matrices are interrelated and this may help to explain this finding. It has to be mentioned in this connection that noncoplanar PCBs are sensitizers of ryanodine receptor calcium channels (41) found also in OHCs (42,43). The role of noncoplanar PCBs in disturbances of neurodevelopment, including plasticity in rat primary auditory cortex (44), are recently intensely studied (45,46).

When comparing the results of the current study with previous observations, one must consider the serum PCB concentrations. The PCB blood concentrations in the area of the current study exceed the concentrations of PCBs observed in several cohorts in various parts of the world (47). The exposure level can be also compared with data from NHANES (48). The 95th percentile of lipid adjusted PCB 153 serum level was 30.3, while for Michalovce, Svidnik and Bratislava 530.8, 225.8 and 184.0 (ng/g lipids), respectively, was obtained.

There are important differences in the assessment of hearing between the current study and previous animal studies which make direct comparisons difficult. For instance, in the current study, both TEOAEs and DPOAEs were assessed, while animal studies (8,9) employed DPOAEs which included the assessment of thresholds. Due to time constraints we did not examine thresholds for the DPOAE measures. There is an agreement between our observation of deficits in low frequency TEOAEs and published pure tone audiometry and histopathological data (7,49).

In statistical analysis we have evaluated separately the hearing outcomes in the left and right ears and adjusted for age, gender and site of examination. The possible interaction of the latter with OAEs is unclear as OAEs measures are rather slightly influenced by a variety of physiological characteristics (17,19) and in our setting we have been taking account of them. Concerning additional potential confounders, other research indicates that iodine deficiency and severe caloric restriction may impair hearing among children (50–52). However given that the status of iodine nutrition in Slovakia is presently normal (53,54), and that adolescents in our study were of healthy weight, we did not consider measures of iodine deficiency or caloric intake as a potential confounders.

The observed changes in cochlear status are subclinical and are unlikely to interfere with neurobehavioral development and speech comprehension among adolescents. However, in combination with exposure to solvents, ototoxic drugs, and noise, the adverse otological outcomes may be potentiated.

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TABLE 1

Data on number of subjects included into the study, their gender, age and concentration of sum of PCBs in serum. MI, SV and BA stands for Michalovce, Svidnik and Bratislava regions, respectively.

Region	Number	Age (years)	Sum of PCBs serum concentrations (ng/g serum lipids)				
		Mean ± SD	Min	25th percentile	Median	75th percentile	Max
MI - boys	92	12.5±0.61	36.9	221.2	410.8	806.5	4443.8
MI - girls	90	12.6±0.63	38.7	184.2	326.7	545.5	5327.7
SV - boys	83	12.7±0.74	14.5	104.4	151.6	222.9	1597.7
SV - girls	80	12.5±0.78	36.5	78.5	146.1	240.7	1407.3
BA - boys	94	12.8±0.71	44.3	105.2	147.2	232.9	922.9
BA - girls	134	12.7±0.61	34.4	80.4	117.9	213.2	1657.9

TABLE 2

Medians of TEOAE total power and TEOAE powers at various frequencies and DPOAE Average power and DPOAE powers at various frequencies (dB SPL) in ears of adolescents grouped into tertiles with regard to sum of PCBs serum concentration.

TEOAE					**DPOAE**				
Power	**Gender**	**Tertile**	**Left Ears**	**Right Ears**	**Power**	**Gender**	**Tertile**	**Left Ears**	**Right Ears**
Total	Boys	1.	13.15	12.8	Average power	Boys	1.	11.1	10.55
		2.	12.8	13.9			2.	11.05	11.2
		3.	12.4	13.8			3.	10.15	11.4
	Girls	1.	13.3	14.15		Girls	1.	11.65	11.7
		2.	13.3	13.6			2.	10.55	11.75
		3.	13.5	13.7			3.	11.0	11.4
At 1000 Hz	Boys	1.	1.2	1.7	At 1000 Hz	Boys	1.	5.6	7.4
		2.	0.2	1.35			2.	6.3	5.95
		3.	−3.0	0.15			3.	−2.5	1.6
	Girls	1.	1.9	1.5		Girls	1.	6.4	6.4
		2.	−0.05	0.0			2.	2.8	6.05
		3.	−0.9	−0.3			3.	−2.9	2.35
At 1500 Hz	Boys	1.	7.95	6.6					
		2.	6.2	7.15					
		3.	6.5	8.1					
	Girls	1.	7.5	7.95					
		2.	7.3	7.85					
		3.	5.6	7.8					
At 2000 Hz	Boys	1.	4.65	5.1	At 2000 Hz	Boys	1.	8.95	9.0
		2.	5.4	5.4			2.	9.8	10.7
		3.	4.7	6.85			3.	7.0	8.8
	Girls	1.	6.1	7.0		Girls	1.	9.8	9.6
		2.	7.2	5.95			2.	5.8	8.45
		3.	5.6	7.0			3.	9.0	9.8
At 3000 Hz	Boys	1.	5.8	5.5					
		2.	5.6	5.2					
		3.	6.0	6.35					

TEOAE					DPOAE				
Power	**Gender**	**Tertile**	**Left Ears**	**Right Ears**	**Power**	**Gender**	**Tertile**	**Left Ears**	**Right Ears**
At 4000 Hz	Girls	1.	6.1	6.8					
		2.	7.5	6.6					
		3.	7.5	7.7					
	Boys	1.	1.45	3.4	At 4000 Hz	Boys	1.	9.7	9.25
		2.	2.5	3.5			2.	9.7	9.95
		3.	1.0	1.55			3.	10.2	11.5
	Girls	1.	3.5	2.8		Girls	1.	10.95	11.25
		2.	3.3	2.5			2.	10.8	10.4
		3.	3.4	3.0			3.	9.8	10.1

TABLE 3

Estimated coefficients with p-values of PCB grouping subsets in relation to TEOAE total power and power at 1000, 1500, 2000, 3000, and 4000 Hz (dB SPL) in combined children group from Michalovce, Svidnik and Bratislava. Adjustment for gender, age and site of examination. Variables with association p≤0.05 are not listed.

		Left Ears			Right Ears		
Power	**Variables**	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**
Total	Sum-PCB	−0.335	0.65	0.609	0.315	0.72	0.662
	Gender	−1.149	0.41	0.005			
	DL-PCB	−0.585	0.48	0.227	−0.184	0.53	0.726
	Gender	−1.117	0.41	0.007			
	NDL-PCB	−0.294	0.64	0.648	0.329	0.71	0.643
	Gender	−1.153	0.41	0.005			
	THY-PCB	−0.167	0.66	0.801	0.468	0.725	0.519
	Gender	−1.285	0.42	0.003			
At 1000 Hz	Sum-PCB	−3.564	1.3	0.006	−1.58	1.36	0.246
	DL-PCB	−3.018	0.96	0.002	−2.433	0.99	0.014
	NDL-PCB	−3.473	1.28	0.007	−1.425	1.34	0.289
	THY-PCB	−3.064	1.32	0.021	−1.341	1.383	0.333
At 1500 Hz	Sum-PCB	−2.14	1.05	0.042	−0.174	1.17	0.881
	DL-PCB	−2.262	0.77	0.003	−1.21	0.85	0.154
	NDL-PCB	−2.011	1.03	0.052	−0.107	1.15	0.926
	THY-PCB	−1.78	1.065	0.095	0.075	1.183	0.95
At 2000 Hz	Sum-PCB	−0.138	0.85	0.87	0.687	0.95	0.472
	Gender	−1.531	0.53	0.004			
	DL-PCB	−0.559	0.62	0.37	0.219	0.697	0.754
	Gender	−1.525	0.53	0.004			
	NDL-PCB	−0.094	0.83	0.91	0.658	0.94	0.485
	Gender	−1.533	0.53	0.004			
	THY-PCB	0.001	0.863	0.999	0.896	0.968	0.355
	Gender	−1.644	0.551	0.006			
At 3000 Hz	Sum-PCB	0.599	0.76	0.429	0.632	0.86	0.463
	Gender	−1.63	0.48	0.001			

		Left Ears			**Right Ears**		
Power	**Variables**	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**
At 4000 Hz	DL-PCB	0.063	0.56	0.172	0.494	0.629	0.432
	Gender	−1.555	0.48	0.001			
	NDL-PCB	0.606	0.75	0.416	0.601	0.85	0.48
	Gender	−1.636	0.48	0.001			
	THY-PCB	0.667	0.774	0.39	0.76	0.864	0.38
	Gender	−1.754	0.494	<0.001	−1.05	0.544	0.054
	Sum-PCB	0.9	0.81	0.268	−0.285	0.94	0.762
	Gender	−1.783	0.51	0.001			
	DL-PCB	0.293	0.6	0.626	−0.125	0.686	0.856
	Gender	−1.709	0.51	0.001			
	NDL-PCB	0.944	0.8	0.238	−0.29	0.93	0.755
	Gender	−1.792	0.51	<0.001			
	THY-PCB	1.021	0.821	0.214	−0.397	0.948	0.675
	Gender	−1.948	0.524	<0.001			

TABLE 4

Estimated coefficients with p-values of PCB grouping subsets in relation to DPOAE Average power and power at 1000, 2000 and 4000 Hz (dB SPL) in combined children group from Michalovce, Svidnik and Bratislava. Adjustment for gender, age and site of examination. Variables with association p≤0.05 are not listed.

		Left Ears			**Right Ears**		
Variables	**Power**	**β**	**SE**	**p-value**	**β**	**SE**	**p-value**
Sum-PCB	Average	−0.645	3.515	0.427	−1.652	0.88	0.061
	1000 Hz	−3.464	1.59	0.03	−1.703	1.44	0.239
	2000 Hz	−2.502	1.43	0.08	−1.052	1.33	0.428
	4000 Hz	−0.574	1.1	0.6	0.474	1.1	0.665
DL-PCB	Average	−0.516	0.63	0.414	−1.674	0.68	0.014
	1000 Hz	−2.595	1.21	0.033	−2.263	1.11	0.041
	2000 Hz	−2.031	1.08	0.61	−1.368	1.01	0.178
	4000 Hz	−0.973	0.83	0.242	0.041	0.84	0.961
NDL-PCB	Average	−0.604	0.8	0.45	−1.574	0.87	0.071
	1000 Hz	−3.308	1.57	0.036	−1.596	1.42	0.262
	2000 Hz	−2.48	1.41	0.079	−0.957	1.31	0.464
	4000 Hz	−0.515	1.08	0.634	0.510	1.08	0.636
THY-PCB	Average	−0.485	0.81	0.552	−1.547	0.87	0.077
	1000 Hz	−3.227	1.62	0.048	−1.36	1.47	0.355
	2000 Hz	2.415	1.45	0.097	−0.854	1.35	0.527
	4000 Hz	−0.496	1.11	0.654	0.632	1.1	0.568