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PCB exposure and cochlear function at age 6 years [★]

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Abstract

Epidemiological studies have documented adverse associations between exposure to polychlorinated biphenyls (PCBs) and otological outcomes. Previously, we documented decreased distortion product otoacoustic emission (DPOAE) levels in children exposed to PCBs, up to the age of 45 months, amongst a cohort of children in eastern Slovakia. The objective of the present study is to evaluate cochlear dysfunction at 72 months of age in 214 children from this same cohort and to compare the otoacoustic test sensitivity to that of pure tone audiometry (PTA). The association between DPOAE, PTA, and PCBs was estimated by means of multivariate ANOVA (MANOVA) and linear regression models. ROC curves were computed to estimate the DPOAE-test power in children. The DPOAE level at 72 months was related to PCB-153 serum levels. The DPOAE Input/Output function test at mid-frequency (2 kHz) has shown instead nonmonotonic dependence on PCB exposure, for the left ears of children, over the whole growth curve. No significant association was found between PTA hearing levels and PCB-153 concentration. High diagnostic power of the DPOAE-test was found in children, similar to that found by the same authors in adults.

In conclusions the DPOAE-PCB correlation obtained at 72 months is similar to that at 45 months suggesting a permanent and stable ototoxic effect of the PCB exposure. The lack of statistical significance of the PCB-PTA correlation suggests that DPOAEs are sensitive biomarkers of cochlear damage.

[★]The study protocol was approved by the Institutional Review Board at the Slovak Medical University.

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Keywords

Ototoxicity; Polychlorinated biphenyls; Pure tone audiometry; Otoacoustic emissions; Hearing impairment

1. Introduction

Persistent organic pollutants (POPs) are organic compounds of anthropogenic origin that resist degradation and accumulate in the food chain (Agency for Toxic Substances and Disease Registry, 2000). Important members of this group of chemicals are the polychlorinated biphenyls (PCBs) which were used for a variety of industrial processes and purposes. An association between exposure to polychlorinated biphenyls (PCBs) and deficits in the auditory system have been documented in experimental studies on animals (Crofton and Rice, 1999; Crofton et al., 2000a, 2000b; Goldey et al., 1995; Herr et al., 1996; Powers et al., 2006; Crofton and Zoeller, 2005). The link between PCB exposure and hearing impairment in humans has been described in epidemiological studies in infants (Jusko et al., 2014), 8–9-year old children (Trnovec et al., 2008), adolescents (Trnovec et al., 2010), and adults (Min et al., 2014).

In a previous study (Jusko et al., 2014), we observed an association between increased PCB serum concentrations and decreasing DPOAE amplitudes at age 45 months, and that this association was specific to the postnatal rather than maternal or cord PCB concentrations. The present study is a follow-up of these same children at 72 months of age, with the aim of determining whether the observed adverse associations persist into later ages. An additional aim was to examine the usefulness of the DPOAE Input/Output (I/O) test for detection of cochlear dysfunction related to environmental exposure to PCBs. Finally, we aimed to examine the relationship between children's hearing levels (HL) determined audiometrically and the DPOAE amplitudes.

2. Materials and methods

2.1. Subjects

The present study is an extension of a previously described one (Jusko et al., 2014). In that study, 351 children were followed through 45 months of age, and PCB-153 concentrations were measured in cord blood and at children's serum at 6, 16 and 45 months. Auditory function was monitored at 45 months by determination of DPOAEs. Presently, a subgroup of 214 children of the same population were assessed to collect audiological data, DPOAEs, DP growth rate and PCB serum concentration at the age of 72 months. The number of participating children is a result of attrition, diseases as otitis media, or availability of parents on the day of examination. We assume on the basis of the data in Table 1 that characteristics of this subset are similar to the larger cohort. Examination of hearing by pure tone audiometry (PTA) and assessment of the I/O function (DP growth) in a single frequency band (2 kHz) was enabled due to better cooperation of children with the examiner at this age.

2.2. Otologic and audiological assessments

As a means of assessing potential PCB-induced ototoxicity, we have employed, as a marker of cochlear status, the measurement of distortion product otoacoustic emissions (DPOAEs) (Kemp, 2002). Otoacoustic emissions (OAEs) are sounds generated in the cochlea, and measured in the ear canal, as a by-product of the activity of the nonlinear amplifier localized in the cochlear outer hair cells (OHCs), which is responsible for the excellent sensitivity and frequency resolution of human hearing. Invasive physiological experiments on animals (e.g., Robles and Ruggero, 2001) have demonstrated that the OHC electromotility provides an additional force on the basilar membrane (BM) that amplifies the response to low-level tones by as much as 40–50 dB. As the OHCs are the most vulnerable part of the hearing apparatus, cochlear damage results in both decrease of the OAE level and increase of the threshold of hearing, which is audiometrically measured.

Among the different types of OAEs, classified according to the stimulus used to evoke them, DPOAEs were chosen as effective biomarkers of the cochlear function because they had demonstrated particularly high correlations (e.g., Gorga et al., 1993; Sisto et al., 2007) with the hearing level measured by pure tone audiometry (PTA). DPOAEs arise from the nonlinearity of the basilar membrane (BM) response. When a nonlinear mechanical system such as the BM is stimulated at two nearby frequencies, f_1 and f_2 , an OAE response is produced also at other frequencies that are specific linear combinations of the stimulus frequencies. These tones are called distortion products, and the most important of them is that of frequency $2f-f_2$. Due to this peculiar spectral signature, DPOAEs are unambiguously interpreted as signals generated in the cochlea, because the cochlear amplifier is the only nonlinear system involved in their generation.

Otologic and audiological assessments were performed at morning appointments by the staff of the Department of Otorhinolaryngology at the Michalovce district hospital. An otoscopic examination was conducted with the aim of excluding children with ear infections or obstructions of the ear canal. The tympanometric test (GSI 38 Auto Tym; Grason-Stadler Inc., Milford, NH, USA) was used to test the middle ear function. Tympanograms were scored following the Jerger's classification (Jerger, 1970). In PTA, the threshold level of hearing is psychoacoustically determined, for a set of standard frequencies, by administering tones of different amplitude in an acoustically insulated environment (audiometric booth), and asking the subject to manifest perception. For each subject, PTA has been performed both by air and bone conduction (acoustic stimulus delivered through an earphone and mechanical stimulus to the mastoid, respectively). The audiometric HL, expressed in dB HL, was evaluated at 125, 250, 500 Hz, 1000, 2000, 4000, and 8000 Hz, with steps of 5 dB, starting from a minimum level of 10 dB HL. These frequencies are standardly used in clinical practice in Slovakia.

2.3. DPOAE recording

DPOAEs were recorded as described previously (Jusko et al., 2014; Sisto et al., 2015) by the Echoport ILO 292 USB-I Otodynamics Ltd. (Hatfield, Herts, UK) instrument connected to a personal computer equipped with ILO V6 software. In these measurements, a miniaturized OAE probe, housing two loudspeakers and a sensitive microphone, is inserted in the ear

canal. The loudspeakers provide the acoustic stimuli, and the evoked acoustic response is synchronously measured by the microphone. 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. Both the f_1 and f_2 levels were set to 70 dB SPL. The frequency of primary tones was varied in one fourth of octave steps. For each scan step, a signal analyzer picked up the discrete frequency component at the DPOAE $2f-f_2$ frequency, getting amplitude spectra with one fourth of octave resolution. DPOAE findings were presented as fourth of octave amplitudes in dB SPL units for $f_2 = 1000, 1189, 1414, 1682, 2000, 2378, 2828, 3364, 4000, 4757$ and 5657 Hz. Although the signal is actually measured at frequency $2f-f_2$, the response is conventionally referred to f_2 , because, according to cochlear mechanics (e.g., Shera and Guinan, 1999) the DPOAE wave is generated in the cochlear region resonating at frequency f_2 , and associated with perception at f_2 . This choice of frequencies covers the most sensitive range of human hearing, which is also that typically first damaged by ototoxic agents. In the ILO DP design, noise is simultaneously measured as the average power spectrum level at a few off-band frequencies around each DP frequency.

In addition to DPOAE spectral measurements at a constant stimulus level, it is possible to record the DPOAE response at a constant frequency as a function of the stimulus level. This test, called DPOAE growth, or I/O, test, was performed with step sizes of 5 dB. Up to seven stimulus levels from L_2 45–75 dB SPL have been applied with f_2 set at 2 kHz, the default frequency recommended by the manufacturer. The primary-tone level difference was increased with decreasing stimulus level resulting in an L_1/L_2 setting described by $L_1 = 0.4 L_2 + 39$. This setting, usually named ‘scissors’ or ‘optimal’ paradigm, is assumed to yield the highest DPOAE level for each L_2 (Kummer et al., 2000). Due to the cochlear nonlinearity, one does not expect proportionality between stimulus and response amplitude, so different stimulus levels set different “working points” of the hearing system, with different gain and bandwidth.

2.4. PCB exposure assessment

Fifteen PCB congeners [IUPAC (International Union of Pure and Applied Chemistry)] numbers 28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, and 189 were determined in child blood serum samples at 72 months with the same protocol as in Jusko et al. (2014). The procedure for quantification the PCB concentrations and terms of QA were described previously: the serum samples were extracted using solid phase extraction method and cleaned up according to Drobná et al. 2011 and Conka et al., 2005, and the extracts were analyzed applying isotope dilution method by high resolution gas chromatography coupled to high resolution mass spectrometry and using all fifteen ^{13}C labeled PCB congeners (Drobná et al. 2011; Jusko et al., 2014). The fifteen labelled PCB standard recoveries met the USEPA Method 1668B criteria (U.S. Environmental Protection Agency, 2008). Wet-weight concentrations (ng/ml) were determined at the Department of Toxic Organic Pollutants at the Slovak Medical University in Bratislava. This laboratory serves as the National Reference Laboratory for Dioxins and Related Compounds for the Slovak Republic and has been certified by the Slovak National Accreditation Service (ISO/IEC 17025:2005, certification no. S-111). Further, this laboratory regularly participates in interlaboratory comparison tests, such as the Intercomparison Programme (German External Quality

Assessment Scheme) (G-EQUAS 2009) and the Interlaboratory Quality Assessment coordinated by the World Health Organization (2000). Although a “sum” variable can be defined, which was the arithmetic sum of PCB congeners, as in Jusko et al. (2014), the analysis was focused on PCB-153 (ng/ml) only. The reason for this choice is that the PCB-153 is highly correlated with total PCB concentration, and it is easily detectable in all child samples. Indeed, 100% of PCB-153 results were above LODs which varied from 0.00005 to 0.00143 ng/ml serum (depending on analysed serum amount and signal to noise ratio). We report wet weight as well as lipid adjusted concentrations. We estimated total serum lipids using the enzymatic summation method (Akins et al., 1989).

2.5. Statistical analysis

All statistical analyses were performed using the software R (R Foundation for Statistical Computing, Vienna, Austria). Owing to the dependence of the different DPOE frequency bands, a multivariate analysis of variance (MANOVA) was used to estimate the association between and test the statistical significance of the relation between the DPOAE amplitude and the PCB serum concentration. DPOAE levels at all primary tone frequencies f_2 were plotted against the natural logarithm ($\log_e$) of the serum concentration of PCB-153. Coefficients of determination R^2 , regression coefficients β , and t -test statistics for significance of β were calculated. Arithmetic means were calculated from the hearing thresholds data. When studying I/O function medians and 95% Confidence Intervals for the DPOAE data were computed. The interpercentile difference was tested by Kruskal–Wallis one-way analysis of variance.

3. Results

3.1. Study participants and PCB concentrations

Compared with the 351 children examined at 45 months, children at 72 months were 214. Median PCB-153 serum concentrations were 0.67 and 0.45 ng/ml at 45 and 72-months, respectively, (Table 2) and they were strongly correlated ($r = 0.94$) (Fig. 1 top).

3.2. Auditory measurements at 45 and 72 months of age

For the DPOAEs measured at 45- and 72-months, the correlation between the two measurements in the same ear was rather high (Fig. 1, bottom). As reported in Table 3, the average DPOAE amplitudes measured at 72 months correlate well with those measured at 45 months in the same ears. The average audiometric HL at the test frequencies 125, 250, 500, 1000, 2000, 4000 and 8000 Hz is shown in Fig. 2 separately for right and left ears. It can be seen that the hearing thresholds are the same for ear sides between errors and decrease from low frequencies in direction to high frequencies. In Fig. 3 the histograms of the hearing level at 1, 2, 4 and 8 kHz are also shown. A hearing threshold of 20 dB was most common for the 4 frequencies tested (1000, 2000, 4000, and 8000).

3.3. Association between serum PCB 153, PTA, and DPOAEs at 72 months

The result of the MANOVA test applied to the PTA as the outcome and PCB as the explanatory variable found no statistically significant association between the HL assessed by PTA and the PCB serum concentration. The HL determined with PTA (at least with the

paradigm used in this study) seems to be insufficiently sensitive to detect on a statistical basis the early cochlear damage induced by PCB contamination.

On the other hand, a statistically significant relation was observed between the DPOAE amplitude and the blood serum PCB concentration at 72 months. The results of the MANOVA test and of each univariate ANOVA are shown in Table 4. Negative slopes mean decreased DPOAE level (and therefore, impaired hearing) with increasing PCB serum concentration, as expected. The regression slopes found at 72 months are very similar to those found at 45 months, well within the errors. This is not surprising, as, due to the fact that PCBs are very persistent contaminants, the PCB concentrations evaluated in the same subjects at 16, 45 and 72 months are highly correlated. At 72 months, significant regressions were found in the lowest- and highest-frequency bands only. In the mid-frequency range (2–3.36 kHz), a nonmonotonic dependence on the PCB exposure was observed, with lowest DPOAE levels at intermediate PCB concentrations. Due to this non-monotonic behavior, the linear regressions in these frequency bands were not significant.

3.4. Association between serum PCB 153 and DPOAE growth curves at 2 kHz

We display the results of the I/O DPOAE test at 2 kHz for all children of our cohort, and separately for boys with regard to known sex differences in cochlear performance (Pavlovcinová et al. 2010) (Fig. 4). We did not find any differences in DPOAE growth curves in association to PCB exposure with both ear sides of girls and right ears of boys (data not shown). On the other hand, children of both sexes pooled together, and boys of the second PCB exposure tertile demonstrate a significant decrease of DPOAE level over the high-stimulus-level end of the growth curve compared with subjects of the highest exposure tertile. It is remarkable that significant decrease appeared at the highest stimulus levels, because at such high levels, the cochlear amplifier is expected to be fully saturated, and immaturity of its development associated with exposure could account for the above-mentioned nonmonotonic behavior. As mentioned in the above paragraph, this behavior was observed in the mid-frequency bands, and the analysis of the 2 kHz I/O function in Fig. 4 shows that the effect is a robust one, extending over the whole stimulus level range.

3.5. Statistical relation between DPOAEs and PTA at 72 months

As an important by-product of this study, we have evaluated DPOAE power as an objective test of cochlear dysfunction useful in future epidemiological studies of hearing impairment by environmental toxicants. A linear combination of the DPOAE amplitudes in different frequency bands was used to discriminate between two subgroups separated according to their audiometric hearing level at 1, 2, 4 and 8 kHz. The discrimination level for hearing dysfunction was set at the standard clinical level of 20 dB HL. ROC curves report the “hit” (true positive) rate (or sensitivity) against the “false alarm” (false positive) rate (or 1 - specificity), as a function of the threshold value set on the test quantity (in this case, a linear combination of DPOAE levels in selected frequency bands) to discriminate negative from positive outcomes. The area below the curve quantifies the predicting power of the test, with 0.5 representing the power of a useless test, and 1 that of a perfect test. The best performance of the DPOAE test was found in the high-frequency range. A power of 0.80 and 0.81 were obtained respectively for the 4 and 8 kHz hearing level. The ROC curves with

the best performing DPOAE linear combinations are shown in Fig. 5 for the detection of the pathological hearing level at 4 (top) and 8 kHz (bottom).

4. Discussion

In this study, a statistically significant adverse association (β coefficients from 0.84 to 1.32 in the frequency range for f_2 from 1 to 5 kHz) between DPOAE level evaluated at 72 months and PCB blood serum concentration at the same age was found in a sample of children participating in a larger longitudinal epidemiological study. This association is similar to that observed in the same population between DPOAE and PCB at 45 months (Jusko et al., 2014). The observed association seems to remain stable during an extended period of development. In a previous paper (Jusko et al., 2014) a significant association was observed between child PCB measures at 16 and 45 months and DPOAE at 45 months, whilst no significant association between cord blood PCB and DPOAE at 45 months were observed. The current finding, combined with the results of Jusko et al., 2014, supports the hypothesis that PCB ototoxic effects mainly occur in the postnatal development period. On the other hand, it does not exclude the possibility that from exposure in utero, auditory outcomes can result. Indeed, we have found recently (Sisto et al., 2015) that the prenatal exposure to PCB 153 is significantly related to amplitudes of DPOAEs, however, in an opposite sense when compared with postnatal exposures at 16 and 45 months. In other words, cord blood serum PCB concentration is related to increased, while postnatal to decreased OAE amplitudes. In conclusion the results of the present study do not permit to exclude the hypothesis that the main ototoxic effect of PCB are due to exposure in the early months of life, and that the damage on cochlear function is a permanent effect. Moreover, it is not possible to exclude the hypothesis that the ototoxic effect continues as the PCB concentration is rather persistent, being reduced at 72 months only by roughly a factor of two with respect to the contamination at 45 months.

Our study has demonstrated, for the first time, that the DPOAE growth function can be used to successfully detect subtle deviations in cochlear function in association with environmental exposure to organochlorines. Moreover we have shown that the character of this association is nonmonotonic, in other words that the DPOAE level is not necessarily proportional to PCB exposure. In fact, the children in the second PCB 153 exposure tertile exhibited a lower DPOAE level when compared with children in the first or 3rd tertiles, over the whole growth curve at 2 kHz, and also in all the mid frequency bands (2–3.36 kHz) at the constant stimulus level (65–55 dB SPL) used for the DP-grams. Nonmonotonic responses and low-dose effects were rather commonly found in studies on natural hormones and EDCs (Vandenberg et al., 2012; Lagarde et al., 2015).

No significant association was found between the HL (dB HL) and PCB concentrations. This result seems to confirm that DPOAEs are a specific biomarker for early cochlear dysfunction, performing even better than psychoacoustical tests.

With regard to DPOAE testing in environmental epidemiological studies in infants, a strong association was observed between the HL and the DPOAE level. This finding is well represented by the ROC curves (Fig. 5). The OAE-test power level is comparable to (or

slightly lower) than that observed in previous DPOAE and TEOAE studies (Gorga et al., 1993; Sisto et al., 2007) of the detection of mild hearing loss levels in adults. The relatively low performance of the OAE test found in this study is not necessarily an argument against the use of OAE-based hearing tests in children. Indeed, the ROC curve compares OAE data with audiometric data, which are affected by their own uncertainty and reproducibility issues. Therefore, the OAE-test power estimated this way may also be affected by the lower reliability of PTA in children.

Besides screening of neonatal hearing and broad clinical use, examination of OAEs is becoming more prevalent in epidemiological research, namely in studies of auditory outcomes in relation to environmental exposures with suspected ototoxicity. In this context this novel tool in environmental medicine deserves comment. As briefly explained in the Introduction, OAEs are considered an epiphenomenon of the activity of the cochlear amplifier which provide objective, quantitative, and non-invasive diagnostics of the cochlear function. The nonlinear amplification active filter mechanism, providing the high sensitivity and sharp frequency resolution of the healthy human auditory system, is localized in the cochlear outer hair cells (OHCs). OHCs are sensitive to several ototoxic agents, so they are often the first part of the auditory system to be damaged by exposure to ototoxic agents such as noise and chemicals. The presence of an effective cochlear active filter is strictly related to the characteristics of measurable OAEs. For this reason, strong correlations have been observed in several cross-section studies between audiometric levels and OAE levels (e.g., Lucertini et al., 2002; Sisto et al., 2007). OAEs can be therefore used for hearing diagnostics, at least in cross-sectional studies, without having necessarily to rely also on direct audiometric level measurements which, in children, may be impossible or not fully reliable.

On the other hand, the strength of the cochlear amplifier is related to the OAE level in a rather complex way, as described by cochlear mechanics, with different functional dependence of the OAE level on the cochlear gain expected for the different types of OAEs, or, more specifically, for the different OAE generation mechanisms (Shera and Guinan, 1999). This issue is particularly relevant for the DPOAE generation. The $2f-f_2$ DPOAE complex response is due to the vector sum of two backward traveling waves: that generated by a nonlinear distortion source localized near the tonotopic place of the higher frequency primary tone f_2 , and that due to reflection of the forward DP wave from roughness, in a cochlear region close to the f_{DP} tonotopic place. The interference between the two corresponding DPOAE components leads to the spectral fine-structure, which is an additional uncertainty source in low-resolution DPOAE level measurements (Mauermann and Kollmeier, 2004). This uncertainty may be overcome, by separating the two components with suitable data analysis techniques (e.g., Moleti et al., 2012) only using high frequency-resolution techniques, which imply long measurement time, and are not used in large epidemiological studies. Fortunately, at high stimulus levels, the DPOAE reflection component is usually much weaker than the distortion one, so the uncertainty associated with the fine-structure spectral oscillations may be neglected.

5. Conclusions

An association between PCB exposure and deficits in cochlear function in young children exposed to environmental PCBs has been confirmed. Otoacoustic emissions, produced as a by-product of the cochlear OHC motility, were efficiently used as markers of cochlear status. The DPOAE levels assessed at the age of 72 months were significantly associated to the PCB exposure at the same age. In particular, a negative correlation between the DPOAE level and the concentration of PCB in blood serum is confirmed. The association between the HL evaluated in bands of one half of octave by means of pure tone audiometry and the PCB concentration was not statistically significant, suggesting a higher OAE sensitivity with respect to PTA, for the detection of early cochlear damage induced by ototoxic contaminants. The DPOAE measured at 45 and 72 months were found highly reproducible also in a population of young children. These results confirm that reproducible tests of the cochlear function may be reliably based on DPOAEs, which may become a necessity in the case of subjects that are not able to cooperate in psychoacoustic tests.

The degree of correlation between PTA HLs and DPOAE levels observed in this study is typical of those documented in OAE-based tests provided by commercial devices. The ROC curves quantifying the sensitivity and specificity of DPOAEs in discriminating normal from pathological hearing levels gives results very similar to those that the same authors have found in young adults in previous studies.

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References

- Agency for Toxic Substances and Disease Registry. 2000. <http://www.atsdr.cdc.gov/substances/ToxOrganSystems.asp>
- Akins JR, Waldrep KJT, Bernert JR. The estimation of total serum lipids by a completely enzymatic 'summation' method Clin. Chim. Acta. 1989; 184:219–226. [PubMed: 2611996]
- Conka K, Drobna B, Kocan A, Petrik J. Simple solid-phase extraction method for determination of polychlorinated biphenyls and selected organochlorine pesticides in human serum J. Chromatogr. A. 2005; 1084:33–38. [PubMed: 16114233]
- Crofton KM, Ding D, Padich R, Taylor M, Henderson D. Hearing loss following exposure during development to polychlorinated biphenyls: a cochlear site of action Hear. Res. 2000a; 144:196–204. [PubMed: 10831878]
- Crofton KM, Kodavanti PR, Derr-Yellin EC, Casey AC, Kehn LS. PCBs, thyroid hormones, and ototoxicity in rats: cross-fostering experiments demonstrate the impact of postnatal lactation exposure Toxicol. Sci. 2000b; 57:131–140. [PubMed: 10966519]
- Crofton KM, Rice DC. Low-frequency hearing loss following perinatal exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in rats Neurotoxicol. Teratol. 1999; 21:299–301. [PubMed: 10386834]
- Crofton KM, Zoeller RT. Mode of action: Neurotoxicity induced by thyroid hormone disruption during development hearing loss resulting from exposure to PHAHs Crit. Rev. Toxicol. 2005; 35:757–769. [PubMed: 16417043]

- Drobná B, Fabišíková A, Šonka K, Chovancová J, Dömötörövá M, Wimmerová S, Šovčíková E, Košan A. Differences between dioxin-like PCB, non-dioxin-like PCB, polychlorinated dibenzo-p-dioxin and dibenzofuran intake from human milk and infant milk formula by infants in the Michalovce district (Slovakia) *J. Food Nutr. Res.* 2011; 50:106–117.
- Goldey ES, Kehn LS, Lau C, Rehnberg GL, Crofton KM. Developmental exposure to polychlorinated biphenyls (Aroclor 1254) reduces circulating thyroid hormone concentrations and causes hearing deficits in rats *Toxicol. Appl. Pharmacol.* 1995; 135:77–88. [PubMed: 7482542]
- Gorga MP, Neely ST, Bergman BM, Beauchaine KL, Kaminski JR, Peters J, Jesteadt W. Otoacoustic emissions from normal-hearing and hearing-impaired subjects: distortion product responses *J. Acoust. Soc. Am.* 1993; 93:2050–2060. [PubMed: 8473617]
- Herr DW, Goldey ES, Crofton KM. Developmental exposure to Aroclor 1254 produces low frequency alterations in adult rat brainstem auditory evoked responses *Fundam. Appl. Toxicol.* 1996; 33:120–128. [PubMed: 8812251]
- Jerger J. Clinical experience with impedance audiometry *Arch. Otolaryngol.* 1970; 92:311–324. [PubMed: 5455571]
- Jusko TA, Sisto R, Iosif AM, Moleti A, Wimmerová S, Lancz K, Tihányi J, Šovčíková E, Drobná B, Palkovičová L, Jureková D, Thevenet-Morrison K, Verner MA, Sonneborn D, Hertz-Picciotto I, Trnovec T. Prenatal and postnatal serum PCB concentrations and cochlear function in children at 45 months of age *Environ. Health Perspect.* 2014; 122:1246–1252. [PubMed: 25051575]
- Kemp DT. Otoacoustic emissions, their origin in cochlear function, and use *Br. Med. Bull.* 2002; 63:223–41. [PubMed: 12324396]
- Kummer P, Janssen T, Hulin P, Arnold W. Optimal L_1 – L_2 primary tone level separation remains independent of test frequency in humans *Hear. Res.* 2000; 146:47–56. [PubMed: 10913883]
- Lagarde F, Beausoleil C, Belcher SM, Belzunces LP, Emond C, Guerbet M, Rousselle C. Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment *Environ. Health.* 2015; 11(14):13.
- Lucertini M, Moleti A, Sisto R. On the detection of early cochlear damage by otoacoustic emission analysis *J. Acoust. Soc. Am.* 2002; 111:972–978. [PubMed: 11863199]
- Mauermann M, Kollmeier B. Distortion product otoacoustic emission (DPOAE) input/output functions and the influence of the second DPOAE source *J. Acoust. Soc. Am.* 2004; 116:2199–2212. [PubMed: 15532652]
- Min JY, Kim R, Min KB. Serum polychlorinated biphenyls concentrations and hearing impairment in adults *Chemosphere.* 2014; 102:6–11. [PubMed: 24360845]
- Moleti A, Longo F, Sisto R. Time-frequency domain filtering of evoked otoacoustic emissions *J. Acoust. Soc. Am.* 2012; 132:2455–2467. [PubMed: 23039440]
- Pavlovčinová G, Jakubíková J, Trnovec T, Lancz K, Wimmerová S, Šovčíková E, Palkovicová L. A normative study of otoacoustic emissions, ear asymmetry, and gender effect in healthy schoolchildren in Slovakia *Int. J. Pediatr. Otorhinolaryngol.* 2010; 74:173–177. [PubMed: 20018388]
- Powers BE, Widholm JJ, Lasky RE, Schantz SL. Auditory deficits in rats exposed to an environmental PCB mixture during development *Toxicol. Sci.* 2006; 89:415–422. [PubMed: 16317017]
- Robles L, Ruggero MA. Mechanics of the Mammalian Cochlea *Physiol. Rev.* 2001; 81:1305–1352. [PubMed: 11427697]
- Shera CA, Guinan JJ Jr.. Evoked otoacoustic emissions arise by two fundamentally different mechanisms: a taxonomy for mammalian OAEs *J. Acoust. Soc. Am.* 1999; 105:782–798. [PubMed: 9972564]
- Sisto R, Chelotti S, Moriconi L, Pellegrini S, Citroni A, Monechi V, Gaeta R, Pinto I, Stacchini N, Moleti A. Otoacoustic emissions sensitivity to low levels of noise-induced hearing loss *J. Acoust. Soc. Am.* 2007; 122:387–401. [PubMed: 17614498]
- Sisto R, Moleti A, Palkovičová Murínová, Wimmerová S, Lancz K, Tihányi J, Šonka K, Šovčíková E, Hertz-Picciotto I, Jusko TA, Trnovec T. Environmental exposure to organochlorine pesticides and deficits in cochlear status in children *Environ. Sci. Pollut. Res. Int.* 2015; 22:14570–14578. [PubMed: 25989860]

- Trnovec T, Šovčíková E, Husáková M, Wimmerová S, Kocan A, Jurečková D, Langer P, Palkovícová L, Drobná B. Exposure to polychlorinated biphenyls and hearing impairment in children *Environ. Toxicol. Pharmacol.* 2008; 25:183–187. [PubMed: 21783857]
- Trnovec T, Sovčíková E, Pavlovčinová G, Jakubíková J, Jusko TA, Husták M, Jurečková D, Palkovícová L, Kocan A, Drobná B, Lancz K, Wimmerová S. Serum PCB concentrations and cochlear function in 12-year-old children *Environ. Sci. Technol.* 2010; 44:2884–2889. [PubMed: 20384380]
- U.S. Environmental Protection Agency, Method 1668, Revision B, EPA-821-R-08-020. Chlorinated Biphenyl Congeners in Water, Soil, Sediment, Biosolids, and Tissue by HRGC/HRMS, Washington, 2008.
- Vandenberg LN, Colborn T, Hayes TB, Heindel JJ, Jacobs DR Jr., Lee DH, Shioda T, Soto AM, vom Saal FS, Welshons WV, Zoeller RT, Myers JP. Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses *Endocr. Rev.* 2012; 33:378–455. [PubMed: 22419778]

Fig. 1. (Top): scatter plot of the PCB153 concentration at 72 months versus that at 45 months. The linear regression slope and R^2 are reported; (bottom): same for the DPOAE level at 3400 Hz.

Fig. 2.
Average hearing level evaluated at 125, 250, 500, 1000, 2000, 4000, 8000 Hz.

Fig. 3. Frequency distributions of the hearing level in four frequency bands from 1 to 4 kHz.

Kruskal-Wallis H test:

L2: 75 dB – $p=0.042$, results of the post hoc test: 2. vs. 3. tertile $p=0.06$

L2: 70 dB – $p=0.024$, results of the post hoc test: 2. vs. 3. tertile $p=0.045$

L2: 65 dB – $p=0.043$

L2: 60 dB – $p=0.051$

Kruskal-Wallis H test:

L2: 70 dB – $p=0.047$, results of the post hoc test: 2. vs. 3. tertile $p=0.099$, 1. vs. 2. tertile

$p=0.078$

L2: 65 dB – $p=0.034$, results of the post hoc test: 2. vs. 3. tertile $p=0.038$

Fig. 4.

Median DPOAE growth with 95% Confidence Interval. Upper panel: Left ears of all children. Lower panel: left ears of boys.

Fig. 5.

ROC curve representing the power of a test based and a linear combination of DPOAE in different frequency bands in testing the hearing level at 4 (left) and 8 kHz (right). The PTA discriminating level was set at 20 dB HL.

Table 1

Characteristics of the children examined at the 45 months and 72 months of age.

Characteristic	45-Month follow-up n = 351	72-Month follow-up n = 214	P-value ^b
	n (%) ^a	n (%) ^a	
Infant sex			
Male	175 (50)	96 (45)	0.249
Female	176 (50)	118 (55)	
Gestation length (weeks)			
< 37	3(1)	2 (1)	0.973
37–41	333(95)	203 (94.8)	
42	12(3)	8 (3.7)	
Missing	3(1)	1 (0.5)	
Ethnicity of child			
Romani	55 (16)	31 (14)	0.704
Slovak/eastern European	296 (84)	183 (86)	
Marital status			
Married	319(91)	200 (93)	0.847
Never married	20(6)	10 (5)	
Divorced/separated	3(1)	2 (1)	
Missing	9(2)	2 (1)	
Maternal age (years)			
18– < 20	27(8)	10 (5)	0.349
20–30	266(76)	165 (77)	
> 30	58(17)	39 (18)	
Maternal PCB-153 (ng/g lipid)			
Mean ± std (median)	226 ± 183 (168)	223 ± 161 (178)	0.838

^aPercents may not sum to 100 because of rounding.

^bP-value compares the distribution of characteristics among the 214 children at 72-months of follow-up (n = 812) to those with hearing data at 45-months (n = 351). Chi-square test was used for categorical variables; *t*-test for quantitative variables (PCB concentrations).

Table 2

Descriptive statistics for the PCB-153 serum concentration at 72 months for 214 children.

PCB-153	Mean	Min	P10	P25	P50	P75	P90	Max
Wet weight (ng/ml)	0.82	0.04	0.12	0.21	0.45	0.96	1.63	12.24
Lipid adjusted (ng/g lipids)	91.7	4.4	13.5	22.9	48	110.1	192.1	1011.6

Abbreviations: Max, maximum; Min, minimum; P, percentile

Descriptive statistics for the DPOAE levels in the right and left ears at 72 months and the correlation between the DPOAE level in combined data for right and left ears at 72 and 45 months.

Table 3

f (Hz)	1000	1189	1414	1682	2000	2378	2828	3364	4000	4757	5657
Corr 72_45 months	0.19	0.41	0.44	0.58	0.54	0.48	0.61	0.63	0.55	0.51	0.55
Right 72 months											
Mean (dB SPL)	7.5 ± 5.7	8.8 ± 6.2	11.3 ± 6.7	12.3 ± 6.8	11.7 ± 7.2	10.8 ± 7.0	9.2 ± 7.2	8.4 ± 6.8	10.9 ± 6.7	12.9 ± 8.3	15.7 ± 8.7
Max	20.1	23	24.5	28.4	27	25.6	25.2	24.4	24.4	29.9	31.1
Min	-10.2	-7.7	-7.7	-7.9	-5.8	-14.1	-12.1	14.4	12.6	13.3	14.1
Left 72 months											
Mean (dB SPL)	7.5 ± 6.0	7.9 ± 6.2	9.7 ± 6.9	10.6 ± 6.7	10.7 ± 7.1	10.7 ± 7.2	8.6 ± 7.3	9.2 ± 6.8	10.0 ± 7.5	12.7 ± 8.3	15.4 ± 9.2
Max	23.6	19.8	22.9	25.4	24.2	23.9	22.6	22.4	25.6	31.1	29.5
Min	-6.6	-6.5	-7.5	-7.7	-10	-11.4	-15.3	-14.1	-14.8	-12.9	-20.4

Abbreviations: f, frequency; Corr, correlation; Max, maximum; Min, minimum; SPL, sound pressure level.

Table 4

Regression (slope) coefficients in each frequency band for the relation between the DP amplitude and the PCB-153 blood serum concentration at 45 and 72 months. The statistical significance and errors are also reported. The regression coefficients are reported only for the frequency bands in which statistically significant results were found.

DP f ₂ Hz	β [dB/(log _e (wet weight (ng/ml)))]	Standard error	p-value
MANOVA(DP45m ~log(PCB153_45m) $p = 0.02$)			
1189	- 0.63	0.29	0.03
2828	- 0.93	0.30	0.002
3364	- 0.93	0.32	0.003
4000	- 0.85	0.33	0.01
4757	- 1.01	0.37	0.006
MANOVA (DP72m ~log(PCB153_72 m) $p = 0.01$)			
1189	- 0.99	0.39	0.01
1414	- 0.84	0.43	0.05
1682	- 1.01	0.42	0.02
4000	- 0.97	0.45	0.03
5657	- 1.32	0.56	0.02