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Environmental exposure to organochlorine pesticides and deficits in cochlear status in children

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Abstract

The aim of this study was to examine the hypothesis that organochlorine pesticides (OCPs), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p′-DDT) and its metabolite 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (p,p′-DDE) are ototoxic to humans. A Multivariate General Linear Model was designed, in which the statistical relation between blood serum concentrations of HCB, β-HCH, p,p′-DDT or p,p′-DDE at the different ages (at birth, 6, 16 and 45 months) and the DPOAEs were treated as multivariate outcome variables. PCB congeners and OCPs were strongly correlated in serum of children from our cohort. To ascertain that the association between DPOAEs at a given frequency and concentration of a pesticide is not influenced by PCBs or other OCP also present in serum, we calculated BMCs relating DPOAEs to a serum pesticides alone and in presence of confounding PCB-153 or other OCPs. We found that BMCs relating DPOAEs to serum pesticides are not affected by confounders. DPOAE amplitudes were associated with serum OCPs at all investigated time intervals, however in a positive way with prenatal exposure and in a negative way with all postnatal exposures. We observed tonotopicity in the association of pesticides with amplitude of DPOAEs as its strength was frequency dependent. We conclude that exposure to OCPs in infancy at environmental concentrations may be associated with hearing deficits.

Keywords

organochlorine pesticides; polychlorinated bihenyls; otoacoustic emissions; hearing impairment; infancy; mixture effects

Introduction

Data reporting on hearing impairment associated with exposure to environmental chemicals have steadily grown during the last decades. Most knowledge on this topic is dealing with hearing deficits associated with exposures to organohalogen compounds and from this class of chemicals, most research has focused on polychlorinated biphenyls (PCBs) (Crofton et al. 2000a; Crofton et al. 2000b; Crofton and Rice 1999; Crofton and Zoeller 2005; Powers et al. 2006; Min et al. 2014; Trnovec et al. 2008; Trnovec et al. 2010; Jusko et al. 2014) and their metabolites (Meerts et al. 2004). Furthermore, from the group of brominated flame retardants, the hexabromocyclododecane (Lilienthal et al. 2009) and tetrabromobisphenol A (Lilienthal et al. 2008), were found to have effects on brainstem auditory evoked potentials and a co-exposure to subthreshold doses of polybrominated diphenyl ethers (PBDEs) and PCBs has shown an additive effect on cochlear function (Poon et al. 2011). The mode of action of the most studied PCBs on hearing is still an open issue. The animal data indicate developmental disorders of cochlea due to an interference of PCBs with thyroid homeostasis during lactation (Crofton and Zoeller 2004) while human data show that current exposures are as well important (Jusko et al. 2014).

However, there is no information about the effect on hearing of organochlorine pesticides (OCPs), that are structurally related to PCBs, in spite of their presence in both cord blood and breast milk, exposing fetuses and infants during perinatal period, except one report on ototoxicity of hexachlorobenzene (HCB) in rats (Hadjab et al. 2004). The human exposure

levels to OCPs vary according to the region (Ali et al. 2014), population, and race however their temporal trends do not show a consistent downward trend (Wang et al. 2010). The current background concentration of each of these pesticides is still much higher than that of notorious organic pollutants such as polychlorinated-p-dibenzodioxins and dibenzofurans (PCDD/Fs), PCBs, and PBDEs (Kang and Chang 2011). It has been shown that OCPs can affect the thyroid system through gender specific mechanisms that may differ among compounds (Freire et al. 2013). Exposure to PCBs and/or OCPs, even at background levels, may affect thyroid function during pregnancy. These findings are important, since thyroid hormones of maternal origin may play an essential role in fetal neurodevelopment (Chevrier et al. 2008), development of cochlea included. The aim of our study was to examine the hypothesis on ototoxicity of OCPs at environmental exposures in developing infants with regard to omnipresence of OCPs in environment and interference of OCPs with thyroid hormone homeostasis.

Materials and methods

Study subjects

This work is based on an on-going birth cohort study in eastern Slovakia that enrolled 1134 mother-infant pairs during 2002–2004 (Hertz-Picciotto et al. 2003). Mothers gave informed consent and were enrolled at the time they came to the hospital for delivery. The protocol excluded (1) mothers with more than four previous births, (2) mothers less than 18 years of age, (3) mothers who had resided fewer than 5 years in their district, and (4) mothers with a major illness during pregnancy. Mother-infant pairs were enrolled at birth from two Districts: Michalovce (n=811) and Svidnik (n=323). The Michalovce area was polluted by PCBs which were produced at the chemical plant Chemko Strážske and at the same time by OCPs due to misuse of agrochemicals during the era of socialism. The characteristics of infants and mothers participating in the study were described earlier (Jusko et al. 2012; Jusko et al. 2011; Jusko et al. 2010; Park et al. 2010; Sonneborn et al. 2008a, 2008b).

From the 811 participants enrolled at birth in the Michalovce district, we included into this study 351 infants who had complete exposure and outcome data at the 45-month follow-up up (children in the Svidnik district were not followed-up after 16 months of age). The study protocol was approved by Institutional Review Boards at the University of California, Davis and the Slovak Medical University.

Exposure and outcome assessment

Exposure assessment, otologic, and audiological assessments and measurement of covariates have been previously described (Jusko et al. 2014). We determined serum concentrations of hexachlorobenze (HCB), alpha-hexachlorocyclohexane (α-HCH), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH), 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (p,p′-DDE), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p′-DDT) and 15 PCB congeners [IUPAC (International Union of Pure and Applied Chemistry) numbers 28, 52, 101, 105, 114, 118,123^{+149}, 138^{+163}, 153, 156^{+171}, 157, 167, 170, 180, and 189] (Conka et al. 2005; Kocan et al. 1994; Petrík et al. 2006; Chovancová et al. 2012) in cord serum and in serum collected at the ages of 6, 16 and

45 months. We report lipid adjusted concentrations. We estimated total serum lipids using the enzymatic summation method (Akins et al. 1989). The concentrations below the limit of detection (LOD), were substituted by the LOD value divided by the square root of 2.

Audiological examination at the age of 45 months consisted of otoscopy, tympanometry, and assessment of otoacoustic emissions (OAEs). Distortion product otoacoustic emissions (DPOAEs) were recorded by the Echoport ILO 292 USB-I Otodynamics Ltd. (Hatfield, Herts, UK) connected to a personal computer equipped with ILO V6 software. DPOAEs were measured in response to pairs of primary tones ($f_2 > f_1$), with f_2 set at default frequencies, varied in one fourth of octave steps between 1000 and 5657 Hz. The f_2/f_1 ratio was 1.22 for each primary pair. For each scan step, a signal analyzer picked up the discrete frequency component at the DPOAE $2f_1$-f_2 frequency, getting amplitude spectra, called DP-grams, presented in dB Sound Pressure Level (SPL) units. Although the DP signal was actually recorded at the $2f_1$-f_2 frequency, the response was attributed to f_2 in the DP-gram and in the diagnostic interpretation, because this DP is generated in a cochlear region near the characteristic place tuned at the f_2 frequency. Both the f_1 and f_2 levels were set to 70 dB SPL. This choice is not a standard in DPOAE clinical practice, the most popular setting being 65-55dB, but higher stimulus levels may have some advantages in terms of getting good Signal to Noise Ratio (SNR) in a shorter time, which is a critical issue with children. In the ILO DP design, noise was simultaneously measured as the average power spectrum level at off-band frequencies around each DP frequency. We included all data in the analysis, adopting the choice of attributing half the noise level (noise-6dB) to all signal levels below this threshold. We considered cord serum concentrations excellently correlating with maternal concentrations (Govarts et al. 2008) of studied organochlorines a marker of prenatal exposure and the concentrations at the age of 6, 16 and 45 months as representing the postnatal exposure.

Assessment of mixture effect

To evaluate the confounding effect of mixture components on cochlear status, we proceeded in a similar way as when we have treated mixture effect of PCDD/Fs and DL-PCBs on thyroid volume and FT_4 (Trnovec et al. 2013). For this purpose we calculated the benchmark concentrations (BMCs) for the effect on DPOAEs (combined for both sexes and for left and right ear) for each OCP without and in presence of the most probable combinations of potentially ototoxic organochlorine confounders using CTDB_BMD software (Dedík 2012). The combinations have been set with regard to statistically significant regression coefficients marked in Figs 1–4.

Statistical Analysis

A multivariate analysis of variance (Multivariate General Linear Model) was designed, in which we studied the statistical relation between the base 10 logarithm of the HCB, β-HCH, p,p′-DDE, p,p′-DDT, and PCB-153 blood serum concentration at the different ages (at birth, 6, 16, and 45 months) and the DPOAEs treated as multivariate outcomes variables. The ear sides were considered as confounders. We have chosen this test because the considered outcome variables (OAE levels in fourth of octave bands) are not statistically independent from each other. A significance criterion $p<0.05$ was conventionally adopted. We have

studied univariate and multivariate linear regression models. All statistical analyses were performed using the statistical software SPSS 16 (Softonic International S.L, Barcelona, Spain.). We calculated coefficients of variation (R^2), regression coefficients β, and t-test statistics for significance of slope β. The regression coefficient β thus represents the rate of change of the DPOAE signal amplitude in dB at a specific frequency *f*, as a function of the HCB, β-HCH, p,p′-DDT, p,p′-DDE and PCB-153 concentration at the different ages. We used the Variance Inflation Factor (VIF) to check whether there would be collinearity from prenatal and postnatal concentrations in the same regression model.

Results

Details on study population have been published previously (Jusko et al. 2014; Patayová et al. 2013) and we do not reproduce them in full length as we have found that anthropometric, socioeconomic, and maternal health determinants are not directly related to evaluated hearing outcomes. Romani ethnicity, however, was associated with poorer DPOAEs (Jusko et al. 2014). When Roma children were excluded from the analysis (16 %), estimates of association did not change substantially, therefore, we did not exclude Roma infants from the analysis.

Although we determined concentrations of HCB, 3 isomers of HCH, p,p′-DDT, p,p′-DDE, and 15 PCB congeners in blood serum, we found to be impractical to work with all PCB congeners and HCH isomers. Therefore we have chosen from the PCB congeners the congener CB-153 and from HCH isomers the isomer β-HCH as exposure markers. Both were detectable in the vast majority of child serum specimens. Across the compounds, prenatal concentration of p,p′-DDE was the highest (median, mean: 520.72, 638,08 ng/g lipid) and β-HCH the lowest (7.48, 9.82 ng/g lipid). Maximum concentrations were observed mostly16 months after birth (Table 1). Table 2 has informative value for evaluation of possible combined effect on cochlear status of individual components of the exposure mixture. Correlations across mixture components during the prenatal period show that HCB, β-HCH, p,p′-DDE and PCB-153 are weakly or moderately correlated. p,p′-DDE correlated strongly with PCB-153 (r=0.625) and with DDT (r=0.611). However p,p′-DDT did not correlate with HCB and β-HCH in cord blood serum. This means that these components alone or in certain combination occurring in the fetus prenatally may be associated with deficits observed at 45 months.

There was weak or no correlation across mixture components when comparing the prenatal and later time intervals. The correlation strength decreased with increasing time interval. However some moderate correlation was found between prenatal and 6 and 16 month time interval concentrations of a particular toxicant (p,p′-DDE, p,p′-DDT and PCB 153, marked in bold). Both concentrations across toxicants and particular toxicants at 6 and 16 months were highly interrelated. Still correlated but to lesser extent were concentrations between 6 and 45 months.

It follows from this overview on interrelations between the concentrations of a particular toxicant and across toxicants that a certain deficit in cochlear function measured at the age

of 45 months may be associated with any component of the exposure mixture of organochlorines or their combination.

Most VIFs were around 5 and since a value of 10 has been recommended as a commonly used cut-off (how2stat 2011) multicollinearity does not seem to be a problem.

We have calculated BMCs for effect on DPOAEs of each OCP studied and PCB-153 without and in presence of the most probable combinations of potentially ototoxic organochlorine confounders and we show them in Supplemental material. It can be seen that the addition of PCB-153 or other OCPs to the examined compound had negligible influence on its BMC value.

The results of the multivariate linear regression models are shown in Figs 1–4. A positive and mostly significant relationship was obtained when relating the DPOAE amplitudes against cord blood serum concentrations of HCB, β-HCH, p,p′-DDE, or PCB-153 for most of the frequencies of primary tones (Fig 1.). In contrast p,p′-DDT was inversely related to DPOAEs at low frequencies. A different pattern was obtained when correlating the DPOAEs to organochlorine concentrations measured 6 month after birth (Fig 2.). The regressions were mostly negative and fewer were significant. A similar response was observed when relating the DPOAEs to concentrations measured at the age of 16 and 45 months, however more regressions were statistically significant (Figs 3 and 4).

Discussion

We have examined and confirmed the hypothesis that OCPs at environmental exposure level are in a dose dependent manner associated with deficits in cochlear function in children in a similar way as we have earlier described for PCBs (Trnovec et al. 2008; Trnovec et al. 2010; Jusko et al. 2014). To demonstrate this association we have chosen the measurement of the amplitude of DPOAEs from available markers of the functional status of the inner ear. DPOAEs serves as an objective indicator of normally functioning cochlea outer hair cells. The effectiveness of OAEs as a fast non-invasive and objective diagnostic tool has been suggested by several cross sectional studies (Gorga et al. 1993; Sisto et al. 2007; Sisto et al. 2013), and its applicability to the exposure to ototoxic pollutants (PCBs) has been recently demonstrated (Jusko et al. 2014).We have drawn the conclusion about ototoxicity of OCPs when relating the DPOAE amplitudes measured at the age of 45 months to the current OCPs serum concentrations and the concentrations determined at 3 time intervals in the past. Regression analysis revealed that DPOAE amplitudes were associated with serum OCPs at all investigated time intervals, however in a positive way with prenatal exposure and in a negative way with all postnatal exposures. It is difficult to explain the change of association from positive to an inverse one within first 6 postnatal months. We assume that the great exposure increase during lactation plays a role. The correlations (Table 2) show that the exposure patterns for particular toxicants at 6 months were similar to prenatal as their concentrations in these two time intervals were mostly correlated (correlation coefficients in bold in Table 2, section prenatal vs. 6 months). We have recently shown however (Lancz et al. 2014), that as a result of breast feeding the exposure magnitude at 6 months is about

twice compared to prenatal. The double exposure might have changed the direction of the outcome.

Though the direction of the association, positive or inverse, appeared to be uniform for a given time interval, the strength of association was frequency dependent which is important for identification of place of damage at the basilar membrane and of the time when it occurred, the exposure sensitive window. Tonotopy means that different regions of the basilar membrane in the organ of Corti vibrate at different frequencies. Frequency related deficits then indicate which part of the cochlea is damaged. Therefore when unveiling the behavior of the cochlea under influence of toxic factors we have considered each particular section of the basilar membrane as a distinct unit. There are several reasons for such approach: The development of the cochlea during gestation and especially the differentiation of the hair cells in the cochlea is a very heterogeneous process with regard to time and differently timed exposure may damage different parts of the auditory system (Graven and Browne 2008). In addition intrinsic susceptibility to damaging factors differs among types of cochlear cells. Basal outer hair cells may be more vulnerable than apical outer hair cells (Sha et al. 2001).

The association of OCPs with hearing in infants raises the question about mode of their action. Most of the published review articles (Mrema et al. 2013; Gourounti et al. 2008; Tebourbi et al. 2011) point toward oxidative stress and/or receptor-mediated mechanisms being important determinants, whereas inflammatory and aberrant epigenetic mechanisms caused by pesticide exposure are only in a preliminary stage of development (Alavanja et al. 2013). Many of pesticides do not have Aryl hydrocarbon receptor (AhR) agonistic activity, and AhR agonistic pesticides may be small in number compared with pesticides possessing estrogen receptor and/or androgen receptor activities (Takeuchi et al. 2006; Takeuchi et al. 2008). Oxidative stress has been found a leading mechanism in various types of hearing loss (Tabuchi et al. 2011; Poirrier et al. 2010; Kovacic and Somanathan 2008): age-related hearing impairment in humans (Hwang et al. 2012; Fujimoto et al. 2014; Bielefeld et al. 2010; Coling et al. 2009), noise induced hearing loss (Park et al. 2014; Henderson et al. 2006) and aminoglycosides induced ototoxicity (Guthrie 2008).

With exposures to toxicologically related compounds we have been facing the issue of the so called mixture effect. From the BMC data we have drawn a conclusion that the association of a particular toxicant in this study with DPOAE amplitudes has not been confounded by the presence in serum of another toxicant. The plausibility of this approach was recently discussed (Trnovec 2014). Moreover, we have recently shown that the DPOAE results appear to be quite robust to the choice of control variables, supporting a minimal level of confounding (Jusko et al. 2014). A newly emerging environmental toxicology issue may be the ototoxicity of inert ingredients approved for use in pesticide products applied to foods. However from the list of publicly known substances (Fishel 2014) none is known as ototoxic (EU-OSHA 2009).

In spite of a broad use of DPOAEs in clinical practice we have shown its value in epidemiology which needs some methodical modifications. Both the f_1 and f_2 levels were set to 70 dB SPL. As the detection of hearing impairment is associated with decreased levels

of signal, rejecting data with low SNR introduces a bias in any data analysis. On the other hand, low SNR data have limited (but not null) information content. When signal is lower than noise (SNR in dB below zero), the noise level has still the meaning of an upper limit to the actual unknown signal level. Neglecting this information by excluding from the analysis all data with negative SNR is an apparently conservative choice, which introduces instead a bias, strongly dependent on the noise level of the experimental setup [probe quality, accuracy of fitting in the ear canal, number of averages, behavior of the subject, and acoustic insulation from (and level of) the environmental noise]. Therefore, as in Jusko et al. (2014), our choice, was to include all the data in the analysis, adopting the choice of attributing half the noise level (noise-6dB) to all signal levels below this threshold. This choice typically leads, on average, to overestimation of the signal levels, so it is still a conservative choice.

The key finding of this study is that environmental exposure to OCPs in infancy can impair hearing. DPOAE amplitudes, a marker of the status of the outer hair cells, were associated with serum OCPs in a positive way with prenatal exposure and in a negative way with all postnatal exposures. Tonotopicity was observed in the association of pesticides with amplitude of DPOAEs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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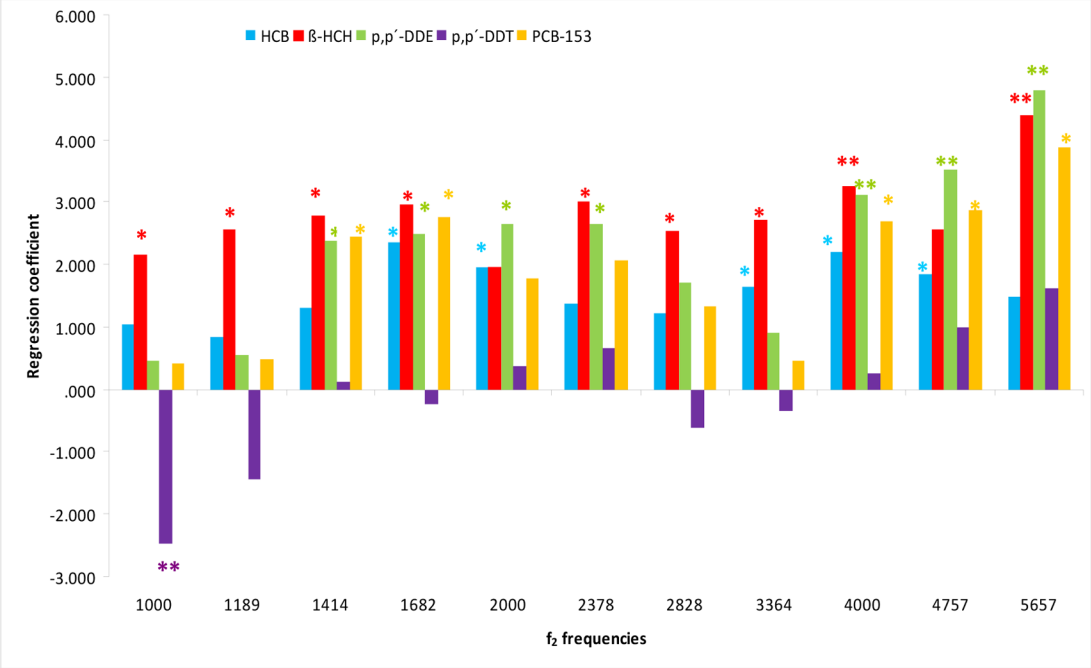

Fig 1.

On the vertical axis are plotted values of regression coefficients obtained when regressing amplitudes of otoacoustic emissions at various f_2 frequencies measured at the age of 45 months against serum concentrations of individual pesticides or PCB-153 in cord blood representing prenatal exposure.

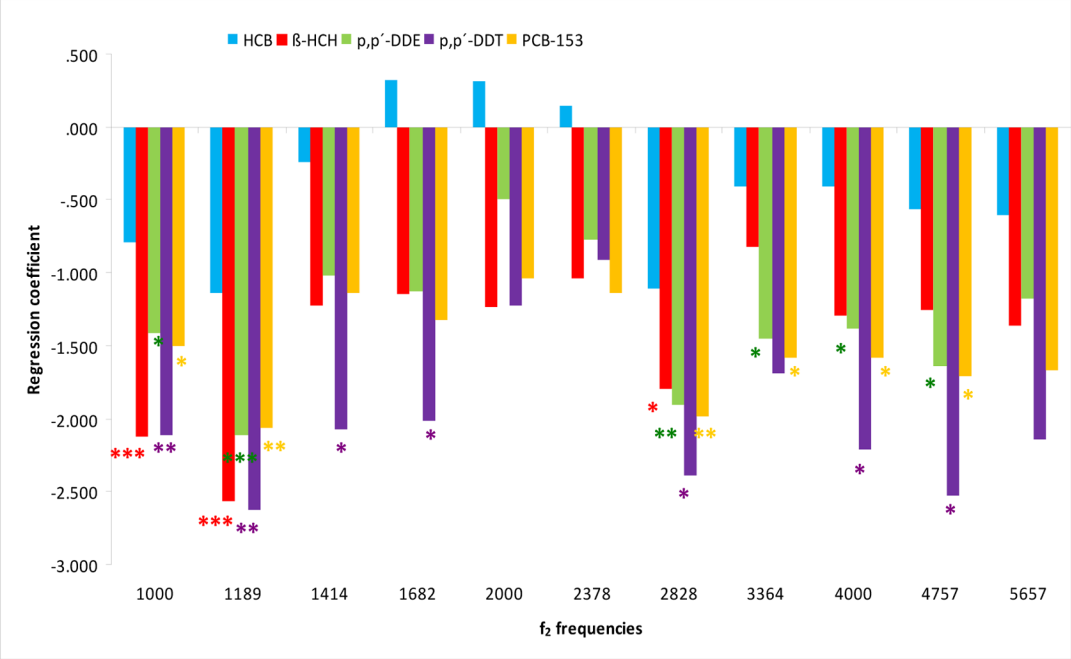

Fig 2.

On the vertical axis are plotted values of regression coefficients obtained when regressing amplitudes of otoacoustic emissions at various f_2 frequencies measured at the age of 45 months against serum concentration of individual pesticides or PCB-153 at the age of 6 months. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

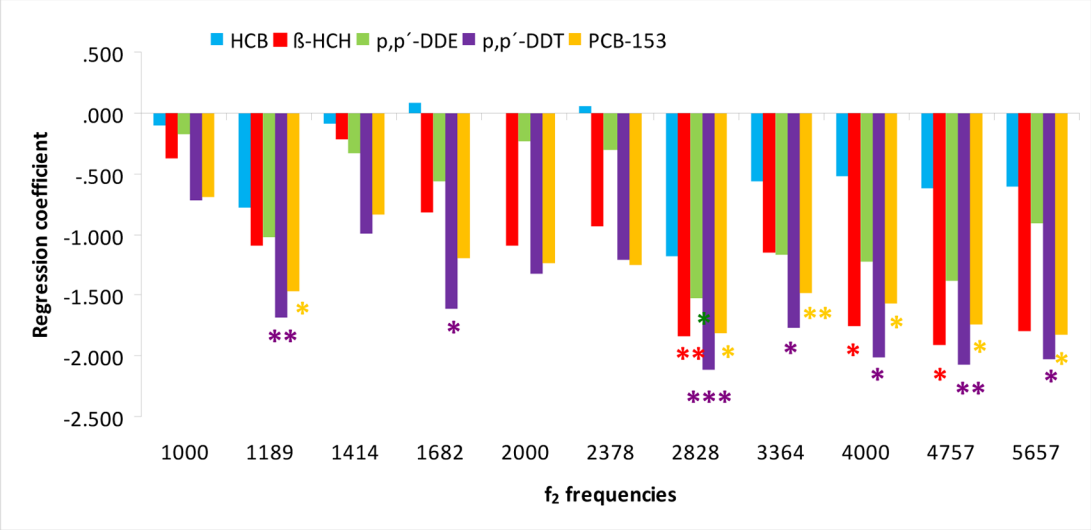

Fig 3.

On the vertical axis are plotted values of regression coefficients obtained when regressing amplitudes of otoacoustic emissions at various f_2 frequencies measured at the age of 45 months against serum concentration of individual pesticides or PCB-153 at the age of 16 months. * p≤0.05, **p≤0.01, ***p≤0.001.

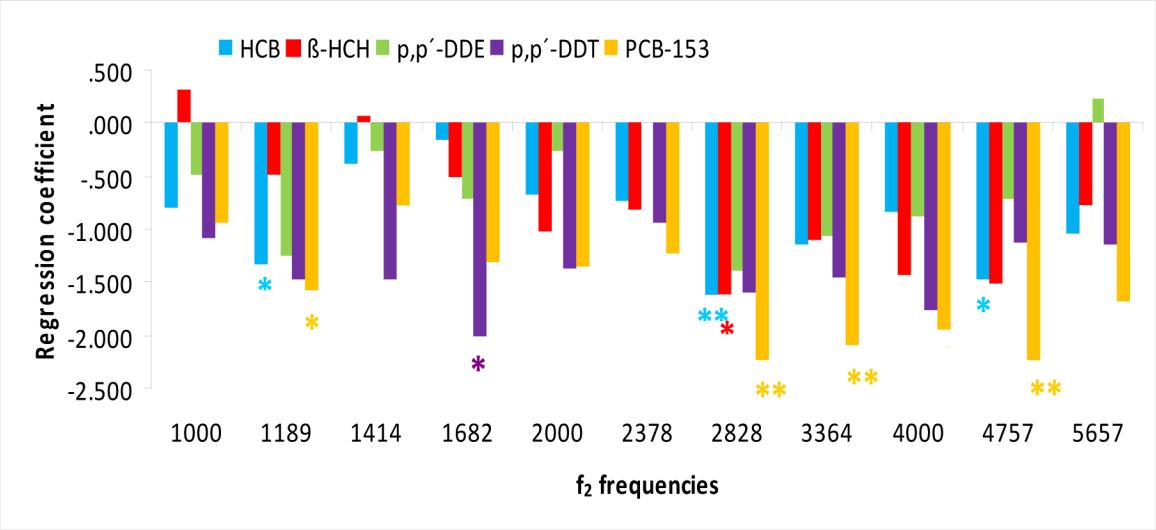

Fig 4.

On the vertical axis are plotted values of regression coefficients obtained when regressing amplitudes of otoacoustic emissions at various f_2 frequencies measured at the age of 45 months against serum concentration of individual pesticides or PCB-153 at the age of 45 months. * p≤0.05, **p≤0.01, ***p≤0.001.

Table 1

Prenatal(cord blood) and three postnatal concentrations (6, 16 and 45 months) of HCB, β-HCH, p,p′-DDE, p,p′-DDT and PCB-153 in Michalovce birth cohort (ng/g lipid).

	N = 351	HCB	β-HCH	p,p′-DDE	p,p′-DDT	PCB-153
	% < LOD	2.6	12.0	0	0.6	0
Cord blood	Mean	122.35	9.84	628.43	28.00	184.36
	Std. Deviation	218.67	8.09	473.67	24.06	175.95
	Median	75.29	7.48	506.69	21.91	129.69
	Minimum	1.13	1.29	21.75	0.76	14.11
	Maximum	3332.76	61.60	3023.44	240.93	1373.70
	% < LOD	2.3	16.8	0.6	0.6	0.3
6 months	Mean	147.23	12.24	700.77	40.37	229.81
	Std. Deviation	353.35	12.9	832.38	40.85	334.94
	Median	72.07	8.19	451.44	30.52	141.49
	Minimum	0.27	0.58	0.33	1.18	1.69
	Maximum	5185.38	106.40	6587.67	265.10	2640.77
	% < LOD	0.9	11.4	0.3	3.1	0.3
16 months	Mean	173.65	13.36	882.84	38.13	268.32
	Std. Deviation	453.3	16.06	1046.58	52.04	436.14
	Median	66.47	6.95	542.61	19.24	137.53
	Minimum	0.13	0.44	1.27	0.42	0.53
	Maximum	5585.48	97.16	7479.88	397.80	3503.70
	% < LOD	3.4	10.3	0	0.9	0
45 months	Mean	113.15	7.70	738.88	27.28	211.0
	Std. Deviation	303.09	9.21	882.49	43.49	280.61
	Median	51.68	4.10	462.14	16.19	121.26
	Minimum	0.16	0.32	5.23	0.23	4.34
	Maximum	4591.50	61.57	7969.23	556.63	1918.81

Table 2

Pearson correlation coefficients for prenatal (cord blood serum) and postnatal concentrations (represented by values for 6, 16, and 45 months after birth) of HCB, β-HCH, p,p′-DDE, p,p′-DDT and PCB-153 in Michalovce birth cohort (ng/g lipid). Correlation coefficients for serum concentration of a particular toxicant across time intervals are in bold. Very weak correlations (0–0.19) are not color marked, weak (0.20–0.39), moderate (0.40–0.59), strong (0.60–0.79) and very strong correlations (0.80–1) are marked with graded intensity of orange.

		Prenatal					6 months					16 months					45 months				
		HCB	**β-HCH**	**p,p′-DDE**	**p,p′-DDT**	**PCB-153**	**HCB**	**β-HCH**	**p,p′-DDE**	**p,p′-DDT**	**PCB-153**	**HCB**	**β-HCH**	**p,p′-DDE**	**p,p′-DDT**	**PCB-153**	**HCB**	**β-HCH**	**p,p′-DDE**	**p,p′-DDT**	**PCB-153**
	HCB	1	0.544	0.517	0.182	0.327	**0.522**	0.290	0.193	0.128	0.062	**0.409**	0.289	0.195	0.110	0.101	**0.160**	0.143	0.041	−0.094	−0.045
	β-HCH		1	0.379	0.063	0.254	0.189	**0.195**	0.055	−0.081	−0.016	0.158	**0.239**	0.034	−0.130	−0.040	0.190	**0.231**	0.039	−0.135	−0.069
	p,p′- DDE			1	0.611	0.625	0.229	0.122	**0.395**	0.178	0.176	0.200	0.135	**0.367**	0.283	0.189	0.099	0.088	**0.356**	0.296	0.193
	p,p′- DDT				1	0.479	0.210	0.114	0.310	**0.406**	0.232	0.174	0.099	0.301	**0.495**	0.212	0.091	0.086	0.368	**0.549**	0.247
Prenatal	**PCB-153**					1	0.134	0.135	0.195	0.026	**0.456**	0.180	0.106	0.175	0.208	**0.378**	0.095	0.049	0.107	0.145	**0.389**
	HCB						1	0.716	0.808	0.628	0.719	**0.858**	0.687	0.718	0.569	0.673	**0.483**	0.539	0.575	0.206	0.552
	β-HCH							1	0.661	0.509	0.663	0.667	**0.700**	0.612	0.509	0.614	0.449	**0.579**	0.545	0.222	0.568
	p,p′- DDE								1	0.688	0.839	0.768	0.694	**0.899**	0.721	0.786	0.448	0.549	**0.762**	0.376	0.701
	p,p′- DDT									1	0.596	0.614	0.583	0.701	**0.698**	0.588	0.345	0.469	0.637	**0.465**	0.553
6 months	**PCB-153**										1	0.714	0.683	0.770	0.710	**0.921**	0.457	0.541	0.650	0.316	**0.873**
	HCB											1	0.831	0.822	0.678	0.776	**0.566**	0.598	0.645	0.264	0.675
	β-HCH												1	0.786	0.658	0.773	0.556	**0.747**	0.737	0.343	0.720
	p,p′- DDE													1	0.816	0.852	0.467	0.633	**0.870**	0.474	0.768
	p,p′- DDT														1	0.804	0.415	0.527	0.806	0.713	0.744
16 months	**PCB-153**															1	0.488	0.607	0.741	0.406	**0.917**
	HCB																1	0.715	0.567	0.336	0.564
	β-HCH																	1	0.719	0.426	0.697
	p,p′- DDE																		1	0.698	0.789
	p,p′- DDT																			1	0.495
45 months	**PCB-153**																				1