DIOXIN-LIKE AND NON-DIOXIN-LIKE PCB EXPOSURE OF MOTHERS AND PSYCHO-MOTOR DEVELOPMENT OF THEIR INFANTS

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Introduction

Polychlorinated biphenyl (PCB) levels are three to ten times higher in the human population living in the Michalovce district) than in that living in other regions of Slovakia[1,2]. The reason is obvious – 21,482 t of technical PCB formulations called Delor, Delotherm or Hydelor were produced in 1959-1984 by the Chemko chemical plant in Strážske town which is situated in the NW part of the Michalovce District. Lipophilicity and resistence to degradation leads to bioaccumulation of these compounds in human tissues and fluids. PCBs, organochlorine pesticides and related compounds have been considered as risk factors because of their potential to act as direct or indirect carcinogens, their estrogenic and anti-estrogenic properties, and their ability to induce cytochrome P450 enzymes[3,4]. In humans, background exposures to PCBs have been associated with decreases of serum thyroid hormones in infants. Data of a study shows potential relationship between PCB exposure and neurobehavioral abilities, thyroid gland dysfunction and immunomodulation of adults and children[5]. These environmental contaminants are dangerous because of damaging not only the immune and hormonal system of adults and children but also the developing foetus in the mother's body. Vulnerable organisms of foetus and neonates exposed across the placenta and via breast milk respectively are in comparison to adults much more sensitive to the environmental exposure since the intensive growth and development of organism, central nervous and immune system as well as endocrine regulation is in progress. The range of impairment is substantially higher as for adults[6,7]. While these changes are also associated with neurological developmental delays, it is uncertain whether these associations are causal[8]. Another studies showed negative effects on neurological and cognitive development in childhood as a consequence of prenatal exposure to PCBs, and that breast-feeding counteracts the adverse developmental effects of PCBs. There was shown greater vulnerability of the prenatally exposed neonates to PCBs who were not breast-fed. It is not clear whether the protection offered by breast-feeding is caused by nutrients in breast milk or better quality of intellectual stimulation often provided by breast-feeding mothers[9,10]. In this study, potential relationships between PCB exposure (especially non-dioxin-like PCBs, ndl-PCBs) and mental and psychomotor development of newborns are investigated.

Material and methods

Recruitment of 143 mothers was realized with administration of all necessary documentation (study object selection criteria, informed consent, questionnaire for mothers, parturition record, Raven test, and breast milk collection report). 143 mother's blood serum (before delivery) and cord blood serum (during delivery) collection for PCB and lipid analysis was carried out in 2006-2007. Study participants performed psychological Raven intelligence tests and Home questionnaires were evaluated by psychologists. 101 ten-month-old infants underwent blood collection, psychological examination, and their psycho-motor development was assessed by the Bayley Mental and Motor Scales. Non-ortho-, mono-ortho- (dioxin-like, dl-) substituted and multi-ortho- (ndl-) substituted PCBs were monitored in study subjects' serum samples.

Analysis of ndl- and dl-PCBs in blood serum

Congener-specific analyses of ndl- and dl-PCBs in maternal and cord blood serum by $^{13}C_{12}$-isotope-dilution method using high resolution gas chromatography – high resolution mass spectrometry (HRGC/HRMS) were performed. Blood serum samples were treated by modified solid-phase extraction (SPE) method published recently[11]. Blood serum (from 3.29 g to 7.55 g) fortified with a known amount of ^{13}C-labelled internal extraction standards was sonicated with a propanol:water mixture and treated on an SPE column (2 g C18, endcapped, Alltech, USA). Analysed compounds were eluted with a hexane:dichloromethane mixture. The extract was cleaned-up on a multi-layer florisil–silica/H_2SO_4 column and eluted with hexane:dichloromethane. ^{13}C-labelled syringe standard solution was added to the concentrated eluate. The eluate was injected (split-less mode, injector

temperature 280 °C) into an HRGC HP 6890 (Hewlett Packard, USA) linked with an HRMS MAT 95 XP (Thermo Finnigan, USA). Separation on a DB-5ms (60 m × 0.25 mm × 0.25 μm) column using carrier gas He (constant flow 0.8 ml.min^{-1}) and a temperature programme of 120 °C (1.5 min), 30 °C.min^{-1}, 200 °C, 3 °C.min^{-1}, 287 °C, 10 °C.min^{-1}, 325 °C was applied (interface temperature was 280 °C). The analyses were performed in the MID (Multi Ion Detection) mode by EI POS 53 eV. Criteria of quality assurance and quality control were realized in accordance with U.S. E.P.A. 1668 method. The samples were treated in sets of 10 with one blank sample.

Lipid determination in blood serum
Total lipid content measurements in serum are needed for PCB concentration evaluation on lipid basis. Total cholesterol, free cholesterol, phospholipids, and triglycerides were determined at the Department of Clinical Biochemistry of TOP-MED General Hospital Bratislava in 143 sets comprising of maternal, and cord serum, and 101 child's serum. Using the concentrations determined the total lipid content was calculated according to Akins[12].

Social-educational atmosphere in family and neuro-behavioural examinations of infants
Psychological Raven test results reflect social and intelligence level of mothers, which has significant influence on child development. The home and emotional environment of infant was characterised by responses of Home questionnaire for 101 children. The psycho-motor development of 10-month-old infants was assessed using the Bailey Mental and Motor Scale. Software SPSS 14.0 and XLSTAT 7.1 were used for statistical data evaluation.

Results and discussion
HRMS results of the analysis of individual PCB congeners and selected organochlorine pesticides in 30 blood serum samples of mothers are presented in Table 1. Levels are reported on lipid basis. Average total lipid content in maternal serum before delivery was: 11.56 mg.ml^{-1}, in cord serum: 2.21 mg.ml^{-1}, and in the serum of 10-month-old child: 6.24 mg.ml^{-1} (high lipid content in maternal serum is caused by physiological changes in mothers' organism during pregnancy). Peaks of tri- (PCB 18, 28, 33, 22), tetra- (PCB 52, 49, 47, 44, 70) and pentachlorinated (PCB 96), rarely for higher chlorinated congeners were observed in blanks so these congeners could not be evaluated. Maximum values only were reported for the statistical evaluation of PCB 95, 92, 101, 110, 136, 149, and 132 congeners for the same reason. Non-ortho substituted dl-PCB 77, 81, 126, and 169 congeners with higher WHO-TEFs were not detected because low serum volumes had been available. Mono-orto-substituted PCBs (PCB 118, 123, 114, 105, 167, 156, 157, 189) are notable from the point of view of toxicity (WHO$_{2005}$ TEF 0.00003). Mean toxic equivalent TEQ for sum of 8 mono-ortho-PCBs was 1.13 pg WHO$_{2005}$ TEQ.g^{-1} lipid, median 0.79 pg WHO$_{2005}$ TEQ.g^{-1} lipid. In comparison with previous studies, PCB levels are lower (e.g., the mean 10.2 pg WHO$_{1998}$ TEQ.g^{-1} lipid for 8 mono-ortho substituted PCBs was 1.5 times lower than in former study of 315 adults of general population from Slovakia[13]) because the objects of our study are young mothers, and PCB concentration in organism increases with age and is higher in men. Time trends unambiguously show decreasing PCB levels in the human population[14]. The mean DDE/DDT ratio of 23.2 shows a long-term metabolization process and low DDT exposure.

Maternal and umbilical cord blood serum samples were analysed in order to assess PCB and organochlorine pesticide exposure of mothers and the transfer of organochlorines to their newborns in a group of 218 mother/child pairs in a previous study[15]. Corresponding lipid adjusted concentrations were observed for mother and cord serum. The median of the sum of the most abundant PCB congeners (118, 138^{+163}, 153, 156^{+171}, 180, 170) for mothers and cords was 815.8 ng.g^{-1} lipid, 628.8 ng.g^{-1} lipid respectively. Statistically significant correlations of PCB 153 (used as biomarker) serum concentrations were found between mother – cord (Spearman r=0.926, p<0.0001), cord – 6-month-old child (r=0.400, p<0.0001), and 6-month – 16-month-old child (r=0.914, p<0.0001) by multiple regression analysis. Besides placental transfer, breast-feeding is the second substantial route of infant exposure. Breastfed infants are a group of high PCB intake which might be two orders of magnitude higher than adult exposure[16]. Close correlations were found between maternal serum and human milk for PCB 153 (r=0.987), p,p'-DDT (r=0.873), and p,p'-DDE (r=0.951) unambiguously confirming organochlorine transfer via umbilical cord and human milk lipids. The results indicate similar correlations and concentrations for the investigated group of 143 mothers of the present study.

Table 1: Di-ortho- and mono-ortho-substituated congeners of PCBs and some organochlorine pesticides in the blood serum of mothers before parturition

Congener	Mean / ng.g⁻¹lipids	Median / ng.g⁻¹lipids	Minimum / ng.g⁻¹lipids	Maximum / ng.g⁻¹lipids
Tetrachlorinated PCBs				
PCB 74	9.76	7.17	0.013	39.3
PCB 66	1.83	1.08	<0.004	9.05
Pentachlorinated PCBs				
PCB 95				60.3
PCB 92				8.99
PCB 101[+84,90,89,113]				78.2
PCB 99	4.71	3.28	0.888	21.7
PCB 117	0.237	0.133	<0.007	1.17
PCB 87	0.893	0.275	<0.009	17.7
PCB 110				52.0
PCB 107	0.204	0.092	<0.002	2.73
PCB 123	0.107	0.068	<0.007	0.645
PCB 118	11.5	7.39	1.73	105
PCB 114	0.427	0.372	<0.018	1.98
PCB 105	1.86	1.15	0.338	15.0
Hexachlorinated PCBs				
PCB 136[+154]				5.10
PCB 151	1.28	0.677	0.219	15.7
PCB 149				35.0
PCB 133	2.05	1.35	0.337	11.4
PCB 146	13.4	7.69	2.03	111
PCB 153	179	126	28.8	1635
PCB 132				11.9
PCB 137	1.67	1.16	<0.028	15.5
PCB 138	102	66.3	16.4	1128
PCB 128	1.81	0.766	<0.016	26.6
PCB 167	4.68	2.84	0.556	53.5
PCB 156	15.2	9.38	2.35	148
PCB 157	1.52	0.934	0.207	16.6
Hepta-chlorinated PCBs				
PCB 176	0.746	0.026	<0.006	19.3
PCB 178	8.10	5.22	1.18	50.9
PCB 187[+182]	97.9	51.2	10.2	781
PCB 183	44.0	29.8	4.80	401
PCB 177	31.2	17.1	3.36	295
PCB 171	17.4	10.7	1.97	176
PCB 172[+192]	15.3	8.53	1.55	142
PCB 180	175	111	24.5	1316
PCB 170[+190]	88.3	53.6	12.1	731
PCB 189	2.39	1.31	0.274	20.2
Octachlorinated PCBs				
PCB 202	2.50	1.60	0.321	10.9
PCB 201	0.010	0.007	<0.001	0.089
PCB 199	30.1	16.3	3.19	215
PCB 196[+203]	19.8	13.2	2.56	109
PCB 195	6.66	4.20	0.819	43.1
PCB 194	19.7	10.8	2.32	157
Nonachlorinated PCBs				
PCB 206	1.77	0.944	0.218	17.0
Decachlorinated PCBs				
PCB 209	0.357	0.253	0.101	1.00
Organochlorine pesticides				
p,p'-DDE	369	294	69.5	1061
p,p'-DDT	15.9	12.0	2.91	67.7
HCB	54.1	40.3	14.7	212
beta-HCH	9.84	7.60	2.58	43.1

Correlation between Raven and Home scores (Spearman r=0.212, p=0.033, N=101) showed significant effect of the social and intelligence level of mother on emotional background and simultaneously on mental and psycho-

motor development of child. Significant correlations between Home score and Mental score of child (Pearson r=0.480, p<0.0001) and between Home score and Mental Developmental Index (MDI, r=0.409, p<0.0001) confirmed this fact. Mean values, medians, minima and maxima of psychological test results of mothers and their 10-month-old children are reported in Table 2. The table shows that the values evaluated for the whole group and for a subgroup of 24 mother/child pairs are similar and the subgroup represents a statistically appropriate group. No significant correlations and associations were found between ndl- and dl-PCB serum levels in mothers versus mental scale score, MDI, motor scale score, and PDI of their children for this number of samples. The PCB serum levels of the whole group of 101 children and their daily intake of ndl- and dl-PCBs via lactation and food are recommended to examine, because of possible direct influence of dietary exposure on infant. Caution should be exercised regarding health implications, and the possibility of subtle effects such as tendency to the poor psychomotor development of children cannot be ruled out.

Table 2: Results of Raven tests of mothers, Home questionnaires, and psychological tests of 10-month-old children assessed using the Bayley Mental and Motor Scale of Infant Development

		Number	Mean	Median	Minimum	Maximum
Score	Raven test of mothers	143/24	46.8/45.9	48/48	13/23	60/60
	Home questionnaire	101/24	31.8/33.5	32/34	20/25	39/39
Score - Bayley Scale	Mental scale - score	101/24	77.3/78.6	77/78	68/73	88/88
	MDI*	101/24	90.1/90.5	93/90	66/77	111/111
	Motor scale - score	101/24	61.9/61.7	62/62	52/52	69/68
	PDI**	101/24	109.4/103.0	110/103	79/79	128/123

* MDI – Mental Developmental Index
** PDI – Psychomotor Developmental Index

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