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)

BEÁTA DROBNÁ – ANNA FABIŠIKOVÁ – KAMIL ČONKA – JANA CHOVANCOVÁ – MILENA DÖMÖTÖROVÁ – SOŇA WIMMEROVÁ – EVA ŠOVČÍKOVÁ – ANTON KOČAN

Summary

Polychlorinated biphenyl (PCB) and polychlorinated dibenzo-*p*-dioxin and dibenzofuran (PCDD/F) intake via nutrition (nursing, infant milk formula) by infants was evaluated. A cohort of mother-child pairs from an area contaminated with PCB by the Chemko chemical plant formerly producing PCB in eastern Slovakia was examined. Breast milk and blood serum were analysed for PCB and PCDD/F by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The median daily intake of total toxic equivalent (TEQ) of dioxin-like PCB (dl-PCB) and PCDD/F from breast milk for fully breast-fed children was 72.8 pg·kg^{-1} body weight per day, assuming 7.6 kg as average body weight of the child. However, daily total intake of TEQ of non-breastfed children during the first 10 month of life was only 0.21 pg·kg^{-1} body weight per day on average. Dl-PCB-TEQ contributed to the total TEQ in breast milk with a higher proportion (68%) than PCDD/F-TEQ (32%) because of a much higher contamination of breast milk with PCB in general. The median of the sum of non-dioxin-like PCB (ndl-PCB) daily intake assuming 7.6 kg body weight was evaluated as 2342 ng·kg^{-1} body weight per day and ranged from 8 ng·kg^{-1} to 13124 ng·kg^{-1} body weight per day.

Keywords

polychlorinated biphenyls; dioxins; breast milk; infant milk formula; daily intake

Polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/F) are halogenated persistent organic pollutants (POP) widely present in the environment. Despite the 1984 ban on the production of PCB at the Chemko chemical plant in Strážske, Michalovce district, Slovakia, the contamination of the environment and locally produced homebred food has remained significant in the Michalovce district [1–3]. In consequence of the bioaccumulation of PCB in particular in animal and human tissues via food chain, elevated human body burden was found in this area [4–6].

POP studied represent groups of compounds with various influences on human organisms especially at various stages of development [7]. Extensive data on endocrine-related effects of PCB are available [8–12].

The effect of high maternal serum PCB levels on birth weight was observed in several studies [13–15]. The higher proportion of teeth affected with enamel defect in children aged 8–9 years was associated with higher PCB serum levels [16]. PCB may affect the outer hair cells of the cochlea, which is consistent with findings from animal studies published to date [17].

Two industrial incidents in Japan in 1968 and in Taiwan in 1979, where cooking rice oil was accidentally contaminated with PCB and PCDF, provided extensive neurodevelopmental information. Persistent growth retardation, movement disorders, generalized slowness, and IQ deficiencies in

Beáta Drobná, Anna Fabišiková, Kamil Čonka, Jana Chovancová, Milena Dömötörová, Eva Šovčíková, Anton Kočan, Department of Toxic Organic Pollutants, Slovak Medical University, Limbová 12, SK – 833 03 Bratislava, Slovakia.
Soňa Wimmerová, Department of Biostatistical Analyses, Slovak Medical University, Limbová 12, SK – 833 03 Bratislava, Slovakia.

Correspondence author:
Drobná Beáta, e-mail: beata.drobna@szu.sk

babies born to mothers exposed during pregnancy were observed [15, 18, 19].

Results of several cohort studies are available in which environmental blood levels of PCB were found to be related to neurobehavioral effects in infants: Michigan, North Carolina, Dutch, Faroe Islands, Düsseldorf cohorts [18]. Perinatal exposure to PCB and dioxins was found to be associated with immune changes in healthy Dutch preschool children [20]. In 9-year-old children of the Rotterdam PCB-dioxin cohort, higher prenatal PCB levels were associated with effects on the developing brain, resulting in e. g. longer response times [21].

For the reason of significant health effects of PCB and dioxins on a developing organism, the estimation of their daily intake by infants is important. The average daily food intake of PCDD/F total toxic equivalent (TEQ) for an American adult weighing 65 kg was estimated between 0.3 pg·kg^{-1} and 3.0 pg·kg^{-1} body weight by SCHECTER et al. [22]. Due to the relatively high levels of PCDD and PCDF commonly found in breast milk of American women and women from other industrial countries, a nursing infant may consume on average 35–53 pg·kg^{-1} body weight per day (expressed as TEQ) during its first year of life, without taking into account dioxin-like PCB (dl-PCB) contribution to the intake.

Substantially lower daily intakes were observed in non-breastfed infants [23, 24]. The estimated daily dioxin and indicator PCB intake of the average infant population due to the consumption of infant formula exceeded neither the tolerable daily intake (TDI) of 2 pg·kg^{-1} body weight per day (expressed as World Health Organization TEQ) recommended by the Scientific Committee on Food [25], nor the threshold value of 10 ng·kg^{-1} body weight per day PCB proposed by the National Institute of Health and Environment (RIVM, Bilthoven, The Netherlands) [26].

Limited exposure data for young children up to six years of age from Europe indicate that the average intake of total six non-dioxin-like PCB (ndl-PCB) is about 27–50 ng·kg^{-1} body weight per day, breastfeeding excluded [27]. The same study concluded that breastfed infants' ndl-PCB intake might be by two orders of magnitude higher than the exposure of adults. In the Czech Republic, medians of estimated indicator PCB daily intakes for infants (evaluated from breast milk contents) of 1.3–6.1 μg·kg^{-1} body weight per day were reported [28].

In general, infants have a relatively high intake of the pollutants of concern, due to their high food consumption per kg of body weight. In this study, breast milk and infant milk formula contents of the pollutants were the basis of daily intake estimation for infants, considering individual consumption of breast milk and infant milk formula from birth to 10th month of age.

MATERIALS AND METHODS

Sample collection

A number of 143 mothers, who resided in the studied area (Michalovce district, Slovakia) for the previous at least five years, were recruited for participation in the study during the years 2006–2008. Only healthy newborns with normal progress of gravidity of mothers and parturition were included. This included newborns born in time between the 38th–42nd weeks of gestation without pre-/peri-/postnatal pathology. Maternal blood collection was carried out before parturition, cord blood after parturition, and child blood at the age of 10 months. The serum was obtained by centrifugation, 0.5 ml stored at –80 °C in microtubes for the determination of lipid content in serum, and the rest stored in glass vials with polytetrafluorethylene (PTFE) sealed caps at –18 °C for the analysis of various PCB congeners.

The mothers participating in the study were required to collect once a week after breast-feeding into glass vials 20 ml of breast milk manually according to instructions and store the milk in a freezer at –18 °C up to the 10th week after childbirth.

Six types of infant milk formula declared in questionnaires by mothers used for their infants in the period of individual infant nutrition were acquired in order to be analysed for PCB and PCDD/F.

Serum sample preparation and analysis

Serum samples were treated by modified solid-phase extraction (SPE) using a previously published method [29]. Each of the serum samples was thawed, then spiked with a known amount of eight ^{13}C-labelled mono-*ortho*-dl-PCB (^{13}C PCB 123, 118, 114, 105, 167, 156, 157, 189), six indicator PCB (^{13}C PCB 28, 52, 101, 153, 138, 180), and five ndl-PCB congeners (^{13}C PCB 170, 178, 194, 206, and 209), and kept overnight at +6 °C. Serum mixed with an equivalent amount of water : 1-propanol (85 : 15, v/v) mixture was applied to a conditioned SPE column (2 g C18, endcapped; Alltech, Deerfield, Illinois, USA). The analytes were eluted with an *n*-hexane : dichloromethane (1 : 1, v/v) mixture, and the eluate was concentrated. The extract was cleaned-up on

a multi-layer florisil–silica/H_2SO_4 column and eluated with hexane : dichloromethane (9 : 1, v/v). The eluate was concentrated under a gentle nitrogen stream just to dryness. ^{13}C -labelled (PCB 32, 188) recovery standard solution was added immediately prior to GC injection.

The measurements were performed by a high resolution mass spectrometer (HRMS, MAT 95 XP; Thermo Finnigan, Bremen, Germany) coupled to an HP 6890 gas chromatograph (Hewlett-Packard, Palo Alto, California, USA) with a DB-5ms column (60 m × 0.25 mm × 0.25 μm) using splitless mode injection. Helium was used as a carrier gas at a constant flow of 0.8 ml·min^{-1}. HRMS was operated at a resolution of 10 000 in the positive ionization mode at 53 eV. The proportion of the two most abundant ions of natural (^{12}C) compounds and ^{13}C-labelled ones monitored in the selected ion monitoring mode together with retention time matching provided sufficient identification criteria according to the US EPA 1668 method [30]. Calibration was completed through the analysis of five calibration standard solutions, each containing the measured ^{12}C and above-mentioned ^{13}C-labelled compounds. The quantification of each congener in the samples applying actual relative response factors (RRF) and meeting the criteria of quality assurance and quality control in accordance with the above-mentioned isotope-dilution US EPA method was completed.

Lipid determination in blood serum

To adjust PCB serum content on the lipid basis, lipid content in the blood serum samples was calculated using "summation enzymatic method" according to Akins et al. [31]. Total cholesterol, free cholesterol, phospholipids and triglyceride contents needed for the calculation were determined by biochemical assays at the Department of Clinical Biochemistry, TOP-MED General Hospital, Bratislava, Slovakia.

Breast milk and infant milk formula analysis

Lipids were isolated from each individual breast milk sample using a liquid-liquid extraction method described previously [32]. The fat from powdered infant milk formulas was extracted using an accelerated solvent extractor (ASE 300; Dionex Corporation, Sunnyvale, California, USA) using hexane : acetone 4 : 1 (120 °C, 10 min/cycle, 2 cycles) and concentrated. Aliquots of about 3–5 g of milk fat were dissolved in *n*-hexane and spiked with the same ^{13}C-labelled PCB congeners as the serum samples and, additionally, with four ^{13}C-labelled non-*ortho*-PCB (^{13}C PCB 77, 81, 126, 169) and seventeen PCDD/F. The milk fat removal on a high-capacity H_2SO_4/silica column was followed by extract clean-up and fractionation on a PowerPrep semi-automated system (Fluid Management Systems, Waltham, Massachusetts, USA) equipped with pre-packed multi-layer silica, basic alumina and carbon columns. HRGC/HRMS analyses were carried out under the same conditions as described above for the serum samples. Recovery standards of ^{13}C-labelled 1,2,3,4-tetra-CDD and 1,2,3,7,8,9-hexa-CDD were added to the fraction containing PCDD/F and non-*ortho*-PCB, and ^{13}C-labelled PCB (PCB 32, 188) were added to the fraction containing mono-*ortho*- and multi-*ortho*-PCB prior to GC/HRMS analysis. PCDD/F and PCB were quantified according to the isotope dilution US EPA 1613 [33] and 1668 methods, respectively.

Quality assurance and quality control

The samples were treated in sets of 10 together with one blank sample. A certified reference material Milk powder BCR-607 (Institute for Reference Materials and Measurements, Geel, Belgium) was used for checking the analytical process accuracy. The laboratory analysing the samples successfully participated in an inter-laboratory study on PCDD/F, dl-PCB and ndl-PCB in human milk (Inter-laboratory comparison on dioxins in food 2006, Norwegian Institute of Public Health, Oslo, Norway).

Evalution of intake of ndl-PCB, dl-PCB-TEQ and PCDD/F-TEQ via breast milk and milk formula by infant during breast-feeding period

Patandin et al. [34] estimated by calculation the daily intake of TEQ from breast milk as an increasing function with an increase in age of infant, assuming a permanent increase in the amount of consumed breast milk. In the first weeks after birth, a significant increase of received breast-milk is observed; however, very often after several weeks, the infant is fed also by infant milk formula. Therefore, these theoretical estimations do not reflect the reality. The duration of full breast-feeding period and the beginning of supplementing, or the total duration of breast-feeding period, are very individual parameters. Usually after weaning the baby, daily intake of PCB and PCDD/F significantly drops because infant milk formulas contain much lower contents of the monitored compounds.

In our study, data on amounts of breast milk consumed by the infants as estimated by their mothers were collected and, in parallel, PCDD/F and PCB contents in breast milk samples were

measured. The period of 10 months was divided into 5 periods: 1st month, 2nd–4th month, 5th–6th month, 7th–8th month, and 9th–10th month. The calculation of the total intake for each child was based on data from questionnaires on infant nutrition during each of the 5 periods.

The total intake of the compounds monitored and TEQ from breast milk up to the age of 10 months was calculated according to the equation:

$$TI = C\,T \sum_i P_i\,V_i\,D_i \quad (1)$$

where TI was total intake (ng) of a compound, C – lipid-adjusted content of a compound in breast milk (ng·g^{-1} lipid), T – lipid concentration in breast milk (g·ml^{-1}), P_i – number of breast feedings per day in a particular period i, V_i – volume of breast milk consumed by an infant during one breast feeding in a particular period i (ml), D_i – number of breast-feeding days in a particular period i.

The infant milk formula TI was evaluated analogously. The daily intake was obtained by dividing the TI by the number of respective days.

Statistical evaluation of data

The statistical evaluation of data was accomplished using SPSS 14.0 (IBM; Somers, New York, USA) and XLSTAT 7.1 (Addinsoft; New York, New York, USA) software.

RESULTS AND DISCUSSION

Information acquired from documentation and questionnaires

A number of 143 parturition reports, including information on newborn's birth weight, length, head circumference, gestational age, Apgar Score etc., were recorded in a database. Maternal questionnaires provided data about mothers and families. The average age of the mothers participating in this study was 26.1 and fathers 29.8 years. None of the mothers indicated in the questionnaire to have worked with chemicals (oil products, plastics, PCB, asbestos, heavy metals). Twelve mothers or fathers of the examined infants had a parent employed in Chemko Strážske Co. for a long time period (6–32 years). The most important information relevant to mother-child exposure and infant development gained from the questionnaires is summarized below. Mothers consuming homebred eggs comprised 44.4%, poultry 26.5% and other homebred meat 30.8%. Homebred food could be a significant source of the studied compounds in this area due to the increased contamination of the environment [2, 3]. Data from the questionnaires revealed a relatively high number of mothers who smoked before their pregnancy (49.6%) and during the gravidity (13.7%). The number of fathers smoking during the mother's pregnancy was 46.2%, so we can assume passive smoking of the mothers, too. Influenza and respiratory diseases were the most frequent illnesses during the pregnancy; 22% of the mothers took antibiotics during gravidity.

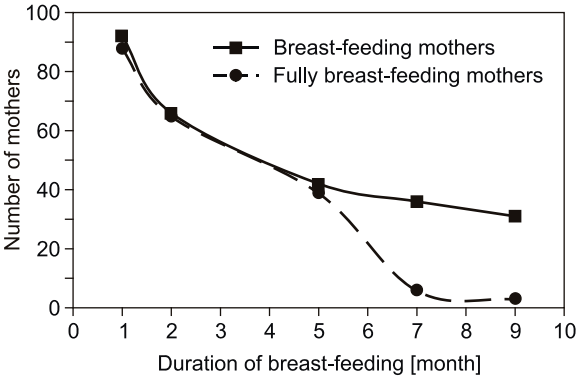

Fig. 1. Number of breast-feeding and fully breast-feeding mothers (from total number N = 101) in dependence on the age of the infants.

A decrease in a number of breast-feeding mothers was strong in the first month after delivery (9%) but the strongest in the 2nd month after delivery (Fig. 1). A percentage of 30.6% of mothers nursed her baby until 9–10 month of age.

Exposure of mothers and infants to PCB

Fifty-five PCB congeners, including all the dioxin-like mono-*ortho*-PCB, were determined in 143 serum samples. It was not possible to quantify planar non-*ortho* substituted PCB congeners having higher TEF [35] in the low amount of blood sera available for analysis (3.29–7.55 g of serum). The WHO 1998 TEF values were used to calculate TEQ of PCDD/F and dl-PCB [35].

Contents of some tri- (PCB 18, 28, 33, 22), tetra- (PCB 52, 49, 47, 44, 70) and pentachlorinated (PCB 96) congeners were not possible to evaluate due to their frequent occurrence in blank samples. If the content of a congener was lower than its limit of detection (*LOD*), the value of half *LOD* was used in all the subsequent procedures of evaluation (sums of contents, TEQ and statistical processing). The highest levels reached were for three (PCB 153, 138, 180) of the six indicator PCB; the sum of which represented approximately 60% of the content of all the multi-*ortho*-substituted PCB.

Mothers participating in the study had noticeably higher median contents of

PCB 138: 59.1 ng·g^{-1} lipid, PCB 153: 125.7 ng·g^{-1} lipid, and PCB 180: 111.4 ng·g^{-1} lipid in comparison with a population of the German study, for which WITTSIEPE et al. presented median values for PCB 138, 153 and 180 being of 33, 60 and 58 ng·g^{-1} lipid, respectively [36]. The serum contents in mothers measured in our study were lower than those found in an earlier study (year 2001) for women of a similar age group living in the Michalovce district and close to those residing in a background area located about 60 km far from the contaminated area (general population of the Svidník and Stropkov districts) [5]. The time trend of PCB had undoubtedly a descending tendency. In serum of Polish general population of mothers, contents of 12.2, 20.9 and 15.1 ng·g^{-1} lipid for the above mentioned 3 indicator PCB were determined by JARACZEWSKA et al. [37] in a time period comparable with our study. This fact confirms that significant contents are still present in the area near the former PCB production in Strážske, Michalovce district, even 25 years after stopping the production. The median value of mono-*ortho*-PCB-TEQ of the cohort was 6.6 pg·g^{-1} lipid. Interesting is the fact that the parents of the mother having the highest serum PCB level were employed at Chemko Strážske company for years.

Contens of PCB in cord blood adjusted to lipid basis were almost the same or slightly lower than in the blood serum of the mothers. Many studies confirmed the trans-placental transfer of PCB [38–41], which was also suggested by our study. Due to about 5-times lower lipid content in cord serum, in comparison with maternal serum, the trans-placental transfer of lipophilic compounds can be assumed as evident but limited. However, the infant "supplements" these persistent compounds through breast feeding. This is probably the reason for the fact that contents in infant sera, after re-calculation on lipid basis, are similar to those in maternal blood serum. Spearman rank correlation coefficients between maternal and infant serum contents of the 3 indicator PCB, sum of ndl-PCB, and TEQ of mono-*ortho*-PCB were 0.469, 0.481 and 0.499, respectively ($p < 0.01$ for all the 3 correlations). The median values of the most abundant PCB were for PCB 138: 48.6 ng·g^{-1} lipid, PCB 153: 118.7 ng·g^{-1} lipid, and PCB 180: 98.2 ng·g^{-1}lipid. The infant with the highest sum of PCB 138, 153, 170, 180 was a child of the mother whose body contained high PCB levels and who fully breast-fed her child for 6 months.

PCB and PCDD/F in breast milk

The advantage of breast milk over blood serum is the higher possibility of analysing not only all the PCB congeners but also PCDD and PCDF in the extracted milk fat. This is because of easier availability of higher volumes of milk samples making possible to determine lower contents of a wider spectrum of compounds. We obtained 75 breast milk samples collected by study participants as mentioned in section "Materials and methods". The extracted breast milk fat content was on average 3.3%, ranging from 1.7% to 6.7%. The average fat content in infant milk formula was almost the same, 3.4%, ranging from 3.2% to 3.6%. Due to the consistency of human milk data with serum results, the ndl- and mono-*ortho*-PCB congeners listed in Tab. 1 that were determinable in the serum samples are presented only. In Tab. 1, levels of analysed individual congeners of PCB, sums of PCB congeners in homologue groups, mono-*ortho*-, and ndl-PCB are presented. A wide scale of analysed compounds in breast milk facilitated the calculation of TEQ originating from all dl-PCB (mono-*ortho*- and non-*ortho*-substituted PCB) as well as from PCDD and PCDF (Tab. 2). Similar levels of PCB (adjusted to lipids) in breast milk and maternal serum indicate the transfer of these compounds into breast milk. High correlation coefficients between summed levels of the 3 most abundant indicator PCB, sum of ndl-PCB, and TEQ of mono-*ortho*-PCB in maternal sera and their levels in breast milk (Spearman test, $r = 0.888$, 0.889, and 0.901, resp., $p < 0.0001$) unambiguously confirm the transport of the monitored compounds into breast milk.

Proportion of TEQ calculated from dl-PCB (TEQ of non-*ortho*- and mono-*ortho*-PCB) to TEQ of PCDD/F in breast milk was found in our study (68% to 32%, resp.) a bit higher than in the German study with a similar design (52% to 48%) [36]. It was probably caused by a higher contamination of the environment and food with PCB in the vicinity of Michalovce in comparison to PCDD/F. West European countries experience a higher contamination caused by PCDD. For example, higher PCDD/F-TEQ than PCB-TEQ was reported in samples from the Netherlands, Belgium, Luxemburg and Spain collected within the 3rd round WHO-coordinated exposure study of human milk. In spite of this fact, the indicator PCB contents were higher in breast milk from Slovakia and the Czech Republic than in all other participating countries [42]. As can be seen in Tab. 2, the median and maximum PCDD/F/dl-PCB-TEQ were 20.8 pg·g^{-1} and 203.7 pg·g^{-1} lipid, respectively, in breast milks from our study. It is interesting to allege that the above-mentioned levels significantly exceed the maximum level stated by Commis-

Tab. 1. Contents of PCB in breast milk of the studied cohort of mothers.

PCB	PCB IUPAC No.	Mean	Median	Min.	Max.
		[ng·g^{-1}]			
Tri-CB	PCB 28	6.58	2.53	0.066	91.0
Sum of tri-CB		6.76	2.62	0.066	91.1
Tetra-CB	PCB 52	0.476	0.181	0.052	7.64
	PCB 47^{+48}	1.03	0.419	0.0009	13.1
	PCB 74	30.3	13.4	0.565	240.8
	PCB 66	5.93	1.71	< 0.0001	143.4
Sum of tetra-CB		37.8	15.1	0.002	382.6
Penta-CB	PCB 95	0.649	0.332	< 0.109	7.72
	PCB 92	0.575	0.255	0.0041	9.05
	PCB 101$^{+84,90,89,113}$	2.66	0.496	0.152	118.9
	PCB 99	10.9	6.20	0.0072	116.2
	PCB 123	0.375	0.144	< 0.0004	9.20
	PCB 118	21.3	12.9	0.021	136.5
	PCB 114	0.984	0.684	0.0007	5.73
	PCB 105	4.159	2.21	0.0018	30.6
Sum of penta-CB		45.1	26.8	0.031	378.3
Hexa-CB	PCB 151	2.05	1.02	0.012	29.8
	PCB 149	1.53	0.717	0.163	16.6
	PCB 133	4.95	2.49	0.0041	67.4
	PCB 146	29.5	15.1	0.029	479.8
	PCB 153	291.2	191.9	0.269	2 097
	PCB 137	3.37	1.92	0.0018	60.6
	PCB 138	152.0	86.8	0.186	2 268
	PCB 128	1.92	0.745	0.0041	50.4
	PCB 167	6.03	3.14	0.0091	77.7
	PCB 156	22.1	14.3	0.018	215.4
	PCB 157	2.03	1.31	0.0010	23.0
Sum of hexa-CB		516.5	327.4	0.523	5 385
Hepta-CB	PCB 178	13.7	8.50	0.015	77.2
	PCB 187^{+182}	27.4	16.9	0.020	220.1
	PCB 183	10.9	6.00	0.010	147.1
	PCB 177	16.7	7.82	0.014	256.6
	PCB 171	9.34	4.29	0.0049	175.4
	PCB 172^{+192}	9.15	3.62	0.0075	152.5
	PCB 180	234.5	131.7	0.222	1 467
	PCB 170^{+190}	113.7	60.2	0.112	908.3
	PCB 189	2.78	1.45	0.0031	23.9
Sum of hepta-CB		438.3	245.1	0.410	3 416
Octa-CB	PCB 202	2.08	1.14	0.00048	11.0
	PCB 200	34.3	17.7	0.031	206.0
	PCB 196^{+203}	21.5	11.9	0.020	136.7
	PCB 195	6.73	3.68	0.0072	44.7
	PCB 194	20.7	11.2	0.019	132.6
Sum of octa-CB		85.3	46.9	0.077	487.8
Nona-CB	PCB 206	1.72	0.871	0.0013	12.7
Deca-CB	PCB 209	0.203	0.110	0.00005	2.24
Sum of all PCB		1 117	651.0	0.098	9 506
Sum of PCB 138, 153 and 180		677.7	406.7	0.677	5 832
Sum of 6 indicator PCB		687.2	416.2	0.677	5 835
Sum of mono-ortho-PCB		59.7	40.1	0.055	490.3
Sum of all ndl-PCB		1 072	620.3	0.988	9 015

Values are expressed per gram of lipid (N = 75).
CB – chlorinated biphenyls.

Tab. 2. Contents of non-*ortho*-PCB, PCDD, PCDF and TEQ of dl-PCB and PCDD/F in breast milk of the studied cohort of mothers.

PCB	Mean	Median	Min.	Max.
	[pg·g^{-1}]			
PCB 77	6.76	4.91	0.849	44.8
PCB 81	7.02	2.93	0.758	112.7
PCB 126	66.3	39.5	11.0	491.5
PCB 169	40.2	26.8	7.54	215.0
TEQ of non-*ortho*-PCB	7.03	4.16	1.21	51.30
TEQ of mono-*ortho*-PCB	15.5	10.7	0.013	138.0
2,3,7,8-tetra-CDD	0.461	0.445	0.057	1.19
1,2,3,7,8-penta-CDD	1.45	1.37	0.048	4.32
1,2,3,4,7,8-hexa-CDD	0.821	0.726	0.216	2.53
1,2,3,6,7,8-hexa-CDD	3.60	3.18	1.30	7.80
1,2,3,7,8,9-hexa-CDD	0.794	0.686	0.147	1.87
1,2,3,4,6,7,8-hepta-CDD	4.43	3.84	0.585	23.4
Octa-CDD	21.2	18.9	8.06	68.0
TEQ of PCDD	2.46	2.38	0.620	6.54
2,3,7,8-tetra-CDF	0.530	0.407	0.106	2.28
1,2,3,7,8-penta-CDF	0.403	0.292	0.026	2.07
2,3,4,7,8-penta-CDF	10.3	7.46	1.98	93.6
1,2,3,4,7,8-hexa-CDF	3.16	2.60	0.613	12.2
1,2,3,6,7,8-hexa-CDF	2.32	2.17	0.570	5.39
1,2,3,7,8,9-hexa-CDF	0.142	0.072	0.014	1.35
2,3,4,6,7,8-hexa-CDF	0.734	0.620	0.191	1.93
1,2,3,4,6,7,8-hepta-CDF	0.993	0.793	0.374	3.10
1,2,3,4,7,8,9-hepta-CDF	0.101	0.054	0.010	0.770
Octa-CDF	0.251	0.149	0.031	1.33
TEQ of PCDF	5.87	4.29	1.22	48.9
TEQ of PCDD/F	8.32	6.72	1.94	52.6
Total TEQ	30.4	20.8	6.95	203.7

Values are expressed per gram of lipid (N = 75).
CDD – chlorodibenzo-*p*-dioxin, CDF – chlorodibenzofuran.

sion regulation 1881/2006: 6.0 pg·g^{-1} lipid for raw cows' milk and dairy products [43].

Intake of ndl-PCB, dl-PCB-TEQ and PCDD/F-TEQ via breast milk and milk formula by infant during breast-feeding period

Fully breast-feeding

The duration (number of days) of full breast feeding without supplementation was taken into consideration for the calculation of daily intakes of the compounds investigated (Fig. 2, Fig. 3). Our study determined the median total TEQ intake to be 553 pg per day and median intake of all PCB to be 18910 ng per day for full breast-feeding period. Daily intake of total TEQ 72.8 pg·kg^{-1} body weight per day assuming an average body weight of 7.6 kg [34] for infant was consequently calculated. The values ranged from 17 pg·kg^{-1} to 524 pg·kg^{-1} body weight per day. The median daily intake of dl-PCB-TEQ was estimated to be 49.8 pg·kg^{-1} body weight per day, which comprises 68.4% of total TEQ. The values of dl-PCB-TEQ ranged from 9.9 pg·kg^{-1} to 364 pg·kg^{-1} body weight per day. The total daily intake determined for our cohort exceeds the TDI of 1–4 pg·kg^{-1} body weight per day recommended by WHO [44], but is similar to values previously found in other industrialized countries. The value of TDI concerns lifetime exposure and is not directly applicable for infants because of a relatively short nursing period, e.g. short period of high intake. Patandin et al. [34] estimated a total TEQ daily intake of 852 pg from breast milk or 112 pg for males and 118 pg for females if referred to kg infant body weight. Focant et al. [45] reported 103 pg·kg^{-1} body weight per day expressed as TEQ from PCDD, PCDF and non-*ortho*-PCB for breast-fed infants from Belgium. Harrison et al. [46] presented an average value of 66 pg·kg^{-1} body weight per day in the period between the 2nd and 10th month of age in the

United Kingdom. PÄPKE [47] estimated a range from 24 pg·kg^{-1} to 145 pg·kg^{-1} of body weight per day, considering 5 kg body weight of an infant in Germany.

The median of the sum of ndl-PCB assuming 7.6 kg body weight was evaluated as 2342 ng·kg^{-1} body weight per day (ranging from 8 ng·kg^{-1} to 13124 ng·kg^{-1} body weight per day). Median daily intakes of individual congeners of PCB 138, 153 and 180 estimated by SZYRWINSKA et al. for breast milk of mothers from the general population in Poland (Wielkopolska region) were 109, 152 and 64 ng·kg^{-1} body weight per day, respectively [48], while in our study, values of 720, 342 and 530 ng·kg^{-1} body weight per day were found. SZYRWINSKA et al. compared estimated daily intakes of summed indicator PCB among various countries. The highest intakes were observed in the Czech Republic and Slovakia (human milk data originating from the 3rd round WHO-coordinated exposure study [42] were used), which can be related to common usage of PCB formulations in former Czechoslovakia. In the present study, median of daily intakes of the sum of six indicator PCB from breast milk collected five years later was calculated as slightly (by approx. 13%) lower value of 1608 ng·kg^{-1} body weight per day in the period of full breast feeding to compare the value reported for Slovakia by SZYRWINSKA et al [48]. In spite of different approach to daily intake estimation, the results of PCB breast milk contents and daily intakes are in a good compliance.

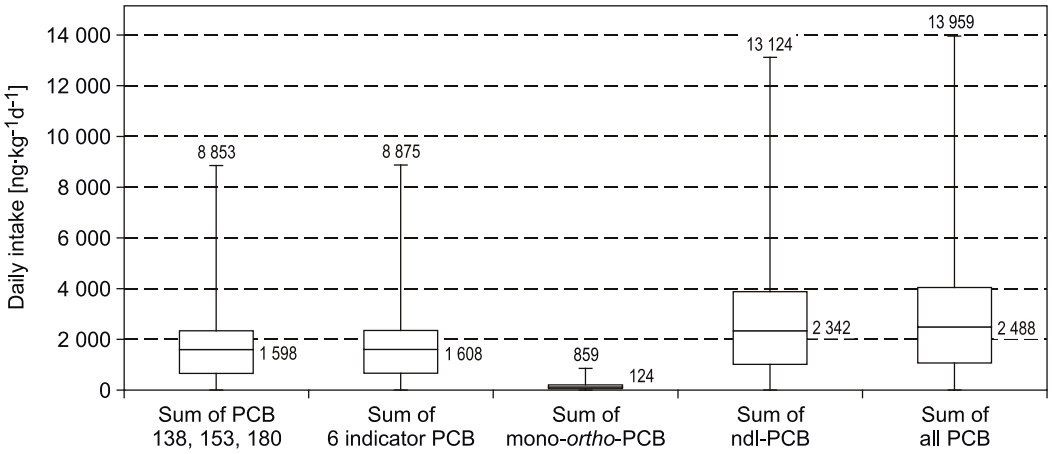

Fig. 2. Daily intakes (per kg body weight) of individual PCB groups calculated for fully breast-fed infants.
Minima, 25th percentiles, medians (values indicated), 75th percentiles and maxima (values indicated) are presented.

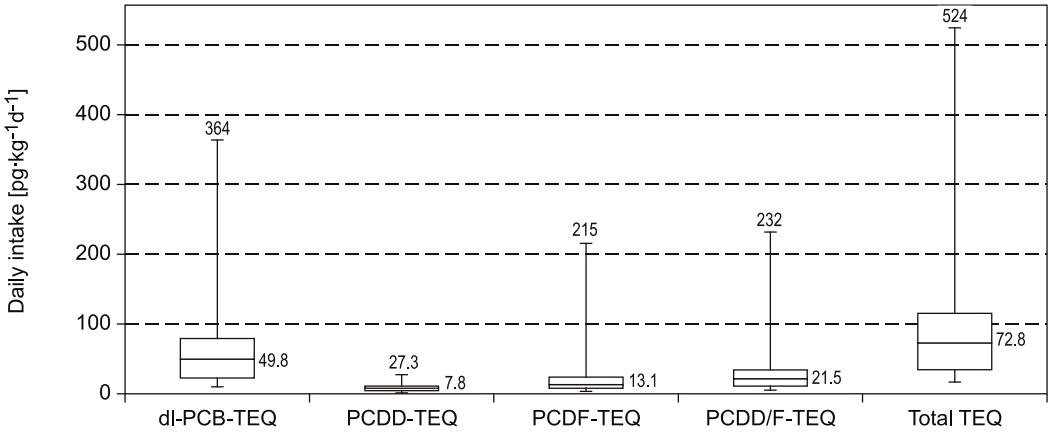

Fig. 3. Daily intakes (per kg body weight) of dl-PCB, PCDD and PCDF expressed as TEQ and total TEQ calculated for fully breast-fed infants.
Minima, 25th percentiles, medians (values indicated), 75th percentiles and maxima (values indicated) are presented.

Breast feeding and infant milk formula supplementation

The cumulative total intake of individual PCB, TEQ of dl-PCB and PCDD/F from breast milk and infant milk formula together during 10 months was almost the same as the total intake from breast milk solely. The values for individual children differed by a hundredth to tenth of percent only. While since the date of birth, the median of cumulative total intake of all the PCB from breast milk without supplementation was 2922 μg, the median was 2932 μg only when the PCB total intake from infant milk formula consumption was added to the total intake from breast milk. The reason for only a 10-μg increase in the intake is the fact that the contents of the investigated compounds in infant milk formulas are much lower than those in breast milk. Six types of the most frequently used infant milk formulas by mothers in our study were analysed. Contents of the compounds were from 500 to 5000 times lower than the contents in the breast milk samples. The median and maximum values of the sum of all PCB in the milk formulas were 1.15 ng·g^{-1} lipid and 1.97 ng·g^{-1} lipid, respectively, which can be considered as very low values. Even in the cases of very high milk formula consumption, its contribution to the total intake was negligible. For comparison, median and maximum values of sum of all PCB in breast milk were 651 ng·g^{-1} lipid and 9506 ng·g^{-1} lipid, respectively. Supplementing the infants by nutrition other than breast milk was the reason why average daily intakes calculated for the whole period of nursing and supplementation (10 months) were lower than those for the fully breast-fed infants (calculated only for the period of full breast feeding). The median of daily intake of total TEQ, assuming 7.6 kg average body weight, and breast feeding combined with infant milk formula, calculated for 10 months of age, was 44.1 pg·kg^{-1} body weight per day.

Nine of the examined infants were not breast-fed at all. The difference between cumulative PCB intake during 10 months from breast milk by nursed infants and not nursed was huge (Tab. 3). The median value of daily intake of total TEQ for a non-nursed 7.6 kg infant was calculated to be as low as 0.21 pg·kg^{-1} body weight per day.

CONCLUSIONS

The purpose of the study was to estimate real daily intakes of ndl-PCB, dl-PCB and PCDD/F from breast milk and infant milk formula in a contaminated area of Slovakia. Although the time trend of maternal serum PCB levels as well as breast milk ones have a declining tendency at the studied area, PCB intake by infants via breast feeding was found to be still significant.

The median daily intake of ndl-PCB, dl-PCB-TEQ and total TEQ, assuming full breast feeding and 7.6 kg infant body weight, was 2342 ng·kg^{-1}, 49.8 pg·kg^{-1}, and 72.8 pg·kg^{-1} body weight per day, respectively. Contents of total PCB (median 1.15 ng·g^{-1} lipid, maximum 1.97 ng·g^{-1} lipid) in the most frequently used infant milk formulas available in Slovakia, in comparison to the median and maximum values of total PCB in breast milk (median 651 ng·g^{-1} lipid, maximum 9506 ng·g^{-1} lipid), were more than 500 times lower. Therefore, PCB and PCDD/F intake from infant formulas can be considered as negligible compared to the intake from breast milk.

Finally, the number of benefits of breast feeding (greater immune health, lower risk of infections, protection from sudden infant death syndrome, higher intelligence, lower probability of developing diabetes mellitus or extreme obesity, and other long-term health effects) most likely exceeds the possible subtle effects of elevated PCB and PCDD/F levels in human milk, so it can be recommended to mothers to nurse their infants.

Tab. 3. Comparison of the cumulative intake of summed PCB and total TEQ taken up by breast-fed and non-breast-fed infants during 10 months after birth from breast milk and infant milk formula.

	Average	Median	Min.	Max.
Total intake of summed PCB	[μg]			
Breast-fed infants (*N* = 59)	4818	2932	14.8	20788
Not breast-fed infants (*N* = 9)	7.85	6.34	2.88	16.4
Total intake of TEQ	[ng]			
Breast-fed infants (*N* = 59)	141	100	5.85	884
Not breast-fed infants (*N* = 9)	0.685	0.472	0.254	1.52

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