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Exposure of breast-fed children in the Czech Republic to PCDDs, PCDFs, and dioxin-like PCBs

Vladimír Bencko[a,*], Milena Černá[b,d], Libor Jech[c], Jiří Šmíd[d]

[a] *First Faculty of Medicine and General University, Hospital Institute of Hygiene and Epidemiology, Charles University in Prague, Studničkova 7, CZ 12800 Prague 1, Czech Republic*
[b] *Center of Preventive Medicine, Charles University in Prague, Czech Republic*
[c] *Axys Varilab Ltd., Czech Republic*
[d] *Center of Environmental Health, National Institute of Public Health, Prague, Czech Republic*

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Abstract

Human milk samples from 81 mothers living in seven selected localities of the Czech Republic collected in 1999–2000 were analyzed for PCDDs, PCDFs, and dioxin-like PCBs. Significant local differences in total WHO-TEQ values were observed (median ranges: 27.8–64.6 pg/g fat) with the highest level in Uherské Hradiště, but the highest PCDD-TEQ value was in Prague. Seven congeners (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, and PCBs 118, 126, 156, and 157) cover about 90–94% of the total TEQ level. The non- and mono-*ortho* PCBs account for approximately 50–70% of the total TEQ levels in individual groups. The calculated median daily intake of the total TEQ for breast-fed infants ranged from 271 pg/kg b.w./day in Uherské Hradiště to 117 pg/kg b.w./day in Liberec and exceeded by about two orders of magnitude a tolerable daily intake (TDI) of 1–4 pg/kg b.w. recommended by the WHO. Our results confirmed significant local differences in the levels of dioxins and suggest that hot spot locations might exist within the country.

Keywords: Human milk; Dioxins; PCDDs; PCDFs; PCB; Breast fed infant exposure

1. Introduction

PCBs, PCDDs, and PCDFs are a class of structurally related compounds with a wide variety of toxic actions including reproductive and developmental effects, neurological and behavioural effects, immunomodulatory and carcinogenic effects (De Rosa et al., 2001; ATSDR Tox Profiles, 2002; Bencko, 2003). All of these compounds are globally distributed in the environment and people are inadvertently exposed to them from numerous sources, of which foodstuffs are the most important. As they concentrate in fat, these compounds can be found in human body fluids and tissues. Breast milk has been the preferred matrix to evaluate human background exposure (Fürst et al., 1994). In the industrialized countries, concentrations of PCBs and other persistent chlorinated organic pollutants have been regularly monitored in human milk and a rather large database on general population exposure is currently available. In the Czech Republic, the levels of indicator congeners of PCBs in human milk monitored since 1994 (Kliment et al., 1997, 2000) showed a significantly decreasing trend in time (Černá et al., 1999, 2003). However, a relevant data concerning the levels of PCDD/Fs and dioxin-like PCBs in human fluids and tissues of the Czech population remains inadequate. The first human background data on the level of PCDD/PCDF in two pooled human milk samples from Czech mothers obtained in a WHO-coordinated study (WHO/ECEH, 1996) did not show any excess of body burden. Similar results were obtained in four pooled human milk samples analyzed within the national-wide Environmental Health Monitoring System (Černá et al., 1999). The objective of the present study was

* Corresponding author. Tel.: +420 2249 19967.
E-mail address: Vladimir.Bencko@lf1.cuni.cz (V. Bencko).

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to produce more reliable and comparable data on levels of PCDD/Fs and PCBs in individual breast milk samples from different regions of the Czech Republic, to improve exposure assessment of breast-fed infants and to promote additional studies, if necessary.

2. Materials and methods

2.1. Breast milk sampling and survey respondents

In seven regions of the Czech Republic, 8–15 breast milk samples of about 50 ml each were obtained from primiparae between the second week and 2 months after delivery. The enrolment of mothers was managed on voluntary basis by local pediatricians. The refusal rate of addressed mothers varied between 25 and 30% according to selected localities. The sampling period was Spring, 2000. The sampling and individual interviews of donors were organized according to the WHO/ECEH protocol (1996). Informed consent was obtained from each subject. Short questionnaires were completed by each donating mother giving information on age, height, weight before pregnancy and after delivery, sampling time, duration of residence in the locality, smoking status, dietary habits (frequency of food consumption of animal origin), occupation history, use of medications, newborn's sex, weight at birth and at time of sampling. The population sample that collected and returned the milk samples constituted a total of 81 mothers.

2.2. Sample analysis

The milk samples collected in glass vessels were stored frozen (−18 °C) until they were analyzed. Fifty milliliters of milk was spiked by ^{13}C labelled internal standards (nine for PCDD/F and eight for PCB) and extracted first by 100 ml hexane–acetone (1:1) and then twice with 30 ml of hexane. Extract was dried and evaporated to constant weight for gravimetric fat determination. Sample was then cleaned up using GPC, layered silica column and carbon column according to EPA 1613 method (EPA, 1994). Two fractions were collected from carbon column. The first fraction eluted by 10 ml of dichloromethane was concentrated to 50 μl. PCB congeners (IUPAC nos. 105, 114, 118, 123, 156, 157, and 189) were analyzed in this fraction. Two microliters was injected to low resolution GC–MS on Varian Saturn. Thirty meters DB-5 column, 0.25 mm i.d. was used for separation. The second fraction eluted by 50 ml of toluene contained non-*ortho* substantiated PCBs (IUPAC nos. 77, 126 and

Table 1
Characteristics of the mothers and infants groups examined

	Number of mothers (*N*)						
	15	15	11	11	11	10	8
	Uherské Hradiště	Prague	Ústí n.L.	Kolín	Liberec	Kladno	Telč
Age (y)							
Mean	26	26	26	26	25	24	24
Median	25	29	25	27	26	25	24
Ranges	20–36	23–30	18–36	21–30	20–31	18–32	20–30
Height (cm)							
Mean	167	168	165	167	169	168	168
Median	168	170	168	170	170	168	169
Ranges	158–176	158–178	153–178	156–176	157–182	155–178	158–176
Weight before pregnancy (kg)							
Mean	60	60	70	61	60	62	63
Median	55	60	70	64	55	56	62
Ranges	45–90	49–72	54–92	51–76	48–75	49–82	55–74
BMI (kg/m^2) before pregnancy							
Mean	21.2	21.1	25.8	21.9	20.9	21.8	22.7
Median	20.9	20.3	24.2	21.6	20.2	20.5	22
Ranges	16.9–31.9	18.2–26.2	19.1–36.7	17.6–26.3	17.2–24.4	17–28.3	19.7–25.8
Weight before delivery (kg)							
Mean	74	74	81	76	75	77	78
Median	72	73	80	73	67	74	76
Ranges	55–107	64–100	64–101	62–95	58–93	61–100	69–94
Weight of child (kg)							
Mean	3.3	3.3	3.3	3.4	3.0	3.3	3.7
Median	3.3	3.4	3.5	3.5	3.1	3.3	3.7
Ranges	2.3–4.0	2.1–4.3	1.8–3.9	2.9–4.1	2.2–3.6	2.8–3.9	2.7–4.5
Gender of child (male/female)	7/8	6/9	7/4	7/4	4/7	4/6	6/2

Table 2
Concentrations of PCDDs/PCDFs(in pg/g fat) in 81 individual human milk samples by location including basic statistical data

Compound	LOQ		Uherské Hradiště	Prague	Ústí n.L.	Kolín	Liberec	Kladno	Telč
2,3,7,8-TCDD	0.2	Mean ± S.D.	1.84 ± 1.25	5.83 ± 3.61	1.94 ± 0.55	1.72 ± 0.51	1.62 ± 0.44	1.43 ± 0.44	1.19 ± 0.31
		Median	1.48	5.97	1.84	1.71	1.66	1.40	1.18
		Ranges	0.73–6.02	1.15–13.9	1.17–2.90	0.80–2.35	0.83–2.38	0.89–2.08	0.78–1.68
1,2,3,7,8-PCDD	0.2	Mean ± S.D.	3.73 ± 1.37	3.86 ± 1.51	3.86 ± 2.16	3.19 ± 1.02	2.21 ± 0.66	2.30 ± 0.53	1.94 ± 0.54
		Median	3.73	3.60	3.42	3.14	1.86	2.41	1.81
		Ranges	1.80–6.17	ND–6.94	1.86–9.37	1.81–5.46	1.44–3.07	1.27–2.74	1.29–2.71
		n < LOQ		2					
1,2,3,4,7,8-HxCDD	0.2	Mean ± S.D.	1.36 ± 0.58	1.85 ± 1.01	2.19 ± 1.94	1.52 ± 0.41	0.89 ± 0.27	1.31 ± 0.42	0.89 ± 0.22
		Median	1.36	1.60	1.52	1.58	0.85	1.38	0.87
		Ranges	0.67–2.57	0.84–4.79	0.75–7.57	0.90–2.13	0.51–1.44	0.63–1.72	0.50–1.14
1,2,3,6,7,8-HxCDD	0.2	Mean ± S.D.	6.63 ± 2.46	9.07 ± 6.08	8.87 ± 8.19	7.59 ± 3.68	4.45 ± 1.03	6.56 ± 4.86	3.95 ± 1.08
		Median	6.28	7.26	6.66	6.74	4.49	4.90	4.21
		Ranges	2.53–10.86	3.34–29.7	3.25–32.6	3.52–15.6	3.10–5.99	2.09–15.9	2.07–5.63
1,2,3,7,8,9-HxCDD	0.2	Mean ± S.D.	1.77 ± 0.82	2.75 ± 1.61	3.26 ± 2.48	2.23 ± 1.30	1.26 ± 0.31	1.99 ± 0.90	1.32 ± 0.31
		Median	1.44	2.40	2.71	1.78	1.14	1.84	1.34
		Ranges	10.8–4.12	ND–7.66	0.98–9.59	0.91–5.01	0.84–1.79	0.97–3.46	0.86–1.72
		n < LOQ		1					
1,2,3,4,6,7,8-HpCDD	0.2	Mean ± S.D.	8.87 ± 4.26	16.03 ± 9.69	12.3 ± 6.33	14.3 ± 6.17	6.30 ± 2.98	9.51 ± 4.78	7.37 ± 3.08
		Median	7.91	14.34	11.9	14.2	6.35	8.31	5.86
		Ranges	3.60–19.21	5.85–43.1	5.58–21.4	5.10–27.2	2.84–12.9	5.08–18.7	4.53–12.2
OCDD	0.2	Mean ± S.D.	41.5 ± 28.1	82.1 ± 53.8	54.1 ± 20.8	75.5 ± 39.1	28.5 ± 6.76	49.2 ± 42.7	31.4 ± 14.3
		Median	30.06	62.1	47.9	67.06	27.6	32.9	28.4
		Ranges	12.7–113.7	23.4–205	31.3–97.4	27.9–144	16.7–39.6	24.3–135	15.0–60.4
2,3,7,8-TCDF	0.2	Mean ± S.D.	2.29 ± 0.31	2.31 ± 1.27	5.95 ± 4.17	1.95 ± 3.55	0.97 ± 0.40	2.36 ± 1.51	3.46 ± 5.23
		Median	2.14	1.75	4.68	0.82	1.05	1.98	1.43
		Ranges	0.47–9.77	0.67–5.63	1.35–12.6	0.33–12.5	0.42–1.46	0.96–4.79	1.09–16.3
1,2,3,7,8-PeCDF	0.2	Mean ± S.D.	1.19 ± 0.61	0.89 ± 0.52	2.97 ± 2.38	0.87 ± 0.5	0.36 ± 0.12	0.84 ± 0.52	2.16 ± 4.32
		Median	1.07	0.80	2.03	0.66	0.37	0.63	0.60
		Ranges	ND–2.57	ND–2.35	0.81–8.80	ND–1.94	ND–0.59	ND–1.85	0.52–12.9
		n < LOQ	5	2		4	2	4	
2,3,4,7,8-PeCDF	0.2	Mean ± S.D.	31.03 ± 30.8	20.5 ± 6.66	22.0 ± 11	17.8 ± 10.6	12 ± 3.99	11.1 ± 2.78	15.3 ± 12.2
		Median	23.3	20.11	19.1	14.3	10.54	11.3	12.1
		Ranges	12.7–133.7	10.03–33.5	10.1–43.9	6.76–45.3	6.22–18.8	7.29–14.7	7.54–44.7
1,2,3,4,7,8-HxCDF	0.2	Mean ± S.D.	9.66 ± 8.13	6.84 ± 2.24	12.6 ± 9.76	5.84 ± 2.82	3.40 ± 0.97	4.10 ± 0.53	6.89 ± 8.55
		Median	7.14	6.59	9.02	5.79	3.12	3.88	3.96
		Ranges	3.53–35.9	3.34–11.5	4.89–39.3	2.17–12.9	2.01–5.45	3.64–4.93	2.81–28.0
1,2,3,6,7,8-HxCDF	0.2	Mean ± S.D.	5.46 ± 2.44	5.15 ± 2.15	6.88 ± 4.66	4.14 ± 1.59	2.49 ± 0.6	2.7 ± 0.46	3.46 ± 2.56
		Median	4.83	4.80	4.88	3.60	2.68	2.92	2.74
		Ranges	2.33–10.86	2.51–11.5	3.02–18.2	2.17–7.08	1.43–3.29	1.93–3.13	1.60–9.67
1,2,3,7,8,9-HxCDF	0.2	Mean ± S.D.	0.60 ± 0.08	0.41 ± 0.35	0.25 ± 0.16	ND	ND	0.28 ± 0.38	0.15 ± 0.11
		Median	0.60	0.23	0.22			0.17	0.12
		Ranges	ND–0.67	ND–0.99	ND–0.58			ND–1.05	ND–0.40
		n < LOQ	11	12	7			4	7
2,3,4,6,7,8-HxCDF	0.2	Mean ± S.D.	2.16 ± 1.07	2.01 ± 0.90	4.42 ± 4.37	1.66 ± 0.75	0.85 ± 0.39	1.34 ± 0.50	2.31 ± 3.25
		Median	2.09	2.02	2.59	1.40	0.77	1.24	1.22
		Ranges	0.94–5.01	0.84–3.83	1.17–15.8	0.79–3.31	0.36–1.58	0.83–2.18	0.71–10.3
1,2,3,4,6,7,8-HpCDF	0.2	Mean ± S.D.	6.38 ± 9.19	6.13 ± 6.59	8.38 ± 10.2	3.34 ± 1.16	1.84 ± 0.91	2.90 ± 1.25	5.14 ± 5.92
		Median	3.05	4.19	2.81	3.02	1.66	2.80	2.73
		Ranges	1.47–29.3	2.09–29	2.43–35.9	1.72–5.75	1.0–4.33	1.29–5.06	1.15–18.4
1,2,3,4,7,8,9-HpCDF	0.2	Mean ± S.D.	0.47 ± 0.12	0.74 ± 0.69	0.86 ± 0.91	0.16 ± 0.10	0.13 ± 0.06	0.46 ± 0.38	0.25 ± 0.14
		Median	0.47	0.51	0.38	0.12	0.11	0.39	0.20
		Ranges	ND–0.60	ND–2.66	0.20–3.17	ND–0.31	ND–0.21	ND–1.20	ND–0.58
		n < LOQ	10	4		10	10	1	4
OCDF	0.2	Mean ± S.D.	2.72 ± 3.63	4.60 ± 9.06	2.48 ± 2.33	2.30 ± 2.76	0.96 ± 0.44	3.01 ± 4.06	0.98 ± 0.44
		Median	1.61	2.35	0.95	1.41	0.88	1.28	0.80
		Ranges	0.52–12.35	0.81–37.0	0.53–7.42	0.44–10.2	0.55–2.08	0.35–11.1	0.56–1.86

Table 3
Concentrations of non-*ortho* (in pg/g fat) and mono-*ortho* PCBs (in ng/g fat) with the established WHO-TEFs in 81 individual human milk samples by location including basic statistical data

Compound	LOQ		Uherské Hradiště	Prague	Ústí n.L.	Kolín	Liberec	Kladno	Telč
PCB 77 pg/g fat	1	Mean ± S.D.	15.6 ± 10.3	18.0 ± 4.89	8.38 ± 3.89	10.5 ± 7.07	12.9 ± 17.1	21.9 ± 14.8	27.7 ± 32.7
		Median	13.4	18.17	7.32	8.38	7.50	15.8	16.2
		Ranges	3.47–42.2	12.4–24.0	3.70–16.5	3.71–27.3	4.23–63.3	8.94–41.3	11.3–108
PCB 126 pg/g fat	1	Mean ± S.D.	256.6 ± 268.5	134.9 ± 97.4	174 ± 113.6	123 ± 76.7	66.1 ± 23.3	76.1 ± 38.2	118 ± 113
		Median	171.3	98.3	144	110	65.0	63.9	87.7
		Ranges	76.3–1123	71.4–458	54.7–362	27.7–291	26.9–104	41.1–131	47.2–395
PCB 169 pg/g fat	1	Mean ± S.D.	111.5 ± 71.2	75.4 ± 32.4	72.5 ± 30.3	54.6 ± 27.5	41.6 ± 13.4	44.7 ± 16.2	39.2 ± 19.3
		Median	86.6	65.55	75.3	53.2	40.0	47.2	34.5
		Ranges	42.9–280	39.3–150.8	27.0–115	27.2–116	19.2–58.8	18.3–66.9	19.4–81.0
PCB 118 ng/g fat	0.5	Mean ± S.D.	69.9 ± 64.5	34.4 ± 19.2	29.1 ± 16.1	22.6 ± 17.6	14.9 ± 6.00	17.1 ± 7.48	18.7 ± 7.37
		Median	41.6	28.4	27.7	16.9	14.0	17.27	17.5
		Ranges	22.5–232	13.2–89.2	11.9–57.1	7.22–70.1	6.12–24.4	8.74–28.0	11.2–30.0
PCB 105 ng/g fat	0.5	Mean ± S.D.	6.47 ± 6.66	3.90 ± 2.55	2.98 ± 1.72	3.43 ± 2.66	1.49 ± 0.78	ND	ND
		Median	4.39	3.09	2.65	2.63	1.31		
		Ranges	0.36–25.3	2.11–11.2	0.89–5.36	0.84–10.5	ND–3.33	LOQ=2	LOQ=2
		n < LOQ					1		
PCB 167 ng/g fat	0.5	Mean ± S.D.	21.3 ± 18.1	8.20 ± 4.60	13.1 ± 6.66	8.73 ± 7.79	4.54 ± 1.48	ND	
		Median	15.5	7.45	11.2	6.75	4.19	LOQ=2	4.34 ± 1.0
		Ranges	5.67–73.9	3.09–20.5	5.33–22.9	3.23–31.6	ND–6.66		4.31
		n < LOQ					1		3.12–5.57
PCB 156 ng/g fat	0.5	Mean ± S.D.	65.5 ± 50.4	23.3 ± 15.0	41.6 ± 19.8	26.6 ± 20.9	16.2 ± 5.09	19.9 ± 7.06	15.2 ± 5.10
		Median	43.5	19.3	35.6	22.1	16.2	21.2	14.1
		Ranges	21–194	10.2–64.2	17.1–75.6	7.39–85.5	8.23–23.4	9.03–28.7	10.2–25.1
PCB 157 ng/g fat	0.5	Mean ± S.D.	5.15 ± 3.53	2.73 ± 1.14	3.42 ± 2.19	2.48 ± 1.50	1.34 ± 0.42	2.36 ± 0.99	2.01 ± 0.74
		Median	3.52	2.70	2.53	2.09	1.44	2.30	2.30
		Ranges	1.71–13.2	1.5–5.74	1.44–8.96	ND–6.23	0.71–1.87	1.02–3.79	0.97–2.79
		n < LOQ	1			1			
PCB 189 ng/g fat	0.5	Mean ± S.D.	7.38 ± 4.76	2.67 ± 1.39	4.04 ± 2.19	2.37 ± 1.58	1.59 ± 0.45	2.37 ± 1.01	1.24 ± 0.60
		Median	6.62	1.96	3.34	2.13	1.71	2.72	1.24
		Ranges	ND–17.8	1.09–5.82	1.36–7.05	0.80–6.71	0.83–2.11	ND–3.16	0.82–1.66
		n < LOQ	2					5	

ND: not detected.

169) and PCDD/Fs (2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF, 1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF). Fraction was concentrated to 20 μl. One mcroliter was analyzed by high resolution GC–MS Autospec Ultima operated at >10 000 resolution. Sixty meters DB-5 column, 0.25 mm i.d. was used for separation. Samples were analyzed in batches of 10 accompanied by blank and reference samples.

2.3. Calculations

Concentrations of most target PCB's and PCDD/F's were significantly higher than limit o.d. detection (LOD, S/N > 10). Exceptions were 1,2,3,7,8-PCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HpCDF, PCB 157 and PCB 189. Number of samples below LOD for these congeners is listed in Tables 3 and 4.

TEQ values were calculated using the TEFs recommended by the WHO (Van den Berg et al., 1998). Concentrations below the detection limit were considered to be half the detection limit for this calculation.

For statistical analysis, non-parametric Kruskall–Wallis one-way analysis of variance was used. A probability value (P) less than 0.05 was regarded as significant. Correlations were assessed with Spearman rank correlation test.

3. Results

The population under study is described in Table 1. The mothers were between 18 and 36 (26 on average) years of age. The average weight of all children was 3.3 (range: 1.8–4.5) kg. No significant demographic differences (maternal age or body mass index, infant birth weight or sex ratio) in individual breast milk donors were found.

Analysis of the effect of smoking habits, dietary habits (consumption of fish, meat and milk and its frequency), and body mass index on PCDD/F and PCB concentrations in human milk did not show significant differences in the levels of analyzed congeners between smokers (including ex-smokers) and non-smokers (only two mothers were current smokers and 18 of them declared they are ex-smokers), or any correlation with BMI. The dietary habits of the mothers did not differed substantially (except two lacto-ovo-vegetarians mothers the rest of the donors indicated a mixed diet).

Table 2 shows the concentrations of analyzed PCDD/Fs (in pg/g fat) in 81 individual human milk samples by location, including basic statistical data. Considerable differences in the concentrations of individual congeners were observed. As the obtained data revealed log-normal distribution, data are expressed also as median values.

Most of the individual PCDD/PCDF congeners were found in concentration of units pg/g fat. Highest levels were

Table 4
Summary statistics (means, medians and ranges) of WHO-TEQ values (in pg/g fat) for PCDDs, PCDFs, and dioxin-like PCBs in human milk

Compound		Uherské Hradiště	Prague	Ústí n.L.	Kolín	Liberec	Kladno	Telč
PCDDs	Mean ± S.D.	6.31 ± 2.05	10.7 ± 3.76	7.37 ± 3.81	6.19 ± 1.85	4.56 ± 1.15	4.82 ± 1.28	3.82 ± 0.86
	Median	6.33	10.7	6.02	6.52	4.07	4.84	3.68
	Ranges	2.89–10.1	5.25–16.4	3.94–17.4	3.79–9.70	2.88–6.28	2.84–6.56	2.46–5.15
PCDFs	Mean ± S.D.	17.59 ± 16.5	12.0 ± 3.84	14.2 ± 7.52	10.33 ± 5.90	6.80 ± 2.19	6.73 ± 1.37	9.42 ± 8.28
	Median	12.0	12.0	11.5	8.43	6.17	6.97	7.21
	Ranges	7.19–72.3	5.91–19.8	6.45–31.4	4.09–25.4	3.60–10.6	4.88–8.59	4.71–29.6
Non-*ortho* PCBs	Mean ± S.D.	26.8 ± 27.4	14.2 ± 9.93	18.1 ± 11.6	12.9 ± 7.91	7.03 ± 2.43	8.06 ± 3.94	12.2 ± 11.5
	Median	17.6	10.5	15.2	11.5	7.09	6.80	9.14
	Ranges	8.06–115	7.66–47.0	6.29–37.1	3.05–30.3	2.89–10.9	4.30–13.7	4.91–40.3
Mono-*ortho* PCBs	Mean ± S.D.	43.5 ± 33.6	16.9 ± 9.58	26.3 ± 12.6	17.4 ± 13.2	10.6 ± 3.38	13.0 ± 4.69	10.1 ± 3.60
	Median	27.4	13.4	23.8	14.2	10.3	13.1	8.86
	Ranges	15.5–127	7.05–39.7	11.0–47.5	5.57–54.9	5.26–14.7	6.06–19.3	6.99–16.9
Total TEQ	Mean ± S.D.	94.2 ± 63.8	53.8 ± 22.8	66.0 ± 32.4	46.8 ± 27.2	29.0 ± 8.67	32.6 ± 9.82	35.6 ± 19.4
	Median	64.6	45.5	55.0	40.3	27.8	31.2	31.4
	Ranges	39.5–234	30.7–113	30.0–122	21.7–118	14.6–40.4	18.1–46.5	20.1–80.4

found for OCDD (median concentrations between 28.3 and 67.1 pg/g fat) followed with 2,3,4,7,8-PeCDF (median concentrations between 10.5 and 23.3 pg/g fat). The concentrations of 2,3,7,8-TCDD in the Prague samples (median: 5.97 pg/g fat) were significantly ($P < 0.05$) higher than in other groups under study. Similarly, most of the other PCDD congeners were present in the Prague samples in higher concentrations levels in comparison with other groups.

The concentrations of non-*ortho* (in pg/g fat) and mono-*ortho* (in ng/g fat) dioxin-like PCBs are presented in Table 3. From the dioxin-like PCB congeners, the most abundant one was IUPAC 156 with median concentrations between 14.1 and 43.5 ng/g fat in individual groups. The concentrations of dioxin-like PCBs in samples from Uherské Hradiště indicated the highest exposure to PCBs for this location.

Table 4 shows descriptive summary statistics of PCDDs, PCDFs, and dioxin-like PCBs expressed on a WHO-TEQ basis. The median of the total WHO-TEQ values ranged from 64.6 pg/g fat in Uherské Hradiště to 27.8 pg/g fat in Liberec. WHO-TEQ for PCDDs contributed from about 10% (Uherské Hradiště) to 23.5% (Prague) to the total TEQ value. The PCDD-TEQ value in Prague was significantly higher than in all other groups. On the contrary, the contribution of non-*ortho* and mono-*ortho* PCBs to the total TEQ value was lowest (53%) in Prague and highest (69%) in Uherské Hradiště. In Uherské Hradiště, the levels of TEQs for PCDF, dioxin like PCBs, as well as the total TEQ value were significantly higher than in other groups except for Ústí n.L. In all analyzed groups, the major contributors to the total TEQ values were PCBs 156 and 126, each of which contributed about 30%. The further main contributing congener, 2,3,4,7,8-PeCDF, was responsible for about 17–21% of the total TEQ value of respective group.

Daily dietary intake of PCDD/Fs and dioxin-like PCBs for breast-fed infants is shown in Table 5. The basis for this calculation was a daily consumption of 120 ml breast milk per kg of body weight with a lipid content of 3.5% (Schutz et al., 1998). The median daily intake of total WHO-TEQs for PCDD/Fs and PCBs for breast-fed infants ranged from 271 pg/kg b.w./day in Uherské Hradiště to 117 pg/kg b.w./day in Liberec and exceeded about two orders of magnitude the tolerable daily intake (TDI) of 1–4 pg/kg b.w. recommended by the WHO.

4. Discussion

The concentration of dioxins in human milk is an indicator of the exposure history of the individual or group of individuals. The results from the Second round of the WHO-coordinated study have shown exceptionally high levels of the indicator PCBs for one particular region (Uherské Hradiště) in the Czech Republic (WHO/ECEH, 1996). However, the levels of PCDDs/PCDFs were relatively low in both exposed and control regions analyzed in the WHO study (I-TEQ values 18.4 and 12.1 pg/g fat, respectively (Bencko et al., 1998).

Table 5
Estimated exposure of breast-fed infants to PCDDs/PCDFs and PCBs expressed as WHO-TEQ values in pg per kg of body weight

Compound		Uherské Hradiště	Prague	Ústí n.L.	Kolín	Liberec	Kladno	Telč
PCDDs	Mean ± S.D.	26.5 ± 8.6	44.9 ± 15.8	30.9 ± 16.0	26.0 ± 7.8	19.2 ± 4.8	20.3 ± 5.4	16.0 ± 3.6
	Median	26.6	44.9	25.3	27.4	17.1	20.3	15.5
	Ranges	12.1–42.4	22.1–69.0	16.5–73.2	15.9–40.7	12.1–26.4	11.9–27.6	10.3–21.6
PCDFs	Mean ± S.D.	73.9 ± 69.1	50.3 ± 16.1	59.8 ± 31.6	43.4 ± 24.8	28.5 ± 9.2	28.2 ± 5.8	39.6 ± 34.8
	Median	50.2	50.3	48.4	35.4	25.9	29.3	30.3
	Ranges	30.2–303	24.8–83.3	27.1–132	17.2–107	15.1–44.5	20.5–36.1	19.8–124
Non-*ortho* PCBs	Mean ± S.D.	112.5 ± 114.9	59.8 ± 41.7	76.1 ± 48.6	54.0 ± 33.2	29.5 ± 10.2	33.8 ± 16.5	51.3 ± 48.4
	Median	73.9	44.1	63.7	48.4	29.8	28.6	38.4
	Ranges	34.0–482	32.3–197	26.5–156	12.6–127	12.2–45.8	18.1–57.7	20.6–169
Mono-*ortho* PCBs	Mean ± S.D.	183 ± 141	70.8 ± 40.2	110 ± 53.0	73.0 ± 55.6	44.5 ± 14.2	54.4 ± 19.7	42.6 ± 15.1
	Median	115	56.4	100	59.7	43.4	55.0	37.2
	Ranges	65.1–535	29.8–159	46.2–200	23.5–231	22.3–61.7	25.5–81.2	29.4–71.0
Total TEQ	Mean ± S.D.	395 ± 268	226 ± 95.8	277 ± 136	196 ± 114	122 ± 36.4	137 ± 41.2	150 ± 81.4
	Median	271	191	231	169	117	131	132
	Ranges	166–983	129–476	126–509	91.0–497	61.4–170	75.9–195	84.3–338

Similar levels ranging from about 10 pg/g fat WHO-TEQs were found in four human milk samples (each pooled from about one hundred of individual samples) analyzed in 1998 (Černá et al., 1999, 2003). This means that the data for the Czech population lower than for a relevant European population (WHO/ECEH, 1996; Päpke, 1998). There is no other comparable individual data relating to the Czech population to assess the exposure of the population and to estimate time trends. However, new information in this field is to be expected from the third round of the WHO coordinated exposure study starting in 2000/2001.

The data obtained in this study have shown local and individual differences in the levels of particular congeners as well as in the exposure of the Czech population to PCDDs/PCDFs and dioxin-like PCBs. The regions significantly differ in the levels of the total TEQs with the highest value in Uherské Hradiště followed by Ústí n.L. This finding corresponds well with previous findings of increased body burden of PCBs in these regions either within the WHO study (WHO/ECEH, 1996), or within the Environmental Health Monitoring System (Černá et al, 2003). Also, in this study, PCBs were found to constitute a major part (about 50–70%) of the pertinent WHO-TEQ value, which documented the significance of PCB body burden of the Czech population (Černá and Bencko, 1999). The highest 2,3,7,8-TCDD levels as well as the highest TEQ value for PCDDs were found in Prague samples and might reflect the intensity of traffic in our capital. The relatively high concentrations of 2,3,4,7,8-PeCDF obtained in our samples correspond with the findings in other European countries, whereas lower levels are typically observed in samples from the United States or Canada (WHO/ECEH, 1996). There was no difference in PCDD/PCDF/PCB levels in human milk between current or former smoking and non-smoking mothers. Only two mothers from 81 participants reported smoking habit and 18 were ex-smokers. Though a declining trend of PCDDs/Fs in breast milk samples has been observed in several European countries, no such data are yet available for the Czech population.

According to our results, the median values of infant exposure to dioxin-like compounds vary between 117 and 271 pg/kg b.w. daily. This means that the exposure of breastfed infants is about two orders of magnitude higher than the WHO tolerable daily intake as well as an average dietary exposure estimated for German adult population to be 2.3 pg of TEQ/kg b.w./day (Beck et al., 1994). However, our findings are in good agreement with those of other studies on human milk from EU countries with estimated average exposure 144 pg I-TEQ/kg b.w./day (EC DG Environment, DETR, 1999). The health consequences of this potential temporary elevation of infant exposure via breastfeeding are uncertain. Therefore, it is important to implement all possible measures to prevent PCDD/PCDF/PCB contamination of the environment and to continue monitoring of such compounds in human body fluids, namely in blood samples.

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References

ATSDR Tox Profiles, Atlanta, 2002, http://www.atsdr.cdc.gov.

Beck, H., Dross, A., Mathar, W., 1994. PCDD and PCDF exposure and levels in humans in Germany. Environ. Health Perspect. 102 (Suppl. 1), 173–185.

Bencko, V., Skulová, Z., Krečmerová, M., Djien Liem, A.K., 1998. Selected polyhalogenated hydrocarbons in breast milk. Toxicol. Lett. 96 (97), 341–345.

Bencko, V., 2003. Risk assessment and human exposure to endocrine disrupters. In: Jedrychovski, W.A., Petera, F.P., Maugeri, U. (Eds.), Molecular Epidemiology in Preventive Medicine. International Center for Studies and Research in Biomedicine in Luxembourg, pp. 315–327.

Černá, M., Bencko, V., 1999. Polyhalogenated hydrocarbons: body burden of the Czech and Slovak populations. Part I. Polychlorinated biphenyls. Centr. Eur. J. Publ. Health 7, 67–71.

Černá, M., Svobodník, J., Čížková, M., Krýsl, S., Šmíd, J., 1999. Levels of PCBs, PCDDs and PCDFs in human milk of mothers living in four districts of the Czech Republic. Cent. Eur. J. Publ. Health 8, 24–25.

Černá, M., Šmíd, J., Svobodník, J., Grabic, R., Crhová, Š., Kubínová, R., 2003. Monitoring of selected polyhalogenated hydrocarbons in breast milk: Czech Republic, 1994–2001. Fresenius Environ. Bull. 12, 203–207.

De Rosa, C.T., Pohl, H.R., Bencko, V., Richter, P., Jones, D.E., 2001. Health risk od xenobiotics modulating the endocrine system. II. Human health implications and a strategy of prevention (in Czech). Prakt. Lék. 8 (11), 619–923.

EPA (1994). Method 1613 *tetra*- through *octa*-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.

European Commission DG Environment, UK Department of the Environmental Transport and the Regions (DETR), 1999. Compilation of EU Dioxin Exposure and Health Data: summary report.

Fürst, P., Fürst, C., Wilmers, K., 1994. Human milk as a bioindicator for body burden of PCDDs, PCDFs, organochlorine pesticides, and PCBs. Environ. Health Perspect. 102 (Suppl. 1), 187–193.

Kliment, V., Kubínová, R., Kazmarová, H., Havlík, B., Šišma, P., Ruprich, J., Černá, M., Kodl, M., 1997. System of monitoring the environmental impact on population health of the Czech Republic. Centr. Eur. J. Publ. Health 5, 107–116.

Kliment, V., Kubínová, R., Kazmarová, H., Kratzer, K., Šišma, P., Ruprich, J., Černá, M., Gregůrková, M., 2000. Five years of the system of monitoring the environmental impact on population health of the Czech Republic. Centr. Eur. J. Publ. Health 8, 198–205.

Päpke, O., 1998. PCDD/PCDF: human background data for Germany, a 10-year experience. Environ. Health Perspect. 106 (Suppl. 2), 723–731.

Schutz, D., Moy, G.G., Kaferstein, F.K., 1998. GEMS/Food International Dietary Survey. Infant exposure to certain organochlorine

contaminants from breast milk–a risk assessment. In: Food Safety Issues, WHO.

Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, P., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larse, J.C., van Leeuwen, F.X.R., Djien Liem, A.K., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

WHO/ECEH, 1996. Levels of PCBs, PCDDs and PCDFs in human milk. In: Environmental Health in Europe Series, vol. 3. Second round of WHO-Coordinated Exposure Study, 121 pp.