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DETERMINATION OF TOXIC PCB CONGENERS IN BIOTIC SAMPLES: HUMAN MILK FROM THE PRAGUE AREA, CZECH REPUBLIC

PAVEL GREGOR, JANA HAJŠLOVÁ* and ROMAN SCHOULA

Department of Food Chemistry and Analysis, Institute of Chemical Technology, Technická 5, 16628 Prague 6, Czech Republic

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This study is concerned with documentation of human exposure to toxic PCBs in the Czech Republic. Samples of human milk were collected in 1995 in the Prague area and were examined for the levels of toxic *non-*, *mono-*, and *di-ortho* PCB congeners. The concentrations of toxic PCBs found are comparable with data from other countries. The main contribution to the toxic equivalents (TEQ) originated from PCB 156 (31.47%), PCB 126 (25.8%), and PCB 170 (20.28%). Strong linear correlation was found for toxic equivalents (TEQs) with two major chlorobiphenyl congeners: PCB 153 and PCB 180 ($R = 0.981$ and $R = 0.985$, resp.).

Keywords: Human milk; PCBs; planar PCBs; toxic equivalents (TEQs); two-dimensional high resolution gas chromatography (2D-HRGC); ECD detection

1. INTRODUCTION

Because of position of human beings on the top of the food chain, relatively high amounts of the organochlorine xenobiotics can be found in their tissues. Depending on the extent of dietary exposure, lipophilic polychlorinated chemicals, such as biphenyls (PCBs), dibenzodioxins (PCDDs) and dibenzo furans (PCDFs) persist especially in the fat rich tissues. They circulate through the human body associated with the lipids and in the case of nursing women they are transferred into milk [1]. Contamination of human milk is of special concern since it is the exclusive diet for new-born infants [2,3]. As it

* Corresponding author.

was documented, breast feeding is a major transfer route of these contaminants from mother to infant besides minor placental transfer [2,4]. Maternal food habits (namely consumption of fish, meat and dairy products) up to the age of reproduction are of great importance determining the degree of foetal exposure and residue levels in the breast milk. Several studies report long-term negative effects of PCBs on behavioural and early cognitive development observed by exposed infants [5–7].

Planar PCBs represent the major toxicological risk from environmental exposure to mixtures of persistent organochlorines and PCB Nos. 77, 126 and 169 (IUPAC) elicit toxic and biochemical responses like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) due to the *non-ortho* substitution pattern in the biphenyl rings [7]. The first report [8] on the determination of the above mentioned highly toxic PCB congeners was published ten years ago (blubber of a finless porpoise, *Neophocoena phocoenoides*) and since this time a number of reports have been published dealing with their occurrence and distribution in various biotic specimens [9–18].

This study presents levels of planar PCB congeners in human milk collected in the Czech Republic. These are the first data with the exception of small set of data from Brno Region (five individual samples, primi- or secundipara) [19] and one study conducted in the part of the former Czechoslovakia, the Slovak Republic (pooled samples from six model areas, primiparae) [20].

2. MATERIALS AND METHODS

2.1. Standards and Other Chemicals

PCB standards were obtained from Dr. Ehrenstorfer (Germany), concentration of each in isooctane was 10 μg/ml. Standard mixture I containing most toxic congeners consisted of PCB No. 77, 81, 126 and 169 (*non-ortho*), 105, 114, 118, 123, 156, 157, 167 and 189 (*mono-ortho*), concentrations of calibration solutions were 20.0, 2.0, 0.2, 0.05 and 0.01 ng/ml isooctane. Standard mixture II contained (i) indicator PCBs: No. 28, 52, 101, 118, 138, 153 and 180, (ii) other major congeners commonly reported [21–23] in human milk samples: No. 66, 74, 99, 110, 149, 170, 183 and 187, (iii) some other congeners: No. 8, 18, 31, 44, 70, 84, 128, 129, 146, 151, 163, 178, 199, 195, 194, 203 and 209. Concentrations of calibration solutions were 400, 100, 40, 10, 4, 1, 0.4 and 0.1 ng/ml isooctane.

Silica gel (70–230 mesh, surface area about 490 m^2/g) was obtained from Merck (Darmstadt, Germany). Prior to use it was activated at 170°C for 5 h, then deactivated with 3% water (w/w). Sulphuric acid (98%) was obtained

from Merck (Darmstadt, Germany). All solvents (Merck, Darmstadt, Germany) used were of organic trace analysis grade.

2.2. Experimental Material

Human milk samples were collected from the Prague area in January 1995 in the co-operation with the Institute for the Care of Mother and Child in Prague. The mothers involved were of the age ranging from 18 to 41 (median 23), primiparae (see Table I). The way of sample collection was carried out according to WHO standardised protocol [24].

2.3. Extraction

Samples of frozen milk were thawed, centrifuged for about 10 min and the fat was mixed with anhydrous sodium sulphate to obtain a free-flowing powder. This was subsequently extracted with *n*-hexane, as described in more details previously [25]. The percentage of lipids in milk was determined gravimetrically.

TABLE I Characteristics of breast milk donors [26]

Sample code	M 3	M 4	M 5	M 6	M 7	M 8	M 10	M 11	M 15	M 21
Mother's age	21	23	23	41	18	27	18	20	26	23
Period of milk collection (days)	4	7	7	7	5	3	4	7	7	7
Total volume collected (ml)	500	230	370	280	430	320	340	320	400	320
Mother's height (cm)	175	168	179	164	178	175	166	170	160	168
Mother's weight (kg)										
before pregnancy	63	48	75	56	60	66	81	56	58	63
prior to delivery	82	71	92	66	85	75	89	68.5	74	67
Quetelet index[†]										
before pregnancy	20.6	17.0	23.4	20.8	18.9	21.6	29.4	19.4	22.7	22.3
prior to delivery	26.8	25.2	28.7	24.5	26.8	24.5	32.3	23.7	28.9	23.7
Child's sex	M	M	M	M	M	M	M	F	F	M
Child's age at the beginning of sampling (days)	7	12	7	7	7	7	7	6	7	7
Weight										
at birth (g)	3310	3940	4000	4460	3540	3540	3850	3390	3500	3610
After collect. (g)	3100	3540	4100	4080	3380	3480	3530	3100	3400	3590
Mother's diet. habits	mix	mix	veg	mix	mix[a]	mix	mix	mix	mix	veg
beef*	2	1/4	0	1	1/2	1/4	3	1	1/2	0
beefsoup*	1/2	7	0	1	1/2	1/2	3	2	1	0
fish*	1/2	1	3	1	1	2	1/2	1/4	1	1
milk and milk product*	7	0	4	0	4	7	7	7	7	7
Smoking habit	No	No	No	No	No	No	No	No	No	Yes

*Quetelet index = weight (kg)/height^2 (m).
[†]Frequency of consumption per week. Mix: mixed diet. Veg: vegetarian with milk and eggs. Mix[a]: mixed diet from the beginning of pregnancy, from 13 to 18 years she was strictly vegetarian.

2.4. Clean-up

The bulk of lipid material was mineralised by concentrated sulphuric acid. For removing of residual lipids, an additional clean-up step employing disposable silica gel column was involved: 2 g of silica gel were packed into the column and conditioned with 30 ml of *n*-hexane. The residues from the first step were dissolved in 1 ml of *n*-hexane, transferred on the top of column and the flask was rinsed with 2 × 1 ml *n*-hexane (the washes were also added at the top of the column). Elution of analytes was carried out with 15 ml *n*-hexane. The eluate was evaporated carefully by rotary evaporation and the residue was transferred to a micro vial by 200 µl of hexane and concentrated before fractionation step to approx. 50 µl under a gentle stream of nitrogen.

2.5. Fractionation

Separation of *non-* and *mono-ortho* congeners from the bulk of the other PCB (and other organochlorine pesticides) was carried out by HPLC utilising Cosmosil PYE column (2-(1-pyrenyl)ethylsilica, 250 × 4.6 mm, 5 µm particles), supplied by Nacalai Tesque (Japan). The mobile phase was *n*-hexane, flow rate 0.5 ml/min. Fractions of *mono-* and *non-ortho* PCBs corresponding to the elution volume in the range of 6–10 ml, and 10–20 ml, resp. were collected. PYE column was operated at 4°C to obtain better separation among *non-ortho* and the other PCBs [27].

2.6. Quantitation

HRGC was used for analysis of the above fractions. Hewlett Packard 5890 ser. II gas chromatograph equipped with electronic pressure control (EPC), split/splitless injector, two ^{63}Ni electron capture detector (2 ECDs) and two parallel columns possessing different selectivities was employed for this purpose. GC conditions are summarised in Table II. The analysis of major PCBs congeners (standard mixture II, see Section 2.1.), was performed by a procedure described elsewhere [25].

2.7. QA/QC Procedure

The method performance was tested for blank impurities and recovery values. For each two samples one "blank run" was carried out. Electronic signals of blank runs (residues-free matrix was not available) were subtracted from sample signals by means of HP GC ChemStation software to ensure results

TABLE II GC conditions used for analysis of toxic PCBs

Parameter	*Description*
Columns type	DB-5 and DB-17 5% and 50%-phenyl-methylpolysiloxane (supplied by J&W Scientific, USA)
Columns size	both: 60 m × 0.25 mm (I.D.) × 0.10 μm (phase)
Injector temperature	300°C
Detectors temperature	300°C
Oven temperature program	60°C (2.5 min), 30°C/min to 220°C, 0.4°C/min to 240°C, 10°C/min to 280°C
Splitless period	2.5 min
Carrier gas	helium
Inlet pressure program	552 kPa (1.5 min), 600 kPa/min–193 kPa, then constant flow 1.5 ml/min, i.e. 191 kPa (60°C)
Linear velocity	27.1 cm/s
Injected volume	3 μl
Data processing	HP GC ChemStation Rev. A.04.02, ©HP 1990–1996

without any interference. Recovery experiments were made twice at spiking level 100 pg/g milk fat for three *non-ortho* congeners. Recovery values for PCB 77, PCB 126 and PCB 169 were 57.3%, 65.3% and 61.9%, resp*.

3. RESULTS AND DISCUSSION

The levels of PCBs in human milk presented in this study are compared with surveys conducted in other countries [29–32] in Table III. Highly chlorinated persistent congeners (PCB 153, 138, 180 and 170) were the most abundant components in examined milk samples, the levels we found were higher in comparison with those reported in foreign studies. These PCBs were the main components of Delor 106, a technical mixture which was the main source of contamination of ecosystem in the Czech Republic. Less chlorinated congeners (such as PCB 28 and 52) were detected at relatively low levels probably because of different dietary food habits of Czech population with a relatively low consumption of fish and other sea foods (which are a common source of persistent organochlorine compounds [33,34]). A typical gas chromatographic pattern of persistent organochlorine compounds isolated from human milk sample and pre-separated into three fractions is shown in

* The values shown here may seem rather low, nevertheless similar results were reported in other studies: 64–83% for PCB 77, 66–70% for PCB 126 and 71–98% for PCB 169 (spiking level 5 pg/ml milk) [28], 32% for ^{13}C-PCB 77, 37% for ^{13}C-PCB 126 and 31% for ^{13}C-PCB 169 [17], 70% for PCB 77 and 72% for PCB 126 [22].

TABLE III Toxic PCBs in pooled human milk samples collected in Prague region – comparison with foreign studies (values expressed in pg/g milk fat)

Country, year [*ref.*] (*No. of samples*)	*Norway*, 1994 [29] ($n=28$)		*The Netherlands*, 1994 [30] ($n=194$)		*The Netherlands*, 1995 [31] ($n=32$)		*Sweden*, 1997 [32] ($n=7$)		*Czech Republic*, 1997 [*this study*] ($n=10$)	
Congener	*mean* (range)	*TEQ* (pg/g)	*mean* (range)	*TEQ* (pg/g)	*mean* (range)	*TEQ* (pg/g)	*mean* (range)	*TEQ* (pg/g)	*mean* (range)	*TEQ* (pg/g)
PCB 77	45.9 (5.4–273.5)	0.02	19.3 (3.7–143.2)	0.01	†	0.00	< 2.38	0.00	6.7 (*–23.7)	0.00
PCB 126	156.0 (36.0–737)	15.60	152.0 (39.4–443.9)	15.20	98.5 (29.0–236.0)	9.85	105 (51.1–151)	10.50	63.3 (15.2–120.8)	6.33
PCB169	191.7 (46.4–1353)	1.92	84.3 (33.2–282.9)	0.84	63.3 (27.0–122.0)	0.63	39.2 (18.6–60.4)	0.39	31.7 (7.8–66.0)	0.32
	TEQ *non-ortho*	17.54		16.05		10.48		10.89		6.65
PCB 105	7700 (n.d.–16800)	0.77	9400 (400–22900)	0.94	7400 (2700–17800)	0.74	3800 (1200–6700)	0.38	1900 (400–4300)	0.19
PCB 114	4000 (n.d.–15200)	2.00	†	†	†	0.00	†	0.00	400 (100–1500)	0.20
POB 118	26200 (9600–56700)	2.62	35500 (9700–94000)	3.55	30500 (12400–80300)	3.05	19400 (7800–27600)	1.94	21200 (4800–42500)	2.12
PCB 156	11600 (5200–18900)	5.80	2100 (2900–78000)	10.50	15800 (6400–31000)	7.90	7900 (3600–10900)	3.95	15400 (400–33700)	7.70
PCB157	1600 (n.d.–4300)	0.80	†	†	2700 (1100–5900)	1.35	†	0.00	1900 (400–5500)	0.95
PCB 167	†	†	†	†	4900 (1700–11900)	0.05	2900 (1300–4100)	0.03	4000 (1000–8000)	0.04
PCB 189	†	†	†	†	†	0.00	†	0.00	1400 (500–2700)	0.14
	TEQ *mono-ortho*	11.99		14.99		13.09		6.30		11.34
PCB 170	24700 (11500–52800)	2.47	37100 (11400–219900)	3.71	†	0.00	†	0.00	49700 (16800–98200)	4.97
PCB 180	50600 (9700–108300)	0.51	76800 (2500–418800)	0.77	70200 (29300–133000)	0.70	44500 (23300–62600)	0.45	149500 (47300–290500)	1.50
	TEQ *di*	2.98		4.48		0.70		0.45		6.47

	Sum TEQ	32.51		35.52		24.27		17.64		24.46
PCB 28	7800 (n.d.–24200)		12100 (200–188600)		7600 (2900–38200)		3700 (1200–6700)		900 (**–2000)	
PCB 52	‡		2600 (200–32700)		5800 (2400–12300)		†		2200 (**–18300)	
PCB 101	1100 (n.d.–4700)		1500 (200–10000)		2100 (1000–7300)		1400 (900–2000)		**	
PCB 138	86800 (74600–105900)		129900 (43800–314300)		101500 (49600–222800)		50800 (30700–84400)		122300 (35700–260800)	
PCB 153	114400 (49600–259400)		186300 (59900–475700)		127800 (57000–267100)		103000 (60800–156000)		229300 (77200–439000)	

N.d.: not detected.
†Not analysed in this study.
‡Analyte was hidden behind contamination peaks.
*Below the limit of quantification (5 pg/g lipids).
**Below the limit of quantification (1 ng/g lipids).

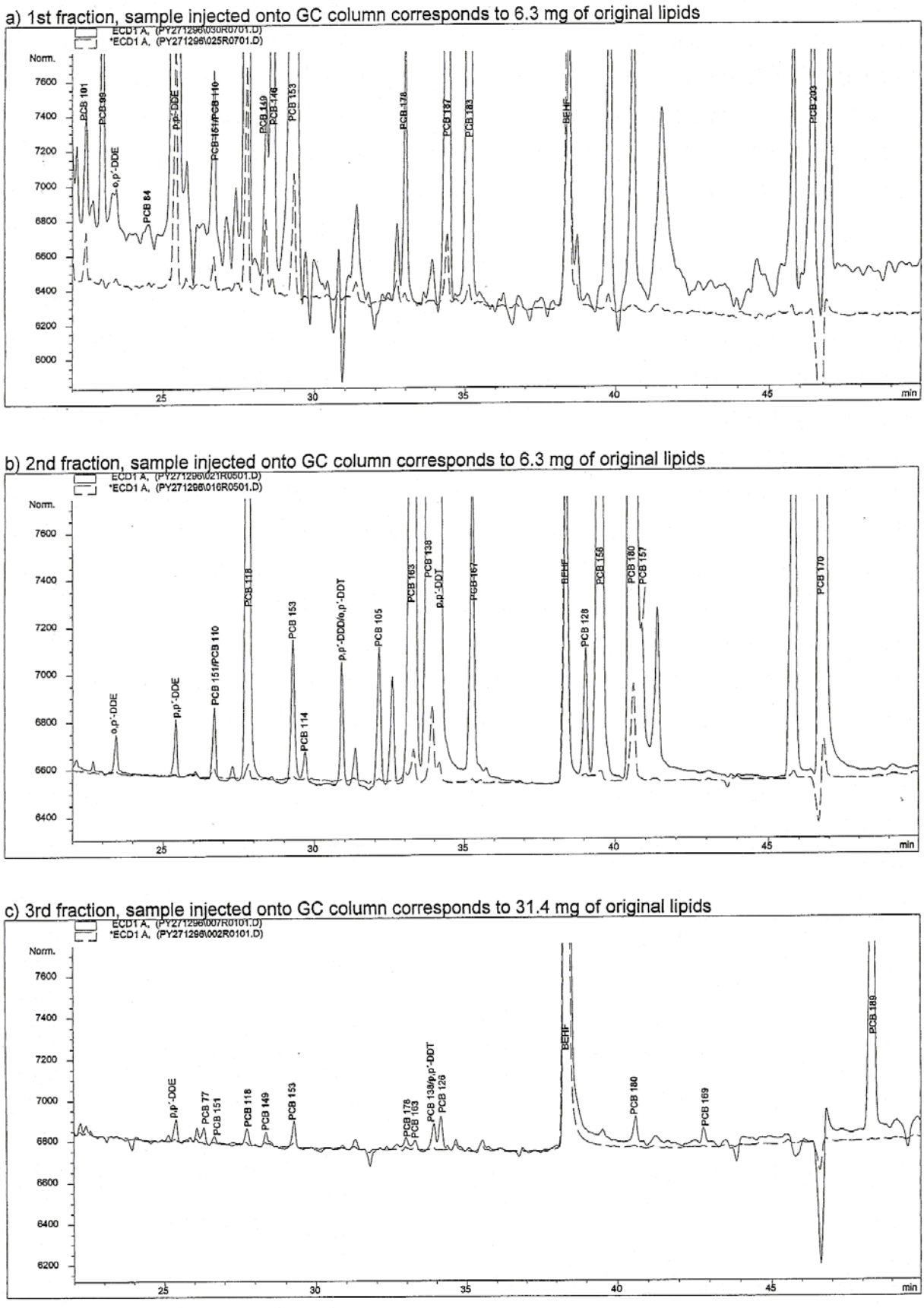

FIGURE 1 HRGC/ECD (DB-17 capillary) of fractions obtained by separation of persistent organochlorine compounds occurring in human milk on PYE column (----procedure blank).

Figure 1. The content of *non-ortho* PCBs follows the trend in other reports: the most abundant congener is PCB 126 which imparts the highest contribution to total TEQ value (mean value 24.46 pg/g milk fat) – 25.83%. The values of other toxic PCBs are summarised in Table IV. Concentration levels of hexachlorobiphenyl 169 were two times lower and that of congener PCB 77 even about ten times lower than levels of PCB 126 (the contribution to the total TEQ value is 1.29% for PCB 169 and 0.01% for PCB 77). The ratios between three *non-ortho* congeners did not differ too much from values reported in Italy [36], Canada [37,38] and the Netherlands [39]. Of other congeners, the main contribution to total TEQ value was from PCB 156 (*mono-ortho* group) and for PCB 170 (*di-ortho* group), similarly to other studies [29–32]. In spite of distinctly higher total PCBs content in Czech samples, absolute levels of *non-ortho* PCBs were lower compared to results reported in foreign studies. Many authors report Arochlor 1254 as a main cause of environmental pollution. Higher exposure of humans may be anticipated in such situation since the levels of toxic PCBs in this technical mixture are higher compared to Delor 106 which in this respect resembles Arochlor 1260 (rather than Arochlor 1254), see Table V. Large differences in concentrations found in the quoted studies can be attributed to very low levels of target analytes which unavoidably results in large analytical error. Moreover, compared to

TABLE IV Relationship between PCBs content and TEQ value (shadowed fields indicate congeners involved in TEF concept [35]

PCB congener No:	*% of total PCB content*	*% of total TEQ value*	*PCB congener No:*	*% of total PCB content*	*% of total TEQ value*
8	0.00	0.00	146	1.50	0.00
18	0.00	0.00	149	0.11	0.00
28	0.13	0.00	151	0.00	0.00
31	0.07	0.00	153	31.95	0.00
44	0.00	0.00	156	2.15	31.47
52	0.30	0.00	157	0.27	3.98
66	0.05	0.00	163	3.84	0.00
70	0.00	0.00	167	0.56	0.16
74	0.52	0.00	169	0.00	1.29
77	0.00	0.01	170	6.92	20.28
84	0.03	0.00	178	0.54	0.00
99	0.73	0.00	180	20.83	6.10
101	0.00	0.00	183	2.77	0.00
105	0.26	0.76	187	3.00	0.00
110	0.00	0.00	189	0.19	0.57
114	0.06	0.87	194	1.58	0.00
118	2.96	8.67	195	0.55	0.00
126	0.01	25.83	199	0.03	0.00
128	0.00	0.00	203	0.98	0.00
129	0.04	0.00	209	0.00	0.00
138	17.05	0.00			

TABLE V Concentrations of toxic PCB congeners in technical mixtures Delor 106, Arochlor 1254 and Arochlor 1260 (values expressed in μg/kg of technical mixture)

PCB No:	*Delor* 106 [41]	*Arochlor* 1254					*Arochlor* 1260				
		[42]	[43]	[44]	[45]	average	[42]	[43]	[44]	[45]	average
77	351	1550	100[a]	1200	300	790	210	100[a]	38	n.d.	87
81	12	174	100[a]	62	n.d.	84	25	100[a]	1[a]	n.d.	32
105	480	69000	20300	47100	29900	47575	450	800	450	2200	975
114	40	4150	100[a]	430	1800	1620	30	100[a]	14	n.d.	36
118	5813	27400	84500	90900	73500	69075	3900	11500	5700	5000	6525
123	n.a.	2170	9300	3300	1500	4068	440	100[a]	1[a]	n.d.	135
126	20	250	100[a]	270	100	180	24	100[a]	4	n.d.	32
156	7409	8360	24000	10700	8200	12815	3130	10500	4800	5300	5933
157	641	2880	200	260	1900	1310	200	700	240	200	335
167	3367	1800	500	450	2700	1363	1560	1500	300	200	890
169	n.d.	n.d.	800	1[a]	n.d.	200	n.d.	500	1[a]	n.d.	125
189	1118	250	100[a]	310	100	190	1060	3500	1300	1100	1740

n.a.: not analysed.
n.d.: not detected.
[a]Limit of determination.

TABLE VI Correlation coefficients between PCBs representing minor (toxic) and major congeners (shadowed areas indicate correlation better than 0.80)

Minor (toxic) PCBs	*Major PCBs*				
	118	138	153	170	180
77	0.067	−0.060	0.002	0.066	0.124
105	0.980	0.932	0.928	0.897	0.848
114	0.685	0.525	0.689	0.762	0.819
126	0.786	0 737	0.847	0.870	0.878
156	0.939	0.879	0.951	0.961	0.964
157	0.312	0.402	0.328	0.257	0.200
167	0.974	0.908	0.984	0.992	0.984
169	0.793	0.707	0.837	0.879	0.926
189	0.929	0.853	0.961	0.984	0.996

determination of major PCBs the analytical procedure is significantly more laborious and time-consuming, thus the number of repeated runs is limited. Considering these facts, the possibility to predict the TEQ values on the basis of routine analysis of PCBs content was tested. The relationship between major PCBs and *non-* and *mono-ortho* planar PCB was investigated by means of statistic methods, and results obtained are presented in Table VI. The best correlation coefficients were, found between planar PCBs and PCB No. 153 and No. 180. Congeners No. 153 and 180 hence may be useful as reference compounds, because they are not measurably subjected to chemical changes in the environment [40]. Thus total TEQ value can be estimated on the basis of

concentration of these congeners using the following equations:

$$\text{Sum TEQ(pg/g lipid)} = 0.0974 \times \text{Conc.}_{\text{PCB 153}}\text{(ng/g lipid)} + 2.2287 \quad (R = 0.981),$$

$$\text{Sum TEQ(pg/g lipid)} = 0.1521 \times \text{Conc.}_{\text{PCB 180}}\text{(ng/g lipid)} + 1.8285 \quad (R = 0.985).$$

Positive correlations was found among PCB 153, PCB 118 and *non-* and *mono-ortho* congeners in the Netherlands [31] (R varied between 0.794 and 0.982). In the other study from the Netherlands [17] correlations were found between PCB 153 and TEQ values derived from *non-ortho* PCBs ($R = 0.54$) and from *mono-ortho* PCB concentrations ($R = 0.86$). The recent study from Sweden [32] reports correlation between PCB 153 and TEQ values characterised by correlation coeffcient $R = 0.980$.

4. CONCLUSIONS

Ten individual human milk samples from the Prague area, Czech republic, were examined both for the levels of *non-*, *mono-*, *di-ortho* and some major PCB congeners. The results are in good agreement with foreign studies. The highest contributions to the TEQ values were observed for PCB 156, PCB 126 and PCB 170 (31.47%, 25.83% and 20.28%, resp.), which are of major toxicological concern. Very good correlations were found among some *non-* and *mono-ortho* congeners, and other PCBs. To estimate total toxicity from dioxin-like PCBs on the basis of routinely monitored indicator PCB congeners, linear equations for PCB No. 153 and PCB No. 180 calculated in this study can be used.

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