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Polychlorinated Biphenyls and Organochlorine Pesticides in Human Milk from the Locality Prague, Czech Republic: A Comparative Study

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Although the use of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) was restricted many years ago, they still remain widely distributed in the environment. Exposure of humans to these hazardous chemicals occurs mainly due to consumption of contaminated diet (Turrio-Baldassarri et al. 1995; Harrison et al. 1998). Thanks to preventive measures that have been taken in developed countries to protect human food chain, the dietary intake of PCBs and OCPs has been gradually decreasing during recent decades.

In European diet, foods of animal origin, mainly fish and seafood may represent the potential source of persistent organochlorine contaminants. Under common conditions these lipophilic chemicals are accumulated in adipose and other fatty tissues; biomagnification of some of them may occur. However, under certain circumstances such as starving and/or breast-feeding, mobilization of deposited contaminants occurs. In the later case this phenomenon results in their excretion into milk. Mother's body burden can be therefore estimated by examination of this bioindicator. The levels of excreted PCBs/OCPs are, of course, influenced by several factors such as the donors' age, number of children and duration of breast-feeding after delivery (Czaja et al. 2001).

Unfortunately, comparison of the most recent data with those obtained in older studies is rather complicated. Approximately up to the middle of 80th, PCB concentrations were expressed as "weight equivalent of technical mixture". Replacement of low-resolution packed GC columns by high performance capillaries enabled determination of individual congeners and, consequently, significantly improved characterization of particular sample contamination pattern (Rivas et al. 1997).

The aim of this study was to compare the levels of PCBs and OCPs in human milk collected in Prague region in the year 2000 with similar studies conducted recently abroad. The generated data were also compared with results obtained at the same locality 6 years ago. The relationship between PCB patterns and personal data of donors was evaluated, too.

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MATERIALS AND METHODS

Human milk samples (26 from primiparous and 17 from secundiparous mothers) were collected in July 2000 from mothers living in the Prague locality in the co-operation with the Gynaecological-maternity Clinic, 3rd Faculty of Medicine (Charles University, Prague).

The age of primiparous donors ranged from 18 to 37 years (median 25.5) and the secundiparous donors from 23 to 30 years (median 27). All of them were healthy. The breast milk was expressed manually into the glass bottles and stored at -10 °C until analysis. Questionnaire protocol was prepared according to WHO methodology (WHO/EURO 1991). The questions were focused on the mothers' background data such as body weight, pre-pregnancy dietary habits, smoking, number of deliveries, etc.

PCB standard containing 10 $\mu g.mL^{-1}$ of analytes in iso-octane was supplied by Dr. Ehrenstorfer GmbH (Germany). Working standard mixtures (in iso-octane) contained following analytes: (i) indicator PCBs: 28, 52, 101, 118, 138, 153, 180; (ii) mono-*ortho* PCBs: 105, 114, 156, 157, 167, 189; (iii) other PCBs: 8, 18, 31, 44, 47, 49, 56, 66, 70, 74, 84, 87, 95, 97, 99, 110, 128, 129, 141, 146, 149, 151, 170, 183, 187, 194, 195, 199, 202, 203, 206, 209. Standard solutions of organochlorine pesticides and their metabolites included: HCB, α-HCH, β-HCH, γ-HCH, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, octachlorostyrene. All of them were obtained by Dr. Ehrenstorfer GmbH (Germany), as solids. Working mixture of OCPs in iso-octane was prepared from these individual standards.

Aliquot of milk sample was transferred into a separatory funnel. After addition of potassium oxalate and ethanol, repeated extraction by *n*-hexane/diethyl ether (1:1, v/v) followed. The combined extracts were dried over anhydrous sodium sulphate; isolated lipids were determined gravimetrically.

Gel permeation chromatography on Bio-Beads S-X3 (50 × 0.8 cm I.D. steel column, mobile phase cyclohexane/ethyl acetate (1:1, v/v), flow rate: 0.6 $mL.min^{-1}$) was used for separation of analytes from the bulk lipids. The residual lipids left in analytes' fraction were removed by concentrated sulphuric acid.

Sample extracts were analysed by two-dimensional high-resolution gas chromatography instrument (HP 5890 ser. II gas chromatograph) employing two ^{63}Ni electron capture detectors for data acquisition. Separation of sample components was carried out on two parallel capillary columns possessing rather different selectivity: DB-5 and DB-17 (60 m × 0.25 mm I.D. × 0.10 μm phase). 1 μL of the purified extract was injected (splitless mode) at 250 °C. The oven temperature was programmed from 60 °C (2.5 min) to 220 °C at 30 $°C.min^{-1}$ and to 280 °C at 2.5 $°C.min^{-1}$. The final temperature was maintained for 10 min. Helium was used as a carrier gas (1.7 $mL.min^{-1}$ flow).

The limits of quantitation (LOQs) were 5 $ng.g^{-1}$ lipids for PCBs and 10 $ng.g^{-1}$ lipids for OCPs.

The method performance was tested for blank and recovery values. External quality control was realised via proficiency testing programme (FAPAS®).

RESULTS AND DISCUSSION

In Table 1, there are summarised levels of persistent organochlorine contaminants determined in our study concerned with examination of the set

Table 1. Mean and median concentration levels of organochlorine contaminants determined in breast milk ($ng.g^{-1}$ lipids)

	Primiparous donors			Secundiparous donors		
	Mean (Range)	S.D.	Median	Mean (Range)	S.D.	Median
β-HCH	56 (10 – 112)	27	57	64 (16 – 207)	45	55
HCB	318 (72 – 1,459)	286	249	425 (101 – 1,272)	333	371
p,p'-DDT	81 (17 – 451)	84	60	86 (28 – 176)	42	74
p,p'-DDE	1,017 (417 – 2,989)	614	822	921 (335 – 1,905)	444	857
PCB 70	9 (< 5 – 21)	4	8	10 (< 5 – 25)	6	9
PCB 74	14 (< 5 – 28)	6	13	17 (6 – 45)	11	13
PCB 99	6 (< 5 – 19)	4	6	8 (< 5 – 25)	6	8
PCB 118	24 (10 – 43)	8	24	26 (6 – 59)	15	20
PCB 129	6 (< 5 – 23)	6	< 5	< 5 (< 5 – 26)	7	< 5
PCB 138	164 (87 – 406)	86	140	156 (67 – 522)	103	136
PCB 146	20 (< 5 – 39)	8	19	27 (9 – 71)	14	23
PCB 153	264 (130 – 712)	131	215	301 (100 – 643)	142	265
PCB 156	23 (10 – 44)	9	20	25 (7 – 53)	13	21
PCB 163	47 (19 – 96)	18	40	56 (16 – 117)	30	42
PCB 170	80 (35 – 186)	37	66	86 (27 – 201)	43	73
PCB 180	182 (78 – 278)	49	150	215 (54 – 281)	61	175
PCB 183	29 (15 – 60)	11	26	49 (17 – 113)	24	45
PCB 187	38 (10 – 87)	20	31	47 (12 – 124)	34	32
PCB 194	17 (7 – 36)	8	14	20 (6 – 54)	12	16
PCB 195	7 (< 5 – 16)	4	6	9 (< 5 – 25)	6	7
PCB 203	7 (< 5 – 21)	5	7	10 (< 5 – 35)	8	8

of 43 breast milk samples. It should be noted that some of target analytes did not exceed limits of quantitation (due to a limited amount of available samples, lower values of LOQs were not attainable). Although mean concentrations of both groups of measured analytes were rather higher in milk samples obtained from secundiparous mothers compared to primiparous donors, no significant differences between these two sets of data were proved.

In Figure 1 is shown a comparison of our data with results obtained in several recent studies in which the same analytes were determined in breast milk samples (Polder et al. 1998; Kiviranta et al. 1998; Liem et al. 1995; Georgii et al. 1995; Malisch 1996; Norén et al. 2000; Lulek et al. 2002; Schoula et al. 1996). Generally, the levels of PCBs in Czech samples are still one of the highest among European countries.

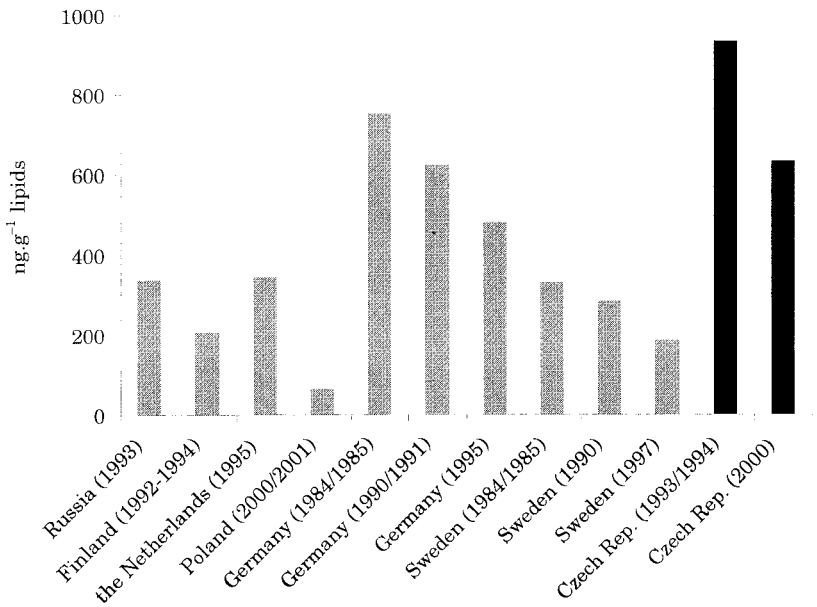

Figure 1. Mean levels of the sum of 7 indicator PCBs in human milk samples (the year in which particular study was conducted is shown in parenthesis)

Worth to notice is the absence of lower chlorinated PCBs (PCB no. 28 and 52 did not exceed LOQ in any of our samples), while the contribution of hexa- and heptachlorobiphenyls to the total PCBs in Czech breast milk was higher compared to samples from other countries, see Figure 2 (She et al. 1998; Newsome et al. 1995; Polder et al. 1998). Fundamentally different sources of dietary exposure might be responsible for this phenomenon.

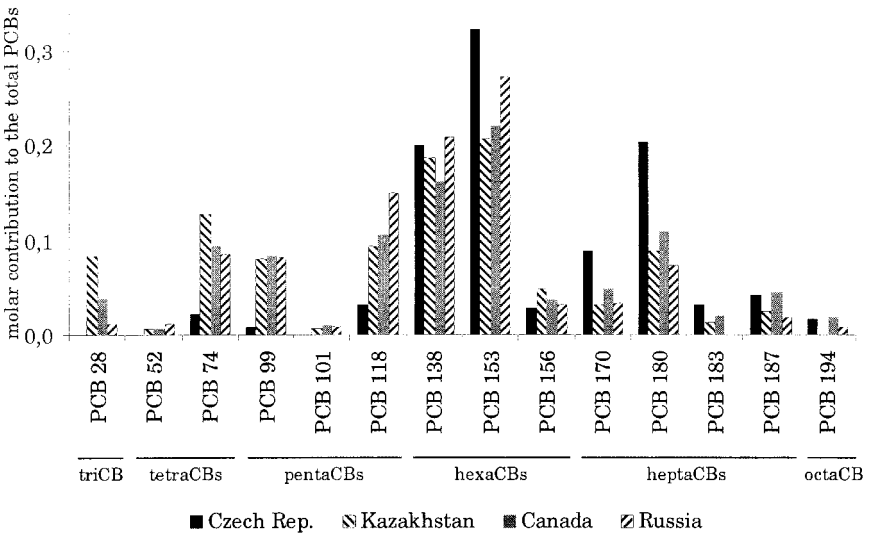

Figure 2. Comparison of the PCBs pattern characteristic for the Czech breast milk samples with data obtained in similar studies realised in other countries

Statistically significant decrease of the PCBs (expressed as sum of indicator congeners) and HCB during recent 6 years occurred (see Figure 1). Overall downward trends in levels of these pollutants were reported by other authors, too.

As shown in our study, *p,p'*-DDE was dominating compound among OCPs practically in all milk samples. In none of them the contribution of *p,p'*-DDT to the sum of DDT compounds exceeded 10 % (w/w), what documents that contamination of food chain is due to "historical" uses, i.e. contamination's input into food chain occurred many years ago.

Figure 3 (Polder et al. 1998; Norén et al. 2000; Schoula et al. 1996) illustrates relatively high dietary intake of OCPs in the Czech Republic. The levels of target analytes in breast milk are several times higher compared to samples obtained from mothers living in unpolluted European areas represented e.g. by Sweden. Moreover, no distinct diminution (contrary to PCBs and HCB) considering inter-annual trends was observed, what may indicate continuing risk of dietary exposure of population to these hazardous chemicals.

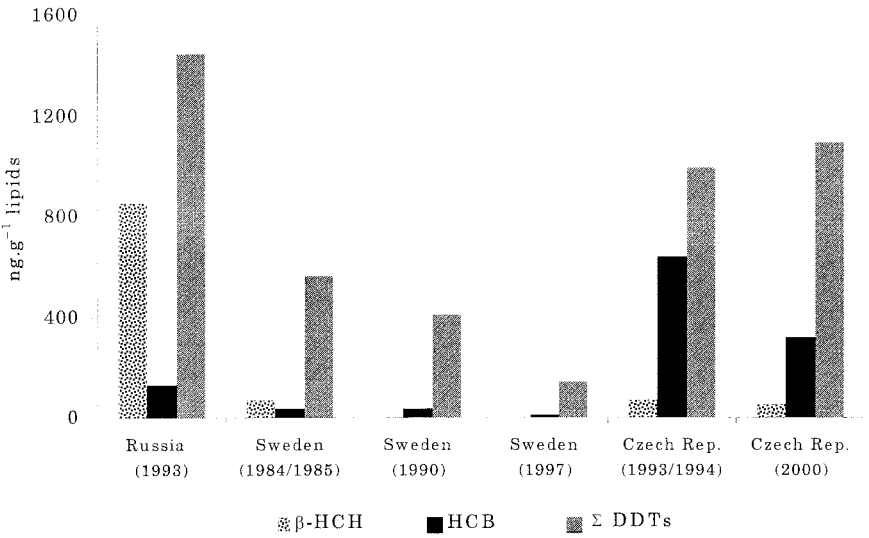

Figure 3. Mean levels of organochlorine pesticides in human milk samples (the year in which particular study was conducted is shown in parenthesis)

As regards assessment of the generated results in context of information collected through questionnaire protocols, no correlation was found between the mothers' age/weight and any of examined group of contaminants.

A positive correlation was found between PCB 153 and the sum of indicator congeners (R = 0,985 and R = 0,994 for primiparous and secundiparous mothers, respectively). Hence for screening purposes, PCB 153 might be used as contamination marker.

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