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Organochlorine Pesticides and PCB Congeners in Human Milk from Two Population Groups in Croatia

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, bioaccumulative and toxic compounds. PCBs have been produced for many applications since 1929, whereas an OCPs have intensively been used in agriculture and public health since Second World War. After the discovery of PCBs and OCPs in biotic and abiotic environments, numerous researches have been done in order to understand their environmental omnipresence and their toxic effects. Consequently, the use of PCBs and OCPs has been banned or restricted, but they are still present in the environment.

General population is exposed to pollutants through inhalation, ingestion or dermal absorption. It has been suggested that the most important intake of OCPs and PCBs is by food (Liem and Theelen 1997). These compounds accumulate in the human adipose tissue and are excreted in human milk. This is why human milk can be used to assess exposure to PCBs and OCPs.

This study analysed the levels of 20 PCB congeners and OCP in human milk samples collected from two geographically different areas of Croatia.

MATERIALS AND METHODS

Human milk samples were collected in 2000 from nursing mothers of the general population in Zagreb and on the island of Krk. Zagreb is the northwestern Croatian capital with about 1,000,000 inhabitants while Krk is an island located in the northern Adriatic. In Zagreb 29 samples were collected from mothers aged 21-43; eight mothers were multiparae, while the others were primiparae. On Krk, 23 samples were collected from mothers aged 20-40; 10 mothers were multiparae while others were primiparae. The milk was manually expressed into pre-cleaned glass bottles and stored at -20 °C until analysis.

Milk (2.5 g) was extracted with a mixture of chloroform and methanol (1:1). Milk fat was weighed and dissolved in *n*-hexane and cleaned up with sulphuric acid (for details see Krauthacker et al. 1986; Zubčić and Krauthacker 2004). The analysis was done on an "ATI UNICAM" 610 SERIES gas chromatograph with ^{63}Ni electron capture detector. Two capillary columns were used simultaneously: 1) 60 m × 0.25 mm, SPB-5 film thickness 0.25 μm, temp. programme 100 °C, then

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4 °C min^{-1} to 240 °C, 50 min isothermally. 2) 30 m × 0.25 mm, SPB-1701 film thickness 0.25 μm, temp. programme 110 °C, then 4 °C min^{-1} to 240 °C, 50 min isothermally. The carrier gas was nitrogen. The injector and the detector temperatures were 250 °C and 270 °C, respectively. Qualitative and quantitative analyses were done by comparison with the external standard. Each sample was analyzed on both columns. Only compounds identified on both columns were evaluated. Determination limits were 1.64 ng g^{-1} milk fat for PCB congeners, β-HCH, DDE, DDD and DDT and 0.98 ng g^{-1} milk fat for HCB, α-HCH and γ-HCH.

The following compounds were analyzed: 20 PCB congeners (PCB-28, PCB-52, PCB-60, PCB-74, PCB-77, PCB-101, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-169, PCB-170, PCB-180, PCB-189 numbered according to IUPAC) and organochlorine pesticides HCB (hexachlorobenzene), α-, β-, γ-HCH (alpha-, beta-, gama-hexachlorocyclohexane), DDE (1,1-dichloro-2,2-di(4-chlorophenyl)ethene), DDD (1,1-dichloro-2,2-di(4-chlorophenyl)ethane, DDT (1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane.

For intralaboratory quality control a pool of 62 individual milk samples collected in 1993/95 was used. Pooling was done on equal-volume basis. Certified reference materials CRM 450 (powdered natural cow's milk), CRM 187 (powdered natural cow's milk), and CRM 188 (powdered cow's milk spiked with OCPs) supplied by the Community Bureau of Reference, Commission of the European Communities, Bruxelles, Belgium were also analysed.

Recovery and repeatability of the method were tested by the addition of known amounts of analyzed organochlorine compounds to sub-samples of human milk pool prior to extraction at levels 4.9-45.4 and 76.2-343.6 ng g^{-1} of milk fat, depending on the compound. For each level, six sub-samples were analyzed. The recoveries of PCBs and OCPs were calculated after subtracting the levels of two non-fortified samples from the fortified ones. The results are summarized in Table 1.

Table 1. Mean method recovery (%) and repeatability expressed as relative standard deviation (RSD;%).

Polychlorinated biphenyls (N=6)			Organochlorine pesticides (N=6)		
Fortification level (ng g^{-1} fat)	Recovery (%)	RSD (%)	Fortification level (ng g^{-1} fat)	Recovery (%)	RSD (%)
7.7-11.5	54-86	5-45	4.9-45.4	41-73	14-57
76.2-96.1	55-90	5-24	99-343	49-94	6-24

The accuracy of the method was determined by use of the certified reference materials CRM 450 (PCB-52, PCB-118, PCB-153, PCB-156, PCB-170, PCB-180 in powdered natural cow's milk), CRM 187 (HCB, α-HCH, γ-HCH, *p,p'*-DDE in powdered natural cow's milk) and CRM 188 (HCB, α-HCH, γ-HCH, *p,p'*-DDE, β-HCH, *p,p'*-DDT in spiked powdered cow's milk). Table 2 shows certified values of organochlorine pesticides in CRM 187 and CRM 188, and the determined

Table 2. Mass fractions (ng g^{-1}dry mass) of organochlorine pesticides in certified reference materials CRM 187 and CRM 188.

Compound	CRM 187		Compound	CRM 188	
	Certified values	Determined values		Certified values	Determined values
HCB	1.5±0.2	1.3±0.1	HCB	37.4±2.7	33.4±2.8
α-HCH	1.8±0.14	3.0±0.2	α-HCH	20.0±0.9	16.3±2.5
γ-HCH	5.7±0.7	16.6±4.7	γ-HCH	45.4±2.9	44.4±4.7
p,p'-DDE	6.6±0.6	8.0±0.8	*p,p'*-DDE	51.3±3.5	59.4±5.9
			β-HCH	12.0±1.2	12.4±1.2
			p,p'-DDT	69.0±4.6	62.6±9.5

Table 3. Mass fraction (ng g^{-1}dry mass) of PCBs in certified reference material CRM 450.

Compound	Certified values	Determined values
PCB-52	1.16±0.17	4.3±0.6
PCB-118	3.3±0.4	3.5±0.3
PCB-153	19.0±0.7	19.4±2.4
PCB-156	1.62±0.2	1.3±0.2
PCB-170	4.8±0.6	4.2±0.4
PCB-180	11.0±0.7	7.8±0.4

values. The HCB level determined in CRM 187 was within the certified range, but DDE was above the range. The concentrations of α-HCH and γ-HCH were outside the certified range for CRM 187. For CRM 188, γ-HCH concentrations were acceptable, and α-HCH were slightly lower than the certified values. This suggests the possibility of interferences during analysis of CRM 187. For CRM 188, β-HCH, HCB and DDT were within the certified range.

Table 3 shows certified and determined values for CRM 450. Analyzed PCB congeners were within the certified range, save for PCB 52 which was higher and for PCB-180 which was slightly lower. Covaci et al. (2001) also analyzed certified reference material CRM 450 and unlike our result, their determined value for PCB-52 was lower than certified.

RESULTS AND DISCUSSION

Table 4 shows the levels of organochlorine pesticides in human milk samples collected in 2000 on the island of Krk and in Zagreb. Organochlorine pesticide levels in human milk samples were between the determination limit and 1287 ng g^{-1} milk fat on both locations. β-HCH, γ-HCH and DDE were detected in all samples. The profile of concentration medians were the same in milk from both locations: DDE>β-HCH>γ-HCH≈DDT>HCB>DDD>α-HCH. Ratios DDE/DDT were prominently higher than unity, which means that there was no recent output of DDT into the environment.

Table 4. Levels of organochlorine pesticides (ng g^{-1} milk fat) collected in Zagreb (N=29) and on the island of Krk (N=23) in 2000.

Compound	ZAGREB			KRK			χ^2
	Range	Median	n (%)	Range	Median	n (%)	
HCB	0-47.2	12.5	93	0-105	7.4	91	0.31
α-HCH	0-43.9	2.4	86	0-26.1	1.5	74	0.31
β-HCH	4.6-57.3	20.0	100	3.8-113	18.7	100	0
γ-HCH	2.7-64.5	18.5	100	0.98-118	15.4	100	1.25
p,p'-DDE	84.8-911.56	257	100	60.6-1287	227	100	0
p,p'-DDD	0-157	2.0	59	0-20.4	5.0	87	1.25
p,p'-DDT	0-424	19.4	90	0-64.2	13.7	87	1.25
α/γ-HCH*	0.03-1.29	0.17	/	0.01-1.01	0.14	/	/
DDE/DDT*	1.3-45.6	10.1	/	6.3-55.6	9.9	/	/

χ^2 – statistic of Median test; 0-below determination limit; * - determined in positive samples; n – percentage of positive samples

Ratios α/γ-HCH were low (below 1), which implies that general population was recently exposed to lindane. Lindane levels in human milk were low, probably due to fast elimination from the body. Mixtures of HCHs and DDT had not been used in Croatia for decades, and higher concentrations of DDE and β-HCH in comparison to other analyzed pesticides confirms their persistence in humans.

Published data (Aune et al. 1999; Polder et al. 1998; Schoula et al. 1996; Harvey Newsome et al. 1995; Burke et al. 2003) and our results show that DDE and β-HCH are the major compounds found in human milk. Organochlorine pesticide levels in human milk from Croatia are not high, and are comparable to those in the UK, Japan (Burke et al. 2003) and Sweden (Aune et al. 1999). These levels could be viewed as a consequence of former pesticide use, and they reflect global biosphere pollution by persistent organochlorine compounds.

Table 5 shows the concentration ranges and medians of PCBs in human milk collected at the two Croatian areas. PCB-138 and PCB-153 were found in all analysed samples, while PCB-157 was below determination limit in all samples. The concentration range of PCBs was between the determination limit and 213 ng g^{-1} of milk fat. Ten PCB congeners were detected in more than 50 % of samples. The concentration median was the same for both locations: the highest concentration was measured for PCB-153, PCB-138 and PCB-180, and the lowest for PCB-60. The median of the sum of six indicator PCBs in samples from Zagreb was 120 ng g^{-1} of milk fat and from Krk 123 ng g^{-1} of milk fat. Co-planar PCB-126 was not found in any of the samples. PCB-77 and PCB-169 were detected in no more than 14% and 3% of positive samples, respectively (max. level 5.4 ng g^{-1} of milk fat).

The island of Krk and Zagreb differ in geographical position, in the level of industrialization and in eating habits. Nonparametric statistics Median test was used to see whether these two independent groups were taken from the same population. Tables 4 and 5 show that all χ^2 values were below the critical value (3.841) with

one degree of freedom and the significance level of 5 %. This suggests that concerning organochlorine pesticide and polychlorinated biphenyl concentrations these two groups of mothers belong to the same population. Taking this into account, the median of the sum of six indicator PCBs for both group was 123 ng g^{-1} of milk fat (range 38-498 ng g^{-1} milk fat).

Table 5. Levels of polychlorinated biphenyls (ng g^{-1} milk fat) collected in Zagreb (N=29) and on the island of Krk (N=23) in 2000.

Compound	ZAGREB			KRK			χ^2
	Range	Median	n (%)	Range	Median	n (%)	
PCB-28	0-24.2	8.3	90	0-39.9	10.5	96	1.25
PCB-52	0-146	12.5	93	0-66.9	10.4	96	0
PCB-101	0-24.5	2.6	59	0-34.3	2.9	70	0.13
PCB-138	10.6-62	33.1	100	3.5-168	32.6	100	0.13
PCB-153	16.7-108.7	41.5	100	16.9-213	38.8	100	0
PCB-180	0-40.1	13.2	93	7.1-82.7	19.4	100	0.31
PCB-105	0-17.3	0	3	0-5.1	0	13	/
PCB-118	0-69.7	4.5	72	0-19.5	5.1	83	0.07
PCB-60	0-16.2	1.7	69	0-8.3	0	48	1.25
PCB-74	0-12.1	2.4	55	0-23.1	4.1	78	2.81
PCB-156	0-16.1	0	48	0-12.2	3.4	83	1.25
PCB-157	0	0	0	0	0	0	/
PCB-167	0-5.1	0	45	0-5.0	0.7	52	0.06
PCB-189	0-4.4	0	7	0	0	0	/
PCB-123	0-12.6	0	31	0-4.83	1.8	61	3.5
PCB-114	0-10.5	0	34	0-12	0	13	/
PCB-170	0-26.9	11.4	90	0-87	10.7	91	0.31

χ^2 – statistic of Median test; 0-below determination limit; * - not determined; n – percentage of positive samples

WHO organized exposure studies of PCB, PCDD and PCDF in human milk samples collected from all over the world, and the 3rd round of studies was conducted between 2000 and 2002 (van Leeuwen and Malisch 2002). Our laboratory participated in this round and collected two pools of samples of human milk in December 2000. One pool of samples (Zagreb) consisted of 12 individual samples, and the other (Krk) of 10 individual samples. All mothers were primiparae. Samples were analyzed in the reference laboratory of the State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany (Malisch and van Leeuwen 2002). The sum of six indicator PCBs ranged from 121 to 150 ng g^{-1} of milk fat, and the median was 135 ng g^{-1} of milk fat. The ranges of the sums of six indicator PCB levels measured in our laboratory in this study (range: 38-498 ng g^{-1} of milk fat; median: 123 ng g^{-1} of milk fat) overlapped with the data obtained for our two pooled samples measured by the Freiburg reference laboratory. Data obtained from all participants of the 3rd WHO round are given in Figure 1. Croatia belong neither to the group with the highest nor with the lowest PCB levels. PCB levels in Croatia are comparable with those in Sweden, Ukraine, Russia, Norway and Egypt.

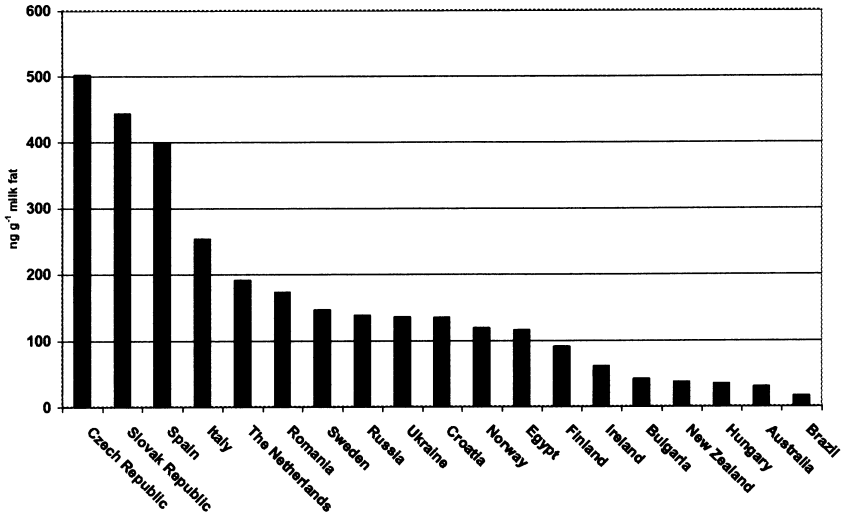

Figure 1. Median of the sum of six indicator PCBs (ng g^{-1} of milk fat) in human milk samples analyzed in the 3rd round of a WHO study (van Leeuwen and Malisch 2002).

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