

## POLYBROMINATED DIPHENYL ETHERS, POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN HUMAN MILK AS MARKERS OF ENVIRONMENTAL EXPOSURE TO THESE COMPOUNDS

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**Abstract:** This study aimed at the generation of preliminary results allowing for the assessment of breastfed infants exposure to polybrominated diphenyl ethers (PBDEs) which constitute important contaminants in places of human habitation. The second goal was to compare the concentrations of these compounds with other contaminants which people are exposed to *via* food chain. 28 breast milk samples from women living in Warsaw and neighbourhood were analyzed for polybrominated diphenyl ethers (BDE-47, BDE-99, BDE-153), polychlorinated biphenyls (CB-77, CB-101, CB-118, CB-126, CB-138, CB-153, CB170, CB-180) and organochlorine pesticides (HCB,  $\beta$ -HCH,  $\gamma$ -HCH, p,p'-DDE, p,p'-DDD, p,p'-DDT). The  $\Sigma$ DDT levels noted in our studies were higher than in other European countries. The concentrations of the examined polychlorinated biphenyls and polybrominated diphenyl ethers did not diverge from the levels presented by other authors and are comparable to the levels noted in other countries in Europe.

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### INTRODUCTION

Persistent organohalogen compounds such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are classified as persistent organic pollutants (POPs) [10]. Due to their lipophilic nature they have a capacity to bioaccumulate in the fat tissue and to biomagnify in food chains. Numerous studies confirm that the main way of eliminating this type of compounds from women's bodies is through breast milk. There is an increasing number of studies which conclude that PBDEs and PCBs can disrupt thyroid hormone balance in animals and humans. In the pre- and postnatal period it can lead, among others, to developmental disorders of the central nervous system.

It was demonstrated that these compounds are neurotoxic, weaken behavioural reflexes, decrease general activity, lower mobility, influence learning and memorizing processes. They also affect the immunological system and induce activity of xenobiotic metabolizing enzymes. Moreover, they have a capacity to influence cytosolic Ah receptor and disturb the activity of estrogen hormones [3, 12, 19, 24, 30, 34].

The most exposed group, for which the daily intake of these compounds is the highest, is the group of breastfed neonates [16]. The exposure to polychlorinated biphenyls and organochlorine pesticides is not without implications for this age group, especially due to the lack of fully developed detoxicating mechanisms [12]. The above data encouraged the authors to undertake the study which aimed at

explaining certain aspects of children exposure to polybrominated diphenyl ethers in the period of their most intensive growth. Because of the physicochemical similarities between PBDEs and PCBs, the latter group of compounds was introduced to the study, as being an important health risk factor for infants in the postnatal period. The analyses include also HCH, HCB and DDT in order to evaluate total exposure to the whole group of persistent organic pollutants.

The following compounds were selected for analysis: BDE-47, BDE-99, BDE-153, which according to literature data constitute 70–80% of the total quantity of PBDEs detected in the human material, PCBs indicator congeners: 101, 138, 153, 180 and PCBs congeners the most frequently found in human tissues: 77, 118, 126, 170. Organochlorine pesticides HCH (hexachlorocyclohexane), HCB (hexachlorobenzene) and DDT were also examined.

## MATERIAL AND METHODS

The study material consisted of 28 samples of breast milk obtained from women living in Warsaw and the surrounding area. After having obtained the consent of a medical ethical committee the samples were collected in one of the obstetric clinics in Warsaw in 2002–2005. The sample donors also provided information about their age, body mass, height, number of deliveries, type of diet and general health condition.

All samples were stored in  $-20^{\circ}\text{C}$  until analysis. Extraction was performed according to the procedure described by Kalantzi *et al.* [19]. Milk samples were thawed and centrifuged for 17 minutes at 3,000 rpm. The water phase was separated from the fat phase. After being centrifuged, the solid phase was boiled with sodium sulfate and hexane for 10 minutes under rotary evaporator with reflux condenser. After cooling, 5 ml of the extract were taken for gravimetric measurement of lipids. The extracts were preliminarily cleaned-up with concentrated sulphuric acid. Next, they were concentrated to 2 ml and underwent further clean-up by gel permeation chromatography (GPC). The GPC column was filled with BioBeads SX3 and the sample was eluted with a mixture of dichloromethane (DCM) and hexane (1:1, v/v). The cleaned-up extracts were analyzed using gas chromatography with electron capture detection (GC/ECD) and mass spectrometry (GC/MS).

For GC/ECD analyses the gas chromatograph (Agilent Technologies 6890N) was used and the following working conditions applied:

column: HP-5 (30 m  $\times$  0.32 mm i.d., 0.25  $\mu\text{m}$ );

injector temperature:  $260^{\circ}\text{C}$ ;

sample volume: 5  $\mu\text{l}$ ;

column oven temperature programme:  $70^{\circ}\text{C}$  (1.7 min),  $30^{\circ}\text{C min}^{-1}$  –  $190^{\circ}\text{C}$ ,  $3^{\circ}\text{C min}^{-1}$  –  $240^{\circ}\text{C}$ ,  $30^{\circ}\text{C min}^{-1}$  –  $280^{\circ}\text{C}$ .

The results were confirmed using the GC/MS technique (Varian 4000) under the following working conditions:

column: DB-5MS (30 m  $\times$  0.32 mm i.d., 0.25  $\mu\text{m}$ );

sample volume: 10  $\mu\text{l}$ ;

column oven temperature programme:  $70^{\circ}\text{C}$  (1 min),  $30^{\circ}\text{C min}^{-1}$  –  $170^{\circ}\text{C}$ ,  $8^{\circ}\text{C min}^{-1}$  –  $300^{\circ}\text{C}$  (15 min);

ion trap temperature:  $200^{\circ}\text{C}$ .

Due to very low levels of polybrominated diphenyl ethers, the confirmation analyses were conducted using tandem mass spectrometry in order to isolate the PBDEs from the peaks from the matrix and to increase the measurement system sensitivity. The following ions were selected as typical parent ion for particular PBDE congeners: BDE-47 – 486 m/z, BDE-99 – 564 m/z, BDE-153 – 484 m/z. Parent ions were further fragmented in non-resonant mode of a mass spectrometer. For all analyzed congeners, the value of qz parameter, responsible for effectiveness of ion trapping was set between 0.2–0.3, depending on the number of attached bromine atoms. The best values of excitation amplitude (EA) for particular congeners were the following: for BDE-47 EA = 66 V, for BDE-99 EA = 73 V, for BDE-153 EA = 81 V. Excitation time was 20 ms for all PBDEs.

## RESULTS AND DISCUSSION

The analytical method applied in this study enabled the obtaining of the limits of quantification (LOQ) for the analyzed compounds at 0.2–2.0 ng/g of lipid content and analyte recoveries of these compounds, obtained at the method validation stage, ranged from 54–95%.

Mean concentrations of the analyzed compounds were calculated on the assumption that results below LOQ equal zero.

Table 1 presents the characteristics of 28 donors of milk samples used in this study.

The mean lipid content in milk was 1.37% (0.1–4.9%), thus was relatively low. This was probably due to sample collection during first days of lactation when the level of lipids in the milk is the lowest. Lipid concentration level in mature milk is higher and its highest level is obtained after 15 days of postpartum [27], which can influence the neonates exposure level to a certain degree. The milk composition as well as the quantity and the quality of its lipid content can depend on the following factors: BMI, diet, physical activity, smoking and body mass loss during the lactation period. Therefore, these factors can also influence indirectly the exposure level which also depends on the

**Table 1.** General characteristic of women participating in this study.

Subject characteristics	Mean $\pm$ SD <sup>a</sup>	Range
Maternal age in years	29.86 $\pm$ 3.56	23–40
Maternal weight before pregnancy [kg]	59.25 $\pm$ 9.44	45–89
Pre-pregnant body mass index [kg/m <sup>2</sup> ]	21.39 $\pm$ 3.18	17–32

<sup>a</sup>SD – standard deviation

**Table 2.** PCBs, OCPs and PBDEs mean concentrations expressed in ng/g lipid weight and in µg/l of milk.

Compound	% > LOQ <sup>a</sup>	Mean [ng/g lipid weight]	Range [µg/l of milk]	Mean	Range
HCB	100	10.6	2.0–66.8	0.161	0.002–1.611
b-HCH	100	31.9	5.9–202.9	0.361	0.005–4.892
g-HCH	96	23.8	< LOQ–200.0	0.126	< LOQ–0.550
p,p'-DDE	100	2,146.9	241.5–12,803.1	19.347	0,037–101.202
p,p'-DDD	100	124.4	2.8–1,883.3	1.829	0.041–45.394
p,p'-DDT	100	383.2	35.0–3,055.5	2.510	0.027–26.590
PCB 77	79	33.6	< LOQ–411.3	0.106	< LOQ–0.475
PCB 101	32	16.9	< LOQ–251.9	0.147	< LOQ–2.854
PCB 118	46	1.9	< LOQ–15.2	0.042	< LOQ–0.391
PCB 126	18	4.5	< LOQ–107.8	0.096	< LOQ–2.598
PCB 138	86	23.6	< LOQ–173.4	0.263	< LOQ–1.464
PCB 153	90	39.8	< LOQ–200.0	0.459	< LOQ–2.613
PCB 170	57	4.6	< LOQ–26.6	0.072	< LOQ–0.425
PCB 180	96	26.1	< LOQ–163.9	0.315	< LOQ–2.239
BDE 99	57	0.8	< LOQ–4.2	0.092	< LOQ–0.912
BDE47	64	1.1	< LOQ–8.6	0.141	< LOQ–0.726
BDE 153	22	0.2	< LOQ–1.9	0.034	< LOQ–0.279

<sup>a</sup> LOQ – limit of quantification

time of exposure, age, ability to eliminate the compounds through metabolism and – in the case of women – intensity of lactation [35, 37].

The presence of BDE-47 and BDE-99 congeners above the LOQ was noted in 64% and 57% of the examined samples, respectively. The mean concentrations of these compounds were 0.14 and 0.09 µg/l of milk respectively. BDE-153 was present in 22% of samples with the mean level of 0.03 µg/l of milk. The highest concentrations of all compounds were noted for p,p'-DDE, p,p'-DDT i p,p'-DDD, for which the mean concentrations reached 19.34, 2.51 and 1.83 µg/l of milk respectively. The highest concentrations among polychlorinated biphenyls (PCBs) were obtained for the following congeners: CB-153 – 0.46 µg/l of milk, CB-180 – 0.32 µg/l of milk and CB-138 – 0.26 µg/l of milk. Mean levels of the remaining PCB congeners in the breast milk were as follows: CB-101 – 0.15 µg/l of milk, CB-126 – 0.09 µg/l of milk, CB-77 – 0.11 µg/l of milk, CB-170 – 0.07 µg/l of milk and CB-118 – 0.04 µg/l of milk, and HCH isomers (β and γ) – 0.36 and 0.13 µg/l of milk, respectively.

Presenting the concentration levels of the analyzed substances in breast milk per volume unit provides a basis for the quantitative assessment of neonate exposure to these substances. For the purpose of comparison, however, it is justified to present the results calculated per lipid content, similarly to how other authors of monitoring studies present their results.

The results of the present study suggest that concentrations of the analyzed compounds calculated per lipids may depend on the lipid content synthesized during the milk

production process of the particular women. Considerable differences in the quantity of lipids in milk were noted among particular donors, yet they were within physiological norm. Since all of the studied compounds are lipophilic, and therefore excreted in the lipid phase of milk, it is justified to state that their concentration in the breast milk fat reflects the general body burden.

The concentrations of the analyzed substances, calculated per lipids contained in milk, were lower in samples with higher lipid content. This tendency was not visible in the case of BDE-153 due to an insufficient number of samples containing this congener above LOQ.

Presenting the results calculated per lipid content facilitates the comparison of levels of the analyzed substances to those obtained by other authors, but can lead to misleading conclusions about the exposure of neonates. An infant takes a specific volume of milk, and the lipid content in this milk influences the volume of milk intake only to a small extent.

Mean concentrations of all the examined compounds in milk samples, expressed in µg/l of milk and ng/g of lipids weight, are presented in the Table 2.

The mean concentrations of organochlorine pesticides were relatively high, which was due to the presence of two milk samples for which the obtained results were significantly higher than the results of the remaining samples. After having conducted the Dixon test, the outliers were excluded from the calculations; this allowed the comparison of the mean concentration values of the analyzed substances with results presented by other authors. Table 3 demonstrates the mean concentrations calculated after the

**Table 3.** Mean concentrations of OCPs excluding outliers expressed in ng/g lipid weight.

Compound	Mean [ng/g lipid weight]	SD	Range [ng/g lipid weight]
HCB	8.5	7.7	2.0–21.8
b-HCH	15.6	8.7	5.9–39.6
g-HCH	8.2	6.4	< LOQ <sup>a</sup> –20.4
p,p'-DDE	1,400.1	934.6	241.5–4,276.8
p,p'-DDD	33.2	16.2	2.8–197.4
p,p'-DDT	187.3	158.8	35.0–681.1

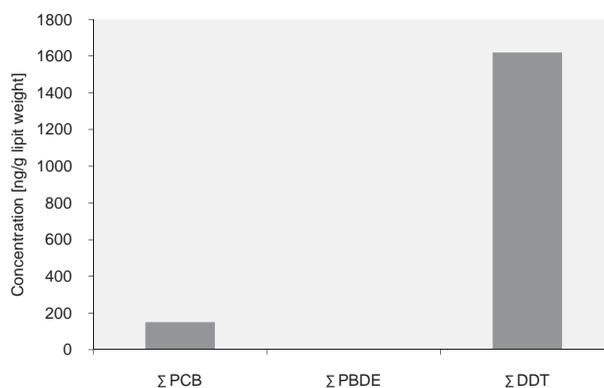
<sup>a</sup> LOQ – limit of quantification

exclusion of the outliers. It should be noted, however, that these results indicate sporadic cases of high exposure to organochlorine pesticides deposited in various environmental compartments.

The profiles of polychlorinated biphenyls in the analyzed breast milk samples did not diverge from those presented by other authors. The highest concentrations were found in case of congeners CB-153, CB-180 and CB-138. The lack of correlation between concentrations of PBDEs and PCBs was also typical. Comparison of the mean concentrations of the analyzed groups of substances in the breast milk is presented in the Figure 1.

The concentrations of PCBs and PBDEs observed in the breast milk samples were similar to the results presented by Jaraczewska *et al.* [17]. The profiles were also similar to those reported in other European studies. The PBDEs profile was dominated by BDE-47, BDE-99 and BDE-153, respectively.

The highest concentrations among all analyzed groups of compounds were noted for organochlorine pesticides, despite the fact that they have not been used for many years in Poland. The PBDEs constituted only a small percentage

**Figure 1.** Distribution of mean concentrations of total PCBs, PBDEs, and DDT (ng/g lipid weight) in human milk samples.

of the analyzed pollutants. A similar tendency was reported by Jaraczewska *et al.* [17] in the study of maternal milk of woman from Poland.

Studies carried out in the last three decades indicate substantial differences in the concentration of the analyzed substances in the milk of European woman. The differences were noted in both the levels of the substances and the time of sample collection (Tab. 4).

As shown in the Table 4, the concentrations of organochlorine pesticides detected in the present study are higher than those reported in the studies of maternal milk conducted by Jaraczewska *et al.* [17]. The levels of these compounds are also higher than in other European countries. In the present study, the samples were collected in the first days of lactation, which resulted in a low lipid content in milk, but a high concentration of the examined substances calculated per lipids content. This is due to the fact that the above mentioned compounds are contained in the milk fat. The lipid content in milk undergoes fairly significant fluctuations during the lactation period; therefore, analogical fluctuations are observed in the concentration of lipophilic compounds [6]. The sample collection performed on

**Table 4.** PCBs, OCPs and PBDEs mean concentrations expressed in ng/g lipid weight in human milk samples from Europe.

Country	Year of collection	N	β-HCH	HCB	p,p'-DDT	p,p'-DDE	Σ DDT	Σ PCB	Σ PBDE	References
Poland	1971	40	–	–	3,920	8,910	12,870	–	–	[20]
Poland	1980	106	–	–	–	–	6,800	490	–	[18]
Poland	2000–2001	12	–	–	–	–	–	77.6	–	[23]
Poland	2004	22	13	32	51	817	868	153	2.5	[17]
Poland	2002–2005	28	15.6	10.6	187.3	1,400.1	1,620.6	151	2.2	Present study
Germany	2005	39	17	27	–	–	180	230	1.9	[28]
Czech Republic	2001	90	22.5	192	36.8	839	884	2,046 <sup>a</sup>	–	[4]
Slovakia	2003	14	19	98	36	659	–	651	–	[37]
Sweden	2000–2001	13	–	–	–	–	–	190 <sup>b</sup>	2.14 <sup>b</sup>	[13]

<sup>a</sup> breast milk samples collected in an exposed region; <sup>b</sup> median.

**Table 5.** Mean concentrations of total DDT reported in breast milk from Europe [Smith 1999].

Country	Year of collection	$\Sigma$ DDT [ng/g lipid weight]	N	References
Poland	1970	11,500	32	[30]
	1980	6,800	106	
	1987	4,600	54	
	2002–2005	1,620.6	28	Present study
Yugoslavia	1973	3,850	14	[21]
	1976	1,790	27	
	1987	1,080	33	[22]
Czechoslovakia	1974	7,300	–	[26]
	1993	1,845	26	
Germany	1980	2,040	836	[29]
	1984	978	144	[11]
	1991	531	113	
France	1991	2,283	20	[2]
Spain	1979	7,200	45	[15]
	1981	10,240	20	[1]
Sweden	1972	2,930	75	[25]
	1980	1,240	199	
	1990	421	40	
Finland	1974	2320	–	[36]
	1982	890	50	

a particular day of lactation is an important factor influencing the level of the analyzed compounds. The studies conducted by Waliszewski *et al.* [35] show that the levels of organochlorine pesticides ( $\beta$ -HCH, *p,p'*-DDE i *p,p'*-DDT) in human milk decrease with lactation. This tendency is also documented in our earlier study comparing two subsequent lactations in the same women. In the second lactations the levels of the examined compounds were lower [6].

Analysis of studies conducted in 1970–1999 (Tab. 5) in Europe leads to the conclusions that the  $\Sigma$ DDT levels (DDT, DDE and DDD) in human milk differed considerably depending on the country. The highest levels among the countries listed in Table 5 were noted in Poland and Spain [32]. All the studies report a decrease in  $\Sigma$ DDT levels with time, and the present concentrations of these compounds are significantly lower than those presented by many authors in the 1970s and 80s.

The studies analyzing human milk with regard to persistent organic pollutants, including polybrominated diphenyl ethers, are still scarce in Poland. It seems necessary to continue this research line with a special focus on examining the persistent organic pollutants in a bigger number of maternal milk samples from the whole of Poland in order to evaluate the postnatal neonate exposure.

## CONCLUSIONS

The presented study reports the presence of organohalogen compounds in maternal women's milk from Poland. The  $\Sigma$ DDT levels noted in our studies were higher than in other European countries. Nevertheless, a decreasing trend in the concentrations of these compounds throughout the period of time was observed, similarly to the findings of studies presented by other authors. Concentrations of the examined polychlorinated biphenyls and polybrominated diphenyl ethers did not differ from the levels presented by other authors, and are comparable to the levels noted in other countries in Europe.

The results obtained in this study confirm the capacity of organochlorine compounds to bioaccumulate and to be eliminated from a woman's body through lactation.

The presence of polybrominated diphenyl ethers found among other persistent organochlorine pollutants in the maternal milk samples inclines the authors to undertake an attempt at a cumulative risk assessment which would take into account the impact of these pollutants on the hormone system development, as it is a common endpoint for the majority of the analyzed compounds.

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