POLYBROMINATED DIPHENYL ETHERS AND CHLORINATED ORGANIC POLLUTANTS IN HUMAN BREAST MILK FROM THESSALONIKI, GREECE

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Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants that are incorporated into a variety of consumer products, such as furniture foam padding, plastics and textiles to slow down combustion. They have a high degree of lipophilicity and are known to be persistent and bioaccumulative through the food chain (Klosterhaus *et al*., 2012). PBDEs have been detected in a variety of environmental compartments and animal/human tissues (Kalantzi and Siskos, 2011), even in remote places like the Arctic (de Wit et al., 2006). Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are organohalogenated compounds, with similar properties to PBDEs. Due to their PBT ("persistent, bioaccumulative, toxic") properties, a growing number of both human health and environmental concerns have led to a ban of the Penta- and Octa-BDE mixtures in the EU (Directive 2003/11/EC). Being persistent chemicals, PBDEs, PCBs and OCPs accumulate in the human body, in adipose tissue and breast milk. The aim of the present study was to analyze POPs (PCBs, PBDEs, HCHs and DDTs) in human breast milk collected in the Thessaloniki region, in Northern Greece.

Materials and methods

A total of 87 human breast milk samples were collected between July 2004 and July 2005 in the Obstetrics and Gynecology Department of the Hippokration General Hospital of Thessaloniki and the European Interbalkan Medical Center of Thessaloniki. Informed consent was obtained from all participants. Samples were shipped to the analytical lab on dry ice and stored at −20 °C until analysis. Questionnaires were given to each participant to obtain information that included their age, weight and weight gained during pregnancy, place of birth and residence, employment, smoking habits, type of diet, number of children, duration of pregnancy and breastfeeding, sex, weight and height of newborn. Table 1 summarizes the characteristics of the women who participated in the study.

Table 1: Characteristics of the pregnant women participating in this study (n=87)

		n	% of total
Age	**<20**	7	8
	21-25	14	16
	26-30	19	22
	>30	47	54
Area of residence	**urban**	67	77
	rural	20	23
Occupation	**housewife**	45	52
	office worker	35	40
	other	7	8
Parity	**primiparous**	34	39
	multiparous	53	61
Diet	**omnivore**	86	99
	vegetarian	1	1
Smoking	**Yes**	13	15
	No	74	85
Duration of pregnancy	**<40 weeks**	50	57
	≥ 40 weeks	37	43

Human breast milk samples were analyzed for the following PBDE congeners: 28, 47, 100, 99, 154, 153 and 183; the following PCB congeners: 101, 99, 105, 118, 146, 153, 138, 187, 183, 128, 174, 177, 171, 156, 180, 170, 199, 196/203, 194, 206 and 209; and the following OCPs: oxychlordane (OxC), trans-nonachlor (TN), Cis-nonachlor (CN), HCB, α-HCH, β-HCH, γ-HCH, p,p'-DDE and p,p'-DDT. A volume of 1 to 3 ml of milk (depending on sample availability) was mixed with 20 g anhydrous sodium sulfate and Soxhlet extracted with hexane:acetone (1:2, v/v). CB 143, BDE 77 and ε-HCH in acetone were used as internal standards. After extraction, an aliquot was used for lipid determination and the rest of the extract was cleaned with 8 g acid silica (44%, w/w). Analytes were eluted with 20 ml hexane:dichloromethane (DCM) (1:1, v/v). The cleaned extract was concentrated with a rotary evaporator, further evaporated under a gentle nitrogen stream until dryness and reconstituted in 100 μl iso-octane. The mixture was transferred to an injection vial for GC –MS analysis.

For the detection of PBDEs, HCHs and chlordanes, an Agilent 6890-5973 gas chromatograph was used, coupled with a mass spectrometer (GC-MS) and equipped with a capillary column (30 m x 0.25 mm x 0.25 μm DB-5). The GC-MS was operated in electron capture negative ionization (ECNI) mode and used in the selected ion-monitoring (SIM) mode. For the measurements of PCBs, DDXs, and HCB a similar GC-MS system was operated in electron ionization (EI) mode and equipped with a 25 m x 0.22 mm x 0.25 μm HT-8 capillary column. Method precision and accuracy was tested by the analysis of certified reference material (CRM) 450 (PCBs in powdered milk) and standard reference material (SRM) 1945 (PCBs, OCP and PBDEs in whale blubber). Values which were lower than the LOQ, had their detection frequency multiplied by the LOQ. SPSS 16 was used for the statistical analysis. Nonparametric tests were used for statistical comparisons between mothers and other parameters tested through the questionnaires (Mann–Whitney U test). Correlations were performed using Pearson correlation on log-transformed data. A p-value less than 0.05 was considered statistically significant.

Results and discussion

PBDEs

All PBDE congeners were detected in the human breast milk samples. BDEs 47 and 153 were the most abundant congeners, both with a detection frequency of 82%, followed by BDE 99 with a detection frequency of 66%. BDE 28 had the lowest detection frequency (14%). Total PBDE concentrations ranged from 0.32 to 13.03 ng/g lipid, with a mean of 2.28 ng/g lipid. BDE 47 had the highest mean concentration of all PBDEs (0.80 ng/g lipid weight). Table 2 summarizes the concentrations of PBDEs detected in the human milk samples.

Table 2: Concentrations of PBDEs (in ng/g lipid weight) in Human Breast Milk Samples from Thessaloniki, Greece (n=87)

	Mean	Median	Range	SD
BDE 28	0.060	0.010	0.01 – 0.73	0.13
BDE 47	0.80	0.48	0.08 – 7.75	1.05
BDE 100	0.39	0.19	0.06 – 3.17	0.49
BDE 99	0.54	0.27	0.0 -2.97	0.69
BDE 154	0.060	0.020	0.0 – 0.41	0.09
BDE 153	0.38	0.30	0.08 – 1.22	0.29
BDE 183	0.060	0.020	0.0 – 0.63	0.10
Σ_7PBDEs	**2.28**	**1.55**	**0.32 – 13.03**	**2.18**

Previous studies in the Philippines also support the dominance of BDE 47 in PBDE congener profiles in human breast milk (Malarvannan *et al*., 2013). Compared to other countries, the results obtained from human breast milk in this study are on the lower end of European studies (Kalantzi and Siskos, 2011), the United States (She *et al*., 2004; Daniels *et al*., 2010; Schecter *et al*., 2010) and most Asian countries, with the exception of Japan (Inoue *et al*., 2006).

PCBs & OCPs

Total PCB concentrations ranged from 18 to 354 ng/g lipid, with a mean of 110 ng/g lipid. CB 153 had the highest mean concentration (29 ng/g lipid weight) of all PCB congeners. CBs 105, 118, 146, 153, 138, 187, 183, 128, 177, 156, 180, 170, 199 and 196/203 were all abundant in the breast milk samples, with a detection frequency of 100%. CB 101 had the lowest detection frequency (76%). p,p'-DDE, p,p'-DDT, OxC, TN and b-HCH all had a detection frequency of 100%, and α-HCH had the lowest detection frequency (22%). p,p'-DDE had the highest mean concentration of all OCPs (527 ng/g lipid weight). Table 3 summarizes the concentrations of organochlorines detected in the human milk samples.

Table 3: Concentrations of PCBs and OCPs (in ng/g lipid weight) in Human Breast Milk Samples from Thessaloniki, Greece (n=87)

	Mean	Median	Range	SD
CB 101	1.3	0.70	0.3 – 7.0	1.5
CB 99	3.9	2.6	0.4 – 18.2	3.3
CB 105	1.9	1.3	0.3 – 11.3	1.7
CB 118	6.1	4.6	0.9 – 22.6	4.3
CB 146	3.4	2.9	0.4 – 12.2	2.4
CB 153	28.9	24.4	3.4 – 97.0	20.6
CB 138	16	12.8	2.0 – 53.7	11
CB 187	5.0	3.7	0.8 – 16.3	3.6
CB 183	2.2	1.8	0.3 – 8.2	1.6
CB 128	2.9	2.3	0.3 – 9.2	2.0
CB 174	0.60	0.40	0.2 – 2.9	0.50
CB 177	1.4	1.1	0.2 – 4.3	0.90
CB 171	0.80	0.60	0.2 – 3.2	0.60
CB 156	2.8	2.1	0.3 – 13.5	2.3
CB 180	18.9	13.1	2.2 – 74.1	15.1
CB 170	8.5	6.3	1.0 – 36.9	6.7
CB 199	1.7	1.2	0.3 – 5.7	1.2
CB 196/203	2.1	1.5	0.3 – 8.5	1.6
CB 194	0.60	0.50	0.2 – 2.9	0.50
CB 206	0.70	0.40	0.2 – 8.2	1.0
CB 209	0.60	0.30	0.2 – 8.7	1.0
Σ_{21}PCBs	**110.4**	**90.5**	**17.6 – 353.7**	**74.1**
a-HCH	0.10	0	0 – 1.9	0.30
b-HCH	72.3	40.1	7.2 – 700.2	103.2
g-HCH	0.90	0.10	0.1 – 10.6	1.5
ΣHCHs	**73.3**	**40.2**	**8.5 – 702.8**	**103.3**
HCB	38.3	19.6	0.8 – 661.8	76.7
pp-DDE	527.3	394.7	25.5 – 2720.1	486.5
pp-DDT	21	14	1.6 – 155.6	23.2
ΣDDTs	**548.3**	**407.8**	**27.1 – 5875.7**	**505.9**
OxC	4.1	3.3	0.5 – 11.9	2.8
TN	3.3	2.4	0.3 – 16.2	2.8
CN	0.40	0.30	0.1 – 2.0	0.40

When comparing the data for PCBs and OCPs from this study with studies from other countries, we can observe that women from Thessaloniki, Greece had lower breast milk PCB concentrations than women in most European countries, but higher PCB levels than Asian women (Malarvannan *et al*., 2013). Compared to the PCB levels measured in a previous study in Athens, Greece in 2006 (mean concentration of 156.65 ng/g lipid) (Costopoulou *et al*., 2006), the levels found in this study were lower, which could be an indication declining PCB/OCP concentrations in the course of a decade. OCP levels (p,p'-DDE, p,p'-DDT, a-HCH, b-HCH, g-HCH and HCB)

were lower in our study than recent European studies, with the exception of b-HCH, which was higher in our study than in Polish and Russian women (Klinčić *et al*., 2014).

As expected, age did not show any correlation with any of the BDE congeners, indicating that human exposure to PBDEs is continuous. On the other hand, age had a positive correlation with most PCBs and OCPs ($p<0.05$), in agreement with previous studies (Hassine *et al.*, 2012). None of the individual PBDEs, PCBs or OCPs was correlated with parity, which has been observed in several studies (Malarvannan *et al*., 2009). None of the other factors such as mothers' weight, weight gained during pregnancy, duration of pregnancy, duration of breastfeeding and sampling time (day and time of sample collection) showed any correlations for any of the POPs measured. Place of residence, smoking, diet, occupation and sampling time (before or after breastfeeding) did not show any significant differences for PBDEs, PCBs or OCPs.

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