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Levels and profiles of brominated and chlorinated contaminants in human breast milk from Thessaloniki, Greece

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ABSTRACT

Human breast milk samples (*n*=87) collected between July 2004 and July 2005 from primipara and multipara mothers from Thessaloniki, Greece were analyzed for six groups of persistent organic pollutants (POPs): polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane compounds (CHLs), hexachlorocyclohexane isomers (HCHs) and hexachlorobenzene (HCB). DDTs [median: 410 ng/g lipid weight (lw)], PCBs (median: 90 ng/g lw) and HCHs (median: 40 ng/g lw) were the predominantly identified compounds in all the breast milk samples. Levels of PBDEs (median: 1.5 ng/g lw) in human breast milk samples from Thessaloniki, Greece were lower compared to other countries. Maternal age had a positive correlation with most compounds, but not with PBDEs. Women with a higher occupational exposure to PBDEs (i.e., working in office environments) had higher PBDE concentrations than all others and showed strong correlations, especially for BDE 47 and BDE 153. None of the analysed compounds showed any correlation with parity. Based on these levels, the daily intake of each group of POPs via human milk was calculated and compared with the tolerable daily intakes (TDI) or the reference doses (RfD). For the majority of samples (85 out of 87) a higher daily intake of PCBs than the TDI was calculated, while 11 out of 87 samples had a higher HCB intake than the TDI. The TDI and the RfD were not exceeded for DDTs and PBDEs, respectively. This is the first report of brominated flame retardants in human breast milk from Greece.

Keywords: Human breast milk, POPs, Brominated flame retardants, Infants, Health risk, Thessaloniki, Greece

1.1 Introduction

Polybrominated diphenyl ethers (PBDEs) are a group of brominated flame retardants (BFRs) 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 are not chemically bound to the polymers to which they are added. As a consequence of substantial long-term use, PBDEs have contaminated humans, wildlife, air, water, soils, and sediments, even in remote areas (Hale et al., 2003; de Wit et al., 2006; Law et al., 2006; Covaci et al., 2011; Kalantzi and Siskos, 2011). In recent years, strict bans have been imposed on the worldwide use of Penta- and Octa-BDE formulations and components of these mixtures have been added to the persistent organic pollutants (POPs) list of the Stockholm Convention (Ashton et al., 2009). Being persistent chemicals, PBDEs accumulate in the human body, in breast milk and adipose tissue (Malarvannan et al. 2009; Covaci et al. 2008; Malarvannan et al. 2013a). Despite regulations, PBDEs continue to leach from existing products that are in service or have been disposed of in landfills. Based on a number of recent studies, it has been observed that human non-occupational exposure to PBDEs occurs mainly via a combination of diet, ingestion of indoor dust, and inhalation of indoor air (Roosens et al., 2009; Harrad et al., 2010). The exact contribution of these three pathways varies substantially on a compound-specific basis and between individuals and within national populations (Roosens et al., 2009; Covaci et al., 2011).

Greece depends largely on agriculture, but at the same time has experienced a rapid degree of urbanization since the 1980s. Previous studies have indicated that the general population of Greece has been exposed to legacy POPs such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) (Schinas et al., 2000; Costopoulou et al., 2006). However, data on POPs levels in humans in Greece is very limited compared to other European countries, and is mostly available for human serum and hair (Kalantzi et al., 2011; Vafeiadi et al., 2014; Covaci et al., 2002; Tsatsakis et al., 2008). Very little information exists on PBDEs in humans in Greece and there is no previous report of PBDEs in human breast milk of Greek women.

In this study, we investigated the levels of PBDEs in human breast milk from Thessaloniki (the second largest urban centre in Greece) in order to assess their contamination status, examine relationships between contaminant levels and parity/age of the mothers and assess intake of contaminants by infants through breast milk consumption. The study also includes data on POPs such as PCBs, dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs) and hexachlorobenzene (HCB) to give a comprehensive picture on the organohalogen contaminants found in breast milk in Greece.

1.2 Materials and methods

1.2.1 Sample collection

Human breast milk samples (*n*=87) from primipara (*n*=34) and multipara (*n*=53) mothers from Thessaloniki, Greece were collected between July 2004 and July 2005 and analysed for PBDEs, PCBs, DDTs, CHLs, HCHs and HCB. The mean age of the mothers was 30 years old and ranged from 18 to 43 years of age. About 20 mL of breast milk was collected using a breast pump to transfer the milk into pre-washed glass containers prepared for every individual. The samples were shipped frozen to the laboratory and stored at -20 °C until analysis. Informed consent was obtained from all the donors and ethical approval for this study was obtained from the Ethics Committee of the University of Thessaloniki. Table 1 presents the demographic characteristics of the cohort.

Table 1: General demographic characteristics of the breast milk donors who participated in this study.

		n	% of total
Maternal age [mean (SD) = 30.2 (6.2) years]	<20	7	8
	21-25	14	18
	26-30	19	21
	>30	47	53
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 status	Yes	13	15

	No	74	85
Duration of pregnancy	<40 weeks	50	57
	≥ 40 weeks	37	43

The distributions of milk lipid content were similar in the two groups (primipara and multipara mothers).

1.2.2 Chemical analysis

The following POPs were targeted: 21 PCB congeners (IUPAC nrs. 99, 101, 105, 118, 146, 153, 138, 187, 183, 128, 174, 177, 171, 156, 180, 170, 199, 196/203, 194, 206 and 209), dichlorodiphenyltrichloroethane and its metabolite (DDTs), three chlordanes (oxychlordane (OxC), trans-nonachlor (TN) and cis-nonachlor (CN), three hexachlorocyclohexane isomers (α-, β-, and γ-HCH), hexachlorobenzene (HCB) and 7 PBDEs (BDE 28, 47, 99, 100, 153, 154 and 183). Analyses of POPs in human breast milk samples were performed according to the methods described elsewhere (Covaci et al. 2008; Malarvannan et al. 2013a) with slight modifications. Breast milk samples (1 to 3 mL) were weighed, mixed with anhydrous sodium sulphate (Na_2SO_4) and then transferred to a mortar and mixed until dry. The samples were transferred to thimbles and were spiked with internal standards (CB 143, BDE 77 and ε-HCH), followed by a 2 h extraction by hot Soxhlet with 100 mL hexane/acetone (1:2, v/v). The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on ~8 g acidified silica (44%, w/w) and eluted with 20 mL hexane:dichloromethane (1:1, v/v). The cleaned extract was concentrated with a rotary evaporator, further evaporated under a gentle nitrogen stream to incipient dryness and reconstituted in 100 μL of iso-octane. The mixture was transferred to an injection vial for GC –MS analysis. Quantification of POPs was done using GC–MS operated in electron-capture negative ionization mode (Covaci et al., 2008; Malarvannan et al. 2013a) (see Supporting Information for technical details, Table SI-1). Abbreviations are expressed as follows: PBDEs as the sum of 7 congeners, PCBs as the sum of 21 congeners, DDTs as the sum of 2 compounds, HCHs as the sum of 3 isomers and CHLs as the sum of 3 compounds.

1.2.3 Quality assurance/quality control

The extraction, clean up, and fractionation steps were evaluated by measurement of the absolute recoveries of the internal standards. The peaks were quantified as target compounds if: (1) the retention time matched that of the standard compound within ±0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. The limit of quantification (LOQ) was calculated as three times the standard deviation of the mean of the blank measurements. Procedural blanks were analysed simultaneously with every batch of seven samples to check for interferences or contamination from solvent and glassware. Procedural blanks were consistent (RSD < 30%) and therefore the mean value was calculated for each compound and subtracted from the values in the samples. Mean ± SD recoveries of the internal standards PCB 143 and BDE 77 were 86 ± 6% and 93 ± 10%, respectively. The analytical procedures were validated through the analysis of certified reference material (CRM 450; PCBs in powdered milk) and standard reference material (SRM 1945; PCBs, OCPs and PBDEs in whale blubber) for which deviations from certified values were < 15% (Table SI-2). To test the accuracy of the experiment, milk samples purchased from a super market with a known lipid percentage were used. The deviation percentage lipid to the theoretical percentage lipid ranged between 90-98% (Table SI-3).

1.2.4 Statistical analysis

Statistical analysis was performed with the SPSS software (SPSS for Windows v.23, SPSS Inc.). Outliers were identified using box plots and confirmed by Grubb's test. 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. For statistical analysis, concentrations below the LOQ were assigned a value equal to the detection frequency multiplied by the LOQ. The Spearman rank correlations were used to examine the strength of associations between parameters. The results are presented as mean and median with minimal and maximum values. Parameters with a probabilistic value of <0.05 were considered as having a significant relationship with contaminant level. The concentration of PCBs, DDTs, HCHs, CHLs, HCB and PBDEs is expressed in ng/g lipid weight (lw), unless otherwise specified.

1.3 Results and discussion

1.3.1 Contamination status

POPs were detected in variable quantities in all the breast milk samples collected from Thessaloniki in the order of DDTs > PCBs > HCHs > HCB > CHLs > PBDEs, indicating maternal exposure to these contaminants. There were no significant differences in the concentrations of all the analysed compounds (PCBs, OCPs and PBDEs) between primipara and multipara mothers and hence the data for both the parity groups were treated together for further discussion.

1.3.2 Residue levels and contamination status of PBDEs

The concentrations of the sum of the 7 congeners (ΣPBDEs) found in breast milk samples varied widely, from 0.32 to 13 ng/g lw, with a median of 1.5 ng/g lw (Table 2). BDE 47 had the highest concentration of all PBDEs (median: 0.48 ng/g lw). 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%). Table 2 summarizes the concentrations of PBDEs detected in the human milk samples. The highest concentrations of ΣPBDEs in breast milk (13 ng/g lw) were found in a sample from a 36-year old woman and who was nursing for the second time. The lowest concentration (0.32 ng/g lw) was observed in a sample from a 38 year old woman and who was nursing for the first time. These are the first reported data for PBDEs in human breast milk from Greece.

Table 2: Concentrations of organohalogen compounds (ng/g lw) in human breast milk samples from Thessaloniki, Greece.

	Primiparae			**Multiparae**			**All donors**		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
Maternal age (years)	28	28	18-58	32	32	19-43	31	31	19-58
Lipid content (%)	1.7	1.3	0.58-6.0	1.7	1.3	0.37-6.0	1.7	1.3	0.37-6.0
Number of samples	34			53			87		
BDE 28	<0.10	<0.10	<0.10-0.42	<0.10	<0.10	<0.10-0.73	<0.10	<0.10	<0.10-0.73
BDE 47	0.81	0.55	<0.10-2.9	0.79	0.39	<0.10-7.7	0.80	0.48	<0.10-7.7
BDE 100	0.34	0.27	<0.10-1.2	0.42	0.19	<0.10-3.2	0.39	0.19	<0.10-3.2
BDE 99	0.72	0.42	<0.10-3.0	0.42	0.26	<0.10-2.9	0.54	0.27	<0.10-3.0
BDE 154	<0.10	<0.10	<0.10-0.41	<0.10	<0.10	<0.10-0.39	<0.10	<0.10	<0.10-0.40
BDE 153	0.44	0.38	<0.10-1.2	0.34	0.28	<0.10-1.2	0.38	0.30	<0.10-1.2
BDE 183	<0.10	<0.10	<0.10-0.38	<0.10	<0.10	<0.10-0.63	<0.10	<0.10	<0.10-0.63
Sum PBDEs	2.5	1.6	0.32-8.0	2.2	1.5	0.34-13	2.3	1.5	0.32-13
CB 101	1.2	0.69	<0.40-5.6	1.4	0.66	<0.40-7.0	1.3	0.67	<0.40-7.0
CB 99	4.7	4.0	1.3-15	3.5	2.3	<0.40-18	3.9	2.6	<0.40-18
CB 105	2.0	1.4	0.35-6.5	1.8	1.2	<0.40-11	1.9	1.3	0.33-11
CB 118	7.1	5.3	0.95-22	5.5	4.3	0.86-18	6.1	4.6	0.86-23

CB 146	3.9	3.0	0.57-12	3.1	2.7	0.42-9.5	3.4	2.9	0.41-12
CB 153	32	26	3.9-83	27	20	3.4-97	29	24	3.4-97
CB 138	18	15	2.4-46	15	13	2.0-54	16	13	2.0-54
CB 187	5.3	3.9	0.76-16	4.8	3.7	0.87-14	5.0	3.7	0.76-16
CB 183	2.4	1.8	0.35-7.1	2.2	1.7	0.34-8.2	2.2	1.8	0.34-8.2
CB 128	3.2	2.6	0.35-8.7	2.7	2.0	0.29-9.2	2.9	2.3	0.29-9.2
CB 174	0.53	0.37	0.18-2.9	0.59	0.40	<0.20-2.9	0.57	0.39	<0.20-2.9
CB 177	1.5	1.2	0.27-3.5	1.4	1.0	0.22-4.3	1.4	1.1	0.22-4.3
CB 171	0.80	0.56	<0.20-2.5	0.73	0.62	<0.20-3.2	0.75	0.59	<0.20-3.2
CB 156	3.0	2.5	0.32-7.9	2.7	2.0	0.29-14	2.8	2.1	0.29-14
CB 180	20	13	2.2-61	18	14	2.9-74	19	13	2.2-74
CB 170	9.0	6.3	0.99-24	8.1	6.7	1.3-37	8.5	6.3	0.98-37
CB 199	1.6	1.1	0.26-5.0	1.7	1.2	0.33-5.7	1.7	1.2	0.26-5.7
CB 196/203	2.1	1.5	0.26-6.1	2.1	1.5	0.38-8.5	2.1	1.5	0.26-8.5
CB 194	0.61	0.48	<0.20-1.9	0.65	0.45	<0.20-2.9	0.63	0.45	<0.20-2.9
CB 206	0.57	0.30	<0.20-3.4	0.75	0.38	<0.20-8.2	0.68	0.36	<0.20-8.2
CB 209	0.52	0.32	<0.20-2.7	0.72	0.33	<0.20-8.7	0.64	0.33	<0.20-8.7
Sum PCBs	120	96	17-280	104	89	26-354	110	90	17-350
p.p'-DDE	510	410	87-1200	540	350	25-2700	530	400	25-2700
p.p'-DDT	22	17	2.0-73	20	11	2.0-160	21	14	2.0-160
Sum DDTs	530	430	90-1300	560	360	27-2900	550	410	27-2900
Oxychlordane	4.0	3.0	1.1-12	4.2	3.7	0.47-10	4.1	3.3	<0.20-12
Trans-nonachlor	3.7	2.8	0.50-16	3.0	2.3	0.32-14	3.3	2.4	0.32-16
Cis-nonachlor	0.49	0.38	<0.20-2.0	0.35	0.31	<0.20-1.4	0.41	0.34	<0.20-2.0
Sum CHLs	8.2	6.1	2.4-30	7.6	6.8	0.89-24	7.8	6.5	0.89-30
α-HCH	<0.20	<0.20	<0.20-1.1	<0.20	<0.20	<0.20-2.0	<0.20	<0.20	<0.20-1.9
β-HCH	82	43	8.8-700	66	31	7.2-560	72	40	7.2-700
γ-HCH	0.66	<0.20	<0.20-4.1	1.0	<0.20	<0.20-11	0.86	<0.20	<0.20-11
Sum HCHs	83	43	11-700	67	33	8.5-560	73	40	7.2-700
HCB	29	20	2.5-140	44	20	0.80-660	38	20	0.80-660

PBDE results from Greece were compared with those from other countries (Table 3). Global comparison indicated that the levels observed in this study were lower than those of other European countries, Australia, Ghana, most Asian countries and the USA and Canada, but higher than India and South Africa (Table 3).

Table 3: Mean concentrations (ng/g lw) of PBDEs in human breast milk samples from various countries.

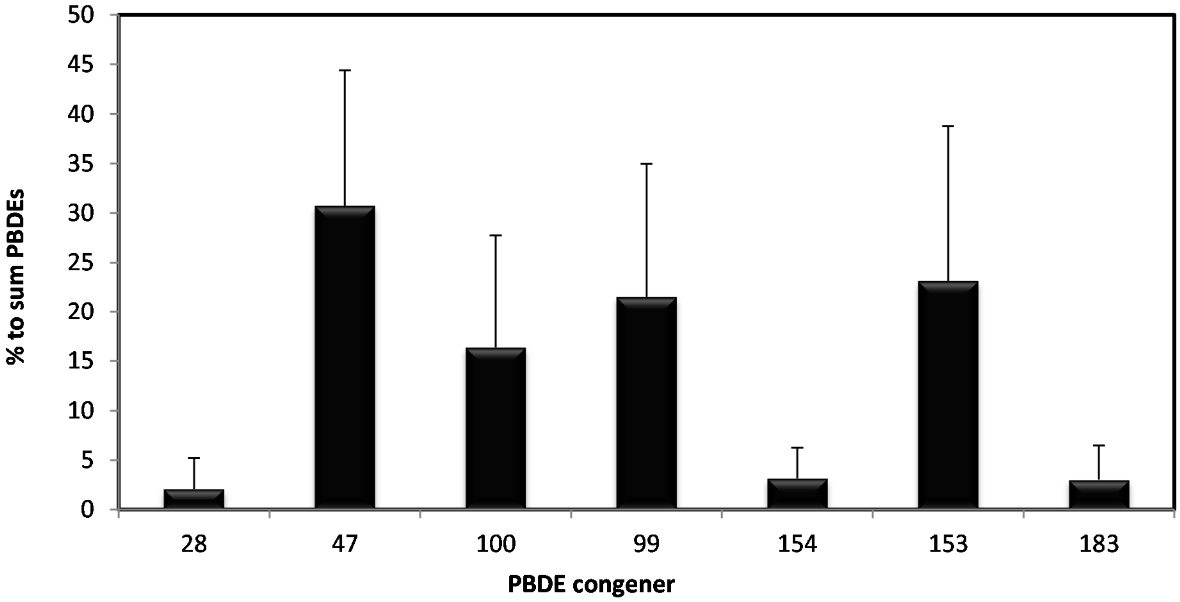
Country	Sampling year	Number of samples (n)	ΣPBDEs	Reference
EUROPE				
Greece	2004-5	89	2.3	this study
Russia (Murmansk/Arkhangelsk)	2000-2	37	0.47/0.71	Polder *et al*., 2008
Italy (Rome)	2000-1	10	4.1	Ingelido *et al*., 2007
Sweden	2000-1	15	2.14*	Meironyte *et al*., 2003
Norway	2000-2	29	4.1*	Polder *et al*., 2008
Germany	2001-3	93	2.23	Vieth *et al*., 2004
UK	2001-3	54	8.9	Kalantzi *et al*., 2004
Spain	2002	15	2.41	Schuhmacher *et al*., 2004
Norway	2003-9	393	3.4	Thomsen *et al*., 2010
Germany	2005	42	1.9	Raab *et al*., 2008
Belgium	2006	22 (pooled)	3.0*	Roosens et al., 2010
Germany	2006-9	2173	1.68	Hoopman et al., 2012
NORTH AMERICA				
Canada (Nunavik)	2001-2	98	22	Pereg *et al*., 2003
USA (Texas)	2002	47	73.9	Schecter et al., 2003
USA, Canada (Pacific Northwest)	2003	40	96	She et al., 2007
Canada (Ontario)	2003-4	39	22	Siddique et al., 2012
USA (California)	2004	16	77.5	She *et al*., 2004
USA (Massachusetts)	2004	38	76.3	Johnson-Restrepo et al., 2007
USA (North Carolina)	2004-6	331	51*	Daniels *et al*., 2009
Canada (Ontario)	2005	34	48.3	Ryan and Rawn, 2014
USA (New Hampshire)	2005-6	40	35.5	Dunn *et al*., 2010
USA (Texas)	2007	30	57.6	Schecter et al., 2010
Canada (Quebec)	2008-9	48	23	Siddique et al., 2012
ASIA				
Indonesia	2001-3	30	2.2	Sudaryanto *et al*., 2008a
Hong Kong	2002-3	238 (10 pools)	3.4	Hedley *et al*., 2010
China	2004	19	2.5	Sudaryanto *et al*., 2008b
Philippines (Malate)	2004	11	6.3/2.6**	Malarvannan et al., 2009
Japan	2005	89	1.56	Inoue *et al*., 2006
China	2005	205 (23 pools)	1.2	Li *et al*., 2008
Japan	2005-6	40	3.4	Fujii et al., 2012
China	2006	80 (pooled)	2.83	Zhu *et al*., 2009
China	2006-7	158	2.24-4.16	Sun *et al*., 2010
Taiwan	2007	20	2.65	Horng *et al., 2010*
Taiwan	2007-8	46	3.59	Chao *et al*., 2010
India	2009	55	1.4	Devanathan et al., 2012
OCEANIA				
Australia	2002-3	157 (17 pools)	10.2	Toms *et al*., 2007
Australia	2003-4	N/A	12.4	Toms et al., 2012
Australia	2006	N/A	9.9	Toms et al., 2012
Australia	2007-8	N/A	2.5-3.0	Toms et al., 2012
Australia	2008-11	164	0.08	Stasinka et al., 2014
AFRICA				
South Africa	2004	14	1.7	Darnerud et al., 2011
Ghana	2009	42	4.5	Asante et al., 2011

*median
** primiparae/multiparae
N/A: not available

The differences between countries can be partially explained by the PBDE concentrations in the diet, especially in food items with higher contribution to the total PBDE intake and different dietary patterns between countries (Roosens et al., 2010). Meat, chicken, pork and fish (on whose consumption food culture in Greece is largely based) appear to be important and common pathways for all contaminants analyzed in the present study (Schecter et al., 2006). Previous studies have reported that dietary intake is the dominant exposure pathway for lower brominated PBDE congeners (Domingo et al., 2008; Fromme et al., 2009).

Among PBDEs, BDE-47, -153, -99, and -100 were the predominant congeners (Figure 1). The dominant congener was BDE 47, accounting more than 31% of all PBDE congeners, followed by BDEs 153 and 99, which accounted more than 23% and 22% of the total PBDE congeners respectively. BDE 100 contributed to 16% of the total PBDE congeners, BDE 154 (3.0%), BDE 183 (3.0%) and BDE 28 (2.0%). This abundance of BDE-47 in human milk may be attributed to commercial penta-BDE mixture exposure, of which BDE-47 is the major component. Previous studies also support the dominance of BDE 47 in various human matrices, such as breast milk (Meironyté et al., 2003; Kalantzi et al., 2004; Malarvannan et al., 2013b), blood plasma (Mazadai et al., 2003) and adipose tissue (Johnson-Restrepo et al., 2005; Malarvannan et al. 2013a).

Figure 1: Congener profile of PBDEs in human breast milk from Thessaloniki, Greece (error bars represent standard deviations)



1.3.3 Residue levels and contamination status of PCBs and OCPs

Total PCB concentrations ranged from 18 to 350 ng/g lw, with a median of 90 ng/g lw (Table 2). All PCB congeners were detected in the human breast milk samples. PCB 153 had the highest concentration (median: 24 ng/g lw) and PCB 194, 206 and 209 had the lowest concentrations (median: 0.45, 0.36 and 0.33 ng/g lw) of all PCB congeners. The highest concentration (350 ng/g lw) was observed from a mother (43 years old) who was nursing for the second time. On the other hand, the lowest concentration (17 ng/g lw) was observed from a mother (20 years old) who was nursing for the first time. When comparing our data to the global PCB data, we can observe that women from Thessaloniki, Greece had lower breast milk PCB concentrations than women in most European countries, such as the UK (mean: 200 ng/g lw; Kalantzi et al., 2004), Poland (mean: 153 ng/g lw; Jaraczewska et al., 2006), Russia (mean: 191 ng/g lw; Polder et al., 2008) and Italy (mean: 240 ng/g lw; Ingelido et al., 2007), but higher PCB levels than women from China (mean: 20 ng/g lw; Kunisue et al., 2004), Indonesia (mean: 27 ng/g lw; Sudaryanto et al., 2006), the Philippines (mean: 65 ng/g lw; Malarvannan et al., 2013b), Ghana (mean: 62 ng/g lw; Asante et al., 2011) and South Africa (mean: 10 ng/g lw; Darnerud et al., 2006). Compared to the PCB levels previously measured in Athens, Greece in 2006 (mean: 97.9 ng/g lw; Costopoulou et al., 2006), the levels found in this study were lower, which could be an indication of declining PCB concentrations in this area.

The PCB congener patterns are summarized in Figure 2, with the standard deviation represented by the error bars. PCB 153 was the most abundant congener accounting for more than 26% of all PCBs. PCBs 180 and 138 were the next most abundant congeners accounting for more than 17 and 15%, respectively. A similar result has been observed in other studies around the world (Polder et al., 2009; Asante et al. 2011; Malarvannan et al. 2013b). The different profiles of PCBs in this study may be attributed to the participants' variation in dietary habits. However, it is difficult to draw a certain conclusion about the exposure pathways of higher and lower chlorinated congeners, as no detailed congener-specific information are available for foodstuffs in Greece.

Figure 2: Congener profile of PCBs in human breast milk from Thessaloniki, Greece (error bars represent standard deviations)

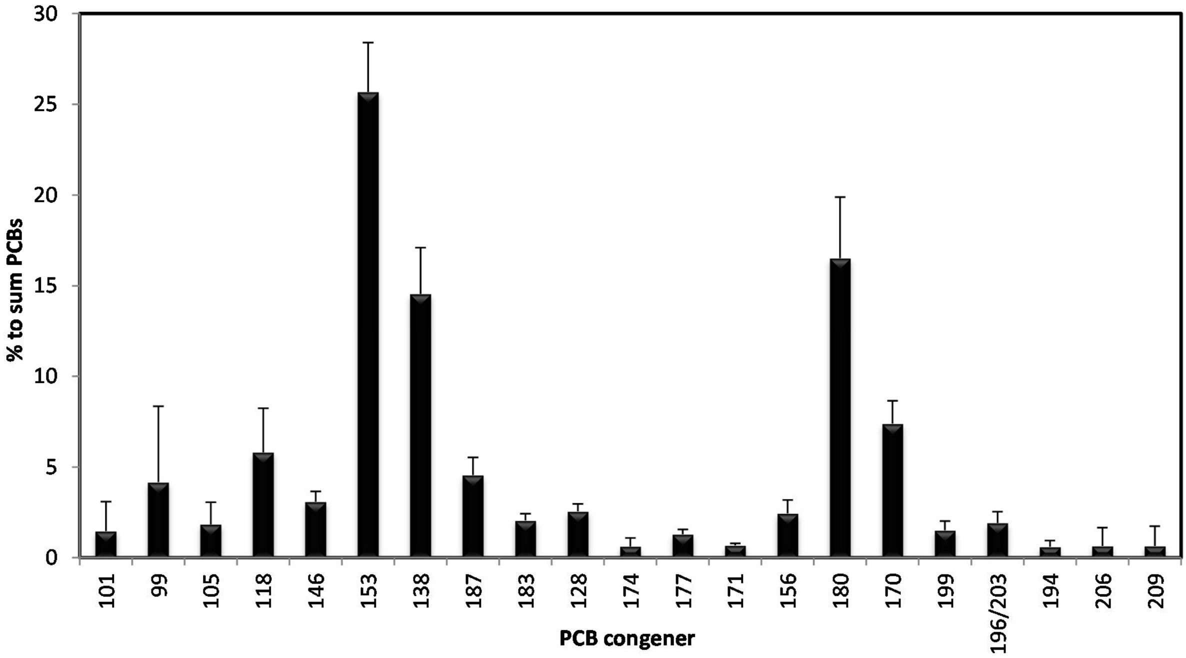


Table 2 summarizes the concentrations of OCPs detected in the human milk samples. Among OCPs, *p,p'*-DDE, *p,p'*-DDT, OxC, TN and β-HCH all had a detection frequency of 100%, and α-HCH had the lowest detection frequency (22%). DDTs had the highest concentration of all OCPs (median: 410 ng/g lw). These results indicate that DDTs are the major contaminants in Thessaloniki, Greece. The highest concentration of DDTs (2900 ng/g lw) was observed from a mother (34 years old) who lives in Thessaloniki and nursing for the second time. On the other hand, the lowest concentration of DDTs (27 ng /g lw) was observed from a mother (30 years old) from Iran, who was also nursing for the second time. Elevated levels of DDTs have also been found in human milk in many countries located in the tropical regions, which have used DDT for malaria control until recently, such as Mexico (mean: 4700 ng/g lw; Waliszewski et al., 2001), China (mean: 3550 ng/g lw; Wong et al., 2002)(mean: 2100 ng/g lw; Kunisue et al., 2004), Indonesia (mean: 630-1300 ng/g lw; Sudaryanto et al., 2006) and Vietnam (mean: 2200 ng/g lw; Minh et al., 2004). Generally, the levels of DDTs in the present study were lower than those in Northern (mean *p,p'*-DDT: 30 ng/g lw; Fytianos et al., 1985) and Southern Greece (mean DDTs: 787 ng/g lw; Schinas et al. 2000), indicating that the concentration of DDTs has been declining in the Greek environment.

Although the results show decreasing trends, levels of DDTs in Greece were comparable with industrialized nations, such as Japan (mean: 340 ng/g lw; Kunisue et al., 2006), the UK (mean: 470 ng/g lw; Harris et al., 1999), Russia (mean: 580 ng/g lw; Tsydenova et al. 2007) and Canada (mean: 244 ng/g lw; Newsome and Ryan, 1999). The general declining trend found in the present study confirms the positive effects of restrictions and prohibitions on the usage of DDT and other measures taken to minimize organochlorine pollution (in Greece DDT was banned in 1972). Among the DDTs, *p,p'*-DDE was the main compound found in the present study, suggesting past usage and long-term accumulation of DDTs in humans. In addition, the DDT/DDE ratio was lower than one (mean: 0.043), which also indicates a historical exposure to this pollutant.

The highest value of HCHs was observed for β-HCH with a median value of 40 ng/g lw, and range from 7.2 to 700 ng/g lw. β-HCH is the most persistent and bioaccumulative of all HCH isomers and has been found to be the most predominant in human milk (Solomon and Weiss, 2002). HCB and CHLs were the least prevalent pollutants detected in all the samples (Table 2). HCB levels ranged between 0.80 and 660 ng/g lw, with a median value of 20 ng/g lw. The presence of HCB in human milk samples might be due to contamination from other pesticide formulations present either as an impurity and/or as a by-product of various chlorination processes and combustion of industrial products. Total CHL concentrations ranged from 0.89 to 30 ng/g lw, with a median value of 6.5 ng/g lw. Among chlordane compounds, the most abundant were oxychlordane and trans-nonachlor (Table 2). This is because CHLs are rapidly breaks down into metabolites such as oxychlordane, γ-chlordane or into impurities such as trans-nonachlor or cis-nonachlor, and these breakdown products persist in the tissues of fish, mammals and birds (Solomon and Weiss, 2002). In comparison to earlier studies from the 1970s, 1980s and 1990s from Thessaloniki and Northern Greece, data from this study is lower for most OC pesticides by about an order of magnitude, indicating a declining time trend (Panetsos et al. 1975; Fytianos et al. 1985; Schinas et al. 2000). Overall, this result indicates specific exposure to OCPs, possibly due to variation in their usage patterns in different regions of Greece, particularly for DDTs and HCHs.

1.3.4 Specific accumulation with maternal characteristics

PCBs, OCPs and PBDEs did not show any correlation with parity (data not shown). This is in agreement with several studies (Hassine et al., 2012; Sudaryanto et al., 2008; Malarvannan et al., 2009). Mothers' weight, weight gained during pregnancy, residence (rural/urban), and smoking did not have any correlation with any of the pollutants measured

(PCBs, OCPs and PBDEs). For employment we had 3 groups: housewives, office clerks and other occupations; 45 out of 87 women were housewives while 35 were office workers and the rest of them were working, but not in an office environment (midwife, nurse, manufacturer, hairdresser, cleaner). Employment was strongly correlated for PBDEs, especially BDE 47 and BDE 153 (data not shown; $p<0.05$), the office group had higher PBDE concentrations than all others.

Maternal age had a positive correlation for most of the PCBs (Table SI-4). PCBs 146, 153, 138, 187, 183, 128, 177, 171, 156, 180, 170, 199, 196/203, 194, 206 and 209 were all positively correlated ($p<0.01$), while PCB 118, was positively correlated ($p<0.05$). CB-99, -105, -174 did not show any correlation with age. We observed that women over 30 years of age had significantly higher concentrations of PCBs and OCPs than women less than 30 years old ($p<0.01$, data not shown), with the exception of BC101, 99, 174, *p,p'*-DDE, *p,p'*-DDT and α-HCH. The correlation between age and PCBs was in agreement with previous studies as age is the most significant factor affecting PCBs (Hassine et al. 2012; Tsydenova et al. 2007). Maternal age had a positive correlation with most of the OCPs (Table SI-5). For instance, HCB, Oxy-CHL, trans-nonachlor and β-HCH were all positively correlated ($p<0.01$). It is reported that the concentrations of OCPs in human breast milk vary with factors such as maternal age and parity (Harris et al. 2001). No significant correlations were found for DDTs as a function of age in Azeredo et al. (2008), Devanthan et al. (2006) and Kunisue et al. (2004). Mes et al. (1993) analysed 412 milk samples from Canadian mothers and observed low correlation between age of the mothers and organochlorine levels. According to Kunisue et al. (2006) older mothers may transfer higher amounts of OCs to the first infant than to the infants born afterwards through breast-feeding.

Maternal age did not show either a positive or a negative correlation with PBDEs (data not shown). Previous studies have also reported that age and PBDE levels are not correlated (Raab et al. 2008; Covaci et al. 2008; Malarvannan et al. 2013b). Linear regression analysis also showed that age was the single most significant factor for most PCB congeners and OC pesticides. This indicates that human exposure to PBDEs is continuous and may occur by breathing contaminated dust or air in indoor environments, where PBDE-containing materials such as in electrical devices and upholstered furniture are present. There may also exist other factors controlling the variability of these contaminants in human breast milk, or they could be related to contemporary exposure to these chemicals in the general population.

1.3.5 Health risk assessment

Daily intakes (DI) of OCPs and PBDEs by infants were calculated based on the assumption that the average breast milk consumption of a 5 kg infant was 700 g/day (Oostdam et al., 1999). The dietary intakes were calculated per compound (or class of compounds, e.g. PCBs) and for each individual in the study. These DI were compared to the tolerable daily intakes (TDI) or with reference doses (RfD), expressed in ng/kg body weight (bw) per day (Table 4). For PCBs, 85 out of 87 samples had values above TDI of 20 ng/kg body weight per day (ATSDR, 2015). This fact may raise greater concern for infant health, because infants are highly susceptible to effects from environmental contaminants. The median DI for individual congeners CB 101, 118, 153, 138 and 180 were 1.6, 10, 49, 27 and 29 ng/kg body weight per day, respectively (Table SI-4). The median DI for the total PCBs was 120 ng/kg body weight per day, while the TDI value by ATSDR was 20 ng/kg body weight per day.

Table 4: Daily intake (ng/kg bw/day) of PCBs, PBDEs and OCPs for infants from Thessaloniki, Greece.

Compounds	TDI (ng/kg bw /day)	Mean	Median	Range	SD	Above TDI (out of 87 samples)
PCBs *						
CB 101		2.7	1.6	0.8-19	3.1	
CB 118		15	10	0.9-62	14	
CB 153		73	49	3.5-300	72	
CB 138		40	27	2.0-170	39	
CB 180		47	29	2.5-230	49	
Sum PCBs	20	180	120	12-710	170	**85**
PBDEs **						
BDE 47	100	1.7	1.0	0.3-7.7	1.8	
BDE 100		0.80	0.50	0.3-3.9	0.90	
BDE 99	100	1.3	0.70	0.3-10	1.7	
BDE 153	200	1.0	0.60	0.3-3.5	0.80	
Sum PBDEs		5.0	3.7	1.2-23	4.5	0
DDTs ***						
p.p'-DDE		1300	810	49-7300	1300	
p.p'-DDT		52	27	2.4-420	66	
Sum DDTs	20000	1300	840	52-7700	1400	0
CHLs ***						
OxC		10	6.6	1.1-73	12	
TN		9.0	4.8	0.4-114	15	
Sum CHLs	500	21	12	2.7-200	27	0
HCB * **	170	97	32	2.2-1100	180	**11**

*ATSDR
** US EPA
***WHO

The calculated DI for PBDEs was compared to the EPA RfD (US EPA, 2008), a benchmark dose operationally derived from the NOAEL (no-observed-adverse-effect level) and expressed in µg/kg of body weight per day. For BDEs 47, 99 and 153, none of the samples had DI values above the RfD. Therefore, it is not clear whether current PBDE concentrations in the Greek human breast milk, can cause any adverse effects on infant health. Even though persistent pollutants are present in human milk, the benefits of breast-feeding are believed to outweigh the potential health risks from exposure to these chemicals during lactation (LaKind et al., 2004).

1.4 Conclusions

The results showed that six POP groups were ubiquitously found in human breast milk from Thessaloniki, Greece, with DDTs and PCBs being the predominantly identified compounds in all samples. Levels of PBDEs in nursing mothers from Greece were lower than those of other European, North American or Asian countries. Mothers' weight, weight gained during pregnancy, residence (rural/urban) and smoking status did not show any correlation with any of the pollutants measured (PCBs, OCPs and PBDEs). Employment showed strong correlations for PBDEs, especially BDE 47 and BDE 153 in women working in office environments. The estimated infant daily intake of OCs shows that the intake of PCBs through lactation exceeded the TDI, which is of concern for infant health. This is the first reported data of PBDEs in human breast milk from Greece. Although the number of samples is low, results of this study provide a useful baseline data for future research on human exposure to brominated flame retardants in Greece, and highlight a need for more detailed investigation of the levels of BFRs and OCs in various environmental matrices and food, as well as other routes of exposure of POPs in the general the Greek population.

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