MULTI-POP ANALYSIS IN BREAST MILK OF FIRST TIME MOTHERS IN IRELAND

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Introduction

Human breast milk has frequently been used as an indicator of the body burden of persistent organic pollutants (POPs). Collaborative studies undertaken by the World Health Organisation (WHO) and other studies have shown a consistent decline in levels of PCDD/F and PCBs in breast milk over the last 20 years [1-5], however, in contrast to the decline in levels of PCDD/F and PCBs in breast milk, the levels of POPs such as brominated flame retardants (BFRs), perfluoroalkylated substances (PFAS) and polychlorinated naphthalenes (PCNs), have been reported to be increasing in the human population in some parts of the world [3, 6-10]. Recent sequential data from Sweden and Norway may however indicate a stabilisation or even a reversal of this trend [9, 10].

This study was conducted to establish a body burden baseline for POPs not previously evaluated in breastmilk in Ireland, including polybrominated diphenyl ethers (PBDEs), polybrominated dibenzo dioxins and furans (PBDD/Fs), polybrominated biphenyls (PBBs), mixed halogenated dioxins and furans (PXDD/Fs), Hexabromocyclododecane diastereomers (HBCDs), Hexabromobenzene (HBB), Bis(246-tribromophenoxy)ethane (BTBPE), Decabromodiphenylethane (DBDPE), PCNs and PFAS and to examine any changes in those that had previously been tested in 2002 (i.e. PCDD/Fs & PCBs). Results of the latter are not reported here and can be found elsewhere [11].

Materials and methods

Eleven pooled breast milk samples were collected from 109 first time mothers at four collaborating centres across Ireland, following the protocol developed by WHO [12], as described in detail together with demographics of the mothers in the eleven pools in an earlier publication [11]. In summary, the mean age of the 109 participants was 32.7, the mean age of each of the individual pools being similar to the overall mean, ranging from 30.2 for Pool 7 to 34.7 for Pool 11. The mean body mass index (BMI) of the participants was 23.3, and the result for each individual pool was similar to this overall mean. All mothers born outside Ireland had fulfilled the WHO protocol requirement of 5 years residence in their current geographical area. The majority of all mothers reported having a mixed diet.

Individual breast milk samples were thawed and homogenised before pooling (10-11 per pool). A total of 11 pooled samples were identified by a unique code, refrozen and shipped to the analytical laboratories in a frozen state. All 11 pooled samples were homogenised, freeze-dried and analysed for PCDD/Fs, PBDD/Fs, PBDEs and PBB/PCBs by the Food and Environment Research Agency (FERA), using previously reported methodology [13-14]. In brief, samples were fortified with 13C-labelled analogues of target compounds and exhaustively extracted using mixed organic solvents. PCDD/Fs, PBDD/Fs and planar PCBs were fractionated from other analytes, on activated carbon exploiting the degree of molecular planarity and further purified using adsorption chromatography on alumina. Analytical measurement was carried out using high resolution gas chromatography/high resolution mass spectrometry (HRGC–HRMS) for the PBDD/Fs, PBDEs, PCDD/Fs and non-ortho substituted PCBs and high resolution gas chromatography/unit resolution mass spectrometry for the ortho substituted PCBs. PBDE/PBB, PBDD/F, PCDD/F and PCB analyses have been accredited to the ISO17025 standard.. A similar method was used for the analysis of PCNs [17], for which extracts of freeze-dried samples were acid hydrolysed, fractionated from potential interferants such as PCBs on activated carbon, purified on alumina and analysed by HRGC-HRMS. PXDD/Fs and PXBs were analysed using more complex methodology, involving dual carbon column fractionation, purification on alumina, and analysis by HRGC-HRMS at 13000-15000 resolution [16].

HBCD and TBBPA were analysed using the method described earlier by Harmer et al. (2008)[18]. In brief, following extraction and acid hydrolysis, extracts were solvent exchanged to a methanol:water system, and analysed by HPLC-MS/MS. Each sample was fortified with ^{13}C-labelled analogues and analysed in duplicate. Similarly, HBB, BTBPE and DBDPE were extracted from the freeze-dried milk samples and subjected to acid hydrolysis, prior to further purification using activated Florisil^{R} and analysed by HRGC-HRMS [19]. For PFAS, replicate aliquots (including unspiked and overspiked portions) of each sample were fortified with labelled internal standards, extracted with methanol, concentrated and solvent exchanged to water, prior to purification on SPE cartridges (weak anion exchange), concentration and analysis by HPLC-MS/MS [20].The analytical standards used, native as well as isotopically labeled, for the individual analyte groups were obtained as solutions either from Cambridge Isotope Labs, Mass. USA or from Wellington Laboratories Inc. Ontario, Canada. The analysis for pesticide POPs was carried out by the Irish Pesticide Control Laboratory using the laboratory standard multi-residue methodology (SOP 101 for analysis by GC–MS/MS, based on Analytical Methods for Pesticide Analysis, 6th Ed., Ministry of Public Health, Welfare and Sport, the Netherlands, and SOP 111 for analysis by LC-MS/MS, standard multi-residue methodology according to Lehotay et al., 2005 [21].

The methodology used for all analyses has been robustly validated. PCDD/F, PCB, PBDE/PBB, PBDD/F, analyses and measurements for HBCD/TBBPA have been accredited to the ISO17025 standard. The analyses include method blanks and reference materials with the sample sets. Where reliable inter-calibration schemes exist for food matrices, the methodology has been further validated by successful participation in these studies.

Results and discussion

This study has investigated concentrations of chlorinated, brominated and fluorinated POPs in 11 pooled samples of breast milk from 109 first-time mothers in Ireland, sampled in 2010. Table 2 provides the lowerbound and upperbound mean and range over the 11 pools for all substances tested.

The mean PCDD/F+ PCB WHO-TEQ over the 11 pools was 10.2 pg g^{-1} fat, with a range of 7.49–13.7 pg g^{-1} fat. The mean uppberbound Σ_{24}PBDEs was 4.9 ng g^{-1} fat, with a range of 2.7 to 9.4 ng g^{-1} fat. BDE-47, BDE-153, BDE-99, BDE-100 and BDE-209 were the congeners found at the highest concentrations. The only PBBs detected consistently above the LOD were BB-77 (mean 0.1 pg g^{-1} fat), BB-126 (mean 0.3 pg g^{-1} fat), BB-153 (mean 0.1 ng g^{-1} fat). Several mixed chlorinated/brominated dibenzodioxins and dibenzofurans (2-B-3,7,8-CDD, 2,3-B-7,8-CDF, 4-B-2,3,7,8-CDF) were also detected in most of the milk samples, 4-B-2,3,7,8-CDF being present at the highest concentration and detectable in the majority of samples. Additionally the mixed halogenated biphenyls PXB 105, PXB 118, PXB 126 and its dibromo and tribromo derivative and PXB 156 were detected in all 11 breast milk pools, PXB 118 being found at the highest concentration (mean UB concentration of 0.54 pg g^{-1} fat) followed by PXB 156 (mean UB 0.37 pg g^{-1} fat) and PXB105 (mean UB 0.33 pg g^{-1} fat). These levels are lower than the concentrations measured in the same study for the chlorinated analogues (PCB 118 mean UB 4.1 pg g^{-1} fat, PCB 156 mean 1.46 pg g^{-1} fat, PCB 105 mean 0.87 pg g^{-1} fat) [11].

The mean ΣHBCD enantiomers over the 11 pools was 3.5 ng g^{-1} fat, with α-HBCD representing over 70% of the total, while of the other brominated flame retardant substances (TBBPA, HBB, DBDPE and BTBPE) examined in this study, only TBBPA was detected above the LOD, and only in two of the 11 pools analysed. 2,3,7,8-Tetrabromo-dibenzodioxin (TetraBDD) was the only PBDD detected in Irish breast milk, while all six of the PBDFs examined could be detected in the majority of the pooled milk samples, with the 1,2,3,4,6,7,8-heptabromo-diobenzofuran (HeptaBDF) being detected at the highest concentration.

No PFAS were detected in the current study above the limits of detection (ranging from 0.5-2 ng g^{-1}fat). Polychlorinated naphthalenes were however detected in all samples, at concentrations ranging from 59 to 168 ng kg^{-1} lipid for Σ_{12}PCNs. Analysis of chlorinated pesticides indicated that none of the pesticides analysed in the multiresidue screen were present at concentrations above the reporting limits of the laboratory. Traces of b-HCH (2.4 and 1.3 ng g^{-1} fat) were detected in pooled samples P7 and P10, and traces of pp-DDE were found in all samples, ranging from 0.5 to 2.7 ng g^{-1} fat, however these values were only estimates as they were below the reporting limits.

Table 2 Mean and range of POPs tested in 11 breast milk samples expressed on fat weight basis (except PFAS)

Substance	Unit	n < LOD	Mean (LB/UB)	Range	Substance	Unit	n < LOD	Mean (LB/UB)	Range
% Fat		0	2.9	2.4-3.4	**PCNs**				
PCDD/Fs					PCN 52/60	ppt	0	57.5	33.1-123.3
TEQ PCDD/F	ppt	0	6.3	4.5-8.7	PCN 53	ppt	0	1.8	1-4.5
non ortho-PCB	ppt	0	2.3	1.8-3.5	PCN 66/67	ppt	0	29.0	21.9-42.9
ortho-PCB	ppt	0	1.7	1.2-2.4	PCN 68	ppt	1	0.23/0.3	<0.2-0.32
TEQ DL-PCBs	ppt	0	3.9	3-5.4	PCN 69	ppt	0	2.9	1.5-5.8
Total WHO TEQs	ppt	0	10.2	7.5-13.7	PCN 71/72	ppt	7	0.14/0.3	<0.24-0.49
6 Marker PCB	ppb	0	41	31.5-59	PCN 73	ppt	0	0.67/0.7	0.52-0.93
PBDEs					PCN 74	ppt	10	0.005/0.05	<0.03-0.07
BDE-100	ppb	0	0.29/0.3	0.13-0.44	PCN 75	ppt	11	0/0.4	<0.32-<0.55
BDE-99	ppb	0	0.30/0.3	0.11-0.6	Sum PCNs	ppt	0	92.9	59.3-168
BDE-153	ppb	0	0.96/1.0	0.72-1.15	**HBB**	ppb	11	0/0.3	<0.29-<0.42
BDE-47	ppb	0	1.2	0.5-2.3	**BTBPE**	ppb	11	0/0.3	<0.29-<0.42
BDE209	ppb	1	1.4/1.5	<0.4-7.2	**DBDPE**	ppb	11	0/2.3	<2.1-<2.5
Sum BDE excl 209	ppb		4.8/4.9	2.7-9.4	**TBBPA**	ppb	9	0.05/0.3	<0.17-0.42
					Total HBCD	ppb	0	3.45/3.5	<2.16-5.94
PBDD/Fs					**PXDD/Fs**				
237-TriBDD	ppt	10	0.02/0.1	<0.03-0.22	2-B-7,8-CDD	ppt	8	0.005/0.02	<0.01-0.03
2378-TetraBDD	ppt	0	0.09/0.1	0.07-0.17	2-B-3,7,8-CDD	ppt	3	0.03/0.04	<0.02-0.08
12378-PentaBDD	ppt	11	0/0.1	<0.04-<0.11	2,3-B-7,8-CDD	ppt	9	0.003/0.02	<0.01-0.03
123478/123678-HexaBDD	ppt	11	0/0.08	<0.08-<0.1	1-B-2,3,7,8-CDD	ppt	11	0/0.01	<0.01-<0.02
123789-HexaBDD	ppt	11	0/0.08	<0.08-<0.1	2-B-1,3,7,8-CDD	ppt	11	0/0.01	<0.01-<0.02
238-TriBDF	ppt	3	0.03/0.05	<0.03-0.08	2-B-3,6,7,8,9-CDD	ppt	9	0.007/0.02	<0.01-0.05
2378-TetraBDF	ppt	0	0.88/0.9	0.72-1.46	2-B-7,8-CDF	ppt	10	0.003/0.02	<0.01-0.04
12378-PentaBDF	ppt	0	0.35/0.4	0.24-0.69	3-B-2,7,8-CDF	ppt	11	0/0.02	<0.01-<0.02
23478-PentaBDF	ppt	0	0.76/0.8	0.5-1.41	2-B-6,7,8-CDF	ppt	11	0/0.01	<0.01-<0.02
123478-HexaBDF	ppt	4	0.18/0.2	<0.09-0.37	2,3-B-7,8-CDF	ppt	6	0.008/0.02	<0.01-0.02
1234678-HeptabromoBDF	ppt	1	1.3	<0.2-2.3	1-B-2,3,7,8-CDF	ppt	11	0/0.005	<0.005-<0.01
PBBs					4-B-2,3,7,8-CDF	ppt	0	0.08	0.04-0.13
PBB-77	ppt	5	0.07/0.1	<0.11-0.14	1,3-B-2,7,8-CDF	ppt	10	0.002/0.009	<0.005-0.027
PBB-126	ppt	0	0.25/0.3	0.2-0.4	**PXBs**				
PBB-169	ppt	10	0.006/0.05	<0.02-0.07	PXB126	ppt	0	0.13	0.09-0.22
BB-15	ppb	11	0/0.02	<0.02-<0.03	PXB126 di-Br	ppt	0	0.09	0.04-0.15
BB-49	ppb	11	0/0.002	<0.002	PXB126 tri-Br	ppt	1	0.056/0.058	<0.02-0.11
BB-52	ppb	11	0/0.002	<0.002	PXB 118	ppt	0	0.54	0.35-1.07
BB-80	ppb	11	0/0.002	<0.002	PXB 105	ppt	0	0.33	0.1-0.92
BB-101	ppb	10	0/0.002	<0.002-0.003	PXB 156	ppt	0	0.37	0.18-0.93
BB-153	ppb	0	0.12	0.06-0.19	**Total PFAS (wet weight basis)**	ppb	10*	0/1.6	<17.5
PBB209	ppb	11	0/0.3	<0.29-<0.42					

PXB126: 4'-B-3,3',4,5-CB; PXB126 di-Br: 3,4-B-3',4',5'-CB; PXB126 tri-Br: 3',4',5'-B-3,4-CB; (PXB 118: 4'-B-2,3',4,5-CB; PXB 105: 4'-B-2,3,3',4-CB; PXB 156: 4'-B-2,3,3',4,5-CB; PFAS: PFOSA, PFBSH, PFHxS, PFOS, PFHxA, PFHpA, PFOA, PFNA, PFDeA, PFUnA, PFDoA ; PBDEs: 17, 28/33, 37, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 155,183, 197, 202, 203, 206, 207, 208 and 209; *only 10 pools analysed

In summary, a very wide range of chlorinated, brominated and fluorinated POPs have been measured in Irish breast milk, the majority of them for the first time. The concentrations measured are comparable or slightly lower than those reported in other European countries [3, 9, 10, 22-25]. The pattern of occurrence of these POPs in Irish breast milk bears a reasonable relationship to their occurrence in food, as reported in a number of

surveillance studies carried out by the FSAI. It can be concluded, therefore that the diet is the principal source of exposure to these pollutants for the Irish population. In the context of the current information on the toxicology of these compounds, the concentrations of brominated and fluorinated contaminants measured in Irish breast milk in this study are considered to have no implications for the health of Irish mothers or their babies.

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