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Concentrations of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in human milk from Ireland: temporal trends and implications for nursing infant exposure

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Abstract

Aim: The elucidation of temporal trends in human exposure to polychlorinated biphenyls (PCBs) and 17 polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs) since the previous Irish human milk surveys and to evaluate the impacts of legislative bans and restrictions on human exposure to these compounds.

Methods: Concentrations of PCBs and 17 PCDD/Fs were measured in 16 pools of human milk collected from 92 Irish primiparas participating in the Irish EPA-funded ELEVATE project between October 2016 and April 2018, using Gas-Chromatography coupled with Mass spectrometry.

Results: The geometric mean upper bound concentration of 16 pooled human milk samples [PCDD/Fs + dioxin-like (dl)-PCB TEQ; 4.5 ng kg^{-1} lipid weight] are on the lower end of those reported internationally. WHO-TEQ PCDD/Fs + dl-PCB are significantly lower ($P < 0.005$) compared to those reported in the previous Irish human milk studies in 2010 and 2002.

Conclusion: Detected concentrations in this study are comparable to those reported for less industrialised countries in the last WHO/UNEP global surveys for PCDD/Fs. This downward temporal trend likely reflects the impact of regulatory bans and restrictions on the emissions of dioxins and PCBs. While mean upper bound WHO PCDD/F PCB TEQ concentrations are lower than those estimated by EFSA to be associated with adverse health effects in children age 9, maximum upper bound concentrations do exceed EFSA reference concentrations. While the positive health benefits of breastfeeding to both mother and child significantly outweigh potential adverse health effects at reported concentrations, continued action to reduce human body burdens of dioxins and PCBs is required.

Keywords: Dioxins, PCBs, POPs, temporal trends, human biomonitoring, breast milk

INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs), commonly referred to as dioxins and polychlorinated biphenyls (PCBs), are highly toxic persistent organic chemicals capable of resisting photolytic, biological, and chemical degradation[1]. Generated mostly from anthropogenic activity, PCDD/Fs are produced as a by-product of numerous industrial and combustion processes, including the manufacture of pesticides and herbicides, chlorine bleaching of paper and waste incineration[1]. Emissions from such anthropogenic activities substantially outweigh those from natural sources such as forest fires and volcanic eruptions[1,2]. PCB manufacture commenced in 1929, finding a variety of uses, including as heat exchange fluids in electrical transformers and as additives in paints and adhesives, until manufacture and new uses were banned across the globe in the late 1970s and early 1980s[1-4]. There are 209 PCB congeners which are divided into two groups based on their toxicity. PCBs which display similar toxicological effects to PCDD/Fs due to their ability to adopt a co-planar structure and interact with Aryl hydrocarbon (AH) receptors, are referred to as dioxin-like PCBs. Binding to the AH receptors induces CYP1 drug metabolising enzymes, and this binding is critical for dioxin-like PCBs' toxic action[3]. Data on non-dioxin like PCBs are often reported as the sum of six PCB congeners (PCB 28, 52, 101, 138, 153, 180) or as the sum of six congeners plus PCB 118[5].

Environmental contamination with PCDD/Fs and PCBs arises as a result of point pollution sources and long-range transportation, resulting in their ubiquitous distribution in the environment, including locations distant from the original source[6,7]. Extensive stores of PCB and dioxin containing equipment and/or waste still remain across the globe, which must be managed or treated in an environmentally sound manner so as to avoid further environmental contamination[4].

Human exposure to PCDD/Fs and PCBs has been linked to a range of both acute and chronic human health effects, including chloracne, immunotoxicity, reproductive, neurodevelopmental, and dioxins are carcinogenic[1]. There has been considerable public, scientific, and regulatory debate, and concern over the adverse impact of PCDD/Fs and PCBs on human health and on the environment, which has resulted in their inclusion under Annexes A (PCBs) and C (PCDD/Fs) of the UNEP Stockholm Convention[4]. Due to the propensity of PCDD/Fs and PCBs to bio-accumulate, the diet, in particular the ingestion of animal-based produce, is recognised as being the main non-occupational human exposure pathway.

Acknowledging the importance of the dietary exposure pathway for dioxin and dioxin-like PCBs, in 2001, the European Commission introduced a strategy to take further action to prevent environmental and adverse health effects from these compounds. This strategy included the introduction of a Tolerable Weekly Intake (TWI) value of 14 pg toxic equivalent (WHO-TEQ)/kg body weight. At that time, daily average European dietary intake was between 1 and 3 pg (WHO-TEQ)/kg body weight, and thus a substantial proportion of the EU population exceeded the TWI[8].

An assessment of Irish adult dietary exposure to Dioxins and Indicator PCBs from data collected in a biomonitoring programme between 2003-10 and food consumption between 2008-10 concluded that dietary exposure of Irish adults was lower than the European average and did not exceed the TWI for dioxins or the NOAEL for PCBs[9].

In 2018, the European Commission, on the advice of the EFSA CONTAM Panel, revised the TWI downward to 2 pg (WHO-TEQ) kg^{-1} body weight/week, to take account of new epidemiological and animal data and protect against reproductive health effects in males. EFSA reported that average and high dietary exposures across the EU were up to five and fifteen times higher than the new TWI in adolescents, adults, and the elderly[10].

WHO has planned to conduct an International re-evaluation of the current Toxic Equivalency Factors (WHO-TEFs) used to estimate the total dioxin-like activity of mixtures of PCDDs, PCDFs and PCBs in 2022, following concerns that the current TEFs may overestimate the toxicity of some PCBs such as PCB-126[11].

Since 1987, WHO has coordinated a global monitoring programme for PCDD/Fs and PCBs in human milk samples[12,13]. The programme aims to evaluate the impact of restrictions and bans implemented under the Stockholm Convention on human exposure and to examine global temporal and spatial trends in exposure. Fifty-two countries, including Ireland, participate in this programme. Results from the most recent, fourth and fifth surveys (2005-2010) show higher concentrations in more industrialised countries, with the highest values reported in Europe. Comparisons between results from the fourth and fifth surveys and those from the third survey are indicative of a downward trend in exposure; however, in all countries studied, total toxic equivalency (TEQ) concentrations of PCDD/Fs and PCBs combined were one to two orders of magnitude higher than levels considered toxicologically relevant for the foetus and breastfed infants, indicating a requirement for continued action to reduce human exposure[14].

Prior to this study, the most recent study of PCDD/Fs and PCBs in Irish human milk was based on samples collected in 2010[15]. Measured concentrations were compared to results from Irish human milk samples included in the third WHO survey that were collected in 2002[12]. Reported concentrations in 2010 (sum of WHO-TEQ, 9.66 pg g^{-1} fat) were lower than values reported in 2002 (≈ 13.0 pg g^{-1} fat total WHO TEQ for urban pools and 8.9 and 11.6 pg g^{-1} fat total WHO TEQ for rural pools), and consistent with some of the lower exposure data reported for Europe.

This study report concentrations of PCDD/Fs and PCBs in 16 pooled samples of Irish human milk collected from 92 Irish primiparas between October 2016 and 2018. This study was designed to facilitate the elucidation of temporal trends in human exposure to PCDD/Fs and PCBs since the last Irish human milk survey in 2010 and evaluate the impacts of legislative bans and restrictions on human exposure to these compounds.

EXPERIMENTAL

Human milk sample collection and pooling

Samples analysed in this study were collected between October 2016 and April 2018 as part of an Irish EPA funded research project (ELEVATE, reference 2016-HW-MS-8). The samples have previously been analysed for concentrations of brominated flame retardants[16] and Perfluoroalkyl substances[17]. The study population, approach to recruitment, sampling, and sample pooling are reported in detail in Wemken *et al.*[16], 2020. In brief, study recruitment and sampling protocols, adhered (with minor deviations) to WHO UNEP guidelines for developing a national protocol for measurement of POPs in human milk[13] and the study protocols of Pratt *et al.*[15], 2012. Comparability of study design with the study of Pratt *et al.*[15] (2012) was important to facilitate elucidation of temporal trends in concentrations of PCDD/Fs and PCBs in human milk in Ireland. Study protocols were approved by the Clinical Research Ethics Committee of the University Hospital Galway (Ref: C.A. 1578) and the Research Ethics Committee of the Coombe Women and Infants University Hospital in Dublin (No. 30-2016).

Mothers were recruited while attending breastfeeding clinics in two Irish maternity hospitals, who also participated in the Pratt *et al.*[15], 2012, namely University Hospital Galway (UHG) and the Coombe Women and Infants University Hospital (Coombe), Dublin. Participants chosen had to meet the following criteria: (1) primiparas exclusively nursing one infant; (2) in good health; (3) 3-8 weeks postpartum; (4) residing in their current address for at least 5 years previously; and (5) 40 years old or younger (in contrast to the WHO guidance of less than 30 years old, this criterion was amended to less than 40 years old for the study, as 65% of Irish mothers are between 30-40 years old[18]). All eligible candidates completed consent forms and provided contextual information via a questionnaire.

Approximately 30-60 mL of milk were collected from each individual donor in clean polypropylene containers and stored at -18 °C before pooling. The total number of samples collected was 92 (UHG = 59 and CIMH = 33). Samples were thawed and homogenised prior to pooling. Data provided from the questionnaires were used to determine how each sample was pooled, with a summary of donor demographics per pool provided in supporting information [Supplementary Table SI-1]. There were a total of 16 sample pools prepared, pools were determined by the following factors: place of birth (e.g., Ireland, UK, EU, non-EU), place of residence for the previous 5 years (e.g., urban or rural *etc.*). Two pools were prepared on the basis of whether participants consumed fish at least twice a week. Each pool contained 30 mL aliquots of milk from each contributing donor (in fish-consumer pools, only 15 mL aliquots were used due to limited sample availability). The individual sample numbers per pool varied between 3 and 10. Pooled milk samples were freeze-dried at -73 °C for approx. One hundred and twenty hours prior to analysis (using a VirTis Bench Top Pro BTP-3X100 freeze drier).

Sample preparation of human milk

Extraction and clean-up

Human milk sample extraction, extract purification/clean-up, and instrument analysis processes were undertaken at the State Laboratory, Backweston Lab Campus, Celbridge, Co. Kildare. Lipids were extracted from a 10 g portion of freeze-dried breast milk from each pool using pressurised liquid extraction (ASE 350, Dionex, Sunnyvale, California, USA), at 80 °C, 1500 psi, and a solvent ratio of 62.5% hexane, 25% dichloromethane, and 12.5% methanol. Lipid content was determined gravimetrically (further details are provided in Supplementary Table SI-2).

2.5 g of the extracted lipid was then spiked with a known quantity of internal standard (details are provided in Supplementary Table SI-3), 1 mL of hexane was added to aid loading the sample to the clean-up column. Clean-up and fractionation were carried out on a Miura GO-6HT sample clean-up system (Miura, Matsuyama, Ehime, Japan), using silica, alumina, and carbon columns. The samples were collected in two

fractions, fraction A containing the Mono-ortho PCB, and Non-dioxin like PCB (MOPIP) congeners and fraction B containing the Dioxin and Non-ortho PCBs (DIOXNOP) congeners. Sample fractions were then concentrated, and reconstituted in either toluene (fraction B) or nonane (fraction A) (depending on fraction), and fortified with recovery standards (13C12-1,2,3,4 TCDD and 13C12-1,2,3,4,6,8,9 HpCDF).

Instrumental analysis of human milk samples

Samples were analysed for 2,3,7,8-chlorinated PCDD/Fs and non-*ortho* substituted PCBs (congeners 81, 77, 126 and 169) using a Thermo DFS system (Thermo Fisher Scientific, Bremen, Germany). GC programme; 90 °C for 3 min, heat to 200 °C at 20 °C/min, from 200 °C to 280 °C at 4 °C/min, hold 20 min, from 280 °C to 300 °C at 5 °C/min, hold 8 min. Samples were analysed for the mono-*ortho* chlorinated PCBs (congeners 123, 118, 114, 105, 167, 156, 157 and 189) and indicator PCBs (congeners 28, 101, 153, 138 and 180) on a Waters Autospec GC-HRMS system (Waters, Milford, Massachusetts, USA). GC programme; 110 °C for 3 min, heat to 200 °C at 20 °C/min, from 200 °C to 300 °C at 4 °C/min, hold 10 min. The GC column used for both analyses was a DB-5MS, 60 m × 0.25 mm × 0.25 mm (Agilent, Santa Clara, California, USA).

Analysis was completed using a validated method, and performed following the criteria laid out in EU Commission Regulation 2017/644, of 5th April 2017[19], with two masses measured for each of the 35 congeners of interest [Supplementary Table SI-4].

Quality control and assurance

All Dioxin sample analysis work is INAB accredited to ISO 17025 and was carried out in the State Laboratory, which is the National Reference Laboratory for persistent organic pollutants (POPs) in food and feed in Ireland. All batches included solvent blanks, solvent standards as system suitability/verification checks, and quality control material (either a certified reference material, or a previously analysed proficiency testing (PT) scheme sample. Method blanks were also included in the batches at regular intervals (further details are provided in Supplementary Table SI-5).

Reporting of Dioxin results - TEF concept

Concentrations of individual PCDD/Fs and dl-PCBs were expressed as a TEQ, calculated by multiplying the concentration of each individual target compound by the corresponding TEF published by WHO in 2005[20] and provided as Supplementary Table SI-6. WHO-TEQs were calculated as upper bound concentrations, using the limit of quantification for the contribution of each non-quantified congener[21]. Summing of TEQ concentrations of all individual congeners for a given sample provided the sum TEQ concentration for that sample.

Statistical analysis was performed using SPSS Statistics for Window version 26. Concentration data were normally distributed, and a *t*-test was used to compare concentrations in the current study with previous data from 2010 and 2002[12,15].

Estimation of infant intake of PCDD/F and PCBs via breast milk

In order to evaluate a nursing infant's dietary intake of PCDD/Fs and dl-PCBs, in this study, estimated daily intake values were calculated using Equation (1)[10,22] [Supplementary Table SI-7]:

$$\text{EDI (pg-TEQ}_{2005}\text{ kg}^{-1}\text{ body weight day}^{-1}) = \frac{CxM}{W} \quad (1)$$

where EDI is the estimated daily intake calculated for breastfed infants below 6 months of age (median age of three months was used as per EFSA, 2018); C geometric average PCDD/F and dl-PCB concentrations in the study groups (pg-TEQ_{2005} g^{-1} milk); M is the estimated average daily milk consumption (g day^{-1}); and W is the mean body weight of a baby aged three months. Body weight of 6.1 kg and an average human milk intake of 800 mL day^{-1}, and high consumption of 1200 mL day^{-1} were used[10]. The median lipid content of the samples analysed was 3.47 g per 100 mL of breast milk, resulting in average and high lipid intakes of 27.76 g day^{-1} and 41.64 g day^{-1}, respectively. Upper bound (UB) and lower bound (LB) geometric average PCDD/F and PCB concentrations were used to calculate exposure intakes in pg TEQ kg^{-1} bw per day.

RESULTS

Concentrations of PCDD/Fs, non-o-PCBs, and mono-ortho-PCBs in Irish human milk samples collected between 2016 and 2018

Tables 1 and 2 respectively [Supplementary Table SI-8, Supplementary Figures SI-8.1-SI-8.9] list concentrations of PCDD/Fs and dl-PCBs and indicator PCBs (expressed as upper bound concentrations per kg lipid weight), measured in the 16 pools of human milk collected in this study. An analysis of individual PCDD/F congener concentrations, showed that OCDD was presented in the highest concentration followed by 2,3,4,7,8-PeCDF and 1,2,3,4,6,7,8-HpCDD, similar to the profile reported in the last Irish study of samples collected between 2009 and 2010[12]. OCDD, 1,2,3,4,6,7,8-HpCDD and 1,2,3,6,7,8-HxCDD are also among the dominant congeners reported by other researchers for previous studies on human milk[22,23]. The dominant dl-PCB congeners reported in this study were PCB 118 followed by PCB-156, also consistent with previous studies[15,22,23]. PCB-153 followed by PCB-180 were the dominant Indicator PCBs detected in this study, similar to the pattern reported in Pratt *et al.*[15], 2012, and Focant *et al.*[23], 2013.

The sum of WHO TEQs (PCDD/F + dl-PCB) concentrations ranged from 3.4-8.0 ng kg^{-1} lipid (GM = 4.5 ng kg^{-1} lipid), overall, PeCDD congeners made the greatest contribution to WHO TEQ_{05} (38%) followed by PCBs (33%) and PCDFs (29%) congeners. An analysis of the congener profiles showed that 1,2,3,7,8-PeCDD, PCB-126, and 2,3,4,7,8-PeCDF were the dominant congeners (24%, 25%, and 22% of total WHO TEQs (PCDD/F + dl-PCB) consistent with Pratt *et al.*[15], 2012, Hernández *et al.*[22], 2020, and Focant *et al.*[23], 2013.

DISCUSSION

Comparison of PCDD/Fs, non-ortho-PCBs, and mono-ortho-PCBs levels in Irish human milk samples collected between 2016 and 2018 to previous surveys

Table 3 (reproduced from Hernández *et al.*[22], 2020) compares concentrations of ∑PCDD/Fs and dl-PCBs in human milk reported in this study with concentrations reported internationally over the period 2012 to 2020. Irish concentrations (GM PCDD/Fs + dl-PCB TEQ; 4.5 ng kg^{-1}) are comparable to studies reporting lower concentrations. Geometric mean values in this study (2016-18) reported by Hernández *et al.*[22], 2020, (GM PCDD/Fs + dl-PCB TEQ; 4.42 ng kg^{-1}) in the Spanish BETTERMILK study for samples collected in 2015. Irish values in this study (PCDD/Fs + dl-PCB TEQ GM 4.5 ng kg^{-1}) are comparable to values reported in the fourth and fifth WHO UNEP survey for less industrialised countries and countries in the southern hemisphere[14] and are much lower than those reported for mainland Europe including Italy (AM value; 13.4 ng kg^{-1})[26] and France (10.74 ng kg^{-1})[31]. They are also less than those reported in the last Irish study for samples collected in 2009-2010 (AM value; 9.66 ng kg^{-1})[15]. Concentrations of the dominant indicator PCBs, (PCB-153; 11.0 μg kg^{-1} PCB-180; 6.4 μg kg^{-1}, PCB-138; 5.9 μg kg^{-1}) are slightly lower than those reported in the last Irish human milk study in 2010 (PCB-153; 12.6 μg kg^{-1} PCB-180; 7.4 μg kg^{-1}, PCB-138; 6.9 μg kg^{-1})[15] and significantly lower than concentrations reported in the French Elfe Pilot study in 2007 (PCB-153; 83.04 μg kg^{-1} PCB-180; 48.34 μg kg^{-1}, PCB-138; 39.97 μg kg^{-1})[23].

Table 1. Descriptive statistics for PCDD/F and PCB concentrations (ng kg^{-1} lipid weight) and WHO-TEQ$_{05}$ (upper bound concentrations ng kg^{-1} lipid weight) in 16 pooled human milk samples

	Arithmetic mean	Geometric mean	Median	Min	Max
PCDD/F					
2,3,7,8-TCDF	0.32	0.31	0.30	0.18	0.59
1,2,3,7,8-PeCDF	0.25	0.22	0.22	0.11	0.49
2,3,4,7,8-PeCDF	3.5	3.3	3.0	2.3	6.6
1,2,3,4,7,8-HxCDF	0.92	0.88	0.86	0.63	1.8
1,2,3,6,7,8-HxCDF	0.96	0.91	0.90	0.63	1.8
2,3,4,6,7,8-HxCDF	0.52	0.49	0.48	0.31	1.0
1,2,3,7,8,9-HxCDF	0.10	0.10	0.11	0.06	0.12
1,2,3,4,6,7,8-HpCDF	0.64	0.62	0.64	0.39	1.0
1,2,3,4,7,8,9-HpCDF	0.08	0.08	0.08	0.06	0.1
OCDF	0.09	0.09	0.09	0.09	0.09
2,3,7,8-TCDD	0.36	0.35	0.33	0.27	0.61
1,2,3,7,8-PeCDD	1.1	1.1	0.96	0.78	2.0
1,2,3,4,7,8-HxCDD	0.43	0.41	0.37	0.28	0.68
1,2,3,6,7,8-HxCDD	2.1	2.0	1.9	1.4	3.4
1,2,3,7,8,9-HxCDD	0.52	0.50	0.45	0.36	0.8
1,2,3,4,6,7,8-HpCDD	2.5	2.4	2.3	1.5	4.2
OCDD	20	19	17	14	40
WHO-PCDD/F-TEQ UB[a]	**3.1**	**3.0**	**2.8**	**2.3**	**5.6**
Non-Ortho-PCBs/ dl-PCBs[b]					
PCB-81	0.85	0.82	0.78	0.61	1.5
PCB-77	5.1	4.5	5.3	2.1	11
PCB-126	12	11	11	8.5	19
PCB-169	6.8	6.5	6.2	4.4	11
WHO-Non-Ortho-PCB-TEQ UB[a]	**1.4**	**1.3**	**1.3**	**1.0**	**2.2**
Mono-Ortho-PCBs/ dl-PCBs					
PCB-123	35	34	34	24	52
PCB-118	2400	2400	2200	1700	380
PCB-114	130	120	110	85	220
PCB-105	550	540	500	410	810
PCB-167	350	320	310	190	700
PCB-156	1000	960	830	650	2600
PCB-157	220	210	180	140	390
PCB-189	93	83	71	52	270
WHO-Mono-Ortho-PCB-TEQ UB[a]	**0.15**	**0.14**	**0.13**	**0.10**	**0.26**
WHO-PCB-TEQ UB[a]	**1.5**	**1.5**	**1.4**	**1.1**	**2.4**
WHO-PCDD/F-PCB-TEQ UB[a]	**4.6**	**4.5**	**4.1**	**3.4**	**8.0**

[a]UB: Upper bound; [b]dl-PCBs: dioxin-like PCBs.

Highest WHO TEQs (PCDD/F + dl-PCB) concentrations are reported for Pool 6 (GM 8.01 ng kg^{-1}) and lowest concentrations for Pool 8 (GM 3.42 ng kg^{-1}). Donors to both pools were recruited from the west of Ireland and lived in suburban (Pool 6), urban, and rural locations (Pool 8). The dietary habits of the donors to both pools were similar (a varied diet and were all fish consumers (fish consumed included sea fish, salmon, cod, and trout). The median donor age for Pool 6 is higher than that of Pool 8 (37 and 33 years, respectively). Correlations between the concentration of persistent organic chemicals such as dioxins in

Table 2. Descriptive statistics for Indicator PCBs concentrations (×g kg^{-1} lipid weight UB[a]) in 16 pooled human milk samples

Indicator-PCBs	Arithmetic mean	Geometric mean	Median	Min	Max
PCB-28	0.44	0.43	0.43	0.35	0.63
PCB-52	0.13	0.13	0.12	0.09	0.21
PCB-101	0.20	0.19	0.20	0.14	0.29
PCB-153	11	10	8.9	6.4	31
PCB-138	5.9	5.5	4.9	3.8	16
PCB-180	6.4	5.6	4.7	3.1	22
∑ indicator PCBs UB[a]	24	22	19	14	69

[a]UB: Upper bound.

Table 3. Comparison of PCDD/F and dl-PCB TEQ concentrations (pg TEQ g^{-1} lipid weight) detected in human milk in this study and comparator surveys elsewhere (Table adapted from Hernández *et al.*[22], 2020).

Ref.	Period	Country	*n*	∑PCDD/Fs	∑PCDD/Fs + dl-PCBs
Current study	2016-2018	Ireland	92	3.1[a,b]	4.63[a,b]
Hernández *et al.*[22], (2020)	2015	Valencian Region (Spain)	75	2.71[a,c]	4.42[a,c]
Schuhmacher *et al.*[25], (2019)	2017	Tarragona (Spain)	20	2.26[a,b]	-
Roberto *et al.*[26], (2018)	2010	Italy	20	7.13[b,d]	13.4[b,d]
Chen *et al.*[27], (2018)	2013-2016	China	25	2.44[a,c]	-
Hue *et al.*[28], (2018)	2014-2015	Vietnam	36	10.2[a,b]	-
Ae *et al.*[29], (2018)	1998-2015	Japan	1194	10.95[a,b]	17.00[a,b]
Rawn *et al.*[30], (2017)	2008-2011	Canada	298	4.9[a,b]	6.3[a,b]
Antignac *et al.*[31], (2016)	2011-2014	France	96	6.16[a,c]	10.74[a,c]
Antignac *et al.*[31], (2016)	1997-2002	Denmark	438	13.01[a,c]	19.86[a,c]
Antignac *et al.*[31], (2016)	1997-2002	Finland	22	8.79[a,c]	13.62[a,c]
Zhang *et al.*[32], (2016)	2011	China	1760	4.9[b,d]	6.7[b,d]
Lu *et al.*[33], (2015)	2011-2012	China	150	5.4[a,b]	8.3[a,b]
Giovannini *et al.*[24], (2014)	2007-2008	Italy	95	6.46[a,b]	-
Focant *et al.*[23], 2013	2007	France	44	9.58[a,c]	17.81[a,c]
Croes *et al.*[34], (2013)	2009-2010	Belgium	84	6.9[a,c]	10.7[a,c]
Wong *et al.*[35], (2013)	2009	China	137	7.48[b,d]	11.27[b,d]
Pratt *et al.*[15], (2012)	2010	Ireland	109	6.32[b,d]	9.66[b,d]

[a]TEQ 2005; [b]mean; [c]geometric mean; [d]TEQ 1998, *n* = samples.

human milk and donor age are likely and have been reported in the literature[22,24]. However, in this study, donor samples were pooled, and so an analysis of associations between concentration and age of donor was not possible.

In terms of total TEQ (PCDD/F + dl-PCB) both pools are characterised by four dominant congeners, the percentage contribution of each congener to TEQ only differs slightly suggesting similar exposure sources. 1,2,3,7,8-PeCDD is the dominant (25%) congener in Pool 6 (followed by 2,3,4,7,8-PeCDF > PCB-126 > 2,3,7,8-TCDD) whereas PCB-126 is the dominant congener (25%) in Pool 8 (followed by 1,2,3,7,8-PeCDD > 2,3,4,7,8-PeCDF > 2,3,7,8-TCDD).

In contrast to total TEQ concentrations (PCDD/F + dl-PCB), for which Pools 6 and 8 had the highest and lowest concentrations respectively, the highest concentrations of indicator PCBs are found in Pool 9 (69 ng kg^{-1}), followed by Pool 1 (36 ng kg^{-1}). Pool 6 ranks third in terms of the highest indicator PCB

concentrations (30 ng kg^{-1}), and Pool 8 has one of the lower concentrations (16 ng kg^{-1}). Both Pools 1 and Pool 9 were two of the four pools (alongside Pools 10 and 11) composed of donors born outside of Ireland. Factors including diet and the proportion of donors born outside of Ireland within a given pool may explain differences in concentration.

Temporal trends in PCDD/F and PCB TEQ concentrations in Irish human milk

Concentrations of individual PCDD/Fs, dioxin-like PCBs and indicator PCBs in the current study (n = 16 pools) were compared with those reported in the last Irish study from 2010 (n = 11 pools)[15] and with Irish data from 2002 (n = 4 pools)[12] [Tables 4 and 5, Supplementary Tables SI-8.1-SI-8.4, Figure 1, Supplementary Figures SI-8.6-SI-8.9]. A full t-test comparison was possible to compare data from the 2010 survey with the current study. However, due to the limited pool size of the 2002 survey (n = 4), only a comparison of average concentrations was possible with this earliest study.

An examination of the temporal trend over the period 2002 to 2010 and from 2010 to 2016-18 shows a decreasing trend in WHO-PCDD/F-PCB-TEQ, consistent with other international studies and results from WHO/UNEP global surveys[14,23,27], reflecting the positive impact of regulatory measures restricting and banning the use of persistent organic chemicals on human exposure[36].

Concentrations of WHO-TEQ PCDD/F + dl-PCB in 2016-18 have declined by > 43% (P < 0.005) since the last Irish study in 2010, reported concentrations in 2016-18 are > 51% lower than the concentrations reported in 2002 (WHO-TEQ PCDD/F + dl-PCB 9.5 ng kg^{-1} lipid weight in 2002, 8.1 ng kg^{-1} lipid weight in 2010 *vs.* 4.6 ng kg^{-1} lipid weight in 2016-18) [Figure 1]. This decline in concentrations has been accompanied by a shift in the contribution of PCDDs relative to PCDFs and PCBs, to the total WHO-TEQ PCDD/F + dl-PCB concentration. The contribution of PCDFs has increased from 24% in 2010 to 29% in 2016-18, while the contributions of PCDDs and PCBs have dropped from 41 to 39% and 35 to 33 %, respectively [Figure 2].

When compared to 2010 data, the percentage decrease in concentrations of individual PCDDs ranges from 48%-57%, while the percentage decrease in concentrations of individual PCDFs ranges from 31%-93%, in line with those observed by Focant *et al.*[23], for French human milk surveys over the period 1998-99 and in 2007. In the last human biomonitoring survey in 2010, concentrations of many individual PCDFs and some dl-PCBs (PCB-77, PCB-126) have unchanged or increased slightly since 2002, however, results from this current study show that concentrations of all congeners (apart from 1,2,3,7,8,9-HxCDF) have decreased since 2010. Concentrations of 2,3,4,7,8-PeCDF are significantly lower (P < 0.001) in 2016-18 compared to values reported in 2010. In 2010, 2,3,4,7,8-PeCDF accounted for 82% of the total WHO-TEQ for PCDFs, however, in the current study, it contributes 78% of the total PCDF TEQ and indicates a statistically significant decrease (P < 0.001). Concentrations of 1,2,3,4,6,7,8-HpCDF have decreased by 68% since 2010, others such as 2,3,4,6,7,8-HxCDF have decreased by 93% since 2010, however concentrations of 1,2,3,4,7,8,9-HpCDF remain unchanged since 2010 but are 50% lower than values reported in 2002. As expected, greater decreases are observed when comparing concentrations in the current study with those reported in 2002, e.g., congeners such as OCDF have dropped by 88% and 2,3,4,6,7,8-HxCDF by 89%.

Apart from PCB-77 (significantly higher in 2016-18 P < 0.005), concentrations of dioxin-like PCBs are significantly lower in 2016-18, decreasing by 41% and 46% for non-ortho and mono-ortho PCBs, respectively, since 2010. Concentrations of most individual congeners have decreased from between 15% and 55% since 2010, with the greatest decreases observed for concentrations of PCB-169 since 2010 (56%) and since 2002 (63%). In contrast, concentrations of PCB-77 have increased by 65% over the same period.

Table 4. Comparison of arithmetic mean concentrations (ng kg^{-1} lipid weight) of individual PCDD/F and dioxin-like PCB congeners in the current study (2016-18) and previous Irish human milk surveys in 2002 and 2010 [Pratt *et al.*[15], (2012)]

PCDD/F	2002 (*n* = 4 pools)	2010 (*n* = 11 pools)	2016-18 (*n* = 16 pools)
2,3,7,8-TCDF	0.32	0.55	0.32
1,2,3,7,8-PeCDF	0.16	0.38	0.25
2,3,4,7,8-PeCDF	4.6	5.1	3.5
1,2,3,4,7,8-HxCDF	1.3	1.5	0.92
1,2,3,6,7,8-HxCDF	1.3	1.5	0.96
2,3,4,6,7,8-HxCDF	0.54	0.89	0.52
1,2,3,7,8,9-HxCDF	0.07	0.06	0.1
1,2,3,4,6,7,8-HpCDF	1.8	2.0	0.64
1,2,3,4,7,8,9-HpCDF	0.04	0.08	0.08
OCDF	0.44	0.17	0.09
2,3,7,8-TCDD	1.0	0.67	0.36
1,2,3,7,8-PeCDD	2.7	1.9	1.1
1,2,3,4,7,8-HxCDD	1.7	0.86	0.43
1,2,3,6,7,8-HxCDD	8.1	4.7	2.1
1,2,3,7,8,9-HxCDD	2.0	0.97	0.52
1,2,3,4,6,7,8-HpCDD	12	5.5	2.5
OCDD	63	40	20
WHO-PCDD/F-TEQ UB[a]	**6.7**	**5.3**	**3.1**
Non-ortho-PCB/ dl-PCB[b]			
PCB-81	1.3	1.0	0.85
PCB-77	3.1	3.1	5.1
PCB-126	20	21	12
PCB-169	18	15	6.8
WHO-Non-Ortho-PCB-TEQ UB[b]	**2.5**	**2.6**	**1.4**
Mono-ortho-PCB/ dl-PCBc UB[b]			
PCB-123	29	47	35
PCB-118	4700	4200	2400
PCB-114	250	230	130
PCB-105	1100	940	550
PCB-167	770	460	350
PCB-156	2300	1600	1000
PCB-157	450	350	220
PCB-189	180	150	93
WHO-Mono-Ortho-PCB-TEQ UB[b]	**0.29**	**0.24**	**0.14**
WHO-PCDD/F-PCB-TEQ UB[b]	***9.5***	**8.1**	**4.6**

[a]UB: upper bound; [b]dl-PCB: dioxin-like PCBs.

Concentrations of ∑ indicator PCBs have declined by 42% since 2010 and are 55% lower than values reported in 2002. Individual congeners such as PCB-28 has decreased by 56% while PCB-52 has increased by 44% over the same period.

Concentrations of WHO-TEQ PCDD/F+ dl-PCB in both Dublin and Galway pools are significantly lower ($P < 0.001$) in 2016-18 than in 2010 and do not display any regional variations. Similar declines in concentrations (over the period 2010-2016-18) of WHO-TEQ PCDD/F + dl-PCB are observed in both

Table 5. Arithmetic mean concentrations (×g/kg) of indicator PCB congeners detected in the current study (2016-18) and in previous Irish human milk surveys in 2002 and 2010 [Pratt *et al.*[15], (2012)]

Indicator-PCB	2002 mean (*n* = 4 pools)	2010 mean (*n* = 11 pools)	2018 mean (*n* = 16 pools)
PCB-28	1.1	1.0	0.44
PCB-52	0.19	0.09	0.13
PCB-101	0.35	0.2	0.2
PCB-153	19	17	11
PCB-138	18	13	5.9
PCB-180	16	9.3	6.4
∑ indicator PCBs g/kg UB[a]	***54***	**41**	**24**

[a]UB: upper bound

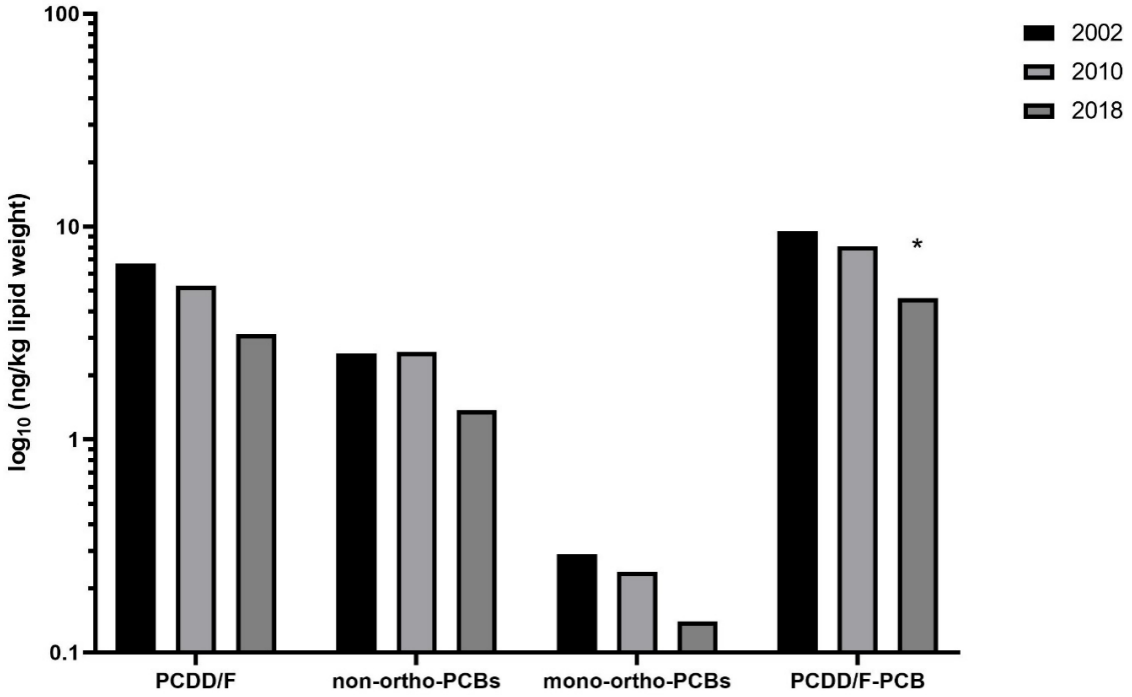

Figure 1. Comparison of mean WHO_{2005}/TEQ concentrations for PCDD/Fs, non-ortho-PCBs, mono-ortho-PCBs and ∑PCDD/F + dl-PCB (lipid weight, upper bound) in Irish breast milk from 2002 (*n* = 4), 2010 (*n* = 11) and 2016-18 (*n* = 16) surveys; *statistical significance between 2010 and 2018, *P* > 0.005. TEQ: Toxic equivalency; PCDD/F: polychlorinated dibenzo-p-dioxins and furans; PCB: polychlorinated biphenyls; dl: dioxin-like.

Dublin and Galway pools (41%-42%) compared to the full data set.

Nursing infants' dietary intake of PCDD/Fs and dl-PCBs

As described in **Estimation of infant intake of PCDD/F and PCBs via breast milk**, estimated daily intake values for infants were calculated using ∑PCDD/F TEQ, ∑PCB TEQ and ∑PCDD/F + PCB TEQ UB GM concentrations based on a daily intake value of 800 mL and 1200 mL breast milk as 14, 6.7, 20, and 21, 10 and 31 pg TEQ kg^{-1} bw per day, respectively [Supplementary Tables SI-7.1 and SI-7.2]. EFSA does not recommend that exposure data for breastfed infants be compared directly to the TWI of 2 pg TEQ kg^{-1} bw per week, as the TWI is based on the mother's intake that would result in concentrations in breast milk leading to a child serum level at age nine (7.0 pg WHO_{2005} TEQ/g fat) potentially causing adverse effects in

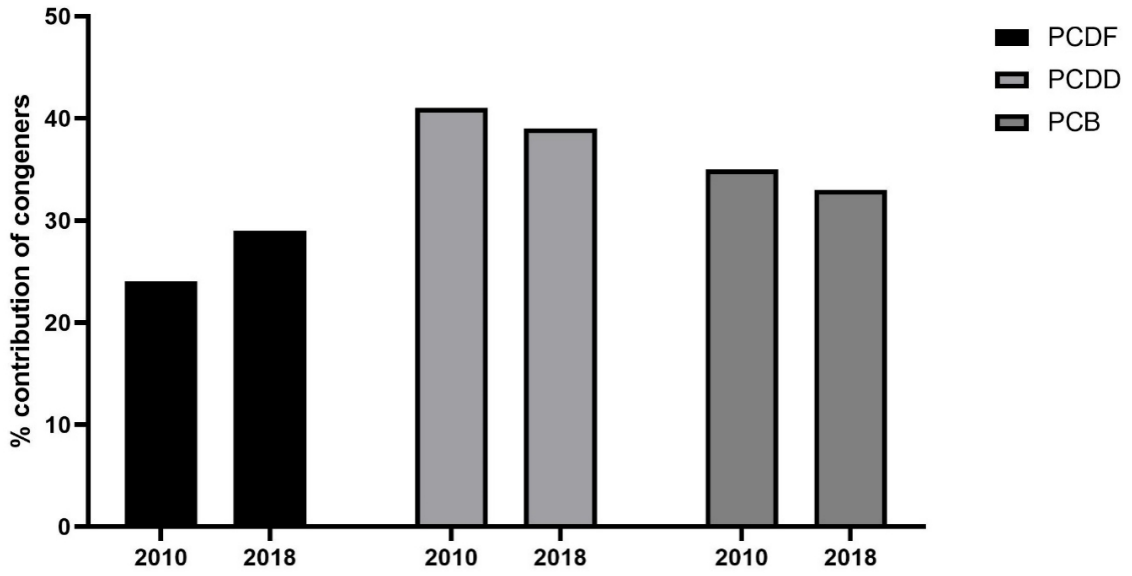

Figure 2. Comparison of percentage contribution of WHO_{2005}/TEQ concentrations for PCDF, PCDD and PCBs (lipid weight, Upper Bound) in Irish breast milk from 2010 (*n* = 11) and 2016-18 (*n* = 16) surveys. TEQ: Toxic equivalency; PCDF: polychlorinated dibenzofurans; PCDD: polychlorinated dibenzo-p-dioxins; PCB: polychlorinated biphenyls.

older children[10]. Instead, it is more appropriate to compare concentrations to 5.9 pg TEQ g^{-1} fat, the human milk concentration likely to result in the NOAEL serum level of 7.0 pg WHO_{2005} TEQ/g fat at age nine (EFSA, 2018). Reassuringly, our mean WHO PCDD/F + PCB TEQ concentrations are less than 5.9 pg TEQ g^{-1} fat. However, both the LB and UB concentrations reported for Pools 6 and 9 exceed 5.9 pg TEQ g^{-1} fat. While this exceedance for a small proportion of the population highlights the need for continued efforts to reduce human exposure to dioxin-like chemicals, we note and emphasise the position of WHO, that the positive benefits of breast feeding for both infant and mother significantly outweigh the health risks due to exposure[14].

CONCLUSION

This study reports concentrations of PCDDs, PCDFs, and PCBs in human milk from first-time mothers in Ireland, sampled from two different maternity hospitals, one in the West of Ireland (Galway) and one in the East of Ireland (Dublin). Concentrations reported are at the low end of those previously reported for Europe and elsewhere.

Significant declining temporal trends are observed when compared to previous Irish milk surveys from 2002 and 2010. There has been a 41% decrease in mean WHO-PCDD/F TEQ values, a 39% decrease in mean WHO-PCDD/F-PCB TEQ values, and a 45% decrease in mean indicator-PCB concentrations. This declining temporal trend concurs with findings elsewhere and likely reflects the beneficial impacts of the measures introduced over the past 16 years under Ireland's National Implementation Plan for POPs under the UNEP Stockholm Convention. WHO-TEF values are due to be re-evaluated in spring 2022, which may result in the lowering of the TEF values for some of the congeners, and in turn, the overall TEQ values.

DECLARATIONS

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Authors' contributions

Made substantial contribution to study design, participant recruitment, data analysis and interpretation, technical writing: Wemken N, Coggins MA, Harrad S, Tlustos C

Made substantial contributions to sample preparation, sample analysis, data analysis and interpretation, technical writing: Houlihan M

Made substantial contributions to participant recruitment and sample collection: Cellarius C, Cleere K, Morrison JJ, Daly S

Made substantial contributions to sample preparation, sample analysis, data analysis and interpretation: Keogh M, Tierney J, O'Riordain C, Noone C

Availability of data and materials

Extra data is published in the Supplementary data.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Study protocols and design were approved by the Clinical Research Ethics Committee of the Galway University Hospital (Ref: C.A. 1578) and the Research Ethics Committee of the Coombe Womens and Infants University Hospital in Dublin (No. 30e2016).

Consent for publication

Not applicable.

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