PCDDS/PCDFS IN HUMAN BREAST MILK FROM ARGENTINA

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Introduction

Due to its relatively high lipid content and non-invasively collection, human breast milk is very appropriate to monitor PCDD/Fs body burdens and has been selected as a preferred matrix in worldwide programs (WHO, 2007; UNEP, 2013). Moreover, breast milk enables the exposure assessment of newborns through lactation. In the last decades several breast milk monitoring programs have been coordinated with standardized sampling and analytical protocols in many countries[1,2,]. However, studies in South America have been scarce and mainly focused on organochlorine pesticides and polychlorinated biphenyls [4,5,6,7].

To the best of our knowledge, this is the first study to evaluate PCDD/Fs levels and congener composition in human breast milk from Argentina.

Methods

Breast milk samples were collected from 174 breast-feeding mothers in public health institutions from Buenos Aires, La Plata, Ensenada and Punta Lara cities in Buenos Aires province and Gualeguaychú city in Entre Ríos province from March 2009 to December 2011. Donors in the age range of 21-40 years were primi and multiparae mothers at different times of lactation. A questionnaire with relevant socioeconomic (i.e. education, occupation, monthly income, residence story), biologic (i.e. age, number of children, breast-feeding period of the donor), diet (fish, meat, vegetables, dairy products frequency consumption) and smoking habits information was completed. Informed consent was obtained from all of the donors.

Samples, collected in ultra-trace cleaned glass recipients with a manual silicone breast-pump, were conserved on ice during transportation and frozen immediately at -20 ˚C in laboratory until analysis. Twenty one pools were created to represent homogenous characteristic in terms of expected low-high exposure [8] and mother´s geographic-residential story [9]

The analytical procedures were based in method EPA 1613.Human milk samples were defrosted, homogenized, spiked with internal standards (LCS 1613; Wellington) and centrifuged at 3000 rpm for 25 minutes to separate the cream from the aqueous phase. The cream was freeze-dried and lipids were ultrasonically extracted with petroleum ether. Lipid content was determined gravimetrically. The extracts were concentrated under nitrogen and treated with sulfuric acid to partially remove the lipid material. The supernatant phase was concentrated under nitrogen and fractionated on successive column chromatography on silica gel and silica gel-charcoal [10]. The PCDD/F fraction was concentrated by gentle stream of dry nitrogen. The final extract (20 µl) was spiked with isotopic labeled internal standard (ISS 1613; Wellington) and then analyzed by high resolution gas chromatography coupled to high resolution mass spectrometer (Agilent 7890-Autospect Micromass Ltd UK). Chromatographic separation was achieved using a DB 5 MS fused silica column (30 m long, 0.25 mm i.d., 0.25 µm film). The mass spectrometer was operated under positive electron impact (35 eV) and selected ion monitoring (SIM) mode at resolving power of 10000 amu. The detection limits (3:1 signal versus noise value) ranged between 2-12 pg g lipid for tetra to octa PCDD/F. Recovery efficiency ranged 13-40% for each individual congener. Field and procedure blanks were analyzed for every batch of ten samples.

Seventeen 2,3,7,8 substituted PCDD/F congeners were quantitated and reported as total ($\sum_{17}$PCDD/F) and as Toxic equivalents (TEQs) using WHO equivalent factors[11] (TEFs).

Results and Discussion

$\sum_{17}$PCDD/F ranged between 39 to 268 pg g^{-1} lipid (average: 140 ± 52 pg g^{-1} lipid; Table 1). On a global context, these values are higher than those reported for human milk from Italy [12](99-131 pg g^{-1}) and France[13].The profile of PCDD/F was dominated by OCDD (56%); 1,2,3,6,7,8-HxCDD (20%);1,2,3,4,6,7,8-HpCDD (7%) and 1,2,3,4,6,7,8-HpCDF (6%). This pattern is normally found in breast milk samples [14,15].

Total TEQs in the 21 pools ranged from <1 to 37 pg g^{-1} lipid (average: 14 ± 12 pg g^{-1} lipid), basically contributed by three congeners: 1,2,3,7,8 PCDD (40-79 %), 1,2,3,6,7,8 HxCDD (6.6-87 %), and 2,3,4,7,8 PCDF (6.3-27 %).

TEQ concentrations found in this study compare well with the values reported in the WHO/EURO 2002–2003 dioxin exposure study[16], where PCDD/F-TEQs among 100 pooled samples from the 26 countries/regions ranged from 2.7 pg TEQ g^{-1} lipid (Brazil) to 51.5 pg TEQ g^{-1} lipid (Egypt). Similarly, Wong et al., 2013[17] found a comparable range of TEQ in human milk from Southern Taiwan (4.2-53 pg TEQ g^{-1} lipid).
Considering the socio-economic and biological information, there was no evident relationship among residence sites, born sites and $\sum_{17}$PCDD/F concentrations. In contrast, $\sum_{17}$PCDD/F TEQ concentrations showed differences respect to residence sites. The lowest TEQ concentration values correspond to small coastal cities (Punta Lara and Ensenada: 1.0±1.7 pg TEQ g^{-1} lipid, <55000 inhabitants), followed by a small rural site (Saladillo: 10.9 pg TEQ g^{-1} lipid, <35000 inhabitants), mid coastal-urban (Gualeguaychu: 15.5 pg TEQ g^{-1} lipid, ~100000 inhabitants) and larger urban sites (La Plata, Florencio Varela, Buenos Aires city and metropolitan area: 17.6±13.4 pg TEQ g^{-1} lipid, >400000 inhabitants). The whole data set insinuate a positive relationship between maternal age and $\sum_{17}$PCDD/F but with very low significance (R^2: 0.14). The correlation improves considerably removing variability introduced by residence sites. Effectively, for Buenos Aires which is the most represented area (11 from 21 pools), the maternal age correlated positively with $\sum_{17}$PCDD/F (R^2=0.42; Figure 1), reflecting the significance of time-accumulated exposure on PCDD/F body burdens.
According to our results, the estimated daily intake (EDI) of PCDD/F for a 5 kg infant [18] average 50±46 pgTEQ/kg body weight. This value is lower than EDIs reported for Belgium [19](76 pgTEQ/kg body weight) and Korea [20](103 pgTEQ/kg body weight), similar to data from Spain[21] (35 pgTEQ/kg body weight), and 5 times higher than EDIs reported for Taiwan [22,23](11-13 pgTEQ/kg body weight, respectively). As is normally observed, these EDI concentrations are significantly higher that the adult whole life exposure tolerable daily intake from WHO (TDI: 4 pgTEQ/kg body weight). These results stress the importance of monitoring studies to identify and reduce predominant exposure pathways of PCDD/Fs.

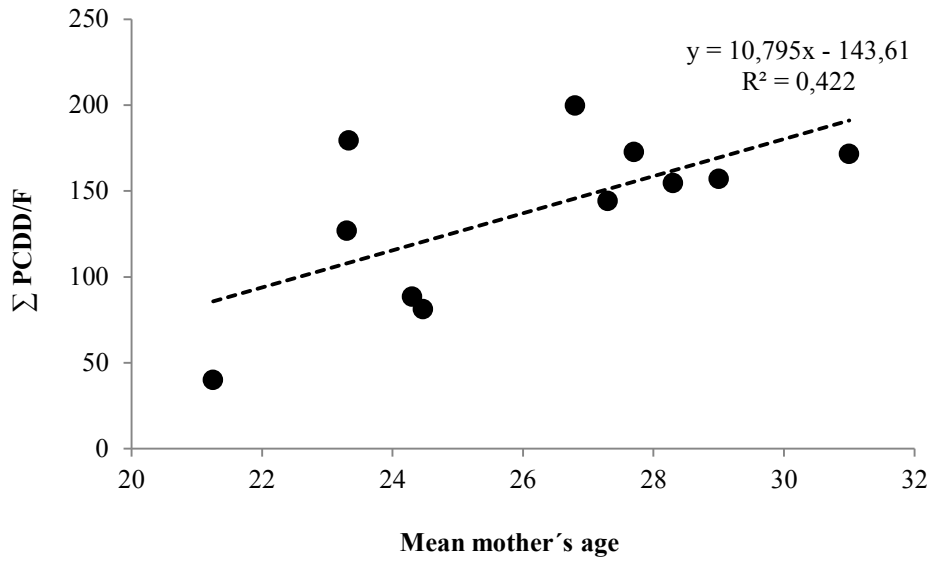

Figure 1. $\sum_{17}$PCDD/F concentration in breast milk versus mother's age from Buenos Aires donors.

Table 1. Breast milk pools details

Pool	Geographic classification			N	Age	Lipid	PCDD/F	TEQ
	Country/Region of birth		Residence		Years	%	pg g^{-1} lipid	
1	Argentina	Buenos Aires province	Saladillo	10	21-37	3.0±1.3	155.7	10.9
2	Argentina	Buenos Aires province	Punta Lara	2	18-24	2.8±3.0	206.2	0.1
3	Argentina	Buenos Aires province	Punta Lara	3	24-28	3.8±1.6	117.4	3.1
4	Argentina	Buenos Aires province	Punta Lara	12	21-40	2.2±0.1	121.6	9.6
5	Argentina	Buenos Aires province	Ensenada	5	21-42	2.7±1.1	84.8	0.03
6	Argentina	Buenos Aires province	Gualeguaychú	6	21-32	1.7±1.6	135.3	7.5
7	Argentina	Buenos Aires province	Gualeguaychú	23	21-35	2.71±1.2	69.2	21.6
8	Argentina	Buenos Aires province	La Plata	4	20-38	2.3±1.6	267.9	17.2
9	Argentina	Buenos Aires province	Florencio Varela	13	18-38	2.0±1.4	124.5	18.1
10	Argentina	Buenos Aires province	Florencio Varela	2	21 -37	2.3±0.8	139.6	37.4
11	Argentina	Northeast	AMBA	3	21-40	3.8±3.0	154.6	26.1
12	Argentina	Northwest	AMBA	8	22-35	2.6±1.0	199.7	7.0
13	Peru		AMBA	3	22 Y 37	3.2±2.0	171.6	0.1
14	Peru		AMBA	7	21-30	2.3±1.4	126.8	15.0
15	Paraguay		AMBA	3	20-32	1.4±1.3	88.5	3.1
16	Paraguay		AMBA	15	21-40	2.1±1.1	144.1	23.3
17	Paraguay		AMBA	4	26 Y 40	2.1±1.5	172.6	31.1
18	Paraguay		AMBA	3	27-31	2.4±1.5	157.1	1.4
19	Argentina	Buenos Aires province	AMBA	6	19-27	2.1±1.4	179.5	36.0
20	Argentina	Buenos Aires province	AMBA	4	18-29	2.5±0.7	39.9	4.0
21	Argentina	Buenos Aires province	AMBA	23	18-38	3.0±1.4	81.3	14.7

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