LEVES OF PCDD/Fs AND DL-PCBs IN PLANCENTA FROM A GENERAL POPULATION IN SPAIN

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Introduction

There is evidence that polychlorinated dibenzo-p-dioxinas (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) may adversely affect the health of wildlife and humans[1]. Humans are exposed daily to complex mixtures of PCDD/Fs and PCBs mainly via trace amounts present in food[2]. A variety of toxic effects in experimental animals exposed to these chemicals, including immunologic, neurochemical, neurotoxic, carcinogenic and endocrine changes have been reported[3]. One of the most significant concerns regarding health effects is the influence of these chemicals on future generations, stemming from prenatal and/or postnatal exposure. Pregnant and nursing women pass these pollutants to their babies both trans-placentally and lactationally[4].

Previous biomonitoring of dioxins and PCBs focused mainly on levels in human samples like serum and breast milk. Although levels in blood and breast milk can represent human body burden and postnatal exposure, levels in placenta provide a measure of the exposure of the fetus[5]. They are few studies on prenatal exposure to PCDD/Fs and dioxin-like PCBs (DL-PCBs). Several studies of lower-level PCBs exposure during pregnancy observed associations with decreased birth weight and other growth parameters[6]. Exposure of pregnant women to these chemicals can lead to toxic effects on mothers and to in utero exposure of the fetus by blood circulating through the placenta.

The main objective of this study was to report the levels of PCDD/Fs and DL-PCBs in placenta samples from INMA "INfancia y Medio Ambiente" (Environment and Childhood) Project; a prospective population-based cohort study in Spain. Results were compared with data from recent studies from different geographical locations.

Materials and methods

50 placenta samples were randomly selected for the INMA study from the population living in five different areas (10 samples in each one): Basque country, Sabadell, Granada, Asturias and Valencia. (Figure 1). A common characteristic of these areas is that the vast majority of the population attends the public health sector. Information from pregnant woman was collected (socio-demographical and occupation characteristics, smoking habits, and maternal diet).

At the laboratory, individual placenta samples were homogenized and freeze-dried s a pretreatment steps to the extraction of the analytes. For PCDD/F and DL- PCB analysis, samples were extracted in a Soxhlet for ~24h with toluene:cyclohexane (1:1) after being spiked with known amounts of mixtures of $^{13}C_{12}$-PCDD/Fs (EPA-1613LCS, Wellington Lab., Guelph, Canada) and $^{13}C_{12}$-DL-PCBs (WP-LCS, Wellington Lab., Guelph, Canada). Next, the extracts were rotary evaporated and kept in an oven overnight (105 ºC) in order to eliminate the solvents prior to gravimetric fat determination. Afterwards, fat residues were redissolved in *n*-hexane. Organic components, fat and other interfering substances were removed by treating the *n*-hexane extracts with silica gel modified with sulphuric acid (44%). The extracts were then rotary concentrated and filtered prior to the next clean-up step. Further sample purification and instrumental analysis by high resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) are described elsewhere[7]. High resolution gas chromatography coupled to high resolution mass spectrometry (HRGC-HRMS) was used for the final instrumental analysis. All analyses were performed on a Trace GC ultra gas chromatograph (Thermo Fisher Scientific, Milan, IT) fitted with a 60m x 0.25 mm i.d. x 0.25 µm film thickness DB-5ms fused silica column (J&W Scientific, CA, USA) coupled to a high resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany) controlled by a Xcalibur data system. Positive electron ionization (EI+) operating in the MID

mode at 10 000 resolving power was used. Quantification was carried out by the isotopic dilution method. Fat determination was performed by gravimetric methods. The criteria for ensuring the quality of dioxin analysis include the application of quality control (QC) and quality assurance (QA) measures, such as continuous monitoring of laboratory contamination based on the determination of a blank sampled throughout the whole analytical procedure, including extraction, clean-up and quantification[8].

Results and discussion:

Table 1 show the concentrations of PCDD/Fs and DL-PCBs in placenta samples for all locations studied. For PCDD/Fs, in terms of WHO-TEQ concentration, the highest levels were found in a sample from Asturias with a value of 20.13 pg WHO-TEQ/g fat, followed by two samples from Basque Country, while the lowest levels were found in a sample from Basque Country. For the case of DL-PCBs, a similar trend was also observed, being a sample from Asturias that showed the highest concentration for these compounds, expressed as total WHO-TEQ concentration (13.13 pg WHO-TEQ/g fat).

As an example Figure 2 show a HRGC-HRMS chromatograms for PCDD/Fs obtained from a placenta sample. As we can observe, in placenta samples the concentration of PCDD/Fs is higher than furans, being the octachloro dioxin the highest concentrated congener. This fact was also observed in serum and whole blood samples analyzed in our laboratory. All the non-toxic congeners in both families were below detection limit (LOD) in all placenta samples.

Levels of chemical residues found in this study were notably lower than those obtained by a Japanese study of 21 placentas, 34.2 pg WHO-TEQ/g lipid[9] and to those found in subjects in a study in domestic areas with ages between 24 and 37 years old that reported a median value of 22.1 pg WHO-TEQ/g lipid[10]. However, these levels are similar to those found in subjects in a study in Taiwanese woman's reporting a median value of 13.6 pg WHO-TEQ/g lipid (n=53)[11].Although determinants of exposure –location, age, parity, lactation, BMI and diet, among others- are still under study, in the meantime, we would recommend placentas as an appropriate tissue for exposure assessment in mother-child cohorts because of technical feasibility and biological significance.

Acknowledgements:

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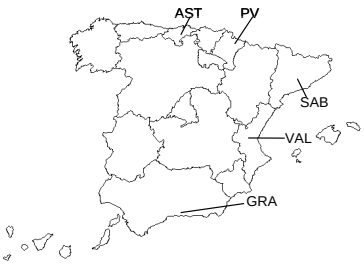

Figure 1. Sampling locations.

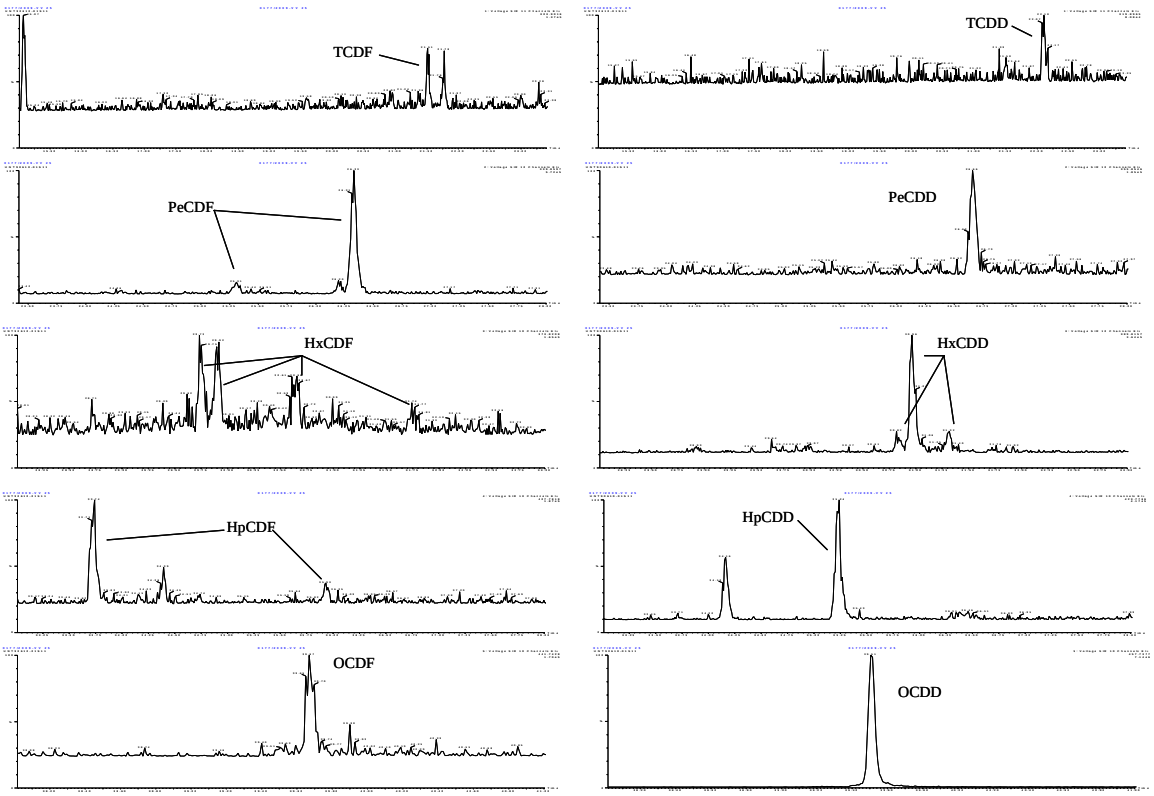

Figure 2. PCDD/Fs HRGC-HRMS chromatogram of a placenta sample

Table 1. Concentration of PCDD/Fs (pg WHO-TEQ/g fat) in placenta samples from general population living in Spain.

	PCDD/F	DL-PCBs		PCDD/F	DL-PCBs
	pg WHO-TEQ/ g lipid	pg WHO-TEQ/ g lipid		pg WHO-TEQ/ g lipid	pg WHO-TEQ/ g lipid
PV1	2,51	1,16	VAL1	7,31	3,32
PV2	6,9	4,44	VAL2	4,99	1,36
PV3	7,46	4,52	VAL3	8,11	2,99
PV4	5,23	2,81	VAL4	5,38	2,25
PV5	15,51	6,23	VAL5	4,65	1,88
PV6	10,65	5,31	VAL6	12,2	2,5
PV7	9,58	5,79	VAL7	9,12	4,16
PV8	8,07	13,13	VAL8	4,42	1,95
PV9	11,14	3,36	VAL9	5,33	1,61
PV10	15,83	6,7	VAL10	5,82	2,31
AST1	5,94	2,49	SAB1	7,81	3,06
AST2	6,94	3,95	SAB2	5,32	2,07
AST3	7,71	2,68	SAB3	4,96	1,74
AST4	7,86	3,16	SAB4	7,86	3,09
AST5	6,49	2,17	SAB5	13,64	5,93
AST6	7,11	3,8	SAB6	9,11	2,88
AST7	7,22	1,68	SAB7	11,1	2,48
AST8	20,13	4,21	SAB8	9,25	4,13
AST9	9,07	6,01	SAB9	12,2	3,67
AST10	8,76	5,61	SAB10	11,86	5,06
GRA1	8,62	1,55			
GRA2	7,22	1,86		PV: BASQUE COUNTRY	
GRA3	5,39	1,63		AST: ASTURIAS	
GRA4	2,93	0,81		GRA: GRANADA	
GRA5	2,86	1,31		VAL: VALENCIA	
GRA6	6,12	2,36		SAB: SABADELL	
GRA7	3,34	1,53			
GRA8	7,3	2,51			
GRA9	4	2,31			
GRA10	4,29	1,47			