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RESEARCH ARTICLE

Polychlorinated biphenyls and their hydroxylated metabolites in placenta from Madrid mothers

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Abstract
Introduction Concentrations and congener profiles of polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in placenta samples from a Madrid population (Spain) are reported. Structure dependent retentions of OH-PCBs are known to occur in both humans and wildlife, making it of interest to assess placental transfer of both parent compounds and their metabolites to the developing foetus.
Results The ΣPCB concentrations found in placenta samples were in the range 943–4,331 pg/g fresh weight (f.w.), and their hydroxylated metabolites showed a 20-time lower concentration level (53–261 pg/g f.w.). The PCB profiles were surprisingly dominated by CB-52 and CB-101 accounting for more than 44% of the total PCB concentration. This is indicating a source of exposure that is not yet identified. The OH-PCB profiles were dominated by 4-OH-CB187 and 4-OH-CB146, representing >50% of the ΣOH-PCB concentration of the placenta samples. Statistical analysis of the data revealed strong correlations between the PCB congeners, among some OH-PCBs, and between OH-PCB metabolites with a *meta*- and *para*- substitution pattern. Both PCB and OH-PCB concentrations presented homogeneous distribution, what allowed the establishment of a partial least squares model that correlated the concentrations of OH-PCB with those of PCBs in placenta samples. In addition, causal correlations were observed between the concentrations of OH-PCBs and those of their corresponding PCB precursors.

Keywords PCBs · OH-PCBs · Placenta · Maternal transfer · Multivariate regression

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1 Introduction

Polychlorinated biphenyls (PCBs) are environmental contaminants of great concern due to their persistency (P), bioaccumulativity (B), and toxicity (T). PCBs are, due to their P and B characteristics, ubiquitous environmental contaminants. The lipophilicity of these compounds and their ability to biomagnify by living organisms make species at the highest positions in food chains, such as humans, to be at potential risk for adverse effects. In general, PCBs, as other persistent organic pollutants (POPs), are metabolised leading to polar metabolites which as such are more easily excreted. PCBs are metabolised by hepatic microsomal oxidases to hydroxylated metabolites (OH-PCBs) which are eliminated via urine or excreta (Letcher et al. 2000). However, studies have shown that some OH-PCB metabolites are strongly bound to plasma proteins, both in wildlife and in humans (Gutleb et al. 2010; Letcher et al. 2000; Sandau et al. 2000). Further, some authors have established that OH-PCBs present agonist or

antagonist interactions with hormone receptors and/or induce hormone-receptor-mediated responses (Braathen et al. 2009; Mortensen et al. 2007) and are able to completely saturate the thyroid hormone transport capacity (Gutleb et al. 2010). So, it is important to consider that the toxic effects observed in a particular individual exposed to PCBs are due to both the toxicity of the PCBs themselves and the toxicity of the metabolites generated by the individual. Therefore, PCB's toxicity is not only due to PCB concentrations but is also due to the presence of PCB metabolites in the same individual (Haraguchi et al. 1998). All of this demonstrates that PCB metabolites should be considered as a secondary class of contaminants of concern.

So, it is important to investigate the accumulation patterns of PCBs and those of their metabolites in tissues that have not been very well studied, such as human placenta, mainly when previous studies have shown that POPs, such as PCBs and PBDEs, are able to cross the placenta barrier (Covaci et al. 2002; Gómara et al. 2007) reaching the foetus. In addition, to investigate the different ratios of PCBs and OH-PCBs in the placental tissue could reveal the possible differences in placental transfer of both parent contaminants and their metabolites, as it has previously been pointed out by other authors by means of the measurement of mother/foetus ratios for serum samples (Guvenius et al. 2003; Kawashiro et al. 2008; Park et al. 2007, 2008; Soechitram et al. 2004).

Placenta plays an important role as an endocrine organ in the development of the foetus and, for this reason, the presence of endocrine disruptor compounds in placenta samples is a cause of concern. Placenta is a two-way monitor and controller of flux for contaminants since different contaminants could diffuse in a different way through the placental membrane and present different behaviours in the maternal and foetal sides due to the presence of different enzymes and transporters in each side (Myllynen et al. 2005). In addition, the influence of the placenta in foetal thyroid hormone supply should increase the concern for the exposure to endocrine-disrupting compounds that affect the thyroid hormones, such as PCBs and their metabolites (Chan et al. 2009). Besides, it is important to highlight that placenta are non-destructive matrices adequate for monitoring human exposure to PCBs, their metabolism and transfer from mother to the foetus through the placenta barrier. There are few published data concerning PCB concentrations in placental samples (Schecter et al. 1998; Wang et al. 2004), but there is no information about their metabolites in this type of samples. Several papers could be found in the literature regarding PCB metabolites in blood and/or serum samples (Axmon et al. 2008; Covaci et al. 2002), and some authors have studied the placental transfer of PCBs and OH-PCBs by means of the correlation between their concentrations in the cord and in the maternal serum (Park et al. 2008; Soechitram et al. 2004). However, neither of them have determined the concentration levels in such placentas.

The main objective of the present work is to study levels and the relationship between concentrations of PCBs and their hydroxylated metabolites (OH-PCBs) in placenta collected from a group of women living in the Community of Madrid (Spain) and to report, for the first time, the concentration levels and accumulation profiles of OH-PCB metabolites in placental samples from the Spanish population. The study includes statistical analysis for the development of a predictive model to estimate concentrations of OH-PCBs in placenta samples based on their PCB concentrations.

2 Materials and methods

2.1 Study design and sampling collection

The study design and the sampling collection were conducted by the public health authorities of the Community of Madrid and the Institute of Health Carlos III (Madrid, Spain). Among all the samples collected between October 2003 and May 2004 from volunteers living in the metropolitan belt of the Community of Madrid, a total of 17 placentas were analysed for PCBs and their hydroxylated metabolites (OH-PCBs).

Placenta samples were obtained after the delivery, kept frozen and, once at the laboratory, freeze-dried and stored at room temperature until analysis. The goals and requirements of the study were explained to all participants, and donors were asked to participate and to sign a consent form. Pregnancies were full term, and no medical problems were reported during pregnancy. All mothers were healthy, primiparas, and the mean age was 30.5 years. None of the donors reported any work-related potential for exposure to POPs. The sample collection was approved by the local committee of medical ethics.

2.2 Reagents and standards

All solvents, acids and salts used for the analyses were of the highest quality available and were obtained from Merck Co. (Darmstadt, Germany). Silica gel, 200–400 mesh, 60 Å, was purchased from Sigma–Aldrich (USA). The synthesis of diazomethane, employed for the methylation of the OH-PCB fraction, was carried out at the laboratory following the method previously described by Sandau (2000) and using *N*-nitroso-*N*-methylurea (Sigma–Aldrich) as precursor compound. All analytical standards were either purchased from Cambridge Isotope Laboratories (Andover, MA, USA) or prepared in house (Bergman et al. 1995).

A total of 15 PCB congeners and 13 OH-PCBs were studied in the 17 samples selected for the study. CB-200 and 4-OH-CB193 (4-hydroxy-2,3,3′,4′,5,5′,6-heptaCB) were employed as internal standards for the PCB and OH-PCB quantification, respectively. Injection standard, added to the vials prior to instrumental determination for recovery calculations, was octachloronaphthalene for both families of compounds. The different PCB and OH-PCB congeners analysed are presented in Table 1; the abbreviated names used in the present paper, according to Letcher et al. (2000), and their chemical structures are given of all of them.

2.3 Sample treatment

The method used for extraction and clean-up of the placenta samples is a slight modification of a method described elsewhere (Hovander et al. 2000) for serum samples. Briefly, the freeze-dried placenta samples (1 g) were reconstituted using 10 mL of Milli-Q water, spiked with the internal standards and mixed on a vortex mixer overnight. The method was based on the denaturalisation of the proteins of the sample using hydrochloric acid and 2-propanol and the later liquid–liquid extraction of the analytes from the matrix using a mixture of *cyclo*-hexane/methyl-*tert*-butyl ether (1:1, *v*/*v*). Lipid content was determined gravimetrically by means of the evaporation of the extraction solvent under a gentle stream of nitrogen. Afterwards, several liquid–liquid extractions were carried out in order to separate the neutral and the phenolic compounds using an ethanolic solution of potassium hydroxide (0.5 M in 50% ethanol). The OH-PCB fraction was methylated using diazomethane to obtain MeO-PCB derivates (Hovander et al. 2000) for their subsequent GC analysis. The clean-up of the two fractions (methylated OH-PCBs and PCBs) was carried out using multilayer columns filled with different amounts of neutral activated silica gel and activated silica gel modified with potassium hydroxide and sulphuric acid as described elsewhere (Hovander et al. 2000; Linderholm et al. 2007). The final fractions were evaporated under a gentle stream of nitrogen before the addition of the injection, standards previously mentioned, and the injection of the extracts in the different GC systems.

Table 1 Abbreviation and chemical structure of the different compounds investigated in this study

Abbreviation	Structure
PCB 28	2,4,2,4′-trichlorobiphenyl
PCB 52	2,2′,5,5′-tetrachlorobiphenyl
PCB 101	2,2′,4,5,5′-pentachlorobiphenyl
PCB 105	2,3,3′,4,4′-pentachlorobiphenyl
PCB 118	2,3′,4,4′,5-pentachlorobiphenyl
PCB 128	2,2′,3,3′,4,4′-hexachlorobiphenyl
PCB 138	2,2′,3,4,4′,5-hexachlorobiphenyl
PCB 146	2,2′,3,4′,5,5′-hexachlorobiphenyl
PCB 153	2,2′,4,4′,5,5′-hexachlorobiphenyl
PCB 156	2,3,3′,4,4′,5-hexachlorobiphenyl
PCB 170	2,2′,3,3′,4,4′,5-heptachlorobiphenyl
PCB 180	2,2′,3,4,4′,5,5′-heptachlorobiphenyl
PCB 183	2,2′,3,4,4′,5′,6-heptachlorobiphenyl
PCB 187	2,2′,3,4′,5,5′,6-heptachlorobiphenyl
PCB 189	2,3,3′,4,4′,5,5′-heptachlorobiphenyl
4-OH-CB107	4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl
4′-OH-CB120	4-hydroxy-2′,3,4′,5,5′-hexachlorobiphenyl
4′-OH-CB130	4-hydroxy-2,2′,3,3′,4′,5-hexachlorobiphenyl
3′-OH-CB138	3-hydroxy-2,2′,3′,4,4′,5-hexachlorobiphenyl
4-OH-CB146	4-hydroxy-2,2′,3,4′,5,5′-hexachlorobiphenyl
3-OH-CB153	3-hydroxy-2,2′,4,4′,5,5′-hexachlorobiphenyl
4′-OH-CB159	4-hydroxy-2′,3,3′,4′,5,5′-hexachlorobiphenyl
4′-OH-CB172	4-hydroxy-2,2′,3,3′,4′,5,5′-heptachlorobiphenyl
4′-OH-CB178	4-hydroxy-2,2′,3,3′,5,5′,6′-heptachlorobiphenyl
3′-OH-CB180	3-hydroxy-2,2′,3′,4,4′,5,5′-heptachlorobiphenyl
3′-OH-CB187	3-hydroxy-2,2′,3′,4,5,5′,6′-heptachlorobiphenyl
4-OH-CB187	4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl
3′-OH-CB188	3-hydroxy-2,2′,3′,4,5′,6,6′-heptachlorobiphenyl

2.4 Instrumental determination of PCBs and methylated OH-PCBs using GC-ECD

The 15 PCBs and the 13 OH-PCBs (previously methylated) selected were identified and quantified by GC coupled to an electron capture detector (ECD) (Varian 3800, CA, USA) and equipped with a CP-8410 auto-sampler. A low bleed GC capillary column CP-Sil 8-CB (25 m×0.15 mm i.d., 0.12 μm film thickness), purchased from Varian, was used for the separation, and hydrogen was employed as the carrier gas at a constant head pressure of 31 psi. The temperature of the ECD was set at 360°C and a make-up flow of 28 mL/min of nitrogen was fixed.

For PCB fraction, standards and samples were injected using a hot splitless injector (0.8 μL, at 240°C; splitless time, 2.0 min) applying a pressure pulse of 41 psi during 2.50 min. The column temperature was set initially at 80°C (2.5 min) and then raised at 20°C/min to 300°C (5 min). On the other hand, for methylated OH-PCB analyses, the standards and sample extracts were injected into a programmable-temperature vaporizing injector in hot splitless mode (4 μL, programmed at 64°C, hold for 0.35 min and then raise to 280°C at 200°C/min; splitless time, 2.5 min) applying a pressure pulse of 41 psi during 2.51 min. The column temperature was programmed as follows: 80°C (2.5 min) at 50°C/min to 200°C, then at 1°C/min to 230°C and then at 100°C/min to 330°C (1 min).

Standard solutions containing all the PCBs and OH-PCBs selected for the study plus the internal and injection

standards chosen for both families of compounds were prepared and injected in the GC-ECD in order to establish the retention time of all the compounds and to construct the calibration curves for the quantification of the sample extracts. Identification and quantification of the analytes in the samples were made in base on relative retention times and relative response (area) factors against the internal standards added at the beginning of the sample treatment.

2.5 Quality control criteria

Analyses of standard solutions and procedure blanks were done in batches and internal standards (prior to the extraction step), and injection standards (prior the instrumental determination for recovery calculations) were used, in order to comply with the adequate QA/QC for the determinations of PCBs and their metabolites in real-life samples. Satisfactory intermediate precision was achieved when analysing standard solutions, obtaining relative standard deviations (RSDs) between 1% and 8% for all congeners investigated. Recoveries of the internal standards added at the beginning of the extraction step were always higher than 70% for both PCB and OH-PCB fractions. Procedure blanks were prepared using 10 mL of a solution of 1% of potassium chloride in Milli-Q water. Each procedure blank was spiked with the internal standards, vortexed overnight and extracted and fractionated as the placenta samples. A procedure blank was analysed with each batch of four samples, and no methodology interferences were found.

2.6 Statistical analysis

Statgraphics Centurion 15.1 (Statpoint Technologies Inc., Warrenton, VA, USA) and The Unscrambler 9.6 (CAMO Software AS, Oslo, Norway) programmes were used for statistical data analysis and regression procedures. The PCB and OH-PCB congeners that presented a concentration in placenta samples below the limit of detection in more than 60% of the samples were not considered for the statistical analyses (i.e. 3′-OH-CB188 and 4′-OH-CB159).

3 Results and discussion

3.1 Concentration levels and profiles of PCBs and their hydroxylated metabolites (OH-PCBs) in human placenta samples

Concentrations of the 15 PCB and 13 OH-PCB congeners, expressed as median and concentration range and mean ± standard deviation, in picogrammes per gramme of fresh weight (pg/g f.w.), in the human placenta samples analysed are summarised in Tables 2 and 3, respectively. Although in the present paper, the results are expressed in picogrammes per gramme of fresh weight, the lipid content was determined for each sample obtaining a mean value of 0.81% (median of 0.84%) of lipids in the placenta samples (RSD=13%). The minimum lipid content was 0.49%, and the maximum was 0.92%.

Table 2 Concentrations of PCBs (expressed in picogrammes per gramme fresh weight) in the placenta samples from the Spanish population

	Median (n=17)	Range	Mean±SD
PCB 28	169	45–294	169±80
PCB 52	658	81–1,530	638±386
PCB 101	409	56–1,231	488±312
PCB 118	100	35–189	112±54
PCB 146	51	29–126	59±26
PCB 153	258	140–664	309±132
PCB 105	56	22–164	74±41
PCB 138	198	105–451	226±94
PCB 187	93	51–229	103±48
PCB 183	38	16–215	56±48
PCB 128	12	4.6–38	14±8.3
PCB 156	26	13–62	31±15
PCB 180	150	96–335	182±76
PCB 170	70	38–153	82±34
PCB 189	1.4[a]	ND[a]–3.5	1.6±1.2
Total PCBs	2,292	943–4,331	2,546±1,058

[a] For the calculations of the median values, ND was replaced by 0

ND non detected

Table 3 Concentrations of OH-PCBs (expressed in picogrammes per gramme fresh weight) in the placenta samples from the Spanish population

	Median (n=17)	Range	Mean ± SD
4′-OH-CB120	0.38[a]	ND[a]–4.9	1.0±1.4
4-OH-CB107	6.1	1.3–22	7.5±5.5
3′-OH-CB188	ND[a]	ND[a]–1.3	0.089±0.32
3-OH-CB153	12	2.2–35	14±8.6
4-OH-CB146	24	12–56	28±13
3′-OH-CB138	12	1.8–30	11±6.4
4′-OH-CB130	ND[a]	ND[a]–5.9	0.51±1.4
3′-OH-CB187	4.3[a]	ND[a]–18	5.0±4.0
4′-OH-CB178	2.2[a]	ND[a]–7.7	3.2±2.3
4-OH-CB187	50	22–87	53±18
4′-OH-CB159	ND	–	–
3′-OH-CB180	3.3	0.40–10	4.0±2.5
4′-OH-CB172	8.7	4.6–18	9.7±3.3
Total OH-PCBs	123	53–261	137±54

[a] For the calculations of the median values, ND was replaced by 0

PCBs showed higher concentrations than OH-PCBs in all the samples analysed in this study. Regarding PCBs, all of the 15 congeners were detected in all placenta samples, except CB-189. This PCB congener had concentrations below the limit of detection in two of the 17 samples. The most abundant congener was CB-52, contributing 25% to the total PCB concentration, followed by CB-101 (19%) and CB-153 (12%). The contribution of the other PCB congeners was between 1% and 8% except for CB-128 and CB-189, which were the less abundant congeners, contributing 0.6% and 0.1% to the total concentration, respectively. This profile, with CB-52 and CB-101 as the major contributors, is rare in species that are at high levels of the food chains, such as humans. Both these PCB congeners are very rapidly metabolised in a living organism (Boon et al. 1989; McFarland and Clarke 1989), so this pattern must indicate recent exposure to PCBs containing low chlorinated PCB congeners. However, to the best of our knowledge, there is no specific source of exposure to PCBs close to where the sampled individuals lived. Therefore, it is not possible to explain the PCB profile determined in the placentas from these women. Studies aimed to assess concentrations of brominated flame retardants in different types of human samples have also shown that the placenta presents a completely different profile to the one observed in serum samples (Gómara et al. 2007).

Seven of the 13 OH-PCB congeners were detected in all the samples analysed, i.e. 4-OH-CB107, 3-OH-CB153, 4-OH-CB146, 3′-OH-CB138, 4-OH-CB187, 3′-OH-CB180 and 4′-OH-CB172, and three of them (4′-OH-CB120, 3′-OH-CB187 and 4′-OH-CB178) were above the limit of detection in almost all the samples. On the other hand, 4′-OH-CB159 was not detected in any of the placenta samples; 3′-OH-CB188 was only detected in two samples, and 4′-OH-CB130 was detected in seven. Taking into account the contribution of each congener to the total OH-PCB concentration, two congeners were the major contributors, 4-OH-CB187 (38%) and 4-OH-CB146 (21%), accounting for more than half of the total concentration. The rest of the congeners, except 3′-OH-CB188 (0.1%), 4′-OH-CB130 (0.4%) and 4′-OH-CB120 (0.8%), contributed between 2% and 10% to the total OH-PCB concentration.

These results show that the most abundant hydroxylated metabolites in placenta samples were those that come from generally abundant PCB congeners in both commercial mixtures and biotic samples, such as 3-OH-CB153, 4-OH-CB146 and 3′-OH-CB138 (which come from PCBs 138 and 153) and 3′-OH-CB180 and 4′-OH-CB172 (which come from PCB congeners 180 and 170). The case of 4-OH-CB187 should be highlighted, in which the PCB precursor is not an abundant congener, neither in commercial PCB mixtures nor in biotic samples. The explanation for the high concentration found for this metabolite in the placenta is not clear, but this fact has been also observed by other authors in human samples, such as maternal and cord serum (Guvenius et al. 2003; Kawashiro et al. 2008; Park et al. 2008) and breast milk (Guvenius et al. 2003). However, in other human tissues such as the umbilical cord (Kawashiro et al. 2008), liver and adipose tissue (Guvenius et al. 2002), the trend changes, and the most abundant congener is 4-OH-CB146.

It has been previously described in the literature that several POPs can cross the placenta barrier due to the binding of these compounds to transthyretin (TTR), a thyroid hormone transport protein capable of crossing the placenta (Gutleb et al. 2010), so the specific interaction between TTR and each group of POPs would be mainly responsible of their transfer and/or accumulation in the placenta. Some authors have studied the placental transfer of PCBs and their hydroxylated metabolites by means of the measurement of their concentrations in maternal and foetus serum and the calculation of the ratios of cord-to-maternal serum concentrations of OH-PCBs and PCBs (Kawashiro et al. 2008; Meerts et al. 2002; Park et al. 2008; Soechitram et al. 2004). Since these ratios are higher for OH-PCBs than for PCBs, they conclude that there is a higher placental transfer for OH-PCBs than for PCBs. So, PCBs are less likely to bind to TTR, or, as could be drawn from the results obtained in the present study analysing placenta samples, PCBs are specifically accumulated in the placenta because the concentration ratios between PCBs and OH-PCBs are, in general, higher in placenta samples than in both maternal and cord serum samples (Guvenius et al. 2003; Kawashiro et al. 2008; Park et al. 2007, 2008; Soechitram et al. 2004). These results agree with the conclusions reached by other authors measuring OH-PCB/PCB ratios in both maternal and foetus serum and show that a different placental transfer occurs for both groups of compounds.

Regarding the comparison with other studies, it must be highlighted that there are few data on PCBs in placental samples. Anyhow, the concentration levels found in the Madrid population (2,292 pg/g f.w., calculated as the sum of the 15 PCB congeners analysed) are higher than those reported previously in Taiwan (294 pg/g f.w. including 18 PCBs, Wang et al. 2004). Data comparison for OH-PCBs is not possible since, to the best of our knowledge, there are no published data on the concentration levels of OH-PCBs in placenta samples, the present results being the first study carried out for PCB metabolites in such kind of samples.

3.2 Statistical analysis of PCB and OH-PCB concentrations

As previously mentioned, those congeners (i.e. 3′-OH-CB188 and 4′-OH-CB159) with a concentration below the limit of detection in more than 60% of the samples were not

considered for the statistical analysis of the concentrations found in placenta samples.

Preliminary descriptive statistics revealed a strong skewness in the data, so they were log-transformed to approach a normal distribution and to be able to apply parametric statistical procedures (Sandau et al. 2000). Therefore, all the subsequent statistical data analyses were carried out on log-transformed data.

First of all, strong correlations (Pearson $R>0.8$) among several PCB concentrations (data not shown) should be highlighted. For example, PCBs 28, 52 and 101 were strongly correlated among them, and the same behaviour was observed for the group of PCBs 138, 146, 153, 156, 170, 180 and 187. In addition, some strong correlations could be found among few OH-PCB congeners in the placenta samples studied (e.g. 3′-OH-CB138, 3-OH-CB153, 4′-OH-CB172 and 3′-OH-CB172). The existence of this collinearity is a very important fact from a statistical point of view and should be considered in order to avoid misleading conclusions and developing ill-conditioned regression models. On the other hand, as shown in Fig. 1, an important correlation ([*para*-OH-PCBs]=0.38+0.42 [*meta*-OH-PCBs], $R=0.76$) has also been established between hydroxylated metabolites with the OH– group in *meta*- and *para*- positions. The metabolic pathway of PCBs in living organisms is well known, being those congeners with no chlorine substitution in *meta*- and *para*- positions susceptible to metabolism (Letcher et al. 2000). There are three possible pathways for OH-PCB formation; one of them involves the direct insertion of OH– group in a *meta*-position, and the other two require the formation of an arene oxide and the subsequent opening of the epoxide followed by the rearrangement of the OH– and Cl– groups leading to a *meta*- or *para*- position by means of the NIH shift in the PCB arene oxide or without it (Letcher et al. 2000). The results obtained in the present study suggest that these three metabolic pathways are correlated, leading to a significant correlation between the concentrations of OH-PCB metabolites with the hydroxyl group in *meta*- and *para*- positions.

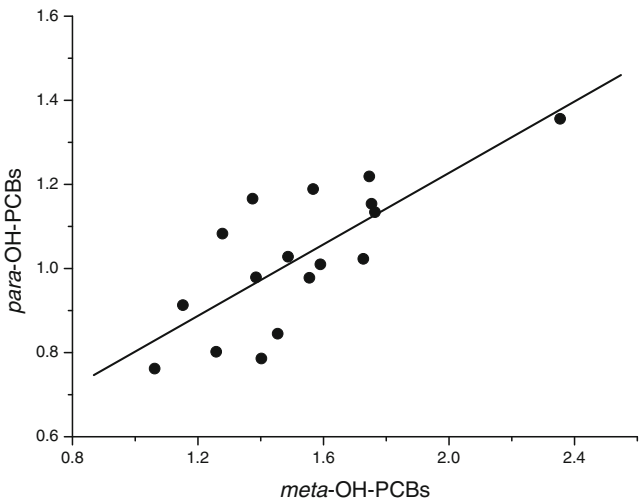

Fig. 1 Correlation found between log-transformed concentrations of *para*- and *meta*- OH-PCBs in Spanish placenta samples

Relationships between the log-transformed concentrations of precursor PCBs and their corresponding hydroxylated metabolites were investigated. As previously mentioned, collinearity was observed in both the PCB and the OH-PCB data, so some spurious correlations were also expected among several OH-PCB/PCB pairs, even with no metabolic path relationship between them. For example, 4-OH-CB146 and 4-OH-CB187 showed significant correlations with CB-156 (Pearson $R=0.72$ and 0.69, respectively), although CB-156 is not described in the literature as precursor of neither of the hydroxylated metabolites mentioned. The possible reason for this correlation is that concentrations of CB-156 were strongly correlated (Pearson $R>0.8$) with those of PCBs 146 and 153 (precursors of 4-OH-CB146) and CB-187 (precursor of 4-OH-CB187). For this reason, the selection of the pairs was based on the literature (Hovander et al. 2006; Letcher et al. 2000; Ohta et al. 2008; Soechitram et al. 2004; Weijs et al. 2008). Thereby, CB-118 and CB-101 were correlated with 4′-OH-CB120, CB-146 with 4-OH-CB146 plus 3-OH-CB153, CB-128 with 4′-OH-CB130 plus 3-OH-CB153, CB-138 with 3′-OH-CB138 plus 4-OH-CB146 plus 4′-OH-CB130, CB-153 with 4-OH-CB146 plus 3-OH-CB153, CB-170 with 4′-OH-CB172, CB-180 with 4′-OH-CB172 plus 3′-OH-CB180, CB-183 with 4-OH-CB187, and finally, CB-187 with 4-OH-CB187. These causal correlations obtained for placenta samples were significant in all cases (p value< 0.05, $n=17$), varying from 0.41 to 0.73. As an example, Fig. 2 shows the correlation found between CB-187 and its metabolite, 4-OH-CB187 ([4-OH-CB187]=0.67+0.62 [CB-187]).

In addition, a principal component analysis was carried out on the standardised log-transformed concentration values, revealing that the data presented a homogenous distribution for both PCBs and OH-PCBs (Fig. 3), indicating a data composition similar enough to carry out a reliable regression model. The results indicated that, for PCBs, the first two principal components accounted for 96% (85% PC1+11% PC2) and, for OH-PCBs, they accounted for 91% (81% PC1+8% PC2) of the total variability.

Taking into account that there are no data concerning OH-PCB concentrations in placenta samples in the literature, it could be interesting to develop a predictive model that allows the calculation of OH-PCB concentrations in placenta samples from their corresponding PCB levels. Due to the homogenous distribution of variability for both PCB

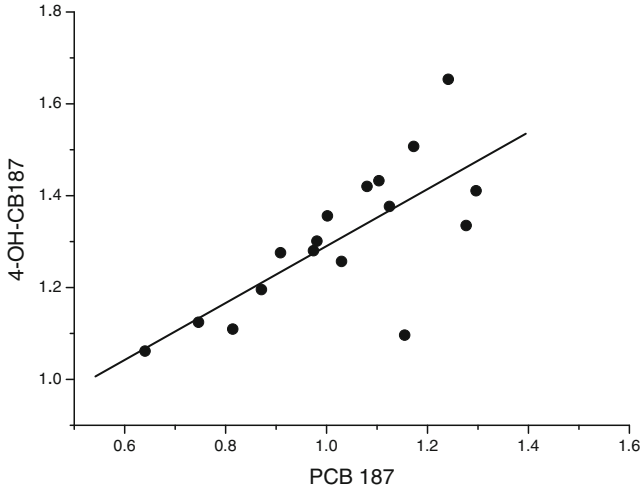

Fig. 2 Correlation between log-transformed concentrations of PCB 187 and its corresponding metabolite, 4-OH-CB187, in the placenta samples analysed

and OH-PCB samples, a reliable and consistent model could be expected. For this task, and taking into account the high collinearity previously mentioned among several PCB concentrations and among some OH-PCB concentrations, a partial least squares (PLS) procedure was accomplished, and the results obtained are gathered in Fig. 4. The optimum regression model was developed with the three first principal components and validated by a tenfold cross-validation procedure. The developed model uses only three principal components, agreeing with the high collinearity between PCB concentrations, and shows an acceptable capacity of prediction, as can be seen in Fig. 4, where the relationship between the measured and estimated total concentrations of OH-PCBs ([predicted total OH-PCBs]= 0.28+0.68 [measured total OH-PCBs]) produce a correlation coefficient of 0.78.

4 Conclusions

This manuscript reports, for the first time, the concentration levels and profiles of PCBs and OH-PCBs found in placenta samples in the Madrid population (Spain). The results show that although PCBs and their hydroxylated metabolites can cross the placenta barrier, the speed of the process and the retention in the placenta matrix seem to differ for each family, PCBs being more retained in the placenta than their corresponding hydroxylated metabolites. The concentration levels of PCBs found in Spanish placenta samples were higher than those found previously in Taiwan, and, contrary to the profiles found usually in other human tissues such as serum and breast milk, the PCB profiles for Spanish placenta samples were dominated by low chlorinated congeners (i.e. PCB 52). In general, the most abundant hydroxylated metabolites in placenta samples were those that come from generally abundant PCB congeners in both commercial mixtures and biotic samples, such as 3-OH-CB153, 4-OH-CB146, 3′-OH-CB138, 3′-

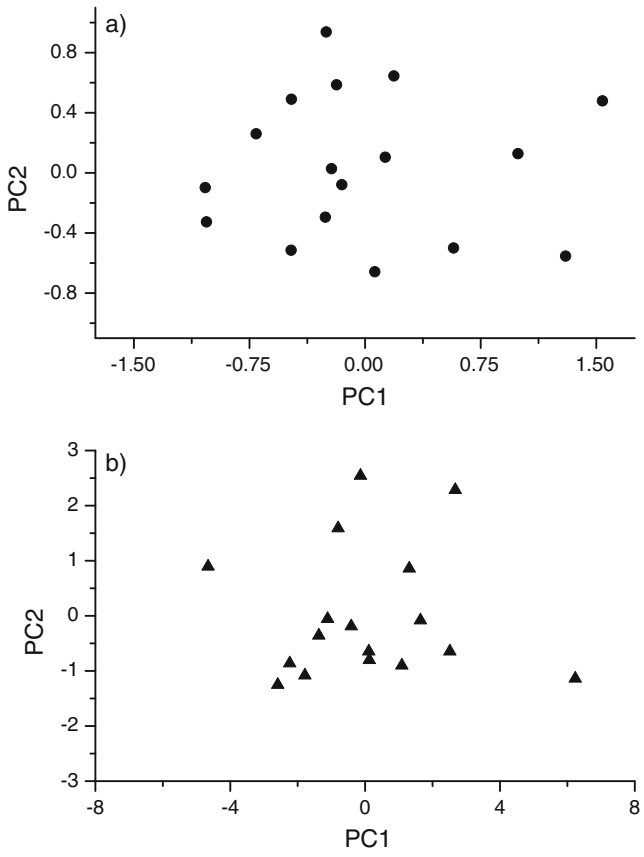

Fig. 3 Two-dimensional principal components score plots for log-transformed **a** PCB and **b** OH-PCB concentrations in placenta samples from Spain

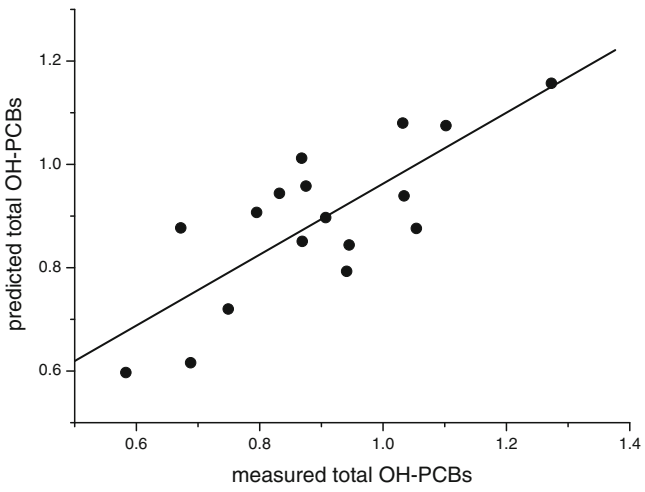

Fig. 4 Relationship between the measured and estimated total log-transformed concentrations of OH-PCBs obtained from the PLS regression model

OH-CB180 and 4′-OH-CB172. Special attention should be paid to 4-OH-CB187, which is the most abundant hydroxylated metabolite in all cases. The application of a PLS model revealed a significant correlation between PCB and OH-PCB concentrations in the placenta samples, and a predictive model was developed for the estimation of OH-PCB concentrations from the PCB concentration levels.

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