Enantioselective determination of chiral PCBs in Spanish breast milk samples by heart-cut MDGC

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INTRODUCTION

Polychlorinated biphenyls (PCBs), due to their persistence and lipophilic character, bioaccumulate through the food chain and persist in the human body for years. The ingestion of contaminated food is the principal pathway of human exposure, and in particular, foodstuffs of animal origin. Levels in breast milk derive from those previously accumulated in the body fat and are of special concern because of the health risk they pose to the nursing baby. Nineteen out of the 209 possible PCB congeners, were predicted to exist as stable atropisomers at room temperature [1], and nearly all are present in commercial PCB mixtures. Those PCBs were released into the environment as racemates, and therefore the non-racemic composition could be evidence of enantioselective bioaccumulation and/or metabolism. Moreover, the enantioselective analysis would provide a more comprehensive understanding of the real toxicity associated with the presence of those congeners in real samples, since different toxicity has been demonstrated for each of the enantiomers [2]. Up to now, studies dealing with the determination of the enantiomeric composition of chiral PCBs in breast milk samples have reported a racemic composition on a Chirasil-Dex GC column for PCB 149 and an enantioenrichment of the 1st and 2st eluted enantiomer of PCBs 95 and 132, respectively [3, 4], while data for other chiral PCBs are up to now not available.

In this study, the concentration of the most relevant PCB congeners and the enantiomeric fraction (EF) of 10 chiral PCBs were determined in eleven Spanish breast milk samples. Heart-cut multidimensional gas chromatography was employed for the unambiguous determination of both enantiomers, using two different chiral columns, *i.e.*, Chirasil-Dex and BGB-172.

MATERIALS AND METHODS

Sampling

Eleven breast milk samples (LM-1 to LM-11) were collected during 2004 from mothers living in Madrid, the surroundings of the city and Alicante. Samples were collected at different times of lactation, stored at –20ºC and once at the laboratory lyophilised and stored at room temperature until analysis.

Sample preparation

The procedure has been previously described [5]. Briefly, extraction was carried out by a matrix solid phase dispersion of the sample and further clean-up steps were performed using activated and modified multilayer silica columns. The extract was fractionated with carbon SPE cartridges. Three fractions were obtained, the first one containing the bulk of PCBs, which was further used for the enantioselective analysis.

Instrumental analysis

GC-ECD analysis

The list of PCBs analysed included the 8 mono-*ortho* PCBs, the set of seven indicator congeners usually used for monitoring purposes and some other congeners present in commercial PCB mixtures and food samples as well as those chiral (PCBs 28, 45, 52, 84, 91, 95, 101, 105, 114, 118, 123, 131, 132, 135, 136, 138, 149, 153, 156, 157, 167, 170, 171, 174, 176, 180, 183, 189 and 194). Analyses were carried out on an Agilent 6890 (Palo Alto, USA) equipped with a DB-5 (60m × 0.25 mm I.D., 0.25mm film thickness, J&W, USA). Further details on the

chromatographic conditions have been described elsewhere [6].

Heart-cut Multidimensional gas chromatography (Heart-cut MDGC)

Enantioselective determination of chiral PCBs was done by heart-cut MDGC (Varian Ibérica, Spain) equipped with two independent ovens. Column switching was achieved by means of a Deans valve placed in the first oven. A DB-5 column (30m × 0.25mm I.D., 0.25mm film thickness, J&W Scientific, USA) was used as pre-column in the first oven. In the second oven, 2 enantioselective columns were used as main columns: Chirasil-Dex (2,3,6-tri-*O*-methyl ß-CD, 25m × 0.25mm I.D., 0.25mm film thickness, Varian-Chrompack,Middelburg, The Netherlands) for the analysis of PCBs 91, 95, 132, 149, 174 and 176, and BGB-172 (25% 2,3,6-*tert.*-butyldimethylsilyl ß-CD, 30m × 0.25mm I.D., 0.18mm film thickness, BGB Analytik, Adliswil, Switzerland) for the analysis of PCBs 84, 135, 171 and 183. Details of the chromatographic conditions are described elsewhere [6].

RESULTS AND DISCUSSION

Total PCB concentrations ranged from 25.9 to 243 ng/g on a fat weight basis. Congener specific profile was dominated by PCBs 138, 153 and 180, although in four samples the contribution of low chlorinated PCBs such as congeners 52, 84, 95, 101 was of relevance. Mono-*ortho* PCBs accounted for between 4% and 7% of the total PCB content, congener 118 being the most abundant. This profile is similar to that previously found in Spain and in other countries [7,8]. The most abundant chiral PCBs in the samples were PCBs 95, 84, 171 and PCB 183, each accounting for 2% of the total PCB content.

Table 1 shows the enantiomeric fractions (EF) obtained for the 11 samples analysed. PCBs 91, 95 and 149 showed a nearly racemic composition in all samples, although for the last of these, an enantioenrichment of the 1st eluted enantiomer has been reported for 5 out of 10 samples analysed in a previous study carried out in Germany [4]. For PCB 84, half of the samples showed a racemic composition, while the rest presented enrichment either of the 1st or the 2nd eluted enantiomer. Something similar was observed for PCB 174, although most of the breast milk samples showed a racemic composition, 3 of them presented a slight enantioenrichment of the 1st eluted enantiomer. PCBs 132, 135 and 176 showed in most of the samples an enrichment of the 2nd eluted enantiomer. For the first of these, this result is in good agreement with those previously published, in which Chirasil-Dex was also used as enantioselective column [3]. In the case of PCBs 171 and 183, a clear enrichment also of the 2nd eluted enantiomer was observed, especially for the former, as shown in Figure 1.

Table 1. Enantiomeric fractions (EF = area 1st eluted enantiomer/(area 1st eluted enantiomer + area 2nd eluted enantiomer) of the selected chiral PCBs analysed.

	LM-1	LM-2	LM-3	LM-4	LM-5	LM-6	LM-7	LM-8	LM-9	LM-10	LM-11
PCB 84	0.52	0.37	0.36	0.60	0.72	0.51	0.51	int	0.34	0.45	0.46
PCB 91	0.53	0.55	int	0.58	int	0.53	0.57	0.58	0.54	0.54	0.65
PCB 95	0.52	0.55	0.60	0.52	0.57	0.50	0.49	0.45	0.49	0.49	0.49
PCB 132	0.41	0.44	0.35	0.36	0.44	0.42	0.44	0.41	0.42	0.48	0.47
PCB 135	0.68	ND	0.34	0.35	int	0.52	int	int	0.43	0.46	0.38
PCB 149	0.37	0.48	0.47	0.47	0.49	0.49	0.49	0.50	0.47	0.48	0.48
PCB 171	0.14	0.003	0.02	0.03	0.01	0.01	0.05	0.05	0.03	0.03	0.01
PCB 174	0.61	0.51	0.56	0.66	0.65	0.58	0.51	0.50	0.55	0.51	0.53
PCB 176	ND	ND	ND	0.35	ND	0.37	0.45	0.42	0.41	0.46	0.40
PCB 183	0.38	0.29	0.36	0.40	0.31	0.35	0.36	0.38	0.40	0.34	0.26

int; not determined due to interferences

ND; not detected

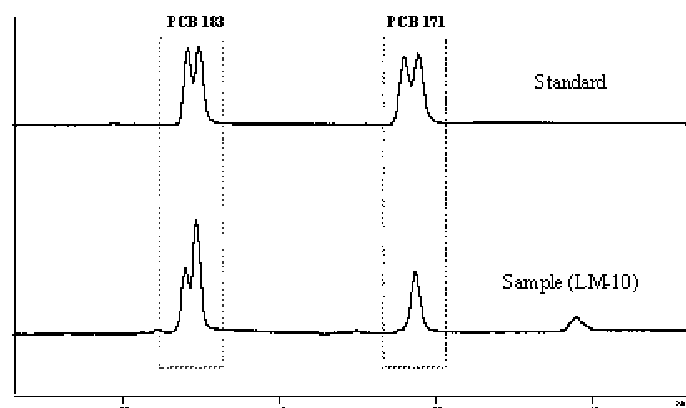

Figure 1. Chromatogram obtained for a breast milk sample and for a standards using BGB-172 as enantioselective column.

The mechanisms involved in the enantioselective accumulation and/or degradation processes are still unknown and can affect both enantiomers in different ways. Some authors have characterised PCBs according to the presence or absence of vicinal H atoms in the *meta*/*para* or *ortho*/*meta* positions of the biphenyl ring [9]. Congeners 84, 132, 171, 174 and 176 are readily metabolisable PCBs, and this is a possible explanation for the non-racemic composition found in some samples. Nevertheless, PCB 183, regarded as a difficult to metabolise congener because it lacks vicinal hydrogen atoms in *meta*/*para* and *ortho*/*meta* positions, showed a clear enrichment of the 2nd eluted enantiomer. On the other hand, it should be taken into account that the composition could already be non-racemic before the PCBs enter the human body, if enantioselective transformations have previously occurred.

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