HEXABROMOCYCLODODECANE IN HUMAN BREAST MILK: LEVELS AND ENANTIOMERIC PATTERNS

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Introduction

Hexabromocyclododecane (HBCD), a brominated alicyclic hydrocarbon, is the principal flame retardant in polystyrene foams and is used as thermal insulation in the building industry [1]. Technical 1, 2, 5, 6, 9, 10-HBCD is produced industrially by addition of bromine to *cis-trans-trans*-1,5,9-cyclododecatriene, with the resulting mixture containing three predominant diastereoisomers α-, β- and γ-HBCD. Normally, the γ-isomer is the most dominant in the commercial mixtures (ranging between 75 and 89%), followed by α- and then β-isomer (10-13% and 1-12%, respectively). But, the α-, β- and γ- HBCD diastereoisomers are chiral and because of that must be present as enantiomeric pairs. The physical-chemical properties of HBCD are similar to PBDEs and other persistent organic pollutants; in fact the log K_{ow} of HBCD is 5.6 and that valour places in the optimum range for bioaccumulation. Due to their lipophilic and persistent character HBCDs accumulate in the human body. But, little information is available regarding HBCD concentrations in human samples. Moreover, although limited data on human exposure to HBCD exists, no information is available on its enantiomeric pattern.

The aim of this study was to determine HBCD isomer levels in human breast milk from Spain. Moreover, for the first time, enantiomeric fractions (EFs) will be calculated in order to investigate potential selective enantiomeric enrichment in human bodies.

Materials and Method

Sample treatment: Human breast milk samples were collected in 2007, and corresponded to mothers living in Catalonia (NW of Spain). Samples were frozen and sent in frozen status to the laboratory. Before extraction, samples were lyophilised. The extraction of 0.5 g dry weight of milk was carried out by pressurized liquid extraction (PLE) method, using CH_2Cl_2:hexane (2:1) as solvent extraction, at 100ºC and 2000 psi of pressure. After extraction, the crude extracts were subjected to a purification step via acid attack with H_2SO_4 conc. Samples were finally concentrated to 50 µL prior to the analysis by LC-QqLIT-MS.

Diastereoisomer analysis: The LC system used was an Agilent HP 1100 binary pump (Agilent Technologies, Palo Alto, CA, USA) with a Symmetry C_{18} column (2.1 mm x 150mm, 5µm) preceded by a C_{18} guard column (2.1 x 10 mm) supplied by Waters (Massachusetts, USA). Experiments were carried out in negative ionization mode using H_2O:methanol (3:1 v/v) as eluent A and methanol as eluent B, at a flow rate of 0.25 mL/min. The injection volume was set at 4 µL. The elution program started at an initial composition of 100% A and was ramped to 0% A in the first minute, then eluent A increased to 10% in 17 min and initial conditions were reached again in three min and returned to the starting conditions in 15 min. Mass spectrometric analysis was performed with a hybrid triple quadrupole/linear ion trap Applied Biosystem MSD Sciex 4000QTRAP™ (Applied Biosystems, Foster City, CA, USA) instrument equipped with an electrospray (ESI) Turbospray interface. For target quantitative analyses, data acquisition was performed in SRM. The $[M - H]^- \rightarrow Br^-$ transitions at *m/z* 640.6 → 78.9 and 640.6 → 80.9 were monitored for unlabelled HBCD. The labelled HBCD were monitored at the 652.6 → 78.9 and 652.6 → 80.9 transitions. The MS/MS detection conditions were previously optimized to afford the highest relative intensity: curtain gas (CUR) at 50 psi, collision gas (CAD) at 4.5 x 10^{-5} Torr , temperature of the turbo gas in the TurboIonSpray™ source (TEM) at 350ºC, ion source gas 1 (GS1) at 50 psi and ion source gas 2 (GS2) at 10 psi [2].

Enantiomeric analysis: A chiral chromatographic column, NUCLEODEX β-PM (4.0 x 200 mm x 5µm), was used to afford the enantiomer-specific determination. The optimal separation of enantiomers was achieved using methanol, acetonitrile and water as mobile phase. Experiments were carried out using 70% H_2O: 30%MeOH as eluent A and 70% AcN: 30% MeOH as eluent B, at a flow rate of 0.50 mL/min. The injection volume was set at

10 µL. The elution program started at an initial concentration of A at 50% decreased to 0% along the first 8 minutes, and was maintained for 17 minutes and initial conditions were reached again in 5 min and maintained for additional 12 min. [3]. Mass spectrometric analysis was performed using the same conditions as for diastereoisomer analysis. The enantiomeric composition was expressed as enantiomeric fraction (EF), which is normally calculated from the peak areas of the enantiomeric pairs by Eqn. 1:

$$EF = \frac{A_+}{(A_+ + A_-)} \qquad \text{(Eqn.1)}$$

where A_+ and A_- are the peak area of eluting enantiomers.

It is well know that electrospray ionization is subject to sample matrix effects that can cause enhancement or suppression of the target analytes signal and can adversely affect their quantification. In order to avoid this effect that can affect in EF calculations, Marvin *et al.* [4] introduced the corrected EF values, calculated by the equation 2. This correction is based on the use of isotopic labelled standards (d_{18}-HBCDs) since d_{18}-labelled enantiomeric analogues behave in an identical manner to their native counterparts.

$$EF_{corrected} = \frac{([A_+] / [A_{+d_{18}}]) \times pg\ A_{+d_{18}}}{([A_+] / [A_{+d_{18}}]) \times pg\ A_{+d_{18}} + ([A_-] / [A_{-d_{18}}]) \times pg\ A_{-d_{18}}} \qquad \text{(Eqn. 2)}$$

Results and Discussion

Diastereoisomer levels: HBCD was detected in 13 out 15 human milk samples, at concentration levels ranging between 8 and 188 ng/g lw (Table 1). Although there are few reports regarding HBCD concentrations in human breast milk, our values are higher than those available for other countries such as Sweden, Norway, Canada, USA or Japan, which are always below 20 ng/g lw.

Table 1 Concentration levels and EF values for human breast milk samples.

Sample Code	% lw*	Concentration level (ng/g lipid weight)				$EF_{corrected}$	
		α-HBCD	β-HBCD	γ-HBCD	Total HBCDs	α-HBCD	γ-HBCD
L-1	4.0	12.0	NQ	176	188	-	0.602
L-2	2.6	1.59	NQ	141	143	-	0.494
L-3	5.8	NQ	NQ	66.5	66.5	-	0.507
L-4	4.2	0.13	NQ	68.7	68.8	-	0.554
L-5	6.4	0.25	NQ	7.80	8.05	-	0.595
L-6	7.1	5.35	NQ	22.6	28.0	-	0.490
L-7	3.7	NQ	NQ	27.0	27.0	-	0.553
L-8	5.7	2.24	NQ	16.4	18.6	-	0.508
L-9	4.1	2.82	NQ	13.7	16.5	-	0.507
L-10	5.3	NQ	NQ	7.90	7.90	-	0.344
L-11	3.1	2.21	NQ	23.1	25.3	-	nc
L-13	4.3	NQ	NQ	21.7	21.7	-	nc
L-14	1.7	ND	ND	ND	ND	-	-
L-16	5.0	ND	ND	ND	ND	-	-
L-18	2.8	71.5	0.10	NQ	71.6	0.102	-

* Lipid weight percentage referred to wet weight
ND: Below limit of detection
NQ: Below limit of quantification
nc: not calculated

As regards the isomeric pattern, there is a predominance of the γ-isomer, with a low contribution of the α-isomer, and the β-isomer being below the limit of quantification. Only in one sample, this isomeric pattern was different, showing a clear dominance of the α-isomer versus the γ-isomer. This inconsistency in the isomeric pattern may be due to individual variability in metabolizing capacity or other unknown factors such as the frequency of exposure to HBCD.

Enantiomeric fractions: For the first time, HBCD enantiomeric analysis was carried out in human breast milk samples, showing the presence of the two pairs of enantiomers, (-)α- and (+)α-, and (-)γ- and (+)γ-HBCD (Figure 1).

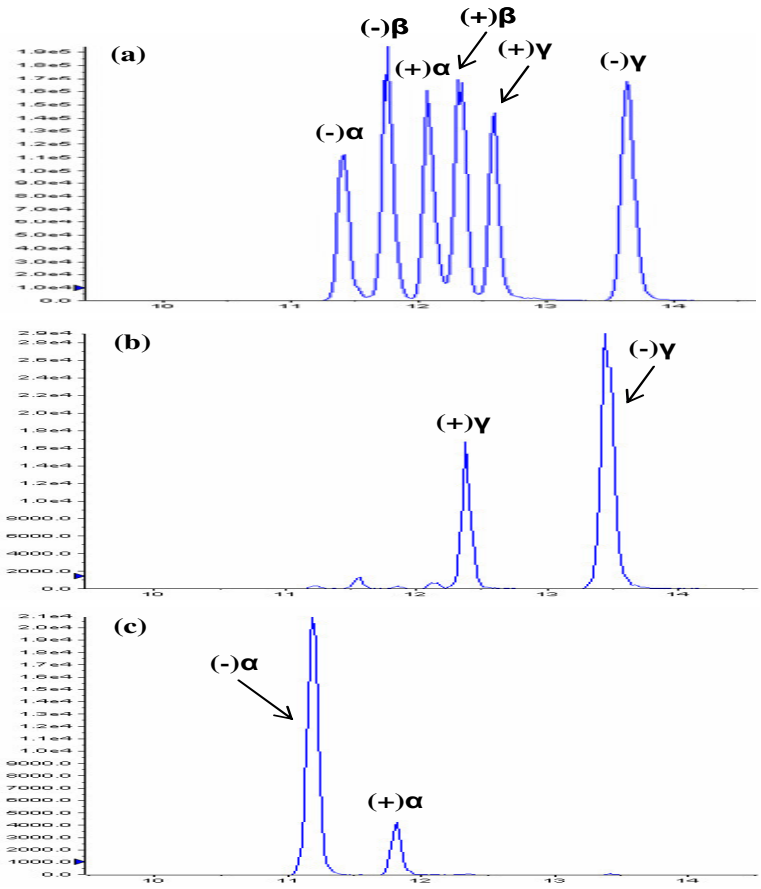

Figure 1. Enantiomeric analysis for (a) a standard solution, and (b) and (c) human breast milk samples

EF corrected values were calculated for the different samples (Table 1). In the case of γ-HBCD, it was observed that EF values are between 0.344 and 0.602, whereas EF for α-HBCD was calculated only for one sample, with a value of 0.102. If we compare these $EF_{corrected}$ values with those obtained with standard solutions (Figure 2), we can deduce that no significant differences were observed for γ-HBCD; but, in the case of α-HBCD, an important decrease of EF value was observed in the human breast milk with respect to the standards. Thus, we can assume that an (-)α-HBCD enrichment was occur in this sample, showing a selective enantiomeric enrichment in human body. These data has not been described before in the literature. However, further studies in a great number of human breast milk samples are required in order to check this differential enantiomeric behaviour.

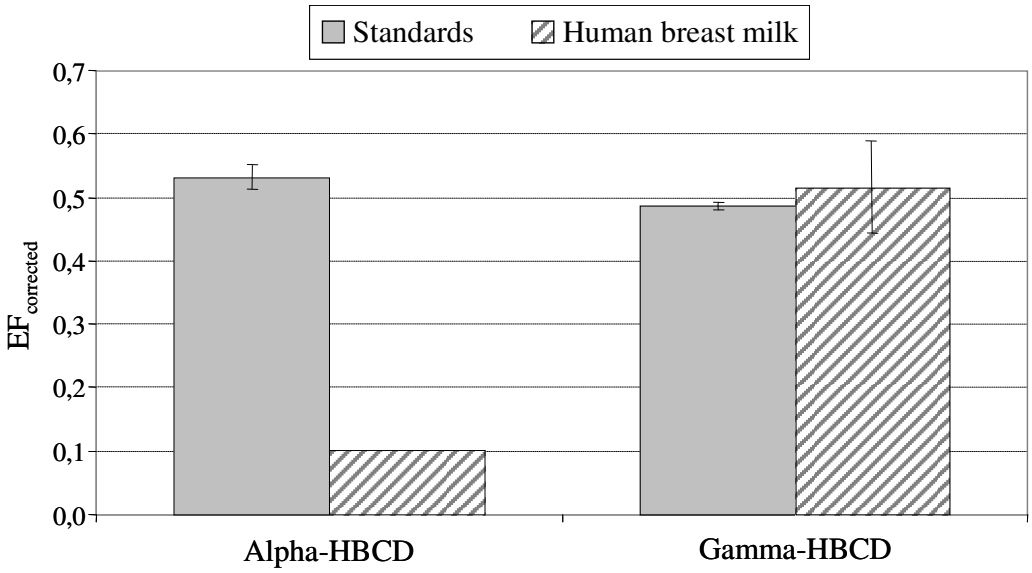

Figure 2. Enantiomeric fraction values (mean ± standard deviation) in standards and human breast milk samples

References

[1] Tanabe, S.; Kunisue, T. *Environ. Pollut.* **2007**, 146, 400-413.
[2] Guerra, P. ; de la Torre, A. ; Martínez, M.A. ; Eljarrat, E.; Barceló, D. *Rapid Commun. Mass Spectrom.*, **2008**, 22, 916-924.
[3] Guerra, P. ; Eljarrat, E. ; Barceló, D. *J. of Chromatogr.,* (submitted).
[4] Marvin, C.; MacInnis, G.; Alahee, M.; Arsenault, G.; Tomy, G. *Rapid Commun. Mass Spectrom.*, **2007**, 21, 1925.