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Hexabromocyclododecane in Human Breast Milk: Levels and Enantiomeric Patterns

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Samples of human breast milk ($n = 33$) from A Coruña (NW of Spain) collected in 2006 and 2007 were analyzed for HBCD diastereoisomers and enantiomers. HBCD was detected in 30 out of 33 human milk samples, at concentration levels ranging between 3 and 188 ng/g lw, with a median value of 27 ng/g lw, showing higher levels than those published for other countries. Diastereoisomeric pattern shows the predominance of the γ-isomer, with low contribution of the α-isomer and the β-isomer being below the limit of quantification. However, in six samples, there is a dominance of the α-isomer versus the γ-isomer. For the first time, HBCD enantiomeric analysis was carried out in human breast milk samples, showing a selective enantiomeric enrichment in human body. As regards α-HBCD, an enrichment of (−)-enantiomer was observed. However, in the case of γ-HBCD, no clear preference for one or the other enantiomer was found. Finally, and based on the calculated HBCD concentrations in human breast milk from Spain, the daily ingestion rate of HBCD was estimated. The nursing infant dietary intake for HBCD was set at 175 ng (kg of body weight)$^{-1}$ day^{-1}.

Introduction

Hexabromocyclododecane (HBCD) is a brominated aliphatic cyclic hydrocarbon used as a flame retardant in thermal insulation building materials, upholstery textiles, and electronics. In 2001, the world market demand for HBCD was 16 700 tins, from which 9500 tons was sold in the European Union (*1*). These figures make HBCD the second highest-volume brominated flame retardant (BFR) used in Europe, after tetrabromobisphenol-A and before decabromodiphenyl ether (*2*). The production and use of penta- and octabromodiphenyl ethers has been restricted in Europe (*3*). HBCD may be used as an alternative for polybrominated diphenyl ethers (PBDEs) in some applications (*4*). To date, there are no restrictions on the production or use of HBCD. As a result of their widespread use and their physical and chemical properties, HBCD are now ubiquitous contaminants in the environment and humans (*5*).

When all elements of symmetry are considered, 16 different HBCD diastereoisomers, including six pairs of enantiomers as well as four meso forms, are possible theoretically. Technical HBCD is produced industrially by addition of bromine to *cis-trans-trans*-1,5,9-cyclododecatriene, with the resulting mixture that contains three predominant diastereoisomers known as 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 β-isomers (10−13% and 1−12%, respectively) (*6*). Moreover, α-, β-, and γ- HBCD diastereoisomers are chiral, leading to three pairs of enantiomers.

Biomonitoring studies on persistent organic pollutants (POPs) in human tissues and fluids provide a direct measure of the internal dose of these substances. At present, human biomonitoring data represent the most reliable estimate of human exposure resulting from multiple sources to be used in risk assessment. The chemico-physical stability of POPs is responsible for their long persistence in humans, whereas their high lipophilicity determines their accumulation and storage in the lipid fraction of tissues. The relatively rich lipid content of human milk (compared to blood or urine) makes it an ideal matrix for measurement of POPs. Moreover, breast milk is a noninvading way of taking samples. In addition, levels in human milk reflect accumulation in the mother but also indirect exposure of the fetus and direct exposure of the breast-fed infant at a sensitive stage at the beginning of life. Breast milk monitoring studies of POPs are of primary importance for carrying out an adequate risk assessment at the actual levels of human exposure and represent a major source of information on infant perinatal exposure. During the past few decades, numerous investigations on pollution of POPs in human breast milk have been conducted in various countries (*7, 8*). As regards BFRs, a considerable number of studies of PBDEs in human breast milk have been undertaken (*9, 10*). Moreover, PBDEs were also included on the Breast Milk Monitoring Program (BMMP) managed by the WHO, in which many countries are participating (*11*). However, very few studies have reported the occurrence of HBCD in human samples, and little information is available regarding HBCD concentrations in human breast milk. Moreover, HBCD consists of several diastereoisomers, but the majority of published works did not given information on isomeric HBCD content. To our knowledge, there are only two studies reporting the isomeric HBCD distribution in human breast milk samples (*12, 13*).

The objective of this study was to determine HBCD diastereoisomer levels in human breast milk from Spain and to provide valuable information to evaluate the body burden of mothers and the exposure to this flame retardant in infants via breast-feeding. Moreover, enantiomeric fractions (EFs) were calculated in order to investigate potential selective enantiomeric enrichment in human bodies. To our knowledge, this is the first time that enantiomeric HBCD patterns have been investigated in human breast milk.

Experimental Procedures

Sample Collection. The experimental protocol was approved by a local ethical committee in accordance with Spanish regulation, and the informed consent of all participating subjects was obtained. The mothers were asked to complete a questionnaire for information about residence, maternal

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age, number of infants previously breast-fed, newborn weight, and newborn sex.

The breast milk (about 10 mL) was collected either by use of a breast pump or by hand-expressing the milk into prewashed glass containers 40 days postpartum at the hospital. After milk collection, samples were stored in the freezer at −22 °C until analysis in the laboratory.

Sample Preparation. Before extraction, samples were lyophilized, homogenized, and stored at −22 °C. Samples of 0.5 g dry weight (dw) of milk were spiked with 50 μL of the labeled standard γ-HBCD-d_{18} at 125 pg/μL. Spiked samples were keep overnight to equilibrate. Extraction was carried out by the pressurized liquid extraction (PLE) method on a fully automated ASE 200 system (Dionex, Sunnyvale, CA). An 11 mL extraction cell was loaded by inserting two cellulose followed by the human milk sample. The dead volume was filled with anhydrous sodium sulfate for residue analysis previously activated at 150 °C for 4 h (Panreac Quimica S.A., Montcada i Reixac, Barcelona, Spain). Extraction conditions were as follow: dichloromethane/hexane (2:1) mixture as solvent extractor, temperature of 100 °C, pressure of 14 MPa, two static extractions of 5 min and 100% flush. After extraction, the crude extracts were concentrated to 3 mL and then subjected to a purification step via acid attack with concentrated H_2SO_4 (95−97% purity) (3 × 2 mL). Samples were finally concentrated to incipient dryness and redissolved with 50 μL of the labeled standard α-HBCD-d_{18} at 125 pg/μL, prior to instrumental analysis by LC-MS.

All milk material was used for HBCD analyses, and no extra sample was available for the determination of lipid content on additional subsample. That is because calculations were carried out through the concentration to dryness of crude extracts and final determination of lipid content gravimetrically.

Diastereoisomer Analysis. The LC system used was an Agilent HP 1100 binary pump (Agilent Technologies, Palo Alto, CA) with a Symmetry C_{18} column (2.1 mm × 150 mm, 5 μm) preceded by a C_{18} guard column (2.1 × 10 mm) supplied by Waters. Experiments were carried out with water/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 was increased to 10% in 17 min, and initial conditions were reached again in 3 min and returned to the starting conditions in 15 min. Mass spectrometric analysis was performed with a hybrid triple-quadrupole/linear ion trap MSD Sciex 4000QTRAP (Applied Biosystems, Foster City, CA) instrument equipped with an electrospray (ESI) Turbospray interface, working in negative ionization mode. For target quantitative analyses, data acquisition was performed in selected reaction monitoring (SRM). The $[M - H]^- \rightarrow Br^-$ transitions at m/z 638.7 → 78.9 and 638.7 → 80.9 were monitored for unlabeled HBCD. The labeled HBCD were monitored at the 655.8 → 78.9 and 655.8 → 80.9 transitions. The MS/MS detection conditions were optimized to obtain the highest relative intensity: curtain gas at 50 psi, collision gas at 4.5 × 10^{-5} Torr, temperature of the turbo gas in the TurboIonSpray source at 350 °C, ion source gas 1 at 50 psi, and ion source gas 2 at 10 psi (*14*).

Quality Assurance/Quality Control. Method blank samples were performed to check for interference or contamination from solvents and glassware. No presence of analytes of interest (α-, β-, and γ- HBCD diastereoisomers) was observed.

Spiked samples with native compounds (α-, β-, and γ-HBCD) were analyzed by the described methodology to check recoveries. These samples were concentrated to incipient dryness and redissolved with 50 μL of labeled compounds mixture, containing α-HBCD-d_{18} and γ-HBCD-d_{18} at 125 pg/μL, prior to instrumental analysis by LC-MS. Recovery values ranged from 79.% to 119%, with a relative standard deviation (n = 5) values between 7% and 10%. Detection limits, defined as the minimum amount of analyte that produces a peak with a signal-to-noise ratio equal to 3, were 0.1 ng/g lw for α- and γ-isomers and 1.0 ng/g lw for β-HBCD. Limits of quantification, defined as the minimum amount of analyte that produces a peak with a signal-to-noise ratio equal to 10, were 0.2, 0.3, and 3.3 ng/g lw for α-, γ-, and β-HBCD, respectively.

Enantiomeric Analysis. A chiral chromatographic column, Nucleodex β-PM (4.0 mm × 200 mm × 5 μm), was used to afford the enantiomer-specific determination. The optimal separation of enantiomers was achieved with methanol, acetonitrile, and water as mobile phase. Experiments were carried out with 70% water/30% methanol as eluent A and 70% acetonitrile/30% methanol 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 eluent A at 50%, decreased to 0% along the first 8 min, and was maintained for 17 min, and initial conditions were reached again in 5 min and maintained for additional 12 min (*15, 16*). Mass spectrometric analysis was performed under the same conditions as diastereoisomer analysis.

Enantiomeric composition was expressed as enantiomeric fraction (EF), which is normally calculated from the peak areas of the enantiomeric pairs by

$$\mathrm{EF} = \frac{A_+}{(A_+ + A_-)} \quad (1)$$

where A_+ and A_- are the peak areas of eluting (+)- and (−)-enantiomers, respectively.

It is well-known that ESI 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, which can affect EF calculations, Marvin et al. (*17*) introduced the corrected EF values, calculated by eq 2. This correction is based on the use of isotopic labeled standards (HBCD-d_{18}) since d_{18}-labeled enantiomeric analogues behave in an identical manner to their native counterparts.

$$\mathrm{EF}_{\mathrm{corrected}} = \frac{(A_+/A_{+d_{18}})[\text{picograms of (+)-HBCD-}d_{18}]}{(A_+/A_{+d_{18}})[\text{picograms of (+)-HBCD-}d_{18}] + (A_-/A_{-d_{18}})[\text{picograms of (−)-HBCD-}d_{18}]} \quad (2)$$

Results and Discussion

Diastereoisomer Levels. HBCD was detected in 30 out 33 human milk samples, at concentration levels ranging between 3 and 188 ng/g lw (Table 1), with the mean and median concentrations being 47 and 27 ng/g lw, respectively. The lipid content ranged from 0.6% to 7.1%.

In comparison to PBDEs, much less information is available on the HBCD concentrations in human tissues such as human breast milk. Thus, a comparison of our results with other available data is hardly achievable. The number of published studies related to HBCD concentration levels in human breast milk is limited (Table 2). HBCD was detected in human milk from primiparous Swedish women collected in 2001, with the mean and maximum concentrations being 0.5 and 2.4 ng/g lw, respectively (*18*). Similar results were obtained in subsequent monitoring carried out in 2002 and 2003 (*19*). HBCD was also found in Norwegian human milk at concentrations between 0.3 and 20 ng/g lw (*20, 21*). HBCD levels have been reported in breast milk samples from Mexican mothers at concentrations ranging from 0.8 to 5.4 ng/g lw, with a mean concentration of 1.2 ng/g lw (*22*). Another breast milk study in North America reported the HBCD contamination in samples collected in Ontario

TABLE 1. Concentration Levels and EF Values for Human Breast Milk Samples[a]

		concentration level (ng/g of lipid weight)				$EF_{corrected}$	
sample code	% lw[b]	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	NQ	NQ	68.7	68.7		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-12	4.3	NQ	NQ	21.7	21.7		nc
L-13	1.7	ND	ND	ND	ND		
L-14	5.0	ND	ND	ND	ND		
L-15	2.8	71.5	NQ	NQ	71.5	0.102	
L-16	2.1	7.51	ND	28.9	36.4		
L-17	3.2	17.5	ND	53.9	71.4		
L-18	4.1	10.4	ND	25.2	35.6		
L-19	3.0	19.1	ND	22.8	41.9		
L-20	5.1	3.94	ND	9.49	13.4		
L-21	2.4	1.10	ND	61.9	63.0		
L-22	1.7	13.8	ND	9.13	22.9		
L-23	2.0	1.77	ND	0.95	2.72		
L-24	0.6	4.95	ND	60.5	65.5		
L-25	6.0	14.7	ND	134	148		0.268
L-26	1.5	3.56	ND	NQ	3.56		
L-27	2.5	9.49	ND	17.5	27.0		
L-28	2.8	2.11	ND	23.4	25.5		
L-29	5.5	NQ	ND	12.9	12.9		
L-30	5.4	0.76	ND	5.43	6.19		
L-31	5.3	NQ	ND	NQ	NQ		
L-32	2.6	2.76	ND	NQ	2.80		
L-33	0.8	122	ND	14.2	136	0.204	

[a] ND, below limit of detection; NQ, below limit of quantification; nc, not calculated. [b] Lipid weight percentage referred to wet weight

TABLE 2. HBCD Concentrations in Human Breast Milk from Different Countries[a]

country	year	α-HBCD	β-HBCD	γ-HBCD	total HBCD	positive	ref
Sweden	2001	nr	nr	nr	ND−2.4	12 ($n = 33$)	*18*
	2002−2003	nr	nr	nr	ND−1.5	24 ($n = 30$)	*19*
Norway	2001	nr	nr	nr	0.25−2.0	nr ($n = 9$)	*20*
	1993−2001	nr	nr	nr	0.4−20	49 ($n = 85$)	*21*
Mexico	nr	nr	nr	nr	0.8−5.4	7 ($n = 7$)	*22*
Canada	2002−2003	3.8	nr	nr	0.4−19	nr ($n = 8$)	*12*
United States	2002	0.5	nr	nr	0.2−0.9	nr ($n = 9$)	*12*
Japan	1973−1988	ND	ND	ND	ND	nr	*13*
	1988−2006	0.43−1.9	ND	ND−2.6	0.43−4.0	11 ($n = 11$)	*13*
Russia	2000−2002	nr	nr	nr	ND−1.67	11 ($n = 37$)	*23*
France	2005	ND−5	ND	ND	ND−5	7 ($n = 23$)	*24*
Spain	2006−2007	ND−122	ND	ND−176	ND−188	30 ($n = 33$)	this study

[a] Results are expressed in nanograms per gram of lipid weight. ND, below limit of detection; nr, not reported.

(2002−2003) and Texas (2002), with mean concentration values of 3.8 and 0.5 ng/g lw, respectively (*12*). HBCD have been also detected in human samples from Japan (1988−2006), with levels ranging from 0.4 to 4 ng/g lw (*13*). Finally, two recent studies reported the first values for HBCD concentrations in human breast milk from Russia (*23*) and France (*24*), with values up to 1.7 and 5 ng/g lw, respectively. It should be pointed that our values are higher than those available for other countries (Sweden, Norway, Mexico, Canada, United States, Japan, Russia, or France), which are always below 20 ng/g lw. However, it is important to notice that the majority of published data are based on sampling campaigns carried out some years ago. There is a lack of information related to recent HBCD levels in human breast milk. Since usage of HBCD is increasing, it is expected that HBCD levels in human samples also increased. This could be one explanation of our higher results.

As regards the diastereoisomeric pattern, there is a predominance of the γ-isomer (from 54% to 100% of total contribution), with a low contribution of the α-isomer and the β-isomer being below the limit of quantification. However, in six samples, this diastereoisomeric pattern was different, showing dominance of the α-isomer (from 60% to 100% of the total contribution) versus the γ-isomer. This inconsistency in the diastereoisomeric pattern may be due to individual

variability in metabolizing capacity or other unknown factors, such as the frequency of HBCD exposure.

Some HBCD diastereoisomeric pattern studies have been carried out in human samples. Some reports have suggested that β- and γ-HBCD diastereoisomers can be more extensively metabolized in organisms than can α-HBCD diastereoisomer. Weiss et al. (*25*) found that the most abundant stereoisomer in serum samples from Sweden was α-HBCD. In contrast, and similar to our findings, Johnson-Restrepo et al. (*26*) found that γ-HBCD was the dominant isomer present in human adipose tissue samples from the United States, accounting for 83% of the total HBCD concentrations, and followed by α-HBCD (17%). β-HBCD stereoisomer was not detected in any of the human adipose tissue samples analyzed. However, 35% of the human tissue samples contained α-HBCD concentrations higher than the concentrations of γ-HBCD, suggesting that some individuals are exposed to elevated concentrations of α-HBCD. Thomsen et al. (*27*) also found higher concentrations of γ-HBCD than the other HBCD diastereoisomers in serum of workers from an industrial polystyrene plant in Scandinavia.

As regards information related to HBCD diastereoisomeric patterns in human breast milk, there are only two published works reporting data. The first study was carried out analyzing breast milk samples from North America (*12*). In these samples, the α-diastereoisomer predominated, with concentration levels ranging between 0.2 and 19 ng/g lw. The second study reported the diastereoisomeric pattern in human breast milk from Japanese women (*13*). In the majority of these samples, the α-diastereoisomer also predominated, but some exceptions were observed in which the contribution of the γ-diastereoisomer was higher than that of α.

As the literature regarding diastereoisomer distribution of HBCD in human milk is very scarce, further studies are needed to elucidate the relationship between HBCD diastereoisomers found in humans and those found in source materials.

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-HBCD and (−)-γ- and (+)-γ-HBCD (Figure 1). $EF_{corrected}$ values were calculated for different samples (Table 1). In the case of γ-HBCD, it was observed that EF values are between 0.268 and 0.602, whereas EF for α-HBCD was calculated only for two samples, with values of 0.102 and 0.204. If we compare these EF values with those obtained with standard solutions (Figure 2a), we can observe that no significant differences were detected between EF mean values for γ-HBCD. The EF mean value obtained for standard solutions was 0.487 [1.14% of relative standard deviation (RSD)] and 0.493 for human breast milk samples. However, the RSD for milk samples was set at 20.6%, indicating that we can sort all the samples into three different groups (Figure 2b). The first one corresponded to samples with no significant differences from standards. This group was formed by five different samples, with an EF mean value of 0.501 and RSD of 1.70%. The second one, formed by four milk samples, showed an increase of EF value compared to standard value: mean value of 0.576 and 4.54% of RSD. Thus, samples included in this second group showed an enrichment of the (+)-γ-HBCD enantiomer in the human body. Finally, the third group consisted of samples ($n = 2$) where EF values are lower than those obtained for standard solutions: 0.268 and 0.344. In this case, the lowering of EF value indicates an enrichment of the (−)-γ-HBCD enantiomer in human body.

In the case of α-HBCD, it was possible to calculate the EF value only for two human breast milk samples. Results showed an important decrease of EF values (0.102 and 0.204) in these samples with respect to the standards (mean value of 0.531). Thus, we can assume that an (−)-α-HBCD enrichment occurs, showing a selective enantiomeric enrichment in human body.

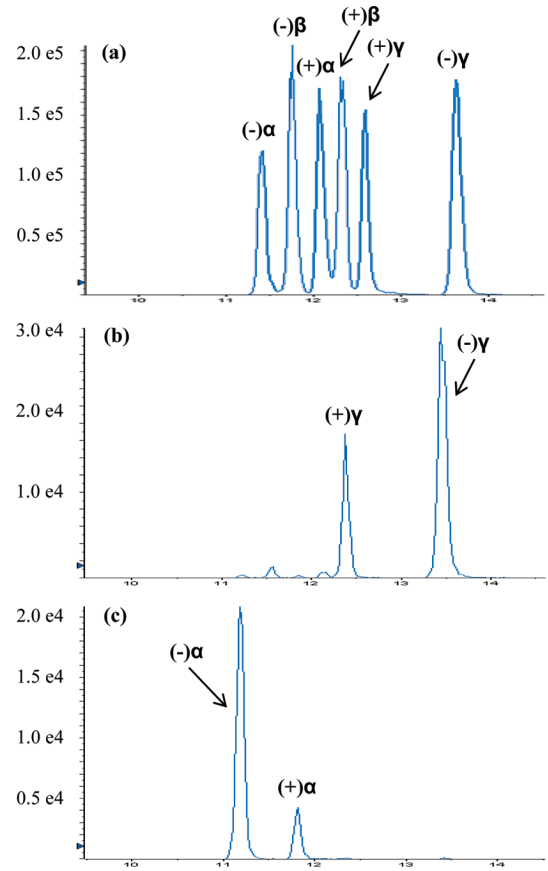

FIGURE 1. Enantiomeric analysis for (a) standard solution and (b, c) human breast milk samples.

In a published work on marine species from the Western Scheldt estuary (*28*), the authors have reported for the first time selective accumulation of different HBCD enantiomers in marine fish. In two further studies, the database of EFs for α-HBCD has been extended to herring and several predatory birds (*29*) and dolphins (*30*). In all cases, a deviation from the racemic mixture (EF = 0.5) has been observed, suggesting that an enantioselective uptake and/or metabolism of (+)- and (−)-α-HBCD must occur. Interestingly, no clear preference for one or the other α-HBCD enantiomer was found. Herring muscle and falcon eggs were clearly enriched in (−)-α-HBCD, whereas in whiting liver and sea eagle eggs (+)-α-HBCD dominated.

Our data in human samples have not been described before in the literature. Further studies in a great number of human breast milk samples are required in order to check this differential enantiomeric behavior.

Nursing Infant Dietary Intake Estimation. POPs may accumulate for a long period in the body of the mother and then be partially transferred to the nursing infant via mother's milk. It is realized that the presence of POPs in human breast milk has been of great concern because these lipophilic chemicals are readily transferred and absorbed to infants. In the case of dioxins, it is reported that 1- to 3-month-old infants absorb more than 90% of most dioxin congeners containing in their mothers' milk (*31*). Hence they may be exposed to relatively high levels of POPs during this period.

To understand the magnitude of exposure to HBCD by infants, we estimated the daily intake (EDI). EDI of contaminants by infants via breast milk can be calculated as

$$EDI_i = C_i FMb \quad (3)$$

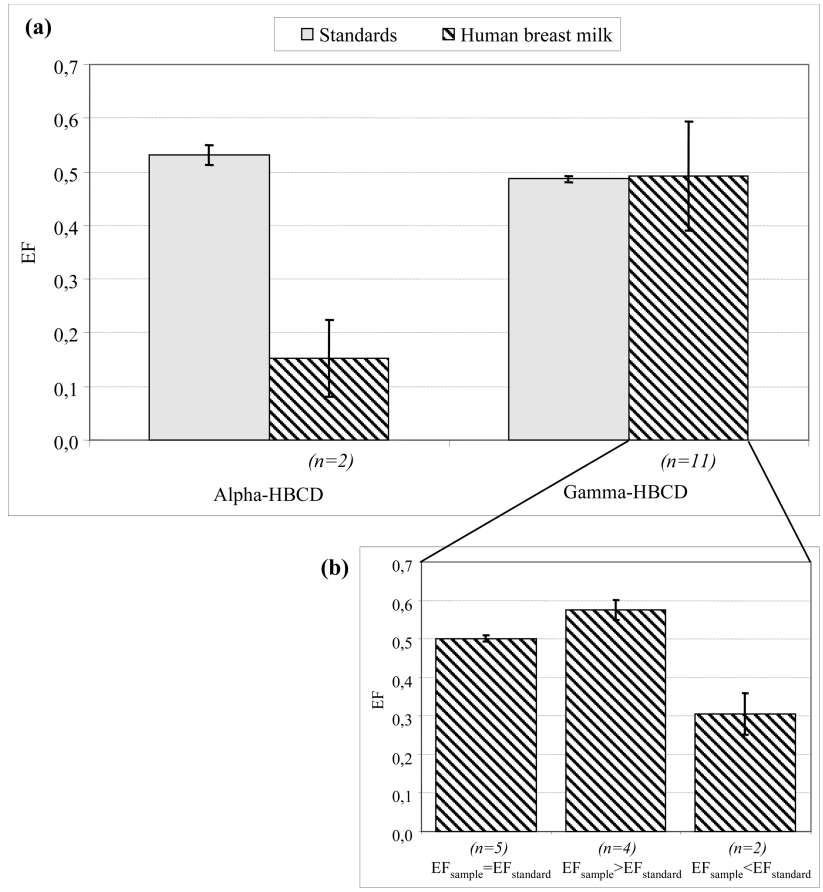

FIGURE 2. Enantiomeric fraction (EF) values (mean ± standard deviation) in standards and human breast milk samples: (a) mean values of all samples analyzed; (b) breakdown into samples with EF equal to, lower than, or higher than standard EF.

where EDI_i is the estimated daily intake [micrograms per kilogram of body weight (bw) per day]; C_i is the median concentration of HBCD in milk samples (micrograms per gram of lipid weight); F is the lipid content in milk samples (grams of lipid per 100 g of milk); and Mb is the daily consumption of milk (grams per kilogram of body weight per day). For this calculation, guidelines suggested by the U.S. EPA (*32*), such as the daily intake rate of breast milk and body weight for a 1-month-old infant were on average 702 mL of milk per day (723 g of milk per day) and 4.14 kg, respectively, were used to calculate an infant's average milk consumption, which was (Mb) 175 g of milk (kg bw)$^{-1}$ day^{-1}. The mean value of lipid content in analyzed samples was used for F estimation, with a value of 3.71 g of lipid per 100 g of milk; and the median concentration of HBCD in analyzed samples was applied for C estimation, with a value of 0.027 μg/g lw. The infant's daily intake rate estimated for HBCD was 175 ng (kg bw)$^{-1}$ day^{-1}. The possible toxicological impact of such an EDI should certainly be investigated.

Our daily dietary intake estimation through breast-feeding was compared with published data on human adult dietary intake through the diet (Figure 3). There are few data available on the dietary HBCD intake. A median intake of 141 ng of HBCD per day [2.15 ng (kg bw)$^{-1}$ day^{-1}] was estimated for the Swedish population, with a maximum of 1076 ng per day [21.5 (kg bw)$^{-1}$ day^{-1}] (*33*). In another study (*34*), an estimation of the fish-related dietary exposure of HBCD for the average Dutch population was done. The median bound intake in that study was estimated at 8.3 ng/day for a 70-kg person [0.12 ng (kg bw)$^{-1}$ day^{-1}]. The estimated adult dietary intake of HBCD arising from the United Kingdom diet was in the range of 5.9−7.9 ng (kg bw)$^{-1}$ day^{-1} (*35*). Finally, dietary exposure to HBCD in the Norwegian population was established at 0.3 ng (kg bw)$^{-1}$ day^{-1} (*36*). As can be seen, all

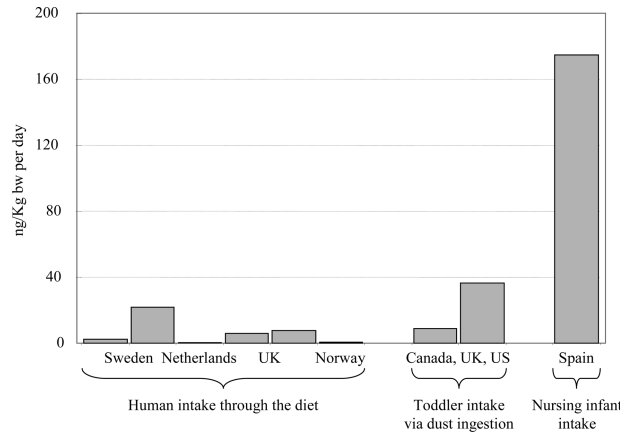

FIGURE 3. HBCD dietary intake evaluations (expressed as nanograms per kilogram of body weight per day) through different routes of exposition and for different groups of population.

these studies presented estimated HBCD dietary intake very much lower than those obtained in our study for nursing infants. Our daily intake of HBCD by Spanish infants via breast-feeding value is 25−1458 times greater than the estimated adult dietary intakes reported from Sweden, Netherlands, United Kingdom, and Norway. However, it should be pointed that EDI for infants changes when body weight increases (POP diluting effect), whereas estimation for adults is based on a lifelong exposure.

Human exposure to HBCD occurs through multiple routes. For nonoccupationally exposed persons, the major intake of HBCD is probably from food and indoor air or dust. However, the relevance of human HBCD exposure originating

from house dust versus food-based HBCD exposure is still unknown. Recently, Abdallah et al. (*37*) estimated the exposure of toddlers (6−24 months) to HBCD via dust ingestion in three different countries (Canada, United Kingdom, and United States) if 100% absorption of intake was assumed. The median exposure for mean dust ingestion (50 mg/day) was established between 19 and 37 ng/day. However, for high dust ingestion (200 mg/day), the values reached up to 150 ng/day. By comparison of orders of magnitude obtained from dust ingestion intake with those obtained with our daily dietary intake estimation through breast-feeding, it seems that, in Spain, food intake represents the main HBCD exposure for a nursing infant (Figure 3). However, it should be pointed that a correct comparison between different routes of exposure must be done with data coming from the same place. But actually, there is a lack of data in the same location.

Although discussions on the safety of breast-feeding have come to the firm conclusion that exclusive breast-feeding should continue to be strongly supported and promoted, the HBCD and POP contamination problem should not be ignored. Continued breast milk monitoring programs are important exercises as surveillance and early warning systems for abnormal exposure of the population to HBCD and other POPs.

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Literature Cited

(1) Bromine Science and Environmental Forum. http://www.bsef.com/.

(2) Alaee, M.; Arias, P.; Sjödin, A.; Bergman, Å. An overview of commercially used brominated flame retardants, their applica tions, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* **2003**, *29*, 683–689.

(3) Directive 2003/11/EC of the European Parliament and of the Council of 6 Feburary 2003 amending for the 24th time Council directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (pentabromodiphenyl ether and octabromodiphenyl ether). *Off. J. Eur. Union L: Legis. (Eng. Ed.)* **2003**, *L 042*, 4546.

(4) Sellström, U.; Kierkegaard, A.; de Wit, C.; Jansson, B. Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. *Environ. Toxicol. Chem.* **1998**, *17*, 1065–1072.

(5) Covaci, A.; Gerecke, A. C.; Law, R. J.; Voorspoels, S.; Kohler, M.; Heeb, N. V.; Leslie, H.; Allchin, C. R.; de Boer, J. Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. *Environ. Sci. Technol.* **2006**, *40*, 3679–3688.

(6) Tomy, G.; Budakowsky, W.; Halldorson, T.; Whittle, D.; Keir, M.; Marvin, C.; MacInnis, G.; Alahee, M. Biomagnification of α- and γ-hexabromocyclododecane isomers in a Lake Ontario food web. *Environ. Sci. Technol.* **2004**, *38*, 2298–2303.

(7) Abballe, A.; Ballard, T. J.; Dellatte, E.; di Domenico, A.; Ferri, F.; Fulgenzi, A. R.; Grisanti, G.; Iacovella, N.; Ingelido, A. M.; Malisch, R.; Miniero, R.; Porpora, M. G.; Risica, S.; Ziemacki, G.; De Felip, E. Persistent environmental contaminants in human milk: concentrations and time trends in Italy. *Chemosphere* **2008**, *73*, S220−S227.

(8) Tanabe, S.; Kunisue, T. Persistent organic pollutants in human breast milk from Asian countries. *Environ. Pollut.* **2007**, *146*, 400–413.

(9) Li, J.; Yu, H.; Zhao, Y.; Zhang, G.; Wu, Y. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from Beijing, China. *Chemosphere* **2008**, *73*, 1852–186.

(10) Ingelido, A. M.; Ballard, T.; Dellatte, E.; di Domenico, A.; Ferri, F.; Fulgenzi, A. R.; Herrmann, T.; Iacovella, N.; Miniero, R.; Päpke, O.; Porpora, M. G.; De Felip, E. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in milk from Italian women living in Rome and Venice. *Chemosphere* **2007**, *67*, S301−S306.

(11) Hooper, K.; McDonald, T. A. The PBDEs: An emerging environmental challenge and another reason for breast-milk monitoring programs. *Environ. Health Perspect.* **2000**, *108*, 387–392.

(12) Ryan, J. J.; Wainman, B. C.; Schecter, A.; Moisey, J.; Kosarac, I.; Sun, W. F. Trends of the brominated flame retardants, PBDEs and HBCD, in human milks from North America. *Organohalogen Compd.* **2006**, *68*, 778–781.

(13) Kakimoto, K.; Akutsu, K.; Konishi, Y.; Tanaka, Y. Time trend of hexabromocyclododecane in the breast milk of Japanese women. *Chemosphere* **2007**, *71*, 1110–1114.

(14) Guerra, P.; de la Torre, A.; Martínez, M. A.; Eljarrat, E.; Barceló, D. Identification and trace level determination of brominated flame retardants by LC-quadrupole linear ion trap (Qq-LIT) mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 916–924.

(15) Guerra, P.; Eljarrat, E.; Barceló, D. Enantiomeric specific determination of hexabromocyclododecane by LC-quadrupole linear ion trap (Qq-LIT) mass spectrometry in sediment samples. *J. Chromatogr.* **2008**, *1023*, 81–87.

(16) Eljarrat, E.; Guerra, P.; Barceló, D. Enantiomeric determination of chiral persistent organic pollutants and their metabolites. *Trends Anal. Chem.* **2008**, *27* (10), 847–861.

(17) Marvin, C.; MacInnis, G.; Alahee, M.; Arsenault, G.; Tomy, G. Factors influencing enantiomeric fractions of hexabromocyclododecane measured using liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 1925–1930.

(18) Aune, M.; Barregård, L.; Claesson, A.; Darnerud, P. O. Organic environmental pollutants in breast milk from Gothenburg, Sweden, 2001; Report 219 0108 to the Swedish EPA; The Swedish National Food Administration: Uppsala, Sweden, 2002; http://www.imm.ki.se/National/Datavard/PDF/sakrapport%20brostmjolk%202001%20Gbg.pdf.

(19) Lignell, S.; Darnerud, P. O.; Aune, M.; Törnkvist, A. Persistent organic pollutants (POP) in breastmilk from primiparae women in Uppsala County, Sweden 2002−2003; Report to the Swedish Environmental Protection Agency, 2003-11-05, 2003.

(20) Thomsen, C.; Froshaug, M.; Leknes, H.; Becher, G. Brominated flame retardants in breast milk from Norway. *Organohalogen Compd.* **2003**, *64*, 33–36.

(21) Thomsen, C.; Froshaug, M.; Broadwell, S. L.; Becher, G.; Eggesbo, M. Levels of brominated flame retardants in milk from the Norwegian human milk study: HUMIS. *Organohalogen Compd.* **2005**, *67*, 509–512.

(22) López, D.; Athanasiadou, M.; Athanassiadis, I.; Yáñez estrada, L.; Díaz-Barriga, F.; Bergman, A. Third international workshop on brominated flame retardants, Toronto, Canada, 2004; pp 482−487.

(23) Polder, A.; Gabrielsen, G. W.; Odland, J. O.; Savinova, T. N.; Tkachev, A.; Loken, K. B.; Skaare, J. U. Spatial and temporal changes of chlorinated pesticides, PCBs, dioxins (PCDDs/PCDFs) and brominated flame retardants in human breast milk from Northern Russia. *Sci. Tot. Environ.* **2008**, *391*, 41–54.

(24) Antignac, J. P.; Cariou, R.; Maume, D.; Marchand, P.; Monteau, F.; Zalko, D.; Berrebi, A.; Cravedi, J. P.; Andre, F.; Le Bizec, B. Exposure assessment of fetus and newborn to brominated flame retardants in France: preliminary data. *Mol. Nutr. Food Res.* **2008**, *52*, 258–265.

(25) Weiss, J.; Wallin, E.; Axmon, A.; Jonsson, B. A. G.; Akesson, H.; Janak, K.; Hagmar, L.; Bergman, A. Hydroxy-PCBs, PBDEs, and HBCDDs in serum from an elderly population of Swedish fishermen's wives and associations with bone density. *Environ. Sci. Technol.* **2006**, *40*, 6282–6289.

(26) Johnson-Restrepo, B.; Adams, D. H.; Kankan, K. Tetrabromobisphenol A (TBBPA) and hexabromocyclododecanes (HBCDs) in tissues of humans, dolphins, and sharks from the United States. *Chemosphere* **2008**, *70*, 1935–1944.

(27) Thomsen, C.; Molander, P.; Daae, H. L.; Janak, K.; Froshaug, M.; Liane, V. H.; Thorud, S.; Becher, G.; Dybing, E. Occupational exposure to hexabromocyclododecane at an industrial plant. *Environ. Sci. Technol.* **2007**, *41*, 5210–5216.

(28) Janák, K.; Covaci, A.; Voorspoels, S.; Becher, G. Hexabromocyclododecane in marine species from the Western Scheldt Estuary: Diastereomer- and enantiomer-specific accumulation. *Environ. Sci. Technol.* **2005**, *39*, 1987–1994.

(29) Janák, K.; Sellström, U.; Johansson, A. K.; Becher, G.; de Wit, C.; Lindberg, P.; Helander, B. Enantiomer-specific accumulation pattern of hexabromocyclododecane in eggs of predatory birds breeding in Sweden. *Organohalogen Compd.* **2005**, *67*, 204–207.

(30) Peck, A. M.; Tuerk, K. J. S.; Keller, J.; Kucklick, J. R.; Schantz, M. M. Hexabromocyclododecane diastereomers and enantiomers in white-sided dolphin blubber and liver tissue. *Organohalogen Compd.* **2005**, *67*, 1259–1262.

(31) McLachlan, M. S. Digestive tract absorption of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in a nursing infant. *Toxicol. Appl. Pharmacol.* **1993**, *123*, 68–72.

(32) U.S. Environmental Protection Agency. *Child-specific exposure factors handbook*; National Center for Environmental Assessment: Washington, DC, 2002; Chapt. 2 and 11, p 20.

(33) Lind, Y.; Aune, M.; Atuma, S.; Becker, W.; Bjerselius, R.; Glynn, A.; Darnerud, P. O. Food intake of the polybrominated flame retardants PBDEs and HBCD in Sweden. *Organohalogen Compd.* **2002**, *58*, 181–184.

(34) van Leeuwen, S. P.; de Boer, J. Brominated flame retardants in fish and shellfish - levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD. *Mol. Nutr. Food Res.* **2008**, *52*, 194–203.

(35) Fernandes, A.; Dicks, P.; Mortimer, D.; Gem, M.; Smith, F.; Driffield, M.; White, S.; Rose, M. Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: methodology, occurrence and human dietary exposure. *Mol. Nutr. Food Res.* **2008**, *52*, 238–249.

(36) Knutsen, H. K.; Kvalem, H. E.; Thomsen, C.; Froshaug, M.; Haugen, M.; Becher, G.; Alexander, J.; Meltzer, H. M. Dietary exposure to brominated flame retardants correlates with male blood levels in a selected group of Norwegians with a wide range of seafood consumption. *Mol. Nutr. Food Res.* **2008**, *52*, 217–227.

(37) Abdallah, M. A. E.; Harrad, S.; Ibarra, C.; Diamond, M.; Melymuk, L.; Robson, M.; Covaci, A. Hexabromocyclododecanes in indoor dust from Canada, the United Kingdom, and the United States. *Environ. Sci. Technol.* **2008**, *42*, 459–464.

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