Environment International 37 (2011) 443–448

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: Relationship to external exposure

Mohamed Abou-Elwafa Abdallah [a,*], Stuart Harrad [b]

[a] Department of Analytical Chemistry, Faculty of Pharmacy, Assiut University, 71526 Assiut, Egypt
[b] Division of Environmental Health and Risk Management, School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom

ARTICLE INFO

Article history:
Received 21 September 2010
Accepted 17 November 2010
Available online 17 December 2010

Keywords:
TBBP-A
HBCD
Degradation products
Human breast milk
Human exposure

ABSTRACT

Tetrabromobisphenol-A (TBBP-A), hexabromocyclododecane (HBCD) and its degradation products were determined in 34 human milk samples from Birmingham, UK. TBBP-A was detected in 36% of samples (average = 0.06 ng g^{-1} lw), with HBCDs detected in all samples (average ΣHBCDs = 5.95 ng g^{-1} lw). α-HBCD comprised 62–95% ΣHBCDs while β- and γ-HBCD constituted 2–18% and 3–33% respectively. Enantioselective enrichment of (−)-α-HBCD (average enantiomer fraction = 0.29) was observed indicating potential enantioselectivity associated with HBCD absorption, metabolism and/or excretion. The degradation products pentabromocyclododecenes (average = 0.04 ng g^{-1} lw; $n = 9$) and tetrabromocyclododecadienes (average = 0.15 ng g^{-1} lw; $n = 25$) were detected for the first time in human tissues. Average exposures of a nursing infant to ΣHBCDs and TBBP-A (35 and 1 ng kg^{-1} bw day^{-1} respectively) via breast milk exceeded upper-bound dietary intakes of both UK adults and toddlers. Using a simple pharmacokinetic model, intakes of UK adults via inhalation, diet and dust ingestion were converted to predicted body burdens. Predictions compared well with those observed for HBCDs but observed body burdens of TBBP-A exceeded predictions. This may indicate the human half-life of TBBP-A is greater than observed previously, that intakes may be underestimated, or that concentrations reported here reflect recent elevated episodic exposure.

1. Introduction

Tetrabromobisphenol-A (TBBP-A) and hexabromocyclododecane (HBCD) are widely used brominated flame retardants (BFRs) with reported global market demands of 170,000 and 16,700 metric tonnes in 2004 and 2001 respectively (BSEF, 2009). Several studies have reported different levels of both BFRs in various human tissues including serum, placenta, liver, adipose tissue and breast milk from different European and North American countries in the last few years (Covaci et al., 2006, 2009; Frederiksen et al., 2009). These biomonitoring data provide a direct measurement of the human body burden of BFRs resulting from various external exposure pathways (e.g. inhalation, ingestion of dust, diet and water) and contribute to the risk assessment of such compounds. However, the only available information on BFRs in UK human samples is for tri- to hexa-BDEs (major components of the pentabromodiphenyl ether commercial product) where the median concentrations for Σtri- to hexa-BDEs in human milk and serum samples collected in 2003 were 6.3 and 4.18 ng/g lw respectively (Kalantzi et al., 2004). In addition, BDE-209 was detected in 11 out of 153 serum samples at concentrations from 15 to 240 pg g^{-1} lw) (Thomas et al., 2006). Recently, we have reported on the presence of the HBCD degradation products pentabromocyclododecenes (PBCDs) and tetrabromocyclododecadienes (TBCDs) in UK indoor dust (Abdallah and Harrad, 2009a; Abdallah et al., 2008b). Despite this, we are unaware of any reports of such HBCD degradation products in human tissues worldwide.

Currently, very little is known about the extent to which the known contamination of indoor environments with BFRs influences human body burdens. While some studies have managed to establish significant positive correlations between the levels of BFRs in food or indoor dust and their concentrations in human milk or serum (Roosens et al., 2009a; Thomsen et al., 2008; Wu et al., 2007); such correlations could not be established in other studies (Roosens et al., 2009b; Toms et al., 2009). Recently, an alternative approach was adopted by Lorber (Lorber, 2008) who applied a simple pharmacokinetic model to predict the body burdens of PBDEs in American adults using intake data from different exposure pathways. The predicted body burdens were then compared to the reported levels of PBDEs in human milk and serum and the relation between external and internal exposure were discussed (Lorber, 2008).

To address the dearth of information related to the levels of TBBP-A, HBCD, and its degradation products in human tissues from the UK, this study determines the concentrations of these contaminants in human milk samples. Human milk is selected due to its high lipid content (compared to blood) which makes it an ideal matrix for measurement of internal exposure to lipophilic POPs. Moreover,

* Corresponding author. Tel.: +20 88 241 1009; fax: +20 88 2332776.
E-mail address: mae_abdallah@yahoo.co.uk (M.A.-E. Abdallah).

0160-4120/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2010.11.008

breast milk is a non-invasive medium for biomonitoring the external exposure of breast-fed infants to BFRs. Therefore, this study reports – for the first time – on the concentrations of the three main HBCD diastereomers and their degradation products in addition to TBBP-A in human breast milk samples ($n=34$) from Birmingham, UK. In addition, the diastereomeric and enantiomeric patterns of HBCDs in the studied milk samples are discussed. Concentrations of the studied compounds in breast milk are then used to estimate the dietary exposure of nursing infants using different scenarios. Finally, a simple, one-compartment pharmacokinetic model is applied to predict the body burdens of the studied BFRs in UK adults (using indoor air and dust levels reported elsewhere for Birmingham, UK (Abdallah and Harrad, 2010; Abdallah et al., 2008a)) and the model predictions compared to the concentrations of target compounds measured in the analyzed human milk samples.

2. Materials and methods

2.1. Sample collection

Breast milk samples (each comprising 50–100 mL) were obtained from 34 adult healthy volunteers via Birmingham Women's Hospital Milk Bank after the study protocol was approved by Warwickshire Research Ethics Committee and the R&D Department in Birmingham Women's NHS foundation trust. Informed consent was obtained from all the participants before sample collection. Samples were kept in clean screw-capped glass containers and transferred from the Milk Bank to the laboratory in special ice boxes then stored at −20 °C until the time of analysis. Due to ethical regulations, the samples were collected in a completely anonymous fashion with all participant information kept strictly confidential.

2.2. Sample extraction

Accurately weighted aliquots of the freeze-dried samples (~2 g) were loaded into pre-cleaned 66 mL Accelerated Solvent Extraction (ASE 300, Dionex Inc., UK) cells containing 1.5 g florisil, 3 g alumina, 5 g anhydrous Na_2SO_4 and hydromatrix (Varian Inc., UK) to fill the void volume of the cells, spiked with 25 ng of each of ^{13}C-labelled TBBP-A, α-, β- and γ-HBCD as internal standards. The ASE cells were extracted with hexane:dichloromethane (1:9, *v/v*) at 90 °C and 1500 psi. The heating time was 5 min, static time 4 min, purge time 90 s, flush volume 50%, with three static cycles. The lipid weight of the studied samples was determined gravimetrically on separate aliquots using a standard procedure (The European Standard EN 1528-2, 1996; See Supplementary data for more details).

2.3. Sample clean-up

The crude extracts were concentrated to 0.5 mL using a Zymark Turbovap® II (Hopkinton, MA, USA) then washed with 3 mL of 98% sulfuric acid. After phase separation, the hexane layer was transferred onto a florisil column topped with sodium sulfate and eluted with 25 mL of hexane:dichloromethane (1:1, v/v). The eluate was evaporated to dryness under a gentle stream of N_2 and the dried extract was reconstituted in 200 μL of d_{18}-γ-HBCD (25 pg μL^{-1} in methanol) used as recovery determination (or syringe) standard to determine the recoveries of internal standards for QA/QC purposes.

2.4. LC–ESI–MS/MS analysis

Sample analysis was carried out using an LC–MS/MS system composed of a dual pump Shimadzu LC-20AB Prominence liquid chromatograph equipped with SIL-20A autosampler, a DGU-20A3 vacuum degasser coupled to a Sciex API 2000 triple quadrupole mass spectrometer. Details of the multi-residue analytical methodology used for separation and quantification of the studied BFRs can be found elsewhere (Abdallah et al., 2008a, 2008b; Harrad et al., 2009). (A brief description is given in the supplementary data section).

2.5. Comparison of BFR intake to human body burdens

We have previously estimated UK adult intake of the target BFRs and degradation products via inhalation, dust ingestion and diet (Abdallah and Harrad, 2009b; Abdallah et al., 2008a) (A summary of the methods used to generate these estimations can be found in the Supplementary information section). To compare the estimated intakes to the body burdens measured in human milk samples, a simple one-compartment, first order pharmacokinetic (PK) model was used. The studied BFRs were hypothesized to accumulate in lipids (the single compartment in the model). Therefore, the change in BFR lipid concentration over time can be expressed by Eq. (1) (Lorber, 2008).

$$\frac{\delta C_{BFR}}{\delta t} = \frac{I_{BFR}(t) \times AF_{BFR}}{BL(t) - K_{BFR} \times C_{BFR}(t)} \quad (1)$$

where C_{BFR} is the compound specific concentration in lipids (ng g^{-1} lw); I_{BFR} is the daily intake of the target BFR (ng day^{-1}); AF_{BFR} is the absorption fraction; BL is body lipid mass (g) and K_{BFR} is the compound specific first order dissipation rate (day-1).

If K_{BFR} is assumed to be constant over time then Eq. (1) can be solved into:

$$C_{BFR}(t) = C_{BFR}(0) \times e^{(-K_{BFR} \cdot t)} + \left[\frac{(I_{BFR}(t) \times AF_{BFR})}{BL(t)}\right] \times \left[\frac{(1 - e^{(-K_{BFR} \cdot t)})}{K_{BFR}}\right] \quad (2)$$

where $C_{BFR}(0)$ is the studied BFR body lipid concentration at time 0 (initial concentration before intake).

Assuming a constant dose over time at constant body lipid mass, the steady state BFR lipid concentration can be calculated from Eq. (3).

$$C_{BFR} = \frac{(I_{BFR} \times AF_{BFR})}{BL \times K_{BFR}} \quad (3)$$

While Eq. (3) is used to predict the body burdens of the target BFRs, it is stressed that the assumption of steady state conditions is an inherent uncertainty with this approach.

2.6. Quality assurance/quality control

Good recoveries (71–103%) of the ^{13}C-labelled internal standards were obtained for all the studied compounds (Table SI-4). Further inspection of the method extraction/clean up performance was achieved via spiking milk samples ($n=5$) with d_{18}-α-HBCD prior to freeze drying and excellent recoveries (>90%) were obtained (Table SI-5).

No TBBP-A or HBCDs were detected in method blanks ($n=5$; consisting of 2 g pre-extracted anhydrous sodium sulfate treated exactly as a sample) or field blanks ($n=5$; consisting of ~2 g of broken pieces of the glass milk containers treated exactly as a sample). Therefore, there was no need for blank correction of the results and method limits of detection (LOD) and quantification (LOQ) were estimated based on a 3:1 and 10:1 S:N ratios respectively.

In the absence of an appropriate standard reference material for TBBP-A and HBCDs, the accuracy and precision of the analytical method for HBCDs was assessed via replicate analysis ($n=7$) of NIST SRM 2585. The results obtained compared favourably with the indicative values reported elsewhere (Keller et al., 2007) (Table SI-6a). For TBBP-A, a standard addition or "matrix spike" method at 3 concentration levels ($n=5$ at each level) was used to assess the