BFRs IN UK HUMAN MILK: RELATIONSHIP TO EXTERNAL EXPOSURE

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Introduction

Brominated flame retardants (BFRs) constitute a diverse group of compounds widely used in various consumer products to prevent or minimize fire hazard. However, the persistence and bioaccumulative characters of these compounds have resulted in increasing concern over their potential adverse effects to human health. Animal studies have shown BFRs to be potential endocrine disruptors, neurotoxic, immunotoxic and possibly carcinogenic[1]. The level of concern about this situation is illustrated by the fact that several BFRs are either included or under consideration for inclusion under the UNEP Stockholm Convention on POPs[2-3]. 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[4-6]. 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 Σtri-hexa BDEs (major components of the penta BDE commercial product) where the median concentrations in human milk and serum samples collected in 2003 were 6.3 and 4.18 ng g^{-1} lw respectively[7]. In addition, BDE-209 was detected in 11 out of 153 serum samples at concentrations from 15-240 pg g^{-1} lw)[8]. 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 [9-11]; such correlations could not be established in other studies [12-13]. An alternative approach was adopted by Lorber[14] who applied a simple pharmacokinetic model to predict the body burdens of PBDEs in American adults using intake data from different exposure pathways. To address the dearth of information related to the levels of BFRs in UK human matrices, this study reports on concentrations of TBBP-A, BDE-209, Σtri-hexa BDEs (Penta-BDE), HBCDs and HBCD degradation products in 34 human milk samples from Birmingham, UK. 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 by our research group for Birmingham, UK [15-18] and the model predictions were compared to the concentrations of target compounds measured in the analyzed human milk samples.

Materials and Methods

Sample collection: Breast milk samples (50-100 mL) were obtained from 34 adult healthy volunteers via Birmingham Women's Hospital Milk Bank after the study protocol was approved by the Warwickshire Research Ethics Committee. Informed consent was obtained from all the participants before sample collection. Samples were transferred to the laboratory in special ice boxes then stored at -20ºC until the time of analysis. Due to ethical regulations, samples were collected in a completely anonymous fashion with all participant information kept strictly confidential.

Sample Analysis: Milk samples were freeze-dried, accurately weighed then spiked with 25 ng of each of ^{13}C-labelled TBBP-A, BDE-47, BDE-99, BDE-153, BDE-209, α-, β- and γ-HBCDs prior to pressurized fluid extraction (ASE 300, Dionex Inc., UK). The crude extract was concentrated then washed with 98% sulfuric acid followed by further clean-up using florisil-packed columns. The eluate was evaporated under a gentle stream of nitrogen then reconstituted in 200 µL of methanol containing d_{18}-γ-HBCD and ^{13}C-BDE-100 as recovery determination standards. TBBP-A, HBCDs and HBCD degradation products were analyzed using LC-ESI-MS/MS while PBDEs were analyzed using LC-APPI-MS/MS. Further details can be found elsewhere[19-20].

Results and Discussion

Levels of BFRs in human milk: Table 1 shows the average concentrations of target BFRs in the analyzed milk samples compared to the average levels reported from other countries. TBBP-A was detected in 36% of the studied samples with concentrations ranging from <0.04 to 0.65 ng g^{-1} lw. Given the phenolic structure of this

compound and its reported short human half-life, detection of TBBP-A in some of the studied milk samples is likely to reflect recent rather than past exposure.

BDE-47 was quantified in all the analyzed samples contributing 34-93% to Σtri-hexa BDEs (sum of congeners 47, 49, 85, 99, 100, 153, 154). While the levels of Σtri-hexa BDEs in this study are slightly lower than those reported in UK human milk samples collected in 2003, these concentrations are still at the high end of those reported from other European countries. On the other hand, Σtri-hexa BDEs in UK human milk are substantially lower than those reported from USA and Canada which is in agreement with the extensive production and usage of the Penta-BDE formulation in North America.

BDE-209 was above LOQ in 69% of samples ranging from <0.05-0.92 ng g^{-1} lw. Interestingly, these levels are at the lower end of BDE-209 concentrations reported in human milk from other European countries despite the significantly higher levels of this BFR reported in UK indoor dust compared to the rest of Europe[21]. Given the poor bioavailability of BDE-209[6, 22], this may indicate that while indoor dust ingestion is the major pathway of external human exposure to BDE-209[14, 18], the high levels of this compound in indoor dust do not significantly contribute to internal body burdens which will be dominated by dietary exposure.

Table 1: Average concentrations (ng/g lw) of target BFRs in human milk

	This study (UK)	Norway	France	Spain	Sweden	USA	Canada	Australia	China
TBBP-A	**0.06**	0.067[23]	0.47[24]						0.93[25]
∑tri-hexa BDEs	**5.95**	2.34[26]	2.51[27]	2.14[28]	3.57[29]	34.00[30]	42.80[31]	7.6[32]	2.53[33]
BDE-209	**0.31**	0.61[34]	1.62[27]	2.9[35]		0.92[30]	0.43[36]	0.31[32]	3.00[33]
∑HBCDs	**5.95**	1.7[34]	2.2[37]	47[38]	0.45[4]	0.5[39]	3.8[39]		2.4[25]

HBCDs were quantified in all samples (1-22 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. Given the previously reported racemic chiral signatures of HBCDs in indoor dust[40] and diet[10], This indicates the presence of potential enantioselective processes associated with the absorption, metabolism and/or excretion of HBCDs.

HBCD debromination products pentabromocyclododecenes (PBCDs, 3 isomers; average = 0.04 ng g^{-1} lw; n=9) and tetrabromocyclododecadienes (TBCDs, 2 isomers; average = 0.15 ng g^{-1} lw; n=25) were detected for the first time in human tissues. Due to the lack of native or ^{13}C-labelled standards for TBCDs and PBCDs, semi-quantitative estimation of their concentrations in the analysed milk samples was performed using the average of response factors for α-, β- and γ-HBCDs. While our results confirm the presence of lower brominated HBCD derivatives in humans, it is not yet clear whether the detected TBCDs and PBCDs originate from *in vivo* biotransformation or exist as a result of intake via ingestion of indoor dust[41].

Nursing infants' dietary intake of BFRs via breast milk: To estimate the nursing infants' dietary intake of the studied BFRs via breast milk, equation 1 was used.

$$Di = \frac{C_{BFR}\ x\ F_{lipid}}{Bw} \quad ....(1)$$

Where *Di* is the estimated dietary intake (ng kg^{-1} bw day^{-1}); C_{BFR} is the concentration of target BFR in milk (ng g^{-1} lw); F_{lipid} is the daily lipid intake via breast milk (g day^{-1}) and *Bw* is the body weight (4.14 kg)[42]. The infant's daily lipid intake via breast milk ($\boldsymbol{F_{lipid}}$) was calculated based on U.S. EPA guidelines[42]. Table 2 shows the estimated dietary intake of target BFRs via breast milk using different exposure scenarios.

Interestingly, the average exposures of a nursing infant to ΣHBCDs, Σtri-hexa BDEs and TBBP-A via breast milk exceeded upper-bound dietary intakes of both UK adults and toddlers[16].

Table 2: Exposure (ng kg^{-1} bw day^{-1}) of a 1 month old infant to the target BFRs via breast milk.

	5th %ile	Average	Median	95th %ile
α- HBCD	6.44	28.62	18.49	89.02
β- HBCD	0.5	1.84	1.75	3.88
γ- HBCD	0.87	4.24	3.25	12.33
Σ HBCDs	9.77	34.71	22.32	104.97
Σ PBCDs	<0.04	0.6	0.48	1.04
Σ TBCDs	<0.04	1.08	0.91	2.05
TBBP-A	<0.03	0.98	0.63	2.74
Σtri-hexa BDEs	3.12	35.08	29.88	78.01
BDE-209	<0.05	2.56	2.52	4.91

Comparison of BFR intake to human body burdens: We have previously estimated UK adult intake of target BFRs via inhalation, dust ingestion and diet[16, 18]. To compare the estimated intakes to the body burdens measured in human milk samples, a simple pharmacokinetic model was used[14]. Assuming a constant dose over time at constant body lipid mass, the steady state BFR lipid concentration can be calculated from equation 2.

$$C_{BFR} = \frac{(I_{BFR}\ x\ AF_{BFR})}{BL\ x\ K_{BFR}} \quad \text{.....(2)}$$

Where C_{BFR} is the compound specific concentration in lipid (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 $^{-1}$).The bioaccessible fractions of each target compound derived from our PBET model[43] were used to substitute for AF_{BFR} in case of exposure via dust ingestion or diet, while the inhalable fraction was assumed to be 100% bioavailable. Body lipid mass was estimated based on a 25% body fat for a 70 kg adult[44]. Finally, K_{BFR} was calculated as $0.693/t_{0.5}$; where $t_{0.5}$ is the half-life in the body lipid compartment. Half-lives of target BFRs reported by Geyer et al.[22] were used in this exercise. Reported values for the $t_{0.5}$ of ΣHBCDs in human adipose tissue vary from 23-219 days[22]. Several studies have reported on the higher bioaccumulation potential and longer $t_{0.5}$ of α-HBCD in marine biota and mammals[4, 45-46]in addition to preferential biotransformation of the β- and γ- isomers in marine mammals[47]. Therefore, we selected a $t_{0.5}$ of 165 days (75% of the maximum $t_{0.5}$ of 219 days[28]) for α-HBCD, while a $t_{0.5}$ = 55 days was used for the β- and γ- isomers (25% of 219 days).

Good agreement was observed between the estimated and the measured body burdens of target BFRs (figure 1) indicating that air, dust, and food are the main human exposure pathways for the studied compounds. Given the dearth of information regarding the $t_{0.5}$ of HBCDs in various tissues and the bioavailability of the studied compounds from human GIT, the good agreement between the observed and predicted body burdens for HBCD diastereomers (figure 1) supports the previous reports of higher bioaccumulation potential and longer half-life of α-HBCD than the β- and γ-isomers.

However, more research is required for assessment of the bioavailability of various BFRs via different exposure routes and determination of $t_{0.5}$ of BFRs in various human tissues.

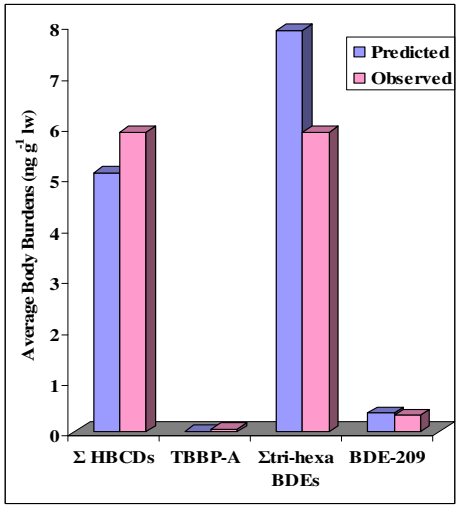

Fig. 1: Comparison of estimated BFR intakes to human body burdens

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