

Emerging and legacy flame retardants in UK human milk and food suggest slow response to restrictions on use of PBDEs and HBCDD

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INTRODUCTION

Following recent bans and restrictions on the manufacture and new use of legacy flame retardants (LFRs) like polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCDD), concerns have emerged over the increasing use of emerging flame retardants (EFRs). We recently showed concentrations of LFRs to be decreasing in UK indoor air and dust over the last decade, while those of EFRs have increased (Tao et al., 2016). Combined with reports of the presence of the bioaccumulative character of some EFRs manifested by their presence in biota, this suggests that concentrations of EFRs in the human diet and tissues may increase in the future. Despite emerging health concerns and evidence of exposure via indoor air and dust, very limited information on levels of EFRs in the human diet and human tissues exists to date (e.g. Fernandes et al., 2010; Sahlström et al., 2015). BTBPE, α -DBE-DBCH and β -DBE-DBCH were found in two Swedish pooled human milk samples collected in 2009-2010, while Zhou et al. (2014) measured several EFRs including EH-TBB, BEH-TEBP, BTBPE, and DBDPE in paired human maternal serum (n = 102) and breast milk (n = 105) samples collected in Canada in 2008-2009. EH-TBB was detected in > 55% of both serum and milk samples, while BEH-TEBP, BTBPE, and DBDPE were also present but less frequently detected in both matrices. In the present study, 16 EFRs were measured in 14 groups of composite food samples covering meat, liver, oily fish, eggs, and cheese to provide a preliminary estimate of UK dietary exposure. Concentrations of 8 PBDEs and 3 HBCDD diastereomers were measured in the same samples and compared with those reported in previous UK studies to evaluate the impact of regulations and restrictions on these LFRs. Also reported are the concentrations of EFRs in UK human milk. Comparison of concentrations of our target EFRs and LFRs in human milk sampled in 2010, with those in samples collected in 2014-15, we examine the impact of restrictions on PBDEs and HBCDD on human body burdens of both LFRs and EFRs.

MATERIALS AND METHODS

Target FRs FRs investigated in this study comprise: 8 PBDEs (BDEs # 28, 47, 99, 100, 153, 154, 183 and 209), 3 HBCDDs (α -, β - and γ -HBCDD) and 16 EFRs (α -DBE-DBCH, β -DBE-DBCH, EH-TBB, BTBPE, BEH-TEBP, DBDPE, TBBPA-BDBPE, PBT, HBB, PBBz, TBCT, TBX, PBEB, TBP-DBPE, as well as *syn*- and *anti*-dechlorane plus (DDC-CO).

Food samples. Samples of 14 different food groups were collected from two supermarkets representing national chains and one local market in Birmingham, UK during May and June

2015. Three samples of each food group were collected per retail outlet. Following purchase, equal weights of each of the 3 samples comprising each food group taken from each outlet were homogenized to provide a composite sample. Following homogenization, all composite samples were freeze dried and stored at -20 °C prior to analysis.

Human milk samples. Donors of all human milk samples were primiparas. Archived human milk samples (n=25, each comprising ~50 mL) for which LFR data have been reported previously (Abdallah and Harrad, 2014, 2011) were obtained from the milk bank of Birmingham Women's Hospital. Contemporary human milk samples (n=10, each comprising ~50 mL) were collected from two hospitals in Southampton and London, UK, between August 2014 and May 2015.

Analytical protocols In summary, following extraction and clean-up, instrumental analysis was conducted on a Trace 1310 GC coupled to an ISQ™ single quadrupole mass spectrometer (Thermo Scientific, TX, USA) operated in ECNI mode. After GC/MS analysis, the samples were evaporated and reconstituted in 200 µL of methanol for determination of HBCDDs by LC-MS/MS using a previously reported method (Harrad et al., 2009).

RESULTS AND DISCUSSION

Concentrations of FRs in food

EFRs Of all target 16 EFRs, only α -DBE-DBCH, β -DBE-DBCH, EH-TBB, BTBPE, BEH-TEBP, and DBDPE were detected in food. β -DBE-DBCH was detected in all samples (accounting for $65\% \pm 30\%$ of Σ EFRs), followed by α -DBE-DBCH and EH-TBB (detected in 97% and 77% of samples, respectively), with DBDPE the least detected EFR - detection frequency (DF) = 33%.

LFRs Concentrations of Σ HBCDD (<0.48-20 ng/g lw; <22-830 pg/g ww) in our food samples were comparable to those detected in similar foodstuffs in two previous UK studies (<LOD-300 pg/g ww (Driffield et al., 2008) and 65-680 pg/g ww (Food Standards Agency, 2006), respectively). Interestingly, concentrations of Σ PBDEs in our 2015 study exceed those recorded in previous UK studies conducted in 2003-2004 and 2006 (Food Standards Agency, 2006; FERA, 2009). In contrast, we reported recently a temporal decline in concentrations of BDE-209 in dust and of BDE 47 and 99 in air in UK offices (Tao et al., 2016). This apparent contradiction may be attributable to a gradual shift over time of PBDEs from the indoor to the outdoor environment of which one manifestation may be increasing concentrations of PBDEs in the human diet (Harrad and Diamond, 2006).

Concentrations of FRs in human milk

EFRs Table 1 summarises concentrations of target EFRs in archived human milk samples collected in 2010 and 2014-2015. Similar to food samples, only α -DBE-DBCH, β -DBE-DBCH, EH-TBB, BTBPE, BEH-TEBP, and DBDPE were found in human milk. Of our target EFRs, β -DBE-DBCH showed the highest DFs and concentrations in both human milk groups. To our knowledge, this is the first report of β -DBE-DBCH in human milk samples worldwide. Sahlström et al. (2015) detected only α -DBE-DBCH in two pooled breast milk samples in Sweden, at an average of 4.0 pg/g ww, well below the average concentrations detected in our study (41 and 24 pg/g ww in 2010 and 2014-15, respectively).

While no statistically significant differences were found between concentrations of individual EFRs in the two groups ($p > 0.05$), the DFs of all EFRs in 2010 were lower than those in 2014-15. This may indicate increased usage of these EFRs currently than hitherto, although the higher median concentrations of Σ EFRs in 2010 than 2014-15 (albeit driven by β -DBE-DBCH) suggests longer-term monitoring is required to elucidate the existence and nature of any temporal trends.

While both DBDPE and BTBPE display high bioaccumulation potential in fish (He et al.,

2012; Tomy et al., 2007), findings in mammals such as rats (Wang et al., 2010) and chickens (Zheng et al., 2015) suggest low bioaccessibility and relatively high biotransformation potential, consistent with the low DFs of these chemicals in our human milk samples. Interestingly, very high levels of BTBPE (56 and 54 ng/g lw) were found in two 2010 human milk samples, which may reflect elevated exposure to BTBPE of the individual donors concerned - plausible given our detection in one UK dust sample of BTBPE at a concentration of 4,700,000 ng/g (Tao et al., 2016).

In our study, EH-TBB was more frequently detected than BEH-TEBP in line with a previous study of EFRs in human milk from Canada (Zhou et al., 2014). This may be associated with higher bioaccessibility of EH-TBB compared to BEH-TEBP (Fang and Stapleton, 2014). Similar observations were made by Liu et al (2016) i.e. EH-TBB was detected more frequently than BEH-TEBP in human hair, fingernails, toenails and serum. In Canada, concentrations of EH-TBB (nd-24 ng/g lw) in human milk samples (n=105) (Zhou et al., 2014) exceeded those in our study, while concentrations of BEH-TEBP (nd-6.6 ng/g lw) and DBDPE (nd-25 ng/g lw) were comparable to those reported here. In the USA, concentrations of EH-TBB and BEH-TEBP in human hair, fingernails and toenails (EH-TBB: 7.6-4540 ng/g; BEH-TEBP: 13-2600 ng/g) as well as serum samples (TBB: 1.3-54 ng/g lw; BEH-TEBP: 19-69 ng/g) (Liu et al., 2016), greatly exceeded those reported here for human milk.

LFRs Concentrations of Σ tri-hexa-BDEs, BDE-209 and Σ HBCDDs in human milk are summarized in Table 1. Concentrations of Σ HBCDDs in 2014-15 were slightly - albeit not statistically significantly - lower than those in 2010 (Abdallah and Harrad, 2011). While concentrations of Σ HBCDD in our food samples were comparable to those in two previous UK studies (Driffield et al., 2008; Food Standards Agency, 2006); Σ HBCDDs in UK indoor air and dust collected between 2013 and 2015 were lower than in samples collected between 2006 and 2007 (Tao et al, 2016). This may account for the slight downward trend we observed for Σ HBCDDs in UK human milk.

Concentrations of both Σ tri-hexa BDEs and BDE-209 in 2010 (Abdallah and Harrad, 2014) and 2014-15 were not statistically distinguishable ($p > 0.05$). It is thus intriguing that while concentrations of Σ tri-hexa BDEs in our UK food samples exceed those reported in two previous UK food surveys; no significant temporal change was observed in concentrations of Σ tri-hexa BDEs in UK dust between 2006-2007 and 2013-2015 (Tao et al., 2016). This may indicate the importance of dust relative to diet as a vector of exposure to Σ tri-hexa BDEs. No statistically significant change in concentrations of BDE-209 in human milk was evident between 2010 and 2014-15 ($p > 0.05$). In contrast, while concentrations of BDE 209 in UK office dust decreased significantly between 2006-2007 and 2013-2015 (Tao et al., 2016), those in diet appear steady. This implies that concentrations of BDE-209 in dust exert a relatively minor influence on body burdens.

Dietary intakes

EFRs Estimated high-end and average dietary intakes of Σ EFRs in the UK were 26 and 89 ng/day for adults and toddlers, respectively. Of this, 14 and 50 ng/day respectively were to β -DBE-DBCH. By comparison, a Swedish study on dietary exposure to EFRs, reported they were detected only in fish (Sahlström et al., 2015). Estimated median daily intakes of EFRs were 6.8 and 3.3 ng/day for Swedish mothers and toddlers, similar to our estimated daily intakes of EFRs through fish consumption (10 and 2.2 ng/day, respectively).

LFRs Estimated average daily intakes of Σ PBDEs in our study are 42 and 124 ng/day for toddlers and adults, respectively; lower than in a previous study by the UK Food Standards Agency (2006). For Σ HBCDDs, estimated average daily dietary intakes for UK adults and toddlers are 8.8 and 31 ng/day respectively.

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Table 1: Concentrations of EFRs and LFRs in UK Human Milk (ng/g lw)

BFR	2010 Samples (n=25)			2014-15 Samples (n=10)		
	DF (%)	Range	Median	DF (%)	Range	Median
α-DBE-DBCH	20	<0.13-1.7	<0.13	100	0.30-1.1	0.60
β-DBE-DBCH	76	<0.13-38	3.1	100	0.43-10	1.2
EH-TBB	44	<0.01-2.1	<0.01	90	<0.01-0.48	0.16
BTBPE	28	<0.1-56	<0.1	40	<0.1-0.71	<0.1
BEH-TEBP	36	<0.1-4.6	<0.1	50	<0.1-0.73	<0.1
DBDPE	4	<0.78-250	<0.78	10	<0.78-58	<0.78
ΣEFRs	-	0.57-260	7.9	-	1.9-59	3.1
Σtri-hexa-BDEs	-	0.20-26	5.0	-	1.7-14	5.8
BDE-209	69	<0.06-0.92	0.25	40	<0.22-0.67	<0.22
ΣHBCDDs		1.0-22	3.8	-	0.69-7.1	2.9