Hammel *et al. J Environ Expo Assess* 2024;3:8
DOI: 10.20517/jeea.2023.51

Journal of Environmental Exposure Assessment

Research Article

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Novel and legacy brominated flame retardants in human breast milk and house dust from Denmark

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How to cite this article: Hammel SC, Vorkamp K, Nielsen JB, Sørensen LS, Knudsen LE, Frederiksen M. Novel and legacy brominated flame retardants in human breast milk and house dust from Denmark. *J Environ Expo Assess* 2024;3:8. https://dx.doi.org/10.20517/jeea.2023.51

Received: 18 Dec 2023 **First Decision:** 15 Jan 2024 **Revised:** 2 Feb 2024 **Accepted:** 18 Feb 2024 **Published:** 29 Feb 2024

Academic Editors: Judy LaKind, Stuart Harrad **Copy Editor:** Fangyuan Liu **Production Editor:** Fangyuan Liu

Abstract

A suite of novel brominated flame retardants (NBFRs), hexabromocyclododecanes (HBCDDs), polybrominated diphenyl ethers (PBDEs) and dechlorane plus were measured in matched samples of house dust (n = 47) and human breast milk (n = 40) from Denmark sampled in 2007, i.e., shortly after PBDE restrictions were implemented in Europe providing a valuable reference point. The most abundant BFR in breast milk was BDE-153 with a median concentration of 0.79 ng/g lipid followed by BDE-47 (median: 0.61 ng/g lipid) and BDE-209 (median: 0.53 ng/g lipid). Levels of bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP) were comparable (median: 0.78 ng/g lipid); however, they were based on fewer samples. α-HBCDD was the most abundant HBCDD in breast milk (median: 0.24 ng/g lipid) and detected in all samples. The house dust samples were dominated by BDE-209 (median: 432 ng/g) and γ-HBCDD (median: 177 ng/g); among the NBFRs, the highest levels were found for decabromodiphenyl ethane (DBDPE) (median: 86.2 ng/g) and BEH-TEBP (median: 26.7 ng/g). BDE-28, 47 and 153 were significantly correlated between breast milk and serum (r_s = 0.73-0.81, P < 0.0001); however, this was not the case for any of the NBFRs. Intake estimates for a 3-month-old infant indicated that for most of the flame retardants, breast milk was by far the dominant route of exposure, whereas dust only contributed substantially for BDE-209, γ-HBCDD, and to some extent α-HBCDD. This study is the first to report on human exposure to NBFRs in Denmark

and shows that internal exposure levels of NBFRs were similar to those of most PBDEs already at the time of PBDE restrictions.

Keywords: PBDE, NBFR, HBCDD, dechlorane plus, indoor environment, exposure

INTRODUCTION

Brominated flame retardants (BFRs) have been used in consumer products (e.g., home furnishings, electronics, and building materials) to reduce their flammability and slow down or prevent fires. Polybrominated diphenyl ethers (PBDEs) were among the first classes of additive flame retardants (FRs) used worldwide since the 1970s and typically applied in one of three technical mixtures: Penta-, Octa-, and Deca-BDE[1]. Due to evidence of persistence, bioaccumulation, long-range transport, and toxicity, the Penta- and Octa-BDE mixtures were banned in the EU and phased out in the USA from the mid-2000s[2], with their eventual global ban under the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009[3]. The use of DecaBDE in electronic applications has been restricted in the European Union (EU) since 2008, and DecaBDE was added to the Stockholm Convention in 2017[4]. In the early 2000s, hexabromocyclododecane (HBCDD) was the second most prominent BFR on the EU market. It was mainly used in polystyrene, but also as a replacement for PBDEs[5]. It was banned in the EU and worldwide in 2013 due to its POP- characteristics. The ban of PBDEs and HBCDD in consumer products led to an increased demand for alternatives, which include other brominated chemicals often referred to as novel brominated FRs (NBFRs) and the chlorinated flame retardant dechlorane plus (DP, also known as DDC-CO), which was recently added to the Stockholm Convention[6]. The use patterns of the different FR classes has varied between regions with a notable preference for PentaBDE in North America, HBCDD in Europe, TBBPA in Asia, and DecaBDE in all three regions[7]. However, there have also been temporal changes in consumption of the commercial mixtures of PBDEs, and later export of waste from more to less industrialized countries has altered the emission patterns[8]. Similar information on use patterns has not been identified for NBFRs.

Due to their persistence and the long lifetime of many consumer products, PBDEs and HBCDDs are still detected in the environment, including wildlife and abiotic environmental media such as air, soil, and indoor dust[9-11]. At the same time, NBFRs have also been widely detected in environmental samples, consumer products, and food[12,13]. In humans, PBDEs have been measured all over the world in a variety of matrices, including breast milk, blood, placenta, and adipose tissue, while reports on HBCDDs are less frequent and only few measurements exist on NBFRs in human matrices[14-18]. NBFRs have also been detected in upholstered furniture foam and house dust, and a urinary metabolite of 2-ethylhexyl 2,3,4,5-tetrabromoethylhexylbenzoate (EH-TBB) has been frequently found in both adults and children[19-22]. However, breast milk measurements of NBFRs have been rare and have only been conducted in the UK, USA, and China to date[23-26]. Overall, both legacy and current-use BFRs are omnipresent in our environment, suggesting chronic exposure and consequently concern about potential adverse health impacts.

PBDE congeners have been associated with a variety of negative health outcomes such as thyroid hormone disruption and neurodevelopmental deficits in both human and animal studies[27-30]. In particular, prenatal and early-life PBDE exposure has been linked to various adverse effects on neurobehavioral development in young children, such as poorer attention, fine motor coordination, and cognition as well as lower IQ[28,31,32]. Much less is known about the NBFRs[13] and their potential toxicity, but for several of the better-studied NBFRs, neurotoxic potential has been shown, possibly linked to endocrine disruption[33]. Specifically, components of the Firemaster® 550 mixture, which includes both brominated and organophosphate

compounds, have been associated with metabolic and endocrine disruption and activation of nuclear receptors regulating adipogenic pathways in *in vitro* studies[34-36]. Given the long-term effects of PBDE exposure and the growing prominence of NBFRs in consumer product use and environmental presence, there is a need to elucidate dominant pathways of exposure for these brominated compounds. This is of particular concern since children tend to have higher levels of exposure to chemicals including PBDEs, compared to adults[37].

Here, we report concentrations of PBDEs, HBCDDs, as well as NBFRs and dechlorane plus in breast milk collected from women in the Copenhagen area in 2007-2008. This was a critical point in BFR history with the phase-out and global ban of PBDEs in 2009 changing the use pattern of BFRs. Further, the breast milk concentrations were compared to house dust concentrations of HBCDDs and NBFRs collected at the same time as well as and to previously published paired PBDE concentrations in placenta tissue as well as maternal and cord blood. Exposure estimates for breast milk and dust were investigated and compared for the infants. These data present a retrospective assessment of breast milk concentrations in Denmark immediately following the international PBDE ban and prior to the HBCDD-ban, thus providing a benchmark for more recent and future measurements of BFR concentrations in breast milk.

EXPERIMENTAL

Study population and sample collection

Study participants were recruited among healthy, pregnant women scheduled for caesarean section at Copenhagen University Hospital from March to December 2007; further details are given elsewhere[15,38]. Caesarean section might have subtle influences on nutrient composition in breast milk[39], but potential influences on contaminant levels are unknown. In combination with a relatively high rate (approximately 20%) of caesarean sections in Denmark[40], the samples are likely to represent typical Danish FR levels at the time. The women were asked to sample breast milk once a week for 3 months after giving birth using a manual breast milk pump supplied by the study; after sampling, the milk was transferred to a pre-clean amber glass. The weekly samples were combined in a composite sample stored in the participants' household freezer. Each participant recorded the dates of sampling. Three months after birth, both milk samples and the participants' vacuum cleaner bags were collected. The content of the bags was sieved to < 75 µm, as also described in Vorkamp *et al.* (in which the current samples correspond to the "second dust sample")[41]. All samples were then stored at -20 °C until analysis. The study was approved by the Regional Ethics Committee of the Capital Region of Denmark (H-KF-327603) and the Danish Data Protection Agency. PBDE concentrations are also available for placental tissue, maternal and umbilical cord plasma as well as air and dust from the participants' homes sampled before giving birth[15,41-43]; an overview of the biological matrices is given in Supplementary Table 1. PBDE data for placenta, fetal blood, and maternal blood were used in this study to investigate associations with levels in breast milk and dust.

Chemical analysis

The samples were first analyzed for PBDEs and then later for NBFRs, DPs, and HBCDDs; due to a limited sample volume, a few samples were only analyzed for PBDEs; an overview of the different analyses is given in Supplementary Figure 1. For the analysis of PBDEs in breast milk, 20 mL of breast milk was dried with diatomaceous earth, spiked with ^{13}C-labelled BDE-209 and octachlorobiphenyl (PCB-198) and extracted using Soxhlet with hexane:acetone (4:1). The milk extracts were cleaned on a multilayer column including sulfuric acid-impregnated silica eluted with 250 mL hexane as described in Vorkamp *et al.*[44]. The cleaned extract was reduced in volume by rotary evaporator, and the final volume was approximated to 0.5 mL and analyzed by gas chromatography with electron capture negative ionization-mass spectrometry (GC-ECNI-MS). The analysis included 19 PBDEs (BDE-17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 156, 154, 183, 197, 203, 206, 207, 208 and 209); however BDE-154 was not separated from the polybrominated biphenyl

BB-153 in this analysis. Octa to deca PBDEs were quantified using ^{13}C-BDE-209, whereas PCB-198 was used for quantification of tri-hepta PBDEs. The dust samples (0.5 g) were extracted as the milk samples and cleaned up on a column consisting of 2 g activated aluminum oxide, 2 g sulfuric acid impregnated silica, and some Na_2SO_4, eluted with 60 mL hexane and analyzed by GC-ECNI-MS for the PBDEs given above.

The chemical analyses of HBCDDs and NBFRs included α-, β-, and γ-HBCDD, EH-TBB, 2,3-dibromopropyl 2,4,6-tribromophenyl ether (DPTE, also known as TBP-DBPE), bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), decabromodiphenyl ethane (DBDPE), as well as syn- and anti-DP. Their analysis in breast milk and dust followed the method described in Vorkamp *et al.*, with some modifications[45]. In brief, sub-samples of approximately 0.5 g of house dust or 20 mL breast milk (after heating to 37 °C) were dried with diatomaceous earth, spiked with isomer-specific internal standards of ^{13}C-HBCDD as well as ^{13}C-BEH-TEBP (where relevant) and extracted by Pressurized Liquid Extraction (dust) or Soxhlet (milk) using hexane:dichloromethane (1:1). Dust samples were cleaned up on a column consisting of 2 g activated aluminum oxide, 2 g deactivated silica and some Na_2SO_4 and eluted with 60 mL hexane:dichloromethane (1:1). For the milk extracts, the column clean-up was the same described in Vorkamp *et al.*[44,45], but applied twice and eluted with 250 mL hexane:dichloromethane (1:1). For both dust and milk extracts, the eluates were split equally in two for analysis of NBFRs and HBCDDs, respectively. Each of the milk extracts was evaporated to dryness, using silicone-coated vials for NBFRs[45] and reconstituted in 200 µL isooctane or methanol for analysis of NBFR and HBCDD, respectively. For NBFRs in dust, the extracts were reduced in volume by rotary evaporation and under nitrogen, using 300 µL isooctane as a keeper, and adjusted to a precise volume of 1 mL in isooctane. The second dust extract was evaporated to dryness and reconstituted in 500 µL methanol for HBCDD analysis. NBFRs were analyzed by GC-ECNI-MS using the parameters described in Vorkamp *et al.*, but combining all NBFRs in one analytical run on a 15 m DB-1 GC column (0.25 mm i.d., 0,25 µm film thickness, J&W Scientific, Folsom CA, USA)[45]. HBCDD-isomers were analyzed by LC-MS-MS as previously described[46]. The NBFR BEH-TEBP was analyzed separately in a subset of milk samples (n = 5), using Gel Permeation Chromatography for clean-up as described in Vorkamp *et al.* because BEH-TEBP is not stable to the acid treatment used for clean-up of the milk samples[45]. BEH-TEBP was included in all dust analyses.

Each analytical batch included QA/QC measures such as procedural blanks, internal or standard reference material (NIST SRM 2585), as described elsewhere[15,41]. For PBDEs in breast milk, a sample of human breast milk from the ring test "Dioxins in Food, 2006" was used (with consensus values for BDE-28, 47, 99, 100, 153, 154, 209)[47]. The obtained results were within ±20% of the consensus values for all congeners except for BDE-209 in one batch, which was 37% above the consensus value, and BDE-154, which was generally overestimated due to the co-elution with BB-153 described above. NIST SRM 2582 was also used for quality control of HBCCDs and NBFRs, based on indicative values published in the literature[22,48]. For the NBFR analyses, PCB-198 [and, in some batches, ^{13}C-polychlorinated naphthalene (PCN)-27] were added for recovery determination prior to extraction. The recovery rate for dust was 91.5% on average, with a range of 68%-116%. The recovery standards were difficult to integrate in the milk samples, leading to an average of 85% (range 52%-120%). However, the low numbers are likely caused by chromatographic challenges due to matrix effects, rather than indicating losses in the process.

Statistical analyses

Analyses were conducted using SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, USA). Based on examination of distributions of all PBDEs and NBFRs in breast milk and the biological matrices previously analyzed (placenta, fetal and maternal blood), the majority of which were right-skewed, non-parametric statistics or $\log_{10}$-transformed concentrations were used for all analyses. Statistical analyses were conducted only for chemicals with detection frequencies > 65% overall. For any concentrations below the

method detection limit (MDL), values were imputed as MDL/2 and normalized in a matrix-specific manner (i.e., breast milk, serum, and placenta by lipid concentration and dust by dry mass).

We evaluated associations between breast milk and dust for NBFRs and between breast milk and dust, placenta, fetal blood, and maternal blood for PBDEs using Spearman correlations. Relationships among NBFRs and PBDEs, separately, were assessed within breast milk samples, also with Spearman correlations. We examined additional relationships between $\log_{10}$-transformed concentrations and various predicting factors using regression analyses. These factors included mother's age, pre- and term-pregnancy body mass index, and pregnancy weight gain (evaluated as continuous variables) and parity and previous breastfeeding (evaluated as categories, dichotomized based on median reported value). For evaluating associations with breast milk PBDE concentrations, maternal serum and house dust concentrations were split into tertile categories. Beta coefficients from all of these models were exponentiated and used as an estimate of multiplicative change in concentrations for each unit of increase for all continuous variables or relative to the reference category for any categorical variables.

Exposure estimate calculations

Estimates of daily intake were calculated based on breast milk and house dust concentrations of NBFRs and PBDEs with > 65% detection in a matrix. These estimates were calculated for a 3-month-old infant, given that the house dust concentrations were collected in homes at 3 months post-delivery. Estimates are presented in units of ng/day for comparison of exposure pathways and were not normalized to body mass. The US Environmental Protection Agency Exposure Factors Handbook was used for recommended estimated daily consumption of breast milk and for unintentionally ingested house dust for 3-month-old infants. For human milk intake, the weighted mean intake value for exclusively breastfed infants was 758 mL/day, with breast milk density estimated to be 1.03 g/mL[49]. Dust ingestion for children < 6 months was estimated to be 20 mg/day[50]. The intake equations are shown below:

$$Breast\ milk\ daily\ intake = \frac{FR\ ng}{g\ milk}\left(\frac{758\ mL\ milk\ consumed}{day}\right)\left(\frac{1.03\ g}{mL\ milk}\right) = \frac{FR\ ng}{day}$$

$$Dust\ daily\ intake = \frac{FR\ ng}{g\ dust}\left(\frac{0.02\ g\ dust\ consumed}{day}\right) = \frac{FR\ ng}{day}$$

Furthermore, intake estimates of PBDEs from air were calculated based on air measurements in the same homes days prior to birth as previously presented in Vorkamp *et al.*[41]. A long-term exposure value for inhalation of 3.5 m^3/d was estimated for infants 1-3 months of age[51]. The equation for intake by inhalation was then:

$$Inhalation\ daily\ intake = \frac{FR\ ng}{m^3\ air}\left(\frac{3.5\ m^3\ inhaled\ air}{day}\right) = \frac{FR\ ng}{day}$$

RESULTS & DISCUSSION

Study population

A total of 51 women were recruited for the overall study. Of these, four women did not participate in the follow-up, and another seven did not wish to or were not able to deliver a breast milk sample. This resulted in a total of 40 breast milk samples and 47 dust samples collected 3 months after birth; details about the overall study population have been described previously[15,41,42]. The mean age of mothers of the subset of participants providing breast milk samples was 32.7 years (range: 24-43 years) [Supplementary Table 2]. The average number of previous children was one (range: 0-3 children) and mothers had previously breastfed for about a year prior to starting the sample collection (mean: 11 months; range: 0-30 months). Further details on descriptors are given in Supplementary Table 2.

Breast milk

Levels

BFRs have only previously been reported in breast milk in Denmark in two studies collecting samples between 1997 and 2002, which included PBDEs in milk and placenta samples[52] and PBDEs and HBCDDs in milk[53]; NBFRs had not previously been measured in breast milk from Denmark. In the current study, we measured 19 PBDE congeners, 3 HBCDD diastereomers, 5 NBFRs, and 2 DP isomers. Of these chemicals, 8 PBDEs, 2 HBCDD diastereomers, and 4 NBFRs were detected in over 65% of the breast milk samples [Tables 1 and 2]. Overall, the most abundant BFR measured in breast milk was BDE-153. It had a median concentration of 0.79 ng/g lipid and was detected in all samples [Table 1], which could be expected given that the half-life of BDE-153 is among the longest of the PBDE congeners[54]. This was, however, not observed in Danish breast milk collected 5-10 years earlier where BDE-47 was the dominating congener[52,53]. BDE-47 was the congener with the second highest median concentration in our study (median = 0.61 ng/g lipid), followed by BDE-209 (median = 0.53 ng/g lipid), which were detected in 100% and 95% of the samples, respectively. PBDE congeners 99, 100 and 154 were also detected in more than 95% of the samples, but with median concentrations < 0.25 ng/g lipid. However, the high detection frequency of BDE-154 may have been affected by the co-elution of BB-153, which was more frequently detected in the corresponding blood samples where the two BFRs were quantified separately[42]. These concentrations were lower than in those sampled in Denmark 5-10 years earlier with the exception of BDE-209[53]. However, the levels were similar to those measured in a large-scale study in Norway between 2003 and 2005, although the concentration of BDE-209 in this study was slightly higher (Norwegian study median = 0.32 ng/g lipid)[55]. The concentrations were also comparable to breast milk samples collected in Sweden around the same time period[56]. Finally the $\sum_6$PBDE is within the range of other European countries for the same sampling period as reported by the World Health Organization (WHO), though the number of participating countries was low. The observations also match the decreasing trend observed in Western Europe since the year 2000[57].

Of the HBCDD diastereomers, α-HBCDD was detected in all samples and was the most abundant diastereomer (median = 0.24 ng/g lipid), with γ detected in 65% and β detected in only 6% of samples [Table 2]. α-HBCDD was also the most abundant non-PBDE flame retardant that was measured in the breast milk samples if disregarding BEH-TEBP that was only analyzed in five samples. α-HBCDD has also previously been the dominant HBCDD diastereomer in diastereomer-specific analyses of breast milk[58,59]. Compared to a previous Danish study conducted between 1997 and 2002 (n = 435), our study observed slightly lower concentrations of α-HBCDD[53]. Although it is difficult to determine trends in a region from just two time points, data suggest that HBCDD concentrations could have decreased in Denmark in the 2000s, which is in line with Swedish studies indicating decreasing concentrations in the same time period[37]. Other studies examining breast milk samples in Europe from the mid to late 2000s detected similar albeit slightly higher concentrations, including a cohort of Norwegian women[60] and studies conducted in Ireland[61], as well as the recent WHO/UNEP overview[57]. However, other studies conducted in the UK showed substantially higher concentrations of HBCDDs[62,63], whereas HBCDDs were not detected in a study on breast milk from Sweden, possibly related to a GC-MS method, which is less sensitive than the commonly applied LC-MS/MS method[56,64].

Detected in nearly all samples (DF = 91%), the most abundant NBFR in our study was DPTE (median = 0.11 ng/g lipid) [Table 2]. DPTE was the main component of the BFR mixture Bromkal 73-5PE that was manufactured until the mid-1980s[65,66]. Although production past the 1980s is unknown, it has been measured in house dust, in addition to Arctic air, seawater, and biota, indicating persistence, bioaccumulation, and long-range transport[12,67,68]. Previous studies conducted in the Netherlands, China,

Table 1. Descriptive statistics of PBDEs in milk and dust

		Breast milk (n = 40) ng/g lipid			House dust (n = 47) ng/g dry weight			
Chemical	**% Detect**	**Mean**	**Median**	**Range**	**% Detect**	**Mean**	**Median**	**Range**
BDE-17	85	0.035	0.024	< 0.014-0.13	60	0.36	0.22	< 0.022-3.09
BDE-28	85	0.076	0.047	< 0.036-0.43	83	0.66	0.35	< 0.022-6.63
BDE-49	43	0.039	n.a.	< 0.012-0.13	79	1.18	0.67	< 0.022-8.09
BDE-71	0	n.a.	n.a.	n.a.	0	n.a.	n.a.	n.a.
BDE-47	100	1.20	0.61	0.19-9.92	100	35.4	13.7	1.12-316
BDE-66	38	0.021	n.a.	< 0.008-0.042	96	1.34	0.75	< 0.082-6.17
BDE-77	0	n.a.	n.a.	n.a.	0	n.a.	n.a.	n.a.
BDE-100	95	0.45	0.23	< 0.10-8.14	100	8.56	3.53	0.270-60.0
BDE-99	100	0.28	0.18	0.065-1.48	100	44.7	19.2	1.27-325
BDE-85	18	0.031	n.a.	< 0.013-0.10	89	1.80	0.78	< 0.024-13.5
BDE-154[$]	98	0.21	0.15	< 0.095-0.81	96	3.85	1.83	< 0.029-26.5
BDE-153	100	1.55	0.79	0.44-25.3	98	5.98	2.71	< 0.057-45.7
BDE-183	30	0.054	n.a.	< 0.023-0.43	100	7.19	4.14	0.36-31.2
BDE-197	58	0.098	0.046	< 0.051-0.28	100	3.56	2.14	0.27-15.3
BDE-203	3	n.a.	n.a.	n.a.	100	2.38	1.84	0.30-13.5
BDE-208	35	0.058	n.a.	< 0.022-0.47	87	8.12	2.92	< 0.55-175
BDE-207	43	0.13	na.a	< 0.045-1.21	94	22.8	5.47	< 0.95-628
BDE-206	3	n.a.	n.a.	n.a.	96	65.2	12.0	< 1.95-2,220
BDE-209	95	2.70	0.53	< 0.061-49.8	100	2641	432	54.5-79,800
$\sum PBDE_6$[*]	100	3.73	2.21	0.98-45.2	100	105	53.6	4.64-672

[*]$\sum PBDE_6$: ∑BDE-47, -99, -100, -153, -154, and -183; [$]co-eluting with BB-153.

Table 2. Descriptive statistics of NBFRs, HBCDD and Dechlorane Plus in milk and dust

		Breast milk (n = 37) ng/g lipid			House dust (n = 43[a]) ng/g dry weight			
Chemical	**% Detect**	**Mean**	**Median**	**Range**	**% Detect**	**Mean**	**Median**	**Range**
DPTE	91	0.13	0.11	< 0.028-0.34	93	1.36	0.99	< 0.21-5.76
EH-TBB	43	0.12	n.a.	< 0.018-0.60	100	12.0	6.26	1.75-89.1
BTBPE	100	0.087	0.064	0.026-0.28	100	31.7	7.23	2.15-293
BEH-TEBP	100[b]	0.92	0.78	0.53-1.50	100	39.1	26.7	1.79-200
DBDPE	19	0.21	n.a.	< 0.088-2.06	100	141	86.2	10.4-801
Syn-DP	49	0.062	n.a.	< 0.008-0.44	98	9.77	1.57	< 0.21-332
Anti-DP	84	0.13	0.073	< 0.022-1.85	100	8.23	4.54	0.55-54.3
α - HBCDD	100	0.53	0.24	0.11-5.68	100	140	92.1	11.4-548
β - HBCDD	6	0.051	n.a.	n.a.	100	52.4	32.1	5.52-293
γ - HBCDD	65	0.10	0.051	< 0.020-1.13	100	431	177	18.8-3,310

[a]in one sample it was not possible to quantify BEH-TBEP, DBDPE, Syn- and Anti-DP due an interference in the chromatogram; [b]only measured in 5 samples with different clean-up technology because BEH-TEBP degraded in the acid treatment.

and Tanzania have analyzed DPTE in breast milk samples, and while concentrations seem similar, detection frequencies were much lower (< 50%)[25,69,70]; however, this may be highly method-dependent. Given that Given that manufacture and distribution data is unknown, it is unclear why DPTE is still wide-spread in environmental and human samples, with relatively high concentrations in this study, similar in concentration to BDE-99.

Although BEH-TEBP was only measured in a subset of five samples due to degradation in the standard clean-up procedure, the concentrations were among the highest of any BFR measured in the breast milk samples (median = 0.78 ng/g lipid). High levels despite moderate detection frequency were also observed for milk samples collected in Norway, Slovakia, and the Netherlands around the same time[71], which collectively calls for inclusion of this compound in future studies, despite the analytical challenges, as it could be an important pathway of exposure for infants.

The levels of syn- and anti-DP detected here were slightly lower than previously observed across Europe[71] but higher than in Canada[72]. However, the literature on NBFRs in human milk is still very limited and thus limits the comparison of our results to others; a recent review of contaminants in human milk by Chi *et al.* included several groups of flame retardants but not any of the NBFRs investigated here[73].

Intramatrix correlations

In general, PBDEs, particularly the lower molecular weight congeners and congeners of the commercial PentaBDE mixture, were highly correlated in breast milk [Table 3]. Likewise, the two diastereomers of HBCDD (α and γ) were also highly correlated (r_s = 0.64, $P < 0.0001$). DPTE in breast milk was positively and significantly correlated with BTBPE and anti-DP, but not with the HBCDD diastereomers. Anti-DP was further moderately correlated with the two HBCDD diastereomers, whereas BTBPE was correlated with BDE-28. α-HBCDD was also positively correlated with BDE-28, -47, -99, and -100 (r_s = 0.34-0.47, $P < 0.05$, Table 3). Given their concurrent use as flame retardants, this indicates that HBCDDs and PBDEs could have similar routes of exposure or sources.

Relationship with age

Several of the BFR compounds in breast milk were positively associated with the mother's age. Two NBFRs (BTBPE and anti-DP), α-HBCDD, and BDE-154 concentrations increased by almost 10% per one-year change in the mother's age [Figure 1]; however, for BDE-154 it is worth noting that it co-elutes with BB-153, which is very persistent and its use dates back further than the PBDEs.

Dust

Among the PBDEs, the dust samples were dominated by BDE-209 (median: 432 ng/g), followed by BDE-99 (median: 19.2 ng/g) and BDE-47 (median: 13.7 ng/g) [Table 1], as previously observed in other European studies[16]. The median level of the ΣHBCDDs was comparable to BDE-209, though the maximum values were not as pronounced for ΣHBCDDs. DBDPE was also found in high levels though not as high as BDE-209, which at the time of sampling was still in use in some applications. Substantial amounts of EH-TBB, BTBPE, and BEH-TEBP were also detected. Although median levels in the literature span many orders of magnitude[13], the medians and ranges in the current study were very similar to levels reported by Sahlström *et al.*, who sampled a similar population in Sweden two years later, particularly for the PBDEs[74].

Intermatrix correlations of breast milk

Dust

Of the BFRs analyzed in both breast milk and house dust, three of the seven PBDEs in paired samples were significantly correlated (r_s = 0.35-0.42, $P < 0.05$; Table 4), i.e., BDE-28, -47, -99. They are all lower molecular weight PBDEs and prominent congeners of the Penta-BDE commercial mix. This suggests that house dust could be a recent source of exposure together with toxicokinetic processes, which may favor the transfer to and accumulation of lower brominated molecules in milk, despite higher exposure to BDE-209[75]. Positive and significant correlations between milk and house dust have been observed previously for PBDE congeners, although the strength of correlation varied by congener in different geographical regions. Significant associations between breast milk and house dust have been observed in Sweden for BDE-47 and -99[56], in the U.S. for summed PBDEs[76], and in New Zealand for BDE-209[77], but not in a study performed in Australia[78].

Table 3. Correlation table within milk for flame retardants detected in > 65% of samples (PBDEs: *n* = 40, NBFRs: *n* = 37)

	BDE-17	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209	DPTE	BTBPE	Anti-DP	α - HBCDD	γ - HBCDD
BDE-17	1												
BDE-28	0.15	1											
BDE-47	0.06	0.84***	1										
BDE-99	0.34*	0.69***	0.82***	1									
BDE-100	0.03	0.73***	0.85***	0.63***	1								
BDE-153	0.27	0.17	0.18	0.17	0.35*	1							
BDE-154	0.30	0.24	0.18	0.24	0.11	0.33*	1						
BDE-209	0.29	-0.16	-0.23	-0.11	-0.13	-0.13	-0.06	1					
DPTE	0.07	0.19	0.08	0.14	0.14	0.00	-0.03	0.00	1				
BTBPE	0.10	0.38*	0.25	0.31	0.25	0.11	0.24	-0.02	0.55***	1			
Anti-DP	-0.04	0.17	0.23	0.17	0.04	-0.31	0.13	0.22	0.44**	0.13	1		
α - HBCDD	0.12	0.45**	0.43**	0.34*	0.47**	-0.02	0.20	0.00	0.23	0.20	0.38*	1	
γ - HBCDD	0.14	0.18	0.14	0.23	0.08	-0.12	-0.01	0.07	0.23	0.06	0.35*	0.64***	1

*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

In general, breast milk concentrations of NBFRs were not significantly correlated with house dust [Supplementary Table 3], although the associations were generally positive with the exception of DPTE being notably negatively correlated (r_s = -0.32, P = 0.07). This is unlike PBDE exposure, which has historically been linked fairly strongly to house dust[79]. It may suggest other exposure pathways than dust; however, the transformation processes of NBFRs are not well-understood and might differ from those of the relatively persistent PBDEs. Only one other study was identified addressing correlations between HBCDDs in milk and dust, but the correlation analysis was not possible due to the low detection frequency in breast milk[56].

Maternal serum

Concentrations of PBDEs in maternal serum of the same individuals were previously described and reported in Frederiksen *et al.*[42]. In brief, five PBDEs were detected in the majority of the samples, and BDE-209 was the most abundant, although it was only measured in 18 samples total, with BDE-153 being the second most abundant PBDE in serum [Supplementary Table 1]. PBDEs in breast milk and maternal serum were significantly and positively correlated for the three PBDE congeners with full sample overlap (i.e. DF > 65%) between milk and serum (r_s = 0.73-0.81, $P < 0.0001$; Table 4), but not for BDE-209. When relationships were adjusted for parity and mother's age, these relationships were still clearly observed, with mothers who had the highest tertile concentration

Table 4. Spearman correlation (r_s) between milk *vs.* dust, placenta, fetal plasma, and maternal plasma for flame retardants detected in > 65% of samples

		Breast milk							
House dust (*n* = 40)	PBDEs	BDE-17	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-209
	BDE-28		0.35*	0.39*	0.36*				
	BDE-47			0.39*	0.37*				
	BDE-99			0.39*	0.42*				
	BDE-100			0.37*	0.39*	0.19			
	BDE-153			0.37*	0.37*		0.01		
	BDE-154			0.38*	0.42*			0.19	
	BDE-209								-0.12
Placenta (*n* = 39)	BDE-47		0.56**	0.53**		0.58**			
	BDE-99		0.37*	0.32*	0.09	0.36*			
	BDE-100		0.42*	0.45*	0.38*	0.58**			
	BDE-153					0.42*	0.73***		
	BDE-154							0.59***	
	BDE-209								0.12
Fetal blood (*n* = 31)	BDE-28		0.83***	0.77***	0.58***	0.67***			
	BDE-153						0.64***	0.37*	
Maternal blood (*n* = 39)	BDE-28		0.81***	0.74***	0.55**	0.62***			
	BDE-47		0.65**	0.76***	0.57**	0.67***			
	BDE-153						0.73***		
	BDE-209[#]						-0.59*		0.39

[#]analyzed in only 15 samples. *P < 0.05, **P < 0.001, ***P < 0.0001.

of BDE in their blood having between 2 to 4 times as high concentrations in breast milk, depending on the congener [Figure 2].

Cord blood

Concentrations of PBDEs in cord blood were previously reported in Frederiksen *et al.*[42]. Only BDE-28 and -153 were detected in > 65% (cut-off set for statistical analyses) of both the cord blood and breast milk samples. Both congeners were highly correlated between cord blood and breast milk (r_s = 0.83 and 0.64, P < 0.0001; Table 4). This is to be expected given the strong correlations previously observed between maternal serum and cord blood[42]. Notably, the concentrations in fetal serum reflect *in utero* PBDE exposure, while PBDEs in breast milk will continue adding to infant exposure post-partum.

Placenta

PBDE concentrations in placenta were previously described in Frederiksen *et al.*, with further investigations comparing placenta and dust concentrations in Vorkamp *et al.*[15,41]. Positive and significant correlations were observed for 4 of the 6 PBDEs detected in both placenta and breast milk (BDE-47, -100, -153, and -154 (r_s = 0.53-0.73; P < 0.0001)), and notably, no association was observed for BDE-99 and -209.

Exposure estimates

Exposure estimates for 3-month-old infants in 2007 were calculated based on breast milk and house dust measurements for NBFRs, HBCDDs, DPs, and PBDEs; for PBDEs, intake via air was also calculated. For the NBFRs and PBDEs, with the exception of BDE-209, breast milk contributed substantially more to the daily exposure than house dust [Table 5]. This was in agreement with Toms *et al.*, who reported an intake of BDE-47 from breast milk for Australian infants very similar to our calculations (10-440 ng/day) for samples

Table 5. Exposure estimates for 3-month-old infant (ng/day), reported if > 65% detection

	Breast milk			House dust			Air*		
Chemical	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
DPTE	2.71	2.55	1.18-14.8	0.03	0.02	< 0.21-5.76	n.a.		
EH-TBB				0.24	0.13	1.75-89.1			
BTBPE	1.90	1.34	0.85-5.49	0.63	0.15	2.15-293			
BEH-TEBP	24.4	21.5	20.3-50.0	0.78	0.53	1.79-200			
DBDPE				2.82	1.72	10.41-801			
Syn-DP				0.20	0.03	< 0.21-332			
Anti-DP	3.28	1.14	< 0.17-60.5	0.17	0.09	0.55-54.3			
α - HBCD	15.2	4.67	2.69-248	2.80	1.84	11.35-548			
β - HBCD				1.05	0.64	5.52-293			
γ - HBCD	2.74	0.95	< 0.429-37.0	8.62	3.54	18.82-3,310			
BDE-17	0.59	0.52	< 0.19-1.70	0.01	0.004	< 0.001-0.06	0.05	0.04	< LOQ-0.09
BDE-28	1.77	1.14	< 0.19-18.1	0.02	0.01	< 0.001-0.13	0.05	0.04	0.01-0.16
BDE-49				0.03	0.01	< 0.001-0.16	0.03	0.02	0.01-0.12
BDE-47	28.9	15.0	1.65-419	0.67	0.26	0.02-6.32	0.67	0.48	0.17-2.61
BDE-66				0.03	0.01	< 0.001-0.12	0.02	0.01	< LOQ-0.05
BDE-100	13.6	4.73	< 0.48-344	0.16	0.07	0.01-1.20	0.05	0.05	0.01-0.13
BDE-99	6.08	4.01	1.03-62.7	0.86	0.42	0.03-6.50	0.29	0.23	0.06-1.3
BDE-85				0.04	0.02	< 0.001-0.27			
BDE-154[$]	4.40	3.23	< 0.81-14.4	0.07	0.03	< 0.001-0.53			
BDE-153	44.8	17.1	6.56-1,070	0.11	0.05	< 0.001-0.91			
BDE-183				0.08	0.08	0.01-0.62			
BDE-197				0.07	0.04	0.01-0.31			
BDE-203				0.05	0.04	0.01-0.27			
BDE-208				0.17	0.06	< 0.01-3.50			
BDE-207				0.48	0.11	< 0.01-12.6			
BDE-206				1.36	0.24	< 0.04-44.4			
BDE-209	70.9	10.2	< 0.48-1,190	49.4	8.52	0.37-1,596	0.81	0.42	< LOQ-6.41

*Based on pre-birth home air data from Vorkamp *et al.* (2011)[41]; [$]not separated from BB-153.

taken the same year[78]. For BDE-209 and α-HBCDD, median exposure estimates were more similar for dust and breast milk, although slightly higher in breast milk, while the opposite trend was observed for γ-HBCDD. Exposure through inhalation only contributed marginally to the total exposure of PBDEs but was comparable to the exposure via dust for PBDEs with up to four bromine atoms [Table 5]. These data suggest that in addition to *in utero* exposures, exposure through breast milk plays a dominant role in contributing to the cumulated infant exposure to BFRs compared to house dust (and air), and that this trend also includes the NBFRs. Although these samples were collected in the late 2000s, they provide a valuable point of reference and insight for exploring exposure pathways and prioritizing preventive initiatives, particularly for NBFRs, for which much is still unknown. PBDE and HBCDD levels are expected to continue to decline in the future due to global restrictions, as was recently demonstrated for breast milk BDE-47 and BDE-99 concentrations[80], whereas the expected trend for NBFR is less clear as these chemicals are not yet restricted.

The final exposure pathway, dermal uptake, was not assessed directly in the current study as several of the required input data such as dust loadings and skin lipid thickness were not available for infants, making the estimates highly uncertain. For infant skin in particular, the stratum corneum is thinner than adult skin[81] so the permeability coefficients determined on adult skin may not apply. However, estimates of skin

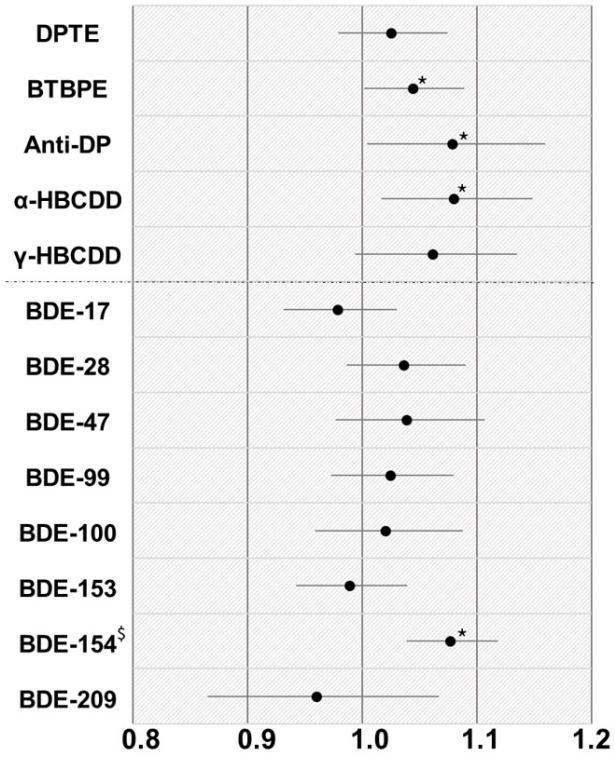

Figure 1. Multiplicative change in NBFR and PBDE concentration (log_{10}-transformed) in breast milk and 95% confidence intervals for a one-year change in mother's age. *$P < 0.05$; $^{\$}$not separated from BB-153.

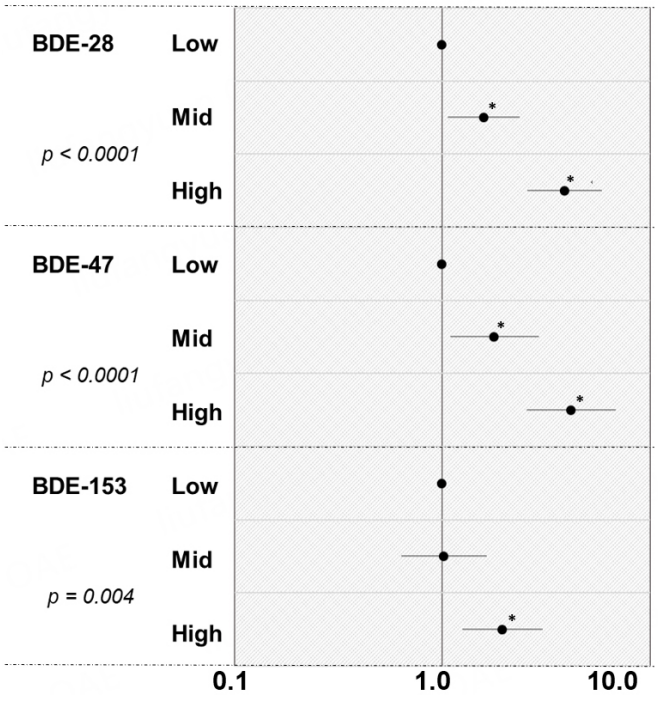

Figure 2. Tree plot of relationships between PBDEs in maternal blood (as shown in tertiles) compared to multiplicative change in breast milk concentrations. These models were adjusted for parity and mother's age. *$P < 0.005$.

permeability of BFRs have shown that dermal uptake of NBFRs does occur, though much is initially trapped in the epidermis[82]. The highest dermal uptake is expected for the BFR with lower log K_{ow} such as DPTE, HBCDD, and the least brominated PBDEs, and it cannot be ruled out that dermal uptake may be a relevant exposure pathway.

CONCLUSION

Despite attention to BFRs over the last few decades, NBFRs have only scarcely been studied with regard to human external and internal exposure. This study shows that the internal exposure levels of NBFRs were similar to those of most PBDEs already at the time of PBDE restrictions. As opposed to PBDEs, NBFRs were not correlated in breast milk and house dust, indicating other prominent exposure pathways than dust ingestion and/or transformation and toxicokinetic processes which are not yet understood. These data add to the knowledge of exposure pathways and estimated total intake for infants that may be a valuable reference point for future BFR studies and assessment of current and future restrictions.

DECLARATIONS

Acknowledgments

The authors would like to thank all study participants for volunteering, Marie Pedersen for the initial recruitment, and Birgit Groth for skillful analyses of NBFRs, DPs, and HBCDDs.

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Visualization, formal analysis: Hammel SC
Investigation: Frederiksen M

Availability of data and materials

Fully anonymized data can be shared upon justified request.

Financial Support and sponsorship

The study was supported by the Danish Agency for Science, Technology and Innovation (271-06-0355) and Danish Council for Independent Research (DFF - 1333-00034), the data analyses and publication were further supported by FFIKA (Focused Research Effort on Chemicals in the Working Environment) from the Danish Government.

Conflict of Interest

All authors declare that there are no conflicts of interest.

Ethical approval and consent to participate

The study was approved by the Regional Ethics Committee of the Capital Region of Denmark (H-KF-327603) and the Danish Data Protection Agency. All participants provided written consent to participate.

Consent for publication

Not applicable.

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