Linking exposure pathways to internal concentrations of brominated flame retardants in Swedish mothers and their toddlers

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ABSTRACT

Brominated flame retardants (BFRs) have been used for decades in a variety of consumer products to enhance their fire safety. This has resulted in exposure of the environment, wildlife and humans causing risks of endocrine disruption and effects on neurobehavioral development. The bans or use restrictions of many BFRs have resulted in the introduction of other brominated chemicals (emerging BFRs) on the market. Humans are exposed to BFRs primarily via diet and dust ingestion, but the importance of the different exposure pathways for different BFRs has scarcely been studied. Children in particular have not been studied well due to lack of biomonitoring data.

In this thesis, a mother-toddler (11-15 months of age) cohort (n=24) from Uppsala was studied for their exposure to a broad range of BFRs including tri-decabrominated diphenyl ethers (tri-decaBDEs), isomer-specific hexabromocyclododecanes (HBCDs) and emerging BFRs (EBFRs). A clean-up and fractionation method was developed for determination of the above-mentioned BFRs in the same sample without dividing the sample. The samples analyzed included matched serum (mothers and toddlers), feces (toddlers), dust from their homes, dietary items and two pooled breast milk samples. Measures of external exposure from dietary intake and dust ingestion were estimated. These were compared to internal concentrations (serum, feces) to determine which exposure pathways were most important for explaining the different BFR concentrations and patterns found in mothers and toddlers.

Serum BDE concentrations (BDE-47, -100, -197, -207, -208 and -209) in toddlers were significantly higher than those in their mothers. Taking all the results into account, the toddlers' higher serum levels of tetra-pentaBDEs seem to be the result of previous breastfeeding and those of octa-decaBDEs from exposure to house dust. For mothers, diet, and in particular meat and fish intake, was estimated to be the main exposure route of tri-hexaBDEs (probably important for hepta-nonaBDEs as well) and HBCDs. Dust ingestion was estimated to be the main route for BDE-209 exposure in mothers.

Significant correlations were found between the tetra-decaBDE concentrations in matched serum and feces samples indicating that feces could be used as a non-invasive sample matrix for biomonitoring of PBDEs in toddlers. The ratios between the concentrations in serum and feces indicated efficient uptake and accumulation of tetra-hexaBDEs from the human gut while BDE-209 was taken up less efficiently and much of the ingested amount was excreted in the feces.

HBCDs were not detected in the serum samples but four HBCD isomers were detected in feces of toddlers. EBFRs were detected in the feces of toddlers and in a few serum samples from both mothers and toddlers indicating that exposure to these replacement chemicals occurs. The estimated dietary and dust intakes of HBCDs and EBFRs were lower than those of PBDEs.

SAMMANFATTNING

Bromerade flamskyddsmedel (BFR) har använts i flera årtionden i ett brett sortiment av konsumentvaror för att öka deras brandsäkerhet. Detta har lett till att miljö, djur och människor har exponerats för dessa kemikalier, vilket innebär risker för endokrina störningar och neurologiska beteendeeffekter under barnets tidiga utveckling. Flera BFR har förbjudits vilket har resulterat i att andra bromerade kemikalier (”nya” BFR) har introducerats på marknaden. Människan exponeras för BFR huvudsakligen genom kost- och dammintag, men betydelsen av de olika exponeringsvägarna har inte studerats speciellt väl. Det saknas särskilt sådan information för små barn på grund av brist på biomonitoringdata.

I denna avhandling har exponering för flera BFR studerats i 24 svenska mammor och deras 11-15 månader gamla småbarn. De BFR som studerades var tri-dekabromerade difenyletrar (tri-dekaBDE), isomer-specifika hexabromcyklododekaner (HBCD) och en rad ”nya” BFR (EBFR). En upprenings- och fraktioneringsmetod utvecklades för att kunna bestämma dessa BFR i ett och samma provextrakt. De analyserade proverna var matchade serum (mammor och småbarn), fekalier (småbarn), damm från deltagarnas hem, matprover och två poolade bröstmjölksprover. S.k. externexponering från mat- och dammintag uppskattades och jämfördes med de koncentrationer som fanns i serum och fekalier. Detta för att förstå vilka exponeringsvägar som är viktigast för att förklara de olika BFR-koncentrationer och mönster som påvisades i mammorna och deras småbarn.

Serumkoncentrationerna av BDE-47, -100, -197, -207, -208 och -209 i barnen var signifikant högre än i deras mammor. En sammanfattning av alla resultat visar att de högre serumhalterna av tetra-pentaBDE i barnen verkar komma från tidigare bröstmjölksintag och de högre halterna av okta-dekaBDE från exponering för damm. För mammorna verkar matintaget, särskilt av kött och fisk, vara den viktigaste exponeringsvägen för tri-hexaBDE (förmodligen viktig också för hepta-nonaBDE) och HBCD. Dammintaget verkar för mammorna vara den viktigaste exponeringsvägen för BDE-209.

Tetra- dekaBDE-koncentrationer i matchade serum- och fekalieprover från barnen visade signifikanta korrelationer. Detta indikerar att fekalier skulle kunna användas som en icke-invasiv provmatris för biomonitoring av PBDE i småbarn. Kvoterna mellan koncentrationerna i serum och fekalier indikerar att tetra-hexaBDE tas upp effektivt från tarmen medan BDE-209 tas upp mindre effektivt och att mycket av den intagna mängden utsöndras i fekalierna.

HBCD påvisades inte i serumproverna men fyra HBCD-isomerer detekterades i barnens fekalier. EBFR detekterades i barnens fekalier och i några få serumprover från både mammor och barn vilket antyder att de exponeras för dessa ersättningskemikalier. De uppskattade intagen av HBCD och EBFR från mat och damm var lägre än intagen av PBDE.

TIIVISTELMÄ

Brominoituja palonestoaineita (BFR:t) on vuosikymmenien ajan käytetty eri kuluttajatuotteissa lisäämään niiden paloturvallisuutta. Tästä johtuva ympäristön, eläinten ja ihmisten altistuminen lisää riskiä endokrinologisiin häiriöihin ja vaikuttaa neurologiseen kehitykseen. Monien BFR:ien käyttöä koskevat kiellot ovat johtaneet uusien brominoitujen kemikaalien (emerging BFRs, EBFRs) markkinoilletuontiin. Ihmisten altistuminen BFR:ille tapahtuu pääasiallisesti ruoan ja pölyn välityksellä, mutta näiden eri altistumisreittien tärkeyttä on tutkittu vain vähän. Erityisesti lasten altistumista ei ole tutkittu hyvin, koska biomonitorointitietoa pitoisuuksista lapsissa ei ole saatavilla.

Tässä väitöskirjaprojektissa tutkittiin 24 ruotsalaisen äidin ja heidän pikkulastensa (11-15 kk) altistumista tri-decabrominoiduille difenyylieettereille (tri-decaBDE:ille), heksabromisyklo-dodekaani (HBCD)-isomeereille ja useille EBFR:ille. Puhdistus- ja erotusmetodi kehitettiin yllä mainittujen BFR:ien määritystä varten tehtäväksi samasta näyteuutteesta jakamatta sitä. Analysoidut näytteet olivat veriseerumi (äidit ja pikkulapset), uloste (pikkulapset), pöly äitien ja lasten kotoa, elintarvikenäytteet ja kaksi yhdistettyä rintamaitonäytettä. Altistuminen ruoan ja pölyn välityksellä arvioitiin ja niitä verrattiin äitien ja pikkulasten sisäisiin pitoisuuksiin (seerumi, uloste), jotta voitiin määrittää eri altistumisreittien tärkeys.

Seerumin BDE-pitoisuudet (BDE-47, -100, -197, -207, -208 and -209) olivat merkitsevästi korkeammat pikkulapsissa kuin heidän äideissään. Ottaen huomioon kaikki tulokset, korkeammat tetra-pentaBDE pitoisuudet lasten seerumissa näyttävät olevan seurausta aiemmasta rintamaidon saannista ja korkeammat octa-decaBDE pitoisuudet johtuvat altistumisesta pölylle. Äitien kohdalla ruoan, ja varsinkin lihan ja kalan syönnin, arvioitiin olevan tärkein altistumisreitti tri-hexaBDE:ille (luultavasti myös hepta-nonaBDE:ille) ja HBCD:ille. Pölyn välityksellä tapahtuva altistuminen oli tärkein reitti BDE-209:lle altistumiselle äideissä.

Merkitseviä korrelaatioita löydettiin seerumin ja ulosteen tetra-decaBDE-pitoisuuksien välillä osoittaen, että ulostetta voitaisiin käyttää ei-invasiivisena näytematriisina pikkulasten PBDE-biomonitoroinnissa. Seerumi- ja ulostepitoisuuksien suhteet osoittivat, että tetra-hexaBDE:t imeytyvät tehokkaasti ja kertyvät ihmisen suolesta. BDE-209 imeytyy heikommin ja suuri osa niellystä määrästä eritetään ulosteessa.

HBCD:itä ei havaittu seerumissa, mutta neljä HBCD-isomeeria havaittiin pikkulasten ulosteessa. EBFR:iä havaittiin pikkulasten ulosteessa ja muutamassa seeruminäytteessä äideistä ja lapsista osoittaen, että altistumista näille korvaaville kemikaaleille tapahtuu. Altistumiset ruoan ja pölyn välityksellä arvioitiin olevan alemmat HBCD:ille ja EBFR:ille kuin PBDE:ille.

LIST OF PAPERS

I. Clean-up method for determination of established and emerging brominated flame retardants in dust

Sahlström L, Sellström U, de Wit CA (2012). Analytical and Bioanalytical Chemistry 404:459-466.

II. Brominated flame retardants in matched serum samples from Swedish first-time mothers and their toddlers

Sahlström LMO, Sellström U, de Wit CA, Lignell S, Darnerud PO (2014). Environmental Science & Technology 48 (13):7584-7592.

III. Feasibility study of feces for non-invasive biomonitoring of brominated flame retardants in toddlers

Sahlström LMO, Sellström U, de Wit CA, Lignell S, Darnerud PO (manuscript). Submitted to Environmental Science & Technology

IV. Exposure to brominated flame retardants via diet and dust compared to internal concentrations in a Swedish mother-toddler cohort

Sahlström LMO, Sellström U, de Wit CA, Lignell S, Darnerud PO (manuscript).

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CONTRIBUTION TO PAPERS

I. I was involved in developing the idea and was responsible for all experimental work including development and validation of the methods, and took the lead in writing the paper.

II, III and IV. I was responsible for all experimental work, except for sampling, including laboratory work, instrumental, data and statistical analyses, and took the lead in writing the paper.

Dust samples used in Paper I were obtained from a previous study on indoor environments in Stockholm (Thuresson et al. 2011).

All other sampling (Papers II, III and IV) was performed by The Swedish National Food Agency (SLV) in Uppsala.

LIST OF ABBREVIATIONS

BATE	2-bromoallyl 2,4,6-tribromophenyl ether
BDE	Brominated diphenyl ether
BDE-28	2,4,4’-tribromodiphenyl ether
BDE-47	2,2’,4,4’-tetrabromodiphenyl ether
BDE-99	2,2’,4,4’,5-pentabromodiphenyl ether
BDE-100	2,2’,4,4’,6-pentabromodiphenyl ether
BDE-153	2,2’,4,4’,5,5’-hexabromodiphenyl ether
BDE-183	2,2’,3,4,4’,5’,6-heptabromodiphenyl ether
BDE-196	2,2’,3,3’,4,4’,5,6’-octabromodiphenyl ether
BDE-197	2,2’,3,3’,4,4’,6,6’-octabromodiphenyl ether
BDE-203	2,2’,3,4,4’,5,5’,6-octabromodiphenyl ether
BDE-206	2,2’,3,3’,4,4’,5,5’,6-nonabromodiphenyl ether
BDE-207	2,2’,3,3’,4,4’,5,6,6’-nonabromodiphenyl ether
BDE-208	2,2’,3,3’,4,5,5’,6,6’-nonabromodiphenyl ether
BDE-209	2,2’,3,3’,4,4’,5,5’,6,6’-decabromodiphenyl ether
BEH-TEBP	bis(2-ethylhexyl)tetrabromophthalate
BFR	Brominated flame retardant
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane
DBDPE	decabromodiphenyl ethane
DBE-DBCH	tetrabromoethylcyclohexane
DBHCTD	hexachlorocyclopentenyl-dibromocyclooctane
DDC-CO	Dechlorane Plus
DecaBDE	Technical DecaBDE mixture
EBFR	Emerging brominated flame retardant
ECNI	Electron capture negative ionization
EFSA	European Food Safety Agency
EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate
GC	Gas chromatography
HBBz	hexabromobenzene
HBCD	Hexabromocyclododecane
IQ	Intelligence quotient
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
OBTMPI	octabromotrimethylphenylindane

OctaBDE	Technical OctaBDE mixture
PBBz	1,2,3,4,5-pentabromobenzene
PBDE	Polybrominated diphenyl ether
PBEB	pentabromoethylbenzene
PBT	pentabromotoluene
PentaBDE	Technical PentaBDE mixture
SD	Standard deviation
SLE	Supported liquid extraction
SPE	Solid phase extraction
SRM	Standard reference material
TBCT	tetrabromo-*o*-chlorotoluene
TBP-DBPE	2,3-dibromopropyl 2,4,6-tribromophenyl ether
TBX	2,3,5,6-tetrabromo-*p*-xylene
UPLC	Ultra-performance liquid chromatography
ΣHBCD	sum of α-, β- and γ–HBCD stereoisomers
ΣpentaBDE	sum of BDE congeners occurring in the PentaBDE technical mixture
ΣoctaBDE	sum of BDE congeners occurring in the OctaBDE technical mixture
ΣdecaBDE	sum of BDE congeners occurring in the DecaBDE technical mixture

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INTRODUCTION

Brominated flame retardants

Brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) have been used as flame retardants in various consumer products for decades (de Wit 2002) (see Figure 1 for molecular structures). These chemicals have been added to textiles, carpets, plastics used in electronic device housing and in building materials to fulfill the safety requirements stated by governments or regulatory agencies in different countries. PBDEs have been used as three different commercial mixtures: Penta-, Octa- and DecaBDE. The PentaBDE mixture contains mainly a few tetra-pentabrominated BDEs (BDE-47, -99, -100) but also smaller amounts of tri- and hexaBDEs (BDE-28, -153, 154). The OctaBDE mixture contains hexa-decaBDEs (BDE-153, -183, -196, -197, -203, -206, -207 and -209) and the DecaBDE mixture consists mainly of the fully brominated BDE-209 and smaller amounts of nonaBDEs (BDE-206, -207, -208) (La Guardia 2006). The HBCD technical product contains primarily three stereoisomers, α-, β- and γ-HBCD, in the proportions 11.8, 5.8 and 81.6 %, respectively (Heeb et al. 2005). The annual production and use of all BFRs in 2005 was estimated to be a total of 311000 metric tons per year in North America, Europe and Asia (Fink et al. 2008). The use of BFRs has resulted in widespread exposure of the environment, wildlife and humans (de Wit 2002).

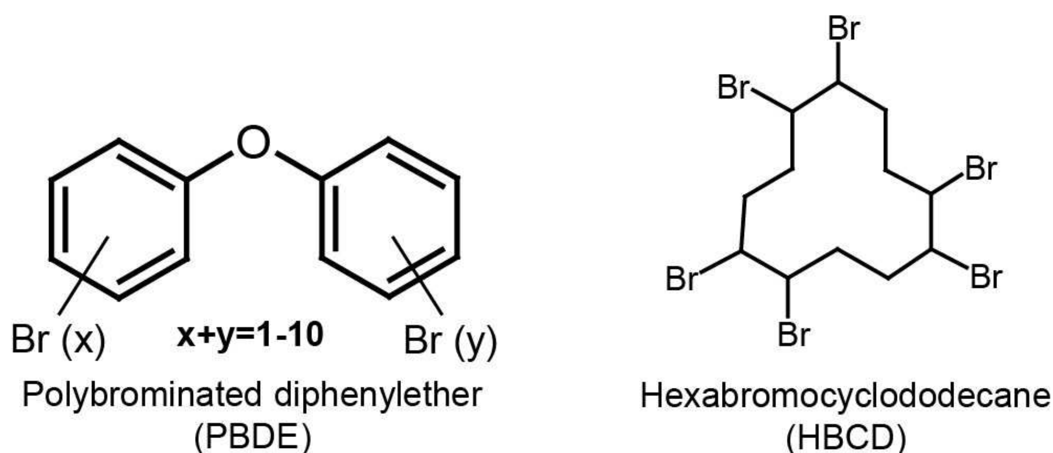

Figure 1. Molecular structures of PBDE and HBCD

PBDEs and HBCD have been shown to cause endocrine disrupting effects in animals and humans (Darnerud 2008). Effects on neurobehavioral development in children have been associated with exposures to congeners present in the PentaBDE mixture (Eskenazi et al. 2013; Gascon et al. 2011; Herbstman et al. 2010). A recent study reported that prenatal exposure to BDE-47 was associated with lower IQ and higher hyperactivity scores in children (Chen et al. 2014).

In light of evidence of PBDEs and HBCD causing toxicological effects in humans and animals, regulatory agencies started to act. Penta- and OctaBDE technical mixtures were recently banned (UNEP 2009), the DecaBDE mixture has been phased out (USEPA 2009) in several countries and HBCD is currently being phased out (UNEP 2013). These regulatory actions to protect human health have resulted in the application of a number of emerging BFRs (EBFRs) to replace the banned or phased-out chemicals (Betts 2008). The names, abbreviations and molecular structures of the EBFRs included in this thesis are listed in Table 1. Physical-chemical properties of EBFRs are presented elsewhere (Bergman et al. 2012). Various EBFRs have already been detected in both abiotic and biotic environments (Covaci et al. 2011). However, to date very limited information is available about the toxicological effects of EBFRs (EFSA 2012). A recent study showed indications of endocrine disrupting effects in rats exposed to a flame retardant mixture (Firemaster 550) containing two EBFRs, EH-TBB and BEH-TEBP (Patisaul et al. 2013).

Table 1. The names, abbreviations and molecular structures of the EBFRs included in this thesis

Compound name (abbreviation)	**Molecular Structure**	**Compound name (abbreviation)**	**Molecular Structure**
2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB)		1,2,3,4,5-pentabromobenzene (PBBz)	
bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP)		tetrabromo-o-chlorotoluene (TBCT)	
bis(2,4,6-tribromophenoxy) ethane (BTBPE)		pentabromotoluene (PBT)	
decabromodiphenyl ethane (DBDPE)		pentabromoethylbenzene (PBEB)	
Dechlorane Plus (DDC-CO)		2,3-dibromopropyl 2,4,6-tribromophenyl ether (TBP-DBPE)	
α- and β-tetrabromoethylcyclohexane (DBE-DBCH)		hexabromobenzene (HBBz)	
2,3,5,6-tetrabromo-p-xylene (TBX)		hexachlorocyclopentenyl-dibromocyclooctane (DBHCTD)	
2-bromoallyl 2,4,6-tribromophenyl ether (BATE)		octabromotrimethyl-phenylindane (OBTMPI)	

Human exposure to BFRs

Humans are exposed to BFRs via several different routes such as air inhalation, dermal absorption from dust or consumer products and diet and dust ingestion (Daso et al. 2010), where the two latter are generally considered as the most important ones (Frederiksen et al. 2009; Covaci et al. 2006). Dust ingestion occurs via hand-to –mouth activity and also via mucociliary clearance of inhaled particles which are eventually swallowed (Jones-Otazo et al. 2005). BFRs are taken up from the gastrointestinal tract by simple diffusion and with help of bile salt micelles (Kelly et al. 2004).

Previous Swedish studies detected tri-decaBDEs and total HBCD in air and dust from different indoor environments (Thuresson et al. 2011) and used these data to estimate intakes from inhalation and dust ingestion for adults and toddlers (de Wit et al. 2012). The estimated intakes were compared to dietary intakes based on Swedish food items (Darnerud et al. 2006; Törnkvist et al. 2011) to see which exposure routes were the most important ones (de Wit et al. 2012). However, there was no dietary data available for octa-decaBDEs and also no data on EBFRs in diet or dust, so the comparison was only possible for BDE congeners occurring in the PentaBDE technical mixture and for total HBCD. The study (de Wit et al. 2012) concluded that both air inhalation and dust ingestion played minor roles compared to intake from diet but when using maximum dust concentrations detected in the different microenvironments (homes, offices, day care centers and cars), dust ingestion was the major contributor for toddler intake of ΣpentaBDE (sum of BDE-28, -47, -99 and -153) and total HBCD. Other studies have also suggested dust ingestion as a more important pathway than diet for children's exposure to PBDEs, particularly for BDE-209 (Fischer et al. 2006), due to children's higher hand-to-mouth activity (Stapleton et al. 2008).

A few studies exist on associations between external exposure to BFRs and internal concentrations in adults. Wu et al. (2007) reported correlations between breast milk and dust concentrations of tetra-hexaBDEs and between breast milk levels of tri-decaBDEs and dietary habits of female adults. Björklund et al. (2012) also found a correlation between dust and breast milk concentrations for BDE-47. In a Belgian study, exposure via dust, but not diet correlated with serum concentrations of HBCDs in adults (Roosens et al. 2009). No such studies exist for children or for EBFRs in adults or children.

PBDEs and HBCD have been detected in human blood in different parts of the world (Athanasiadou et al. 2008; Frederiksen et al. 2009; Gari and Grimalt 2013; Linderholm et al. 2010). Only a few studies have reported PBDEs in serum of young children but when included the concentrations were higher in children compared to adults (Ali et al. 2013; Fischer et al. 2006; Toms et al. 2009). These findings have raised concerns about the possible higher risks of BFR exposure for the young population compared to adults but risk assessments are hindered by the lack of biomonitoring data for young children (Birnbaum and Cohen Hubal 2006).

Biomonitoring of BFRs

Blood is commonly used as a sample matrix for determining body burden of pollutants and risk assessments and health studies are usually based on serum concentrations. Blood is well distributed in the whole body and relatively easy to sample in adults (Esteban and Castano 2009). However, due to ethical considerations, blood is very difficult to obtain from young children. Sample amounts available for analysis are also usually much smaller than those obtained from adults. Pooling of serum samples from several children or the use of non-invasive sample matrices, such as urine have been applied for biomonitoring in children (Heffernan et al. 2013). However, urine is not applicable for analysis of persistent hydrophobic chemicals such as BFRs (Hakk and Letcher 2003) and pooled samples only give information about the mean concentrations in a population and as BFRs often occur in highly skewed distributions in humans (Lignell et al. 2009; Schecter et al 2003), the mean concentration obtained from pooled samples might not be a good representative of the population. Another disadvantage of pooled samples is that they cannot be used in individual biomonitoring aiming to link internal exposure to effects. Other non-invasive matrices, such as saliva, hair and breast milk have been used for analyses of environmental pollutants in adults (Esteban and Castano 2009). Hair is difficult to relate to internal concentrations as it is in direct contact with the surroundings (air and dust) and saliva is not an adequate sample matrix for analysis of persistent hydrophobic chemicals. Breast milk is a very good sample matrix for nursing women giving information about their internal exposure as well as providing a measure of external exposure for nursing infants/toddlers. However this is valid only for the contaminants that readily partition into breast milk and that are absorbed by the child. Very few studies have used feces as a sample matrix for biomonitoring but fecal concentrations of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) have been shown to correlate well with those in blood from adults (Moser and McLachlan 2001; Rohde et al. 1999). Several studies have compared PCDD/F, PCB and HCB concentrations in feces of nursing infants to those in breast-milk to determine uptake and fecal excretion rates (Abraham et al. 1994; Dahl et al. 1995). Recently, tri-heptaBDEs were reported in feces from an infant and a toddler from Australia and the BDE levels in the two feces samples were compared to BDE levels in a pooled blood sample from Australian children 0-4 years of age (Chen et al. 2013). No such studies exist for higher brominated BDEs, HBCDs or EBFRs and there are no studies of associations between serum and feces concentrations in toddlers.

THESIS OBJECTIVES AND HYPOTHESES

Objectives

- develop a method for determination of tri-decaBDEs, isomer-specific HBCDs and EBFRs in the same sample using reasonable amounts of solvents and without dividing the sample (Paper I)
- apply the developed method to the analysis of blood serum (Paper II), feces (Paper III), and house dust, breast milk and dietary items (Paper IV)
- determine and compare serum concentrations of BFRs in mothers versus their toddlers (Paper II)
- investigate if feces could be used as a non-invasive sample matrix for biomonitoring of BFRs in toddlers (Paper III)
- determine BFR concentrations in the two most important exposure media, diet and house dust (Paper IV)
- study associations between the internal concentrations and external exposures to determine the most important exposure pathways of tri-decaBDEs, HBCDs and EBFRs in mothers and their toddlers (Paper IV)

Hypotheses

1. Dust ingestion plays a major role in total exposure to octa-decaBDEs and HBCD for toddlers and plays a measurable role in adult exposure whereas dietary intake is more important for exposure to tri-hexaBDEs.
2. Correlations exist between dust and blood concentrations of BFRs in women as well as in their toddlers.
3. BFR concentrations in blood and feces from toddlers are correlated.

METHODS

Samples

In order to link exposure pathways to internal concentrations of BFRs in mothers and toddlers, a number of samples were collected. Study participants were recruited among first-time mothers who delivered one healthy child in Uppsala University Hospital in 2009-2010 and were included in the POPUP study (Persistent Organic Pollutants in Uppsala Primiparas) soon after delivery (Lignell et al. 2009). When their children were about 11 months old, they were re-contacted and asked to participate in this (present) follow-up study. Possible exposure routes for these children were breastfeeding, food and dust primarily from their homes as they had not yet started daycare. For those that agreed to participate, blood samples from the mothers and blood and feces samples from their toddlers and settled house dust samples from their homes were collected. This resulted in 20 complete matched sets of all sample types. The mothers also filled in a questionnaire on individual dietary habits. The sampling methods are described in Papers II (serum), III (feces) and IV (dust). Furthermore, representative dietary samples of five different food categories (fish, meat, vegetable oils, dairy products and eggs) were analyzed (Paper IV). These samples were from the market basket survey conducted in 2010 by the Swedish National Food Agency (NFA 2012). The market basket samples are collected based on per capita food consumption data every 5 years. Two pooled breast milk samples from the participants in the POPUP study from the years 2009 and 2010 were also analyzed. Individual breast milk samples were not available for the participants in this study. More information about the compilation of the pooled breast milk and dietary samples is given in Paper IV. The sampling was organized by the Swedish National Food Agency in Uppsala, Sweden. This study was approved by the Regional Ethics Committee in Uppsala, Sweden (Permit 2004:M-177) and informed consent was obtained from the participants.

Extraction

Dust samples were extracted according to Thuresson et al. (2011) with minor modifications. In short, the dust was extracted with 2 x 15 mL dichloromethane (DCM) in an ultrasonic bath, the extracts were combined and the solvent was changed to *n*-hexane (*n*-Hx) before further clean-up (Paper I). The solvent consumption in the extraction was decreased to 2 x 10 ml DCM (instead of 2 x 15 ml) in Paper IV. DCM was chosen because it extracted all compounds of interest efficiently from the dust and contained only very low background levels of BDE-209.

The serum samples were assumed to contain rather low concentrations of BFRs and the sample volumes available were sometimes small, especially from the toddlers. In an attempt to reduce the solvent consumption, a supported liquid extraction (SLE) method was tested as

an alternative to the liquid-liquid extraction (LLE) that was finally used (Paper II). For the SLE method development, aliquots (0.8 g) of human serum obtained from the Karolinska University Hospital (Karolinska University Laboratory) were spiked with ^{13}C-BDE-155, -197, -207 and -209 standards, 100 µL isopropanol was added and proteins were denatured in a water bath (70℃, 2 min). The sample was diluted to 2 mL total volume with 0.1 % formic acid in water and applied to an SLE column (Isolute SLE+ 2 mL, Biotage, Uppsala, Sweden) using vacuum suction for 30 seconds and allowed to equilibrate with the column packing material (diatomaceous earth) for 10 min. The samples were eluted with 10 mL methyl-*tert*-butyl ether (MTBE), evaporated and the solvent changed to *n*-Hx prior to further clean-up. The recoveries of the ^{13}C-labelled BDEs ranged from 42 to 91 %, indicating that SLE could be a useful method. The sample preparation was also faster than with LLE because a manual phase separation step was not needed.

The SLE method above and the LLE method (described in Paper II) were then compared by extracting aliquots of the standard reference material (SRM 1958, Organic contaminants in fortified human serum from the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA) using both methods.

The SLE method gave consistently lower PBDE concentrations than the LLE method and the latter results agreed well with the certified values (Figure 2). Therefore, extraction of the serum samples was performed using LLE as described in Paper II, in spite of the larger solvent consumption with this method.

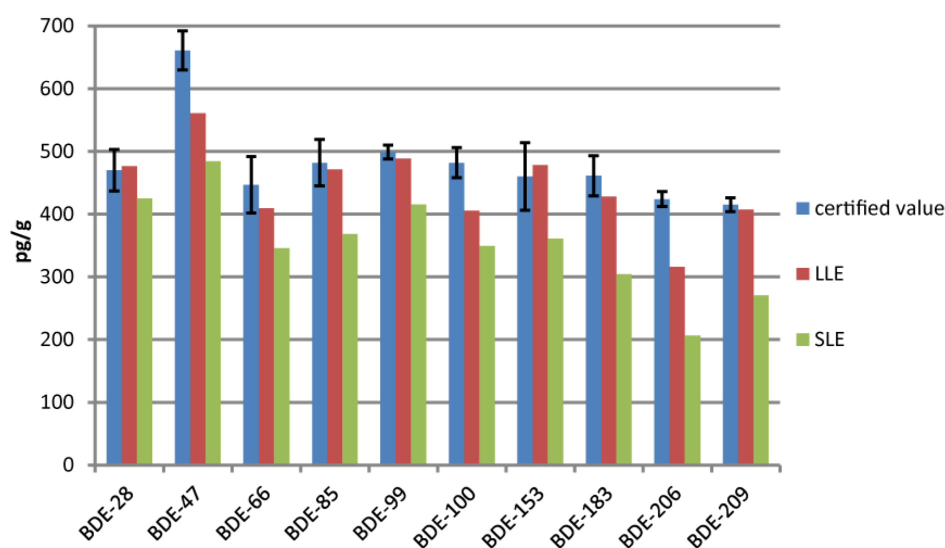

Figure 2. PBDE concentrations in human serum (SRM 1958) standard reference material obtained with liquid-liquid extraction (LLE) and supported liquid extraction (SLE), compared with the certified values from NIST.

All dietary items (Paper IV) were extracted according to modification II in Jensen et al. (2003). This is a well-established method and it has been shown to extract lipids quantitatively and is comparable to the Bligh and Dyer (1959) extraction method.

Clean-up and fractionation method

The development of the clean-up and fractionation method in Paper I was motivated by the need to include emerging compounds in the BFR analyses. Some of these could be expected to be present at low levels and some are degraded by strong acids (e.g. EH-TBB and BEH-TEBP). Furthermore, the sample sizes available were often limited and therefore the detection limits would benefit if the entire sample could be utilized for the analysis of every single compound, i.e. to separate different compound classes from each other before further clean-up rather than to analyze different subsamples for different analytes. Therefore a fractionation method to enable determination of tri-decaBDEs, EBFRs and HBCDs in the same sample without dividing the sample was developed (Paper I).

Other fractionation methods for determination of EBFRs in dust have recently been reported (Ali et al. 2011; Van den Eede et al. 2012). The Ali et al. (2011) method used activated silica to separate the acid sensitive EH-TBB and BEH-TEBP in a fraction that was subsequently cleaned-up on a florisil SPE column while the other fraction was cleaned-up on an acidified silica SPE column. However, this method did not include PBDEs or HBCDs in the analysis. The Van den Eede et al. (2012) method used florisil to separate a number of legacy and emerging flame retardants (FR) into two fractions but HBCD eluted in both fractions and thus, after the determination of the other analytes, the fractions had to be recombined for determination of the HBCDs.

Dust

For the fractionation of the sample extracts, different amounts of florisil and silica packed into SPE cartridges were tested. Two grams of activated silica, (activated overnight at 450° C, deactivated with 2.5 % H_2O) gave the best separation of the compound classes. The method was developed for dust samples and is described in detail in Paper I. A scheme of the fractionation is shown in Figure 3. In short, PBDEs and DBDPE were eluted in the first fraction and this was subsequently treated with concentrated sulfuric acid. In fraction II the acid sensitive analytes (EH-TBB and BEH-TEBP) and BTBPE were eluted. This fraction was further cleaned-up on an aminopropyl column. The elution of BTBPE in this fraction was fortunate because BTBPE co-elutes with a heptaBDE (unknown congener) on the GC column and also produces the same m/z trace from the mass spectrometer (MS). The HBCDs eluted in fraction III, which were further cleaned-up with concentrated sulfuric acid. The isomer-specific determination of HBCDs required a separate analysis and the use of an additional analytical instrument. Therefore the elution of the HBCDs in a separate fraction was convenient.

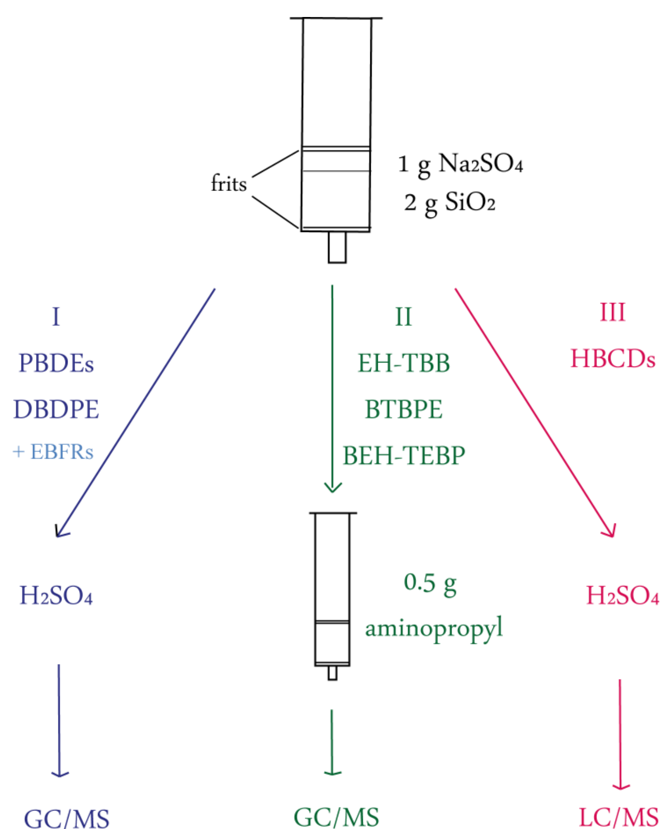

Figure 3. Clean-up and fractionation method (Paper I)

The developed method was validated for a number of PBDEs, DBDPE, BTBPE, EH-TBB and BEH-TEBP and HBCDs in dust (Paper I). Later, several other EBFRs were included in the analyses (Papers II-IV) and all of these eluted in fraction I.

Serum

The serum sample extracts contained only small amounts of lipids, and the clean-up and fractionation method described above could be applied to the serum samples without modifications.

Dietary items

Large amounts of lipids in the sample extract influence the separation of the analytes on the silica gel column. Previous analyses of the dietary samples by the Swedish NFA indicated that the BFR levels could be rather low (NFA 2012). The sample intakes required in order to obtain detectable levels of BFRs resulted in about 0.3-1 g extracted lipids. An additional clean-up step was therefore needed before the silica gel column fractionation.

Concentrated sulfuric acid is commonly used for lipid removal in biological samples but the inclusion of the acid sensitive EBFRs, EH-TBB and BEH-TEBP in the analyses motivated an attempt to use a non-destructive lipid removal method. Non-destructive methods based on gel

permeation chromatography (GPC) (Nortsrom et al. 1985) and semipermeable membranes (Strandberg et al. 1998) have been applied in the analysis of environmental contaminants in biological matrices. However, these methods require relatively large amounts of solvents enhancing the risk of blank problems and thus higher detection limits.

A method described by Hong et al. (2004) where the lipids were precipitated and filtered off from the extract was tested. In short, 1 – 2 g of fish oil was spiked with a variety of BFRs and dissolved in 50 mL acetonitrile (ACN), which has a rather low solubility for lipids. The samples were then put in a freezer at -25℃ for 30 min and the precipitated lipids were filtrated using glass fiber filters and vacuum suction. Eighty to 90 % of the lipids were trapped in the filter and the recoveries of the BFRs varied from 60-90 %, except for BDE-209 and DBDPE, which had much lower recoveries (30-40 %), probably due to their lower solubility in ACN than in lipids. Since good recoveries of these two compounds were important, this method was not applied in our study. Interestingly, a recent study has reported on the development of such a method for determination of PBDEs in fish with the recoveries ranging from 52 to 116 % for mono-decaBDEs. (Shin et al. 2012).

Sulfuric acid was therefore used for the lipid removal step of the dietary items, knowing that this would lead to lower recoveries of the acid sensitive EH-TBB and BEH-TEBP. However, at this point we had obtained isotopically-labelled standards for these two compounds and these were added to the samples to help accurately quantify levels, or in case of non-detects, to determine the method recoveries of these EBFRs.

Feces

Feces is a complex matrix and the clean-up and fractionation method described above and in Paper I was not sufficient for the analysis of the feces samples in Paper III. Therefore, the raw extracts were treated with concentrated sulfuric acid prior to fractionation on the silica gel column. Furthermore, the first 5 mL of fraction I from the silica gel column was discarded because of poor chromatography of the analytes in this fraction on the analytical (GC) column.

Instrumental methods

GC/ECNI-MS

PBDEs, especially the fully brominated congener BDE-209, are subject to thermal degradation during GC analysis, both during injection and separation on the GC column. The use of a programmable temperature vaporizer (PTV) injector reduced the degradation in the injection port (Tollbäck et al. 2003). For the studies included in this thesis (Papers I-IV), the PTV injector parameters were optimized (including injection and transfer temperatures, transfer pressure and cleaning temperature), to maximize the signal-to-noise ratio for the different tri-decaBDEs. The optimized parameters are reported in Paper I.

Electron capture negative ionization using a moderating gas (e.g. ammonia or methane) is a common and very sensitive method for the analysis of brominated compounds. For PBDEs, dissociative electron capture is the dominating ionizing process producing mainly bromide ions (m/z 79 and 81). Bromide ions are not compound-specific and thus identification of a compound is only based on the retention time from the GC column (and the compound's appearance in a certain fraction from the clean-up procedure). Isotopically-labelled surrogate standards cannot be used when quantification is based on bromide ions since these are identical for labelled and native compounds.

Some PBDE congeners have been shown to produce phenoxide ions [$C_6Br_xO^-$, x=4-5] when introduced to ECNI (Björklund et al. 2003). The use of these heavier mass fragments for detection instead of only bromide ions enhances selectivity and the signal-to-noise ratio in the analyses. Therefore a GC/MS method recording heavier mass fragments was developed which enabled the use of isotopically-labelled surrogate standards in the analyses of PBDEs. Ionization parameters such as electron energy, emission current, ion source temperature and the moderating gas flow rate (ion source pressure) were optimized to maximize formation of brominated phenoxide ions (Paper I). Hepta- and octaBDEs produced phenoxide ions with four bromines and the nona-decaBDEs with five bromines attached. The sensitivity of the phenoxide ions or other heavier mass fragments produced by the lower brominated BDEs was very low and thus bromide ions were used for the detection of these compounds.

UPLC/ESI-MS

GC/ECNI-MS can be used in the analysis of HBCD, but only for determination of total HBCD because the HBCD stereoisomers cannot be resolved on a GC column (Abdallah et al. 2008). However, LC/MS methods have been reported for the determination of isomer-specific HBCDs (Abdallah et al. 2008; Morris et al. 2006). In this thesis the chromatographic separation of the α-, β-, and γ-HBCD isomers was obtained on a C18 UPLC column and the instrument system was optimized regarding column temperature and mobile phase gradient and flow rate. No additives were needed in the mobile phase (consisting of methanol and water). The use of an UPLC instrument allowed for a very short analysis time (only 7 minutes including conditioning of the column) and the solvent consumption was kept low.

Electrospray ionization in negative mode was applied for the ionization of the analytes, and the MS was run on multiple reaction monitoring mode, measuring the quasi-molecular ions $[M-H]^-$ as parent ions and bromide ions as daughter ions. The method is described in detail in Paper I and was used unmodified in all of the papers (I-IV).

Quality control

The use of isotopically-labelled standards enabled accurate determination of target compounds and minimized possible matrix effects. For the analytes lacking isotopically-labelled equivalents, the relative recovery to the surrogate standard was used to correct the results.

In Paper I, ^{13}C-labelled BDE-183, -197, -207, and -209 were added as surrogate standards to the samples. When ^{13}C-BDE-155 later became available, this replaced ^{13}C-BDE-183 as surrogate standard for tri-heptaBDEs due to its much greater tendency to form phenoxide ions (Papers II-IV). Also, in order to be able to correct for degradation of BDE-209 to nonaBDEs in every sample individually, ^{13}C-BDE-207 was excluded and ^{13}C-BDE-197 was used as surrogate also for the nonaBDEs (Papers II-IV).

In Papers I-II, ^{13}C-BTBPE was used as surrogate standard for all compounds eluting in fraction II from the silica column. When isotopically-labelled EH-TBB and BEH-TEBP became commercially available, these were used instead (Papers III-IV). At the same time, due to the rather low sensitivity for BTBPE when measuring heavier mass fragments, ^{13}C-BTBPE was no longer used and BTBPE was analyzed measuring the bromide ions using the isotopically-labelled EH-TBB as surrogate.

^{13}C-labelled α-, β-, and γ-HBCDs were used as surrogate standards and ^{2}H-labelled β-HBCD as recovery standard for HBCD determination throughout the study. The ^{2}H-labelled β-HBCD disturbed the m/z trace of the ^{13}C-labelled β-HBCD but this could be controlled by addition of only a low level of the deuterated standard to each sample.

One to two laboratory solvent blanks and quality control (QC) samples were processed together with each batch of 10 samples. The standard reference materials SRM 1958 (Organic contaminants in fortified human serum) and SRM 2585 (Organic contaminants in house dust) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) were used as QC samples in the analyses of serum and dust, respectively. A pooled, in-house salmon homogenate used as QC sample within the national monitoring program at our department was used as QC sample in the food analyses. No SRM or well established QC sample was available for feces. Instead, six replicates of a pooled feces sample were analyzed as QC samples.

BFR EXPOSURE IN A MOTHER-TODDLER COHORT

Internal concentrations

BFRs in serum of mothers and their toddlers

Tetra-hexaBDEs

Significant correlations (Pearson's, $p < 0.05$) were found for concentrations of the tetra-hexaBDEs between the blood serum of the mothers and their toddlers (Paper II). This is probably due to the strong link to *in-utero* exposure and breastfeeding; the exposure of the child is directly determined by the mother's body burden. The concentrations of tetra-pentaBDEs detected in the toddlers were significantly higher (Student's paired comparisons t-test, $p < 0.05$) than in their mothers (Paper II) indicating accumulation from breast milk. The serum concentrations of BDE-153 in toddlers were significantly positively correlated to duration of exclusive breastfeeding and total time breast-fed (Paper II) supporting the importance of breastfeeding as a source of lower brominated BDEs for toddlers. A previous study also found correlations between serum concentrations of BDE-153 and duration of breastfeeding (Stapleton et al. 2012) and a study from Spain reported that breastfeeding was the determining factor for the body burden of BDE-47 and -99 in children, still at 4 years of age (Carrizo et al. 2007). The significant correlations found between tetra-nonaBDEs in the mothers' serum suggest similar exposure sources for most of the PBDEs for mothers.

Octa-decaBDEs

The serum concentrations of nona-decaBDEs were higher in toddlers compared to their mothers but no correlations were found for octa-decaBDEs between the serum of mothers and their toddlers (Paper II) indicating different exposure routes for these compounds for mothers and toddlers. These findings suggest that breast milk cannot be the source of octa-decaBDEs in toddlers. Other studies have shown that the higher brominated BDEs do not partition to breast milk to the same extent as the lower brominated ones (Jakobsson et al. 2012; Schecter et al. 2010). BDE-197 proved to be an interesting congener when studying congener-to-congener correlations within the mothers or toddlers. The BDE-197 concentrations correlated with those of tetra-hexaBDEs in mothers and with BDE-209 concentrations in toddlers indicating that sources of BDE-197 for mothers were similar to those of lower brominated congeners (probably diet) but in toddlers the sources of BDE-197 were similar to sources of BDE-209 (probably dust).

Other FRs

DDC-CO and DBE-DBCH were detected in a few serum samples from both mothers and toddlers indicating that exposure to these emerging contaminants occurs. DDC-CO has recently been reported in 10 serum samples from Norwegian adults (Cequier et al. 2013) but to my knowledge no data exist on DBE-DBCH in humans. No other EBFRs or HBCDs were detected in the serum samples of the mother-toddler cohort from Uppsala (Paper II).

BFRs in toddler feces

Tetra-decaBDEs, 4 isomers of HBCD (α-, β-, γ- and possibly δ-HBCD) and 14 EBFRs were detected in feces of toddlers (Paper III). The BDE concentrations in feces were significantly correlated to those in the matching serum samples indicating that feces could be used as a non-invasive sample matrix for estimation of tetra-decaBDE body burdens in toddlers. The ratios between the serum and feces concentrations indicate efficient uptake and accumulation of tetra-hexaBDEs from the human gut while the same processes seem to be less efficient for BDE-209. Much of the ingested amount of the fully brominated BDE congener is excreted in feces. Similar results have previously been reported in rats showing accumulation of tetra-hexaBDEs while BDE-209 was extensively excreted in the feces (Huwe et al. 2008). The detection of HBCDs and EBFRs in toddler feces shows evidence of these contaminants being ingested by the toddlers. The absence of HBCD in the serum but high detection frequency in feces could be an indication of low uptake and/or fast excretion and/or rapid metabolism. The human half-lives for HBCD have been reported to be much shorter than those for PBDEs (Geyer et al. 2004).

Exposure via diet and house dust

Tri-decaBDEs, α-, β- and γ-HBCD and several EBFRs were detected in dietary items and house dust samples from the participants' homes in Uppsala, Sweden (Paper IV). The concentrations in the two exposure media were used to estimate individual daily intakes for the study participants. Two pooled breast milk samples collected in 2009 and 2010 were analyzed to give an overview of the levels found in Swedish breast milk but could not be used in the estimation of the toddlers' dietary intake as 88 % of the toddlers had already stopped breastfeeding. The median daily intakes (ng/d) of ΣpentaBDE (sum of BDE-28, -47, -99, -100 and-153) for both exposure pathways combined were higher for the mothers compared to their toddlers, ΣoctaBDE (sum of BDE-153, -196, -197, -203, -206, -207 and-208) intake levels were similar for both groups, while the ΣdecaBDE (sum of BDE-206, -207, -208 and-209) intake was higher for toddlers than mothers. Diet was the main contributor to intake of ΣpentaBDE and α-DBE-DBCH for both mothers and toddlers. For ΣoctaBDE, ΣHBCD and PBBz, dietary intake was more important for mothers while house dust ingestion was more important for toddlers. House dust was the main exposure route for ΣdecaBDE, DBDPE, EH-TBB, BEH-TEBP, BTBPE and PBT for both mothers and toddlers. These findings support the hypothesis stated in Paper II based on the BDE-197 correlations to other BDE congeners suggesting that BDE-197 in mothers comes from the same sources as tetra-hexaBDE (diet) and in toddlers from same sources as BDE-209 (dust). Most EBFRs were detected in fish in concentrations similar to those of individual PBDE congeners and HBCD stereoisomers indicating that the replacement BFR chemicals have reached the human food web. The estimated daily intakes of ΣHBCD (sum of α-, β- and γ-HBCD) from diet and dust were roughly half and a third, respectively, of those for PBDEs, and intakes of EBFRs were even lower. The lower intakes of EBFRs than PBDEs could explain why EBFRs were not detected

in high frequency in the serum samples, the concentrations are probably not yet high enough to be detected.

Relations between internal concentrations and exposures via diet and dust

Associations were studied between the tri-decaBDE concentrations in internal (serum and feces) and external (diet and dust) exposure media (Paper IV). The median percent compositions of tri-decaBDEs in the different sample matrices included in the study (serum of mothers and toddlers, feces of toddlers, house dust, pooled breast milk and 5 different dietary items) are shown in Figure 4 to illustrate the overview of the BDE congener patterns in the different sample matrices related to human exposure. Similar congener patterns were seen in the dietary items originating from the terrestrial food chain (dairy products, vegetable oils, meat, eggs) but the pattern in fish was very different from the other matrices with tri-hexaBDEs accounting for over 95 % of the total BDEs. Fish and meat consumption were significantly correlated with the serum BDE concentrations in mothers but no correlations were found between dietary intake and serum or feces concentrations in toddlers (Paper IV). The BDE congener patterns in house dust and toddler feces were very similar and the octa-decaBDE concentrations in these two matrices were significantly correlated (Paper IV). Octa-decaBDE concentrations in the house dust were also correlated with those in toddler's serum. The larger number of significant correlations found between matched house dust samples and toddler's serum compared to mother's serum suggests that dust exposure plays a larger role for the octa-decaBDE body burden in toddlers than in their mothers.

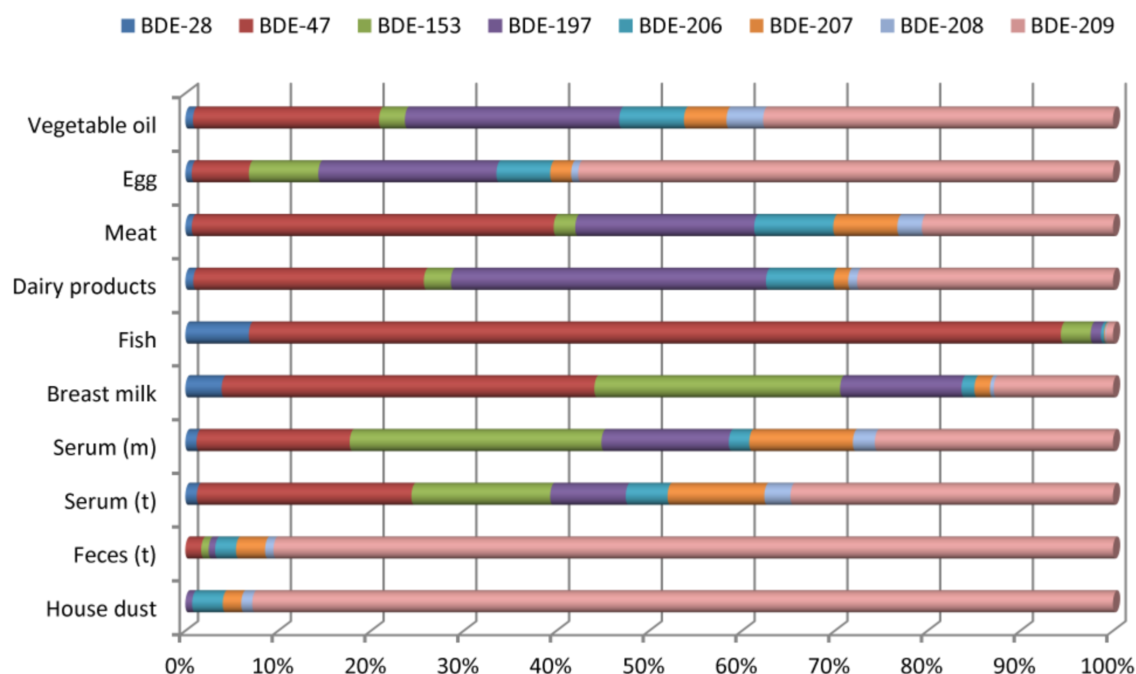

Figure 4. The median percent composition of tri-decaBDEs in different sample matrices related to human exposure (m = mother, t = toddler)

HBCD was not detected in serum, but was found in feces. Figure 5 illustrates the mean abundances of α-, β- and γ-HBCD in toddler feces, house dust, fish and pooled breast milk. The isomer pattern in the pooled breast milk samples was similar to that seen in the fish samples suggesting diet as an important exposure route of HBCDs for mothers while the isomer patterns in house dust and toddler feces were almost identical indicating dust ingestion as an important route of exposure for toddlers. However, no statistically significant correlations were found between the HBCD concentrations in toddler feces and house dust.

An additional isomer of HBCD was detected in toddler feces and also in fish and egg samples. The isomer was not identified but after comparing the retention order (from the LC column) with a previous study on fish in England (Harrad et al. 2009) we suggested that the isomer was δ-HBCD.

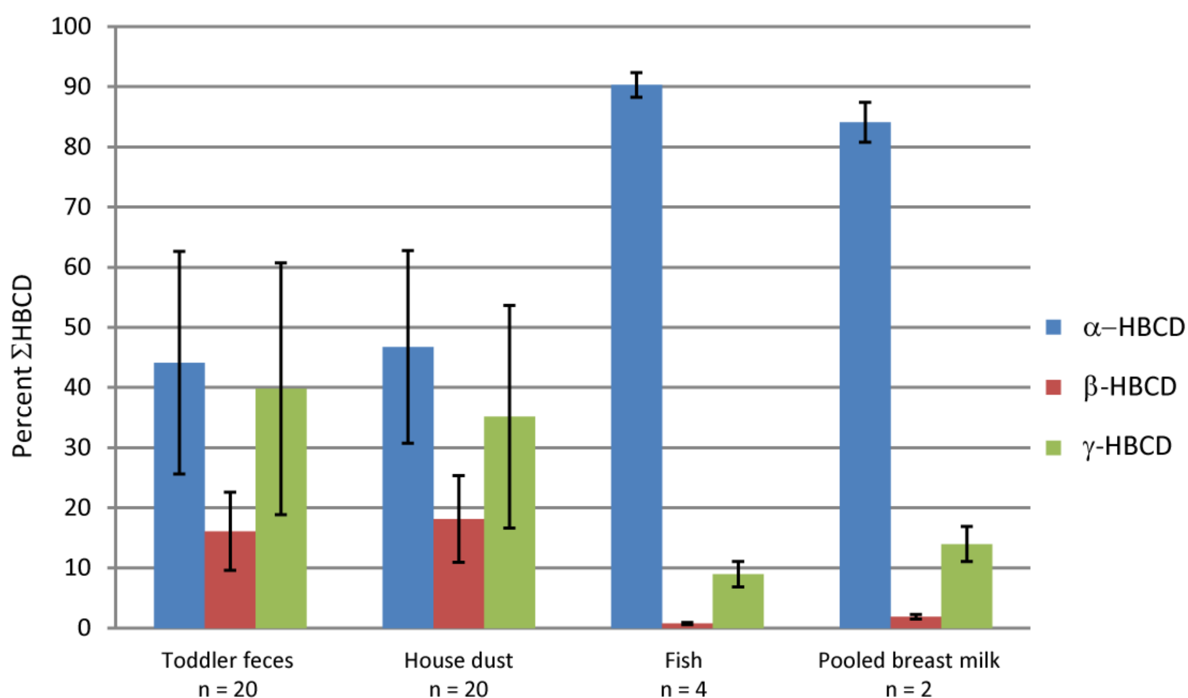

Figure 5. Mean (SD) abundance of individual HBCD stereoisomers in toddler feces, house dust, fish and breast milk from Uppsala, Sweden

CONCLUSIONS AND FUTURE PERSPECTIVES

The clean-up and fractionation method developed in Paper I can be used to determine tri-decaBDEs, isomer-specific HBCDs and a variety of EBFRs in the same sample without dividing the sample. The method was successfully applied to house dust (Paper I and IV), human serum (Paper II), breast milk (Paper IV), dietary items (Paper IV) and human feces (Paper III). However, for the three latter matrices, treatment with concentrated sulfuric acid was required prior to the fractionation on the SiO_2 column to remove lipids. The serum concentrations of many PBDEs were higher in toddlers than in their mothers indicating that toddlers are more exposed to BFRs than adults. Taking all the results into account, the toddlers' higher serum levels of tetra-pentaBDEs seem to be the result of previous breastfeeding and those of octa-decaBDEs from exposure to house dust. Fecal concentrations of PBDEs correlated with those in serum showing that feces can be used as a non-invasive sample matrix in biomonitoring BDEs in toddlers. For mothers, diet, and in particular meat and fish intake, was estimated to be the main exposure route of tri-hexaBDEs (probably important for hepta-nonaBDEs as well) and HBCDs, and dust ingestion was estimated to be the main route for BDE-209 exposure in mothers. We confirmed our three hypotheses concerning PBDEs, except that we did not find as many correlations between dust and serum concentrations in mothers as hypothesised. Our hypothesis of dust ingestion being the major route of HBCD exposure for toddlers was not possible to test, as HBCDs were not detected in their serum. However, α-, β- and γ-HBCD and an additional HBCD isomer were detected in toddler feces indicating ingestion by toddlers. The similarity of the HBCD isomer pattern between feces and house dust but lack of detection in serum suggest low uptake from the gut and/or metabolism/fast excretion via feces. Several EBFRs were detected in food and house dust indicating that human exposure to these occurs. Some EBFRs were also found in toddler feces and DBE-DBCH was found in a few serum samples of mothers and toddlers. Thus there is a need to monitor these replacement chemicals in food and indoor environments as well as in humans.

Additional clean-up methods that do not require the use of concentrated sulfuric acid and allow use of low solvent volumes for more complex sample matrices such as feces and dietary items need to be developed to be able to quantify the acid-sensitive contaminants in the future. Gel permeation chromatography (GPC) is a well-established non-destructive clean-up method, but requires relatively large amounts of solvents enhancing the risk of blank problems and thus higher detection limits. The feasibility of using feces for biomonitoring of a wider range of contaminants and also in children with varying age should be studied; sample amounts are not a problem in this approach.

In future, the data obtained in this project will be used together with a human bioaccumulation model (Czub and McLachlan 2004) to study the feasibility of modelling individual BFR body burdens in the mother: toddler pairs. Further comparisons between measured body burden and modelled intakes via dust and diet could also provide the possibility of calculating more accurate dust ingestion rates than are currently available.

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