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Migration of hazardous chemicals to the indoor environment – "Horizon Scanning" for flame retardants present in consumer goods

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Abstract

The present work is a study about the migration of FR (flame retardant) chemicals into the indoor environment, from the items they are contained in. These chemicals are added to consumer products and construction materials to impart flame retardancy, so as to decrease the risk of fire-related injuries and deaths.

The monitoring of these compounds is important since many of the FRs used in the past, but also in the present day, have been shown to pose serious health risks.

The approach was to firstly explore indicators of indoor contamination with FRs, such as air and house dust. The next step was to investigate the possible sources of FRs, such as consumer products, electronics, textiles and toys. To aid in this, an untargeted screening procedure was also developed to screen for hitherto unknown/unstudied FRs. Lastly, the exposure to these chemicals was assessed using both theoretical and experimental models.

A procedure to simplify the analysis of FRs in dust was developed, and house dust from the USA (California) and Norway was analysed for a wide range of FRs. Paired air samples were also analysed alongside the dust samples from Norway.

A new analytical method for new / emerging PFRs (organophosphate flame retardants and plasticisers) was developed for dust, textiles and plastic on UPLC-MS/MS. To detect and identify hitherto unknown FRs, a non-targeted screening method was developed on LC-(Q)TOF-MS, with a focus on halogenated analytes.

BFRs (brominated FRs) and PFRs were determined in textile samples from the Belgian market, employing a comprehensive multi-technique approach. The samples were firstly screened by Direct Probe-TOF-MS, then underwent quantitative analysis by GC-MS and then further investigation using an array of Environmental Forensic Microscopy techniques. Using XRF, GC-MS and LC-MS/MS, FRs were also investigated in plastics collected from electronic devices. FRs were also investigated in various types of children's toys, a consumer product not thoroughly investigated for FR levels in the literature so far.

The FR exposure that children may get from playing with toys was then assessed using models from the literature. It was determined that for the most vulnerable age group, the infants, the exposure through mouthing is the most significant. Consequently, the mouthing exposure to PBDEs (polybrominated diphenyl ethers), the FRs for which CRMs (certified reference materials) were available, was simulated and more accurately assessed, thus filling a gap in the general knowledge base about FR exposure.

Samenvatting

Het huidige werk is een studie over de migratie van FR (vlamvertrager) chemicaliën vanuit de objecten waaraan ze werden toegevoegd naar de binnenhuisomgeving/interieur omgeving. Deze chemicaliën worden toegevoegd aan consumentengoederen en bouwmaterialen om dankzij hun vlamvertragende werking het risico op brandschade en sterfgevallen terug te dringen.

Het belang van de controle van deze chemische stoffen komt voort uit het feit dat veel FR's gebruikt in het verleden, maar ook in het heden, ernstige gezondheidsrisico's bleken te hebben.

De aanpak was om eerst huisstof en binnenhuislucht als indicatoren van binnenhuis contaminatie met FRs te analyseren. De volgende stap was om de mogelijke FR bronnen, zoals consumentengoederen, elektronica, textiel en speelgoed te onderzoeken. Er werd een niet-gerichte screening procedure ontwikkeld om te screenen voor tot nu toe onbekende / niet-onderzochte FRs. Ten slotte werd op basis van zowel theoretische en experimentele modellen de blootstelling aan deze stoffen bepaald teneinde essentiële onderzoek lacunes op te vullen.

Een procedure ter vereenvoudiging van de analyse van de FRs in stof werd ontwikkeld en huisstof uit de VS (California) en Noorwegen werd geanalyseerd voor een brede waaier van FRs. Samen met de stofstalen uit Noorwegen werden ook gepaarde luchtstalen geanalyseerd.

Een nieuwe analysemethode voor nieuwe / opkomende PFRs (organofosfaat vlamvertragers en weekmakers) werd ontwikkeld voor stof, textiel en plastic op UPLC-MS/MS. Om tot nu toe onbekende FRs te detecteren en te identificeren werd een niet-gerichte screening methode ontwikkeld op LC-(Q)TOF-MS, met nadruk op gehalogeneerde analyten.

BFRs (gebromeerde FRs) en PFRs werden met behulp van een uitgebreide multi-techniek aanpak in textiel stalen van de Belgische markt bepaald. De stalen werden eerst gescreend door middel van Direct Probe-TOF-MS, vervolgens ondergingen ze een kwantitatieve analyse met behulp GC-MS en tenslotte werden ze verder onderzocht met een reeks milieu Forensische Microscopie technieken. Met behulp van XRF, GC-MS en LC-MS/MS werden FRs eveneens onderzocht in plastieken afkomstig van elektronische apparaten. FRs werden ook onderzocht in verschillende soorten speelgoed voor kinderen, een item dat tot nu toe nog niet grondig voor FR spiegels/aanwezigheid werd onderzocht in de literatuur.

De FR blootstelling die kinderen door het spelen met speelgoed kunnen ondervinden, werd vervolgens beoordeeld met behulp van modellen uit de literatuur. Er werd vastgesteld dat voor de meest kwetsbare leeftijdsgroep, de kinderen, de blootstelling via mondgedrag het belangrijkste is. Bijgevolg werd de blootstelling aan PBDEs (polybroomdifenylethers), de FRs waarvoor CRMs (gecertificeerde referentiematerialen) beschikbaar waren, via mondgedrag gesimuleerd en nauwkeuriger bepaald, waardoor een gat in de algemene kennis over de blootstelling aan FRs werd opgevuld.

List of general abbreviations

For the abbreviations of the analytes studied in this thesis, see Appendix 1 (at the end of the manuscript)

ABS	Acrylonitrile butadiene styrene
Ace	acetone
AJS	Agilent JetStream
APCI	atmospheric pressure chemical ionisation
BFRs	brominated flame retardants
CFRs	chlorinated flame retardants
CRM	certified reference material
DAPs	dialkyl phosphates
DCM	dichloro methane
DFs	detection frequencies
ECNI	electron capture negative ionisation
EI	electron ionisation
EPFRs	emerging organophosphate flame retardants
EPS	Expanded polystyrene
ESI	electrospray ionisation
EtOAc	ethyl acetate
FRs	flame retardants
FTIR	Fourier transform infrared spectroscopy
FWHM	Full width at half maximum
Hex	*n*-hexane
HIPS	High impact polystyrene
HLMs	human liver microsomes
HPV	high production volume
HR	high resolution
i.d.	internal diameter
IS	internal standard
LD_{50}	the individual dose required to kill 50 percent of a population of test animals
LOD	limit of detection
log K_{oa}	logarithm of octanol-air partition coefficient
log K_{ow}	logarithm of octanol-water partition coefficient
LOQ	limit of quantification
m/z	mass to charge ratio
MAPs	monoalkyl phosphates
MeOH	methanol
NFRs	new/novel flame retardants
OCs	organochlorines
PBT chemical	Persistent, Bioaccumulative and Toxic
PBT polymer	polybutylene terephthalate
PC	polycarbonate

PET	polyethylene terephthalate
PFRs	organophosphate flame retardants and plasticisers
POPs	persistent organic pollutants
PP	polypropylene
ppm	parts per million
PS	polystyrene
PTV	programable temperature vaporiser
PUF	polyurethane foam
PVC	polyvinyl chloride
QA/QC	quality assurance / quality control
REACH	"Registration, Evaluation, Authorisation and Restriction of Chemicals" EU regulation
RfD	reference dose, as defined by the US EPA
RoHS	"Restriction of Hazardous Substances" EU Directive
rpm	rotations per minute
RSD	relative standard deviation
SEM-EDS	scanning electron microscopy with dispersive x-ray spectroscopy
SIM	selected ion monitoring
SPE	solid phase extraction
SRM	standard reference material
TOF	time-of-flight mass spectrometer
TPs	transformation products
TSH	thyroid stimulating hormone
UAE	ultrasound assisted extraction
v/v	volume/volume
w/w	weight/weight
WEEE	"Waste Electrical and Electronic Equipment Directive" EU directive
WHO	world health organisation
XPS	Extruded polystyrene foam
XRF	X-ray fluorescence

Chapter 1

Introduction

1.1. Human exposure and health risks of selected flame retardants

Based on the following publications:

Covaci A, Geens T, Roosens L, Ali N, Van den Eede N, Ionas AC, Malarvannan G and Dirtu AC. Human exposure and health risks to emerging organic contaminants, The Handbook of Environmental Chemistry (2012), 20, 243-305.

Dirtu AC, Van den Eede N, Malarvannan G, Ionas AC, Covaci A. Analytical methods for selected emerging contaminants in human matrices – a review, Analytical and Bioanalytical Chemistry (2012), 404(9):2555-81.

To decrease flammability and meet fire safety regulations, FRs are frequently added to consumer products ranging from upholstered furniture to textiles, electronics and building materials. All these items serve useful day-to-day functions within society, but are also "point sources" of release and exposure to FRs. Many of these FRs are manufactured in high production volumes (>1000 tonnes/year) (Cooper et al., 2011; van der Veen 2012) and their content in the materials can reach percentage amounts by weight (Kajiwara et al., 2011; Alaee et al., 2003).

Human exposure and body burdens are generally of key concern for these contaminants precisely due to their broad applications. Unfortunately, most of these compounds have a negative impact on the environment and pose a potential risk for animal and human health. Several FRs are Persistent, Bioaccumulative, and Toxic (PBT) compounds (Lyche et al., 2015; Wei et al., 2015), while others are associated with endocrine disruption (Lyche et al., 2015; Vonderheide et al., 2008) and adverse effects on the central nervous system and the reproductive system (Lyche et al., 2015).

Current understanding of these contaminants often contains significant gaps, including their toxicity (towards humans), bioaccumulation, occurrence, transport, and transformation mechanisms.

1.1.2. Human exposure to current-use brominated flame retardants (BFRs)

Due to their adverse health effects, several restrictions and bans have been imposed on the usage of polybrominated diphenyl ethers (PBDEs), namely the Penta-, Octa- and Deca BDE formulations, in Europe, China, North America and Japan (www.bsef.com). The restricted usage of these PBDEs increased the market demand for substitute brominated flame retardants (BFRs) including hexabromocyclododecanes (HBCDDs) (EFSA, 2006), tetrabromobisphenol A (TBBPA) (Covaci et al., 2009) and a range of novel BFRs (NBFRs) (Covaci et al., 2011).

As an additive FR, HBCDD easily reaches the environment through production, usage as FR in a wide variety of household consumer products and recycling of HBCDD-containing materials (Covaci et al., 2006). Although its usage is not restricted, HBCDD has been included in the PBT list of the European Chemical Substance Information System.

Currently, TBBPA is the BFR with the largest production volume worldwide (Hakk and Letcher, 2003). TBBPA can be used as an additive BFR and as an alternative to Octa-BDE in television casings, printer components, fax machines, photocopiers, coffee makers and plugs/sockets (Covaci et al., 2009). The increased production volume is reflected by an ongoing increase of their environmental detection frequencies (Law et al., 2006; Vorkamp et al., 2011; Covaci et al., 2011). However, an extensive review of the existing literature revealed no similar temporal trend for TBBPA which might be due to its shorter half-life in humans (Covaci et al., 2009). Similar to PBDEs, these chemicals reach the environment through the manufacture, use and disposal of various BFR-containing consumer products. In addition, various monitoring studies have indicated their presence in wildlife and in humans making them ubiquitous contaminants (Covaci et al., 2006; Law et al., 2006; Covaci et al., 2011). Their accumulation in humans primarily occurs through dietary intake, indoor dust ingestion and indoor air inhalation (Table 1.1.1). Dermal uptake is considered to be less important as the log K_{ow} of these compounds are > 5 (ECHA, Part II, 2006).

Although produced in lower volumes than the major BFRs, NBFRs have started to be observed in the environment as well. DBDPE is a high production volume chemical produced in the US and China that has been proposed as replacement for BDE 209 (WHO, 1997). As concerns mount about the gradual debromination of BDE 209 to lower and more toxic brominated congeners (Stapleton et al., 2006), the use of DBDPE has consequently increased (Watanabe and Sakai, 2003). BTBPE has been produced since the mid-1970s, in a high production volume in the US and a low production volume in the EU (ECSIS, 2012). In the US, it was marketed by the Great Lakes Chemical Corporation as FF-680 with the intended use as additive FR for thermoplastic and thermoset resin systems (e.g. ABS, HIPS, polycarbonate, etc.). As of 2005, Chemtura used the FF-680 as a replacement for the Octa-BDE formulation (Hoh et al., 2005). EH-TBB and BEHTBP are produced by the Chemtura Chemical Corporation and used together in two FR formulations, namely Firemaster 550 (EH-TBB:BEHTBP in a ratio of 4:1, by mass) (Stapleton et al., 2008) and Firemaster BZ-54 (2.5:1 ratio, by mass) (Bearr et al., 2012). Firemaster 550 was first introduced as a replacement for the Penta-BDE mixture (Stapleton et al., 2008). BEHTBP is also used in the DP-45 formulation, also marketed by Chemtura. In the US, this compound is classified as a high production volume chemical (HEH, 2004).

1.1.2.1. Levels and profiles in human matrices

In general, compared to the extensive database of PBDEs in human tissues, less information is available on the levels of substitute BFRs in humans. In recent years, the levels and the detection frequency of HBCDDs in human tissues have increased significantly, which was paralleled by the higher usage of the HBCDD technical mixture (Glynn et al., 2011). Whereas Covaci et al. reported in

2006 a low detection frequency of HBCDDs in serum and human milk (3-50%), these have mounted up to 70% in serum samples (Kalantzi et al., 2011) and 100% in human milk samples (Abdallah et al., 2011). Moreover, the median detected HBCDD level in serum have caught up with the total median PBDE concentration (Kalantzi et al., 2011), which used to be the dominant BFR in human tissues (Covaci et al., 2006). HBCDD levels in serum and human milk samples are generally below 5 ng/g lipid weight, but can mount up to 100 ng/g lipid weight in occupationally exposed populations (Table 1.1.2). HBCDD levels in European samples are generally higher compared to those detected in the US and Asia, although a lack of regulatory measures in China, combined with a high density of e-waste recycling facilities, may lead to future higher levels in this region (Gao et al., 2011).

A note should be made on the dominance of α-HBCDD in human samples, such as human serum (Covaci et al., 2006; Roosens et al., 2009). In contrast, some studies reported γ-HBCDD to contribute to a higher percentage of the total HBCDDs in human tissues (Thomsen et al., 2007; Johnson-Restrepo et al., 2008). An increase in the percentage of γ-HBCDD has been reported in highly exposed population and in occupationally exposed workers, with γ-HBCDD making up to 40% of ΣHBCDDs (Thomsen et al., 2007; Eljarrat et al., 2009). Although the reasons for the different isomer profiles in human tissues from different studies are not yet clear, it is reasonable to hypothesize that they arise from a combination of differences in external exposures (e.g., α-HBCDD predominated in both dust and diet of the present study) and inter-individual variations in metabolism.

Table 1.1.1: Literature survey regarding human exposure to brominated flame retardants

Compounds	Country	Detection frequency	Population	Exposure estimate ng/day	Particularities	References
HBCDDs	Belgium			1.2-20	Duplicate diet	Roosens et al., 2009
	Belgium			59.4	Market basket	Goscinny et al., 2011
	The Netherlands			174	Market basket	De Winter-Sorkina et al., 2003
	UK			413	Market basket	Driffield et al., 2008
	Belgium			1.1-15	Indoor dust	Roosens et al., 2011
	UK			32.5	Indoor dust	Abdallah et al., 2008a
	US			2-60	Indoor dust	Abdallah et al., 2008b
	UK			3.9	Indoor air	Abdallah et al., 2008a
TBBPA	The Netherlands			0.8	Diet	De Winter-Sorkina et al., 2003b
	Belgium			0.2	Indoor dust	Geens et al., 2009a
	UK			1.3	Indoor dust	Abdallah et al., 2008a

Compounds	Country	Detection frequency	Population	Exposure estimate ng/day	Particularities	References
	UK			0.3	Indoor air	Abdallah et al., 2008a
BTBPE	Belgium and UK	Homes = 85 Class rooms = 86 Office = 100	Adult (working) Adult (non working) Toddler	<0.01[a,b] <0.01[a,c] 0.05[a,d]	Indoor dust (Belgian homes, office and UK day care centres)	Ali et al., 2011a
	New Zealand	44	Adult Toddler	<0.01[a] 0.01[a]	Indoor dust (homes)	Ali et al., 2011b
	China	97.4	Adult Toddler	1.00 4.00	Indoor dust (homes in e-waste area)	Wang et al., 2010
	China	96.3	Adult Toddler	0.32 1.29	Indoor dust (homes in urban area)	Wang et al., 2010
	UK		Adult Toddler	0.23[e] 1.00[f]	Indoor dust (homes, cars and offices)	Harrad et al., 2008
DBDPE	Belgium and UK	Homes = 100 Class rooms = 75 Office = 100	Adult (working) Adult (non working) Toddler	0.18 [a,b] 0.11[a,c] 1.89[a,d]	Indoor dust (Belgian homes, office and UK day care centres)	Ali et al., 2011a
	New Zealand	88	Adult Toddler	0.01[a] 0.16[a]	Indoor dust (homes)	Ali et al., 2011b
	China	100	Adult Toddler	3.15 12.6	Indoor dust (homes in e-waste area)	Wang et al., 2010
	China	100	Adult Toddler	137 547	Indoor dust (homes in urban area)	Wang et al., 2010
	UK		Adult Toddler	2.3[e] 5.5[f]	Indoor dust (homes, cars and offices)	Harrad et al., 2008
EH-TBB	Belgium and UK	Homes = 31 Class rooms = 92 Office = 67	Adult (working) Adult (non-working) Toddler	<0.01[a,b] <0.01[a,c] 0.08[a,d]	Indoor dust (Belgian homes, office and UK day care centres)	Ali et al., 2011a
	New Zealand	74	Adult Toddler	<0.01[a] 0.04[a]	Indoor dust (homes)	Ali et al., 2011b
BEHTBP	Belgium and UK	Homes = 97 Class rooms = 97 Office = 100	Adult (working) Adult (non-working) Toddler	0.02[a,b] 0.01[a,c] 0.40[a,d]	Indoor dust (Belgian homes, office and UK day care centres)	Ali et al., 2011a
	New Zealand	91	Adult Toddler	0.01[a] 0.19[a]	Indoor dust (homes)	Ali et al., 2011b

[a]Concentrations in ng/kg bw/day
[b]Assuming working dust ingestion is 78.9% home and 21.1% office.
[c]Assuming non-working adult dust ingestion is 100% home.
[d]Assuming toddler dust ingestion is 79.9% home and 20.1% classroom.
[e]Assuming adult dust ingestion is 72% home, 23.8% office, and 4.2% car.
[f]Assuming toddler dust ingestion is 95.8% home, and 4.2% car.

Table 1.1.2: Overview of HBCDDs, TBBPA and NBFRs levels in various human matrices.

Compounds	Matrix	Country	Levels (ng/g lw)	Particularities	References
HBCDDs	serum	Belgium (16)	1.7		Roosens et al., 2009
		The Netherlands (78)	1.1		Covaci et al., 2006
		Sweden (50)	0.46		Covaci et al., 2006
		Greece (61)	1.32		Kalantzi et al., 2011
		Norway (41)	4.1	highly exposed	Thomsen et al., 2008
		Norway (10)	101	occupationally exposed	Thomsen et al., 2007
	human milk	Japan	4.0		Kakimoto et al., 2008
		UK (34)	5.95		Abdallah et al., 2011
		Sweden (64)	0.3		Glynn et al., 2011
		China (24)	<LOD-2.8		Shi et al., 2009
	adipose tissue	US (20)	0.02		Johnson-Restrepo et al., 2008
TBBPA	serum	France (91)	16.1		Cariou et al., 2008
		Sweden (4)	1.1-3.8	occupationally exposed	Hagmar et al., 2000
		Norway (5)	1.3	occupationally exposed	Thomsen et al., 2001
		Norway (5)	0.54		Thomsen et al., 2001
		Norway (5)	0.34		Thomsen et al., 2001
	cord serum	France (91)	54.8		Cariou et al., 2008
	human milk	UK (34)	0.06		Abdallah et al., 2011
		China (24)	<LOD-5.1		Shi et al., 2009
		France (77)	0.48		Cariou et al., 2008
		Norway (9)	0.07		Thomsen et al., 2002
	adipose tissue	France (44)	<LOD		Cariou et al., 2008
		US (20)	0.05		Johnson-Restrepo et al., 2008
BTBPE	serum	Sweden (5)	<1.3		Karlsson et al., 2007
		China (128)	<LOD		Zhu et al., 2009
DBDPE	serum	Sweden (5)	<1.0		Karlsson et al., 2007
		China (128)	<LOD		Zhu et al., 2009

Levels and detection frequency of TBBPA are generally lower than those of PBDEs and HBCDDs in human samples. Consistent with its phenolic structure that can be rapidly conjugated in human liver and subsequently excreted in bile (Schauer et al., 2006), TBBPA has a short human half-life (< 2 days) in human plasma (Hagmar et al., 2000; Hakk et al., 2000). In addition, HBCDD and PBDEs are additive BFRs, while TBBPA is a reactive BFR, meaning TBBPA is chemically bound to the polymer structure and, thus, the leaching or release of TBBPA into the environment is limited (de Wit et al., 2002). Therefore, levels of TBBPA are often lower and detection of this flame retardant is likely to reflect recent rather than past exposure (Sjödin et al., 2009; Covaci et al., 2009), which might explain high TBBPA levels found in certain studies such as by Cariou et al., 2008 (Table 1.1.2). Contrarily, Chinese and Japanese studies often reported higher TBBPA levels in human tissues compared to HBCDDs which can be explained as TBBPA was manufactured and used in greater quantity in Asia (Kawashiro et al., 2008).

TBBPA concentrations in human plasma in the low ng/g lipids range have been reported (Table 1.1.2). Concentrations and detection frequency of TBBPA in adipose tissue is even lower due to the relatively low lipophilic properties of TBBPA (K_{ow} = 5), and the the metabolic fate of this BFR.

Few risk assessments and biomonitoring studies concerning NBFRs have been conducted in humans. Therefore, data concerning human exposure to NBFRs are scarce. Karlsson et al. (2007) and Sjödin et al. (2009) studied DBDPE and BTBPE alongside PBDEs in serum samples collected from workers in Swedish electronic recycling plants. BTBPE and DBDPE were not detected in the workers' serum. Analytical methods used by Karlsson et al. (2007) and Sjödin et al. (2009) were primarily based on PBDE analysis, so the apparent absence of BTBPE and DBDPE could be due to sub-optimal analytical conditions for those compounds. Zhu et al. (2009) screened a number of BFRs in 128 human serum samples from China. Although PBDEs were present, DBDPE and BTBPE were not detected in all serum samples.

1.1.2.2. Health effects

The commercial HBCDD has shown low acute toxicity (Covaci et al., 2006). The minimum lethal dose is greater than 20 g/kg for dermal and oral administration (ECHA, Part II, 2006). Repeated dose toxicity was assessed by Van der Ven et al., 2006 and revealed enhanced endocrine and immune parameters and recorded the liver as target organ with an increased liver weight probably due to enzyme induction as from doses of 100 mg/kg/day. Liver weight increase was slowly reversible upon cessation of exposure. Secondary to the liver effects, disruption of the thyroid hormone system has been noted. Reproductive and developmental toxicity were detected (Eriksson et al., 2006; Ema et al., 2008).

No information is available on the effects of single exposure to TBBPA in humans. Based on animal studies it can be concluded that TBBPA is of low acute toxicity by all routes of exposure. It is not a skin or respiratory sensitiser. Chronic exposure was assessed in a 90-day rat study which showed no toxicologically significant effects following oral exposure up to 1000 mg/kg TBBPA (Saegusa et al., 2009). A decrease in serum T4 levels was observed, however in the absence of changes in other parameters (TSH, T3 and changes in the liver, thyroid, parathyroid or pituitary gland) of thyroid homeostasis in a species that is very sensitive to perturbations in thyroid hormone levels, these decreases are not considered to be adverse. There are no studies in humans or animals on the carcinogenic potential of TBBPA. However, there are no indications to raise concerns for carcinogenicity. Neurodevelopmental toxicity of TBBPA was assessed, but no convincing evidence of an adverse effect on neurodevelopment at dose levels up to 1000 mg/kg/day could be provided (ECHA, Part II, 2006). The WHO conducted a similar risk assessment and concluded that TBBPA has little potential for bio-accumulation and the risk for the general population is considered to be insignificant (http://www.icl-ip.com).

No particular toxicological studies are reported for NBFRs in humans. Based on lab animal studies various NBFRs have no to low acute and chronic toxicities. BTBPE have shown low toxicity for laboratory animals, this might be due to the limited accumulation and absorption and rapid excretion of BTBPE in human, which has been indicated in rats when given orally (Nomeir et al., 1993; Hakk et al., 2004). However, Hakk et al. (2004) identified the hydroxylated metabolites of BTBPE which suggest biotransformation by cytochrome P450 enzymes. In an *in vitro* study BTBPE has shown porphyrinogenic properties on chick embryo liver cultures but only after pre-treatment with betanaphtoflavone (Koster et al., 1980). Li et al. (2004) observed no evidence of skin sensitization properties in 200 professional workers during a repeated application of DBDPE in petroleum during three weeks. Lack of toxicity for DBDPE is believed due to the poor bioavailability which is due to its high molecular weight and poor water solubility (Hardy et al., 2002). However, McKinney et al. (2011) using an *in vitro* system based on liver microsomes from three Arctic marine-feeding mammals and laboratory rat observed oxidative and reductive biotransformation of DBDPE. A depletion of 44-74% of 90 pmol was observed for DBDPE in individuals from all species. In another *in vivo* and *in vitro* toxicity study Nakari and Huhtala (2009) revealed that DBDPE is bioavailable, is acutely toxic to water fleas (*Daphina magna*) and have injurious effects on the reproduction physiology of zebrafish (*Danio rerio*). Scientific literature on human health monitoring of 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB), bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEHTBP) and hexachlorocyclopentadienyl-dibromocyclooctane (HCDBCO) is scarce (Harju et al., 2009). Despite the absence of lethality or overt sign of toxicity, Bearr et al. (2010) observed genotoxicity in feathhead minnows over the exposure of Firemaster® BZ54 and 550 mixtures, of which EH-TBB and BEHTBP are important components.

1.1.3. Human exposure to organophosphate esters employed as FRs (PFRs)

Organophosphate esters (PFRs) are used as flame retardants and/or plasticisers in a wide range of polymers, in furniture upholstery, insulation, wall coverings and floor finishing products. Triethyl phosphate (TEP), tri-*n*-butyl phosphate (TnBP), tri-*iso*-butyl phosphate (TiBP), tris(2-ethylhexyl) phosphate (TEHP), and tris(2-butoxyethyl) phosphate (TBOEP) are commonly used as plasticisers in polymers, such as vinyl resins, cellulose esters and lacquers, and sometimes rubber (WHO, 1991; OECD, 1998; WHO, 2000). Another typical use of TBOEP is floor polish (WHO, 2000).

Chloroalkyl phosphates, such as tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris(chloropropyl) phosphate (TCPP) are more often used in textile backcoatings, in rigid and flexible polyurethane foams, which are used for thermal insulation and for furniture and upholstery, respectively (WHO, 1998). In contrast, tris(2-chloroethyl) phosphate (TCEP) is now more frequently used in PVC, unsaturated polyester resins and in textile backcoatings (WHO, 1998).

Aryl phosphates, such as tricresyl phosphate (TCP), triphenyl phosphate (TPhP), 2-ethylhexyl-diphenyl phosphate (EHDPhP), but also TBP are used in flame retardant hydraulic fluids (WHO, 1997). TPhP and TCP are further used as plasticisers, mainly in vinyl polymers and electric cables (WHO, 1990; 1991b). EHDPhP and TEHP are also applied in vinyl food packaging. The consumption of PFRs has increased sharply in the European Union after the restriction in use of PBDEs (EFRA, 2007). Also in the United States and Japan, an increase in the use of PFRs has been observed as PBDEs have been phased out (Saito et al., 2007; Stapleton et al., 2009). The rise in consumption has resulted in increasing levels of these contaminants in the indoor environment with air and dust being the more important sources of exposure for humans because in this environment people generally spend most of their time. Other plausible routes of exposure are the intake of PFRs *via* drinking water and food, though according to recent measurements these matrices seem of minor contribution to human body burdens. They are thus emerging contaminants in the indoor and outdoor environment, have a low hydrophobicity (Table 1.1.3) which enhances their uptake into living organisms, and are strongly and rapidly metabolised (WHO 1991b; WHO 1998; UNEP 2002).

Considering the extent of occurrence of PFRs, concerns rise about possible health effects related to constant exposure to these chemicals.

Table 1.1.3: Physico-chemical properties of the most common organophosphate flame retardants and plasticisers (Wei et al., 2015)

Abbreviation	S	P	H	log K_{ow}	log K_{oc}	BCF	$t_{1/2}$
TCEP	7×10^{3}	1.1×10^{-4}	3.3×10^{-6}	1.44	2.48	1.37	17.5
TCPP	1.6×10^{3}	1.9×10^{-6}	6×10^{-8}	2.59	2.71	42.4	8.6
TDCPP	1.5	7.4×10^{-8}	2.6×10^{-9}	3.8	2.35	13.5	21.3
TEP	5×10^{5}	0.29	3.5×10^{-6}	0.8	1.68	3.88	-
TPhP	827	2.9×10^{-2}	8.2×10^{-6}	2.67	2.83	63.1	-
TnBP	280	1.1×10^{-3}	1.5×10^{-7}	4	3.28	1.03×10^{3}	<1
TiBP	3.72	1.3×10^{-2}	1.1×10^{-4}	3.6	3.05	391	4.3
TCP	0.36	1.8×10^{-7}	9.2×10^{-7}	5.11	4.35	8.56×10^{3}	-
TPhP	1.9	1.2×10^{-6}	3.3×10^{-6}	4.59	3.72	113	-
TBOEP	1.2×10^{3}	2.1×10^{-7}	3.3×10^{-11}	3.65	4.38	1.08×10^{3}	3
TEHP	0.6	2×10^{-6}	9.6×10^{-5}	9.49	6.87	1×10^{6}	-
EHDPhP	1.9	6.5×10^{-7}	2.5×10^{-7}	5.37	4.21	6.49×10^{4}	-

S: solubility (mg/L) in water at 25 ºC; P: vapor pressure (mmHg) at 25 ºC; H: Henry's law constant (atm/m^3/mol) at 25 ºC; log K_{ow}: *n*-octanol/water partition coefficient; log K_{oc}: soil adsorption coefficient; BCF: bioaccumulation factor; $t_{1/2}$ (h) for photodegradation in the atmosphere (5×10^{5} OH/mol)

1.1.3.1. Routes of exposure

Settled dust. Preliminary results on indoor and car dust show that levels of PFRs in dust collected in public buildings and cars are higher than the levels in dust collected from home environments (Marklund et al., 2003; Bergh et al., 2011a; Brommer et al., 2011). There is also a shift in the PFR profile: levels of TPhP and TDCPP increase moderately in the office dust, but a remarkable rise in

TDCPP levels is observed in car dust samples. Table 1.1.4 gives an overview of the sum of analysed PFRs in the countries and the most dominant PFRs in the analysed samples. There seems to be a region specific consumption of PFRs as higher levels of e.g. TPhP and TDCPP were observed in house dust from the US and New Zealand (Stapleton et al., 2009; Ali et al., 2011b).

Table 1.1.4: Levels of PFRs in matrices relevant for human exposure

Country	Nr. samples	Σ PFRs (medians)	Dominant PFRs	Particularities of the study	References
			Dust (µg/g)		
US	50	9.82	TPhP	Homes, 3 PFRs	Stapleton et al., 2009
Belgium	15	16.5	TBOEP, TCPP, TPhP	Shops, 9 PFRs	Van den Eede et al., 2011
Belgium	33	13.1	TiBP, TBOEP, TCPP	Homes, 9 PFRs	Van den Eede et al., 2011
Germany	216	0.40	TCPP	Homes, only TCPP	Ingerowski et al., 2001
Germany	356	0.60	TCEP	Homes, only TCEP	Ingerowski et al., 2001
Germany	10	11	TBOEP, TPhP	Offices, 9 PFRs	Brommer et al., 2011
Germany	12	37	TDCPP, TBOEP	Cars, 9 PFRs	Brommer et al., 2011
Germany	1	3	TCPP, TBOEP	Home, 9 PFRs	Brommer et al., 2011
Japan	40	5.4	TBOEP, TCPP	Homes, 11 PFRs	Kanazawa et al., 2010
Japan	8	48.5	TBOEP, TCPP, TCP	Hotel, 11 PFRs	Takigami et al., 2009
New Zealand	50	4.3	TBOEP, TPhP	Homes, 8 PFRs	Ali et al., 2011
Romania	47	7.5	TBOEP, TCPP	Homes 8 PFRs	Dirtu et al., 2011
Spain	8	21.5	TBOEP, TCPP	Homes, 8 PFRs	García et al., 2007a
Spain	9	24	TBOEP, TCPP	Homes, cars, car wash area, 7 PFRs	García et al., 2007b
Sweden	10	21	TBOEP, TCEP	Homes, 10 PFRs	Bergh et al., 2011a
Sweden	10	1600	TBOEP (TDCPP)	Day care center, 10 PFRs	Bergh et al., 2011a
Sweden	10	140	TBOEP, TCPP	Workplace, 10 PFRs	Bergh et al., 2011a
Sweden	15	59	TBOEP, TPhP	homes and public locations, 12 PFRs	Marklund et al., 2003
			Air (ng/m³)		
Germany	50	10		Homes, only TCEP	Ingerowski et al., 2001
Japan	14	6.6		Offices, TnBP	Saito et al., 2007
Japan	18	4.0		Houses, TnBP	Saito et al., 2007
Japan	40	62.3	TCPP, TEP, TnBP	Homes	Kanazawa et al., 2010
Sweden	10	78	TCPP, TnBP	Homes, 10 PFRs	Staaf et al., 2005
Sweden	10	40	TiBP, TnBP,	homes, 11 PFRs	Bergh et al., 2011a
Sweden	10	140	TBOEP, TCEP	Day care, 11 PFRs	Bergh et al., 2011a
Sweden	10	160	TCPP, TDCPP	offices, 11 PFRs	Bergh et al., 2011a
Sweden	169	14		Apartments, TCPP	Bergh et al., 2011b
Sweden	169	11		Apartments, TiBP	Bergh et al., 2011b
Sweden	5	145	TCPP, TCEP, TiBP	Schools, daycare center, office, 7 PFRs	Carlsson et al., 1997
Sweden	12	121.2 (mean)	TBOEP, TCEP, TCPP	Electronics recycling plant, dismantling hall, 8 PFRs	Sjödin et al., 2001
Sweden	17	160	TCPP, TnBP	Public locations, 10	Marklund et al.,

Country	Nr. samples	Σ PFRs (medians)	Dominant PFRs	Particularities of the study	References
				PFRs	2005
Switzerland	16	41	TCEP, TEP, TCPP	Cars, stores, offices, 8 PFRs	Hartmann et al., 2004
Other matrices					
Sweden		410 ng/g lw	TCPP, TPhP, TCEP	Marine perch	Marklund et al., 2010
Sweden		720 ng/g lw	TCPP, TPhP, EHDPhP	Freshwater perch	Marklund et al., 2010
Sweden		110 ng/g lw	TCPP, TPhP, EHDPhP	Marine herring	Marklund et al., 2010
Sweden		1900 ng/g lw	TCPP, TBOEP, EHDPhP	Freshwater perch (STP)	Marklund et al., 2010
US		15 ng/L	TBP	Finished drinking water	Stackelberg et al., 2007
US		4 ng/L	TCEP	Finished drinking water	Stackelberg et al., 2007
US		12 ng/L	TDCPP	Finished drinking water	Stackelberg et al., 2007
Germany	1	6.25 ng/L	TCPP, TDCPP, TCEP	Finished drinking water, 8 PFRs	Andresen et al., 2006

Indoor air. The most abundant PFRs in indoor air samples from homes were TEP, TiBP, TnBP, TCEP and TCPP, which is outlined in Table 1.1.3. Heavier PFRs such as TBOEP, TPhP, TCP, TDCPP and TEHP are usually present in low concentrations or even below the detection limit. However, in some work environments and cars high levels of TBOEP and TDCPP were observed (Staaf and Östman, 2005; Saito et al., 2007; Bergh et al., 2011a). The higher air concentrations were associated with a higher dust concentration though it is hard to establish a significant correlation due to the high particle-adsorbed fraction of the PFR concentrations in air (Marklund et al., 2005; Bergh et al., 2011a).

Drinking water. PFRs have been detected at the ng/L level in drinking water, resulting from an incomplete removal from wastewater (Meyer and Bester, 2004) or from groundwater polluted with surface water leachate (Regnery et al., 2011). Chlorinated PFRs such as TCEP and TCPP in particular are more persistent to biodegradation and are not easily removed by bank filtration due to their low soil adsorption coefficient (Regnery, 2011). However, activated carbon filtration did remove TCEP and TCPP according to Andresen and Bester (2006). This also appears in the maximum concentrations of TBOEP (350 ng/L) and TDCPP (250 ng/L) reported in finished drinking water by Stackelberg et al. (2004) in contrast to the mean concentrations displayed in Table 1.1.3 (Stackelberg et al., 2004; Andresen and Bester, 2006).

Food. So far only a handful of studies have investigated the presence of PFRs in food samples. In a market basket study performed by the US FDA (2006) most of the PFRs (EHDPhP, TCEP, TCPP, TnBP, TPhP, and TCP) were found only at the ng/g level. More than 91 % of the results were below the quantification limit in most types of samples. Higher detection frequencies were observed for TPhP in margarine and caramels (mean 45 ng/g).

The presence of PFRs in fish samples was investigated by Camponi et al. (2010), but no PFRs were detected (detection limits of 0.2 to 9 ng/g). Marklund et al. (2010) investigated fish from Swedish rivers and the Baltic Sea (Table 1.1.3) and observed higher levels of TBP and TBOEP near point sources such as sewage treatment plants and airports. Brandsma et al. (2011) analysed different samples representative for the pelagic and the benthic food web in the western Scheldt. For most PFRs, concentrations in sediment were much higher compared to lipid-normalised levels in invertebrates and fish, indicating trophic dilution. TBOEP and TCEP and TCPP were found to be more bioaccumulative as levels in flounder and herring exceeded those in sculpin and poulting. Malarvannan et al. (2015) found PFRs in eels sampled from all sampling sites (26 locations) in Belgium. The PFR levels and lipid contents of the fish did not correlate, so the PFRs do not seem to primarily associate with the lipids. Due to this fact, the consumption of eels seems to only be of minor importance compared to other sources of human exposure to PFRs.

Dermal exposure. Dermal contact to PFR treated fabrics or dust containing PFRs could lead to additional exposure. Furthermore, Weschler and Nazaroff (2008) suggested that air concentrations of PFRs may cause a significant exposure through dermal absorption. Since most PFRs possess a log K_{ow} of 2 to 5, the hypothesis of dermal absorption seems acceptable and indeed some of them, namely TBP, TCP, TDCPP were reported to be absorbed in animal and *in vitro* studies (EHC 110; EHC 112; EHC 209). However, other studies report a low toxicity following dermal exposure to TEP, TPhP and TCPP (SIDS TEP TPhP TCPP). TBOEP was also poorly absorbed from skin but proved in contrast to the previously named PFRs to cause serious skin irritation (EHC 218). Only a few studies have shown the extent of dermal exposure by taking hand washing samples or hand wipes. Makinen et al. (2009) found by average a total of 3.5 µg and 34 µg for people working in a circuit board factory and furniture workshop, respectively. TPhP and TCP were the dominating compounds in the samples: Cooper and Stapleton (2011) detected up to 200 ng TPhP and up to 2000 ng of TDCPP in hand wipes after leisure activities. In a later study Stapleton et al. (2014) found that increasing house dust levels and age were associated with higher levels of FRs in handwipes and that the exposure to alternative FRs, such as PFRs, was similar to PBDE exposure. Hoffman et al. (2015) found correlations between the TPhP and TDCPP levels in handwipes and urine, indicating that hand-to-mouth contact and dermal absorption are important pathways of exposure to these chemicals.

1.1.3.2. Levels and profiles in human matrices

PFRs appear to be readily metabolised and so the parent compounds are not frequently detected in human samples (Van den Eede et al., 2015). TBP and TDCPP have been detected in a few adipose tissue samples at the ng/g level (WHO, 1991; 1998). TDCPP has been detected in semen as well (Hudec et al., 1981). Some studies could detect TPhP in blood, but it originated from the PVC packaging (Shah et al., 2006). Marklund et al. (2010) detected PFRs in pools of human milk samples

collected from the 1990s to now. In Table 1.1.4 the most important compounds are mentioned, namely TBOEP, TBP and TCPP. Other PFRs were determined as 5 ng/g lipid weight or lower.

In urine, PFR metabolites have been detected, but the median concentrations of the diaryl and dialkyl phosphates remained mostly below the quantification limit. Ranges of concentrations of dialkyl and diaryl phosphates are shown in Table 1.1.4. In the study of Schindler et al. (2009a,b) the detection frequency was highest for BCEP (50%), followed by DPhP (30%), BCiPP (12%) and DBP (3%). Di-*m*-cresyl and di-*p*-cresyl phosphate were not found in any sample, probably due to lower exposure in the indoor environment. Reemtsma et al. (2011) found also monoaryl and alkyl phosphates in human urine, of which the monobutyl phosphate was the dominant congener (Table 1.1.5).

Table 1.1.5: Concentrations of PFRs and PFR metabolites in human samples

Compounds	Country	Number of samples	Concentration range	Matrix	References
DBP	Germany	25	ND – 0.26 µg/L	urine	Schindler et al., 2009b
DPhP	Germany	30	ND – 4.1 µg/L	urine	Schindler et al., 2009a
BCEP	Germany	30	ND – 27.5 µg/L	urine	Schindler et al., 2009a
BCiPP	Germany	25	ND – 0.85 µg/L	urine	Schindler et al., 2009b
BDCiPP	US	9	0.05 – 1.7 µg/L	urine	Cooper et al., 2011a
DPhP	US	9	0.3 – 7.4 µg/L	urine	Cooper et al., 2011a
MBP	Germany		ND – 158 µg/L	urine	Reemtsma et al., 2011
DBP	Germany		ND – 0.52 µg/L	urine	Reemtsma et al., 2011
DPhP	Germany		ND – 28.6 µg/L	urine	Reemtsma et al., 2011
TCPP	Sweden	6 pooled samples	22 – 82 ng/g lw	milk	Marklund et al., 2010
TBP	Sweden	6 pooled samples	11 – 57 ng/g lw	milk	Marklund et al., 2010
TPhP	Sweden	6 pooled samples	3.2 – 11 ng/g lw	milk	Marklund et al., 2010
TDCPP	US	16	5 – 50 µg/L	semen	Hudec, 1981
BCEP	US	16	ND – 2.1 µg/L	urine	Dodson et al., 2014
BCiPP	US	16	ND – 0.97 µg/L	urine	Dodson et al., 2014
BDCiPP	US	16	ND – 3.9 µg/L	urine	Dodson et al., 2014
DPhP	US	16	ND – 6.8 µg/L	urine	Dodson et al., 2014
DBP	US	16	ND – 0.45 µg/L	urine	Dodson et al., 2014
BBOEP	US	16	ND – 0.71 µg/L	urine	Dodson et al., 2014
DPhP	Australia		<0.3 – 727 µg/L	urine	Van den Eede, Heffernan et al., 2015
BDCiPP	Australia		<0.15 – 8.9 µg/L	urine	Van den Eede, Heffernan et al., 2015
BCiPhiPP	Australia		0.37 – 9.43 µg/L	urine	Van den Eede, Heffernan et al., 2015
TCEP	Australia		<0.35 – 24.5 µg/L	urine	Van den Eede, Heffernan et al., 2015
DBP	Australia		<0.43 – 2.15 µg/L	urine	Van den Eede, Heffernan et al., 2015
BBOEP	Australia		<0.35 – 0.53 µg/L	urine	Van den Eede, Heffernan et al., 2015
BCEP	Belgium		ND – 9.5 µg/L	urine	Van den Eede et al., 2013
BCiPP	Belgium		ND – 6.2 µg/L	urine	Van den Eede et al., 2013
BDCiPP	Belgium		ND – 15 µg/L	urine	Van den Eede et al., 2013
DBP	Belgium		ND – 3.5 µg/L	urine	Van den Eede et al., 2013
DPhP	Belgium		ND – 13 µg/L	urine	Van den Eede et al., 2013
BBOEP	Belgium		ND – 7 µg/L	urine	Van den Eede et al., 2013

1.1.3.3. Health effects

Suspected and observed effects in animals. Acute toxicity of PFRs is associated with typical cholinergic symptoms, such as salivation, diarrhoea, piloerection, tremor, ataxia and respiratory depression. The LD_{50} values were in the range of 1 to 5 g/kg body weight, as a result the PFRs were classified as ”safe“. Concerning subchronic exposure to PFRs, adverse effects included an absolute or relative increase in liver and kidney masses, and a smaller increase in body mass for young animals compared to controls. No teratogenicity was observed for PFRs in rodents. However, exposure before and during mating resulted in a decrease in the number of litters and live pups per litter, which is an indication for reproductive toxicity. The safety of PFRs following chronic exposure can also be questioned: TDCPP caused a development of carcinomas in the liver, kidney, testes and other organs (WHO, 1998). Neurotoxic and carcinogenic properties were also observed for TCEP (WHO, 1998).

Possible adverse health effects in humans. So far only a limited number of studies found associations between adverse health effects and exposure of humans to PFRs, but few have been completely proven. There were some cases of TCP poisoning in the late 19th and early 20th century. This compound causes delayed neuropathy, but is now only used as a minor component in TCP isomer mixtures (WHO, 1990). Camarasa and Serra-Baldrich (1992) reported allergic contact dermatitis after repeated contact with TPhP treated plastics. The presences of TCPP and TDCPP in floor dust was associated with atopic dermatitis, while TBP was associated with increased prevalence of asthma and allergic rhinitis (Araki et al., 2014). In a test employing human nuclear receptors, Kojima et al. (2013) found that several PFRs, such as TBP, TEHP, TDCPP, TPhP and TCP may have potential endocrine disrupting effects via ERα, ERβ, AR, GR and PXR receptors. Fang et al. (2013), Meeker and Stapleton (2010) confirmed this by indicating endocrine disruptive properties for TPhP and TDCPP, through a negative correlation with semen quality and thyroid hormone levels, respectively. Wang et al. (2015a) found TDCPP to be an estrogenic endocrine disruptor that impairs fish reproduction in zebrafish. This same compound was found to be neurotoxic in studies in PC12 cells (Dishaw et al., 2011) and zebrafish (Wang et al., 2015b). Also by using zebrafish as a model species, Du et al. (2015) found that aryl PFRs (especially TPhP and CDP) disturb expressions of key regulators in heart development.

Kanazawa et al. (2010) associated mucosal symptoms of the sick building syndrome with high indoor exposure to TBP. These symptoms include irritation to the eyes, nose and throat symptoms such as flushing, and mucosal symptoms such as irritation to the eyes, nose and throat, the latter symptoms were strongly associated with TBP levels in air and dust.

1.2. Transformation products of emerging contaminants in the environment

Based on the following publication:

Dirtu AC, Ionas AC, Malarvannan G, Covaci A. Transformation Products of Brominated Flame Retardants (BFRs), Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks (2014), ISBN: 978-1-118-33959-6.

Dirtu AC, Van den Eede N, Malarvannan G, Ionas AC, Covaci A. Analytical methods for selected emerging contaminants in human matrices – a review, Analytical and Bioanalytical Chemistry (2012), 404(9):2555-81.

Due to their spread in the environment and humans and to their persistent and toxic properties, FRs have been under scientific scrutiny during the last decade. Some FRs have been classified as contaminants of concern (e.g. PBDEs, TDBPP, and TCEP). BFRs prevent the spread of fire by quenching the radical oxidation reactions which take place during combustion by reacting with the H· and HO· radicals. Since these reactions release Br atoms or radicals from the BFR molecule, it is expected that debromination processes will take place also in environmental or biological systems. Yet, other transformation pathways, such as oxidative metabolism, have been recently reported for BFRs to better explain their fate and behaviour into various environmental compartments, including biota. PFRs contain phosphate ester bonds which can be hydrolysed in a multitude of conditions in the environment, potentially releasing mono- and diesters.

1.2.1. Transformation products of BFRs

The potential of BDE 209 and other higher brominated PBDEs to form lower PBDEs through debromination is of concern. Regarding the environmental behaviour of PBDEs and of their TPs, a few issues need to be taken into account: 1) the exposure to PBDEs occurs via multiple pathways; 2) the absorption of PBDEs in organisms can occur without degradation, leading to bioaccumulation; 3) biotransformation of PBDEs can follow debromination or oxidative metabolism pathways; and 4) TPs may significantly accumulate and/or have higher toxicity. The main transformation or metabolic pathways in which PBDEs are involved in abiotic and biotic media (Tables 1.2.1 and 1.2.2), together with the most important conditions which influence such processes, are discussed here below.

The commercial HBCDD mixture consists of three major isomers, α-, β-, and γ-, in a ratio of 10, 10, and 80% of the mixture, respectively. Despite its small contribution to HBCDD global production and usage, α-HBCDD is the major stereoisomer found in biota. This finding has driven the research in the direction of elucidation of the potential pathways which would explain such behaviour.

TBBPA is a non-volatile FR (Hakk and Letcher, 2003) of widespread use, which degrades very slowly photochemically (Eriksson et al., 2004). Its detection in dust, sediments and biota has led to increasing concerns regarding its effects on wildlife and humans (Covaci et al., 2009). Since it was measured in humans, TBBPA may be a matter of concern to human health (Cariou et al., 2008). Therefore, it is imperative to know the environmental fate of the parent molecule. Several studies on rats, aquatic organisms, and/or microorganisms have indicated that metabolic Phase II conjugation and debromination of TBBPA occur in biota and thus, the literature addressed in the following paragraphs is focused on its environmental degradation and metabolism (Tables 1.2.1 and 1.2.2).
Since the NBFRs (such as DBDPE, BTBPE, EH-TBB and BEHTBP) have been produced in lower volumes than the major BFRs, not so many studies are published about their TPs.

1.2.1.1. Degradation of BFRs in abiotic matrices

PBDEs. Several studies reported potential degradation of PBDEs, especially for BDE 209, through debromination during sample preparation and instrumental analysis (De Boer and Wells, 2006). Multiple factors were suggested to be responsible, including thermal stress or sunlight (Björklund et al., 2004; WHO, 1994). Consequently, a question arose, whether the debromination of BDE 209 could also occur in the environment. Several studies have proven the potential of BDE 209 to decompose in photolytic conditions due to both natural sunlight, as well as artificial UV light exposure (Table 1.2.1). The degradation of BDE 209 was observed independently of the exposure conditions or tested media, such as solvent/water mixtures, silica-gel, sand, sediment or soil samples (Ahn et al., 2006; Eriksson et al., 2004; Söderström et al., 2004). Even if the profile of TPs consistently indicated formation of on tri- to nona-BDEs (Ahn et al., 2006; Söderström et al., 2004), the photodegradation kinetics were highly variable among different matrices. The calculated half-live for BDE 209 varied from 15 min in solvents (Söderström et al., 2004) up to 24000 h on mineral surfaces (Ahn et al., 2006), most probably due to differences in incident radiation intensities, wavelengths or availability of light co-absorbers from tested matrices (e.g. humic substances) (Stapleton and Dodder, 2008).

Nona-BDEs may undergo rapid photodegradation (half-lives ranging from 4.25 to 12.8 min in methanol, toluene, and tetrahydrofuran) leading to the formation of octa- and hepta-BDE congeners (Davis and Stapleton, 2009).

The major congeners usually found in environmental samples (BDE 28, 47, 99, 100, 153 and 183) have the same breakdown potential (Eriksson et al., 2004; Fang et al., 2008), even in hexane.

Table 1.2.1: Transformation pathways of selected BFRs in various abiotic media.

FR	Transformation pathways & products			Conditions	Comments	Reference
	Debromination	Hydroxylation	Other			
BDE 28	BDE15	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 47	BDE 28, 15	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 99	BDE 28, 49, 47, 66	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 100	BDE 28, 75, 47	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 153	BDE 28, 47, 118, 49, 66, 99	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 183	BDE 28, 47, 99, 154, 138, 49, 66, 118, 153	–	–	Irradiation with UV light in the sunlight region	Experiments conducted in *n*-hexane	Fang et al., 2008
BDE 206, 207, 208	octa- and hepta-BDEs	–	–	Exposure to natural sunlight, while compounds kept in different solvents	BDE207 degraded most rapidly, BDE206 degraded the slowest	Davis and Stapleton, 2009
BDE 209	lower brominated BDEs	–	–	Bacterial aerobic biotransformation by *Lysinibacillus fusiformis* strain DB-1	carbon sources (lactate, pyruvate, acetate) were used	Deng et al., 2011
	nona-BDEs: 206, 207, 208 octa-BDEs: 196, 197, 201, 202203/200	–	–	Exposure to direct sunlight for over 200h	Degradation study conducted in house dust	Stapleton and Dodder, 2008
	nona- and octa-BDEs	–	tetra- to octa-brominated dibenzofurans	Exposure to natural sunlight	BDE 209 incorporated into high-impact polystyrene and TV casings	Kajiwara et al., 2008
HBCDDs	–	–	75% of bromine is released as HBr	thermal degradation was carried out in nitrogen and in air at moderate heating rates (10 °C/min)	Oxygen have a negligible influence on the HBCDD decomposition	Barontini et al., 2001
	–	–	γ-HBCDD isomerization to α-HBCDD	Process influenced by temperature increase to above 110 °C	Process observed in toluene	Kajiwara et al., 2009
DBDPE	–	–	bromotoluenes	Pyrolysis experiments in a quartz tube (600 °C)	High-impact polystyrene samples containing DBDPE	Jakab et al., 2003
	hepta- to nona-BDPEs	–	–	Irradiation with UV light	Experiments conducted in acetone/tetrahydrofuran/toluene (20/30/50 %) mixture	Wang et al., 2010
BEHTBP	Debrominated products	–	–	Exposure to natural sunlight	Test compound kept in different solvents	Davis and Stapleton, 2009
EH-TBB	Debrominated products	–	–	Exposure to natural sunlight	Test compound kept in different solvents	Davis and Stapleton, 2009
BTBPE	–	–	2,4,6-tribromophenol; vinyl 2,4,6-tribromophenyl ether	Isothermal conditions at 240 and 340 °C	Inert atmosphere	Balabanovich et al., 2003
TBBPA	–	–	4-isopropyl-2,6-dibromophenol, 4-isopropylene-2,6-dibromophenol, 4-(2-	Irradiation with UV light	Experiments conducted in aqueous solutions at different pH values	Eriksson et al., 2004

FR	Transformation pathways & products			Conditions	Comments	Reference
	Debromination	Hydroxylation	Other			
			hydroxyisopropyl)-2,6-dibromophenol			
	5, 5'-propane-2,2-diyldibenzene-1,2,3-triol, 5, 5'-(1,3-dihydroxypropane-2,2-diyl)dibenzene-1,2,3-triol		2,6-dibromo-4-isopropenylphenol, 2,6-dibromo-4-isopropylidenecyclohexa-2,5-dien-1-one, 2,6-dibromo-4-(1-hydroxy-1-methylethyl)phenol	Photocatalytic degradation by irradiation with UV-VIS light	In the presence of bismuth oxybromide (BiOBr)	Xu et al., 2011

The photodegradation of BDE 209 in dust led to the formation of all nona- and several octa-BDE congeners (BDE 196, 197, 201, 202 and 203/200) (Stapleton and Dodder, 2008). Since the Octa-BDE mixture may contain BDE 201 only in a very small amount (< 0.8%) and BDE 202 is usually not present, it was therefore suggested that the presence of BDE 201 and 202 in house dust is a marker of environmental debromination of BDE 209. Additionally, the ratio of BDE 197 to BDE 201 is indicative of BDE 209 degradation, as their ratio appeared to reach a steady-state value of ~1 (Stapleton and Dodder, 2008).

HBCDDs. Since HBCDD is used as FR, the thermal stability and susceptibility to undergo photolytic degradation, but also the nature/toxicity of the generated products, become important for safe usage in consumer products. In general, two mechanisms were evidenced for the HBCDD degradation: ***debromination*** with generation of tetra-bromocyclododecene, di-bromocyclododecadiene, and cyclododecatriene; ***isomerisation*** processes in which stereoisomer interconversion takes place without the loss of any other secondary products (Table 1.2.1).

Increased temperatures (> 110 °C) during fabrication processes were already reported to influence the isomerization of HBCDDs leading to higher percentages of α-HBCDD (Kajiwara et al., 2009). This is relevant from the perspective of the implications for human exposure to HBCDDs via dust ingestion since α-HBCDD was already shown to possess the highest bioaccumulation potential and longest half-life compared to β- and γ-isomers (Abdallah and Harrad, 2011).

As they bind strongly to solid particles (dust, soil, sediment or sewage sludge), HBCDDs were detected in several matrices, following the general rule: the *closer* to the emission source, the *higher* the expected levels. Sequential degradation of ^{14}C-HBCDDs was tested with activated sludge, digester sludge, soil or freshwater aquatic sediments in microcosms (Davis et al., 2006). Based on the identified TPs, the proposed mechanism implied debromination via dihaloelimination with the subsequent formation of a double bond (Davis et al., 2006). Microorganisms naturally occurring in

aquatic sediments and anaerobic digester sludge might thus mediate complete debromination of HBCDDs.

In indoor dust, an important matrix for human exposure, photo-degradation and/or isomerization of HBCDDs have been reported to occur possibly via elimination of HBr. Using separation (GC or LC) and detection (MS) techniques, the TPs were identified as pentabromocyclododecenes (four isomers) and tetrabromo-cyclododecadienes (two isomers) (Abdallah et al., 2008).

TBBPA. Eriksson et al (2004) investigated the photochemical transformation of TBBPA in an aqueous medium, and proposed that the primary photochemical reaction was the cleavage between one of the benzene rings and the isopropyl group. The photodegradation of TBBPA in the environment may result from direct photolysis as well as reaction with sunlight-generated reactive oxygen species, such as singlet oxygen. Previous studies have shown that there are environmental sources of singlet oxygen, such as the humic acids (Han et al., 2009) which could contribute to the degradation of TBBPA.

The combustion of domestic products containing TBBPA may lead to formation and release of polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs). Large amounts of brominated and mixed chloro-bromo dioxins and furans can be formed in accidental fires where BFRs are present (Söderström and Marklund, 1999). Brominated light hydrocarbons, as well as phenolic derivatives, were observed during thermal decomposition (combustion and pyrolysis) of TBBPA (Ortuño et al. 2011). TBBPA processing at temperatures above the melting point (180 °C) may cause the release of TBBPA in the environment due to evaporation (Marsanich et al., 2004).

At low and neutral pH, TBBPA is virtually insoluble in water and therefore, soil mobility is expected to be minimal. However, at higher pH, the solubility of TBBPA increases significantly. The biodegradation of TBBPA was measured in various soil matrices under aerobic conditions (WHO, 1995). An 80% reduction of TBBPA was observed over 45 days of anaerobic degradation of a highly contaminated soil (Ronen and Abeliovitch, 2000). TBBPA converted into bisphenol-A (BPA) by reductive debromination under anaerobic conditions. Voordeckers et al. (2002) observed complete debromination of TBBPA to BPA under both methanogenic and sulfate-reducing conditions. During the anaerobic stage, TBBPA is reductively debrominated to BPA, which is completely mineralised under bacterial aerobic conditions (Ronen and Abeliovich, 2000). Among other possible microbial biotransformation products identified, there are a number of brominated phenols, including 2,4,6-tribromophenol.

DBDPE. Similar to BDE 209, DBDPE also undergoes thermal degradation (Table 1.2.1) at a comparable temperature, but besides debromination or intermolecular ring closure, it also generates bromotoluenes (Jakab et al., 2003). Two nona-brominated degradation products of DBDPE were observed even in the technical mixture (Kierkegaard, 2007). When exposed to strong UV radiation

(125 W), DBDPE degraded to a number of hepta- to nona-BDPEs (Wang et al., 2010). Under natural sunlight condition, this degradation was not observed in plastic matrices (Kajiwara et al., 2008).

BTBPE. The main decomposition products of BTBPE found through rapid removal by pyrolysis were TBP and vinyl 2,4,6-tribromophenyl ether (Balabanovich et al., 2003) (Table 1.2.1). However, a prolonged contact with a heat source can also produce hydrogen bromide, ethylene bromide, polybrominated vinyl phenyl ethers, poloybrominated diphenyl ethers and dibenzodioxins. Most of them are even more toxic and reactive than BTBPE and therefore the follow up of these TPs is mandatory.

EH-TBB and BEHTBP are prone to photodegradation in organic solvents (toluene, methanol and tetrahydrofuran) and the photodegradation TPs were the debrominated analogues, with two or three bromine atoms (Davis and Stapleton, 2009). Some TPs of BEHTBP lost also both alkyl side chains (Table 1.2.1). These chemicals degrade photolytically slower than the PBDEs, suggesting that their persistence in the environment might be higher

1.2.1.2. Biotransformation pathways for BFRs

PBDEs. The main biotransformation pathways can be initially assessed by *in vitro* research using microsomes together with a wide variety of enzymes, while *in vivo* exposure studies are valuable in identifying major pathways of metabolism. Further, results obtained through either *in vitro* or *in vivo* research might be extrapolated when PBDEs or TPs are measured in biota or human samples (Table 1.2.2).

Table 1.2.2: Transformation pathways of selected BFRs in various biotic matrices

FR	Transformation pathways & products			Conditions	Comments	Reference
	Debromination	Oxidation	Other			
Penta-BDE	BDE 47, 100	11 HO-PBDEs	–	Dietary exposure studies in common carp (*Cyprinus carpio*)	Low ratio of HO-PBDEs to their precursor PBDEs	Stapleton et al., 2004; Zeng et al., 2012
Octa-BDE	BDE 154, 155, 149, 153	–	–	Dietary exposure studies in common carp (*Cyprinus carpio*)		Stapleton et al., 2004; Zeng et al., 2012
Deca-BDE	BDE 154, 155, 149, 188, 179, 202	–	–	Dietary exposure studies in common carp (*Cyprinus carpio*)		Stapleton et al., 2004; Zeng et al., 2012
BDE 47	BDE 4	5 isomers of HO-BDE47	2 isomers of HO-tri-BDE	Orallt dised Jumbo Cornish × Rock cross male chickens at 7 weeks of age		Hakk et al., 2010
	–	5 HO-BDEs, among of which more important were 4′-OH-BDE-49 and 3-OH-BDE-47	–	*In vitro* testing with rat hepatic microsomes	Oxidative metabolism of BDE 99 was greater than that of BDE-47	Erratico et al., 2011
BDE 99	BDE 47	–	–	Experiments on intestine microflora and intestine and liver microsomes of	Intestinal microflora are not responsible for BDE-99 debromination	Benedict et al., 2007

FR	Transformation pathways & products			Conditions	Comments	Reference
	Debromination	Oxidation	Other			
				common carp (*Cyprinus carpio*)		
	BDE 49	–	–	Experiments on Chinook salmon (*Onchorhynchus tshawytscha*) liver fractions	Process not NADPH-dependent, indicating a lack of cytochrome P450 involvement	Browne et al., 2009
	–	7 HO-BDEs, among of which more important were 4-OH-BDE-90 and 6′-OH-BDE-99	–	*In vitro* testing with rat hepatic microsomes	Oxidative metabolism of BDE 99 was greater than that of BDE 47	Erratico et al., 2011
	–	several HO-BDEs and diHO-BDEs	2,4,5-tribromophenol	*In vitro* testing with human hepatic microsomes		Lupton et al., 2009; Erratico et al., 2012
BDE 209	BDE 202	–	–	*In vivo* exposure on common carp (*Cyprinus carpio*) and rainbow trout (*Onchorhynchus mykiss*)		Stapleton et al., 2006
	octa- and nona-BDEs	–	–	*In vitro* tests on liver microsomal fractions from rainbow trout (*Onchorhynchus mykiss*)	Up to 22% by mass of the BDE 209 was biotransformed	Stapleton et al., 2006
	hexa- to nona-BDEs	–	–	*In vitro* tests on liver microsomal fractions from common carp (*Cyprinus carpio*)	Up to 65% by mass of the BDE 209 was biotransformed	Stapleton et al., 2006
HBCDD	PBCDs, TBCDDs (unclear if they are metabolic products in humans)	–	enantioselective enrichment of (−)-α-HBCDD	Observation on human milk samples		Abdallah and Harrad, 2011
	–	–	Stereoisomer interconversion from γ- to α-HBCDD	Repeated exposure in adult female C57BL/6 mice	Shorter half-life recorded for from γ- compared to α-HBCDD	Szabo et al., 2010; Szabo et al., 2011
	Dehydrobromination products through the loss of [-HBr] and [-2HBr], [-3HBr]	–	–	*In vitro* hepatic assay using microsomes extracted from: polar bear, beluga whale, ringed seal, rat	Regardless of species, the rate of γ-HBCDD metabolism is more rapid than α-HBCDD	MacInnis et al., 2010
	–	mono HO-HBCDDs; diHO-HBCDDs only in case of α- and γ-HBCDD	–	*In vitro*, rat liver microsomes from male Spargue-Dawley rats preinduced with phenobarbital/β-naphtoflavone	Preferential metabolism of γ-HBCDD	Esslinger et al., 2011
	PBCDDs as minor metabolites	HO-HBCDDs as major metabolites	HO-PBCDD as minor metabolites	*In vitro*, phenobarbital induced rat microsomes		Roosens et al., 2010; Roosens et al., 2011
	PBCDDs and TBCDDs	mono HO-HBCDDs and diHO-HBCDDs	–	Observations on wildlife species (tern egg, seal, and flounder) and in rats exposed to 30 and 100 mg HBCDD/kg bw/day for 28 days	Differences in metabolism observed among species	Brandsma et al., 2009
DBDPE	Lower BDPEs followed by phase I and phase II metabolism	–	$MeSO_2$-nona-BDPE, $EtSO_2$-nona-BDPE	Oral exposure of male Sprague-Dawley rats (21 days old)	Oral administration of 100 mg/kg bw/day for 90 days	Wang et al., 2010
BEHTBP	–	–	mono(2-ethylhexyl) tetrabromophthalate	*In vitro*, rat and human liver microsomes	Enzymes involved in metabolism: carboxylesterases	Roberts et al., 2012
EH-TBB	–	–	2,3,4,5-tetrabromobenzoic acid	*In vitro*, rat and human liver microsomes	Enzymes involved in metabolism: carboxylesterases	Roberts et al., 2012
	–	–	2,3,4,5-tetrabromomethylbenzoate	In hepatic subcellular fractions (i.e., S9, microsomes and cytosol) in the	Differences in metabolism observed among species	Bearr et al., 2012

FR	Transformation pathways & products			Conditions	Comments	Reference
	Debromination	Oxidation	Other			
				fathead minnow, common carp, mouse and snapping turtle		
BTBPE	Multiple metabolites: monoHO-, monoHO- with debromination, diHO-/debrominated on a single aromatic ring, monoHO- on each aromatic ring with accompanying debromination, and cleavage on either side of the ether linkage to yield tribromophenol and tribromophenoxyethanol			Single dose administrated to male Sprague-Dawley rats	Limited absorption and metabolism occur by ingestion in mammals	Hakk et al., 2004
TBBPA	–	–	TBBPA-glucuronide, TBBPA-glucuronide-sulfate, TBBPA-sulfate, TBBPA-disulfate	*In vivo*, exposure of *Xenopus laevis* tadpoles	Metabolism of > 94% of ^{14}C-TBBPA within 8 h	Fini et al., 2012
	–	–	TBBPA-glucuronide, TBBPA-sulfate	Studies of the TBBPA metabolism in mammals, including humans	Predominance of phase II biotransformation pathway products	Knudsen et al., 2007; Kuester et al., 2007; Schauer et al., 2006
	TribromobisphenolA	–	–	^{14}C-TBBPA intraperitoneally exposure of female Wistar rats	High exposure doses of 250 and 1000 mg/kg	Szymanska et al., 2001

The main transformation pathways reported for the PBDEs in biota are: a) ***reductive debromination*** in which hepta- to deca-BDE congeners lead to tetra- to hexa-PBDEs; b) ***oxidative metabolism*** leading to the formation of hydroxylated PBDEs (HO-PBDEs) or other phenolic compounds (Figure 1.2.1, from Erratico et al., 2012). Other metabolic pathways may include Phase II conjugation (glucuronidation or sulfation). However, dietary studies on fish indicate that at least 15% of administered PBDE congeners produced Phase I and II metabolites (hydroxylated, methoxylated, glucoronidated and glutathione substituted), other than the debrominated congeners (Hakk and Letcher, 2003).

Reductive debromination of PBDEs in biota. A number of studies have found that the profile of TPs formed through reductive debromination is species-specific (Benedict et al., 2007; Noyes et al., 2010; Roberts et al., 2011) and therefore any findings would be difficult to extrapolate to other species.

Oxidative metabolism. Through oxidative metabolism, more polar compounds are formed such as HO-PBDEs and other phenolic compounds. Their biological activity is typically greater than that of the parent compounds (Butt et al., 2011; Erratico et al., 2011, Meerts et al., 2001), with effects such as competing with thyroid hormones for binding to serum transport proteins (Marchesini et al., 2008), inhibiting hepatic thyroxine metabolism (Butt et al., 2011), and aromatase activity (Cantón et al., 2008).

The presence of HO-PBDEs was already reported in blood collected from humans environmentally exposed to PBDEs (Athanasiadou et al., 2008; Qiu et al., 2009; Yu et al., 2010), but also in bile, urine or faeces of rats and mice exposed to PBDEs (Chen et al., 2006; Malberg et al., 2005; Qiu et al., 2007; Staskal et al., 2006).

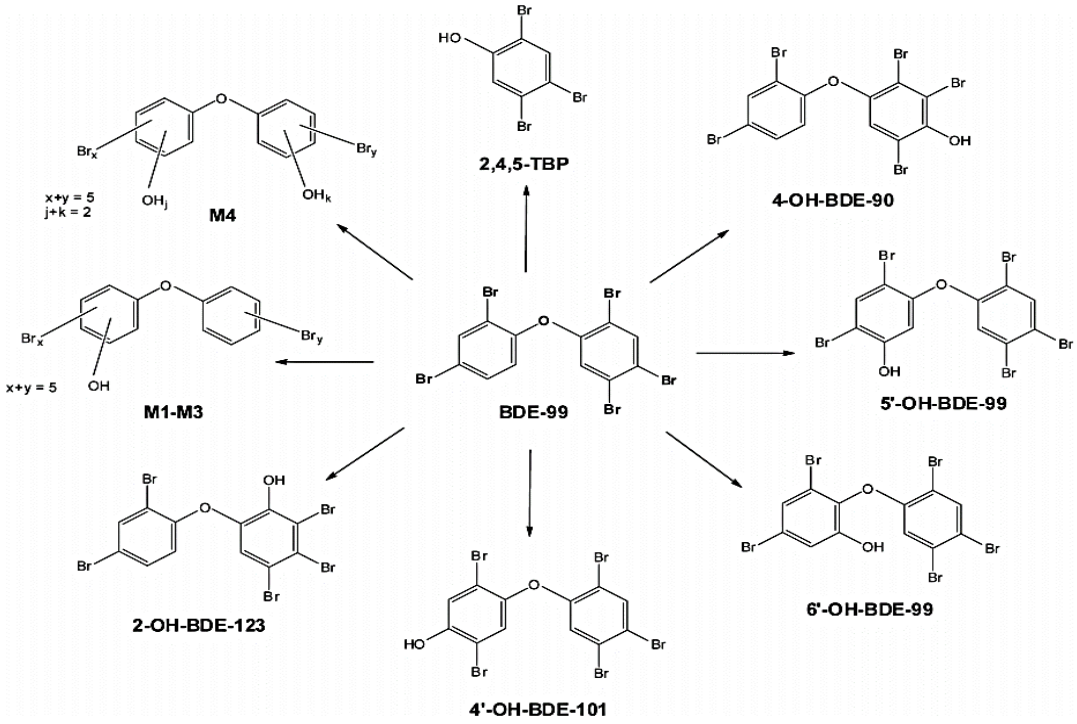


Figure 1.2.1: Scheme showing the chemical structures of metabolites formed following the incubation of human liver microsomes with 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99). General structures for the metabolites M1–M4 are also shown. Reproduced with permission from the publisher of Erratico et al. (2012).

In vitro tests conducted on animal (mostly rat) and human liver microsomes (HLM), by using a panel of human recombinant CYP enzymes and CYP-specific antibodies, evidenced the formation of several HO-PBDEs (mono and dihydroxy-) and 2,4,5-tribromophenol (TBP) for lower BDE congeners (Dong et al., 2010; Erratico et al., 2010; Hamers et al., 2008; Lupton et al., 2009, 2010; Stapleton et al., 2009). As for BDE-209, the absorption rate is very low (>90% of it is excreted with 72 h from exposure) and mono-OH- and *ortho*-MeO-BDEs are the only metabolites formed in rats (Hakk and Letcher, 2003; Mörck and Klasson-Wehler, 2001). But, due to the variable composition of CYP enzymes in liver of different species, chemicals might be metabolised in different ways and interspecies differences in the profiles of TPs is to be expected. As such, Stapleton et al. (2009) did not observe any hydroxylated or debrominated metabolites in the HLMs exposed *in vitro* to BDE 209. So, either BDE 209 is not metabolised by HLMs, or non-extractable, covalently protein-bound metabolites were formed, or the exposure time (24–72 h) was just not long enough for BDE 209 to diffuse into the cells (Stapleton et al., 2009).

HBCDDs. Research in this field evidenced a change in the diastereoisomeric HBCDD patterns from the domination of γ-HBCDD in the technical mixture (Heeb et al., 2005) and sediments (Janák et al., 2005) to the domination of α-HBCDD in biota (Covaci et al., 2006). Although HBCDD standard

mixtures are mostly racemic, the composition of biotic samples often showed a non-racemic pattern (Abdallah and Harrad, 2011; Eljarrat et al., 2009). An overview of HBCDD metabolism is presented in Table 1.2.2.

Oxidative metabolism. Bacteria which can express haloalkane dehalogenases can typically biotransform HBCDDs (with (+)β- and (+)γ-HBCDDs being degraded fastest) to pentabromocyclododecanols (HO-PBCDDs) and to tetrabromocyclododecadiols (di-HO-TBCDDs) (Heeb et al., 2012).

By using subcellular rat liver fractions, phase I metabolites of α- and γ-HBCDDs were formed, mediated by the CYP P450 enzyme system. The major metabolites were reported to be OH-HBCDDs, followed by intermediates like HO-PBCDDs and also PBCDDs as minor metabolites (Esslinger et al., 2011; Roosens et al., 2011).

Debromination. *In vitro* hepatic assays using microsomes extracted from different animal species (polar bear, beluga whale, ringed seal, and rat) and separate HBCDD isomers revealed debromination as the predominant metabolic pathway (MacInnis et al., 2010).

Stereospecific metabolism (bioisomerization). Enantioselective enrichment of (−)-α-HBCDD (average EF = 0.29) was observed in human milk samples, indicating the potential for enantioselectivity associated with HBCDD absorption, metabolism and/or excretion (Abdallah and Harrad, 2011). This type of isomeric interconversion also occurs in the organisms of mice (Szabo et al., 2010) and can be a possible explanation for the higher contribution of α-HBCDD to the total HBCDDs measured in biota samples.

Although the toxicological literature of the TPs of HBCDDs is scarce, higher binding affinities of PBCDDs to human transthyretin receptor (hTTR) than the parent HBCDDs and even T4 were reported (Weber et al., 2009).

TBBPA. The half-life of TBBPA in blood serum of occupationally-exposed Swedish workers (Hagmar et al., 2000) was estimated at 2.2 days, so TBBPA is rapidly depleted from the human body. A single oral dose of 0.1 mg/kg TBBPA was administered to five human subjects (Schauer et al., 2006). TBBPA-glucuronide and TBBPA-sulfate were identified as metabolites of TBBPA in blood and urine of the human subjects. In blood, TBBPA-glucuronide was detected in all human subjects, whereas TBBPA-sulfate was only present in two individuals. Maximum plasma concentrations of TBBPA-glucuronide (16 nmol/L) were obtained within 4 h after administration. In two individuals where TBBPA-sulfate was present in blood, maximum concentrations were obtained at the 4h sampling point; the concentrations rapidly declined below the limit of detection (LOD) after 8 h. Parent TBBPA was not present in detectable concentrations in any of the human plasma samples. TBBPA-glucuronide was slowly eliminated in urine to reach the LOD in 124 h after administration. Absorption of TBBPA from the GI tract and rapid metabolism of the absorbed TBBPA by conjugation result in a low systemic bioavailability of TBBPA. Biotransformation studies of TBBPA

by human and rat subcellular liver fractions have shown that TBBPA undergoes oxidative cleavage at the quaternary carbon atom to give brominated phenols, including 2,6-dibromohydroquinone (Zalko et al., 2006).

NBFRs. DBDPE. In a study on rats, Wang et al. (2010) compared peaks corresponding to the main photodegradation products of DBDPE with peaks from an extract of DBDPE-exposed rat liver tissue. They concluded that debromination alone is not the main biotransformation pathway (Table 1.2.2). By using GC-EI/MS, two metabolites were tentatively identified: a methyl sulfone substituted nona-BDPE and the ethyl sulfone analogue. Definite identification was not possible, though, due to lack of standards for the above compounds. Apparently, reductive debromination was followed by phase I and phase II (glutationation + transformation to methylsulfone) metabolism.

BTBPE. The elimination of BTBPE from rats was studied by administering one single oral dose of ^{14}C-BTBPE and subsequently estimating the amount of radioactivity remaining in tissues and eliminated in faeces (Hakk et al., 2004). Most of the BTBPE (~94%) was found in faeces within 72 h. Tissue retention was minimal, in the most lipophilic tissues, and the metabolites were excreted in urine, bile and faeces, at very low levels. The most significant TPs were the faecal ones (Table 1.2.2). The predominant compounds were those resulted after hydroxylation, debromination, and cleavage on either side of the ether linkage.

EH-TBB and BEHTBP. EH-TBB and BEHTBP have shown a slightly different metabolism during *in vitro* studies (Table 1.2.2); the first compound was rapidly metabolised in rat microsomes and HLMs to 2,3,4,5-tetrabromobenzoic acid (TBBA) (Roberts et al., 2012). However, for TPBH, no significant loss of the initial amount added at the beginning of the experiment was observed, and no metabolites were detected.

After addition of NADPH, no significant difference in the formation rate of TBBA was observed, so a different metabolic pathway than oxidation was involved. Roberts et al. (2012) hypothesised that the enzymes involved in EH-TBB cleavage were carboxylesterases and tested this hypothesis successfully by incubating EH-TBB with porcine carboxylesterases. The same class of enzymes also cleaved one of the ester bonds in BEHTBP, forming mono(2-ethylhexyl) tetrabromophthalate.

However, when Bearr et al. (2012) exposed fathead minnows to Firemaster BZ-54, ten potential metabolites were observed. Only two of them were identified: 2-ethylhexyl dibromobenzoate and 2,3,4,5-tetrabromo methylbenzoate. No TBBA was detected. The authors hypothesised that TBBA undergoes methylation by methyltransferase enzymes, and the process occurs very rapidly, thus preventing the accumulation of TBBA.

1.2.2. Transformation products of PFRs

Organophosphate triesters (PFRs) are used as additive flame retardants (FRs) and plasticisers in polymers, such as cellulose esters, polyvinylchloride (PVC), polyurethane foams (PUF), and also in paints, lacquers, etc. (WHO 1990; WHO 1991a; WHO 1991b; WHO 1998; WHO 2000).

These additives are high production volume (HPV) chemicals, being produced in amounts larger than 100000 tonnes as early as 1992 (OECD 1995; World Health Organization 1997). In nearly a decade, the production increased by more than 80% (Marklund et al., 2005), reaching 186000 tonnes in 2001. This process accelerated, with an additional production growth of more than 60% in only 3 years, reaching the 300000 tonnes in 2004 (Makinen et al., 2009; Moller et al., 2012). Of this amount, 70% were used as FRs (Wei et al., 2015) and out of the total FR consumption in 2006, 20% were PFRs (European Flame Retardant Association; Reemtsma et al., 2008).

PFRs are employed in items present both in the indoor and outdoor environment. They are added to consumer goods (such as electronics, toys, etc.), to home furnishings and to construction materials. In the outdoor environment, they find uses as antifoaming agents in concrete, in engine oil for a variety of vehicle, in hydraulic fluids for heavy machinery and even in the plastic films used in greenhouses (Wei et al., 2015). And as the PFRs tend to be added in reasonably high amounts (up to 30% in some consumer products and polymeric materials), all of the items containing these additives can act as potential sources of PFR contamination to the environment.

1.2.2.1. Degradation of PFRs in abiotic matrices

Due to the presence of an ester bond in their structure, PFRs can undergo hydrolysis in acidic or alkaline conditions, yielding a hydrogen phosphate and an alcohol. However, pH conditions which cause this process to occur naturally are rare in nature. Most of these chemicals do not absorb light with wavelengths longer than 290 nm (Marklund 2005; Regnery & Püttmann, 2010), but are more prone to photodegradation in an aqueous environment (such as TBOEP, TiBP, and TnBP) than others (TCEP, TCPP) (Regnery & Püttmann, 2010). Furthermore, TCEP and TDCPP have been detected in comparable levels in the influents and effluents of sewage treatment plants and waste water treatment plants, indicating that these chemicals persist in treatment plants and likely accumulate in the environment (Takahashi et al., 2013).

Only a combination of harsher conditions seems to degrade these chemicals, such as oxidising agents such as O_3 and H_2O_2 in combination with UV radiation (Echigo et al., 1996, Watts & Linden, 2008). However, in real-life ecosystems some of these conditions can sometimes occur: the solar radiation can be absorbed by the dissolved organic matter in the water and generate reactive oxygen species (e.g. OH-radicals, H_2O_2) (Scully et al., 1996; Moran and Zepp, 1997). These species can react with other molecules in the water, such as the PFRs, degrading them (Regnery & Püttmann, 2010).

1.2.2.2. Biotransformation pathways for PFRs

In the presence of fungi, bacteria and other microorganisms, the main degradation pathway seems to be stepwise enzymatic hydrolysis of the ester bond, yielding di- and mono-esters and alcohols/phenols (Saeger et al., 1979; Marklund et al., 2005). The chlorinated PFRs seem to be more resistant to microbial degradation than the halogen-free counterparts (Marklund et al., 2005; Regnery & Püttmann, 2010) and the larger the alkyl side-chains are, the lower the biodegradation potential is (Saeger et al., 1979).

But even TCEP and TDCPP can be completely degraded by certain types of bacteria. Takahashi et al. determined that the main microbial degradation product of TCEP is 2-chloroethanol and for TDCPP it is 1,3-dichloro-2-propanol (Takahashi et al., 2008). If specific bacterial strains are present, these metabolites can further be degraded. The mechanisms proposed for this process are listed in figure 1.2.4. Otherwise, they can remain in the environment, and can pose a risk of genotoxicity, carcinogenicity, teratogenicity and cardiotoxicity (NTP & NIEHS, 2005; NTP 1985).

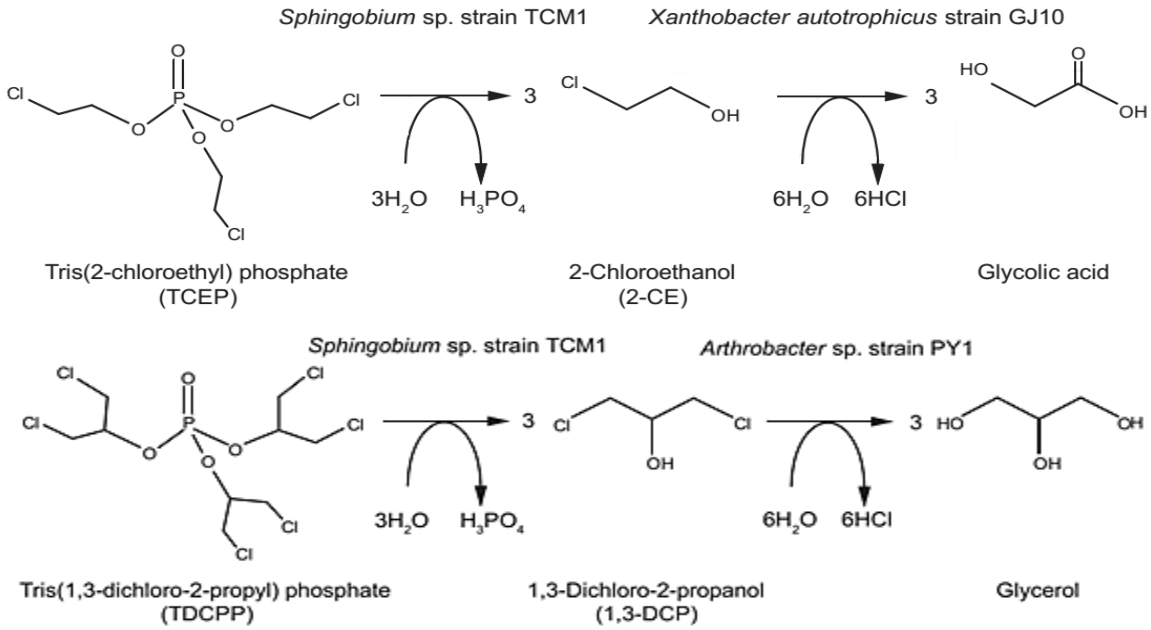
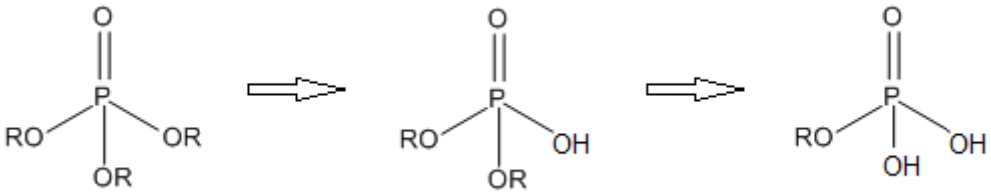


Figure 1.2.4: Two-step biodegradation of TCEP and TDCPP, firstly by Sphingobium sp. strain TCM1 to 2-chloroethanol and 1,3-dichloro-2-propanol, and secondly by Xanthobacter autotrophicus strain GJ10 and Arthrobacter sp. strain PY1, resulting in halogen-free chemicals, naturally present in the environment (Takahashi et al., 2013).

Other bacteria that degrade 2-chlorethanol are Pseudomonas putida strain US2 and P. atutzeri strain JJ and 1,3-dichloro-2-propanol can also be degraded by Arthrobacter sp. Strain AD2, A. erithii H10a, Agrobacterium radiobacter strain AD1, and Coryne-bacterium sp. strain N-1074 (Takahashi et al., 2013).

In animal studies, the main PFR metabolites were identified as dialkyl phosphates (DAPs) and monoalkyl phosphates (MAPs) (Figure 1.2.5). Both the parent compounds and the metabolites are

susceptible to hydrolysis at low pH and to oxidation, which poses challenges for their selective extraction from biological matrices. Care should be taken to perform protein denaturation steps using organic solvents such as methanol or acetonitrile, instead of an acid, since PFRs may be hydrolysed under acidic conditions.



PFRs **DAPs** **MAPs**

Figure 1.2.5: Scheme of metabolisation of organophosphate triesters (PFRs) to dialkyl phosphates (DAPs) and monoalkyl phosphates (MAPs).

Some metabolic pathways are common across different species. This fact allows certain animals, such as mice, rats, zebrafish, etc. to be used as model species and provide information about how a certain chemical would interact with the human organism. But in between different organisms, there are differences in how chemicals are metabolised. A well-known example are "requisite carnivores", like cats, hyenas, the Northern Elephant seal, etc. where evolution led to certain enzymes that were no longer needed (such as varieties of Uridine 5'-diphospho-glucuronosyltransferases or UGTs) to be less produced. Thus, these animals cannot generate glucuronidated metabolites in the same way that humans do.

The major site where exogenous chemicals are metabolised is the liver, through the microsomal enzyme systems found in the hepatocytes. Formulations of human liver microsomes (HLM), cytosol and liver S9 fraction (a mixture of HLM and cytosol) are available for purchase and can be used to predict which metabolites can be expected when a chemical enters the human body. The metabolism of TCEP, TCPP, TDCPP, TBOEP and TPhP is studied using this procedure in Van den Eede et al., (2013).

For TCEP, only bis(2-chloroethyl) phosphate (BCEP), hydroxyethyl 2-chloroethyl hydrogen phosphate and a glutathione conjugate of TCEP with a loss of one Cl atom. But as TCEP is minimally transformed to BCEP and even less in the other metabolites, it can be used in biomonitoring studies (Van den Eede et al., 2015a).

For TCPP, the observed metabolites are depicted in figure 1.2.6. All of them are Phase-I metabolites and no additional Phase-II metabolites were detected.

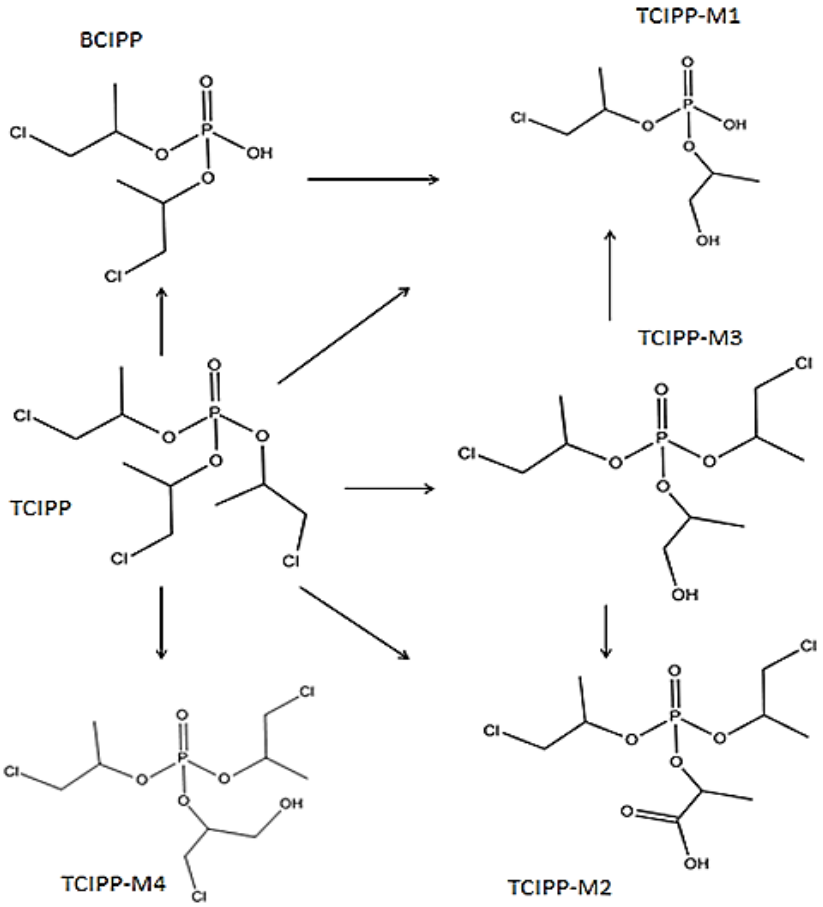


Figure 1.2.6: TCPP metabolites generated through incubation with human liver fractions. Only the isomer with the isopropyl sidechain is considered here as it comprises most of the technical mix. The numbering corresponds to the chromatographic elution order. Only one isomer of all possibly formed isomers is represented for TCIPP-M1 and M4 (Van den Eede et al., 2013).

In biomonitoring studies, the two main metabolites encountered in urine are: 1-Hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (TCIPP-M3) and bis(1-chloropropan-2-yl) hydrogen phosphate (BCIPP) (Dodson et al., 2014; Van den Eede et al., 2015a).

For TDCPP, most of the metabolites are the result of Phase-I metabolism, with the exception of the glutathione conjugate (TDICPP-M3), which was formed by the substitution of Cl with a glutathione moiety (Figure 1.2.7). BDCIPP was the major metabolite observed in rodents, followed by the TDICPP-M3. In biomonitoring studies, BDCIPP is usually enough to reveal the magnitude of TDCPP exposure in a population (Meeker et al., 2013; Butt et al., 2014; Hoffman et al., 2014; Van den Eede et al., 2015a).

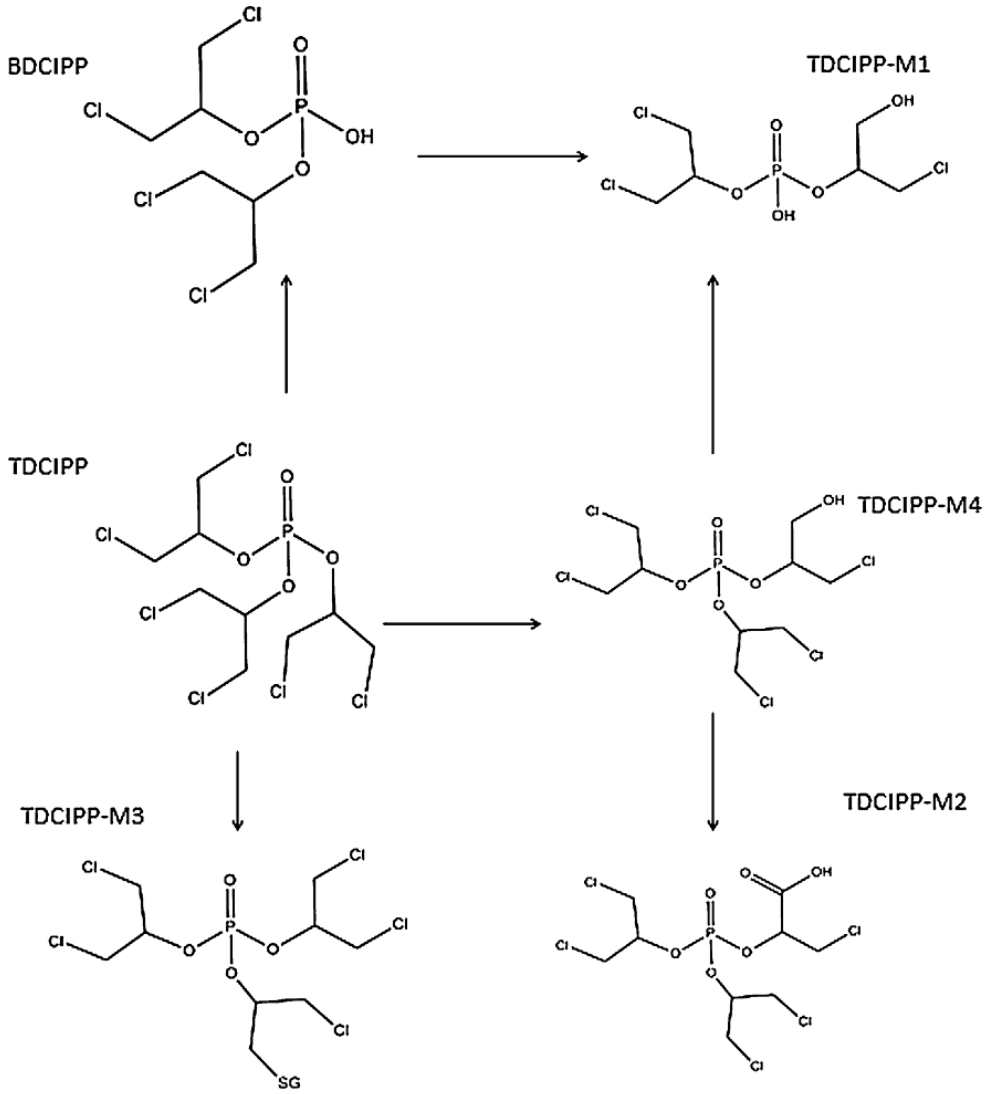


Figure 1.2.7: TDCPP metabolites generated through incubation with human liver fractions. Only the isomer with the isopropyl sidechain is considered here as it comprises most of the technical mix. The numbering corresponds to the chromatographic elution order. The structures for TDICPP-M1 (2 isomers) and TDCIPP-M4 (one isomer) are only tentative, as the precise position of the hydroxyl cannot be determined through mass spectrometry alone (Van den Eede et al., 2013).

For TBOEP, due to the presence of three ether bonds, a multitude of metabolites are generated in the human body. BBOEP, TBOEP-M6, TBOEP-M8, TBOEP-M9, TBOEP-M10 and TBOEP-M12 are the result of Phase-I metabolism. TBOEP-M1, TBOEP-M2, TBOEP-M3, TBOEP-M4, TBOEP-M7 and TBOEP-M11 are the result of secondary Phase-I metabolism and only TBOEP-M5 is the result of Phase-II metabolism (likely a glucuronide conjugate of M5).

The most abundant metabolites, which can be used in biomonitoring studies are BBOEP, TBOEP-M9 and TBOEP-M10 (Van den Eede et al., 2015b).

For TPhP, DPhP, TPhP-M1, TPhP-M6 and TPhP-M7 were Phase-I metabolites, while TPhP-M2 and 3 (glucuronide conjugates), TPhP-M4 and 5 (sulphate conjugates) and TPhP-M7 were Phase-II metabolites (Figure 1.2.8).

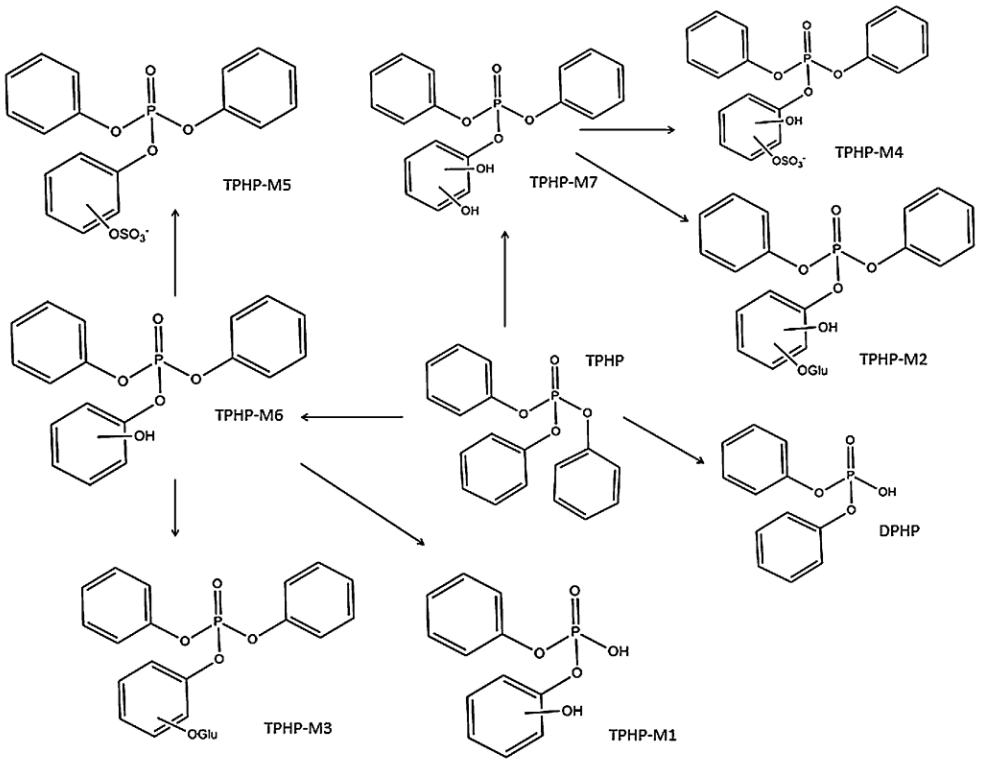


Figure 1.2.8: TPhP metabolites generated through incubation with human liver fractions. The numbering corresponds to the chromatographic elution order (Van den Eede et al., 2013).

DPhP was the main metabolite observed in earlier studies. Yet, it is not the most suited metabolite to be used in biomonitoring studies, as DPhP is also used industrially, with much of the same uses as the parent compound. Furthermore, DPhP can also be generated as a breakdown compound or byproduct of the manufacturing process of a number of other PFRs, such as EHDPhP, IDPhP or RDP and BPA-BDP. TPhP-M5 and TPhP-M6 are the most abundant of all the metabolites in Figure 1.2.8 (Van den Eede et al., 2013) and being more specific than DPhP, are more suited for biomonitoring studies (Su et al., 2015).

1.3. Objectives and aims

Most injuries from fires occur in the indoor environment, where people typically spend most of their time nowadays. Current fire regulations are generally developed to strongly diminish the risks of severe injury from fires. However, they do carry the delayed risk of a myriad of possible health issues associated with the chemicals used to impart resistance to fire.

The general aim of this work was to further understand which FRs are used in every-day consumer goods and in what quantities. More specifically, the objectives were to observe whether the FRs migrate to the indoor environment and to investigate certain thus-far-unexplored exposure pathways, in order to have the necessary tools for assessing the risks associated with such exposure.

To accomplish this, a number of alternative techniques had to be developed and tested for easy and rapid screening. Analytical methods also needed to be developed, to assist in the qualitative and quantitative investigation of classical and emerging FRs in commonly-used typically flame retarded consumer goods, such as electronics, furniture, carpets, curtains, plastic toys, etc.

In order to discover whether these chemicals leach out into the indoor environment, indoor air and dust had to be analysed as well.

When chemicals are discovered in such matrices in the indoor environment, it is a good indicator that they will also enter the human body. Therefore, it was necessary to estimate the magnitude of the exposure to such FRs, in order to get an idea whether adverse health effects can be expected at the current exposure levels.

This research will increase the understanding of FR migration to the indoor environments and consequential exposure, will inform on the assessment of risk associated with the current-use FRs and ultimately will lead to more sustainable approaches to meeting fire safety regulations.

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Chapter 2

Dust as indicator of indoor contamination with flame retardants from consumer products

2.1. Simplifying multi-residue analysis of FRs in indoor dust

Based on the following publication:

Ionas, A.C., Covaci, A., 2013. Simplifying multi-residue analysis of flame retardants in indoor dust. Int. J. Environ. Anal. Chem. 93, 1074–1083.

2.1.1. Introduction

Dust is a suitable indicator of the indoor contamination for a large range of organic compounds, including also FRs, but at the same time, it is also a rather complex matrix. Since these organic contaminants have a wide range of polarity, it is advisable to use a combination of polar and non-polar organic solvents for extraction. However, due to matrix complexity, direct analysis of "raw" extracts is not possible, unless a very powerful separation and detection technique is used. Hilton et al. (2010) have extracted indoor dust with *n*-hexane and, without any clean-up, analysed the extract by multidimensional gas chromatography coupled to high resolution MS (GCxGC–TOF-MS). Yet, when simpler separation (monodimensional GC) or detection (quadrupole MS) techniques are used, a sample preparation method to reduce sample complexity is required (Ali et al., 2011; Van den Eede et al., 2011). For environmental monitoring, it is important to conduct comprehensive analyses which include a large number of target contaminants from one sample aliquot. There are several efficient and robust methods for the determination of major FRs in dust, but most of these methods usually investigate one or two FR groups simultaneously. Running a separate extraction, separation and analysis for each class of FRs would be an inefficient use of consumables, samples and time.

In order to address these issues, it is important to 1) reduce matrix complexity and 2) employ sensitive and selective analytical methods (e.g. GC-MS or LC-MS) which allow the simultaneous determination of various classes of FRs, such as BFRs, PFRs and chlorinated FRs (CFRs). Nevertheless, several target compounds may coelute (e.g. TBBPA with BDE-153 or the degradation products of HBCDD with BDE-49 and BDE-99) (Korytár et al., 2005), and therefore special care should be taken when choosing the chromatographic and mass spectrometric detection conditions.

The aim of this work was to develop a fractionation procedure that would reduce the sample complexity, prevent coelutions and thus facilitate FR analysis. This was done by dividing the target FRs in several fractions according to their polarity. A combination of ultrasonic-assisted extraction (UAE) (Van den Eede et al., 2011; Covaci et al., 2011) and solid phase extraction (SPE) was applied for household dust. FRs eluted from the SPE cartridge by using different solvents of increasing polarity. In this way, several key separations could be achieved, such as PBDEs from HBCDD or BFRs from PFRs, thus allowing the simultaneous determination of these FR classes.

Recent studies have been carried out including the study of (Ali et al., 2011), in which silica SPE was used for NBFRs, but the elution was done with *n*-hexane and dichloromethane (DCM). Sahlström et al. (2012) also opted for the use of silica and for *n*-hexane as elution solvent for a non-polar fraction. However, the used solvent volumes were higher and PFRs were not analysed. Van den Eede et al. (2012) also developed a method where the SPE sorbent was Florisil, but in order to quantify HBCDDs, the fractions were recombined and resolubilised in methanol. Recently, Cristale et al. (2012) have investigated BFRs and PFRs in water samples, but the method focussed mostly on the GC-MS analysis part rather than on the sample preparation.

The procedure described in this paper was developed to be as comprehensive as possible, while at the same time maintaining simplicity, low consumable use, and thus achieving increased throughput.

2.1.2. Materials and methods

2.1.2.1 Reagents, standards and other consumables

All solvents used during analysis were of pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, *n*-butyl chloride, ethyl acetate, methanol and *iso*-octane were purchased from Merck (Darmstadt, Germany). Supelclean™ ENVI™-Florisil® SPE cartridges (500 mg/3 mL) were purchased from Supelco (Bellefonte, PA, USA), Bond Elut-Silica from Agilent (500 mg/3 mL, Santa Clara, CA, USA) and Oasis HLB (500 mg/6 mL) from Waters (Milford, MA, USA). Empty polypropylene filtration tubes (3 mL) SPE cartridges were purchased from Supelco and Alumina 60 (active basic, activity stage I, particle size 0.063-0.2 mm) from Merck.

Standards of BDE congeners 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209, α-HBCDD, β-HBCDD, γ-HBCDD, 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), decabromodiphenyl ethane (DBDPE), 5,6-dibromo-1,10,11,12,13,13-hexachloro-11-tricyclo[8.2.1.02,9] tridecene (HCDBCO), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EH-TBB), di(2-ethylhexyl) tetrabromophthalate (BEHTBP), 1,2,3,4,5,6-hexabromobenzene (HBB), 2,2-bis[4-(2,3-dibromopropoxy)-3,5-dibromophenyl]propane (TBBPA-BDBPE), 2,2',6,6'-tetrabromobisphenol A (TBBPA), 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH) isomers, 2,4,6-tribromophenyl allyl ether (ATE), 2-bromoallyl-2,4,6-tribromophenyl ether (BATE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), 1,2,5,6-tetrabromocyclooctane (TBCO) isomers, octabromotrimethylphenyl indane (OBIND) and dechlorane plus (DP) isomers were purchased from Wellington Laboratories (Guelph, ON, Canada). Standards of PBB congeners (80, 103, 153, 155, 180 and 209) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). BDE 77 was obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of tri-isobutyl phosphate (TiBP), tri-*n*-butyl phosphate (TnBP), triphenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), ethyl-hexyl-diphenyl phosphate (EHDPhP), triscresyl phosphate (TCP, mixture of 4 isomers) and tris(1,3-dichloropropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Tris(2-butoxyethyl) phosphate (TBOEP) and

tris(2,3-dibromopropyl) phosphate (TDBPP) were purchased from Sigma Aldrich. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 2 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBOEP (>94%). SRM 2585 was purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA).

2.1.2.2 Instrumentation

The GC systems used are both Agilent 6890 GC coupled to an Agilent 5973 MS. The first one was operated in the electron capture negative ionization (ECNI) mode. It was equipped with a programmable-temperature vaporizer inlet (PTV) which was run in the pulsed cold splitless mode. The initial inlet temperature was 92 °C and pressure 2.69 psi. The inlet temperature program started at 92 °C , hold 0.04 min, ramp 700 °C/min to 295 °C, hold 25 min, ramp 700 °C/min to 310 °C , hold 5 min. One µL of extract was injected on a DB-5 column (15 m × 0.25 mm × 0.10 µm). The GC temperature program was 90 °C, hold 1.5 min, ramp 10 °C/min to 300 °C, hold 4 min, ramp 40 °C/min to 310 °C, hold 15 min. Helium was used as a carrier gas with a ramped flow. The initial flow was 1 mL/min (for 19 min), and then ramped with 10 mL/min² to a final flow of 2 mL/min, which was then held constant until the end of the run. Methane was used as moderating gas. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

The second GC-MS system was operated in the electron ionisation (EI) mode. The PTV was run in the pulsed cold splitless mode. The initial inlet temperature was 80 °C and pressure 13.69 psi. The inlet temperature program started at 80 °C, hold 0.03 min, ramp 700 °C/min to 300 °C, hold 40 min. One µL of extract was injected on a SGE-HT8 column (25 m × 0.22 mm × 0.25 µm). The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 310 °C, hold 20 min. Helium was used as a carrier gas with a constant flow (1 mL/min). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

The LC systems employed were: for quantification purposes, a dual pump Agilent 1100 Series liquid chromatograph coupled to an Agilent 6410 triple quadrupole MS. Electrospray ionisation (ESI) was employed in the negative mode for compounds such as HBCDDs and TBBPA. The column was a Luna C18 reversed phase (RP) column (150 mm × 2 mm i.d., 3 µm particle size, Phenomenex). The mobile phase was: (A) ammonium acetate 2mM in water/methanol (1:1 *v/v*) and (B) methanol at a flow rate of 0.250 mL/min. The gradient is starting at 75% (B) hold for 2 min, then increased linearly to 100% (B) until 9 min; hold until 12 min followed by a linear decrease to 70% (B) over 0.5 min and held for 7.5 min. N_2 was used as drying gas at a flow of 10 L/min and heated to 300 °C. Nebulizer pressure was 35 psi and capillary voltage 4000 V. HBCDD isomers were quantified by

isotope dilution. MS/MS detection operated in the MRM (Multiple Reaction Monitoring) mode was used for quantitative determination of the HBCDD isomers based on m/z 640.6 to 81 and m/z 652.6 to 81 for the native and ^{13}C-labeled diastereomers, respectively. Fragmentor voltage and collision energy were set as 80 and 15 V, respectively.

For exploratory purposes, an Agilent 1290 Infinity LC coupled to an Agilent 6530 Q-TOF MS analyser, with an electrospray ionisation (ESI) source was employed. The gas temperature for the source was 350 °C, gas flow 8 L/min, nebuliser pressure 48 psig, sheath gas temperature was 325 °C and the sheath gas flow 12 L/min. A volume of 2 µL of extract was injected and separation was achieved using a Zorbax C18 column (50 mm x 2.1 mm i.d, 3.5 µm particle size) using a flow rate of 0.4 mL/min and a linear gradient from 85% methanol/water to 100% methanol in 6.5 min, followed by a 6 min hold before returning to the original conditions for 6 min. Each fraction was injected in both negative and positive polarity ESI.

2.1.2.3 Fractionation procedure

An amount between 30 and 100 mg of sieved dust is sufficient for the analysis. The extraction procedure is based on the method described by Ali et al. (2011). The solvent mixture employed in the extraction is *n*-hexane/acetone (3:1, *v/v*). The process consists of consecutive steps of vortexing (1 min) and ultrasonication (5 min, 40 kHz, using a Branson 5510 ultrasound bath) with 2 mL of the aforementioned solvent mixture. This cycle is repeated 3 times and after each cycle, the supernatant is transferred to a clean tube. All tubes are rinsed beforehand with *n*-hexane and acetone. The extracts are then evaporated to near dryness using a gentle nitrogen stream and the solvent is exchanged to *n*-hexane (1 mL). The obtained extracts are fractionated on silica, with a particle size of 40 µm and an average pore diameter of 60 Å (Agilent Bond Elut-SI, 500 mg/3 mL cartridges), using a non-destructive clean-up (e.g. without sulphuric acid) to ensure that target FRs were not degraded. All cartridges were pre-cleaned and conditioned with 6 mL ethyl acetate followed by 6 mL *n*-hexane.

Organic solvents of increasing polarity were employed to elute various groups of FRs. As such, PBDEs and non-polar NBFRs are eluted with 8.5 mL of *n*-hexane, HBCDDs, TBBPA and BEHTBP with 8 mL of *n*-butyl chloride, the PFRs with 8 mL of ethyl acetate and compounds more polar than PFRs with 8 mL of methanol (Table 2.1.2). This last fraction was collected mainly for exploratory purposes. The whole analysis workflow is given in Figure 2.1.1. The obtained fractions were concentrated to dryness and then resolubilised in 100 µL *iso*-octane, or 100 µL methanol for injection in GC or LC, respectively.

If the method is used for screening/exploratory purposes, most fractions are injected in GC and LC systems (except for the most polar one in GC and the most non-polar one in LC, respectively). For quantitative analyses, the first fraction is injected in a GC-ECNI/MS for analysis of PBDEs and

some NBFRs, the second one in a LC-MS/MS system for HBCDDs and TBBPA and in the GC-ECNI/MS system for EH-TBB and BEHTBP.

Table 2.1.1: Compounds per fraction using silica as SPE sorbent as identified by fractionating the employed mixtures of standards

Fraction	Elution solvent	Compounds
1	*n*-Hexane	PBDEs, PBBs, α,β-TBCO, HBB, BTBPE, syn-DP, anti-DP, OBIND, DBDPE, TBA, BATE, ATE, DPTE, EH-TBB (also in fraction 2), HCDBCO, TBECH (also in fraction 2)
2	*n*-Butyl Chloride	HBCDDs and breakdown compounds, TBBPA, MeTBBPA, TBBPA-BDBPE, BEHTBP, TBECH (also in fraction 1), EH-TBB (also in fraction 1)
3	Ethyl Acetate	PFRs, including TDBPP
4	Methanol	None of the compounds in the FR standard mixtures; these compounds should be more polar than the PFRs

The third fraction was injected on a GC-EI/MS for PFR analysis, except for TDBPP which requested an injection in the GC-ECNI/MS. If any analyte is to be identified in the last fraction, the LC-MS/MS system can also be employed.

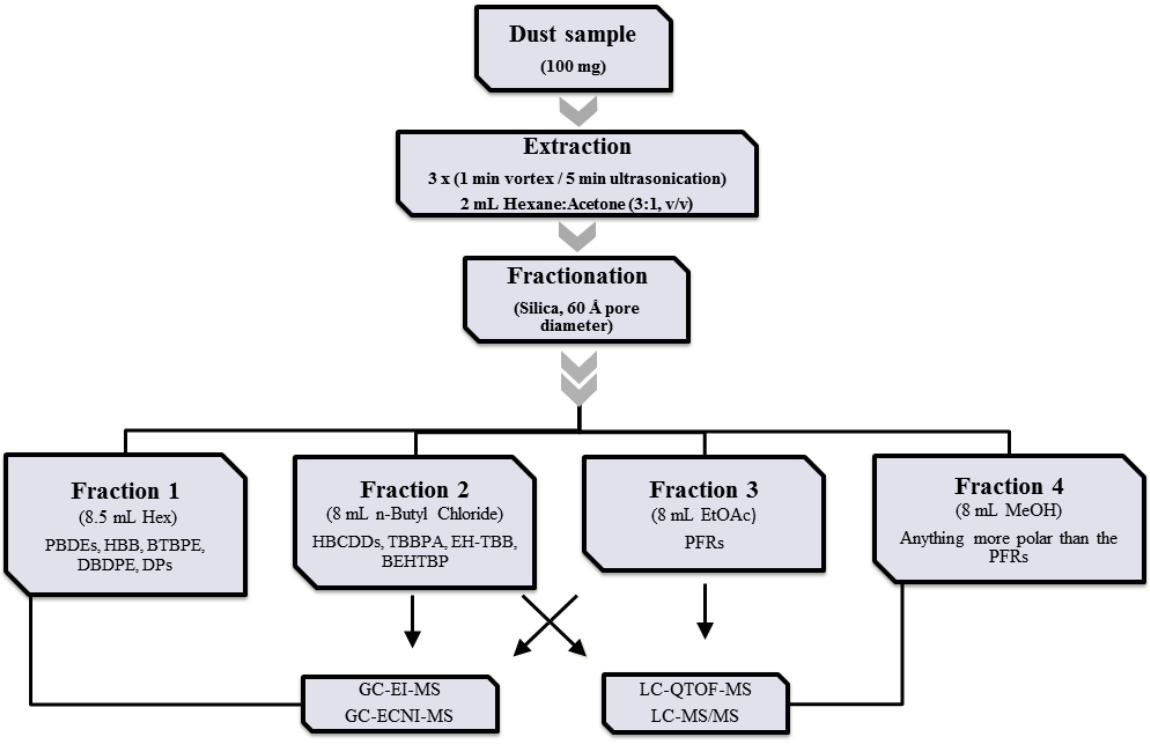

Figure 2.1.1: Schematic representation of the analysis workflow

2.1.3. Results and discussion

2.1.3.1. Method optimisation

For the initial development stages, a sieved dust sample from a carpentry workshop containing components from all major classes of FRs was used. For each experiment, an amount of 100 mg of dust was extracted and the obtained extract underwent fractionation using the different sorbent types, and consequently injection in the above mentioned GC instruments.

To investigate which compounds are to be expected in the various fractions, two mixtures of standards were also prepared. The first mixture contained lower PBDEs (tri-hepta), α-, β- and γ-HBCDD, TBBPA, MeTBBPA, α- and β-TBCO, OBIND, α- and β-TBECH, syn-DP, anti-DP, HBB, BTBPE, DBDPE and BDE-209. The second mixture contained PBBs (80, 103, 153, 155, 180, and 209), ATE, BATE, DPTE, TBA, γ- and δ-TBECH, PFRs, BTBPE, HCDBCO, EH-TBB, BEHTBP and TBBPA-BDPE. The chromatograms of the individual fractions were analysed, the FR compounds were identified and their characteristics (e.g. retention time, characteristic ions) were logged.

Selection of SPE sorbent. Recently, we have shown that Florisil can separate a BFRs from PFRs in clear-cut fractions, except for HBCDD which elutes in both fractions (n-hexane and ethyl acetate) (Van den Eede et al., 2012). Using the same rationale, we have investigated 4 sorbents (Florisil, silica, alumina and Oasis HLB) for their capacity to provide clear-cut fractions for the major classes of FRs from dust extracts. The important requirements were to avoid the elution of one compound in two fractions and to reduce the complexity of the obtained chromatograms. Any type of acid or base clean-up was avoided as it would damage some of the analytes.

Table 2.1.2: Fitness for purpose of different sorbent types tested in this study.

Sorbent	Signal/noise in chromatograms	Clear-cut fractions	Unwanted coelutions	Other issues	Fitness for purpose
Florisil	+++	No	Yes	HBCDDs in two fractions	+
Silica	++	Yes	No	-	+++
Alumina	++	No	No	TPhP present in *n*-BC fraction	++
Oasis HLB	++	No	Yes	HBCDD degradation	-

"+++": most fit for purpose; "-": not fit for purpose

The Florisil sorbent is a highly polar magnesium silicate-based material which offers strong retention for polar compounds and a low background in the chromatograms. Unfortunately, the less polar compounds were not strongly adsorbed and did not elute in clear-cut fractions. The same was observed also for the Alumina sorbent (500 mg). For the latter, a further disadvantage became apparent: the retention on the cartridge varied considerably for compounds in the same class (e.g

trialkyl phosphate esters had higher retention than the triaryl phosphate esters and would elute in different fractions). The only polymeric sorbent tested was the Oasis HLB, which is produced using a hydrophilic (N-vinyl pyrrolidone) and a lipophilic (divinyl benzene) monomer. This sorbent is most efficient for aqueous matrices; in our case (extract in *n*-hexane) it offered a strong retention for non-polar or weakly polar compounds and weak retention for polar compounds, which did not allow the separation of FR classes in clear-cut fractions.

The most adequate sorbent was determined to be underivatised silica (Table 2.1.2), which provided an adequate amount of retention for both non-polar and polar compounds, so that the compounds of interest could be separated in clear-cut fractions and the obtained fractions had no unwanted coelutions. TBECH did not entirely elute in a single fraction and eluted between the *n*-hexane fraction and the *n*-butyl chloride fraction; TBECH was thus excluded from the target analytes of this study.

Selection of elution solvents. Organic solvents of increasing polarity were employed to elute various groups of FRs. The tested solvents were *n*-hexane, *n*-butyl chloride, *n*-hexane/DCM (3/1, *v/v*), DCM, ethyl acetate and methanol. *n*-Hexane, a non-polar solvent, is ideal for the elution of non-polar analytes. For the slightly polar compounds, such as the HBCDDs, the solvent employed needed to be more polar than *n*-hexane, but not that polar as to elute the PFRs. This latter criterion excluded DCM. We tested a mixture of *n*-hexane/DCM (3/1, *v/v*), but it was still too polar, as it also eluted part of the PFRs. Lastly, *n*-butyl chloride was evaluated and it proved to meet all our requirements. For the next fraction, ethyl acetate was employed, based on previous experiments by our group (Van den Eede et al., 2012). Methanol was used to elute very polar compounds still remaining on the employed sorbent after the first three elution steps. All obtained extracts were injected only in GC systems during the development stages.

2.1.3.2. Identification methodology

The target compounds were identified by applying a comprehensive approach: the first step was to compare the spectra of the peaks from the chromatograms obtained in the EI mode to the "Wiley Registry of Mass Spectral Data, with NIST 2008, 9th Edition" mass library. For the identified compounds, the two most abundant specific ions (if present) were logged for future reference. In EI, and also in the ECNI mode, halogen containing compounds are easily distinguished from other compounds by the characteristic ion clusters formed. This gives an indication about the type and number of halogen atoms contained in a molecule.

The standard mixtures of the target FRs were also injected and their retention times and specific ions were logged. In the ECNI mode, the analyte molecules are less fragmented and there are no available spectral libraries. Therefore, a new library was created using the MSD Chemstation

software (Agilent Technologies, Santa Clara, CA, USA). The ECNI spectra for the above mentioned compounds were added to this new library in order to facilitate the identification of unknowns from other extracts. In a monodimensional chromatogram, many can coelute, making identification an even more challenging task. Generally, the use of GC x GC may partly solve this problem (Hilton et al., 2010), but it also generates up to 10,000 different chromatographically-resolved peaks to be identified. To evaluate all generated data in a time- and resource-efficient manner, in twodimensional, but also monodimensional GC-MS analyses, automation is required. One way of implementing automation is through the use of AMDIS (Automated Mass Spectral Deconvolution and Identification System) software provided by NIST to reduce the time used for data analysis and to streamline the process as a whole (Wang et al., 2012). Such identification methodology is especially important in exploratory analyses / screening for "unknowns".

2.1.3.3. Evaluation of the method

To further confirm the distribution of FRs in each fraction, the NIST reference material (SRM 2585 - Organic contaminants in house dust) was analysed using the developed procedure. There are two important advantages in using this reference material: 1) matrix effects are the closest to those in real dust samples and 2) it is certified for several of our target FRs (PBDEs) (NIST 2005). The dust used to prepare this SRM was collected in 1993-1994 from US homes, cleaning services, motels and hotels, and, as such, it provides a good indication of the PBDE contamination from that period. However, the current levels of PBDEs are considerably lower in European dust than in US dust (Harrad et al., 2010), which makes SRM 2585 a less suitable material for European investigations. Interestingly, we have identified TDBPP in SRM 2585 at mean concentrations of 150 ng/g dust. This FR compound was added to clothes (children's garments in particular) and was banned in the US in 1977 by the Consumer Product Safety Commission (CPSC) (CPSC 1977). The fact that TDBPP was found in this reference material for household dust indicates a continued exposure to this harmful compound for the inhabitants of some of the residences from where the dust used to develop the reference material was collected. SRM-2585 is not certified for NBFRs or PFRs, but some of these FRs have been quantified and reported in recent publications (Ali et al., 2011; Bergh et al., 2011; Sahlström et al., 2012; Van den Eede et al., 2012, 2011). The methods employed in the aforementioned studies are similar to the one described in this paper, so results are directly comparable.

Upon analysis of the obtained chromatograms, it was found that the distribution of the analytes in the fractions (Figure 2.1.2) was consistent with our previous observations from the method development stages.

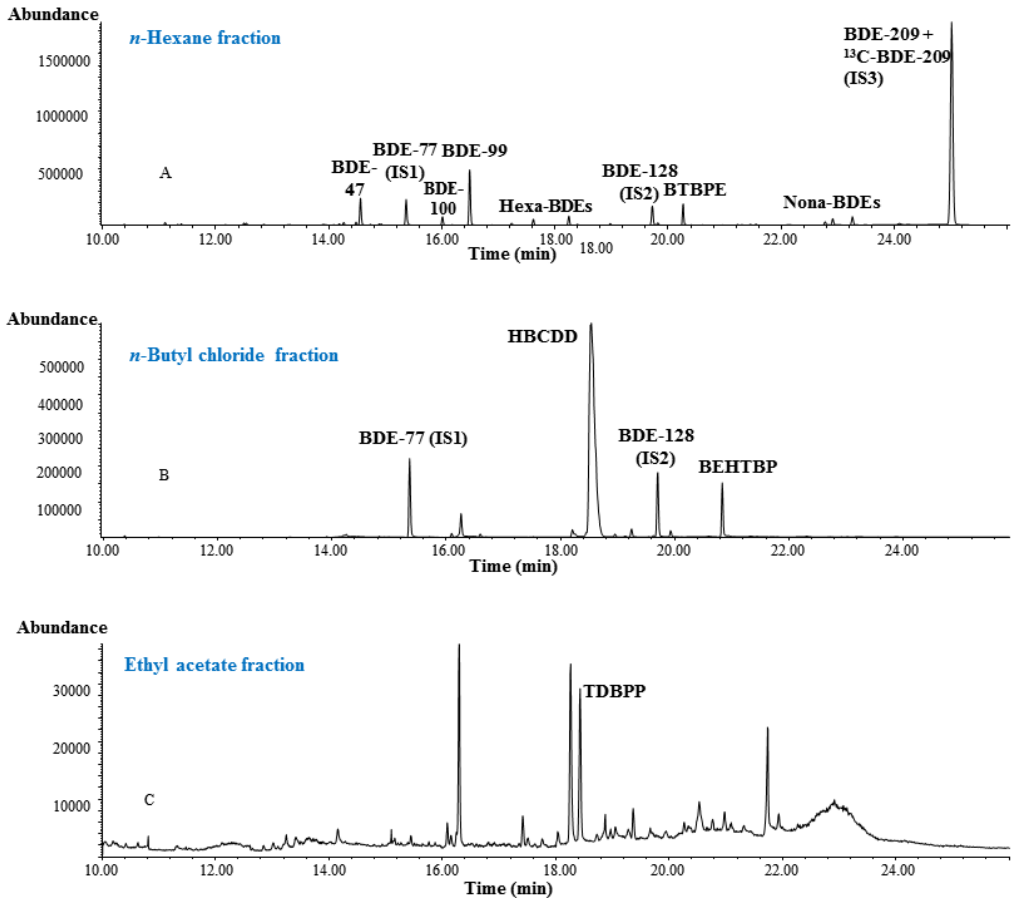

Figure 2.1.2: GC-ECNI/MS total ion chromatograms for the individual fractions (*n*-hexane (A), *n*-butyl chloride (B) and ethyl acetate(C)) of the reference material SRM-2585

However, most likely due to matrix effects, EH-TBB eluted in the *n*-butyl chloride fraction, differently than compared to the mixture of standards injections. The measured concentrations of FRs obtained using this procedure are in agreement with the certified values for the PBDEs and with the values determined in studies for NBFRs and PFRs (Table 2.1.3).

2.1.3.4 Limitations

During a typical analysis, the more polar fractions (n-butyl chloride, ethyl acetate and methanol) are normally also injected on a LC-QTOF-MS with the aim to screen for and to identify unknown compounds or are injected on a LC-MS/MS for the quantification of HBCDDs and TBBPA. However, with the increased sensitivity provided by the QTOF-MS system, extra precautions must be taken. An accidental sample contamination will result in a number of additional peaks in the chromatograms, which can easily mask important target analytes. This has been observed in the present study where polyethoxylates (present in soaps) with different polymerisation degrees were seen in all LC-MS chromatograms. This hampered the investigation of these fractions by LC-MS.

Table 2.1.3: Concentrations of FRs in the SRM-2585 reference material for organic contaminants in house dust (n=3 replicates). All concentrations were blank corrected.

Compound	Mean concentration (ng/g)	SD (ng/g)	Certified values (ng/g)	Uncertainty (ng/g)	Ref.
BDE 28	35	2	47[a]	4	*SRM-2585 certificate*
BDE 47	390	36	497[a]	46	*SRM-2585 certificate*
BDE 66	25	4	30[a]	6	*SRM-2585 certificate*
BDE 100	110	14	145[a]	11	*SRM-2585 certificate*
BDE 99	680	86	892[a]	53	*SRM-2585 certificate*
BDE 85	30	6	44[a]	2	*SRM-2585 certificate*
BDE 154	70	6	84[a]	2	*SRM-2585 certificate*
BDE 153	90	12	119[a]	1	*SRM-2585 certificate*
BDE 183	25	2	43[a]	4	*SRM-2585 certificate*
BDE 197	10	2	n.a.	-	*SRM-2585 certificate*
BDE 203	10	0	37[a]	6	*SRM-2585 certificate*
BDE 196	15	4	n.a.	-	*SRM-2585 certificate*
BDE 209	2480	1000	2510[a]	190	*SRM-2585 certificate*
Compound	**Mean concentration (ng/g)**	**SD (ng/g)**	**Literature values (ng/g)**	**SD (ng/g)**	**Ref.**
HBCDDs	136	5	139 [b]	26	Lankova et al. (2015)
TBBPA	105	10	215 [b]	34	Lankova et al. (2015)
BTBPE	35	4	32[b]	-	Ali et al. (2011)
EH-TBB	40	2	40[b]	-	Ali et al. (2011)
BEHTBP	750	62	652[b]	-	Ali et al. (2011)
HBB	2	0.8	n.a.	-	
TnBP	175	15	180; 190[b]	20	Van den Eede et al. (2011); Bergh et al. (2011)
TCEP	820	30	840[b]	60	Bergh et al. (2011)
ΣTCPP	1190	80	820; 880[b]	140	Van den Eede et al. (2011); Bergh et al. (2011)
TBOEP	59100	500	63000[b]	2000	Van den Eede et al. (2012)
TEHP	370	190	370[b]	40	Bergh et al. (2011)
TPhP	915	35	990[b]	70	Van den Eede et al. (2011)
EHDPhP	915	14	1300[b]	120	Bergh et al. (2011)
ΣTCP	1160	398	1070; 1140[b]	110	Van den Eede et al. (2011; 2012);
ΣTDCPP	1835	84	2020[b]	260	Van den Eede et al. (2011);
TDBPP	150	70	n.a.	-	

a: Certified value (NIST SRM-2585 analysis certificate); b: Indicative values; n.a. – not available

In conclusion, the described method is a simple and time-efficient method to considerably decrease the complexity of the extracts injected and corresponding chromatograms, thus allowing the simultaneous analysis of a wide array of flame retardant compounds. The amount of consumables employed is low and it can also be used as a fast screening method, facilitating the identification of unknown compounds by revealing the polarity intervals in which they fall.

2.2. Analysis of a broad suite of FRs in repeat house dust samples from California

Based on the following publication:

Dodson, R.E., Perovich, L.J., Covaci, A., Van den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R. a, 2012. After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056–66.

2.2.1. Introduction

California house dust contains some of the highest concentrations of PBDEs in the world due to a state-wide furniture flammability standard - Technical Bulletin 117 (Zota et al., 2008). This standard requires the filling (usually PUF) inside products to withstand a 12-second exposure to an open flame. It was applied to upholstered furniture, including juvenile furniture and some items considered to be furniture. As a result, FRs were used even in items not required to meet the standard, such as nap mats and mattress pads, and not just in California, but in the whole of US and even Canada.

PBDEs have been associated with thyroid and other endocrine system disruption and adverse neurological development (Lyche et al., 2015). PBDEs in California homes and residents (Eskenazi et al., 2011; Petreas et al., 2011; Quiros-Alcala et al., 2011; Whitehead et al., 2011; Windham et al., 2010) often exceed risk-based levels for children (Quiros-Alcala et al., 2011; Whitehead et al., 2011; Windham et al., 2010; Zota et al., 2009), raising concerns about exposures to the many other FRs that have not yet been well-characterised. For example, Great Lakes Chemical Corporation, the sole US PBDE manufacturer, introduced Firemaster® 550 to replace the PentaBDE commercial mixture in response to prospective bans in Europe and several US states (Great Lakes 2005). Little is known about the chemical composition, uses, exposure levels and health effects of this mixture or of other brominated, chlorinated and organophosphate chemicals used as FRs. Because additive FRs shed from consumer products, they are found in house dust. Measuring dust concentrations over time can identify exposure trends that result from changes in product formulations.

House dust is the primary route of exposure for PBDEs (Wilford et al., 2005; Lorber et al., 2008) contributing 82%, on average, of a US adult resident's exposure (Lorber et al., 2008). Dust concentrations of PentaBDE were correlated with breast milk levels in 11 women (Wu et al., 2007). Although diet may also contribute (Wu et al., 2007), dust appears to be particularly important in areas, like California, with high concentrations in dust (Whitehead et al., 2011). Dust is a direct exposure pathway through incidental ingestion, inhalation of re-suspended particles, and dermal absorption, and it is a proxy for exposure from product use.

Several PFRs are used as PBDE replacements. In the late 1970s, tris (2,3-dibromopropyl) phosphate (TDBPP or brominated "Tris") was banned from children's pyjamas because of its mutagenic and carcinogenic properties (Blum et al., 1978; US CPSC 1977). Exposure data are limited, although the toxic breakdown product, 2,3-dibromo-1-propanol, was detected in U.S. homes (Rudel et al., 2003). The chlorinated analogue, tris(1,3-dichloro-2-propyl) phosphate (TDCPP), also a carcinogen (US EPA 2005; US CPSC 2005), has been found in U.S. house dust and baby products (Stapleton et al., 2009; Stapleton et al., 2011). TDCPP concentrations in U.S. house dust were recently associated with altered thyroid (free T4) and prolactin hormone levels in men (Meeker et al., 2010). Little information exists on exposure (Stapleton et al., 2009; Van den Eede et al., 2011).

Elevated PentaBDE concentrations in California relative to other parts of the U.S. and world have been well established; however, little is known about levels of other FRs. We expect that FRs used in polyurethane foam, including PentaBDE replacements, may be elevated due to the furniture flammability standard. Exposure patterns for FRs in other applications, such as electronics, are not known because of limited data, including for BDE 209 (Whitehead et al., 2011).

To provide data on a wider range of FRs and on changing exposure patterns, this study measured a broad array of FR chemicals in repeat dust samples collected from 16 California homes. Dust collected in California homes in 2006 and in the same homes in 2011 was analysed for a broad suite of BFRs and PFRs (n= 49). We also measured 13 "legacy" chemicals – persistent organochlorines (OCs) banned long ago (e.g. DDT). Correlation and cluster analysis of simultaneous FR measurements were used to shed light on mixtures and potential sources. Measurement at two time periods allows for the investigation of changes in residential levels, which likely reflect patterns of use. This work contributes to the ongoing characterization of evolving exposures to FR chemicals in homes.

2.2.2. Materials and methods

2.2.2.1. Materials and reagents

Solvents used during analysis were all of pesticide grade. n-hexane (Hex) was purchased from Acros Organics (Geel, Belgium). Acetone (Ac), dichloromethane (DCM), ethyl acetate (EA), iso-octane and methanol (MeOH) were purchased from Merck (Darmstadt, Germany).

Standards of BDE 28, 47, 66, 85, 99, 100, 153, 154, 183, 196, 197, 203 and 209, α-HBCDD, β-HBCDD, γ-HBCDD, BTBPE, DBDPE, HCDBCO, EH-TBB, BEHTBP, HBB, TBBPA-BDBPE, TBBPA, TBECH isomers, ATE, BATE, DPTE, TBCO isomers, OBIND, dechlorane plus (DP) isomers, and labeled internal standards (IS) ^{13}C-BDE 209, ^{13}C-α-HBCDD, ^{13}C-β-HBCDD, ^{13}C- γ -HBCDD, and ^{13}C-TBBPA were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 and 128 (IS) were obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of TEP, tri-isobutyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), triphenyl phosphate (TPhP), tris(2-

chloroethyl) phosphate (TCEP), tri-2-ethyl-hexyl phosphate (TEHP), ethyl-hexyl-diphenyl phosphate (EHDPhP), tricresyl phosphate (TCP, mixture of 4 isomers), tris(1,3-dibromopropyl) phosphate (TDBPP) and tris(1,3-dichloro-isopropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Triamyl phosphate (TAP; IS) was purchased from TCI Europe (Zwijndrecht, Belgium). Labeled TPhP-d15 (IS) and tris(2-butoxyethyl) phosphate (TBOEP) were purchased from Sigma Aldrich. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBOEP (>94%). Standard stock solutions were prepared in iso-octane, except for NBFRs which were prepared in a mixture of iso-octane:toluene (8:2, v/v).

Indoor dust SRM 2585 was purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Silica SPE cartridges (500 mg/3 mL, Bond Elut) were purchased from Agilent, while empty polypropylene filtration tubes (3 mL) SPE cartridges and 500 mg/3 mL Supelclean ENVI- Florisil cartridges were purchased from Supelco (Bellefonte, PA, USA). Silica gel, anhydrous sodium sulfate (Na_2SO_4), and concentrated sulfuric acid (H_2SO_4, 98%) were purchased from Merck.

2.2.2.2. Sample collection

Dust samples were collected in 16 northern California homes in 2006 and again in the same homes with the same participants in 2011. These homes were a subset of 50 homes in two San Francisco Bay Area communities further described in Brody et al. (2009) and Rudel et al. (2010) Samples were collected by trained field staff using a Eureka Mighty-Mite® vacuum cleaner fitted with a specially designed PTFE Teflon crevice tool attachment modified to collect dust into a cellulose extraction thimble (19x90 mm). Samples were collected by slowly dragging the crevice tool for approximately 30 min over surfaces in the living areas of the home. Samples were sieved to <150 µm prior to long-term storage (-16°C ± 10°C) and extraction. Residents were surveyed about the presence of furniture, carpets and electronics, particularly if any items were introduced to the home since the 2006 sample collection. Individual results will be reported to participants.

2.2.2.3. Analyte selection

Analytes were selected based on previous research; current understanding of potential replacements for PBDEs; health concerns; and analytical capability. Based on production volumes, HBCDD and TBBPA are important BFRs. Other potential PBDE-replacements were included. The health effects of chlorinated and brominated PFRs are of concern and recent work suggests they are found at levels similar to PBDEs (Stapleton et al., 2009; Van den Eede et al., 2011). Non-halogenated FRs are expected to be used in various FR mixtures and may be pervasive given their many other uses in the home. Legacy OCs were included to evaluate concentration consistency over time. The 62 target chemicals are listed in Table 2.2.1.

Table 2.2.1. Full and abbreviated nomenclature, identification and quantification ions (bold values), their respective internal standards (IS) used for quantification of targeted analytes, together with instrumental technique employed for their analysis.

Compound	**Acronym**	Identification - **Quantification Ions**	**Internal Standard (IS)**	**Instrument For Analysis**
1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)-ethane	*p,p'*-DDD	248, **71**	CB 143	GC-ECNI/MS
1,1-bis-(4-chlorophenyl)-2,2-dichloroethene	*p,p'*-DDE	318, **316**	CB 143	GC-ECNI/MS
1,1,1-trichloro-2,2-di(4-chlorophenyl)ethane	*p,p'*-DDT	248, **71**	CB 143	GC-ECNI/MS
trans-nonachlor	TN	**444**	CB 143	GC-ECNI/MS
trans-chlordane	TC	410, **408**	CB 143	GC-ECNI/MS
cis-chlordane	CC	410, **408**	CB 143	GC-ECNI/MS
2,2',4,4',5,5'-Hexachlorobiphenyl	CB 153	360, **362**	CB 143	GC-ECNI/MS
2,2',3,4,4',5,5'-Heptachlorobiphenyl	CB 180	396, **394**	CB 143	GC-ECNI/MS
2,2',3,4,5,6'-Hexachlorobiphenyl (IS)	*CB 143*	*360, **362***	n.a.	*GC-MS*
2,4,4'-Tribromodiphenyl ether	BDE 28	81, **79**	BDE 77	GC-ECNI/MS
2,2',4,4'-Tetrabromodiphenyl ether	BDE 47	81, **79**	BDE 77	GC-ECNI/MS
2,2',4,4',6-Pentabromodiphenyl ether	BDE 100	81, **79**	BDE 77	GC-ECNI/MS
2,2',4,4',5-Pentabromodiphenyl ether	BDE 99	81, **79**	BDE 77	GC-ECNI/MS
2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE 154	81, **79**	BDE 77	GC-ECNI/MS
2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE 153	81, **79**	BDE 77	GC-ECNI/MS
2,2',3,4,4',5',6-Heptabromodiphenyl ether	BDE 183	81, **79**	BDE 128	GC-ECNI/MS
2,2',3,3',4,4',6,6'-Octabromodiphenyl ether	BDE 197	81, **79**	BDE 128	GC-ECNI/MS
2,2',3,4,4',5,5',6-Octabromodiphenyl ether	BDE 203	81, **79**	BDE 128	GC-ECNI/MS
2,2',3,3',4,4',5,6'-Octabromodiphenyl ether	BDE 196	81, **79**	BDE 128	GC-ECNI/MS
Decabromodiphenyl ether	BDE 209	485, **487**	^{13}C-BDE 209	GC-ECNI/MS
3,3',4,4'-Tetrabromodiphenyl ether (IS)	*BDE 77*	*81, **79***	n.a.	*GC-ECNI/MS*
2,2',3,3',4,4'-Hexabromodiphenyl ether (IS)	*BDE 128*	*81, **79***	n.a.	*GC-ECNI/MS*
^{13}C-Decabromodiphenyl ether (IS)	*^{13}C-BDE 209*	*497, **495***	n.a.	*GC-ECNI/MS*
α-Hexabromocyclododecane	α-HBCDD**	640.6→78.9	^{13}C-α-HBCDD	LC/ESI-MS/MS
β-Hexabromocyclododecane	β-HBCDD**	640.6→78.9	^{13}C-β-HBCDD	LC/ESI-MS/MS
γ-Hexabromocyclododecane	γ-HBCDD**	640.6→78.9	^{13}C-γ-HBCDD	LC/ESI-MS/MS
^{13}C-Hexabromocyclododecanes (α-, β-, γ-)	*^{13}C-HBCDDs***	*652.8→78.9*	n.a.	*LC/ESI-MS/MS*
Tetrabromobisphenol-A	TBBPA	542.6→78.9	^{13}C-TBBPA	LC/ESI-MS/MS
^{13}C-Tetrabromobisphenol-A	*^{13}C-TBBPA*	*554.6→78.9*	n.a.	*LC/ESI-MS/MS*
Tri-ethyl-phosphate	TEP	**155**	TAP	GC-EI/MS
Tri-*iso*-butyl-phosphate	TiBP	155, **211**	TAP	GC-EI/MS
Tri-*n*-butyl-phosphate	TnBP	155, **211**	TAP	GC-EI/MS
Tris-(2-chloroethyl)-phosphate	TCEP	251, **249**	TCEP-d12	GC-EI/MS
Tris-(1-chloro-2-propyl)-phosphate	TCPP	279, **277**	TCEP-d12	GC-EI/MS
Tri-(2-butoxyethyl)-phosphate	TBOEP	199, **299**	TBOEP-d6	GC-EI/MS
Tris-(1,3-dichloro-isopropyl)-phosphate	TDCPP	379, **381**	TDCPP-d15	GC-EI/MS
Tri-phenyl-phosphate	TPhP	325, **326**	TPhP-d15	GC-EI/MS
Tri-cresyl-phosphate	TCP	367, **368**	TPhP-d15	GC-EI/MS

Compound	**Acronym**	Identification - **Quantification Ions**	**Internal Standard (IS)**	**Instrument For Analysis**
tri-(2-ethylhexyl)-phosphate	TEHP	99, **211**	TBOEP-d6	GC-EI/MS
ethylhexyl diphenyl phosphate	EHDPhP	250, **251**	TPhP-d15	GC-EI/MS
Tri-amyl-phosphate (IS)	TAP	169, **239**	n.a.	GC-EI/MS
Tri-phenyl-phosphate-d15 (IS)	TPhP-*d15*	*339*, ***341***	n.a.	*GC-EI/MS*
Tri-(2-chloro-ethyl)-phosphate-d12 (IS)	*TCEP-d12*	*263*, ***261***	n.a.	*GC-EI/MS*
Tri--(1,3-dichloro-isopropyl-*phosphate-d15 (IS)*	TDCPP-*d15*	*394*, ***396***	n.a.	*GC-EI/MS*
Tri-(2-butoxyethyl)-*phosphate-d15 (IS)*	*TBOEP-d6*	*202*, ***203***	n.a.	*GC-EI/MS*
2-ethylhexyl-2,3,4,5-tetrabromobenzoate	EH-TBB	359, **357**	BDE 77	GC-ECNI/MS
bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate	BEHTBP	515, **384**	BDE 128	GC-ECNI/MS
1,2-*bis*(2,4,6-tribromophenoxy)ethane	BTBPE	81, **79**	BDE 128	GC-ECNI/MS
Decabromodiphenylethane	DBDPE	81, **79**	^{13}C-BDE 209	GC-ECNI/MS
Hexachlorocyclopentadienyl-Dibromocyclooctane	HCDBCO	79, **310**	BDE 77	GC-ECNI/MS
hexabromobenzene	HBB	81, **79**	BDE 77	GC-ECNI/MS
tetrabromobisphenol A - bis(2,3-dibromopropylether)	TBBPA-BDBPE	81, **79**	^{13}C-BDE 209	GC-ECNI/MS
alpha-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane	alpha-TBECH	81, **79**	BDE 77	GC-ECNI/MS
beta-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane	beta-TBECH	81, **79**	BDE 77	GC-ECNI/MS
gamma-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane	gamma-TBECH	81, **79**	BDE 77	GC-ECNI/MS
delta-1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane	delta-TBECH	81, **79**	BDE 77	GC-ECNI/MS
2,4,6-tribromophenyl allyl ether	ATE	81, **79**	BDE 77	GC-ECNI/MS
2-bromoallyl-2,4,6-tribromophenyl ether	BATE	81, **79**	BDE 77	GC-ECNI/MS
2,4,6-tribromophenyl 2,3-dibromopropyl ether	DPTE	81, **79**	BDE 77	GC-ECNI/MS
alpha-1,2,5,6-tetrabromocyclooctane	alpha-TBCO	81, **79**	BDE 77	GC-ECNI/MS
beta-1,2,5,6-tetrabromocyclooctane	beta-TBCO	81, **79**	BDE 77	GC-ECNI/MS
octabromo-1,3,3-trimethyl-1-phenylindane	OBIND	81, **79**	BDE 128	GC-ECNI/MS
tris(2,3-dibromopropyl) phosphate	TDBPP	81, **487**	BDE 128	GC-ECNI/MS
3,3',5,5'-tetrabromo biphenyl	BB 80	472, **470**	BDE 77	GC-EI/MS
2,2',4,5',6-pentabromo biphenyl	BB 103	548, **550**	BDE 77	GC-EI/MS
2,2',4,4',5,5'-hexabromo biphenyl	BB 153	625.5, **627.5**	BDE 77	GC-EI/MS
2,2',3,4,4',5,5'-heptabromo biphenyl	BB 180	548, **550**	BDE 77	GC-EI/MS
decabromo biphenyl	BB 209	81, **79**	^{13}C-BDE 209	GC-ECNI/MS
syn-Dechlorane plus	syn-DP	650, **652**	BDE 128	GC-ECNI/MS
anti-Dechlorane plus	anti-DP	650, **652**	BDE 128	GC-ECNI/MS

n.a. – not applicable

2.2.2.4. Analytical methods

Due to the comprehensive list of target analytes and differences in physical-chemical properties, two different sample preparation methods were used in four extracts per sample (two fractions per method) for chemical analysis. One sample preparation method, which was used to measure the bulk of BFRs, OCs, and PFRs, involved extraction using Hex-Ac (3:1, *v:v*) and fractionation on Florisil (Van den Eede et al., 2012). A sample aliquot (around 50 mg) was accurately weighed and spiked with IS (^{13}C-BDE 209, BDE 77, BDE 128, CB 143, TCEP-d12, TBOEP-d6, TDCPP-d15, TAP, and TPhP-d15). Samples were extracted using 2 mL Hex-Ac (3:1, v/v) by a combination of vortexing and ultrasonic extraction (2 × 1 min vortex and 5 min ultrasonic extraction) which was repeated three times. After each extraction cycle, dust extracts were centrifuged at 3500 rpm for 2 min and supernatants were collected and transferred into clean glass tubes. The pooled supernatants were evaporated until dryness under a gentle nitrogen flow and redissolved in 1 mL Hex. Prior to fractionation, Florisil® cartridges were prewashed with 6 mL of Hex.

The extracts were quantitatively transferred and fractionation was achieved by eluting with 8 mL of Hex (F1) and 10 mL of EA (F2). The 1st fraction (F1) was evaporated until 1 mL and quantitatively transferred onto acidified silica 44% cartridges (prewashed with 6 mL Hex) for a second clean-up. The target analytes were eluted with 10 mL of Hex/DCM (1:1 v/v), and afterwards evaporated until dryness under gentle nitrogen flow and reconstituted in 100 µL of iso-octane.

In the 2nd fraction (F2), IS BDE 128 was added for the quantification of BEHTBP, followed by evaporation until dryness and resolubilised in 100 µL of iso-octane. Fraction F1, contained PBDEs, most NBFRs, OCs and PBBs, was subjected to analysis by GC-ECNI/MS (different acquisition methods) and GC-EI/MS (confirmation of OCs and PBBs). The 2nd fraction (F2), containing PFRs and BEHTBP was subjected to analysis by GC-EI/MS (for PFRs) and GC-ECNI/MS (for BEHTBP and TDBPP). More details about the analytes in Table 2.2.1.

A second sample preparation method (Roosens et al., 2010), involving similar extraction and fractionation on silica, was employed to measure HBCDDs, TBBPA, and to confirm PBDEs. A sample aliquot (typically 50 mg) was accurately weighed and spiked with a mixture containing IS (^{13}C-α-, β-, γ-HBCDD, ^{13}C-TBBPA, ^{13}C-BDE 209, BDE 77, and BDE 128). Samples were extracted using 2 mL Hex-Ac (3:1, v/v) by a combination of vortexing and ultrasonic extraction (2 × 1 min vortex and 5 min ultrasonic extraction) which was repeated three times. After each extraction cycle, dust extracts were centrifuged at 3500 rpm for 2 min and supernatants were collected and transferred into clean glass tubes. The pooled supernatants were evaporated until dryness under a gentle nitrogen flow and redissolved in 1 mL Hex. Prior to fractionation, silica cartridges were topped with 100 mg acid silica (44%) and prewashed with 6 mL of Hex. The extracts were quantitatively transferred and fractionation was achieved by eluting with 8 mL of Hex (Fraction A – FA) and 10 mL of DCM (Fraction B – FB).

Both fractions were afterwards evaporated until dryness under gentle nitrogen flow. Fraction FA, containing PBDEs, was reconstituted in 100 µL of iso-octane and was subjected to GC-ECNI/MS. The 2nd fraction (FB), containing HBCDDs, was resolubilised in 100 µL of methanol and further subjected to LC-MS/MS analysis.

GC-ECNI/MS Analysis. The analysis of F1, containing PBDEs, most NBFRs, and OCs, and the analysis of F2, containing BEHTBP, was performed with an Agilent 6890 GC coupled to an Agilent 5973 MS operated in electrochemical negative ionization (ECNI) mode. The GC system was equipped with electronic pressure control and a programmable-temperature vaporizer (PTV). A volume of 2 µL of cleaned extract was injected on a DB-5 column (15 m × 0.25 mm × 0.10 µm) using solvent vent injection. The injection temperature was set at 90 °C, hold 0.04 min, ramp 700 °C/min to 295 °C. Vent time was 0.02 min and vent flow 75 mL/min. Injection was performed under a pressure of 10 psi until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 300 °C, hold 3 min, ramp 40 °C/min to 310 °C, hold 5 min. Helium was used as a carrier gas with a ramped flow rate of 1.0 mL/min until 20 min and then raised to 2.0 mL/min. The mass spectrometer was employed in selected ion monitoring (SIM) mode, with ions 79 and 81 monitored the whole run time. For BDE 209, ions 487 and 485 were used, while ^{13}C-BDE 209 was monitored using ions 495 and 497. Dwell times were set on 35 ms. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V. Methane was used as moderating gas. An overview of analytes containing detailed nomenclature and applied abbreviation, together with ions acquired for identification and quantification purposes on the GC-EI-MS and GC-ECNI-MS are presented in Table 2.2.1.

GC-EI/MS Analysis. Analysis of PFRs in F2 was performed with an Agilent 6890 GC coupled to an Agilent 5973 MS operated in electron impact ionization (EI) mode. The GC system was equipped with electronic pressure control and a programmable-temperature vaporizer (PTV). One µL of purified extract was injected on a HT-8 column (25 m × 0.22 mm × 0.25 µm) using cold splitless injection. The injection temperature was set at 90 °C, hold 0.03 min, ramp 700 °C/min to 290 °C. Injection was performed using a pressure of1 bar until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.25 min, ramp 10 °C/min to 240 °C, ramp 20 °C/min to 310 °C, hold 16 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The mass spectrometer was run in selected ion monitoring (SIM) mode. Dwell times ranged between 20 and 30 ms in different acquisition windows. The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively, and the electron multiplier voltage was at 2200 V.

LC-MS/MS. The determination of individual HBCDD isomers and TBBPA in the Fraction B (silica fractionation) was achieved using a dual pump Agilent 1100 Series liquid chromatograph equipped with autosampler and vacuum degasser. A Luna C18(2) reversed phase (RP) analytical column (150 mm × 2 mm i.d., 3 μm particle size, Phenomenex) was used for the separation of α-, β-, and γ-HBCDD. A mobile phase of (A) ammonium acetate 2mM in water/methanol (1:1 v/v) and (B) methanol at a flow rate of 0.250 mL/min was applied for elution of HBCDD isomers; starting at 75% (B) held for 2 min, then increased linearly to 100% (b) until 9 min; held until 12 min followed by a linear decrease to 70% (B) over 0.5 min and held for 7.5 min. The target analytes were baseline separated on the RP column with retention times of 4.0, 6.0, 6.8 and 7.4 min for TBBPA, α-, β- and γ-HBCDD, respectively. MS analysis was performed using an Agilent 6410 triple quadrupole MS system operated in the electrospray negative ionization mode. N2 was used as drying gas at a flow of 10 L/min and heated to 300 °C. Nebulizer pressure was 35 psi and capillary voltage 4000 V. HBCDD isomers were quantified by isotope dilution. MS/MS detection operated in the MRM mode was used for quantitative determination of the HBCDD isomers based on m/z 640.6 to 81 and m/z 652.6 to 81 for the native and ^{13}C-labeled diastereomers, respectively. Fragmentor voltage and collision energy were set as 80 and 15 V, respectively. For quantitative determination of TBBPA, the following MRMs were used: m/z 5 to 81 and m/z 652.6 to 81 for the native and ^{13}C-labeled diastereomers, respectively.

2.2.2.5. Quality control

Six procedural blanks were analysed in the same batches as the samples and results are blank corrected. This implies subtraction of mean blank values (in pg) from the raw FR values (in pg) in the samples. Blank values, when detected, were <0.5% of sample values. Compounds consistently detected in the procedural blanks at levels >1 ng (PFRs and BDE 209) or >50 pg (all others) were: TiBP (mean=3.4 ng), TnBP (13.1 ng), TEHP (3.8 ng), β-HBCDD (40 pg), BDE 47 (19 pg), BDE 85 (14 pg), BDE 154 (40 pg), BDE 196 (11 pg), BTBPE (18 pg), and BDE 209 (1330 pg).

Method limits of quantification (LOQ) were calculated as three times the standard deviation of blank values and divided by the amount of dust used for analysis (typically 50 mg). For compounds not detected in the blanks, the LOQ was calculated based on the signal to noise ratio 10/1, taking into account also the chromatogram's characteristics for the respective retention time (co-elution, noisy baseline, etc). LOQs are compound-specific variables and therefore spanned a large range of concentrations (Table 2.2.2).

The method has been recently validated as described by Van den Eede et al., (2012). A series of optimization and spiking experiments were performed for BFRs and PFRs at two concentration levels, QLow and QHigh, and three replicates for each level. Precision between different days were assessed using the same concentration levels spiked on a low contaminated dust sample, using three replicates per level and executed on three different days. Precision was within 12% for each set of

triplicates and all analytes. The recovery was calculated by subtracting the blank concentrations and divided by the calculated concentration of a mixed solution of standards (having the same concentrations). SRM 2585 (Organic Contaminants in House Dust), which has certified values for PBDEs and indicative values for EH-TBB, BEHTBP, HBCDDs, chlorinated PFRs and TBOEP, was used to test the accuracy. Concentrations of PBDEs range between 2 and 30% relative difference from the certified values. EH-TBB, BEHTBP and chlorinated PFRs were within 0 and 56% relative difference; while analytes with lower concentration ranges (e.g. HBCDD) fared worse. Despite the few discrepancies, there does not appear to be a systematic bias to the samples and values were not adjusted.

Inter-laboratory comparisons were conducted using samples collected in 2006 and analysed at two different time periods. In 2006, as part of the Northern California Household Exposure Study, Southwest Research Institute (SWRI) analysed 50 dust for approximately 100 semivolatile organic compounds, including PBDEs and legacy compounds. The 16 homes in this study are a subset of the 50 homes studied in 2006. In 2011, for this study, University of Antwerp analysed stored dust samples (splits of the original samples collected in 2006) for FRs and legacy compounds.

2.2.2.6. Data analysis

Summary statistics were calculated for all analytes within each sampling round. Non-detectable concentrations were left at zero for summary statistics, which results in lower values than if other replacement methods were used. Concentration ratios (2011/2006 concentrations) were calculated to evaluate changes between the two sampling periods. Non-detectable concentrations were set to the LOQ for concentration ratios. Ratios above 1 indicate higher concentrations in 2011 and ratios below 1 indicate higher 2006 concentrations. Spearman rank correlations were used to evaluate associations between absolute concentration differences between rounds (2011-2006 concentrations) and total number of reported new FR-relevant items (e.g. electronics, carpets) in 2011.

Kendall's tau rank correlation estimates were calculated to investigate relationships between analytes within each sampling round and for each analyte across rounds. These estimates were used in cluster analysis to elucidate common mixtures and potential sources. Data analysis was performed in R (version 2.15).

2.2.3. Results and discussion

Overall, 55 compounds were detected and 41 were found in at least 50% of the 32 samples (Table 2.2.2). Detected chemicals were 13 PBDE congeners, 3 components of Firemaster 550, 15 other BFRs, 4 halogenated PFRs, 7 non-halogenated PFRs, and 2 Dechlorane-plus isomers. Table 2.2.3 summarizes information on usage and health concerns of these FRs grouped by common formulations (related to exposure patterns) and chemical structure (often related to use and toxicity). These FR group names are used throughout the paper.

Table 2.2.2: Concentrations (ng/g dust) of flame retardants and legacy organohalogens in California house dust from 16 homes sampled in 2006 and 2011.

Analyte	LOQ	2006 Samples (Round 1; n=16)				2011 Samples (Round 2; n=16)			
		% > LOQ	Min.	Median	Max.	% > LOQ	Min.	Median	Max.
Polybrominated Diphenyl Ethers									
BDE 28	2	100	5	26	270	100	3	14	310
BDE 47	2	100	270	2,300	23,000	100	140	1,000	17,000
BDE 66	2	100	8	64	520	100	4	23	1,800
BDE 85	3	100	13	110	1,300	100	9	66	6,000
BDE 99	2	100	280	2,200	24,000	100	190	1,100	25,000
BDE 100	2	100	56	520	4,900	100	37	240	11,000
BDE 153	3	100	2	250	2,400	100	21	150	7,800
BDE 154	3	100	22	240	1,800	100	17	110	6,700
BDE 183	4	100	9	28	770	100	3	18	920
BDE 196	4	88	<4	7.5	240	56	<4	4	180
BDE 197	4	81	<4	9	530	56	<4	4	230
BDE 203	4	81	<4	5	130	50	<4	2	110
BDE 209	10	100	580	1,400	15,000	100	110	1,200	8,500
Firemaster® 550									
EH-TBB	2	100	4	48	740	100	45	100	5,900
BEHTBP	2	100	36	140	1,900	94	<2	260	3,800
TPhP	20	100	580	3,000	14,000	100	790	2,800	36,000
Tetrabromobisphenol A									
TBBPA	10	94	<10	260	3,400	100	22	200	2,000
Hexabromoxyclododecane									
α-HBCDD	5	100	31	62	710	100	17	62	910
β-HBCDD	5	100	8	18	330	100	7	16	230
γ-HBCDD	5	100	29	94	6,700	100	13	73	790
Σ HBCDD	5	100	82	190	6,800	100	39	160	1,800
Other Brominated Flame Retardants									
HBB	2	50	<2	1	8	31	<2	<2	13
HCDBCO	5	6	<5	<5	9	25	<5	<5	72
BTBPE	2	100	7	30	220	100	3	12	130
DBDPE	10	94	<10	51	430	100	18	140	2,800
TBBPA-BDBPE	10	75	<10	22	180	50	<10	7	560
α -TBECH	2	6	<2	<2	13	19	<2	<2	25

Analyte	LOQ	2006 Samples (Round 1; n=16)				2011 Samples (Round 2; n=16)			
		% > LOQ	Min.	Median	Max.	% > LOQ	Min.	Median	Max.
β -TBECH	2	6	<2	<2	11	12	<2	<2	16
γ -TBECH	2	0	--	--	--	6	<2	<2	3
δ -TBECH	2	0	--	--	--	0	--	--	--
ATE	2	0	--	--	--	0	--	--	--
BATE	2	0	--	--	--	0	--	--	--
DPTE	2	6	<2	<2	2	6	<2	<2	11
α-TBCO	2	6	<2	<2	2	0	--	--	--
β-TBCO	2	0	--	--	--	0	--	--	--
OBIND	5	44	<5	<5	130	25	<5	<5	62
Halogenated Organophosphate Flame Retardants									
TCEP	20	100	610	5,100	160,000	100	330	2,700	110,000
TCPP	20	100	340	2,100	120,000	100	490	2,200	140,000
TDCPP	20	100	730	2,800	24,000	100	920	2,100	44,000
TDBPP	20	62	<20	35	8,900	38	<20	<20	310
Non-halogenated Organophosphate Flame Retardants									
TEP	20	56	<20	28	410	31	<20	<20	250
TiBP	80	56	<80	84	180	19	<80	<80	120
TnBP	80	50	<80	32	1,800	38	<80	<80	1,800
TBOEP	300	100	2,300	12,000	68,000	100	790	11,000	170,000
TEHP	200	19	<200	<200	3,700	12	<200	<200	340
EHDPhP	100	100	180	610	3,000	100	140	560	1,500
TCP	20	100	330	1,000	4,400	100	180	680	10,000
Dechlorane Plus									
syn-DP	2	81	<2	3	22	44	<2	<2	7
anti-DP	2	100	3	7.5	35	75	<2	3	8
Σ DP	2	100	3	10	47	75	<2	4.5	15

LOQ – Limit of Quantification; -- indicates insufficient number of detects to calculate summary statistics

Table 2.2.3: Major flame retardant classes investigated in this study, their uses and health effects.

Flame Retardant Class	Main uses	Health Concerns[a]
Polybrominated diphenyl ethers (PBDEs)		
PentaBDE[b]	• Additive FR in polyurethane foams (Birnbaum 2004) • US High Production Volume (HPV) chemical • Phased-out in US in 2004	• Endocrine disruption in men and women, animals, and *in vitro* assays (Hamers et al., 2006; Stapleton et al., 2011; Stoker et al., 2004; Turyk et al., 2008; Zhou et al., 2001); impaired sexual development (Stoker et al., 2004; Kuriyama et al., 2005); decreased birth weight in humans (Chao et al., 2007) and delayed physical and mental development in children (Herbstman et al., 2010); EPA Action Plan Chemical
OctaBDE[c]	• Additive FR in polymers for plastic housings and office equipment (Birnbaum 2004) • US HPV chemical / • Phased-out in US in 2004	• Endocrine disruption (Zhou et al., 2001) and neurotoxicity (Viberg et al., 2009); • EPA Action Plan Chemical
DecaBDE[d]	• Additive FR in electrical and electronic equipment, textiles and fabric backings; 80% of total PBDE production (Birnbaum 2004) • Volunteer phase-out in US by 2014 • US HPV chemical	• Impaired reproductive function (Tseng et al., 2011); neurotoxicity (Rice et al., 2007; Viberg et al., 2003); endocrine disruption (Rice et al., 2007); decreased birth weight in humans (Chao et al., 2007); carcinogenicity (NTP 1986; US EPA 2008); • EPA Action Plan Chemical
Firemaster® 550	• Replacement for PentaBDE in foams	• Endocrine disruption and reduced sperm concentration in men (Meeker et al., 2010); DNA damage (Bearr et al., 2010); • BEHTBP structurally similar to DEHP, a reproductive and developmental toxicant and listed carcinogen on CA's Proposition 65 List (CECBP 2009; US EPA 2005) • Lack of health studies on cancer, 2 generation reproductive, and developmental effects (IEPA 2007)
HBCDDs	• Additive FR in thermoplastic (mouldable) polymers and styrene resins (Birnbaum 2004) • Used in building insulation, upholstery textiles and electrical equipment housing (Covaci et al., 2006) • US HPV chemical	• Neurotoxicity (Eriksson et al., 2006; Mariussen et al., 2003); endocrine disruption (Hamers T, 2006; Ema et al., 2008; Marvin et al., 2011); decreased viability of offspring (Ema et al., 2008); • REACH Substance of Very High Concern (SVHC); • EPA 2010 Action Plan Chemical to review potential reproductive, developmental and neurological effects (US EPA 2010)
TBBPA	• Most widely used flame retardant; reactive in circuit boards; additive FR in polymers (Birnbaum 2004) • US HPV chemical	• Endocrine disruption (Hamers T, 2006; Meerts et al., 2001; Van der Ven et al., 2008) immunotoxicity (Pullen et al., 2003); neurotoxicity (Mariussen et al., 2003)

Flame Retardant Class	Main uses	Health Concerns[a]
Other brominated flame retardants (BFRs)		
HBB	• Additive FR in paper, wood, textiles, electronic and plastics; not used in Europe (Covaci et al., 2011)	• Systemic toxicity: disruption of heme formation (Szymanska et al., 2000); increased liver/body ratio and increased carboxylesterase (US EPA 1988) • Lack of health studies
BTBPE	• Replacement for OctaBDE (Covaci et al., 2011) • US HPV chemical	• Endocrine disrupting metabolites (Hamers T, 2006); behavioural, gastrointestinal, and respiratory changes, dermatitis, and gross metabolic changes (NPCA 2009) • Lack of health studies, particularly chronic animal studies (NPCA 2009)
DBDPE	• Alternative to DecaBDE (Covaci et al., 2011)	• Developmental effects - decreased viability of offspring (Nakari et al., 2009); Structural similarity to BDE 209 • Lack of health studies
Organophosphate flame retardants (PFRs)		
Halogenated PFRs	• Used in polyurenthane foams (Van den Eede et al., 2011) • TCEP: banned from children's products in NY in 2011(NY Senate 2011) • TDBPP: banned in 1977 for use in children's clothing (CPSC)	• TCEP: Neurotoxicity (IPCS 1998); Listed as carcinogen on CA Prop. 65; REACH SVHC and persistent, bioaccumulative, and toxic (PBT) chemical for reproductive toxicity (ECHA 2009) • TDCPP: Carcinogenicity (IPCS 1998); associated with Sick Building Syndrome in men and women (Kanazawa et al., 2010); endocrine disruption (Meeker et al., 2010) and neurotoxicity (Dishaw et al., 2011); Listed as carcinogen on CA Prop. 65 • TCPP: structurally similar to TCEP • TDBPP listed as carcinogen on CA Prop. 65; IARC 2A carcinogen
Non-halogenated PFRs	• TEP, TnBP, and TiBP used for plasticizing (Van den Eede et al., 2011) • TCP used as FR plasticisers (Van den Eede et al., 2011) • TnBP and TCP used as lubricants in hydraulic fluids (Van den Eede et al., 2011) • TBOEP used in floor wax and rubber stoppers (Van den Eede et al., 2011) • TnBP: US HPV chemical	• TnBP: associated with Sick Building Syndrome in men and women (Kanazawa et al., 2010) • TCP: Reproductive and developmental toxicity (Carlton et al., 1987) • Lack of health studies
DP	• FR in electronics (Sverko et al., 2011) • US HPV chemical	• Systemic toxicity: gross morphologic changes (US EPA 2011); Shares structural similarities with pesticides/insecticides/acaricides dieldrin, chlordane, heptachlor, endrin, and endosulfan (DiGangi et al., 2010) • Lack of health studies

[a] From laboratory or animal studies unless otherwise indicated.
[b] congeners BDE 28, BDE 47, BDE 66, BDE 85, BDE 99, BDE 100, BDE 153, and BDE 154
[c] congeners BDE 183, BDE 196, BDE 197, and BDE 203
[d] congener BDE 209

The highest concentrations, greater than 0.1 mg/g or 0.01%, were for two chlorinated PFRs, including TCEP, which is listed as a carcinogen under California's Proposition 65, and TCPP, and one non-halogenated PFR (TBOEP). Over the five years between the sample collection periods, Firemaster 550 components increased, while PentaBDE levels decreased. Legacy pollutants like DDT also decreased, suggesting that the PBDE reduction may be due to decreased loading and/or possibly to differences in sample collection between 2006 and 2011. Figure 2.2.1 shows ratios of 2011/2006 concentrations; ratios >1 suggest increasing concentrations with time. Detailed findings are presented below by chemical group.

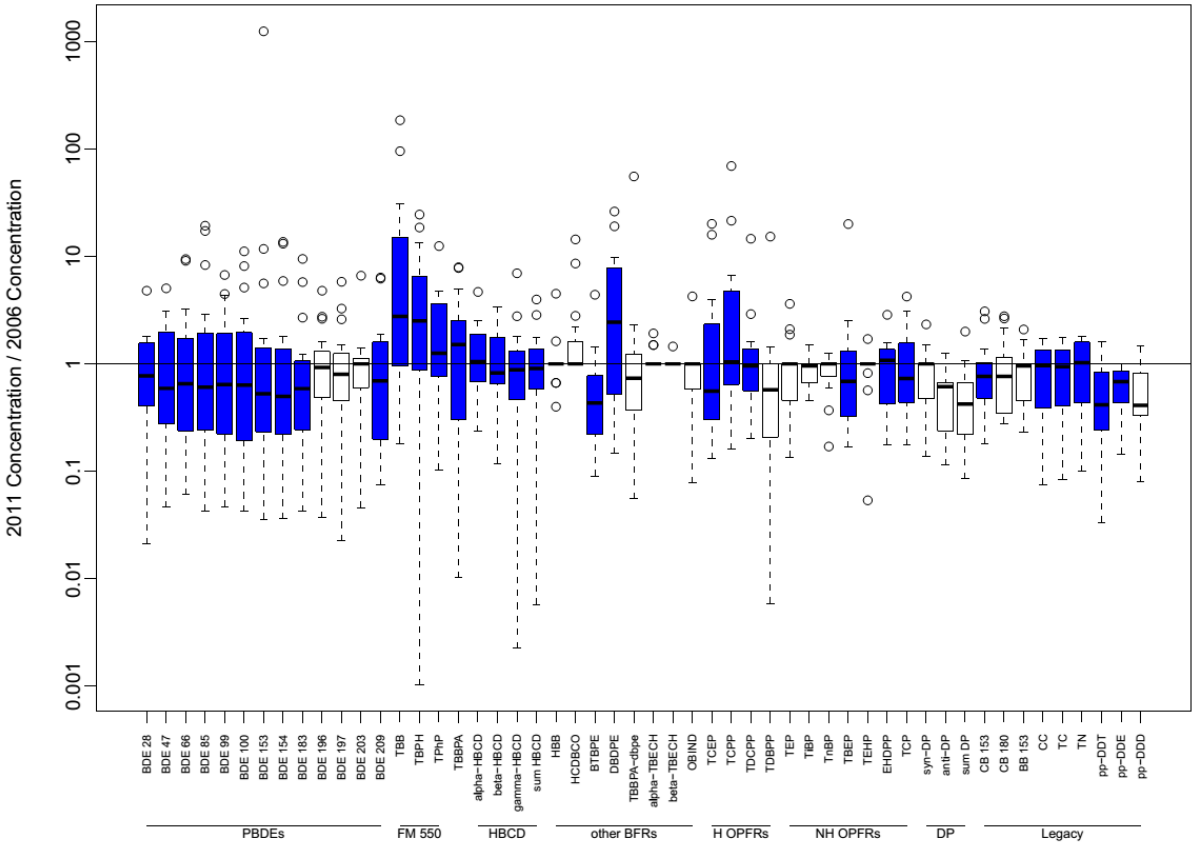

Figure 2.2.1: Distributions of concentration ratios (2011/2006) in dust collected from 16 homes. Non-detectable levels set to detection limit. Chemicals with median ratios above 1 (to right) were higher in 2011 samples compared with 2006 samples. Darker shaded boxes used for chemicals with >75% simultaneous detects.

2.2.3.1. Concentrations in House Dust

PBDEs. We found all targeted PBDE congeners in at least 50% of samples, with the components of PentaBDE (BDE 47 and BDE 99) and DecaBDE (BDE 209) mixtures in 100% of samples. Median concentrations for all PBDE congeners decreased from 2006 to 2011 (Table 2.2.2); however, not all of the means decreased, with exceptions likely driven by two homes with substantial increases in the congeners of PentaBDE mixture. Exposed furniture foam was noted in one of these homes. Ratios of 2011/2006 concentrations are used to evaluate relative concentrations from the two sampling periods.

Median concentration ratios were less than 1 for all congeners (Figure 2.2.1), suggesting a decrease in concentrations between 2006 and 2011, which could reflect decreased use. However, since we saw decreases for legacy OCs, which should generally have minimal changes between 2006 and 2011, the PBDE reduction may reflect some unidentified but systematic difference in sample collection (see Legacy chemicals below).

Substantial decreases (up to 20-fold) in concentrations of PentaBDE were observed in three homes where participants reported remodelling or acquiring new furniture and/or rugs/carpet between 2006 and 2011. In fact, there was a significant statistical association between concentration reductions and participant-reported new furniture, electronics, and flooring ($p<0.05$), suggesting that PentaBDE is no longer present in new household items. Reductions are likely the result of phase-outs (2004) (Great Lakes 2005) and bans (2006 in CA) (State of California 2006) of PentaBDE and OctaBDE. Substantial decrease (14-fold) in BDE 209 was observed in a home where the participant did not report changes in electronics and furnishings; possibly some relevant changes were not reported.

We detected BDE 47 and BDE 99 at median concentrations >1,000 ng/g in both sampling rounds, which is consistent with previous research showing higher PentaBDE concentrations in California than elsewhere due to the unique furniture flammability standard (Whitehead et al., 2011). In comparison to other studies within California, the median concentration of BDE 47 in 2006 is similar (within 30%); whereas median concentrations of BDE 99 and 100 in 2006 (2,200 ng/g and 520 ng/g, respectively) were lower (up to 2x) than other California studies, which used slightly different vacuum sampling techniques (Quiros-Alcala et al., 2011; Hwang et al., 2008).

Correlation and cluster analysis were used to evaluate mixtures and common sources. PBDE congeners measured in each sampling round correlate/cluster together in the three commercial formulations (PentaBDE, OctaBDE, and DecaBDE). OctaBDE levels correlate between sampling rounds, suggesting relatively stable concentrations in the homes over time; however, PentaBDE and DecaBDE levels were not significantly correlated over time, likely due to a few homes with substantial changes.

Firemaster® 550. Chemtura introduced Firemaster® 550 in 2004 as a replacement for PentaBDE in polyurethane foam (Stapleton et al., 2008). Besides TPhP, the other constituents of Firemaster 550 were only recently identified as two brominated compounds: EH-TBB and BEHTBP (Stapleton et al., 2008). Subsequently, Chemtura developed additional products, with undisclosed composition, including Firemaster 600, Firemaster 800, and Emerald Innovation™, with claims of increased efficiency. Firemaster 550 is genotoxic (Bearr et al., 2010) and TPhP was associated with altered prolactin levels and decreased sperm concentration in men (Meeker et al., 2010). To our knowledge, carcinogenicity, reproductive and development studies have not been conducted on the brominated components of Firemaster 550.

We detected EH-TBB, BEHTBP and TPhP in all but one sample. Concentrations of EH-TBB and BEHTBP increased across rounds (median ratio > 1; Figure 2.2.1), except in one home where BEHTBP was found at 1,935 ng/g in the 2006 sample and not detected (<2 ng/g) in 2011. This home also had lower 2011 EH-TBB and TPhP concentrations. The generally increasing trend for EH-TBB and BEHTBP suggests that Firemaster 550 is being used as a PentaBDE replacement.

We compared our 2006 results to two sets of dust samples collected in the Boston area - 50 vacuum bag samples collected between 2002 and 2007 and 20 field technician collected dust samples collected in 2006 (Stapleton et al., 2008; Stapleton et al., 2009; Meeker et al., 2010), and vacuum bag dust collected in Vancouver, Canada in 2007-2008 (Shoeib et al., 2012). The 2006 EH-TBB and BEHTBP levels in our study were similar to, if not slightly lower than, levels in Boston (Stapleton et al., 2008; Stapleton et al., 2009). Our 2006 EH-TBB levels were lower than levels in Vancouver whereas the 2011 levels are comparable (Shoeib et al., 2012). In contrast, the levels of BEHTBP at both time periods in our study were higher than those in Vancouver (Shoeib et al., 2012). The concentrations in our 2006 samples of TPhP were lower than in Boston (Meeker et al., 2010).

EH-TBB and BEHTBP were significantly positively correlated within each sampling round (tau=0.4-0.5; $p<0.05$), which is expected since they are both in Firemaster 550. We compared the observed ratio of EH-TBB/BEHTBP in our samples with the ratio of the commercial mixture and Boston-area samples to evaluate if Firemaster 550 is the sole source and if EH-TBB and BEHTBP have different fates once applied to a product. We observed a mean EH-TBB/BEHTBP ratio of 0.6 (0.04 to 3.1) in the 2006 samples and 1.5 (0.8 to 11) in the 2011 samples. These ratios are lower than the reported ratio in Firemaster 550 (4) and in Boston dust (mean 4.4; range 0.5 to 50) (Stapleton et al., 2008). This suggests other sources of BEHTBP in California or a different fate of the chemicals. TPhP, also present in Firemaster 550, was not significantly correlated with either EH-TBB or BEHTBP in either sampling round, although TPhP concentrations increased in homes with substantial increases in EH-TBB and BEHTBP. This suggests that, in addition to Firemaster 550, there are other sources of TPhP, e.g. as a FR in other formulations or applications or as a plasticiser.

HBCDD, the third most used BFR, is used mostly in polystyrene foams in building materials and consumer products (US EPA 2010). It is being considered for addition to the list of Persistent Organic Pollutants (POPs) under the Stockholm Convention, which would substantially limit its production and use (Marvin et al., 2011). In 2010, U.S. EPA released an Action Plan for HBCDD citing its wide use, presence in humans, bioaccumulation potential, persistence, toxicity to aquatic organisms and concerns about reproductive, neurological and developmental effects in humans (US EPA 2010). The Action Plan was followed by a proposed Significant New Use Rule (SNUR) for HBCDD in textiles, where it is often used to meet furniture flammability standards. The SNUR would limit HBCDD in U.S. furnishings.

We detected all HBCDD isomers (α-, β-, and γ-HBCDD) in all samples, and they were significantly correlated (tau = 0.4-0.8; $p<0.05$) within each sampling round. Total HBCDD (sum of three isomers) concentrations were similar across time periods, ranging from 82 to 6,800 ng/g (median 190 ng/g) in the 2006 samples and 39 to 1,800 ng/g (median 160 ng/g) in 2011. It is unclear whether the phase-out of PentaBDE and OctaBDE mixtures influenced the pattern of HBCDD use. Median concentrations were similar to those reported for U.S. and Canadian samples, but less than for UK samples (Shoeib et al., 2012; Abdallah et al., 2008). However, our maxima (2006: 6,800 ng/g; 2011: 1,800 ng/g) were substantially lower than those reported in Boston living room dust (130,200 ng/g) and UK samples (110,000 ng/g) (Stapleton et al., 2008; Abdallah et al., 2008). Commercial mixtures of HBCDD mainly consist of γ-HBCDD (75-89%), while α- and β-HBCDD are found at lower amounts (Covaci et al., 2006). However, we observed relative abundances of 45-50%, 40-45%, and approximately 10% for γ-, α-, and β-HBCDD, respectively. This is likely the result of thermal rearrangement at high temperatures in production and processing of HBCDD-added materials (Kajiwara et al., 2009) or photolysis (Harrad et al., 2009). This raises cautions about using only source composition information and not evaluating fate and transport of chemicals in products to evaluate potential exposures.

Tetrabromobisphenol A. TBBPA, the most commonly used BFR (Birnbaum et al., 2004), is employed as a reactive FR in circuit boards, plastics, paper and textiles as a plasticiser, in coatings and adhesives, and as an intermediate in the synthesis of other FRs (Covaci et al., 2009). It has been associated with effects on the immune system, reproductive and development effects, and neurotoxicity (see Table 2.2.3 for details and references). TBBPA was detected in nearly all homes in both rounds with concentrations ranging from <10 to 3,400 ng/g in 2006 and 22 to 2,000 ng/g in 2011 (Table 2.2.2). We found a significant association between concentration reductions and new electronics suggesting that new electronics contain less TBBPA (rho=-0.69; p=0.003). Concentrations are higher (17-22x at median) than reported in European homes (D'Hollander et al., 2010) and similar to Michigan, USA, offices (Batterman et al., 2010).

Other BFRs. Dust samples were analysed for 15 other BFRs. BTBPE, in production since the 1970s and now used to replace OctaBDE (Covaci et al., 2011), and DBDPE, introduced in mid-1980s and available as a replacement for DecaBDE (Covaci et al., 2011), were detected in nearly 100% of samples. The concentrations of BTBPE, for which limited toxicity data are available (see table 2.2.3), were similar between 2006 and 2011. In contrast, concentrations of DBDPE, structurally similar to BDE 209 and associated with reproductive and developmental toxicities (Nakari et al., 2009), were generally higher in 2011 (table 2.2.2 and figure 2.2.1), and two homes had substantial (> 20-fold) increases.

Another commonly detected FR was the TBBPA derivative tetrabromobisphenol A-bis(2,3-dibromopropylether) (TBBPA-BDBPE), which is being studied by the National Toxicology Program (NTP) because of the structural similarity with the carcinogenic TDBPP (brominated "Tris"). Levels of TBBPA-BDBPE appear fairly stable over time (Table 2.2.2 and Figure 2.2.1) and lower than levels reported in Belgium (Ali et al., 2011).

Hexabromobenzene (HBB), an additive FR used in paper, wood, textiles, plastics and electronics, and not used in Europe (Covaci et al., 2011), was detected in 50% of 2006 samples and 31% of 2011 samples. Octabromo-1,3,3-trimethyl-1-phenylindane (OBIND) was infrequently detected and one home had substantial (10-fold) reductions over the 5 years. Studies on exposures and health effects of these BFRs are limited.

Halogenated PFRs. Chlorinated and brominated PFRs have a long history of use in polyurethane foam and textiles and an equally long history of concerns about health effects, particularly cancer.

TDBPP or brominated "Tris" was banned from children's sleepwear in the U.S. in 1977 due to carcinogenicity concerns and detection of its mutagenic metabolite in children (Blum et al., 1978). It is listed as a carcinogen in California's Proposition 65. It is reported to be used as a FR in polyurethane and polystyrene foams, acrylic furnishings, polyvinyl and phenolic resins, paints and lacquers, styrene-butadiene rubber, and latexes (NIEHS 2011). We detected TDBPP in 62% of 2006 samples and 38% of 2011 samples. As far as we know, this is the first report of TDBPP in house dust, although we previously detected its mutagenic metabolite, 2,3-dibromo-1-propanol, in about 10% of indoor air samples from Cape Cod, MA (Rudel et al., 2003). Dust concentrations were much lower in 2011 (mean 40 ng/g; maximum 310 ng/g) compared with 2006 (mean 1,000 ng/g; maximum 8900 ng/g), though this may be due to whatever factor led to lower concentrations of legacy pollutants (see below).

We also detected 3 chlorinated PFRs: TCEP, TCPP, and TDCPP (chlorinated "Tris"), which are used in polyurethane foams as replacements for PentaBDE. TDCPP was voluntarily withdrawn from children's pajamas after metabolites 1,3-dichloro-2-propanone and 1,3-dichloro-2-propanol were found to be mutagenic (Gold et al., 1978). The Consumer Product Safety Commission (CPSC) said TDCPP was a potential hazard to consumers, based on cancer and non-cancer end points (US CPSC 2006). The CPSC estimate of children's exposure from treated furniture was 5× higher than the agency's acceptable daily intake, with most of the exposure from inhalation of the chemicals volatilised from treated furniture. TDCPP was the most commonly detected FR (36%) in a U.S. sample of child care products (Stapleton et al., 2011). Our reported concentrations of TDCPP comprise tris(1,3-dichloro-2-propyl)phosphate, which makes up approximately 90-95% of TDCPP, and tris(2,3-dichloropropyl)phosphate. Both TCEP and TDCPP are listed as carcinogens under California's Proposition 65. TCEP is slated to be banned from children's products in New York by

2014, and a bill is currently being considered that would expand the ban to TDCPP (NY Senate 2011). TCPP is structurally similar to TCEP.

Median concentrations of all chlorinated PFRs were above 1,000 ng/g, or 1 μg/g, in both sampling rounds, and maxima were >100,000 ng/g or 0.01%, making these the most abundant FRs in this study (Table 2.2.2). Levels in some homes changed dramatically. For example, between 2006 and 2011, one home with a new roof installed between sampling rounds had 20-fold increase in TCEP concentration and another home with substantial remodelling had a 14-fold increase in TDCPP. TCPP means (2006 mean 1,200 ng/g; 2011 mean 1,700 ng/g) and medians increased (Table 2.2.2), suggesting an increase in use between 2006 and 2011. People who reported new furniture between sampling rounds showed increases in TCPP concentrations (rho=0.6; p=0.02), suggesting that TCPP is a PentaBDE replacement (Van der Veen and de Boer 2012).

Based on limited comparison data, concentrations of chlorinated PFRs observed in this study are some of the highest in the world; only concentrations in Japan are consistently higher (Kanazawa et al., 2010). Generally lower levels have been reported for homes in Boston (Stapleton et al., 2009), Belgium (Van den Eede et al., 2011), Spain (Garcia et al., 2007), Sweden (Bergh et al., 2011), and Germany (Ingerowski et al., 2001), except for higher TCPP in Spain (Garcia et al., 2007) and TDCPP in Sweden (Bergh et al., 2011). The highest concentrations of chlorinated PFRs were found in a study of 41 Japanese homes, which reported median concentrations 2- to 25-fold higher than seen in our California samples (Kanazawa et al., 2010). The levels in Japan are likely a result of a voluntary phase-out of PentaPBDE in the early 1990s (Saito et al., 2007).

TDCPP concentrations were correlated across sampling rounds (tau=0.54). Concentrations of the chlorinated PFRs are not correlated with each other, likely because TCPP has been reported as a replacement for TCEP and is often used in the same types of products as TDCPP, which is typically used only when a more efficient FR is needed, since it is more expensive (van der Veen and de Boer 2012).

Non-halogenated PFRs. Non-halogenated PFRs are used as FRs and often as plasticisers. We analysed 8 non-halogenated PFRs. The highest concentrations were for TBOEP, used as FR as well as in antifoam agents, floor polish, lacquers, plastics, rubbers and solvents (van der Veen and de Boer 2012). It had the highest median concentration, two-fold higher than the next highest, of any analyte (2006: 12,000 ng/g; 2011: 11,000 ng/g) and the highest concentration of any analyte in 2011 (170,000 ng/g). In addition to TBOEP, we detected EHDPhP and TCP (sum of 4 isomers)–used in hydraulic fluids and PVC–in all samples. TBOEP and TCP generally decreased, whereas EHDPhP concentrations generally increased between sampling events (Figure 2.2.1).

Concentrations of TBOEP were higher than in dust samples collected in Belgian and Spanish homes, although lower than Japanese homes (1,570,000 ng/g) (Van den Eede et al., 2011; Kanazawa et al., 2010; Garcia et al., 2007). TCP concentrations were higher than those found in Belgian homes

(Van den Eede et al., 2011). Concentrations of several non-halogenated PFRs (TBOEP, TEP, TnBP, and TCP) were correlated across sampling rounds (tau=0.37-0.69), indicating that these compounds have temporal stability.

Dechlorane-plus. Dechlorane-plus (DP), a chlorinated FR, is used in electronics and is an alternative to DecaBDE. It is pervasive in the environment and has high potential for long-range transport (Sverko et al., 2011; Xian et al., 2011). DP, measured as two isomers (syn and anti), was detected in all of the homes, although levels were lower than other FRs in this study and may have decreased over time. Total DP concentrations were generally lower than those reported in Ottawa in 2002-2003 and 2007 samples (Zhu et al., 2007), whereas concentrations of individual isomers are comparable to those reported in Vancouver in 2007-2008 (Shoeib et al., 2012).

Legacy chemicals. To evaluate whether our dust collection methods produced consistent results between the two sampling rounds, we analysed samples for several legacy compounds that were banned years ago. These chemicals would not be introduced in new products between sampling rounds, though they could possibly increase or decrease with a change in an old item. Despite being banned for many years, legacy compounds were frequently detected. PCBs, chlordane, and DDT were detected in almost all homes, with DDT at the highest concentration (2006 median 530 ng/g; 2011 median 160 ng/g). Polybrominated biphenyls (PBBs) were infrequently detected except for congener BB 153, which was detected in about half of the homes. Concentrations of legacy chemicals were generally significantly correlated across sampling rounds, indicating that the rank order was consistent over 5 years. However, the average concentration ratio (2011/2006) was 0.8, which means that 2006 concentrations were generally higher than 2011 concentrations. This may be due to degradation or depletion. However, it may also be due to some unidentified but systematic difference in sample collection between the two sampling rounds, which could also influence results for other chemicals. For example, PentaBDE levels went down between 2006 and 2011, which may reflect decreasing use or may simply be due to the same factor causing decrease in legacy pollutant concentrations. In light of this, the Firemaster 550 increase may be underestimated. Two homes had substantial (10-30×) decreases in DDT and DDD; one of these homes had significant renovations between rounds, while no explanation was identified for the other household.

2.2.3.2. Co-occurrence of Flame Retardants

We were interested in learning which FRs co-occurred, suggesting common sources, so we conducted correlation analysis for analytes within each sampling round, and also used these correlation estimates in cluster analysis to visualize relationships. As expected, many compounds known to co-occur in commercial formulations were correlated in both rounds. We saw strong

correlations for: PBDE congeners comprising the PentaBDE and OctaPBDE mixtures, DDT and its breakdown products, the legacy pesticides *cis*- and *trans*-chlordane and *trans*-nonachlor, PCB 153 and PCB 180, and the DP isomers. Interestingly, the brominated Firemaster 550 chemicals, EH-TBB and BEHTBP, were also clustered consistently, but the third Firemaster 550 constituent, TPhP, did not cluster with them, suggesting other sources. TPhP was correlated with TDCPP and PentaBDE congeners in 2006 samples. TPhP has reportedly been used in PentaBDE commercial mixture (van der Veen and de Boer 2012).

2.2.3.3. Limitations

As far as we know, this is the first study to analyse for such a broad range of FRs in house dust and to analyse samples collected in the same home at two different time periods. This design allowed us to evaluate time trends in concentrations; however, rigorous longitudinal analysis was not possible due to the small sample size (n=16 pairs). The sample size also limits assessment of generalizability of our findings. Since our study began in 2006, we did not fully capture the effects of the 2004 PBDE phase-out, and although many participants reported some changes in their homes over the 5 year period, larger differences in FR concentration might be seen in a longer study. We observed differences in concentrations in many homes that reported acquiring furniture, carpets, and electronics; however, our ability to link chemical concentrations with characteristics of products and residences was limited, because our questionnaire relied on residents' recollections. Residents may have introduced additional chemical sources that were not identified by our questionnaire, removed major sources without replacing them with new items, or failed to report on changes that we did ask about. These limitations raise cautions about relying on questionnaires to classify FR exposures. Finally, while our analyte list is extensive, it is not exhaustive. There are probably additional FRs used in consumer products that are not included because they have not been disclosed by manufacturers.

2.2.3.4. FR Burden in California Homes

We found that PBDEs; components of Firemaster 550; other BFRs, such as HBCDD, TBBPA, BTBPE, DBDPE; and PFRs, including the carcinogenic TCEP and TDCPP, were abundant and commonly detected, and we hypothesize that they are likely to be found in nearly all California homes. In our study, the levels of individual FRs in dust exceeded 0.01%, with a cumulative level of all FRs almost 0.03% in one home. Such concentration of FRs in dust is expected to lead to 30 μg/day FR ingestion in a typical child. The average total load of FRs in house dust was approximately 80 to 90 μg/g.

For six chemicals, dust concentrations exceeded risk-based screening levels for residential soil (US EPA 2012) in at least one of the homes, indicating exposure is potentially of health concern. Specifically, concentrations of BDE 47, BDE 99, TCEP, TDCPP, and DDT exceed screening levels,

with 13 of 16 homes exceeding at least one chemical screening level in either sampling round. Exposure pathways for residential soil are similar to house dust.

Our previous work showed that elevated PentaBDE levels in California house dust and serum are likely the result of the state's unique furniture flammability standard (Zota et al., 2008). The present study shows California homes still have higher levels of PentaBDEs than the rest of the world and that California also has some of the highest concentrations of halogenated PFRs, which are also used in furniture foam. The only location with consistently higher PFR concentrations is Japan, where the elevated PFRs levels are likely due to the early phase-out of PentaBDE almost 20 years ago (Saito et al., 2007). PFR levels in Japan may foreshadow levels in California.

We also observed that Firemaster 550 concentrations are increasing in California homes, suggesting that Firemaster 550 is being used as a replacement for PentaBDE, which was phased-out in 2004, shortly before our first sample collection. Continued monitoring in California and other locations is warranted because we anticipate levels will continue to increase unless manufacturing practices change.

2.3. Occurrence of a broad range of legacy and emerging flame retardants in indoor environments in Norway

Based on the following publication:

Cequier, E., Ionas, A.C., Covaci, A., Marcé, R.M., Becher, G., Thomsen, C., 2014. Occurrence of a broad range of legacy and emerging flame retardants in indoor environments in Norway. Environ. Sci. Technol. 48, 6827–6835.

2.3.1. Introduction

To determine the level of FR contamination of an indoor environment, samples of air and dust from that environment can be collected and analysed. Different strategies have been assessed in order to collect representative samples from various microenvironments: dust from vacuum cleaner bags (Johnson-Restrepo et al., 2009; Marklund et al., 2003), collected dust from a delimited floor area (Batterman et al., 2010; Muenhor et al., 2010; van den Eede et al., 2011) or the entire floor area (Allen et al., 2008; Takigami et al., 2009) and dust from elevated surfaces (Bergh et al., 2011) using nylon socks (Harrad et al., 2008) or forensic filters (Björklund et al., 2012) with a vacuum cleaner. However, there is no further assessment of which type of dust is more representative to evaluate the indoor exposure. For air sampling, passive samplers have been employed (Muenhor et al., 2010; Takigami et al., 2009) although most studies use active samplers (pumps). Typically, polyurethane foam (PUF) has been used to trap volatile PBDEs (Fromme et al., 2009) and PFRs (Hartmann et al., 2004) from air, while polytetrafluoroethylene (Batterman et al., 2010) or glass fiber (Björklund et al., 2012) filters have been used to separate the FRs adsorbed onto suspended particles.

Some studies point out that indoor dust is a major source for human exposure to PBDEs (Johnson-Restrepo et al., 2009; Dodson et al., 2012; Allen et al., 2007; Harrad et al., 2010) and PFRs (Takigami et al., 2009). It is likely that this is also valid for other FRs used in consumer products. Nevertheless, the daily intakes of PBDEs from dust reported in several studies are lower than the oral reference dose (RfD) established by the U.S. Environmental Protection Agency (USEPA) (Harrad et al., 2008; Chen et al., 2010) and PFRs and plasticisers present the same tendency in different risk assessment studies (van den Eede et al., 2011; Hartmann et al., 2004). However, in highly contaminated environments, and in particular for small children, for whom the dust intake rates can be high, this exposure can potentially be hazardous (Dodson et al., 2012).

The objectives of this study were: 1) to undertake a comprehensive monitoring of a wide range of BFRs, PFRs and dechloranes in indoor air and dust from 48 Norwegian households and two schools, 2) to explore the sources of exposure to these FRs in the households and 3) to assess the total intake of FRs from indoor environments for the mothers and their children living in the household. In addition, we attempted to explore two novel objectives 4) to compare the contents of FRs in settled

dust from elevated surfaces with floor dust collected from the same residences and 5) to investigate the feasibility of estimating FR concentrations in air from measured concentrations from dust, or vice versa.

2.3.2 Materials and methods

2.3.2.1. Chemicals and reagents

The organophosphate standards TnBP, TPhP, TDCPP (2 isomers), TCEP and TCP (4 isomers)) were purchased from Chiron A.S. (Trondheim, Norway). TPhP-d15 and TBOEP were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). TCPP (3 isomers) and TAP were purchased from Pfaltz & Bauer (Waterbury, CTY, U.S.) and TCI Europe (Zwijndrecht, Belgium), respectively. TCEP-d12, TDCPP-d15 and TBOEP-d6 were synthesised by Dr. Vladimir Belov. Standard reference material (SRM2585) was purchased from U.S. National Institute of Standards and Technology (NIST, Gaithersburg, MD, U.S.A.). ^{13}C labelled and native HBB and Dechlorane Plus® (DP; syn- and anti-isomers) were purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.), Dechlorane 602 and Dechlorane 603 (both powder) were purchased from Toronto Research Chemical Inc. (North York, Ontario, Canada) and PBDEs (except BDE-77 and BDE-128, purchased from AccuStandard Inc. (NewHaven, CT, U.S.A.)). EH-TBB, BEHTBP, DBDPE, TBECH, ATE, BATE, DPTE, BTBPE, HCDBCO, TBCO, OBIND, TBX, PBT, PBBz, PBEB and PBBzA were purchased from Wellington Laboratories (Guelph, ON, Canada). All purities were higher than 99%, except TBOEP and Dec602 > 94%.

Solvents were of analytical grade. Hexane was purchased from Acros Organics (Geel, Belgium). Iso-octane, toluene, dichloromethane, ethyl acetate and acetone were purchased from Merck Chemicals (Darmstadt, Germany).

2.3.2.2. Sample collection

Forty eight households and 6 classrooms from two primary schools in the greater Oslo area (Norway) were selected for the study. Collection of dust and air from the indoor environments was performed between January and May 2012. Participants were visited twice on consecutive days; the first day pumps were deployed for air collection and the second day dust samples were taken. The temperature and humidity of the rooms were recorded when sampling. During the first day, participants answered a questionnaire regarding characteristics of the household, such as information on building and consumer goods. Considered demographic information from the participants was age, height and weight. Parameters of the households considered in the questionnaires were: age of the women, construction year of the building, years lived in the household, number of inhabitants in the household, size of the apartment in m^2, size of the living room in m^2, number of cathode ray tube TVs in the household, number of flat screen type TVs, number of DVD and Video players, number of

consoles, number of CD and MP3 players, number of PCs, number of laptops, number of radios, number of telephones, total electronic devices in the household, number of vacuum cleaning in the living room per week, distance of the sampling equipment from TV in the living room (m), humidity in the living room (%), temperature in the living room (°C), location of the house (Rural/Urban), type of household (non-attached/attached), electric heating system (no/yes), fireplace in the living room (no/yes), carpets in the living room (no/yes), chairs made of PUF in the living room (no/yes), renovation of areas of the house in the last 5 years (no/yes). Floor materials and habit of smoking were not assessed because 100% of the participants presented the same conditions (wooden floor and no smoking).

Indoor air. Air sampling was carried out during winter and partially in spring in order to have minimal ventilation in living rooms and classrooms during the sampling period. Air from the living room of 47 households (in one the pump failed) and the classrooms (n=6) were sampled using a Leland Legacy pump (SKC Limited, Dorset, U.K.) at a flow rate of 12 L/min for 24 hours. Four stainless steel holders containing each one quartz filter and two cylinders of PUF (1 cm diameter and 1.5 cm length) hung from a tripod pointing downwards at approximately 1.2 m height. All holders were connected to the same pump with Tygon® R3603 tubing (VWR International, Vienna, Austria). The total volume of air drawn was approximately 17 m^3. Before sampling, holders were thoroughly cleaned with dichloromethane, PUFs were pre-cleaned with toluene for 24 hours in a Soxhlet system and subsequently with acetone for 48 hours. Finally, PUFs were dried in an oven at 50°C and stored in amber glass bottles. Quartz filters were baked at 450°C overnight and wrapped in aluminum foil after cooling.

Indoor dust. Collection of dust was performed using forensic filters (KTM Krim. Teknisk Materiel AB, Bålsta, Sweden) coupled to a GM80 vacuum cleaner (Nilfisk, Morgantown, WV, U.S.A.). Floor dust was collected using a nozzle with a polyethylene grid (1-3 mm pore size) and settled dust was collected without the grid. Settled dust (n=12) was taken from elevated surfaces in the living rooms (>40 mg). Floor dust was collected from the available floor in the living rooms or classrooms (>100 mg). Large particles and hair were removed with stainless steel tweezers. The dust was kept on the forensic filters, wrapped in aluminum foil, put into a low density polyethylene sealed bag and stored in the freezer at -20°C.

2.3.2.3. Selection of flame retardants

Organic brominated, chlorinated and phosphorous-based FRs were chosen for the study of indoor environments due to their proven ubiquity and potential hazard for human health (Cooper et

al., 2011; van der Veen et al., 2012). Table 2.3.1 summarizes the FRs and their abbreviations, as well as the method limits of detection (LOD) and the octanol-air partition coefficients (K_{oa}).

Table 2.3.1: Abbreviations and [a]physico-chemical properties of flame retardants in this study

Abbreviation (previously used)	**Vapour pressure (Torr)**	**Log [b]K_{ow}**	**[c]Air LOD (pg/m^3)**	**[c]Dust LOD (ng/g)**
[d]TBECH	2.2 E-5	4.82	0.88	0.20
[d]TBCO	3.6E-5	5.28	nd	nd
ATE	1.8 E-4	5.04	3.5	nd
TBX	4.4E-5	6.20	1.2	0.27
(PBBz)	9.3E-6	6.44	1.5	0.20
PBT	4.5E-6	6.25	0.59	0.20
PBEB	1.2E-6	6.76	0.59	0.13
DPTE	9.5E-8	5.82	1.2	0.20
EH-TBB	2.8E-9	7.73	7.4	2.0
BEHTBP	1.2E-13	9.34	2.9	4.0
DBDPE	6.0E-15	11.1	14	12
HBB	8.5E-7	6.11	2.9	0.67
[d]DP (DP)	1.0E-13	10.12	2.1 syn 4.7 anti	0.47syn 1.1anti
PBDE	6.7E-7 4.7E-12	5.8 12.11	0.59-1.8	0.13-0.47
HCDBCO	6.2E-9	7.62	nd	nd
BTBPE	2.9E-12	8.31	nd	0.93
Dec 602	1.1E-11	8.3	nd	nd
Dec 603	6.9E-12	9.14	nd	nd
OBIND	1.3E-14		nd	nd
(PBBzA)	1.3E-7	6.89	nd	0.53
(BATE)			nd	nd
[f]TnBP (TiBP)	1.13E-03	4.0	29	37
TCEP	1.08E-4	1.47	29	32
[g]TCPP	2.02E-05	2.59	29	5.3
[f]TBOEP (TBOEP)	2.50E-08	3.75	44	22
TPhP (TPhP)	6.28E-06	4.59	18	2.7
(EHDPhP)	2.55E-6	6.64	18	3.7
([h]TCP)	6.00E-07	5.11	35	64
[d]TDCPP ([e]TAP)	4.07E-8	3.27	18	7.6

[a] Collected from different sources (EPISuite® and Chemdraw software, material safety data sheets, etc); [b] K_{ow} = octanol water partition coefficient; [c]LOD obtained using 17 m^3 for air and 0.075 g for dust; [d] mixture of two isomers, [e] internal standard; [f] used as a plasticiser; [g] Mixture of 3 isomers; [h] Mixture of 4 isomers; nd, not detected

2.3.2.4. Analytical method and quality control

The analytical method for the analysis of the dust has been published elsewhere (van den Eede et al., 2012), and the same method, with slight modifications, was also applied to the air samples. Briefly, 40 to 75 mg of non-sieved dust or one air sample consisting of 8 PUFs and 4 quartz filters were placed in 25 mL glass tubes. Amounts ranging from 1.25 to 150 ng of internal standards were added (^{13}C-HBB, ^{13}C-syn-DP, ^{13}C-anti-DP, ^{13}C-BDE-209, BDE-77, BDE-128, TCEP-d12, TPhP-d15, TBOEP-d6, TDCPP-d15 and triamyl phosphate (TAP)). The dust and air samples were extracted 3 times (1 min vortex and 10 min sonication) using 2 and 8 mL, respectively of hexane/acetone (3:1; v/v). The supernatant was collected and evaporated to near dryness. One mL of

hexane was added and fractionation of FRs was performed on Florisil® (500 mg, 3 mL, Supelco, Bellefonte, PA, U.S.A.). The first fraction containing BFRs and dechloranes, was obtained by elution with 8 mL of hexane. The second fraction containing PFRs, BEHTBP, PBBzA and partially BTBPE and EH-TBB, was obtained by elution with 10 mL of ethyl acetate. BDE-128 was added to this fraction for quantification of the BFRs. Fraction 1 was further purified using a SPE cartridge loaded with approximately 600 mg of silica/sulphuric acid 44% (w/v). The analytes were eluted with 10 mL of hexane/dichloromethane (1:1; v/v). Finally, both fractions were evaporated and the first fraction was reconstituted with 100 µL of *iso*-octane and the second fraction with 100 µL of *iso*-octane/ethyl acetate (1:1; v/v).

BFRs and dechloranes were determined on a GC-ECNI/MS (Agilent Technologies, Santa Clara, CA, U.S.A.) using a DB5-MS column (15 m x 0.25 mm x 0.1 µm; Agilent Technologies). PFRs were determined on a GC-EI/MS (Agilent Technologies) using a HT-8 column (25 m x 0.22 mm x 0.25 µm; SGE Analytical Science Pty. Ltd., Victoria, Australia).

Air and dust were analysed in batches of 20 samples, together with 2 procedural blanks and 2 field blanks which consisted of holders with quartz filters and PUFs for air samples and cellulose filters for dust samples. Both field blanks followed the same procedure as the samples, but without drawing air or vacuuming, respectively. Quality control of the analytical method was performed by analysis of 5 replicates of SRM2585 (1 replicate/batch). The accuracy for the determination of PBDEs in the certified SRM2585 ranged from 94 to 112% (mean 102%; RSD 6%) and the accuracy for PFRs with respect to reference values (van den Eede et al., 2011) from SRM2585 ranged from 78 to 107% (mean 95%; RSD 12%; excluding TCP) (Table 2.3.2).

Table 2.3.2: Accuracy of PBDEs and PFRs in certified dust SRM2585 (n=5)

	Mean (ng/g)	RSD (%)	Certified value	Accuracy %
PBDEs				
BDE-28	41.7	6.6	46.9	112
BDE-47	502	9.2	497	99
BDE-85	142	8.3	145	102
BDE-99	891	8.6	892	100
BDE-100	38.8	8.8	43.8	113
BDE-153	88.7	8.1	83.5	94
BDE-154	123	9.4	119	97
BDE-183	43.9	18	43.0	98
BDE-209	2456	22	2510	102
	Mean (ng/g)	RSD (%)	[a]Reference value	Accuracy %
PFRs				
[b]TnBP	197	14	180	91
TCEP	899	14	700	78
TCPP	1063	11	820	94
[b]TBOEP	45795	7	49000	107
TPhP	1052	13	990	94
TDCPP	1933	14	2020	105
TCP	nd		1070	

[a] van den Eede *et al.* (2011); [b]Main use as plasticiser

Field blanks showed higher background contamination of FRs than procedural blanks. Therefore, when FRs were detected in more than 50% of the field blanks, mean blank values were subtracted from the content of the sample (Table 2.3.3).

Table 2.3.3: Amounts of FRs (ng) and RSD (%) in parentheses in the air and dust field and procedural blanks.

	Air blanks			**Dust blanks**		
	Field (n=13)	[a]Conc. (ng/m^3)	Procedural (n=10)	Field (n=8)	[a]Conc. (ng/g)	Procedural (n=10)
BFRs						
PBBz	0.006 (65)	$3.53x10^{-4}$	0.011 (27)	0.015 (81)	0.2	0.007 (45)
HBB	0.048 (61)	$2.82x10^{-3}$	0.061 (12)	0.064 (15)	0.853	0.062 (10)
BDE-85	0.003 (117)	$1.76x10^{-4}$	0.008 (19)	0.004 (121)	0.053	0.005 (94)
BDE-209	0.745 (64)	0.044	0.231 (85)	1.14 (115)	15.2	0.586 (108)
BDE-99				0.016 (127)	0.213	
DBDPE				1.08 (133)	14.4	
PFRs						
TnBP	2.58 (309)	0.152	0.394 (128)	3.1 (12)	41	1.89 (96)
TCPP	9.33 (99)	0.549	0.520 (58)	11.3 (169)	150	
TCEP	3.70 (81)	0.218		8.44 (60)	112	
TBOEP	1.24 (111)	0.073		71.08 (166)	947	
TPhP	1.42 (170)	0.083		0.340 (194)	4.53	
EHDPhP	6.40 (94)	0.376		0.201 (150)	2.68	
TCP	0.406 (158)	0.024				

[a]Reference concentration of field blanks using 17 m^3 for air and 0.075 g for dust.

Recoveries of the internal standards of BFRs and dechloranes from air ranged from 43% to 83% (mean 68%, RSD 16%; n=12) and from dust from 75% to 111% (mean 89%; RSD 12%; n=8). Recoveries of internal standards of PFRs (except TBOEP-d6) from air ranged from 75% to 95% (mean 82%, RSD 12%; n=11) and from dust from 74% to 100% (mean 88%; RSD 13%; n=11). The recoveries of TBOEP-d6 (n=11) in air and dust were 172% (RSD 15%) and 268% (RSD 27%), respectively (Table 2.3.4).

Table 2.3.4: Recoveries of internal standards in dust and air samples calculated employing CB-207 as recovery standard

	[a]Air sample		**Dust sample**	
Internal standard	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
BFRs and dechloranes	n = 12		n = 8	
BDE-77 (2 ng)	78	6	85	11
BDE-128 (2 ng)	77	6	98	13
^{13}C-BDE-209 (37.5 ng)	55	14	111	15
^{13}C-HBB (1.25 ng)	43	27	79	11
^{13}C-syn-DP (1.25 ng)	72	25	75	13
^{13}C-anti-DP (1.25 ng)	83	22	88	11
PFRs	n = 11		n = 11	
TAP (75 ng)	75	6	86	22
TCEP-d12 (75 ng)	79	8	100	24
TBOEP-d6 (150 ng)	172	15	268	27
TPhP-d15 (75 ng)	77	7	74	27
TDCPP-d15 (75 ng)	95	8	92	25

[a]Extraction of analytes from spiked PUF

The LOD was defined as S/N≥3 and determined experimentally. LOD for BFRs in air and dust samples ranged from 0.59 to 14 pg/m^3 and from 0.13 to 12 ng/g. LOD for PFRs in air and dust samples ranged from 18 to 44 pg/m^3 and from 2.7 to 64 ng/g (Table 2.3.1).

2.3.2.5. Statistical analysis

SPSS v.20 (Chicago, IL, U.S.A.) was used to perform the statistical analyses for compounds with detection frequencies ≥40% (minimum of 19 samples). Non-detects were replaced by half of the LOD. An independent t-test was performed to compare the median concentrations of FRs in households and schools, as well as in floor and settled dust. Differences in the medians were considered statistically significant at *p*-values <0.05.

Concentrations of the analytes were log transformed to achieve normal distributions (Shapiro-Wilk test), but this was only the case for 47% of the FRs. Therefore, Spearman rank correlation was employed to calculate associations between FRs, and FRs and indoor parameters. Correlations between FRs were statistically significant when *p*-values were <0.05. To feed the multiple linear regression analysis with the most relevant indoor parameters, a Spearman rank correlation test was performed between the log transformed concentrations of FRs and indoor parameters accepting *p*-values <0.15. Finally, only those multiple linear regression models having two or more significant parameters were discussed.

2.3.3. Results and discussion

The concentrations of FRs in dust and air (sum of FRs in the gas phase and in the suspended particles) have been expressed on weight basis for easy comparison with other studies, despite the large differences in molecular weights of the target analytes.

2.3.3.1. Air samples

Twenty nine out of the 37 measured FRs were detected in indoor air samples from households and schools, of which 13 and 12, respectively were detected in at least 90% of the samples (Table 2.3.5).

BFRs. In residential living rooms and primary school classrooms, 4-(1,2-dibromoethyl)-1,2-dibromocyclohexane (TBECH), pentabromotoluene (PBT), BDE-28, 47, 99 and 100 were detected in at least 96% of the samples. In both indoor environments, the highest median concentrations were for BDE-47 (128 pg/m^3 and 131 pg/m^3, respectively) followed by TBECH (77.9 pg/m^3 and 46.6 pg/m^3, respectively) and BDE-99 (21 pg/m^3 and 23 pg/m^3, respectively). In the living rooms, PBEB and ATE were detected frequently (>50%), while in the classrooms both medians were below LOD. Furthermore, in living rooms BDE-209 presented a low median (3.76 pg/m^3), but a maximum

concentration of 4150 pg/m^3, whereas in the classrooms median of BDE-209 was below LOD and maximum was 101 pg/m^3. The reason for the detection of a compound with such a low vapor pressure in the gas phase is because BDE-209 is adsorbed to the fine particles suspended in the air (Webster et al., 2009). Those particles are retained on the glass filter which was analysed as part of the air sample.

Table 2.3.5: FR concentrations, detection frequency (% detect) and percentage of total mass of FRs (% mass) in air samples from households and primary schools from Norway

	Residential living rooms (n = 47)					School classrooms (n = 6)				
	Median	Mean	Max.	%detect	% mass	Median	Mean	Max.	%detect	% mass
BFRs and dechloranes (pg/m^3)										
TBECH	80	220	4120	100	28	45	100	400	100	19
[a]ATE	5	5	70	70	2	<LOD	<LOD	3	0	<1
[b]TBX	<LOD	65	2830	38	<1	<LOD	<LOD	3	17	<1
[c]PBBz	5	10	50	100	2	2	3	5	83	1
[d]PBT	10	15	210	100	3	3	3	4	100	1
[e]PBEB	1	1	30	45	<1	<LOD	<LOD	<LOD	0	0
[f]DPTE	1	5	130	40	<1	1	3	10	50	<1
BEHTBP	<LOD	2	25	19	<1	<LOD	2	5	33	<1
DBDPE	<LOD	40	960	47	<1	10	10	20	50	3
HBB	4	10	300	70	3	4	4	5	83	4
Syn-DP	<LOD	0.2	5	2	<1	<LOD	<LOD	<LOD	0	0
Anti-DP	<LOD	0.3	10	4	<1	<LOD	<LOD	<LOD	0	0
BDE-28	10	10	55	98	3	5	10	20	100	2
BDE-47	130	180	720	100	46	130	180	460	100	55
BDE-100	5	10	85	96	2	10	10	20	100	3
BDE-99	20	40	410	100	8	25	25	50	100	10
BDE-85	1	1	20	49	<1	1	1	1	50	<1
BDE-154	0.4	2	60	15	<1	1	1	1	17	<1
BDE-153	1	10	260	81	<1	1	1	2	67	<1
BDE-183	<LOD	10	530	6	<1	<LOD	<LOD	<LOD	0	0
BDE-209	4	320	4150	51	1	<LOD	25	100	33	1
$\sum_8$PBDEs	170	260	2100			180	220	560		
PFRs (ng/m^3)										
[g]TnBP	5	10	120	100	10	3	3	5	100	13
TCEP	2	3	10	98	4	4	5	20	100	16
TCPP	40	85	460	100	83	10	10	25	100	42
[g]TBOEP	1	1	20	100	1	15	20	50	100	29
TPhP	0.3	0.4	2	89	1	0.1	0.1	0.2	100	<1
[h]EHDPhP	0.1	0.5	4	62	<1	0.1	1	5	67	<1
[i]TCP	<LOD	0.1	1	57	<1	<LOD	<LOD	<LOD	0	0
TDCPP	0.1	0.4	10	98	<1	0.05	0.1	0.1	100	<1

[a]2,4,6-tribromophenyl allyl ether; [b]1,2,4,5-tetrabromo-3,6-dimethylbenzene; [c]pentabromobenzene; [d]pentabromotoluene; [e]pentabromoethylbenzene; [f]2,4,6-tribromophenyl-2,3-dibromopropyl ether; [g]used as plasticiser; [h]2-ethylhexyl diphenyl phosphate; [i]tricresyl phosphate.

TBECH was the second most abundant FR in both indoor environments. There is no production of TBECH in Norway and, to the best of our knowledge; these are the highest concentrations of TBECH reported in indoor environments. As TBECH has been identified as a potential endocrine disruptor (Khalaf et al., 2009), the relatively high abundance in indoor air raises concern about this emerging FR. Another interesting finding is HBB, detected in this study with median concentrations around 4 pg/m^3 in households and schools. HBB is used in indoor materials and consumer goods (wood, textiles, electronic and plastics) (Cooper et al., 2011), and it has previously been detected in outdoor air in Norway as well (Arp et al., 2011).

In general, levels of non-PBDEs FRs in air were higher in households than in schools, although the statistical comparison (independent t-test) of the median concentrations did not show significant differences between these two indoor environments ($t(42)=0.185$, $p=0.854$). However, it must be kept in mind that sampling was performed in only 6 classrooms compared to 46 living rooms, which limits the statistical analysis.

Concentrations in Norwegian households were lower than levels reported in other studies from the U.S.A. (Michigan) (Batterman et al., 2010), comparable to Denmark (Vorkamp et al., 2011) and higher than in households in Sweden (Björklund et al., 2012) (Figure 2.3.1).

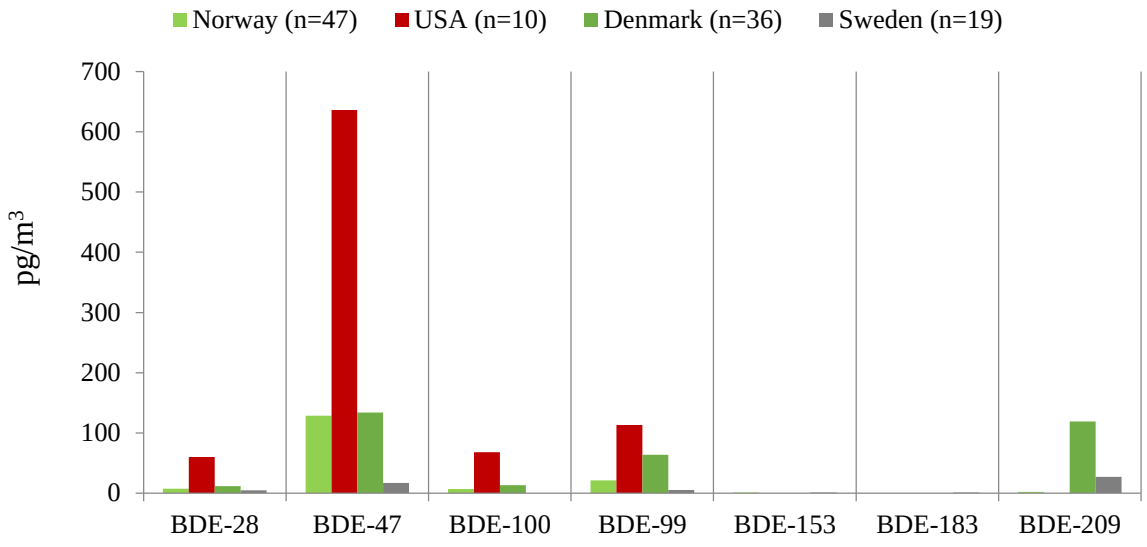

Figure 2.3.1: Comparison of levels of PBDEs in indoor air from studies conducted in Norway, U.S.A. (Michigan; offices), Denmark and Sweden.

PFRs. Overall concentrations of PFRs in air were around two orders of magnitude higher than BFRs (Figure 2.3.2), as expected due to the typically higher vapor pressures of PFRs (Table 2.3.1) and higher production volumes as well.

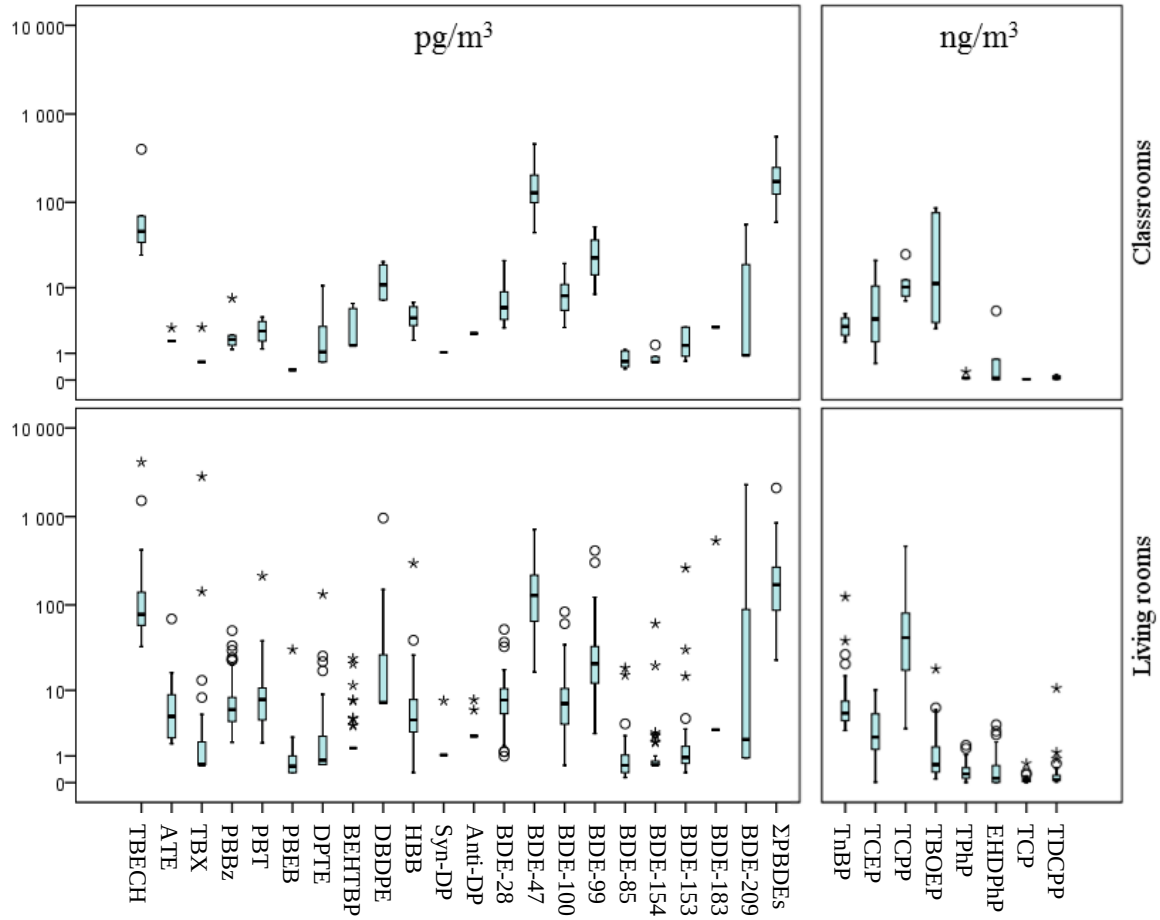

Figure 2.3.2: Box plots of air concentrations of the BFRs, dechloranes and PFRs in living rooms (n=47) and classrooms (n=6).

TBOEP was the most abundant PFR in classrooms with a median concentration of 12.9 ng/m^3, approximately 20 times higher than in the households. Such difference might be attributed to the use of TBOEP as an additive in floor polishing in the schools (van der Veen et al., 2012; Marklund et al., 2003). In living rooms, TCPP had the highest median concentration (42.3 ng/m^3), whereas in classrooms the median was 10.2 ng/m^3. Median concentrations of TnBP, TPhP, EHDPhP and TDCPP were higher in living rooms than in classrooms (Table 2.3.5). Nonetheless, PFRs were not statistically different in these two environments (t(8)=0.609, *p*=0.559).

Levels of PFRs in Norway are in the same range as those found in one study from Sweden (Bergh et al., 2011), except TCPP for which they were almost one order of magnitude higher in Norway. Nevertheless, the PFR concentrations were much lower than the levels detected in residential dwellings in Japan (Kanazawa et al., 2010) (TCPP 89.2 ng/m^3, TnBP 27.1 ng/m^3 and TCEP 15.5 ng/m^3) (Figure 2.3.3).

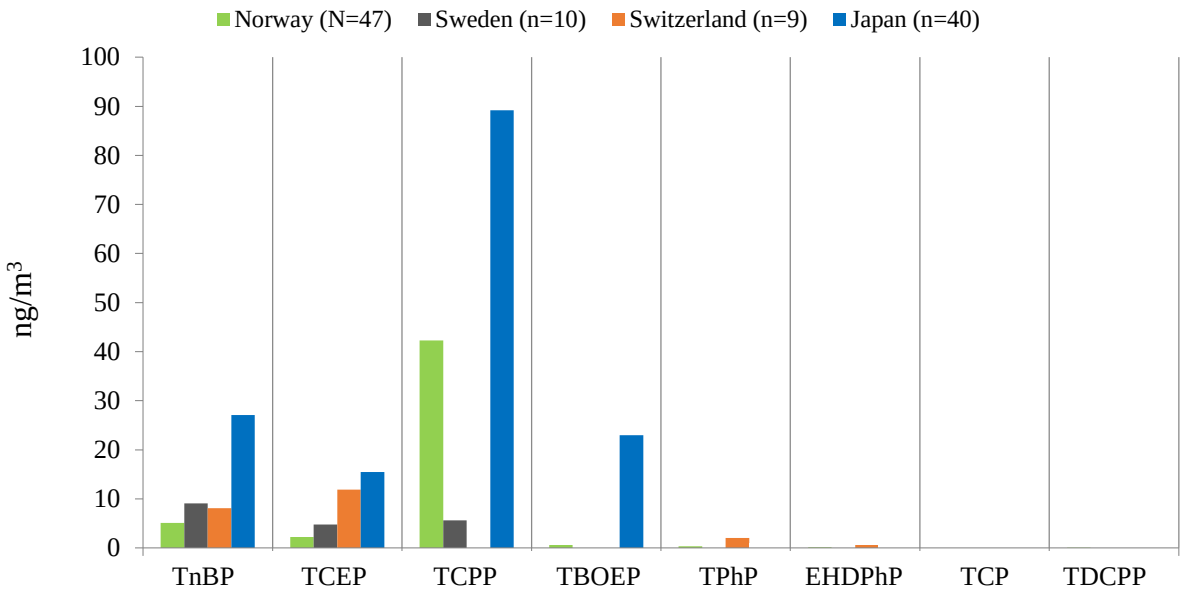

Figure 2.3.3: Comparison of concentrations of PFRs in indoor air from studies conducted in Norway, Sweden, Switzerland and Japan.

Correlations between FRs in air. Correlations among the PBDEs (BDE-28 up to BDE-153) ranged from 0.95 to 0.5 (p<0.01) indicating the presence of the banned PentaBDE formulation. PBT was also correlated (R>0.37; p<0.01) with the more volatile PBDEs (BDE-28, 47, 99 and 100). This association is likely to occur because these FRs have been used together in acrylonitrile butadiene styrene (ABS) polymer (Cooper et al., 2011; WHO 1992). Many other correlations were found between FRs, but it was difficult to assess whether the association was causal or accidental.

2.3.3.2. Dust samples

Thirty one out of the 37 target FRs were found in floor dust samples (Figure 2.3.5). In households and schools, 23 and 19 analytes, respectively were detected at least in 90% of the dust samples (Table 2.3.6).

BFRs and dechloranes. In living rooms, BDE-209 showed the highest median concentration (325 ng/g) followed by BDE-99 (171 ng/g), DBDPE (147 ng/g), BDE-47 (126 ng/g) and BEHTBP (78.5 ng/g). BDE-209 was also the most abundant BFR in classrooms, (507 ng/g), followed by DBDPE (156 ng/g) and BEHTBP (103 ng/g). These three median concentrations were higher in classrooms than in living rooms. In contrast, the median concentrations of BDE-47, 100, 99, 85, 153 and 154 in the living rooms were higher than in the classrooms. These differences suggest that the materials used in the classrooms contain lower amounts of PentaBDE than in the living rooms. Nevertheless, the overall mean concentrations of BFRs and dechloranes in dust between schools and households were not statistically different (t(40)=0.067; p=0.947).

Table 2.3.6: FR concentrations (ng/g), detection frequency (% detect) and percentage of total mass of FRs (% mass) in dust samples from households and primary schools from Norway

	Residential living rooms (n=48)					School classrooms (n=6)				
	Median	Mean	Max.	% detect	%mass	Median	Mean	Max.	% detect	%mass
BFRs and dechloranes										
TBECH	2	5	170	96	<1	2	3	10	100	<1
[a]TBX	<LOD	2	90	6	<1	<LOD	<LOD	<LOD	0	0
[b]PBBz	0	0	5	40	<1	0	0	1	50	<1
[c]PBT	1	1	15	94	<1	0	0	1	67	<1
[d]PBEB	<LOD	0	10	33	<1	<LOD	0	0	0	<1
[e]DPTE	1	2	20	69	<1	0	0	1	50	<1
[f]PBBzA	<LOD	0	10	13	<1	<LOD	<LOD	<LOD	0	0
EH-TBB	3	15	250	58	<1	3	3	5	67	<1
BEHTBP	80	130	810	100	8	100	100	150	100	11
BTBPE	4	10	40	92	<1	5	15	55	100	<1
DBDPE	150	510	4500	96	16	160	180	360	83	17
HBB	1	1	10	50	<1	1	2	5	67	<1
Syn-DP	1	10	310	92	<1	1	1	3	83	<1
Anti-DP	4	20	590	92	<1	3	4	10	100	<1
BDE-28	1	1	5	94	<1	0	0	1	100	<1
BDE-47	130	200	1500	100	13	45	60	200	100	5
BDE-100	35	60	440	98	4	10	10	20	100	<1
BDE-99	170	350	2600	98	18	40	45	95	100	5
BDE-85	10	20	120	98	1	4	5	15	100	<1
BDE-154	15	25	180	98	1	5	5	25	100	<1
BDE-153	25	40	250	98	3	10	10	35	100	1
BDE-183	3	20	270	94	<1	5	5	15	100	<1
BDE-209	330	6800	204000	98	34	510	1200	5300	100	56
$\sum_8$PBDEs	430	710	5100			170	150	290		
PFRs										
[g]TnBP	55	120	980	58	<1	45	45	750	50	<1
TCEP	410	800	4600	98	2	1200	2100	6200	100	1
TCPP	2700	5700	40100	100	14	2000	1900	2700	100	2
[g]TBOEP	1300	18700	128000	100	71	87200	89800	163000	100	91
TPhP	980	1200	4900	100	5	1500	2400	6200	100	2
[h]EHDPhP	620	880	5900	100	3	2300	1600	79000	100	2
[i]TCP	310	1100	16200	92	2	55	100	330	50	<1
TDCPP	500	800	6900	100	3	1500	2700	6100	100	2

[a]1,2,4,5-tetrabromo-3,6-dimethylbenzene; [b]pentabromobenzene; [c]pentabromotoluene; [d]pentabromoethylbenzene; [e]2,4,6-tribromophenyl-2,3-dibromopropyl ether; [f]pentabromobenzyl acrylate; [g]used as plasticiser; [h]2-ethylhexyl diphenyl phosphate; [i]tricresyl phosphate.

PBDE concentrations in dust from Norwegian households were much lower than in dust from U.S.A. (California) (Dodson et al., 2012), but higher than those reported in urban areas from China

(Zheng et al., 2011), Belgian homes and classrooms from the U.K. (Ali et al., 2011). The only exception was BDE-209 in the studies from U.K. and China, which showed the highest concentrations (5000 ng/g and 4040 ng/g, respectively). Levels of DBDPE were similar among the studies conducted in the mentioned countries with the exception of China where the average concentration of DBDPE was 20 times higher (2730 ng/g) (Figure 2.3.4a). The ratio of BDE-209 to DBDPE gives an indication of the level of replacement between BDE-209 and DBDPE. Interestingly, the lowest ratio was seen in the study from China (1.5) followed by Belgium (2.1), Norway (2.2), U.S.A. (California) (8.6) and U.K. (51).

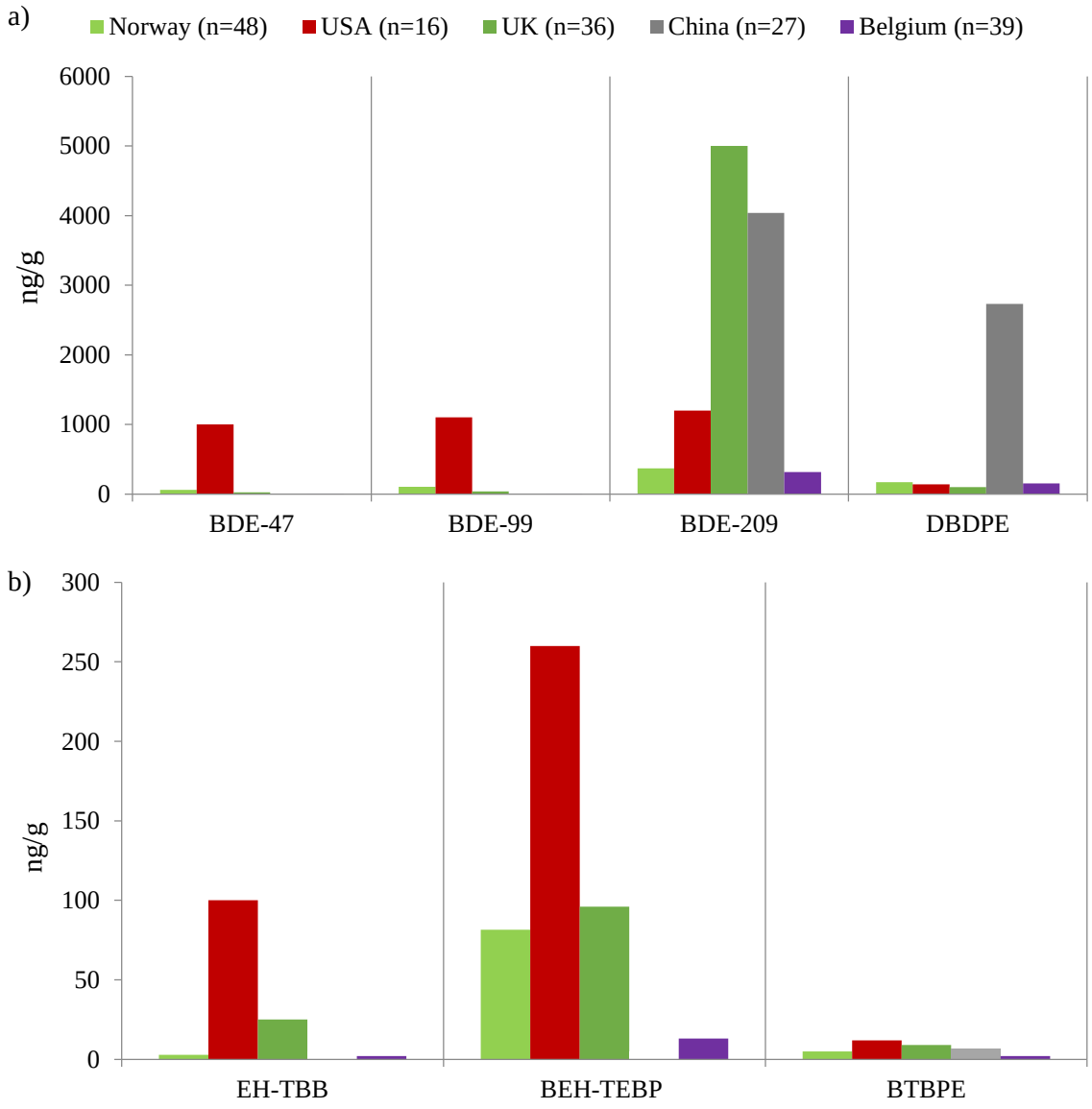

Figure 2.3.4: Comparison of levels of BFRs in indoor dust from studies conducted in Norway, U.S.A. (California), U.K., China and Belgium.

BEHTBP was present in Norwegian dust in comparable concentrations as in dust from other studies conducted in U.K. classrooms, higher than in households from Belgium (Ali et al., 2011) and much lower than in California (Dodson et al., 2012) (Figure 2.3.4b).

The high levels of EH-TBB and BEHTBP seen in the study from California are probably due to the replacement of the banned PentaBDE formulation with certain technical FR mixtures, such as the Firemaster 550, that contain EH-TBB in excess with respect to BEHTBP. Norwegian dust contained a substantial excess of BEHTBP. Therefore, assuming similar persistence of EH-TBB and BEHTBP in the indoor environment, the occurrence of BEHTBP in our study might also come from other sources than Firemaster commercial mixtures, which might reflect a different usage pattern of BEHTBP compared to the U.S.A. Considerable amounts of BTBPE (~5 ng/g), TBECH (~2 ng/g), EH-TBB (~3 ng/g) and DP (*anti*, ~3 ng/g and *syn*, ~1 ng/g) were detected in living rooms and classrooms (Figure 2.3.5). For DP, the fraction of the *anti* isomer was within the range reported for the technical mixture (0.65-0.75) (Sverko et al., 2011; Tomy et al., 2007), confirming the presence of Dechlorane Plus® in consumer products in Norway.

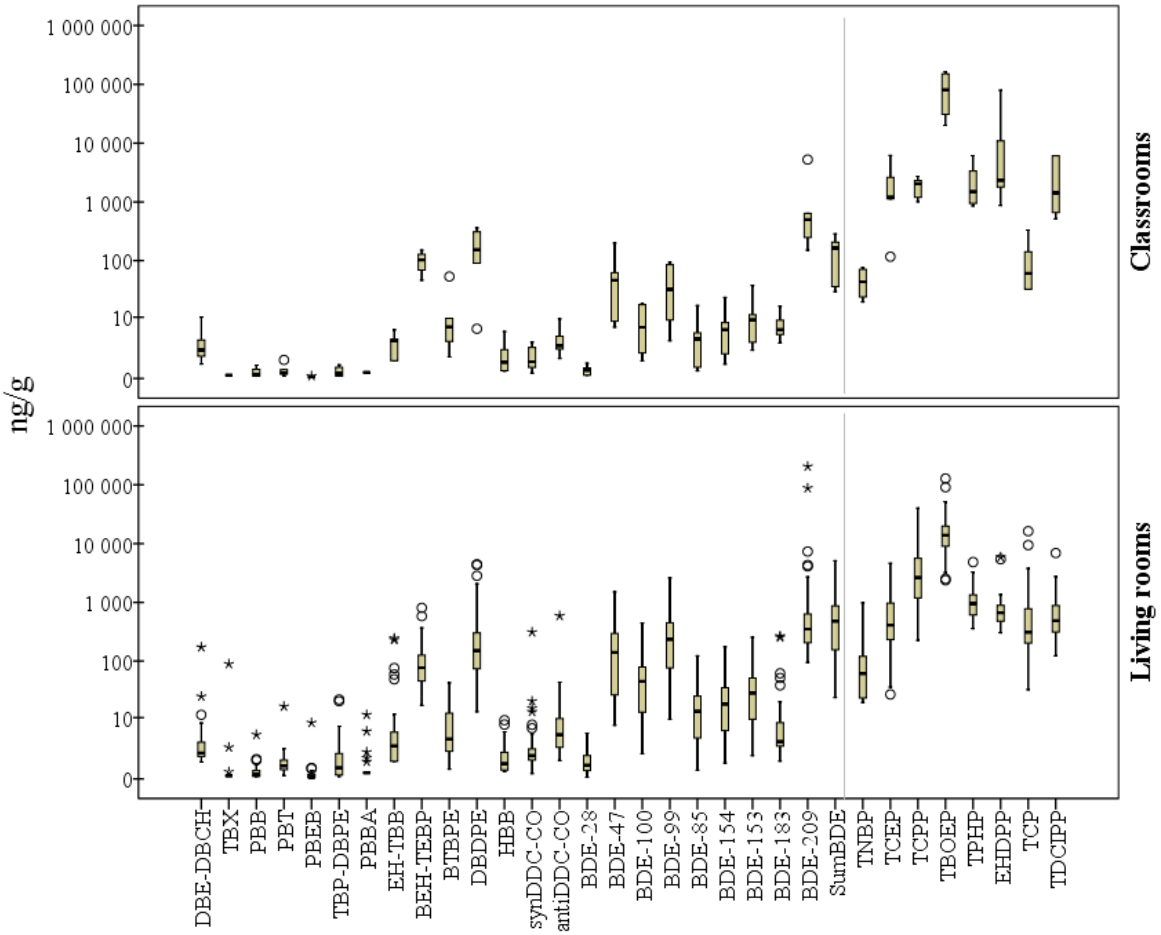

Figure 2.3.5: Box plots of dust concentrations (ng/g) of the BFRs, dechloranes and PFRs in living rooms (n=48) and classrooms (n=6). Y-axis is in log scale.

PFRs. The overall concentrations of PFRs in dust were approximately one order of magnitude higher than BFRs (Figure 2.3.5). TBOEP was detected at highest median concentrations in classrooms (87200 ng/g) and living rooms (13400 ng/g). The large amount of TBOEP found in dust from primary schools can be explained, as in the case of the air samples, by its probable use as an additive in floor polishing (van der Veen et al., 2012; Marklund et al., 2003). TCPP was the second most abundant PFRs in both environments with similar median concentrations (2680 ng/g and 2040 ng/g in households and classrooms, respectively). The median concentrations of EHDPhP, TDCPP, TPhP and TCEP were significantly higher in classrooms than in living rooms (Table 2.3.6) suggesting higher use of these FRs in materials from the school. Nonetheless, the PFRs concentrations between the two groups did not differ statistically ($t(7)=-0.964$, $p=0.366$).

Levels of TnBP, TCEP, TCPP, TPhP and TDCPP from dust of Norway were similar to other studies conducted in Belgium (van den Eede et al., 2011), Sweden (Bergh et al., 2011) or Spain (García et al., 2007) and only TBOEP and EHDPhP were slightly above the overall mean values. Nevertheless, all PFRs were found in much lower concentrations than in dust from a Japanese study (TBOEP and TCPP were 1570000 ng/g and 18700 ng/g, respectively) (Kanazawa et al., 2010) (Figure 2.3.6).

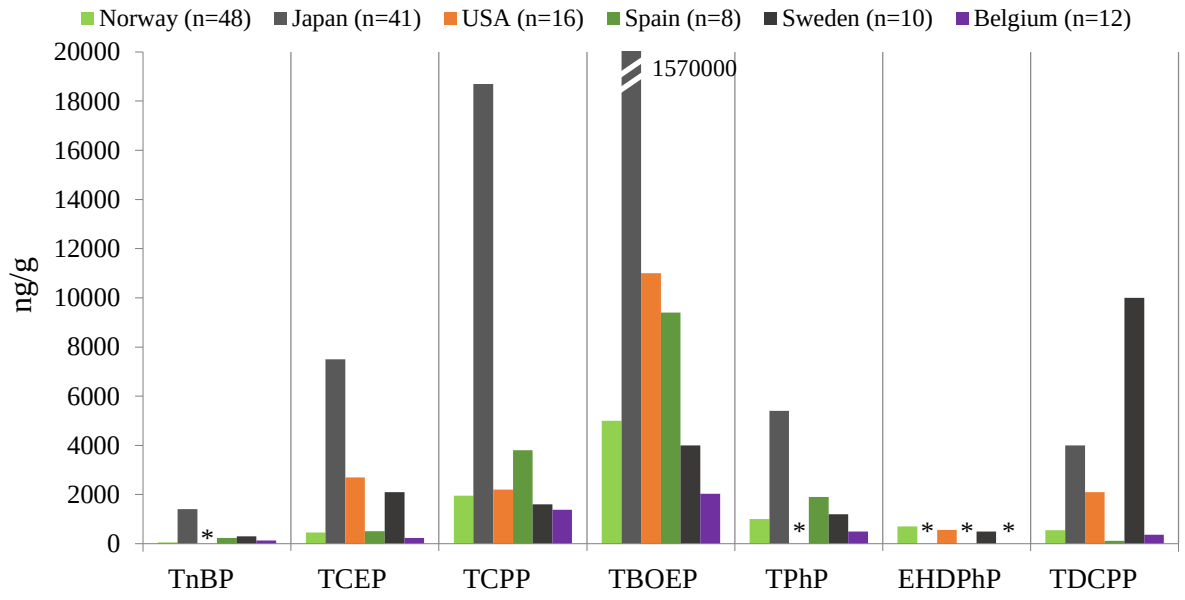

Figure 2.3.6: Comparison of levels of PFRs in indoor dust from studies conducted in Norway, Japan, U.S.A (California), Spain, Sweden and Belgium. (*) indicates no analysis performed.

Correlations between FRs in dust. As already observed in air samples, high correlations between tetra to hexaBDEs were obtained ($R>0.9$; $p<0.01$) and BDE-183 was also correlated with BDE-153 ($R=0.41$; $p<0.01$). These associations suggest the same use of these PBDEs, which most probably arise from the banned PentaBDE and OctaBDE formulations. PBT correlated again with PBDEs from the PentaBDE formulation as observed in air, as well as with BTBPE and anti-DP. BEHTBP and EH-

TBB were moderate to highly correlated (R=0.51; p<0.01), which indicates that these two compounds are used in the same applications or technical formulation, but other than Firemaster 550 due to the different ratio of EH-TBB/BEHTBP in the dust samples. Interesting correlations (0.38<R<0.44; p<0.01) were obtained for HBB with DP, PBBz, DBDPE, BDE-183 and TnBP. In addition, HBB correlated less strongly with TCEP, TCPP, TCP and TDCPP (0.30<R<0.36; p<0.05). These numerous associations suggest that HBB is a common FR in household products.

Comparing content of FRs in floor and settled dust

During the sampling campaign, we collected mainly dust from the floor of the households because it was abundant, but we were also able to obtain 12 samples of settled dust in different households from elevated surfaces (shelves, tables, chairs, electronics, etc). This gives the opportunity to investigate differences between floor and settled dust (Figure 2.3.7) which is important in order to decide from where to sample the dust.

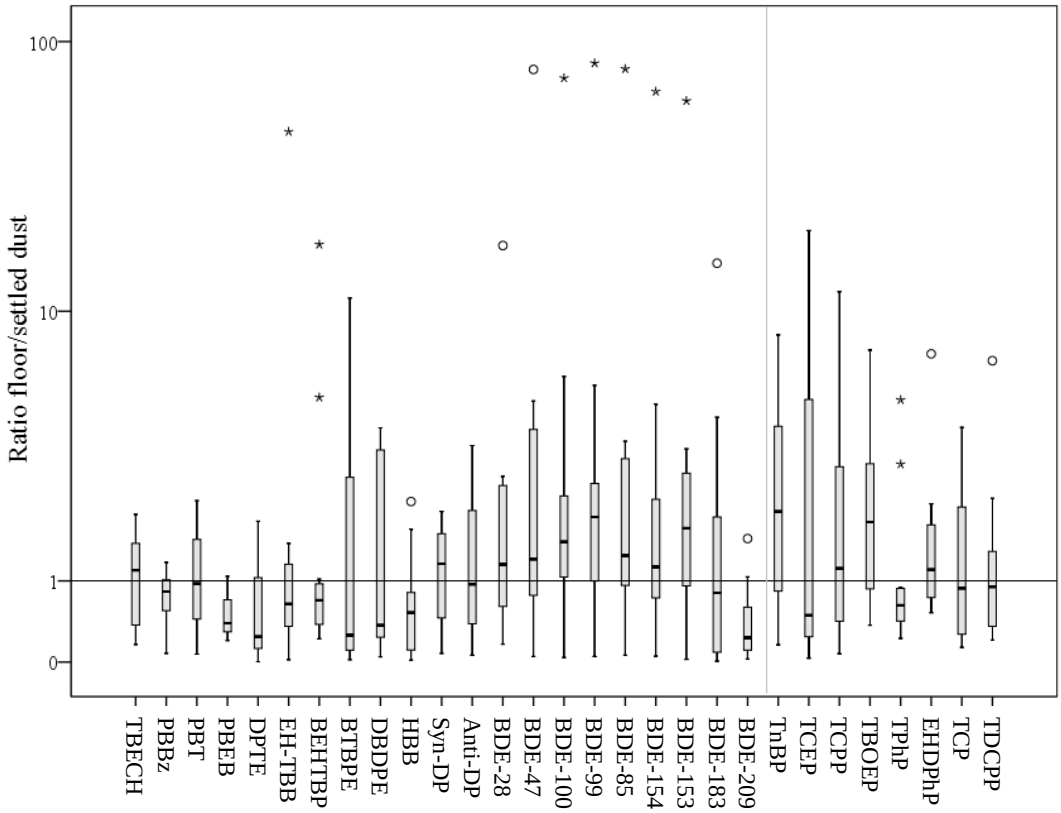

Figure 2.3.7: Box plot of concentration ratios of BFRs, dechloranes and PFRs in floor and settled dust for (n=12). Note Y-axis is in log scale.

The concentration ratios of PFRs in floor and settled dust (Figure 2.3.7) seem to be randomly distributed. While the median ratios for BDE-209 and most of the non-PBDE FRs are slightly <1, i.e., median concentrations in settled dust are higher than in floor dust, the ratios for the lower PBDEs are >1. The wide distribution of the values might be attributed to the spatial variability of FRs in

households (Harrad et al., 2008) and two factors could explain why concentrations in settled dust are higher than in floor dust: 1) settled dust is collected directly from elevated areas, including from the surface of products likely to contain FRs (e.g., electronics, plastics, etc.) and 2) dust from the floor may contain considerable amounts of not contaminated outdoor particles and human and animal cells. Nevertheless, the statistical analysis of the median concentrations of FR in floor dust indicates that they are not significantly different than the median concentrations in settled dust ($t(64)=0.276$, $p=0.784$). Hence, either settled dust or floor dust can be considered equally representative of the indoor contamination.

2.3.3.3. Correlations between FR concentrations in air and dust

Spearman rank analyses gave good correlations between concentrations in air and dust ($0.36<R<0.76$) and highly significant linear regression correlations were obtained for more volatile FR, e.g., TBECH, PBT and HBB, even when the mass percentages in dust were as low as 0.1%. Levels in air and dust of a second group of FRs with lower vapor pressures (BDE-28, 47, 99 and 100) were also significantly correlated in the linear regression models. Similar relationships were observed by Fromme et al. (2009) Levels in air and dust of the PFRs TCPP, TnBP, TBOEP and TCEP were also significantly correlated in the linear regression models. Again, similar relationships were observed by Bergh et al. (2011) for TnBP, TCEP and TCPP. The good correlations between FRs in air and dust found in our study might result from the fact that our air samples also contained suspended particles. At equilibrium conditions, the partition coefficient of FRs between dust and air ($K_{dust\text{-}air}$) is expected to be proportional to the K_{oa}, which has been used to describe the sorption of FRs to organic matter in dust (Weschler et al., 2010). In Figure 2.3.8, the averages of the $K_{dust\text{-}air}$ for the FRs are plotted against their K_{oa}.

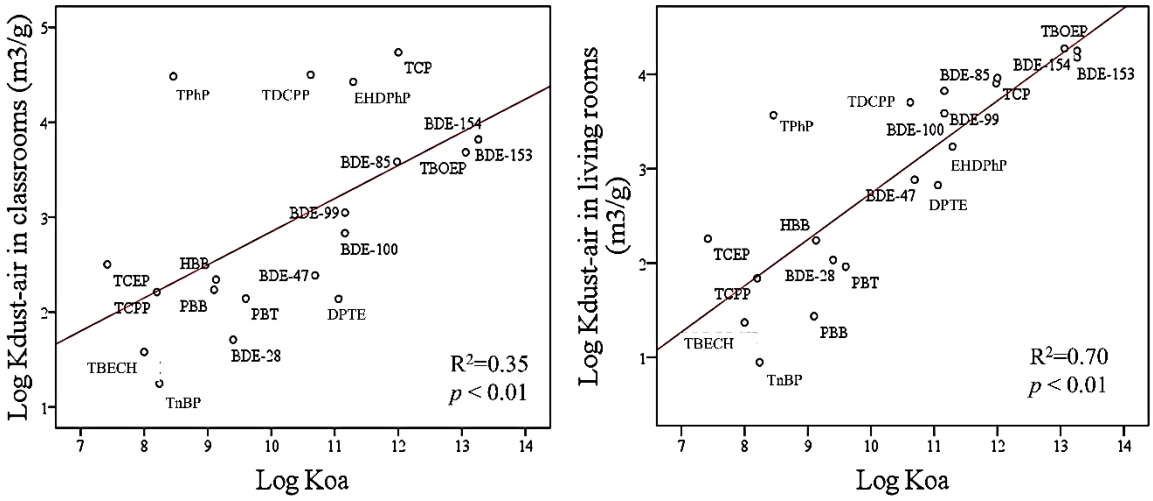

Figure 2.3.8: Log $K_{dust\text{-}air}$ plotted against log K_{oa} of the FRs in living rooms (below) and classrooms (above).

The significant high or moderate R^2 values obtained for the 20 FRs in the living rooms and classrooms, suggest that equilibrium conditions were reached between the two phases for the majority of the FRs. Consequently, by analysing FRs with a known K_{oa} only in dust, it would be possible to estimate the concentration of the BFR in air, or vice versa (Weschler et al., 2010). The fact that R^2 was substantially lower in classrooms compared to the living rooms might be related to the low number of samples (n=6) or that equilibrium between phases was not obtained for some FRs in the classrooms due to forced ventilation.

2.3.3.4. Sources of exposure in indoor environments

Chemicals in consumer goods and household materials may pollute the indoor environment. Some FRs are additives not chemically bounded in the different products (EFRA). Consequently, they can leach into the surrounding air and adsorb to dust. The knowledge of building characteristics and type and number of consumer goods can help to identify exposure sources of the FRs in the indoor environment. The participants in this study answered a questionnaire that assessed 27 factors that may affect the indoor concentrations of FRs.

Results of the bivariate analysis of these factors (Spearman rank correlation) indicated which of them were relevant to study in more detail (data not shown). Associations with $p<0.15$ from the bivariate analysis were evaluated by multiple linear regressions, as some factors are likely to be dependent. The parameter most often related to FR concentrations in air and dust from the living rooms was the number of vacuum cleanings per week (multiple correlations for 10 FRs), followed by the area of the living room in m^2, distance of the sampling equipment from the TV in the living room and the use of electric panel heaters (all parameters had multiple correlations for four FRs). Only occasional associations were seen for other parameters in the multiple linear models (data not shown).

Positive correlations between Σ_8PBDEs in air (also individually for BDE-47, 99 and 100) were obtained with number of vacuum cleaning per week in the living rooms. However, negative correlations were obtained in dust for TBECH, PBBz, HBB, TnBP and TCP. A likely explanation might be that when vacuuming, small particles in the dust are not trapped in the vacuum cleaner bags, increasing the concentration in air. The "aged" dust which has been longer exposed to FRs, is removed by vacuum cleaning and is replaced by "fresh" dust in the room and therefore the levels are likely to be lower when vacuuming more frequently. All PentaBDE components and Σ_8PBDEs in air were positively correlated with the distance of the sampling equipment from the TV, i.e., the further the sampling equipment was from a likely source of FRs (TV), the higher the concentrations. This seems unlikely, and what we probably measured was the distance to other sources of PBDEs, e.g., in adjacent rooms. Use of electric panel heaters negatively correlated with PBT and BDE-85 in air and BDE-28 and BDE-47 in dust, suggesting that households without this heating system contain higher

levels of these FRs. Size of living rooms also correlated positively in dust with TCPP and negatively with BDE-28 and BDE-47 in dust and DPTE in air.

Since only few consistent correlations were seen, the dispersal of FRs is probably highly affected by the spatial variability within the home (Allen et al., 2008) or we simply obtained some accidental correlations due to the relatively limited size of the study. Therefore, despite some relevant correlations (i.e., vacuum cleaning and "TV distance from sampling site"), the sources of FRs in the indoor environment could not be unequivocally identified in this study.

2.3.3.5. Estimated intakes of FRs for children and women

The daily intake of FRs from the indoor environment was estimated for women (median of 41 years) in the households ("occupational" intake could not be assessed) and for children (median of 10 years) in primary schools and households in order to assess whether the levels detected in the Norwegian indoor environments are of concern. The daily intakes of FRs have been calculated according to US EPA risk assessment guidance (US EPA 2012) (Table 2.3.7).

Table 2.3.7: Equations and parameters used for the calculations of the daily exposure dose (DED)

	Daily exposure dose
Air inhalation	[a] $DED = \frac{\text{C x IR x ED}}{\text{BW}}$
Dust ingestion	[a.b] $DED = \frac{\text{C x DI x ED}}{\text{BW}}$
Dermal absorption	$DED = \frac{\text{C x SA x DA x AF x ED}}{\text{BW}}$

[a]C = concentration of FR in air [pg/m^3]; IR = inhalation rate children/adult was 10.9/13.3 [m^3/day];(US EPA 1994) ED = exposure duration [h/day] and body weight = body weight [Kg]. assessed by the questionnaires.
[b]DI = dust ingestion was 0.1 [g/day] for groups older than 6 years old. (US EPA 1994)
[c]SA = skin surface area (arms. hands and legs) children/adult was 4970/8620 [cm^2/day] (US EPA 1994); DA = dust adherence was 0.096 [g/cm^2]; (US EPA 1997) AF = absorption factor was 0.03 and 0.17 [%] for HFRs and PFRs. respectively. (US EPA 1997; Ulsamer et al., 1978).

A more recent *in vitro* study (Abdallah et al., 2015), employing 3D-human skin equivalents to simulate absorbtion through human skin have shown AF values for selected FRs (HBCDD and TBBPA) to be 100-200 times higher than the values estimated by the US EPA and Ulsamer et al.

BFRs. As expected, the main route of exposure for less volatile FRs (vapour pressures $>10^{-7}$ Torr) was dust ingestion for both, women and children, followed by dermal contact and to a small degree by inhalation (Figure 2.3.9).

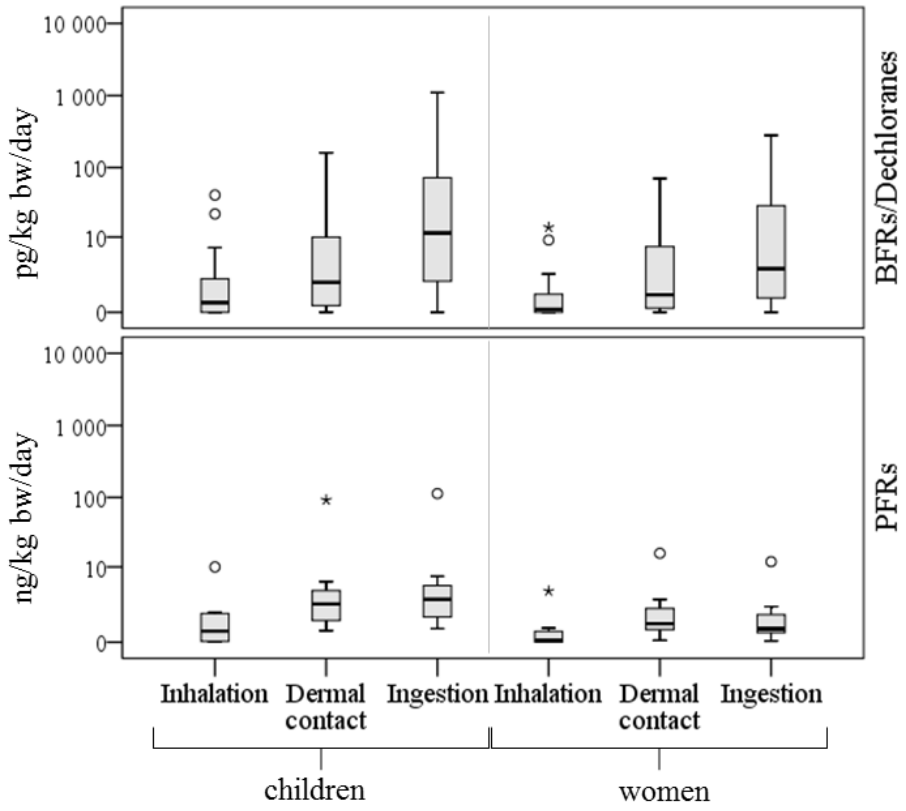

Figure 2.3.9: Comparison of the intake of PFRs (ng/kg bw/day) and BFRs and dechloranes (pg/kg bw/day) for children and women through the inhalation of air, dermal contact and ingestion of dust from indoor environments. Note Y-axis is in log scale.

In contrast, for the more volatile compounds, e.g., TBECH and pentabromobenzene (PBBz), the main source of exposure was air (~80%), while dermal contact accounted for only ~3% of the total exposure. Intake of FRs for children in the homes is higher than for women (Table 2.3.8).

Table 2.3.8: Daily intake of FRs for children and women in the households

	Children intake						Women intake					
	[a]Inh	%	Dermal contact	%	[b]Ing	%	[a]Inh	%	Dermal contact	%	[b]Ing	%
BFRs and dechloranes (pg/Kg bw/day)												
TBECH	17	82	0.48	2	3.3	16	9.0	83	0.37	3	1.48	14
ATE	0.97	100	0	0	0	0	0.53	100	0	0	0	0
PBBz	1.2	75	0.05	3	0.34	21	0.65	77	0.04	5	0.15	18
PBT	1.6	54	0.18	6	1.2	40	0.88	56	0.14	9	0.55	35
PBEB	0.11	100	0	0	0	0	0.06	100	0	0	0	0
DPTE	0.17	13	0.14	11	0.98	76	0.09	14	0.11	17	0.43	68
EH-TBB	0	0	0.70	13	4.9	88	0	0	0.54	20	2.2	80
BEHTBP	0	0	22	13	152	87	0	0	17	20	68	80
BTBPE	0	0	1.0	12	7.3	88	0	0	0.80	20	3.2	80
DBDPE	0	0	41	13	285	87	0	0	32	20	127	80
HBB	0.87	37	0.19	8	1.3	55	0.47	39	0.14	12	0.58	49
Syn-DP	0	0	0.40	13	2.8	88	0	0	0.31	19	1.3	81
Anti-DP	0	0	1.1	12	8.0	88	0	0	0.89	20	3.6	80
BDE 28	1.6	52	0.19	6	1.3	42	0.86	54	0.15	9	0.59	37
BDE 47	27	9	35	11	244	80	15	10	27	18	109	72
BDE 100	1.4	2	9.2	12	64	86	0.78	2	7.1	19	29	79
BDE 99	4.4	1	47	12	331	87	2.4	1	37	20	148	79
BDE 85	0.12	1	2.6	13	18	87	0.07	1	2.0	20	8.0	79
BDE 154	0.08	<1	3.5	12	25	87	0.04	<1	2.7	20	11	80
BDE 153	0.20	<1	7.2	13	50	87	0.11	<1	5.6	20	22	79
BDE 183	0	0	0.89	13	6.2	87	0	0	0.69	20	2.8	80
BDE 209	0.44	<1	90	13	628	87	0.24	<1	70	20	280	80
ΣPBDE	35	4	118	12	824	84	19	4	91	19	367	77
Total	57	3	262	12	1834	85	31	3	203	19	818	78
PFRs (ng/Kg bw/day)												
TnBP	1.1	85	0.09	7	0.11	8	0.58	83	0.07	10	0.05	7
TCEP	0.47	25	0.65	34	0.8	41	0.25	23	0.50	45	0.36	32
TCPP	8.9	49	4.2	23	5.2	28	4.8	47	3.2	31	2.3	22
TBOEP	0.13	<1	21	45	26	55	0.07	<1	16	57	12	43
TPhP	0.05	1	1.5	44	1.9	55	0.03	1	1.2	58	0.86	41
EHDPhP	0.02	1	0.97	44	1.19	55	0.01	1	0.75	58	0.53	41
TCP	0	<1	0.48	45	0.59	55	0	0	0.37	59	0.26	41
TDCPP	0.02	1	0.79	45	0.97	54	0.01	1	0.61	58	0.43	41
Total	11	14	30	39	37	47	5.8	13	23	50	17	37

[a]Inhalation; [b]Ingestion.

Table 2.3.9: Comparison of exposure of children and women to FRs in Norwegian indoor environments and reference dose values

	Household and school daily intake of children			Household daily intake of women			
	Air	Dust		Air	Dust		
BFRs and DPs (pg/kg bw/day)	[c]Inhalation	Dermal Contact	Ingestion	[c]Inhalation	Dermal Contact	Ingestion	[a,b]RfD
TBECH	22	0.78	5.4	9.0	0.37	1.5	
ATE	1.0	0	0	0.53	0	0	
PBBz	1.4	0.06	0.45	0.65	0.04	0.15	
PBT	1.9	0.21	1.5	0.88	0.14	0.55	
PBEB	0.11	0	0	0.06	0	0	
DPTE	0.26	0.17	1.2	0.09	0.11	0.43	
EH-TBB	0	1.2	8.2	0	0.54	2.2	
BEHTBP	0	37	$2.6x10^2$	0	17	68	
BTBPE	0	2.0	14	0	0.80	3.2	
DBDPE	0.91	63	$4.4x10^2$	0	32	$1.3x10^2$	
HBB	1.3	0.32	2.2	0.47	0.14	0.58	
Syn-DP	0	0.53	3.7	0	0.31	1.3	
Anti-DP	0	1.5	11	0	0.89	3.6	
BDE-28	2.2	0.24	1.7	0.86	0.15	0.59	
BDE-47	41	42	$2.9x10^2$	14	27	$1.1x10^2$	$1.0x10^5$
BDE-100	2.3	10	72	0.78	7.1	29	
BDE-99	6.9	53	$3.7x10^2$	2.4	36	$1.5x10^2$	$1.0x10^5$
BDE-85	0.19	3.1	22	0.07	2.0	8	
BDE-154	0.14	4.4	30	0.04	2.7	11	
BDE-153	0.28	8.5	59	0.11	5.6	22	$2.0x10^5$
BDE-183	0	1.7	12	0	0.69	2.8	
BDE-209	0.44	$1.6x10^2$	$1.1x10^3$	0.24	70	$2.8x10^2$	$7.0x10^6$
ΣPBDEs	55	$1.4x10^2$	$9.9x10^2$	19	91	$3.7x10^2$	
TOTAL	82	$3.9x10^2$	$2.7x10^3$	31	$2.0x10^2$	$8.2x10^2$	
PFRs (ng/Kg bw/day)							
[d]TnBP	1.4	0.44	0.54	0.57	0.06	0.04	$2.4x10^4$
TCEP	0.91	1.6	2.0	0.27	0.59	0.42	$2.2x10^4$
TCPP	10	5.9	7.2	4.1	2.9	2.1	$8.0x10^4$
[d]TBOEP	1.6	92	113	0.07	16	12	$1.5x10^4$
TPhP	0.06	2.8	3.4	0.03	1.2	0.87	$7.0x10^4$
EHDPhP	0.03	2.9	3.5	0.01	0.93	0.59	
TCP	0	0.53	0.65	0	0.40	0.29	$1.3x10^4$
TDCPP	0.02	2.0	2.5	0.01	0.68	0.48	$1.5x10^4$
TOTAL	14	108	133	5.1	23	16	

[a]Oral RfD values of BFRs extracted from USEPA; [b]RfD values of PFRs extracted from (Ali et al., 2012); [c]FRs from suspended particles are also taken into account; [d]used mainly as a plasticiser.

Children have less skin uptake and smaller inhalation volumes than women (US EPA 2012), but since averaged residence time in the households for children is assumed higher and body weight lower, these parameters drive their exposure.

If estimated oral intakes (dust ingestion) of the most exposed group (children) are compared with oral RfDs (Table 2.3.9) for BDE-47 (US EPA 2008a), BDE-99 (US EPA 2008b), BDE-153 (US EPA 2008c) and BDE-209 (US EPA 2008d), the intake is 344, 267, 3390 and 6151 times lower than the RfDs, respectively. Even when assuming a high-end scenario using maximum concentrations (Table 2.3.6) and double intake factor, the values are still around one order of magnitude lower than the RfDs.

PFRs. Air contributes significantly to human intakes for organophosphates with vapor pressures $>10^{-5}$ Torr. For example, for children and women the intake in households through inhalation of TnBP, TCPP and TCEP is approximately 85, 49 and 25%, respectively (Table 2.3.9). For PFRs with lower vapor pressures (e.g., TDCPP), dermal absorption contributes 45% to the intake for children and 58% for women, whereas ingestion of dust contributes 54% and 41%, respectively. This higher dermal intake compared to the BFRs is notable in Figure 2.3.9 and is attributed to the higher absorption factor used in the calculation (Ulsamer 1978; Ali et al., 2012) because organophosphates are less lipophilic than BFRs. As a result, the dermal exposure to PFRs is in the range of dust ingestion. When comparing total intake of PFRs for children (inhalation, dermal contact and ingestion) with RfD values from Ali et al. (2012) (Table 2.3.8), the PFR daily intakes are some orders of magnitude lower than the RfD values, being TBOEP the highest with a high-end scenario of only 10 times lower intake than its RfD.

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Chapter 3

New analytical methods for the identification and quantification of emerging flame retardants

3.1. Identification strategies for flame retardants employing time-of-flight mass spectrometric detectors along with spectral and spectra-less (high resolution) databases

Based on the following publication:

Ionas, A.C., Ballesteros-Gómez, A., Leonards, P.E.G., Covaci, A., 2015. Identification strategies for flame retardants employing time-of-flight mass spectrometric detectors along with spectral and spectra-less databases. J. Mass Spectrom. 50, 1031–1038.

3.1.1. Introduction

In the last decades, an increasing number of additives were added to consumer products to enhance certain material properties, such as plasticity or resistance to fire. A particular class of additives are flame retardants (FRs), typically added to materials in order to inhibit or slow down the spreading of fire. FRs are present in a multitude of consumer products ranging from electronics to curtains, carpets and furniture. During the lifetime of the products, FRs may leach out into the indoor environment (Saito et al., 2007; Brandsma et al., 2013), where they can be potentially harmful to human health (Lyche et al., 2015). Because of the increasing number of FRs and their potentially harmful (long-term) effects on the environment and on human health, there is a need for the development of wider scope screening techniques to detect and identify these new compounds. This is the first step in formulating a realistic risk assessment.

In the past, gas chromatography-low resolution mass spectrometry (GC-MS) with either an electron ionisation (EI) or electron capture negative ionisation (ECNI) source, in combination with mass spectral databases, e.g., Wiley/NIST, was the main technique used for non-targeted screening for contaminants in environmental samples (Weigel et al., 2001; Wetter et al., 2001; Rosenfelder et al., 2010; van Stee et al., 1999). However, this technique suffers from low sensitivity for some halogenated FRs when using the EI source or low selectivity with the ECNI source due to generation of unspecific low molecular mass fragments (e.g. *m/z* 79/81 for Br^-). Many FRs with high molecular mass FRs are also not GC-amenable. On the other hand, high resolution (HR) MS detectors can provide superior sensitivity and selectivity for (halogenated) FRs by promoting the formation of the molecular ion or higher molecular mass fragments, when the detectors are equipped with a softer ionisation source, such as atmospheric pressure chemical ionisation (APCI) or electrospray ionisation (ESI). The higher resolution translates to superior resolving power, which makes it possible to distinguish between analytes with the same nominal mass (Mol H, 2013) and greatly reduces interferences, thus increasing selectivity. All these factors make HR MS detectors, such as HR time-of flight (TOF), a suitable tool for the identification of unknowns.

Recently, HR TOF detectors have been successfully employed, coupled to either GC or LC systems, in applications such as non-targeted screening for contaminants in food (Canellas et al., 2012; García-Reyes et al., 2007; Mezcua et al., 2009; Hernández et al., 2011), water (Hernández et al., 2011; Hug et al., 2014; Krauss et al., 2010), herbal preparations (Ibáñez et al., 2013; Hao et al., 2008), polar bear plasma (Simon et al., 2013), metabolite discovery (Van den Eede et al., 2013; Ballesteros-Gómez et al., 2015; Van der Kloet et al., 2013) as well as other applications (Hernández et al., 2011). TOF detectors are also fast enough to allow coupling to two-dimensional chromatographic techniques (e.g. GC×GC or LC×LC), which have the advantage of providing "cleaner" mass spectra by increasing the chromatographic resolving power and by separating co-eluting analytes. A number of studies successfully employing GC×GC-TOF-MS have already been published (Pena-Abaurrea et al., 2014; Ballesteros-Gómez et al., 2013; Hoh et al., 2012; Hilton et al., 2010; Alam et al., 2013).

However, up to now, LC in combination with TOF-MS for the screening / non-target analysis of (halogenated) FRs has been scarcely exploited. LC is applicable to compounds with a wider polarity range, including the less volatile, non GC-amenable and highly hydrophobic FRs. Although the main providers of mass spectrometers offer software tools for this purpose, non-targeted screening is still a complex procedure for which instrumental and data processing parameters need to be carefully optimised for obtaining successful results in a reasonable amount of time.

The main aim of this study was to provide a novel systematic workflow for non-target screening and identification of halogenated chemicals that are potentially used as new FRs in consumer products. The NFRs tend to be less volatile than the FRs they had replaced and so GC analysis is no longer feasible. For example, the Penta-BDE mixture, used in polyurethane foam, was replaced by FRs, such as the Antiblaze V6, alternatively abbreviated as BCMP-BBCP (Bergman et al., 2012). This chemical was first detected in 2011 in foam and textile baby care products (Stapleton et al., 2011), in samples produced as early as 2003. Other examples are RDP, BDP and TTBP-TAZ, which are also used as replacements for Deca-BDE in the plastic components of electronics (Ballesteros-Gómez et al., 2014a; Ballesteros-Gómez et al., 2014b). For these NFRs, liquid chromatography (LC)-based techniques are recommended for analysis and (Q)TOF-MS is a suitable tool for the identification of unknown NFRs.

In this study, an LC-(Q)TOF methodology was employed together with "spectra-less" databases to allow detection of high molecular weight and non-volatile compounds and so, to keep up with the trend of the industry of employing ever heavier FRs.

3.1.2. Materials and methods

3.1.2.1. Reagents and materials

All solvents used were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, toluene, dichloromethane (DCM) and iso-octane were purchased

from Merck (Darmstadt, Germany). Modified nylon centrifugal filters with 0.2 µm and 0.45 µm pore size were bought from VWR (Leuven, Belgium).

3.1.2.2. Samples

Dust samples from previous studies, such as house dust from California (n=5) (Dodson et al., 2012) and dust from e-waste storage areas in Thailand (n=6) (Muenhor et al., 2010), along with samples of car interiors (foam and textile materials, n=8) and consumer products (electrical power boards, an LCD television, plastic children's toy and e-waste sample) from The Netherlands (n=5) were investigated for other chemicals than those previously reported (Ballesteros-Gómez et al., 2013).

3.1.2.3. Instrumentation

The instruments employed in the present study were: 1) a microTOF II MS (Bruker Daltonics, Bremen, Germany), with a mass accuracy <2 ppm and resolution >16500 FWHM, equipped with an atmospheric pressure chemical ionization (APCI) source and coupled to an Agilent 1290 LC; and 2) an Agilent 6530 Q-TOF MS (Agilent Technologies, Palo Alto, CA, USA), with a mass accuracy <1 ppm and resolution >20000 FWHM, equipped with an Agilent JetStream electrospray ionization (AJS ESI) source and coupled to an Agilent 1290 Infinity LC.

On the Agilent instrument, the gas temperature for the source was 300°C (negative mode) and 350°C (positive mode), gas flow 10 L/min (negative mode) and 3.2 L/min (positive mode), nebuliser pressure 45 psig (negative mode) and 25 psig (positive mode), sheath gas temperature was 250°C (negative mode) and 400°C (positive mode) and the sheath gas flow was 11 L/min (negative mode) and 10 L/min (positive mode). A volume of 5 µL of extract was injected and separation was achieved using a Phenomenex Kinetex XB-C18 column (150 mm × 2.1 mm i.d., 2.6 µm particle size) with a flow rate of 0.3 mL/min and a linear gradient from 5% methanol to 99% methanol in 30 min, followed by a 5 min hold before returning to the original conditions and hold for 10 min.

For the Bruker instrument, the detector parameters were: capillary voltage 1000 V, end plate offset -1000 V (negative mode) and +500 V (positive mode), corona current -10000 nA (negative mode) and +6000 nA (positive mode), dry gas flow 4 L/min, nebuliser 3 bar, dry heater 285 °C and vaporiser temperature 285 °C. The injection volume employed was 5 µL and the column was a Kinetex Core-shell C_{18} column (100 mm × 2.1 mm i.d., 2.6 µm particle size), with a similar mobile phase program as above.

3.1.2.4. Extraction

The samples were extracted using a combination of ultrasound assisted extraction (UAE) and solvent vortexing. A mixture of *n*-hexane:acetone (3:1) was used for the extraction of dust samples (Dodson et al., 2012). A mixture of DCM:acetone (1:1) was employed for the car interior samples,

such as foam and textile materials (Ionas et al., 2014), while DCM was used for the (hard/soft plastic) consumer products. The samples did not undergo any conventional clean-up to avoid selective removal of possible analytes of interest. The final extracts were filtered through 0.22 µm centrifugal filters, diluted by a factor of 100-1000 and injected.

3.1.3. Results and discussions

3.1.3.1. Ionisation source selection

For non-targeted screening, it is important to efficiently ionise as many analytes as possible. An ion source which can simultaneously ionise analytes through multiple ionisation mechanisms, such as a multimode source, is a possible solution. Unfortunately, this source has a lower sensitivity compared to separate ESI and APCI sources. In this study, we have used both ESI and APCI sources, the latter employed to cover the more apolar FRs. To further extend the range of chemicals, we selected an Agilent Jet Stream ESI source over classical ESI. This source has an added sheath (heated) gas flow, which increases sensitivity by a factor of 5 to 10 times compared to a classical ESI, and a nozzle voltage (electric potential difference applied between the sheath gas nozzle elements, providing a charging electrical field that further focuses the electrospray plume), which makes it possible to ionise less polar analytes and to diminish ion suppression.

We acquired and optimised the sources parameters for some representative analytes of each group of FRs (brominated, chlorinated and phosphorous-containing compounds). The preferred ionisation mode for all PFRs was ESI(+) giving $[M+H]^+$ as major ion. It has been previously reported that the sensitivity for all PFRs is better in standard ESI(+), except for the least polar chemicals, such as RDP and BDP (Ballesteros-Gómez et al., 2014b), better analysed by APCI(+). To achieve good sensitivity for all analytes and avoid having to use two ionisation sources, we tested the Jet Stream ESI source that provided the desired sensitivity for both the classical PFRs and RDP and BDP. In negative mode though, this source can ionise only analytes with a polar functional group, such as TBBPA, TCBPA, TBBPS, or TBBPA-BHEE. So, brominated and chlorinated phenols and in general any halogenated chemical with a hydroxyl group which is not too shielded by other functions can be easily detected by ESI(-) using the Jet Stream source showing $[M-H]^-$ as main ion. HBCDD is an exception, as it ionises well even in the absence of such functions. It is noteworthy to mention that this source is also very well-suited for metabolites of heavy, non-polar halogenated FRs, such as HO-PBDEs, sulphated HO-PBDEs, HO-HBCDDs, etc.

Nevertheless, since most current-use halogenated FRs are rather non-polar, the use of an APCI source in negative mode and thus stronger ionisation mechanisms due to the high corona voltage (Zhou et al., 2010) is required. It is however more challenging to predict which ions will be generated. In general, $[M-H]^-$, $[M-Br+O]^-$ or $[M-Cl+O]^-$ are the major ions for most halogenated FRs in APCI(-). Heavy brominated FRs containing a triazine ring (e.g. TTBP-TAZ) are an exception,

since they can be detected in both APCI(-) and APCI(+), in the last case showing $[M+H]^+$ as major ion. To ensure that the vast majority of compounds were ionised, the APCI source parameters were similar to those used for the least polar analytes, such as the PBDEs.

3.1.3.2. Workflow of non-targeted screening

Once a chromatogram is obtained, it is ran through the screening procedure as described in Figure 3.1.1.

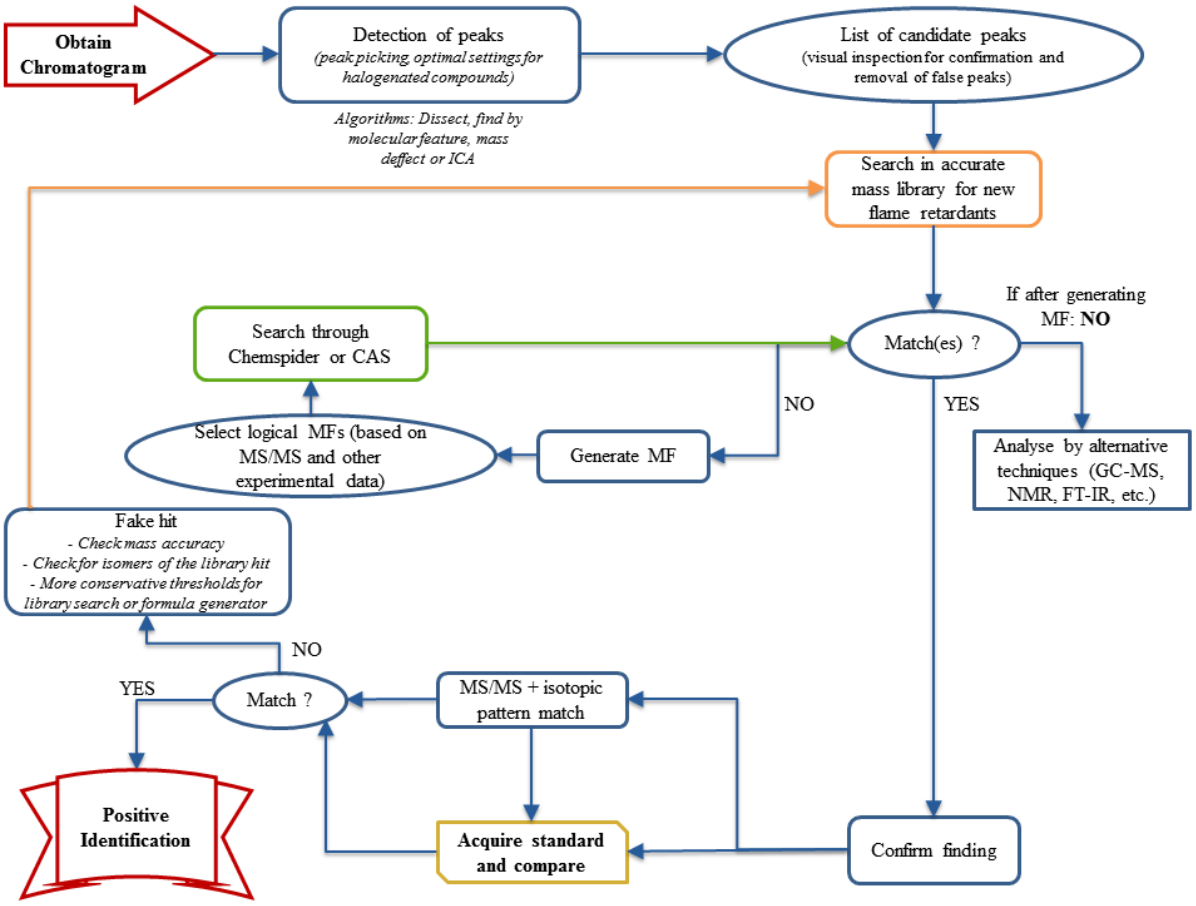


Figure 3.1.1: Structure elucidation flowchart. The round symbols indicate decision processes and processes in which the user is more extensively involved. Abbreviations: molecular formulae (MF)

The first stage in the identification process is peak detection (or "peak picking"), in which compounds are extracted using the "Dissect peaks" and "Molecular Features" tools (Bruker Data Analysis) or "Find by molecular feature" tool (Agilent MassHunter). The software examines the chromatogram by using complex fuzzy-logic algorithms and separates co-eluting peaks without the need for user interaction or any prior information. The parameter input into the software has a big impact on what compounds are extracted. Adding adequate filters is extremely important in diminishing the amount of data through which the analyst needs to sieve (Little et al., 2011). Critical parameter values were optimised for the screening for FRs: *compound detection (peak height) filters*:

higher values of this parameter (10-20) are recommended for compounds expected to be present at high levels in the sample. The lower this parameter, the more compounds are found by the software. A signal-to-noise value under 3 is not recommended. *Ion species/adducts*: it is recommended to monitor $[M+Na]^+$ and $[M+K]^+$ as well, as Na and K ions can originate from the LC system (e.g. from the solvent bottles) and any other adducts (e.g. with NH_4^+) with cations or anions added to the mobile phases used previously. In APCI sources, the formation of complex ions (e.g. $[M-Br]^-$, $[M-Br+O]^-$ or $[M+O_2]^-$) has been described for brominated and chlorinated compounds (Zhou et al., 2010) and therefore these ions were also included for formulae generation. If the employed software features different extraction algorithms, it is advisable to choose a general *small molecule algorithm* and avoid the ones designed for larger molecules such as peptides. Also, if available, it is best to avoid isotope models that favour the extraction of molecular features only for typical organic molecules containing C, H, N, O and S, such as peptides or glycans.

If there is a need to restrict the non-target screening to FRs that are known to be in use, the mass of the fragments extracted can be restricted to the range of 200 to 1400 Da. It is also important to select the appropriate parameters for the complexity of the matrix at hand. For example, for a complex matrix, if the data processing software allows to specify what the desired chromatographic resolving power or maximum number of overlapping compounds can be, it is best to select values closer to the high end of the numeric range for these parameters.

Agilent-specific parameters for molecular feature extraction:

a) *Extraction algorithm*: small molecules (chromatographic)

b) *Retention time* and *m/z restrictions*: the molecular mass of FRs typically varies between 200 and 1400 Da and the retention time varies according to the LC column and parameters used. For non-targeted screening, this filter should not be used. For FR screening, these parameters should be restricted as much as possible to facilitate data analysis.

c) *Isotope model*: the unbiased model proved to work the best for FRs. Among other models, the one for peptides and glycans are unsuited for this purpose and the one for common organic molecules favours the basic organic molecules containing C, H, O, N and S. The same applies when generating formulas.

d) *Quality score*: this parameter is an estimate generated by the algorithm about how likely the extracted compound is to be an actual compound. It takes into consideration factors like signal-to-noise ratio, peak shape, peak width, consistency of retention time, mass difference between ion species, whether it is a single-ion compound and isotope pattern. If the detector is properly calibrated and tuned, this parameter can be set > 80.

Bruker-specific parameters:

a) *Maximum number of overlapping compounds*: to ensure the best results, this value should be set at values as high as possible, especially for complex matrices. For non-targeted screening for FRs from dust samples, this parameter was set at 20. Even for injections of pure analytical standards, this value should be > 3.

b) *Cut-off intensity*: this parameter dictates the relative intensity of mass signals included in the spectrum. To diminish some of the spectral noise, a value of > 0.1 is recommended (the maximum is 10).

c) *Chromatographic resolving power*: this, along with the "maximum number of overlapping compounds" controls if almost co-eluting signals are separated into distinct compounds or combined into one compound. Higher values generate more peaks, if there is any difference in retention time. However, the "dissect peaks" algorithm determines by itself what the most appropriate setting for this parameter is, by factoring in the approximate width of the chromatographic peaks. As the software can select these parameters automatically with good results, we recommend not changing this parameter, unless the output of the "dissect peaks" tool is not the desired one.

d) Proteomics, CHNO: this parameter is similar to the "common organic molecules" isotope model in the Agilent software. It favours the formula generation for typical organic molecules containing mostly C, H, N and O. For (halogenated) FRs, this option needs to be unchecked.

The analyst must then manually review the results looking for the characteristic patterns such as those of chlorine or bromine clusters, with 3 or more atoms, as contained in most current-use halogenated FRs. Next, the fragments of interest can be searched in an in-house accurate mass library (if available). If the software indicates matches, the analyst must verify their validity, so the next step is to confirm the tentative identification by MS/MS experiments. A useful tool for this purpose is the Agilent Molecular Structure Correlator (MSC), which can run the unknown MS/MS spectrum against multiple candidate structures generated using a molecular formula generator (MFG) algorithm, which allows elemental composition parameters to be defined. The correlation scores for each of the candidate structures are automatically calculated, based on mass accuracy and individual scores for each fragment ion signal, and the overall percentage of fragment ion intensity that can be plausibly explained with substructures. This is accomplished by attempting to explain each observed fragment ion into the proposed structure using a "systematic bond-breaking" approach (Hill et al., 2005). There is also the possibility to retrieve all possible structures for the most likely formula from local compound or web-based databases (e.g. ChemSpider). The SmartFormula 3D tool included in the Bruker Data Analysis software package has similar functionality.

The final confirmation is to acquire the presumed chemical standard and to compare the retention times and two mass spectra (Schymanski et al., 2014). However, in the event that they do not match, we are most likely dealing with a false positive hit. In this case, the analyst needs to explore the possibility that the unknown analyte is an isomer of the compound that generated a hit in

the library. If this is not the case, then some of the search parameters need to be adjusted, such as setting more conservative thresholds for the library searches or formula generator tools, or to double-check the mass accuracy of the instrument for that particular analysis.

In the event that no match is found in the in-house accurate mass library, the elemental formulas can be generated using formula generator tools such as the Bruker Smart Formula or Agilent Generate Formula. A number of critical parameters were further optimised for this step to aid in the screening for FRs: *elemental composition expected*: this parameter was optimised for each class of FRs (Table 3.1.1); *adducts*: it is recommended to take into account all possible adducts, as detailed above; *ion electron state/configuration*: it is recommended to allow configurations with both odd and even electron states; *double bond equivalent*: in screening for the known FRs, this parameter varies in between -1 and 23. For non-targeted screening, a maximal value > 23 is recommended. However, this parameter is only a rough estimation of the degree of unsaturation in an organic molecule and it often produces erroneous values, especially for analytes containing multiple halogens, together with S, N and P (Kind et al., 2007); *maximum MS mass error / Tolerance*: for a properly calibrated detector with a resolution greater than 15000 FWHM, a mass error as low as 5 ppm can be used.

Table 3.1.1: Typical elemental composition per flame retardant class

Element	PFRs		CFRs		BFRs		Mixed Cl/Br FRs	
	Min	Max	Min	Max	Min	Max	Min	Max
C	6	39*	5	18*	4	25*	4	39*
H	9	51*	0	36*	0	50*	0	51*
N	0	0	0	0	0	3	0	0*
O	4**	8	0	8	0	6	0	0*
S	0	0	0	0	0	1	0	0*
P	1	2	0	0	0	0	0	0*
Cl	0	12	4	12*	0	0	1	6*
Br	0	9	0	0	3	14*	1	5*

For the CFRs, the values do not take the chlorinated paraffins into account
*Value suited for screening for FRs; higher values are recommended for true non-targeted screening
**The only exception: 9,10-Dihydro-9-oxa-10-phosphaphenanthrene 10-oxide (DOPO) which only contains 2 oxygen atoms

The analyst must select the most likely molecular formulas based on all the available information. The candidate molecular formulas can then be searched through services like the Chemical Abstracts Service (CAS) Registry (>70 million substances) and ChemSpider (>28 million entries). These searches can be automatically performed with Bruker Compound Crawler in a large range of internet databases, including ChemSpider. Alternatively, accurate mass searches can be done directly on the ChemSpider website or even in the NIST Chemistry Webbook. If the generated formula does not produce any hits in the databases, then the analyst must use another technique, such

as NMR or FT-IR. Such complementary analysis techniques can be valuable tools in narrowing down the number of candidate formulas to just one (Little et al., 2013).

3.1.3.3. Steps for automation

As the data processing is the most time-consuming and work-intensive part of non-targeted screening, any operation or resource that can save the analyst's time is invaluable. For instance, the Agilent Qualitative Analysis offers two analysis templates: "Identify Chromatogram Peaks", better suited for simpler matrices and "Find targets by molecular feature extraction (MFE) + Database Search + molecular formula generation (MFG)", which proves more useful with complex chromatograms containing multiple coeluting compounds. Similarly, the Bruker Automation Engine can be used for a wide array of operations by employing Visual Basic scripts.

3.1.3.4. In-house databases

A valuable knowledge base on the screening for FRs is the work of Bergman et al. (2012), where many of the current-use FRs are listed and categorised. To complement this database, a systematic search was performed for NFRs that might be used in the indoor environment and the results were added to an in-house prepared database (not included here / available as Supporting Information 2 from Ionas et al. 2015) to aid in the screening for new/rarely used FRs. Special emphasis was put on the list of restricted / controlled halogenated FRs of big corporations that manufacture electronics, as they are often the main source of FR contamination in an indoor environment (Allen et al., 2008). This database is meant to complete the information provided in (Bergman et al., 2012) and is meant to be used alongside it. The database searches were done on the basis of type of ions generated with our instruments. On the Agilent 6530 QTOF MS with the JetStream ESI source, all PFRs ionised mostly as $[M+H]^+$, with the optimised experimental parameters. PFRs behaved similarly on the APCI(+) source. Brominated and chlorinated FRs are better ionised by APCI, as described above.

3.1.3.5. Considerations for non-targeted screening: the "known-unknown" approach

A major issue in conducting non-target screening experiments is the efficient handling of the sheer amount of generated data. One solution would be to set stricter filters, but relevant data can be lost. Our solution was to set filters more "directed" to FRs. Many chemicals are used as FRs, ranging from inorganic chemicals (hydrated aluminium, magnesium oxides, aluminium diethylphosphinate, etc.), nitrogen FRs (melamine polyphosphate, melamine cyanurate, etc.) to PFRs (ammonium polyphosphate, organophosphate esters, etc.), CFRs (chlorinated organophosphate esters, etc.) and BFRs (PBDEs, TBBPA, HBCDDs, etc.). Since halogenated FRs pose the greatest risk of being persistent, bioaccumulative and toxic (PBT) chemicals (Pena-Abaurrea et al., 2014), we focus our

study on these chemicals which are most likely to be harmful to humans and to the environment. This has been done by selecting the compounds with at least 3 halogen atoms during the manual review of the results from the "peak picking" step. This process can be simplified by using software tweaks, which are discussed in the following subsection.

Another way of obtaining more directed information is to set targeted values when generating formulas for the number of atoms that an unknown may contain, according to the main FR classes (Table 3.1.1). For example, most PFRs typically contain between 4 and 8 O atoms, 1-2 P atoms, additionally up to 9 Br atoms and up to 12 Cl atoms.

3.1.3.6. Tweaks to facilitate the detection of halogenated FRs

Mass defect filtering – Agilent Qualitative Analysis

As most elements commonly encountered in organic molecules (C, H and N) have mass defect close to or slightly above zero, most organic molecules have a positive mass defect. However, halogens have fairly large negative mass defects. Based on this property, halogen-containing molecules can be differentiated from other compounds in complex samples. The exceptions here would be molecules which contain other atoms with negative mass defects (O, S, etc.) in high numbers. The filtering by mass defect is part of the "Find by Molecular Feature" tool of the Agilent Qualitative Analysis software. Among halogenated FRs, one of the mass defects closest to zero is the one of TCEP, with a value of -0.0461 Da, while 1,2,4,5-tetrabromo-3,6-bis(2,3,4,5,6-pentabromophenoxy)-benzene has one of the largest negative mass defects (-1.1534).

Isotope cluster analysis – Bruker Data Analysis

This particular analysis allows searching for chemicals having the same number of a certain atom that shows a noticeable isotopic pattern, such as Cl or Br. There are two main parameters that need to be defined to obtain an Isotope Cluster Analysis Chromatogram: *1) m/z*: the mass difference between the two isotopes. Between ^{35}Cl and ^{37}Cl, there is a difference of 1.99705 Da, and between ^{79}Br and ^{81}Br, the difference is 1.99795 Da. Therefore, the value set was 2, with a tolerance of up to 0.1; 2) Intensity: this parameter is the ratio between the intensity of two fragments containing different halogen isotopes. It is advisable to select the two most abundant isotope peaks from the cluster (Table 3.1.2).

One critical parameter is the tolerance of the intensity ratio, because this can be affected by other atoms with A+2 stable isotopes, such as ^{34}S (4.3%) and ^{18}O (0.2%). To check the impact of these atoms on the theoretical halogen isotope cluster ratios, we have calculated the values for clusters of 3 to 14 halogen atoms, which is the maximum we have encountered for any FR (Table 3.1.3), and compared with the values for several FRs (Table 3.1.3).

Table 3.1.2: The ratios of the intensities between the two most abundant isotope peaks from the cluster

Number of halogens / cluster		3	4	5	6	7	8	9	10	11	12	13	14	Min	Max
Intensity Ratios	Cl	0.96	0.78	0.64	0.8	0.96	0.89	0.78	0.85	0.96	0.94	0.85	0.88	0.64	0.96
	Br	0.97	0.69	0.97	0.77	0.97	0.82	0.97	0.86	0.97	0.88	0.97	0.9	0.69	0.97

The deviation from the theoretical halogen cluster was <5%, so this is a good value for the tolerance of this parameter for general screening of FRs. However, if instrumental variations are expected, we recommend a value of <15%.

Table 3.1.3: Possible deviations from the theoretical halogen clusters due to the presence of multiple O or S atoms in the analyte molecule.

FR name	FR abbreviation	Formula	Halogen	Number	Intensity ratio	% deviation from theoretical halogen cluster
Dodecachlorododecahydro dimethanodibenzocyclooctane	Dechlorane Plus	C18H12Cl12	Cl	12	0.93	1.1
5-(Tetrabromophenyl)-1,2,3,4,7,7 -hexachloro-2-norbornene	Dechlorane 604	C13H4Br4Cl6	Cl/Br	6/4	0.92*	0.4*
2,2-Bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate]	Antiblaze V6	C13H24Cl6O8P2	Cl	6	0.82	-2.5
Tris(2,4,6-tribromophenoxy)-s-triazine	TTBP-TAZ	C21H6Br9N3O3	Br	9	0.98	-1.0
Tetrabromobisphenol S bis (2,3-dibromopropyl ether)	TBBPS-BDBPE	C18H14Br8O4S	Br	8	0.8	2.4
N,N'-Ethylenebis (tetrabromophthalimide)	EBTBPE	C18H4Br8N2O4	Br	8	0.82	0.9
Tetrabromobisphenol S	TBBPS	C12H6Br4O4S	Br	4	0.68	1.4

*To save time in calculating the ratios of all of the possible Cl/Br combinations, we recommend extracting the isotope cluster analysis chromatogram with an intensity of 0.81 and a tolerance of 21%, in order to detect all of the halogenated compounds with more than 3 halogen atoms.

3.1.3.7. Exemplification of the described workflow

We analysed dust, car interiors and consumer products to screen for new FRs and check the applicability of the proposed workflow to real samples.

After the chromatograms and corresponding mass spectra were obtained, they were analysed through the procedure described in the workflow section. The analytes detected in multiple samples were logged and the mass spectra were manually reviewed for specific molecular features. On the identification of new targets, we focused on analytes displaying pattern characteristics of more than 3 Cl or Br atoms.

TBBPA, BDE-209 were detected in 67% of the Thailand dust samples and TCEP and BTBPE in 50% and, respectively, 17% of the samples. As for the US dust, TCPP and TDCPP were present in 80% of the samples, while TCEP and penta BDEs in 60% and 20%, respectively. One particular hexachlorinated compound was detected in 40% of the dust samples and in 63% of the car interior samples. A search in our accurate mass database yielded one possible match with a mass error of 3.95 ppm: 2,2-Bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate]. This chemical is used as FR under the trade names Amgard V6, Antiblaze AB100, Antiblaze V6 or Phosgard 2XC20. Its main uses are in polyether-type polymers, high-resilience and moulded foams, with main applications in automotive furnishings and in upholstery and foam used in furniture.

For confirmatory purposes, the first step was to perform MS/MS experiments and investigate if the obtained product ions match of V6 the possible fragmentation pathways. The two main fragments obtained at *m/z* 234.9680 and 298.9573 corresponded to expected fragmentation pathways (Figure 3.1.2).

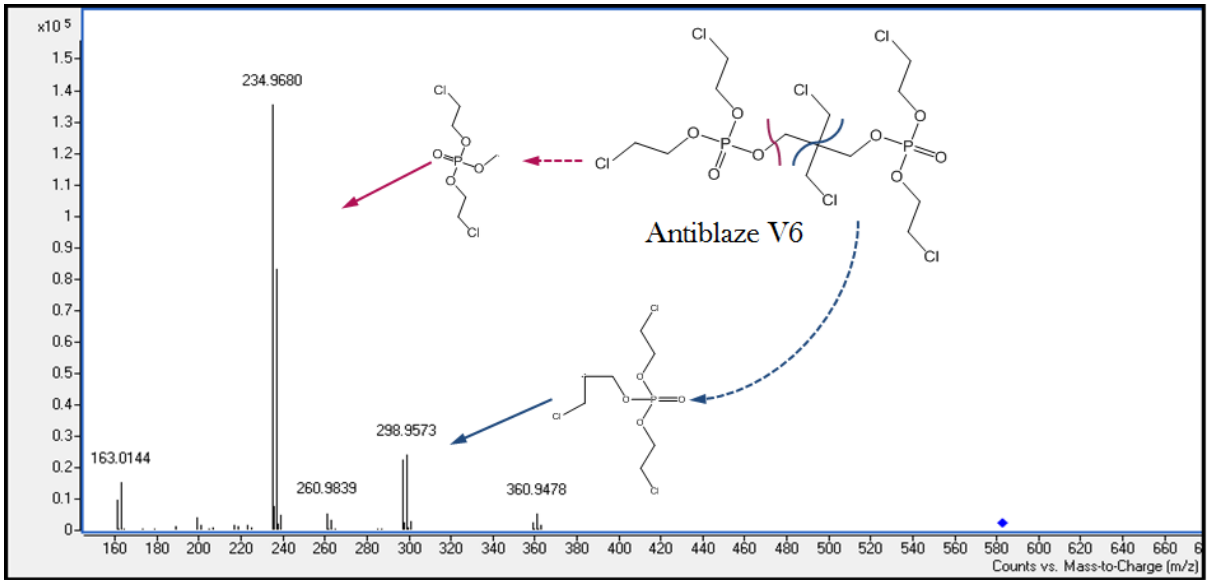


Figure 3.1.2: MS/MS spectrum for V6 indicating the corresponding product ions.

The final confirmation step was done by acquiring the analytical reference standard and comparing the spectra from the sample and the standard, as well as the retention time. Since the retention time and two ion clusters matched in both accurate mass and isotopic pattern (Figure 3.1.3), and also considering the positive outcome of our MS/MS confirmation experiment, we were confident about having positively identified the FR Antiblaze V6.

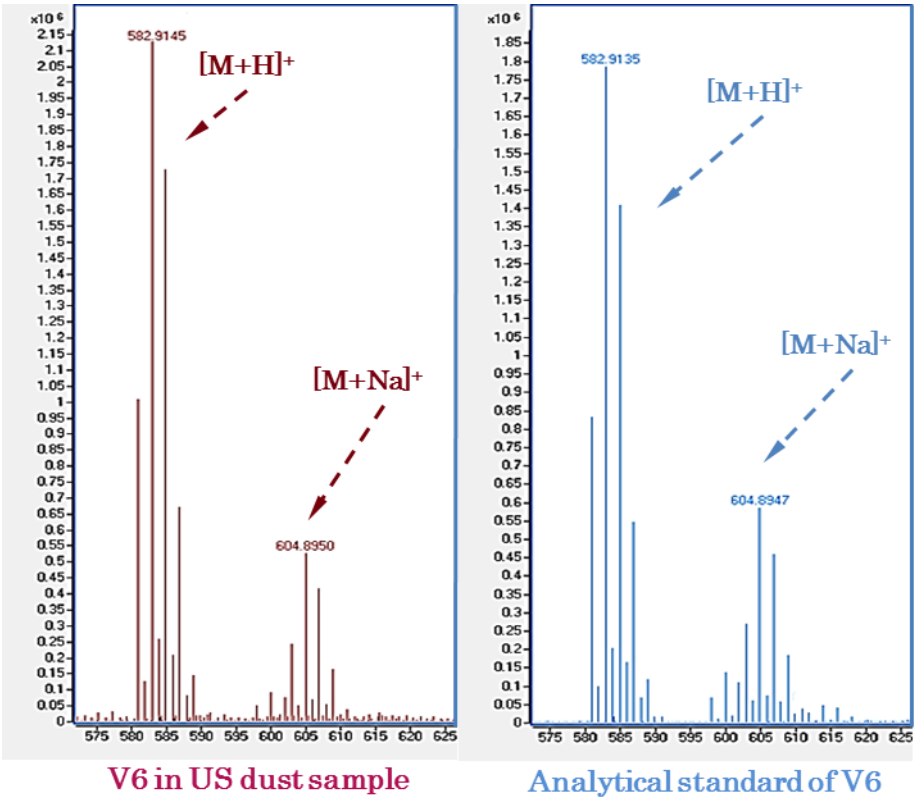


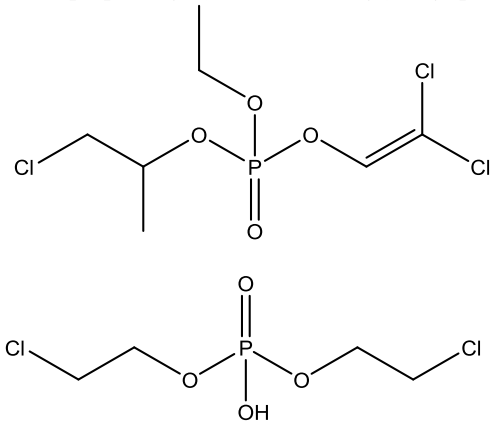
Figure 3.1.3: Final confirmation of the identification of V6

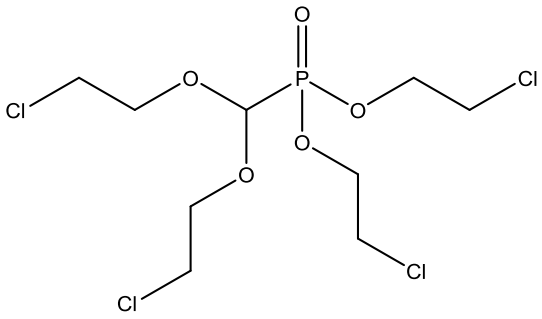
3.1.3.8. Identification of reaction by-products by mass defect filtering: case study for the technical V6 mixture

We used our approach to identify the by-products in the technical V6 mixture. We used the MFE step to filter the peaks according to the mass defect of the compounds, which are the reactants (in this case TCEP) and the reaction products (V6). A critical parameter in this process is the *mass defect tolerance*, which we recommend to keep at ±0.01 Da. As a result, the extracted compounds have a high degree of similarity to either TCEP or V6. Next formulas are generated with targeted element limits: 2-16 O, 1-12 Cl and 1-4 P atoms. The upper element limits were doubled as compared to V6, to ensure that any possible dimers are also detected. The upper threshold for the MFG was set to 80 (out of 100). A score of 90-100 represents a very good match, which can directly be searched in in-house and online databases, while matches with a score of 80-90 should be reviewed carefully.

Following the procedure described in figure 3.1.1, with the amendments above, the compounds with a very good match score were searched in Chemspider and Google and three compounds were tentatively identified (figure 3.1.4).

1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate





Bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate

Bis(2-chloroethyl) hydrogen phosphate

Figure 3.1.4: Reaction by-products and impurities tentatively identified from the Antiblaze V6 technical mixture.

The most abundant was *bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate*, with a mass error of -2.9 ppm and an overall match score of 98.65 (out of 100). Its abundance was close to the one of TCEP, which constitutes 10% of the technical V6 mixture (Stapleton et al., 2011). A peak roughly half the size of the smaller TCEP isomer was tentatively identified as *1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate*, with a mass error of -1.4 ppm and an overall match score of 98.72. And the third and least abundant compound identified is *bis(2-chloroethyl) hydrogen phosphate* (BCEP). A number of other by-products/impurities for which formulas with very good match score were generated are available in table 3.1.4.

We find that this case study is of special environmental significance, because even if the new FRs, which come into use, are safer, some of the reaction by-products and impurities can be significantly more toxic than the main component of the mixture (Leonards et al., 2012).

3.1.3.9. Identification of halogenated analytes by Isotope Cluster Analysis (ICA)

For the unspecific detection of chlorinated and brominated FRs in a very complex sample, ICA can be run, with an intensity ratio of 0.81 and a tolerance of 21%. This puts the intensity with the range of 0.64 to 0.98, which encompasses all isotopic clusters of Cl and Br, from 3 to 14 atoms (Table 3.1.2). The result will be an ICA chromatogram containing just the peaks of analytes containing Cl and Br (Figure 3.1.5), thus simplifying the screening/identification process.

Table 3.1.4: Formulas generated and compounds tentatively identified in the technical mixture of the FR Antiblaze V6 *(In italic: compounds tentatively identified)*

Name	Formula	Total Volume*	Retention Time (min)	m/z	Mass	Mass (MFG)	Mass Difference (MFG, ppm)	Mass Difference (DB, ppm)	Score (DB)	Score (MFG)	Overall match score***
BCEP (Bis(2-chloroethyl) hydrogen phosphate) 2	C4H9Cl2O4P	191,680	7.8	222.9693	221.9621	221.9616	-2.7	-	-	93.9	93.9
BCEP (Bis(2-chloroethyl) hydrogen phosphate) 1	C4H9Cl2O4P	276,887	8.3	222.9687	221.9615	221.9616	0.3	-	-	99.4	99.4
-	C12H22Cl5O6P	389,734	9.3	468.9675	467.9607	467.9597	-2.2	-	-	94.9	94.9
-	C16H29Cl6O12P3	451,010	7.2	733.9344	715.9000	715.9003	0.4	-	-	96.4	96.4
-	C21H31Cl6O5P3	498,228	9.7	666.9574	665.9502	665.9515	2.0	-	-	92.2	92.2
-	C16H30Cl6O10P2	705,432	8.7	654.9536	653.9455	653.9445	-1.4	-	-	97.3	97.3
-	C11H22Cl4O9P2	835,416	5.0	500.9568	499.9494	499.9493	-0.2	-	-	99.7	99.7
-	C18H32Cl8O9P2	1,050,434	10.2	734.9115	733.9039	733.903	-1.3	-	-	97.4	97.4
-	C10H17Cl4O5P	1,091,022	8.2	388.9646	387.9576	387.9568	-2.0	-	-	95.7	95.7
-	C28H32Cl7O12P	1,308,134	9.2	836.9525	835.9459	835.9451	-0.9	-	-	96.4	96.4
-	C19H34Cl8O10P2	1,370,558	10.3	781.9477	763.9135	763.9135	0.0	-	-	99.6	99.6
-	C33H42Cl9O14P	1,715,929	10.1	1008.9583	1007.9513	1007.9509	-0.4	-	-	98.2	98.2
-	C18H34Cl7O13P3	1,888,587	7.6	796.911	795.9042	795.9032	-1.2	-	-	98.8	98.8
-	C11H21Cl5O8P2	2,291,247	6.3	518.9231	517.9159	517.9154	-0.9	-	-	99.4	99.4
-	C34H44Cl10O16P2	2,651,267	9.8	1120.9073	1119.9002	1119.899	-1.1	-	-	96.0	96.0
1-chloropropan-2-yl (2,2-dichlorovinyl) ethyl phosphate	C7H12Cl3O4P	6,765,566	6.7	296.9616	295.9543	295.9539	-1.4	-	-	98.7	98.7
TCEP 2	C6H12Cl3O4P	13,779,816	6.5	284.9621	283.9547	283.9539	-2.9	-2.9	97.3	97.3	97.3
TCEP 1	C6H12Cl3O4P	28,377,846	6.7	284.9619	283.9541	283.9539	-0.8	-0.8	61.9	93.1	77.5
Bis(2-chloroethyl) (bis(2-chloroethoxy)methyl)phosphonate	C9H17Cl4O5P	28,613,420	7.8	376.9659	375.9579	375.9568	-2.9			98.7	98.7
V6	C13H24Cl6O8P2	384,746,848	9.1	580.9171	579.9099	579.9078	-3.6	-3.6	94.3	94.3	94.3

*This parameter is directly proportional with the area of the compound peak and is calculated by the Agilent Qualitative Analysis software as being the total volume (m/z × RT × abundance) of the ions associated with the compound

**The Overall match score is a weighted average of scores obtained by different ID techniques that have contributed to the identification of the hit. In this case the database ("DB") score and the molecular formula generation ("MFG") scores contributed to this number. The MFG score, which is the score for the generated formula designated as the best hit, is calculated as the weighted average of three scores, which the analyst can adjust as contribution to the final MFG score. Here, a mass score of 90 (out of 100), an isotope abundance score of 100 and an isotope spacing score of 80 were used.

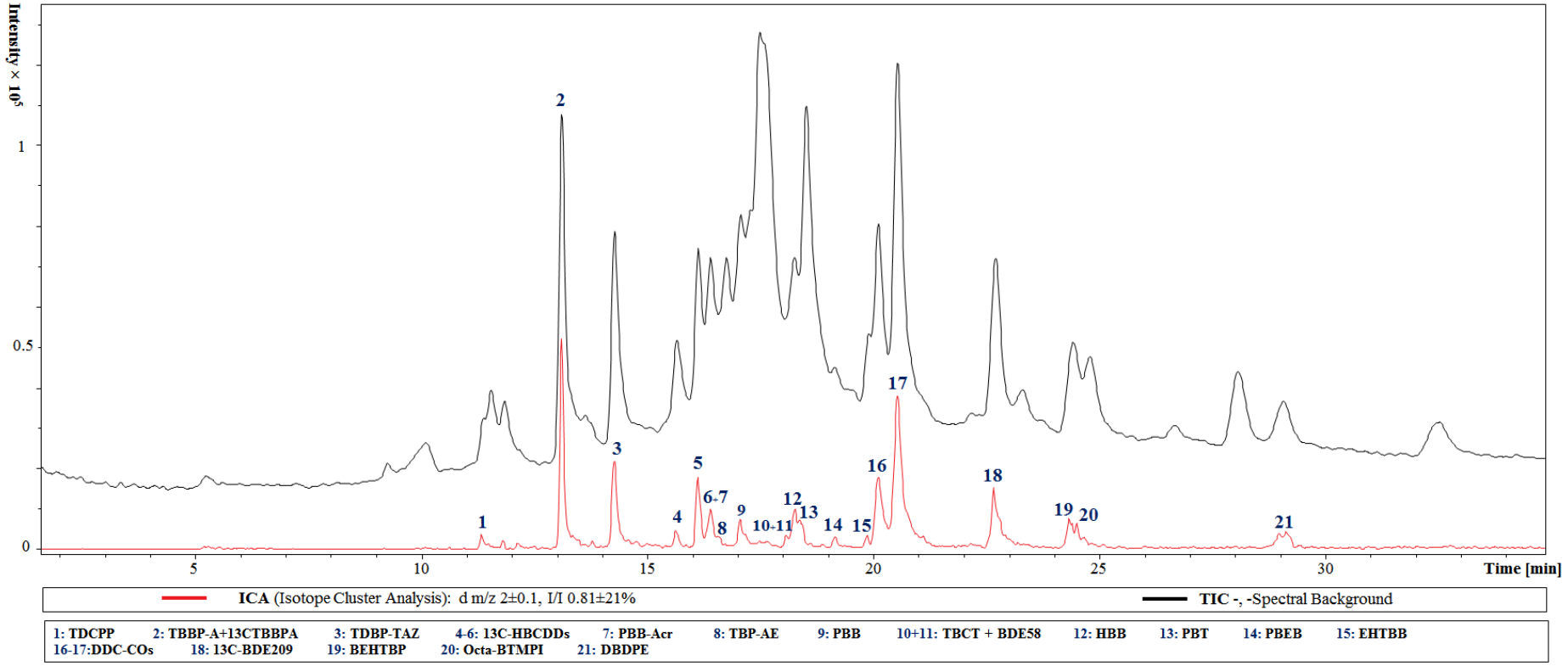


Figure 3.1.5: Isotope cluster analysis (ICA) chromatogram of a complex mixture of standards comprised of both halogenated and non-halogenated FRs.

3.2. Development of a liquid chromatography electrospray ionization tandem mass spectrometry method for the analysis of emerging organophosphate flame retardants in dust, plastics and textiles

Based on the following manuscript:

Ionas AC, Xu F, Neels H, Covaci A. 2015. Development of a liquid chromatography electrospray ionization tandem mass spectrometry method for the analysis of emerging organophosphate flame retardants in dust, plastics and textiles (in preparation)

3.2.1. Introduction

Since BFRs have started to fall out of use due to health concerns (Lyche et al., 2015), PFRs are replacing them in many applications (van der Veen and de Boer, 2012). Since these chemicals are typically used as additives, rather than being chemically bonded to a material, they are released into different compartments of the environment, such as air, water, sediment, soil, biota and dust (Wei et al., 2015). Due to growing concerns for their adverse health effects (see Chapter 1), it is necessary to monitor these chemicals in the environment and in the consumer products they are added to.

In an effort to make the use of PFRs safer, higher boiling point condensed PFRs such as bisphenol A bis(diphenyl phosphate) (BDP), resorcinol bis(diphenyl phosphate) (RDP) and 2,2-bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate] (V6) were developed and put in use, to replace the more volatile classical PFRs and the chlorinated ones (van der Veen and de Boer, 2012). However, these chemicals have started to be detected in the environment as well. V6 was initially detected in baby products (Fang et al., 2013; Stapleton et al., 2011), foam from couches (Stapleton et al., 2012), then in dust from houses and cars (Fang et al., 2013) and more recently in wastewater (Woudneh et al., 2015). In wastewater treatment plants, this FR undergoes very little chemical or biological degradation (Woudneh et al., 2015), so it is very likely that it will be detected in other compartments of the environment as well. RDP and BDP were detected initially in house dust (Brandsma et al., 2013; Matsukami et al., 2010) and then in electronics and plastic consumer products (Ballesteros-Gómez et al., 2014). Recently after, they were also detected in sewage sludge (Brandsma et al., 2014), river sediments (Brandsma et al., 2014; Matsukami et al., 2015) and surface soil from an e-waste recycling area and a rice paddy (Matsukami et al., 2015). RDP has been found to be rapidly mineralised in activated sludge, but this is not the case with BDP as well which is not easily biodegraded (Jurgens et al., 2014).

All of these methods focus on one or two of these emerging PFRs (EPFRs) in one matrix. Since these FRs are becoming more widespread, it is important to be able to efficiently monitor them in a wide variety of matrices, in both the indoor and outdoor environment.

This study therefore aimed to develop a method to rapidly analyse new and emerging PFRs, impurities/breakdown compounds from their technical mixtures and a number of classical PFRs which have been associated with serious health concerns in matrices typically encountered in the indoor environment: plastics, textiles and dust.

3.2.2. Materials and methods

3.2.2.1. Reagents and materials

All solvents used were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone (ACE), toluene, dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc) and iso-octane were purchased from Merck (Darmstadt, Germany). Supelclean™ ENVI™- Florisil® and ENVI-Carb™ SPE cartridges (500 mg/3 mL) were purchased from Supelco (Bellefonte, PA, USA). Standards of triphenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-isopropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway), isodecyl diphenyl phosphate (iDPhP) from Accustandard (New Haven, CT, USA). Trixylenyl phosphate (TXP) was purchased from Chemos (Regenstauf, Germany). Resorcinol bis(diphenyl phosphate) (RDP) and Bisphenol A bis(diphenyl phosphate) (BDP or BDP) were purchased from TRC (Toronto, ON, Canada). Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Labelled TPhP-d15 (IS) was purchased from Sigma Aldrich, while labelled TCEP-d12, TDCPP-d15 were synthesised by Dr. Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). Ultrapure water (18.2 MΩ) was obtained from an Elga LabWater water purification instrument (Saint Maurice, France). Except TXP and iDPhP which were technical mixes, the purity of analytical standards was >98%.

3.2.2.2. Instrumentation

For the instrumental analysis, an Agilent 1290 Infinity liquid chromatography (LC) system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 6460 Triple Quadrupole mass spectrometer (MS) was employed, equipped with a Jetstream® electrospray ionization (ESI) ion source.

The LC parameters were optimised to provide both good chromatographic separation and minimal run tine, in order to maximise sample throughput. A volume of 3 µL of extract was injected on a Phenomenex (Torrance, CA, USA) Kinetex Biphenyl reversed phase column (2.1 x 50 mm, 1.7 µm),

at a column oven temperature of 55 °C. The mobile phases were A: ultrapure H_2O and B: MeOH, both containing 5 mM ammonium formate. Separation was achieved using a flow rate of 0.5 mL/min and a gradient from 55 B to 94% B in 3.4 min, followed by 1 min hold before returning to the initial conditions, making the total run time of 4.5 minutes. The column is re-equilibrated for the next run during a 2.5 min post time.

The source parameters were initially optimised for all main analytes individually and subsequently a set of values for these parameters were selected to provide the best response for all considered analytes. As such, the drying gas temperature was set at 350 °C, the gas flow at 3 L/min, the nebulizer at 25 psi, sheath gas temperature 400 °C, sheath gas flow 12 L/min, capillary voltage 2700 V and nozzle voltage 0 V. The MS was operated in dynamic multiple-reaction monitoring (dMRM) mode, with 2-10 ion transitions for each analyte in their specific retention time (RT) window (RT ± 0.5 min).

The Agilent MassHunter Workstation Software version B.06.00 was used for all aspects of data analysis.

3.2.2.3. Extraction

The dust samples were extracted employing a combination of solid-liquid extraction and ultrasound-assisted extraction, as described in a previous study (Dodson et al., 2012). The plastic samples were cut in small shavings and strong solvents were used to dissolve it (acetone and DCM). The textile samples were cut into smaller pieces and extracted with: *n*-hexane / DCM (1:3, *v/v*). The extraction for the plastic and textile samples is detailed in this previous study (Ionas et al., 2014). The final extract underwent a 1500× dilution, was spiked with internal standards, filtered through 0.22 µm centrifugal filters and injected as such.

3.2.3. Results and discussion

3.2.3.1. Optimisation of mobile phases

Acetonitrile was initially considered as mobile phase, but it was determined that it does not provide good ionisation for some of the analytes (most notably TCEP) and it also has the disadvantage that mobile phase modifiers such as ammonium acetate or formate are not readily soluble. As methanol did not present the same issues, it was chosen as organic mobile phase.

We tested the response and peak shape without adding any modifiers in the mobile phase, with 10 mM or ammonium acetate, 10 mM and 5 mM of ammonium formate. The latter proved to be the best choice as it improved response as compared to no additives, and at the same time favoured the $[M+H]^+$ ions, in detriment of other adducts (which were either not present or very low), while not creating any ion suppression.

3.2.3.2. Selection of stationary phase

A number of different stationary phases were tested for the purpose of this study (Table 3.2.1). Different variations of C18 were the starting point (classical, double endcapped: XDB-C18 and with iso-butyl sidechains and trimethylsilyl endcapping), but this type of stationary phase proved to have a bad elution profile for the analytes of interest. It was also noted that columns wider than 2.1 mm internal diameter and with particles bigger than 2.6 µm typically do not provide narrow enough peaks and good enough resolution and are thus not adequate for fast methods. To improve peak shapes for those analytes, while not compromising on the separation of the other analytes, other phenyl-type stationary phases (Phenyl-hexyl, Pentafluorophenyl and Biphenyl) were tested as well. Interestingly, the analytes which had phenyl and alkyl sidechains eluted in wider tailing and often split peaks on some of the stationary phases.

Table 3.2.1: Columns and stationary phases tested

Column	Stationary phase	Length (mm)	Internal diameter (mm)	Particle size (µm)	Separation	Elution order	Peak Shape	Fitness-for-Purpose
Grace VisionHT	C18	100	2.1	3	passable	passable	inadequate	passable
Phenomenex Kinetex	Biphenyl	100	2.1	2.6	good	passable	passable	passable
Phenomenex Kinetex	PFP	100	2.1	2.6	inadequate	passable	passable	inadequate
Agilent Zorbax Eclipse	XDB-C18	50	2.1	1.8	inadequate	inadequate	passable	inadequate
Phenomenex Kinetex	Phenyl Hexyl	100	2.1	2.6	inadequate	passable	inadequate	inadequate
Phenomenex Kinetex	XB-C18	100	4.6	2.6	inadequate	passable	inadequate	inadequate
Phenomenex Kinetex	Biphenyl	50	2.1	1.7	good	passable	very good	good

We have put emphasis on elution order as well because our initial approach was to maximise sensitivity by employing separate MRM time segments with custom ion source parameters and different transitions. However, when trying to achieve low run times as well, this approach proved to not be very robust.

The column that proved best as separation, peak shape and elution order was the Phenomenex Kinetex Biphenyl (2.1 x 50 mm, 1.7 µm). The short length and small particle size allowed for very short run times and very good peak shapes (Figure 3.2.1).

3.2.3.3. Optimisation of ionisation source parameters

The individual standards of the analytes considered were infused directly into the ionisation source with a KD Scientific syringe pump. The source parameters were adjusted as to favour the $[M+H]^+$ ions. For instance, a value which was too low of the cell accelerator voltage and gas flow

tended to favour the formation of adducts (ammonium and sodium). The fragmentor voltage as well can be optimised to favour either the $[M+H]^+$ ions or the adducts. Increasing the nozzle voltage typically caused a decrease of the response for the $[M+H]^+$ ions, so it was set to 0.

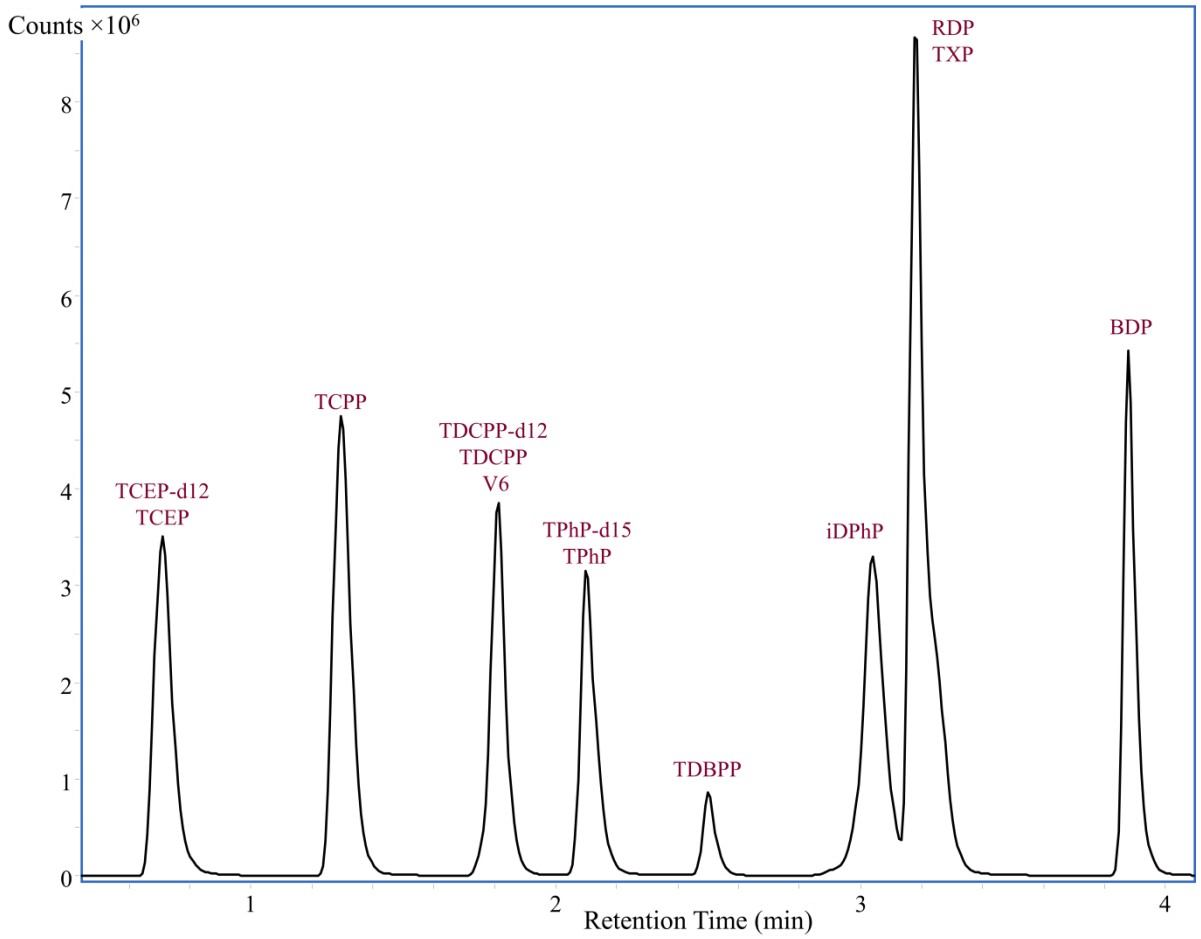


Figure 3.2.1: MRM TIC chromatogram of the analytes of interest (0.6-0.9 ng injected on the column, depending on analyte)

The gas temperature and sheath gas temperature were optimised for the main analytes and the same values were used for the other analytes as well, as changing these parameters during a run takes a considerable amount of time. This data was carefully considered and ion source parameter values which were closest to the optimal values for the main analytes were chosen.

3.2.3.4. Optimisation of MRM transitions

The approach taken for this process was semi-automated. Each analyte was injected, without a column, in scan mode with its optimised source parameters. The $[M+H]^+$ and second most abundant characteristic ion were logged and then product ion scans were taken for these ions. To determine the best values for the collision energy and to further confirm the fragmentor voltage values previously optimised, the Agilent MassHunter Optimizer software was employed. By doing multiple injections

with progressively narrower ranges and then fine tuning of these parameters, optimal values were determined (Table 3.2.2).

Table 3.2.2: Ion transitions included in the dynamic MRM method (in **bold**: most abundant transition per analyte; in *italic*: internal standards)

Analyte	Precursor Ion	Product Ion	Fragmentor Voltage (V)	Collision Energy (V)	Cell Accelerator Voltage (V)	Retention Time (min)	Retention Time Window (min)
TCEP	285	222.8	111	8	1	0.73	0.5
TCEP	**285**	**99**	**109**	**29**	**1**	**0.73**	**0.5**
TCPP	327	175	110	10	1	1.3	0.5
TCPP	**327**	**99**	**110**	**20**	**1**	**1.3**	**0.5**
TCEP-d12	*299*	*67.1*	*128*	*34*	*1*	*0.71*	*0.5*
TCEP-d12	*297*	*102*	*132*	*26*	*1*	*0.71*	*0.5*
TCEP-d12	***297***	***67.1***	***132***	***26***	***1***	***0.71***	***0.5***
TDCPP	**430.9**	**99**	**136**	**10**	**1**	**1.81**	**0.5**
TDCPP	428.9	99	136	10	1	1.81	0.5
V6	586.9	362.8	152	14	1	1.82	0.5
V6	586.9	236.7	156	34	1	1.82	0.5
V6	584.9	360.9	140	14	1	1.82	0.5
V6	584.9	237	144	34	1	1.82	0.5
V6	582.9	361.3	156	14	1	1.82	0.5
V6	582.9	234.9	140	34	1	1.82	0.5
V6	**580.9**	**358.9**	**156**	**14**	**1**	**1.82**	**0.5**
V6	580.9	234.9	156	34	1	1.82	0.5
TDBPP	702.6	99	136	40	1	2.5	0.5
TDBPP	700.6	99	136	40	1	2.5	0.5
TDBPP	**698.6**	**99**	**136**	**40**	**1**	**2.5**	**0.5**
TDBPP	696.6	99	136	30	1	2.5	0.5
TDBPP	694.6	99	136	30	1	2.5	0.5
TDCPP-d15	***446***	***101.9***	***120***	***25***	***1***	***1.78***	***0.5***
TDCPP-d15	*444*	*101.9*	*112*	*30*	*1*	*1.78*	*0.5*
TPhP	327.1	251	130	20	1	2.15	0.5
TPhP	**327.1**	**77**	**130**	**30**	**1**	**2.15**	**0.5**
iDPhP	391.2	251	160	11	5	3.04	0.5
iDPhP	391.2	77.1	160	48	5	3.04	0.5
iDPhP	251	95.1	190	23	1	3.04	0.5
iDPhP	**251**	**77.1**	**190**	**33**	**1**	**3.04**	**0.5**
TXP	411.1	179	192	46	1	3.26	0.5
TXP	411.1	105.1	184	33	1	3.26	0.5
TXP	411.1	79.1	192	57	1	3.26	0.5
TXP	**411.1**	**77.1**	**192**	**90**	**1**	**3.26**	**0.5**
RDP	576.1	77.1	235	65	1	3.18	0.5
RDP	575.1	481.1	244	35	1	3.18	0.5
RDP	575.1	419.1	244	35	1	3.18	0.5
RDP	575.1	405.1	244	39	1	3.18	0.5
RDP	575.1	231	244	47	1	3.18	0.5
RDP	575.1	215	244	85	1	3.18	0.5
RDP	575.1	152	244	85	1	3.18	0.5
RDP	575.1	141	244	55	1	3.18	0.5
RDP	**575.1**	**77.1**	**244**	**83**	**1**	**3.18**	**0.5**
BDP	694.1	368.1	260	33	4	3.88	1

BDP	694.1	367.1	259	34	4	3.88	1
BDP	694.1	179	259	60	4	3.88	1
BDP	694.1	115	267	58	4	3.88	1
BDP	**693.2**	**367.1**	**260**	**33**	**4**	**3.88**	**1**
BDP	693.2	327	262	30	4	3.88	1
BDP	693.2	215.1	262	60	4	3.88	1
BDP	693.2	178	268	60	4	3.88	1
BDP	693.2	165	268	60	4	3.88	1
BDP	693.2	115	268	58	4	3.88	1
TPhP-d15	*342.2*	*223*	*181*	*26*	*1*	*2.11*	*0.5*
TPhP-d15	***342.2***	***82.1***	***181***	***50***	***1***	***2.11***	***0.5***

Since the aim was to rapidly and sensitively analyse the analytes of interest, a multiple time segment dynamic MRM method was preferred over the classical one-segment MRM method, to reduce the scan cycle time and number of concurrent MRMs. Initially, the two must abundant transitions were used as quantifier and qualifier transitions and each analyte or group of similar analytes were separated into different time segments, each with its own transitions and optimal source parameters. The benefit of this approach is that, rather than performing MRM scans for all analytes throughout the method, during any given segment, the triple quad only monitors MRM transitions for the analytes that elute in that segment. The result is that there are fewer MRM transitions during each MS scan, allowing the use of longer dwell times and to reduce the overall cycle time for each MRM scan, which in turn translates to more data points per peak. But the LC gradient must be very well optimised, to ensure that there are no analytes eluting close to the threshold between the two segments, otherwise if, for whatever reason, there is a small retention time shift, the data generated can be compromised. In case any other analytes need to be added to the method, it often requires a complete redevelopment of the LC gradient to introduce some chromatographically "quiet" zones where the segment changes can occur.

This approach ensured good sensitivity for the target analytes, but made the method less robust than desired. To overcome this issue, dynamic MRM mode was employed. This mode is based on only monitoring analytes when they are eluting from the LC and valuable MS duty cycle is not wasted by monitoring them when they are not expected. This is accomplished by storing the transitions and the retention time window for each analyte, which in turn dramatically reduces the number of individual MRM transitions that are monitored during each MS scan. The number of scans/second that the detector can record is thus increased, allowing the collection of sufficient data points even for very narrow peaks (Figure 3.2.2).

3.2.3.5. Optimisation of the SPE step

In other studies (Brandsma et al., 2013; Fang et al., 2013; Matsukami et al., 2015), ENVI™-Florisil® was used to fractionate different EPFRs, in matrices such as dust, soil and sediment. The disadvantages would be the necessity of using solvents which are slow to evaporate (methanol), or

eluting the EPFRs in two separate fractions or employing another SPE sorbent as well. So far, none of the methods described in the literature encompasses all EPFRs included in our study.

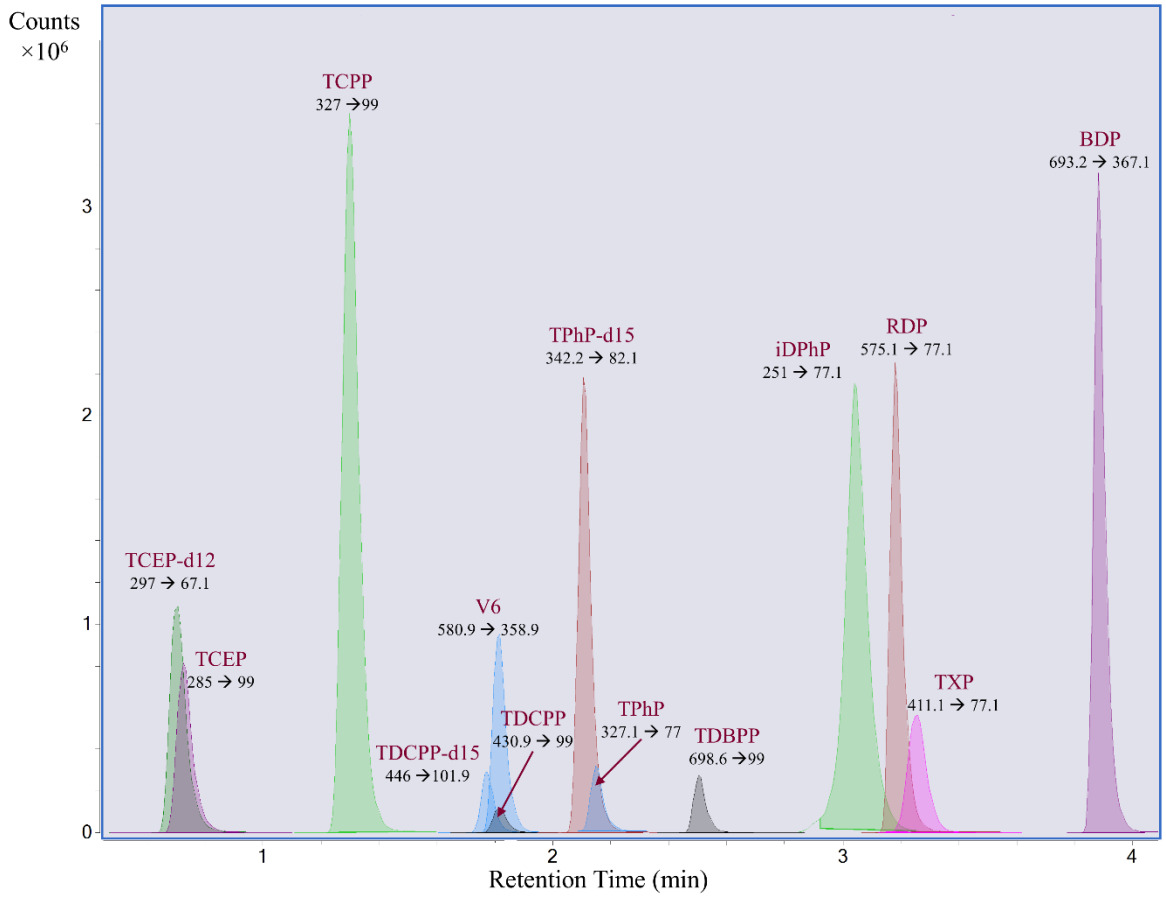


Figure 3.2.2: Most abundant MRM transitions for each analyte (0.6-0.9 ng injected on the column, depending on analyte)

For plastics, the same dilute-and-shoot approach employed in (Ballesteros-Gómez et al., 2014) was used, and since dust is typically a "dirtier" matrix than textiles, we continued the SPE development with dust as matrix. Aliquots of 75 mg of EPFR-free dust were spiked with a mix of EPFRs (50 ng each), extracted with the procedure explained above, and different SPE conditions were tested. The first fraction has been eluted with 10 mL of hexane for all cartridges and for the second fractions, different solvents were tested for the elution of all target analytes. After the second fraction, the cartridges were rinsed with 10 mL of acetone to check for remaining residues.

By employing the Florisil method previously used for the (Dodson et al., 2012) study, V6 was not completely eluted from the cartridge with only 8 mL of ethyl acetate. Other solvents/mixtures were then used, such as a mix of ethyl acetate and isopropyl alcohol (3:1 and 9:1) and acetone. Eluting the second fraction with 10 mL of acetone proved to be most effective, but still did not remove all EPFRs from the SPE cartridge. Next, higher volumes of eluting solvents or stronger

solvents were employed, and three other types of cartridges: Bond Elut NH_2 (APS), Bond Elut-SI (silica) and Isolute ENV+ were included in the testing (Table 3.2.3).

Table 3.2.3: SPE cartridges and elution solvents tested. First fraction, eluted with n-hexane contained no PFRs. The percentages represented are for the PFRs eluting in the second fraction. The rest up to 100% is what remained on the cartridge after elution of the second fraction.

	APS		**Florisil**		**ENV+**			**Silica**		
Trial*	1	2	3	4	5	6	7	8	9	10
Elution solvent / Analyte	10 mL DCM	10 mL EtOAc	12 mL ACE:DCM (1:1)	12 mL ACE	8 mL DCM + 4 mL EtOAc	12 mL ACE:DCM (1:1)	8 mL ACE	10 mL EtOAc	8 mL ACE	12 mL ACE:DCM (1:1)
V6	77%	95%	98%	99%	99%	98%	99%	95%	99%	100%
iDPhP	100%	100%	98%	95%	100%	99%	98%	100%	99%	100%
RDP	100%	100%	97%	96%	99%	98%	97%	100%	99%	100%
TXP	100%	100%	98%	98%	99%	97%	96%	100%	99%	100%
BDP	100%	100%	98%	98%	99%	96%	95%	100%	99%	100%
TCEP-d12	100%	100%	87%	100%	100%	99%	99%	100%	98%	98%
TDCPP-D15	98%	100%	100%	100%	100%	100%	98%	100%	100%	100%
TPhP-d15	100%	100%	98%	96%	100%	99%	98%	100%	100%	100%

**All trials are fraction 2 elutions and were run simultaneously.*

In all tested conditions, no compound was detected in the hexane fraction, while <5% of the compounds were retained in third fractions in some cases. The major proportion of the analytes could be eluted in the second fraction, but with Florisil and ENV+, almost all compounds were found to have certain carry over even when eluted with very polar solvents, such as acetone. Comparably, from silica and APS almost all compounds could be completely eluted with ethyl acetate (trial 2 & 8). The exception here was V6 (>5% carry over for both) which could only be completely eluted by slightly increasing the ethyl acetate volume, with the risk of eluting more of the matrix. The eluates from the APS cartridges demonstrated less matrix effects than the ones from the silica cartridges, and thus were preferred for this analysis. The APS sorbent has some anion-exchange capabilities due to its amino functional group and can retain more interferences, such as pigments (Xu et al., 2015). Trial 2 showed up to 50% higher S/N for most compounds, especially IS and V6, indicating better sensitivity with APS when analysing EPFR-contaminated samples. The same findings were also confirmed for textile matrices.

3.2.3.6 Initial method assessment

The performance of the method was tested for all three considered matrices. Samples with low levels of the analytes of interest and which were homogeneous were chosen for the testing and further spiking. A quality control solution was prepared for high levels of analyte (QH, analyte concentrations from 9.4-13 ng/µL) and one for low levels (QL, levels ranging from 0.46-0.54 ng/µL). The experiments were run over the course of 3 days. Every day, the spiked samples were analysed in

triplicate for the 2 different concentration levels, along with an unspiked replicate. For QA/QC measures, three blanks were run per day of analysis. The analyte levels in the blanks were low and were subtracted from those in the sample extracts.

A number of analytical parameters were monitored to assess the performance of the method: accuracy, precision, recovery (table 3.2.4). The range of accepted values for trueness were between 70-130% and as for precision, values up to 25% were considered acceptable.

Table 3.2.4: Initial method evaluation for the three matrices considered. Green colour = within the range of accepted values / red colour = outside the range of accepted values / yellow colour = on the edge of the range.

Matrix	Parameter	Spike solution	Analyte								
			TCEP	**TCPP**	**TDCPP**	**V6**	**TDBPP**	**iDPP**	**TXP**	**RDP**	**BDP**
Dust	Trueness (%)	**Qhigh**	99	62	82	89	26	47	71	44	104
	Precision (%)		12	20	6	23	9	37	7	64	9
	Trueness (%)	**Qlow**	99	47	84	115	19	35	57	13	96
	Precision (%)		8	9	10	9	26	8	5	29	8
			TCEP	**TCPP**	**TDCPP**	**V6**	**TDBPP**	**iDPP**	**TXP**	**RDP**	**BDP**
Plastic	Trueness (%)	**Qhigh**	128	32	96	121	99	98	91	102	87
	Precision (%)		2	11	10	6	11	15	16	12	44
	Trueness (%)	**Qlow**	129	27	99	173	97	100	77	104	132
	Precision (%)		4	16	18	8	8	12	16	18	13
			TCEP	**TCPP**	**TDCPP**	**V6**	**TDBPP**	**iDPP**	**TXP**	**RDP**	**BDP**
Textile	Trueness (%)	**Qhigh**	122	103	101	102	66	67	108	70	99
	Precision (%)		3	4	12	10	9	21	1	38	6
	Trueness (%)	**Qlow**	128	149	91	91	46	70	139	57	257
	Precision (%)		5	4	25	4	9	6	5	22	5

The precision is calculated as the percentage relative standard deviation (% RSD) from the target value (spiked amount). The trueness is calculated as the percentage of the spiked amount that is measured (recovery).

Some of the analytes considered fall outside of the considered range (Table 3.2.4) and the method performance is highly matrix-dependant. For TCPP, we noticed systematically lower response in the dust spiked samples and especially in the plastic spiked samples. Interestingly, TCEP, an analyte quantified using the same internal standard (TCEP-d12) and very close in volatility with TCPP showed good trueness and precision (even with slight signal enhancement in plastic and textile samples). Likewise was it observed for TDBPP in dust and textile-spiked samples. However, in the plastic spiked samples, the trueness was very close to 100% for both spiking levels. This particular analyte did have somewhat lower sensitivity than the rest and analysis in negative mode would likely provide better sensitivity.

Similarly, RDP is displaying unsatisfactory results in dust and textiles spiked samples. For the heavier oligomeric PFRs such as BDP and especially RDP, we did notice that TPhP-d15 was not the most appropriate internal standard considering the differences in physico-chemical properties and mass, but sadly it was the only one available. Interestingly, the trueness and precision for BDP is for the most part better than for RDP, so this is not the result of a systematic phenomenon leading to the loss of part of the amount of heavier analytes in the samples.

Table 3.2.5: Assessment of extraction procedure through exhaustive extraction

Matrix	Initial spike	Parameter	Analyte								
			TCEP	TCPP	TDCPP	V6	TDBPP	iDPP	TXP	RDP	BDP
Dust	Qhigh	Mean %	1	1	0	0	0	0	0	0	1
		RSD (%)	23	52	0	0	56	67	75	81	96
	Qlow	Mean %	10	9	0	0	1	2	1	0	0
		RSD (%)	56	56	0	0	6	30	30	111	0
Plastic	Qhigh	Mean %	2	1	0	1	0	1	0	0	2
		RSD (%)	115	55	0	174	26	43	42	73	38
	Qlow	Mean %	2	3	0	0	1	1	0	0	0
		RSD (%)	127	131	0	0	22	67	69	0	0
Textile	Qhigh	Mean %	4	5	5	5	3	5	4	3	6
		RSD (%)	78	83	158	168	79	88	91	65	81
	Qlow	Mean %	8	20	0	0	11	14	8	7	11
		RSD (%)	63	31	0	0	14	72	106	71	111

Values calculated based on blank-substracted amounts. A percentage of analyte ≤ 10 % on re-extraction is considered adequate, in between 10-15 passable for the Qlow spike (25 ng), and over 15% inadequate.

As we can see in Table 3.2.5, the percentage of analyte recovered at re-extraction is for the most part less than 10%. Therefore, the extraction step is not the cause of the lower recoveries. Considering all of the above, we conclude that the method requires further development and testing, in order to provide good results for all of the considered analytes.

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Chapter 4

Assessment of flame retardants in consumer products

4.1. Comprehensive characterisation of FRs in textile furnishings by gas chromatography-mass spectrometry, ambient high resolution mass spectrometry and environmental forensic microscopy

Based on the following manuscript:
Ionas AC, Ballesteros Gómez A, Uchida N, Suzuki G, Kajiwara N, Takata K, Takigami H, Leonards PEG, Covaci A. 2015. Comprehensive characterisation of flame retardants in textile furnishings by ambient high resolution mass spectrometry, gas chromatography-mass spectrometry and environmental forensic microscopy. Environ. Res. 142C, 712-719.

4.1.1. Introduction

Deaths from fires and burns are among the leading causes of fatal home injury. For this purpose, flame retardant chemicals (FRs) are added to a wide range of consumer products typically encountered in the indoor environment. Such products are, for example, foam and textile upholstery, carpets, and curtains. Chemicals, such as polybrominated diphenyl ethers (PBDEs), are commonly added to certain types of textiles to impart flame retardancy. In carpet padding, for example, the commercial "Penta-BDE" technical mixture was used. In the back-coating of curtains, the "Deca-BDE" technical mixture is commonly added. Another class of FRs used in textiles are organophosphate FRs (PFRs). These multi-use chemicals are considered to be suitable alternatives for the PBDEs (van der Veen and de Boer, 2012), in applications such as textile back-coatings (Horrocks et al., 2007). However, serious concerns about the possible adverse health effects of the aforementioned FRs have been raised (see chapter 1).

Both BFRs and PFRs are not chemically bound to textiles and the matrix is very soft and flexible, allowing these chemicals to leach out of the materials they were added to (Kajiwara and Takigami, 2013; Kemmlein et al., 2003). The FRs then migrate to indoor air and dust (Kajiwara and Takigami, 2013; Rauert et al., 2014a), and from there are taken-up into the human body (Lyche et al., 2015; Wei et al., 2015).

There has been very little research about the presence of FRs in textiles, their levels, the extent of emissions into the indoor environment and their impact on humans. Kajiwara et al. (Kajiwara et al., 2011, 2009) has investigated the presence of hexabromocyclododecane (HBCDD), BDE-209 and PFRs in textiles from Japan. Similarly, (Shin and Baek, 2012) have described the PBDE levels in flame-retarded textiles present on the South Korean market. In (Kemmlein et al., 2003), BFRs and PFRs were studied in textile samples (among others) and emission studies were undertaken to assess their migration into the indoor environment. Different aspects of this

phenomenon were also studied by (Kajiwara and Takigami, 2013; Rauert et al., 2014a; Rauert et al., 2014b).

Since some FRs have been proven to be harmful to humans (Lyche et al., 2015; van der Veen and de Boer, 2012; Vonderheide et al., 2008), in this study we have investigated the presence and levels of these chemicals in textiles from the Belgian market. To streamline this process, we have investigated the usefulness of the time-of-flight (TOF) high resolution mass spectrometer (direct probe-TOF-MS) technique as a quick, cheap and reliable method to screen for these chemicals in a high number of samples. This technique, along with a gas chromatography-mass spectrometry (GC-MS) and forensic microscopy, can be used as a comprehensive approach to elucidate if and what type of FR is present in a given sample.

4.1.2. Materials and methods

4.1.2.1 Samples

47 curtain samples and 14 carpet samples were collected from four different stores from Antwerp, Belgium in 2013. Environmental contamination was removed from the samples by wiping their surface with paper tissues impregnated with Milli-Q water.

4.1.2.2 Materials

All solvents were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, methanol, ethyl acetate, toluene, dichloromethane and iso-octane were purchased from Merck (Darmstadt, Germany). Modified nylon centrifugal filters with 0.2 µm and 0.45 µm pore size were bought from VWR (Leuven, Belgium). Supelclean ENVI- Florisil cartridges (500 mg/3 mL) were purchased from Supelco (Bellefonte, PA, USA). Capillary melting point tubes (probes) were obtained from Kimble Chase (Vineland, New Jersey, USA).

Standards of BDE 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209, together with ^{13}C-BDE 209 were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 and 128 was obtained from AccuStandard Inc. (New Haven, CT, USA). EH-TBB, BEHTBP, DBDPE, TBECH, ATE, DPTE, BTBPE, HCDBCO were purchased from Wellington Laboratories (Guelph, ON, Canada). Standards of TnBP, TPhP, TCEP, EHDPhP, TCP (mixture of 4 isomers) and TDCPP (mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). TBOEP was purchased from Sigma Aldrich. TCPP (mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBOEP (>94%).

4.1.2.3 Target compounds

FRs and plasticisers that have been known to be added to textile materials were targeted during the Direct Probe-TOF-MS screening (Table 4.1.1). For the analytes which were detected with this procedure, trace analysis by GC-MS was done (Table 4.1.2).

Table 4.1.1: FRs screened by Direct Probe-TOF-MS in textile samples – main ions generated, their mass and the estimated LOD

Analyte	LOD (ng/g)	Type of ion	Ion mass
TriBDEs	n.d.	$[M\text{-}Br+O]^-$	342.8798
TetraBDEs	n.d.	$[M\text{-}Br+O]^-$	420.7903
PentaBDEs	n.d.	$[M\text{-}Br+O]^-$	500.6988
HexaBDEs	n.d.	$[M\text{-}Br+O]^-$	578.6093
HeptaBDEs	n.d.	$[M\text{-}Br+O]^-$	658.5178
OctaBDEs	n.d.	$[M\text{-}Br+O]^-$	736.4283
NonaBDEs	n.d.*	$[M\text{-}Br+O]^-$	816.3367
BDE 209	36500	$[M\text{-}Br+O]^-$	894.2472
BTBPE	n.d.	$[M\text{-}C_8H_6Br_3O]^-$	328.7629
ATE	n.d.	$[M\text{-}Br+O]^-$	306.8787
BATE	n.d.	$[M\text{-}Br+O]^-$	384.7892
DPTE	n.d.	$[M\text{-}Br+O]^-$	466.7133
HCDBCO	n.d.	[M-Cl+O]-	520.7642
HBCDD	n.d.	$[M\text{-}H]^-$	642.6521
EH-TBB	n.d.	$[M\text{-}Br+O]^-$	484.878
BEHTBP	n.d.	$[M\text{-}Br+O]^-$	642.991
DBDPE	n.d.	$[M\text{-}Br+O]^-$	906.2831
TBP	290	$[M+H]^+$	267.1719
TCEP	260	$[M+H]^+$	284.9612
TCPP	170	$[M+H]^+$	327.0081
TDCPP	n.d.	$[M+H]^+$	430.8883
TPhP	280	$[M+H]^+$	327.0781
EHDPhP	280	$[M\text{-}C_8H_{17}+H_2]^+$	251.0468
TEHP	250	$[M+H]^+$	435.3598
TBOEP	180	$[M+H]^+$	399.2506
TCP	190	$[M+H]^+$	369.1250
V6	n.d.	$[M+H]^+$	580.9149
RDP	n.d.	$[M+H]^+$	575.1019
BDP	n.d.	$[M+H]^+$	693.1802

n.d. = not determined, due to the fact that the analytes were not detected by Direct Probe-TOF in any of the samples

*NonaBDEs were detected, but are likely the result of in-source de-bromination of decaBDE, so an LOD could not be assessed for them

4.1.2.4 Direct probe screening (ambient mass spectrometry). Procedure and parameters

The samples were submitted to a rapid screening for FRs employing a direct probe (DP) assembly, mounted on an atmospheric pressure chemical ionisation (APCI) II source, connected to a Bruker Daltonik microTOF II mass spectrometer (mass accuracy <2 ppm and resolution >16500 FWHM).

The procedure followed was similar to the one described in (Ballesteros-Gómez et al., 2014; Ballesteros-Gómez et al., 2013). A small amount of sample was introduced in the glass capillary and a drop of calibration solution was added to the wall of the capillary, which was then slid into the ionisation source. A temperature program was developed in order to gradually vaporise both volatile and less volatile analytes, before the matrix becomes carbonised and background noise increases enough to prevent detection. As such, the initial vaporiser temperature was set at 225 ºC, then increased to 260 ºC after 1 min and 300 ºC after 3.5 min and held at this temperature until 5 min. To prevent cross-contamination, the source was "baked-out" for 5 min at 400 ºC between analyses. The detector had the following parameters: dry gas 2 L/min, nebuliser 4 bar, dry heater 220 ºC, capillary voltage 1000 V (both polarities), corona -8000 nA (negative) and +5000 nA (positive) and end plate offset of -1000 V (positive) and -500 V (negative).

This provided basic information about the FR content of the samples quickly and with minimal consumable use. The limit of detection (LOD) was determined by searching for the lowest level of analyte, determined by GC-MS and detected with the direct probe-TOF-MS. The values were rounded down to the nearest value dividable by 10, if the number was lower than 1000 and down to the nearest value dividable by 100, if the number was between 1000 and 100000 (Table 4.1.1). This approach was used to take advantage of the availability of accurate quantitative levels, determined by GC-MS and to give a more accurate and realistic estimation of the LOD for our analytes of interest in the matrix studied (by compensating for varying spectral noise levels). In some samples which generate less spectral noise during the direct probe-TOF-MS analysis, the analytes can be tentatively detected at even lower levels, but setting the LOD to lower levels can further increase the number of false positive results, some of which can be due to background levels from the environment where the analysis is being conducted.

4.1.2.5 Extraction procedure for GC-MS analysis

The procedure employed for the extraction of the samples was developed in a previous study (Ionas et al., 2014) and it consists of a combination of ultrasound assisted extraction and solvent vortexing, using a mixture of dichloromethane and *n*-hexane (1:3, by volume). The extracts were evaporated to near dryness and re-solubilised in 1 mL of *n*-hexane and added to the ENVI Florisil cartridges. The elution was done in two fractions: the first with 8 mL *n*-hexane to collect the

brominated FRs (BFRs) and the second with 8 mL ethyl acetate to collect the organophosphorus FRs (PFRs). Both fractions were evaporated to near dryness and reconstituted in 100 µL of iso-octane.

4.1.2.6. Instrumental parameters for GC-MS

For the analysis of BFRs, an Agilent 6890 GC coupled to an Agilent 5973 MS was employed and equipped with a chemical ionisation source, operated in negative mode (ECNI). For PFRs, an identical system was used, equipped with an electron ionisation (EI) source. Both systems were equipped with a programmable-temperature vaporizer inlet (PTV), operated in the pulsed splitless mode. The carrier gas employed was He, with a purity of 5.9. The quadrupole and interface temperatures were set at 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V. The injection volume on both systems was 1 µL, but the GC parameters were different.

On the GC-ECNI/MS system, the column employed was an Agilent J&W DB-5 (15 m × 0.25 mm × 0.10 µm) and the GC temperature program started at 90 °C, with a hold of 1.25 min, ramp 10 °C/min to 310 °C and hold for 12 min. The carrier gas (He) was run in a ramped flow mode, and after 20 min, it was increased (20 mL/min^2) from 1 to 2 mL/min. Methane was used as moderating gas and the ion source temperature was set at 250 °C.

On the GC-EI/MS system, a SGE HT-8 column (25 m × 0.22 mm × 0.25 µm) was used. The GC temperature program started at 90 °C, with a hold at 1.50 min, ramp 10 °C/min to 310 °C and hold for 20 min. The carrier gas (He) was run in constant flow mode (1 mL/min). The ion source temperature was set at 230 ºC.

4.1.2.7. Forensic microscopy

A sample area of 10 mm × 10 mm was initially mapped using an energy dispersive micro XRF spectrometer (µEDX-1200, Shimadzu Co.) equipped with a Rhodium X-ray tube and a Nickel primary X-ray filter. The instrumental parameters are similar to the ones from (Rauert et al., 2014a; Suzuki et al., 2009): the tube voltage employed was 50 kV, a tube current of 1000 µA, and a beam diameter of 50 µm. Mapping test were done for Br, Cl and P. Brominated chemicals were more abundant than chlorinated and phosphorus-containing chemicals in the samples considered and the mapping for Br was the most sensitive. The parameter monitored during the mapping was the Kα characteristic X-ray emission lines of bromine. The step sizes were of 50 µm in the x-and y-directions, with a dwell time of 0.5 s at each point within the considered sample area. The detected intensities, in counts per second, were represented as mapping figures. Regions with high bromine content were re-mapped over smaller areas of 4 × 3 mm and 2 × 1.5 mm. By using an optical microscope, a mechanical manipulator (Quick Joy, Micro Support Co., Shizuoka, Japan) and micro tweezers, we attempted to separate the Br hotspots for further characterisation.

A LEXT OLS 4100 3D laser microscope (Olympus, Japan) and a digital optical microscope (VHX-200/100F, Keyence Co.) with a magnification of up to 100 × were employed to observe the

surface of the textile samples around the Br hotspot areas. Both an S2 Ranger Energy Dispersive X-ray Fluorescence spectrometer (Bruker, Billerica, MA, USA) and a Delta DP-2000C (Innov-X Systems Inc, Olympus Corporation, Japan) handheld X-ray fluorescence spectrometer, equipped with a Rh-target X-ray tube were employed to analyse the samples, with a dwell time of 60 sec.

The fibres containing the Br hotspot were further mapped using a Nicolet Continuμm Microscope coupled to a Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Thermo Scientific, Waltham, USA), with an MCT/A detector and an aperture size of 100 × 100 μm, in transmission mode. Spectra were acquired with a resolution of 4 cm^{-1}, 128-256 acquisitions, over a spectral range of 4000 to 650 cm^{-1}. The chemical composition of the sample materials and the position of the hotspots of analytes of interest were identified by conducting searches in the OMNIC Spectra version 9.0 spectral libraries (also provided by Thermo Scientific).

To pinpoint the Br-containing area with more accuracy and to get more surface information about it, a JSM-7600F field emission scanning electron microscope (SEM) (JEOL, Japan), equipped with a retractable backscattering electron detector and energy dispersive X-ray spectrometer (EDS) analyser with silicon drift X-ray detector was employed. Using an accelerating voltage of 10-20 kV, lower secondary electron image (LEI) scans were taken under a magnification of 2000x and the sample was scanned for Br to visualise the Br-rich areas. The presence of Br was only confirmed by monitoring the less abundant, higher energy Kα line at 11.907 keV, because the more abundant Lα line is typically overlapping with the Kα line of aluminium.

4.1.3. Results and discussion

As there is an increasing need for quicker detection of key analytes, we assessed the capabilities of the Direct Probe-TOF-MS (ambient mass spectrometry) for rapid qualitative screening by comparing the results with GC-MS, a validated technique which provides reliable quantitative results. Any anomalous findings were investigated further using forensic microscopy techniques to elucidate the distribution of the compounds on the surface of the textiles.

4.1.3.1. Sample screening by Direct Probe-TOF-MS

After the samples were analysed in both polarities, they were internally recalibrated and the accurate masses of the expected major ions of the analytes of interest were extracted (analytes are provided in Table 4.1.1). The ions of the studied BFRs in APCI negative mode were mainly $[M-Br+O]^-$, while for PFRs, mostly the $[M+H]^+$ ion was expected in positive mode (Table 4.1.1). The threshold error for positive identification was set at 10 ppm as derived from the maximal mass error observed in "injected" standards of the studied FRs. By "injected" in this context, we mean that a drop of analytical standards was added to a clean glass capillary, which was then introduced directly into the APCI source. The accurate masses were thus extracted with a width of ±0.007 mass units. This

range was chosen to correspond to a 10 ppm mass error for the analyte with the largest molecular mass, e.g. bisphenol A bis(diphenyl phosphate) BDP. This analyte, along with resorcinol bis(diphenyl phosphate) (RDP), 2,2-bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate] (V6) and HBCDD were also screened in the samples, but since they were not detected in any sample, they were not further included in the analytes quantified by GC-MS. The analytes thermally desorbed from the textile matrix within just 0.3-1 min of analysis. The extracted ion chromatograms of the target compounds were shaped as wide or tailing chromatographic peaks. The spectra of these "peaks" were averaged to determine mass errors with better reliability. In order to assess the screening method, the analytes detected by Direct Probe-TOF-MS, but not by GC-MS, were considered "false positives". "False positives" were most probably coming from background contamination. On the other hand, the analytes that were quantified by GC-MS at levels over the expected LOD of the direct probe-TOF-MS, but not detected using this technique were considered "false negatives". These limitations were explained by the lower sensitivity of the screening method and the inhomogeneity of some samples as discussed in section 4.1.3.3.

The type of textile material determines the spectral noise level. In APCI negative mode the spectral noise was typically higher than in positive mode, and the LOD of PBDEs was two orders of magnitude higher than for the PFRs (Table 4.1.1). For instance, the LOD for BDE-209 was roughly 37000 ng/g (0.0037%), while the LODs for the PFRs were < 300 ng/g (0.00003%). For halogenated compounds, it is more difficult to get false positive detections, due to the distinctive pattern of their isotope clusters. In positive mode, though, in the absence of characteristic isotope patterns, it is easier to get false positives and their likeliness is higher as the value is closer to the LOD of the Direct Probe-TOF-MS. In this range, the spectral noise is very close to the intensity of the ions of the analytes and the detection can more easily be affected by background contamination which can cause false positives hits. Since background contamination was frequent in the positive mode at around 10-150 ng/g, this was taken into account for the estimation of LODs of PFRs in order to assure a reliable detection. Procedural blanks (unloaded probes) are recommended to be run between samples for avoiding misinterpretation of results due to background contamination.

Since we introduce a small piece of textile directly into the MS source, the sample amount will also greatly influence the detectability of the technique, with higher sample amounts translating to lower LODs, but in some cases also more spectral noise.

In any case this technique does provide reliable detection for FR at the levels required to impart flame retardancy to a textile item.

As a result of the screening with the Direct Probe-TOF-MS, BDE-209 was the main BFR detected in 4 out of the 61 sample analysed, with mass errors from -8.89 to 4.02 ppm. With this technique, we also detected NonaBDEs (mass errors of -2.19 to -4.77 ppm) and OctaBDEs (mass error above 10 ppm) coming from in-source de-bromination of BDE-209. Nona- and OctaBDEs were

not detected by GC-MS confirming the in-source de-bromination of BDE-209 in direct probe-TOF-MS. To the best of our knowledge, the OctaBDE mixture has never been used in textiles.

Among the PFRs, TPhP was the most often detected, in 14 out of the 61 samples, over the LOD of the Direct Probe-TOF-MS, with mass errors from -9.48 to 9.48 ppm. TBOEP is second most often detected, in 13 out of the 61 samples screened, with mass errors between -7.76 to 8.27 ppm.

Overall, this procedure has the advantage of requiring no sample preparation, thus being economical (low consumable use) and "green" as there are no organic solvents used in the process. It is also quick, as the analysis of a sample can be done in about 5 min. Furthermore, it could be advantageous from complex samples from which analytes could be difficult to be extracted by a solvent/clean-up step.

Its limitations are the relatively high limits of detection (LOD) and the possible matrix noise hampering the detection in complex samples. It is useful for a first stage screening at the commonly high levels found in products and prior to further quantitation by conventional techniques.

4.1.3.2. Trace analysis by GC-MS

GC-MS analysis was undertaken, as a validated conventional technique to confirm and assess the results obtained with the direct probe screening and to provide more accurate and in-depth look into the profiles and levels of the FRs. GC-MS is more sensitive, with LODs 1-4 orders of magnitude lower when the selected ion monitoring (SIM) mode is employed. And the chromatographical separation does also provide an extra dimension of selectivity than Direct Probe-TOF-MS, where analytes desorb from the matrix nearly simultaneously. The sample preparation (described in the 4.1.2 section) is however more labour-intensive than the Direct Probe-TOF-MS procedure, with higher use of consumables and more expensive.

Among the analytes of interest, the PFRs were often detected, with detection frequencies up to 72% for TPhP, but at low levels (Table 4.1.2). TPhP also had the highest concentrations in the study (up to 95000 ng/g). However, at this concentration it cannot provide fire retardancy by itself. All other PFRs were present at low levels, with highest levels one order of magnitude lower than the maximum TPhP concentration.

BDE-209 was the most often BFR detected (in 56% of the samples) and with the highest concentrations, while the lower BDE congeners were all in the low ng/g range (Table 4.1.2). Four samples showed much higher levels of BDE-209, 2-3 orders of magnitude higher than the rest (37000, 47000, 78000 and the maximum value of 560000 ng/g). However, these levels are too low to impart flame retardancy, if the Deca-BDE mixture was the only FR added to the material.

The sample containing the highest level of BDE-209, also contained 25000 ng/g DBDPE and 16000 ng/g of TPhP. DBDPE was not detected at this level (0.0025% w/w) during the Direct Probe-TOF-MS screening, probably because it was below the LOD of the technique, which is decreased for highly non polar compounds. It is hard to estimate the flame retardant effect of these chemicals in a

mixture, but considering that 9-10% by weight of BDE-209 is required to efficiently flame retard a textile fabric (Kajiwara and Takigami, 2009; Kajiwara et al., 2013), it is not very likely that even in this mix, the above mentioned FRs would protect the fabric against fire. Similar levels of PBDEs were detected in the only work, to the authors' knowledge, that studies PBDEs in curtains, polymeric foam and textile materials used in car interiors (Shin and Baek, 2012).

Interestingly, in the sample with the highest BDE-209 concentration, the screening results by Direct Probe-TOF were not reproducible in all replicates. Given that environmental contamination was removed from the samples by wiping their surface with paper tissues impregnated with Milli-Q water, we attributed this phenomenon to sample inhomogeneity. Such behaviour was only encountered for BDE-209 among the analytes included in this study.

Table 4.1.2: Overview of the detection frequencies (DF, %) and concentrations of analytes of interest (**ng/g**) measured in the textile samples by GC-MS (n=61). Values rounded to significant digits.

Analyte	DF (%)	10th Percentile	90th Percentile	Max
BDE 28	2	0.3	0.3	0.3
BDE 47	26	0.3	1	3
BDE 66	0	-	-	-
BDE 100	3	0.2	0.4	0.4
BDE 99	28	0.2	2	5
BDE 85	8	0.1	1	2
BDE 154	8	0.3	1	1
BDE 153	16	0.3	2	3
BDE 183	13	0.5	3	4
BDE 209	56	10	25922	560000
EH-TBB	2	10	10	10
DBDPE	13	14	7644	25000
TnBP	18	30	470	1000
TCEP	28	48	686	1800
TCPP*	54	15	368	1000
TBOEP	41	48	2480	4300
TEHP	23	37	1125	1700
TPhP	72	10	2630	95000
EHDPhP	41	34	702	4200
TCPs**	8	94	2820	3300

*Sum of 2 isomers;

4.1.3.3. Environmental forensic microscopy investigations

This approach was employed to determine if a certain element or chemical is homogeneous on the surface of a sample, where the most concentrated areas are, what they look like and what material they are made of. For the IR and XRF spectroscopy techniques, the element or chemical needs to be present in high concentrations in the samples. As a consequence, we could only focus our study on samples with a high concentration of BDE-209.

To completely rule out any possibility of external contamination not removed during the sample pre-treatment, the surfaces of the samples were observed by a laser microscope and a digital optical microscope. For example, one particle was observed on the surface of one of the samples with an optical microscope and later picked-up using a manipulator and micro tweezers and analysed by FTIR. The material was PVC and did not contain bromine.

To test for sample inhomogeneity, the three samples with highest BDE-209 levels were mapped using a Shimadzu µEDX-1200 energy dispersive micro XRF spectrometer (Figure 4.1.1). The Br distribution was not even throughout the fabric, as would be expected for a flame retarded fabric (Figure 4.1.2), but rather in remote hotspots, with very high Br content (Figure 4.1.1 and 4.1.3). However, the intensity of the Br-rich areas was in the same order of magnitude as the FR-containing curtains (see intensity scales in Br-mapping images in Figure 4.1.1 and 4.1.2).

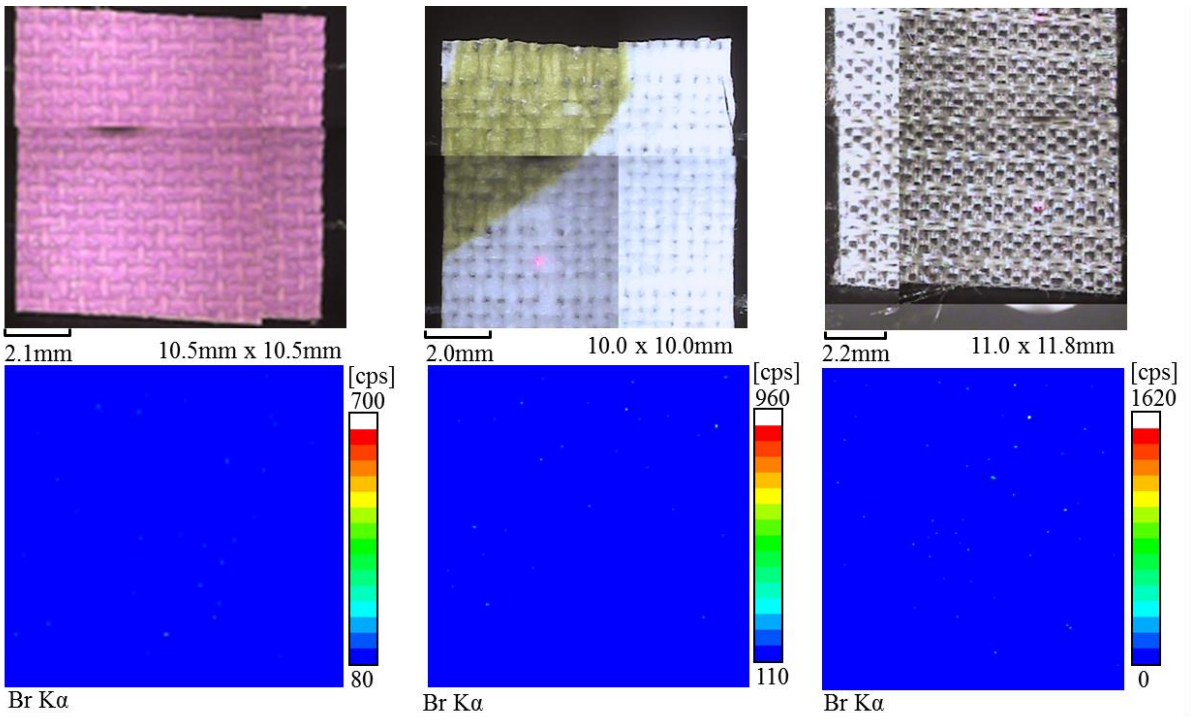

Figure 4.1.1: Br-mapping X-ray fluorescence spectrometry images (lower row) and the images of the corresponding samples. The small, barely discernible white spots are the areas with highest bromine concentration and the areas in blue do not contain bromine. For a more detailed image, see figure 4.1.3.

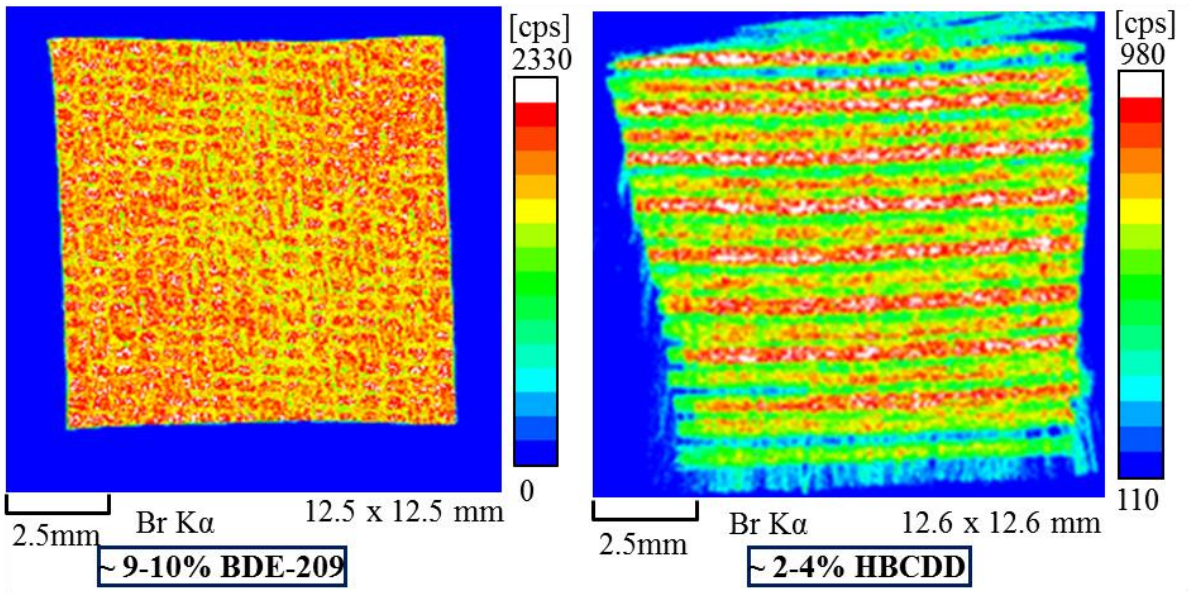

Figure 4.1.2: Br-mapping X-ray fluorescence spectrometry images of curtains (from other studies), flame retarded with BDE-209 and HBCDD. The areas in white followed by the areas in red are spots with highest bromine concentration. The areas in dark blue do not contain bromine.

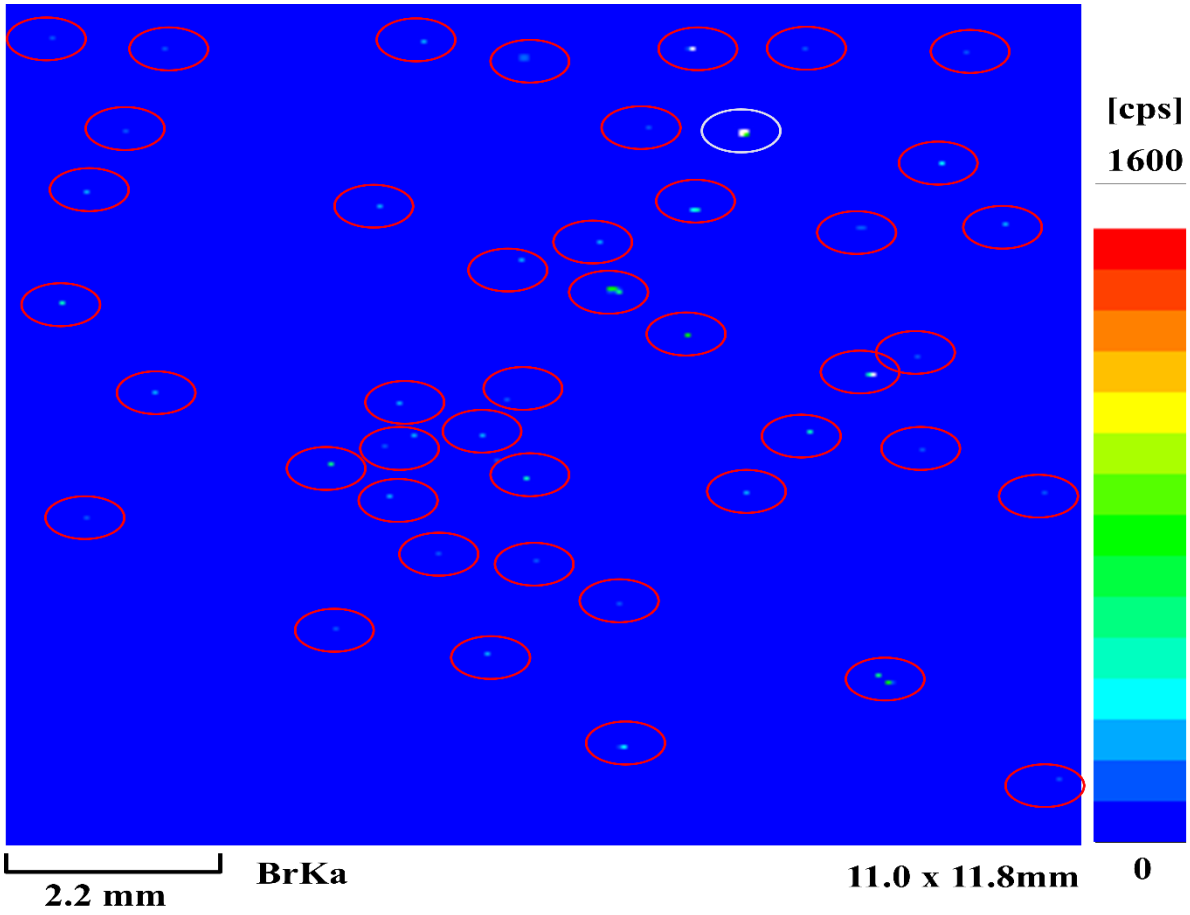

Figure 4.1.3: More detailed Br-mapping image of the samples with highest BDE-209 levels. Highlighted in red are the Br-rich hotspots. Highlighted in white is the most concentrated hotspot, which was cut out and underwent further analysis with other environmental microscopy techniques.

Using a Delta DP-2000C handheld XRF, the same samples were scanned repeatedly, in different areas, on a 10 mm diameter analysis spot. The relative standard deviation of the measurements (taken in different parts of the samples, 3-5 replicates) was 15% for the sample with the lowest levels, 8% for the second most concentrated and 1% for the sample with the highest BDE-209 concentration. So there were more than just a few of these hotspots in just one area of the sample, but rather they were distributed all across the surface of the sample.

Since we saw this pattern of Br-rich hotspots by µEDX, and there was no visible contamination (particles or other traces) on the surface of the samples, at those particular areas, we concluded that the BDE-209 contamination was within the fabric. It is possible that this contamination happened during the manufacturing of the textiles. We then attempted to separate the Br-rich hotspots, to obtain information about the possible origin of this unusual BDE-209 contamination.

The hotspots with the highest intensity were mapped by µEDX with more accuracy - at a higher zoom level and with a smaller step size (5 and 10 µm). They were then cut out using a micro scissor and the fibres were taken apart with micro tweezers and a mechanical manipulator. All these operations were performed under an optical microscope. The area with the Br-rich hotspot was then mapped using the Nicolet Continuµm Microscope coupled to the Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Figure 4.1.4).

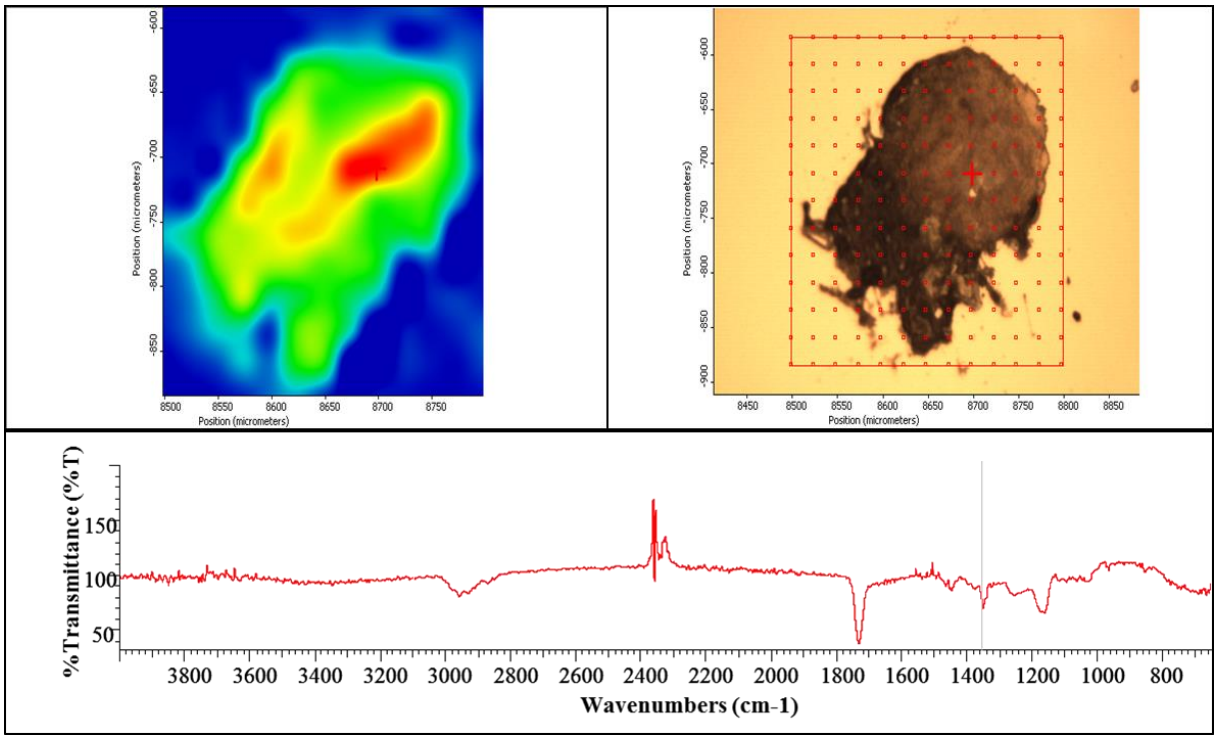

Figure 4.1.4: ***right:*** magnified image of the sample fragment; ***left:*** Br map depicting Br hotspot; to obtain this map, the 1350 cm^{-1} characteristic DecaBDE band (Sato et al., 2010) was monitored. In the image to the left, the colour red depicts the maximum intensity of the Deca BDE band.

To check if there were any differences in the constituent material of the piece, we also did a point analysis of the fibres and of the hotspot separately. After removing the fibres from the material containing the Br-rich hotspot we determined that it was either made of the same material as the fibres of the curtain itself (often polyethylene terephthalate) or covered by the same acrylic resin contained in the curtain – typically a poly(ethylacrylate) blend.

The Br-rich area was then visually inspected with a laser microscope. As visible in Figure 4.1.5, the Br hotspot was inside this piece of textile material, so it was flattened using two SEM specimen mounts ("stubs") and submitted to SEM-EDS analysis.

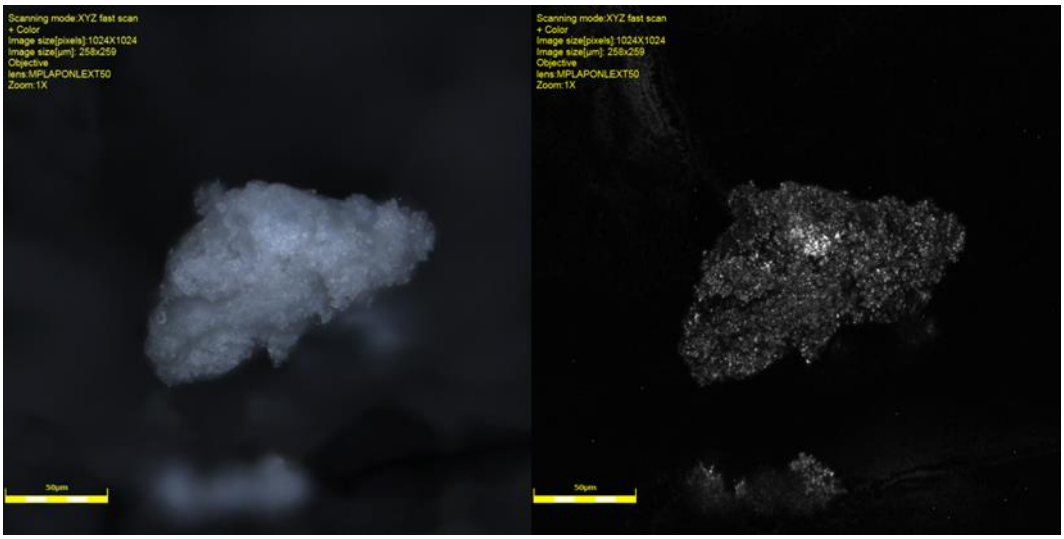

Figure 4.1.5: Optical (left) and laser (right) image of the piece of textile material containing the BDE-209 hotspot

The precise spot of the Br-rich hotspot was pinpointed by mapping the Kα emission line of Br (Figure 4.1.6). Although this line is less abundant than the Lα line of Br, it provides more reliable data, as it does not overlap with any emission line of any other element.

Even at the high level of magnification provided by the SEM, we do not see one unitary area different from the surrounding material which contains the whole Br signal, like we would do with a particle. One possible explanation for this would be that textile manufacturers often produce both flame retarded and non-flame retarded textiles, and there is a possibility that very fine droplets containing BDE-209 may have accidentally gotten on the fabrics that were not meant to be flame retarded during the manufacturing process.

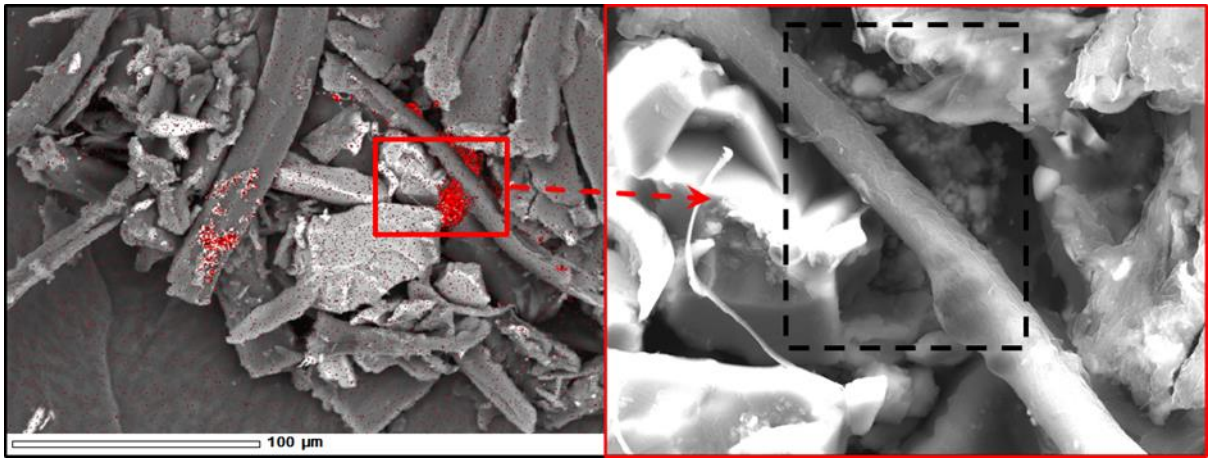

Figure 4.1.6: ***left***: Br map (Kα in red) of the textile material; ***right***: lower (secondary) electron image (LEI) of the Br hotspot, at a magnification of 2000× and an accelerating voltage of 20 kV.

We attempted to estimate the BDE-209 concentration by taking a piece of textile containing a concentrated Br hotspot (determined by µEDX scanning), accurately measuring and weighting it, cutting the hotspot out, measuring its surface and then extracting it with organic solvent and analysing it by GC-MS. This allowed us to calculate two estimates for the BDE-209 concentration. By extrapolating the surface and amount of BDE-209 of the hotspot to the initial sample, for which the weight was known, the percentage of BDE-209 at that spot was 18%. By extrapolating using the signal of the hotspot (in counts per second) returned by the µEDX for a sample containing 9-10% BDE-209 and the signal of this hotspot, the percentage of BDE-209 was estimated at 11.3%. These levels are in accordance with fire retardancy standards and could support the hypothesis of contamination with fire retarded textiles during the processing.

4.1.3.4. Halogen-free / inorganic FRs

When conducting XRF analyses of the samples containing the highest levels of BDE-209 the amounts of other inorganic elements were also determined. It appears that inorganic FRs were also likely used, as Al and Sb were detected in percentage amounts of 1.5-3.5%, which could be the result of aluminium trihydroxide (ATH) and antimony trioxide (ATO) addition (Weil and Levchik, 2009) to impart resistance to flames. Other metallic elements were detected in similar percentages, such as Na or Ca, the salts of which may have been used as fillers (Hornsby, 2007).

4.2. Monitoring of FRs in electronic consumer products

Based on the following manuscript:
Ionas AC, Uchida N, Suzuki G, Takigami H, Neels H, Covaci A. Characterisation of flame retardants in plastics from electronics: levels, elemental and material analysis (in preparation)

4.2.1. Introduction

One of the main sources of FRs in indoor environments are electronics. In electronics, plastic components are in close proximity or even in contact with circuit boards or other components which can get very hot. In case there is a malfunction or if the device is used very intensly it can become a fire hazard. So it is very important to minimise the risk from such devices by ensuring that the plastic materials contained in the electronics have an appropriate resistance to overheating and to fire. For this reason, FRs (particularly BFRs such as PBDEs and TBBPA, and also chlorinated PFRs) have been added to the plastics used in electronics. Unfortunately, these highly efficient FRs come with rather serious health risks associated (Lyche et al., 2015; Wei et al., 2015). Less harmful alternatives to these chemicals have been developed and are being implemented, but it is still very important to monitor these BFRs and PFRs which have proven to be detrimental to human health, in order to be able to assess the exposure arising from using electronics which contain them.

Consequently, the aim of this study was to monitor the plastic contained in current-use electronic devices for these potentially harmful FRs. Analysing plastics poses a number of difficulties, such as the fact that if the extracts are not cleaned-up in the proper way or are not diluted enough, it can cause instrument issues such as damaging of the GC or LC columns. For this reason, it is important to reduce the number of samples which actually undergo chemical analysis, by using non-destructive techniques such as XRF. This has the added advantage that the consumable use is much lower, including of organic solvents, making the whole process is more “green” / eco-friendly.

4.2.2. Materials and methods

4.2.2.1. Reagents and materials

All solvents used were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone (ACE), toluene, dichloromethane (DCM), methanol (MeOH), ethyl acetate (EtOAc) and iso-octane were purchased from Merck (Darmstadt, Germany). Supelclean™ ENVI™- Florisil® and ENVI-Carb™ SPE cartridges (500 mg/3 mL) were purchased from Supelco (Bellefonte, PA, USA). Standards of triphenyl phosphate (TPhP), tris(2-chloroethyl) phosphate (TCEP), tris(1,3-dichloro-isopropyl) phosphate (TDCPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway), isodecyl diphenyl phosphate (iDPhP) from

Accustandard (New Haven, CT, USA). Trixylenyl phosphate (TXP) was purchased from Chemos (Regenstauf, Germany). Resorcinol bis(diphenyl phosphate) RDP and Bisphenol A bis(diphenyl phosphate) BDP were purchased from TRC (Toronto, ON, Canada). Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Labelled TPhP-d15 (IS) was purchased from Sigma Aldrich, while labelled TCEP-d12, TDCPP-d15 were synthesised by Dr. Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany). Ultrapure water (18.2 MΩ) was obtained from an Elga LabWater water purification instrument (Saint Maurice, France). Except TXP and iDPhP which were technical mixes, the purity of analytical standards was >98%.

The certified reference materials ERM-EC680 and EC681 (containing Br, Cl, Al, Sb, As, Ba, Cd, Cr, Cu, Pb, Hg and Ti) in polyethylene were purchased from IRMM (Geel, Belgium).

4.2.2.2. Instrumentation

An S2 Ranger Energy Dispersive X-ray Fluorescence spectrometer (Bruker, Billerica, MA, USA), equipped with an XFlash V5 silicon drift detector, capable of detecting elements as light as sodium and as heavy as uranium was employed for a quick screening. The plastic shavings were added to sample cups and scanned to determine elemental composition.

The samples were then analysed with a Nicolet Continuμm Microscope coupled to a Nicolet 6700 Fourier transform infrared (FTIR) spectrometer (Thermo Scientific, Waltham, USA), with an MCT/A detector and an aperture size of 100 × 100 μm, in transmission mode. Spectra were acquired with a resolution of 4 cm-1, 128-256 acquisitions, over a spectral range of 4000 to 650 cm-1. The chemical composition of the sample materials and the presence of key analytes/classes of analytes of interest were identified by conducting searches in the OMNIC Spectra ver9.0 spectral libraries (also provided by Thermo Scientific).

For the quantification of PFRs, an Agilent 1290 Infinity liquid chromatography (LC) system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 6460 Triple Quadrupole mass spectrometer (MS) was employed, equipped with a Jetstream® electrospray ionization (ESI) ion source.

The LC parameters were optimised to provide both good chromatographic separation and minimal run tine, in order to maximise sample throughput. A volume of 3 μL of extract was injected on a Phenomenex (Torrance, CA, USA) Kinetex Biphenyl reversed phase column (2.1 x 50 mm, 1.7 μm), at a column oven temperature of 55 °C. The mobile phases were A: ultrapure H_2O and B: MeOH, both containing 5 mM ammonium formate. Separation was achieved using a flow rate of 0.5 mL/min and a gradient from 55 B to 94% B in 3.4 min, followed by 1 min hold before returning to the initial conditions, making the total run time of 4.5 minutes. The column is re-equilibrated for the next run during a 2.5 min post time.

The source parameters were initially optimised for all main analytes individually and subsequently a set of values for these parameters were selected to provide the best response for all considered analytes. As such, the drying gas temperature was set at 350 °C, the gas flow at 3 L/min, the nebulizer at 25 psi, sheath gas temperature 400 °C, sheath gas flow 12 L/min, capillary voltage 2700 V and nozzle voltage 0 V. The MS was operated in dynamic multiple-reaction monitoring (dMRM) mode, with 2-10 ion transitions for each analyte in their specific retention time (RT) window (RT ± 0.5 min).

For the analysis of BFRs, an Agilent 6890 GC coupled to an Agilent 5973 MS was employed, equipped with a chemical ionisation source, operated in negative mode (ECNI). The system was equipped with a programmable-temperature vaporizer inlet (PTV), operated in the pulsed splitless mode. The carrier gas employed was He, with a purity of 5.9. The quadrupole and interface temperatures were set at 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V. The injection volume was 1 µL.

The column employed was an Agilent J&W DB-5 (15 m × 0.25 mm × 0.10 µm) and the GC temperature program started at 90 °C, with a hold of 1.25 min, ramp 10 °C/min to 310 °C and hold for 12 min. The carrier gas (He) was run in a ramped flow mode, and after 20 min, it was increased (20 mL/min2) from 1 to 2 mL/min. Methane was used as moderating gas and the ion source temperature was set at 250 °C.

4.2.2.3. Samples

A number of 28 samples were collected by removing plastic shavings with a box cutter, from the back panels of electronic products from offices and homes. The electronics were produced from 2004 to 2014, mostly in China (20 out of the 28 samples). The samples consisted of computer monitors (16 / 28 samples), television sets (4 / 28 samples), computer mice (4 / 28 samples), keyboards (2/28 samples) and others (2 / 28 samples).

4.2.2.4. Extraction

The plastic samples were cut in small shavings and strong solvents were used to dissolve it (acetone and DCM). The extraction for the plastic samples is detailed in this previous study (Ionas et al., 2014). The final extract underwent an 180000× dilution, was spiked with internal standards, filtered through 0.22 µm centrifugal filters and injected as such.

4.2.3. Results and discussion

4.2.3.1. XRF screening

As a first step in the analysis of FRs, the plastic samples were screened using an X-ray spectrometer, in order to determine the amounts of key elements encountered in halogenated FRs and PFRs, some of which are suspected of adverse health effects (Lyche et al., 2015; Wei et al., 2015): Br, Cl and P. Other elements such as Sb, Al, Mg and Zn were also monitored, as their presence can indicate that inorganic FRs (such as ATH - alumina trihydrate, MDH – magnesium hydroxide and zinc borate) were employed to impart flame retardancy.

Organic type FRs are added typically added to plastic products in percent amounts to impart resistance to flames (van Bergen and Stone, 2014). To achieve this effect, for the most effective FRs, only a 1% by mass addition is sufficient (Papazoglou, 2004). Although, if FRs are used in a mixture of multiple chemicals, all meant to increase resistance to flames, the amount can even be less than 1% (Beard, 2007). The minimum amounts of Br, Cl and P contained in current-use FRs is 30% for the BFRs and CFRs (in HCDBCO and TCBPA, respectively) and 3% for P in TTBNPP. So as a screening threshold, we could consider samples with >3000 ppm Br and Cl and with >300 ppm P for further analysis. To test this, we have analysed the ERM EC680 (808 ppm Br, 810 ppm Cl) and 681 (98 ppm Br, 93 ppm Cl) CRMs (Table 4.2.1).

Table 4.2.1: Analysis of Cl and Br from the ERM EC680 and 681 by XRF

Element	CRM	CRM concentration (ppm)	XRF-determined concentration (ppm)	Mean XRF Concentration (ppm)	SD	RSD (%)	Accuracy (%)
Cl	EC680	810	307 310 311	309	2	1	38
	EC681	93	60 59 61	60	1	2	65
Br	EC680	808	597 594 598	596	2	0	74
	EC681	98	59 59 60	59	1	1	61

The Cl and Br were underestimated in the CRMs (accuracy between 38-74%). The main cause for this phenomenon is that the CRMs were plastic beads, similar to the samples (plastic shavings), so there were air gaps in between the sample/CRM pieces during the analysis, which lead to a decreased signal during analysis and an underestimation of the amounts contained. For optimal

results with this instrument, the samples should be perfectly homogeneous, preferably as a uniform cylindrical pellet of known mass and size.

Considering the above mentioned, only 2 samples had Br levels considerable enough to impart some flame retardancy, while 2 others slightly lower levels (Table 4.2.2). 3 of these 4 samples also contain levels of antimony consistent with the use of antimony trioxide as a synergist for halogenated FRs. 4 samples appeared to be flame retarded with phosphorus compounds. Part of the samples which contain higher levels of Br and P, also contain high levels of Al, Mg or Zn, so it is possible that inorganic and organic FRs are used alongside to reach the desired level of resistance to flames.

Table 4.2.2: XRF screening results in electronic consumer products for key elements used in organic FRs (left panel) and inorganic FRs (right panel)

Element / Sample nr.	Br	Cl	P	Sb	Al	Mg	Zn
1	ND	410	130	ND	ND	ND	ND
2	ND	170	65	ND	ND	ND	ND
3	ND	360	10	ND	ND	ND	ND
4	ND	210	30	ND	ND	ND	ND
5	ND	60	35	ND	20	ND	ND
6	ND	70	30	ND	ND	ND	65
7	5	420	150	ND	130	ND	ND
8	70	360	70	ND	70	ND	65
9	ND	310	45	ND	ND	ND	ND
10	ND	4	4	ND	ND	ND	15
11	1100	30	20	420	4500	940	15
12	5	190	55	ND	15	ND	ND
13	2200	10	4	790	4700	620	40
14	35	120	4400	15	200	ND	3
15	ND	160	30	ND	820	ND	ND
16	ND	540	170	ND	ND	350	5
17	830	35	740	ND	1400	2400	ND
18	ND	350	120	ND	ND	ND	ND
19	25	85	45	ND	30	ND	30
20	ND	210	70	ND	ND	ND	ND
21	ND	170	20	ND	ND	ND	ND
22	ND	340	110	ND	ND	ND	4
23	ND	190	45	ND	ND	ND	ND
24	15	290	80	ND	ND	ND	50
25	410	380	140	250	820	ND	ND
26	20	80	3000	15	190	ND	4
27	ND	ND	ND	ND	ND	ND	ND
28	4	300	14800	ND	4300	630	1900

4.2.3.2. FTIR determination of the sample material and screening for selected FRs

The types of polymers used in the samples were determined by FTIR. In most cases, the plastics were a combination of polymers, rather than just one individual polymer (Table 4.2.3). Levels of Cl in the order of hundreds of ppm were found in almost 70% of the samples, which can be indicative that small amounts of PVC are also added to the mixture of polymers used in some of the samples.

Table 4.2.3: Sample database with information about the constituent material, obtained by FTIR analysis

Sample	Device Type	Production year	Production place	Material
1	Computer monitor	2012	China	PET
2	Mouse	2014	China	ABS
3	Computer monitor	2006	China	Styrene / Acrylonitrile
4	Computer monitor	2010	China	ABS
5	Mouse	2012	China	PC + ABS
6	Television	2010	Slovakia	PS
7	Computer monitor	2011	China	PC/ABS + PET
8	Keyboard	-	China	PC/ABS + PS
9	Computer monitor	2005	Malaysia	PC/ABS + poly(acrylonitrile:styrene)
10	Television	2009	Turkey	PS
11	Television	2011	Spain	PS
12	Computer monitor	2012	China	PET
13	Television	2012	Turkey	PS
14	Computer monitor	2007	China	PC/PBT
15	Mouse	2005-2008*	China	PC/ABS + poly(acrylonitrile:styrene)
16	Computer monitor	2009*	-	PC/ABS + poly(acrylonitrile:styrene)
17	Surge-proof multi socket extension cord	-	-	PP
18	Computer monitor	2010	China	PET
19	Keyboard		China	PS
20	Computer monitor	2008	China	PC + ABS
21	Mouse	-	China	PC/ABS + poly(acrylonitrile:styrene)
22	Computer monitor	2014	China	PET
23	Computer monitor	2013	China	poly(acrylonitrile:styrene)
24	Computer monitor	2012	China	PC + ABS
25	Computer monitor	2004	China	PC + ABS
26	Computer monitor	2006*	China	PC
27	Computer monitor	2012	China	PC + ABS
28	Connection cable	-	-	PBT

* Estimated value

FTIR microscopy was then employed as a quick method of checking whether the FRs used in samples with high Br were PBDEs or other FRs and with high were PFRs or elemental phosphorus. A number of key bonds and functions characteristic of BFRs, CFRs and PFRs were monitored (Table 4.2.4).

Table 4.2.4: FTIR bands typically encountered in FRs

Band	Type	Wavenumber (cm-1)
C_{sp3}-Cl	stretch	850－550
C_{sp3}-Br	stretch	690-515
C_{sp2}-H (terminal halides)	wag	1300－1150
C_{sp2}-Br	stretch	1075 - 1030
P-O-R	stretch	900-1050
P-O-C_{sp2}	stretch	905-995 (970 in this study)
P=O	stretch	1100-1350
BDE-209 band*		1350

* From (Sato et al., 2010)

For halogenated FRs, the C-X bands are typically not very intense and are obscured by the bands of the polymer(s) from which the sample is made of. One exception here is the BDE-209 band at 1350 cm^{-1}. For PFRs, most bands are not very intense either or are in very "crowded" areas of the spectrum, with the exception of the P-O-C_{sp2} band at 970 cm^{-1} seen for aryl phosphates.

The BDE-209 band was not clearly present in any of the samples which were determined to have high Br levels by XRF, but the aryl phosphate band was present in the spectra of samples 14, 17 and 26. Interestingly, the spectrum of the sample with highest P content did not present this band or other bands associated with the phosphate function.

4.2.3.3. Trace analysis by GC-MS and LC-MS/MS

The samples with a content of Br close to what would be required to impart flame retardancy in plastic materials were analysed by GC-MS. None of the classical BFRs typically used in plastics, such as PBDEs, HBCDDs and TBBPA were detected. So the source of the Br detected by XRF is likely non-classical BFRs.

The PFRs typically employed as FRs rather than plasticisers were analysed in the samples for which it was determined that the P levels were highest. Interestingly, the sample with the highest amount of P in the XRF screening did not contain any of the PFRs included in this study (Table 4.2.5). So the high levels of P are due to another PFR not analysed in this study or to inorganic red phosphorus, which is also used as FR in some applications. Considering that in FTIR, the typical phosphate bands were not present in the spectrum, it is likely that red phosphorus was used in this sample.

Table 4.2.5: PFR levels determined in the samples pre-selected through XRF screening **(µg/g)**. The samples were arranged in the descending order of the amounts of P determined by XRF.

Sample nr.	TCEP	TCPP	TDCPP	V6	TDBPP	iDPhP	TXP	RDP	BDP	P (ppm) by XRF
28	<LOQ	240	<LOQ	190	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14800
14	<LOQ	<LOQ	<LOQ	<LOQ	3,900	12,500	6,300	59,800	278,000	4400
26	<LOQ	720	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	2,800	233,000	3000
17	<LOQ	<LOQ	<LOQ	440	1,700	790	<LOQ	<LOQ	<LOQ	740

Two of the samples contain BDP, an emerging PFR added to plastics as a replacement for BDE-209, which has seen a rise in use since the aforementioned BFRs has started to be voluntarily phased out in many parts of the world. Sample 14 interestingly seems to contain a cocktail of PFRs including RDP, TXP, iDPhP and even TDBPP. This latter FR, present in sample 17 as well here, saw wide use in plastics and textiles in the past, but was banned in 1977 by the CPSC from clothes and children's garments in particular, due to concerns about its carcinogenicity (US Consumer Product Safety Commission, 1977). This phenomenon can be attributed to improper recycling of flame retarded plastics, among others.

4.3. Downsides of the recycling process: Harmful organic chemicals in children's toys

Based on the following publication:

Ionas, A.C., Dirtu, A.C., Anthonissen, T., Neels, H., Covaci, A., 2014. Downsides of the recycling process: Harmful organic chemicals in children's toys. Environ. Int. 65C, 54–62.

4.3.1. Introduction

An integral part of a child's developmental process is to learn about the world around him by playing. At a young age, this is how the infants spend most of their time. Nowadays, as our society has developed technologically, a very wide array of toys are produced. However, some of these toys may contain harmful chemicals (Greenpeace 2005). PBDEs were added as FRs to toys with a production date before the 1st of June 2007, when the REACH directive limited the use of PBDEs to 0.1% by mass. PBDEs have been shown to have adverse health effects (see chapter 1). Since the use of PBDEs has declined in the last decade, organophosphate flame retardants (PFRs) have been increasingly used in a variety of materials, including toys, to impart flame retardancy or as plasticisers (Van der Veen and de Boer 2012). But serious concerns about the possible adverse health effects of PFRs have been raised (see chapter 1).

The children typically spend a high amount of time playing with toys and may thus be in contact with the chemicals present in the toys. Chen and Mai (2009) investigated the occurrence of BFRs in newly purchased toys made of different materials, such as foam, textile, rubber and plastics. PBDEs were detected in all hard plastic toys (median=53 μg/g with BDE-209 as the main contributor), but also DBDPE (DF=80%, median=5.5 μg/g), BTBPE (DF=67%, median=0.1 μg/g) and PBBs (DF=63%, median=0.03 μg/g) had high detection frequency. The presence of PBBs is surprising, as they were not produced in China and have been banned in the US since the 1970s. A possible explanation is that some toys were manufactured using recycled plastic materials which contained PBBs. If this is indeed the case, the recycling process in its present form allows for the unintentional inclusion of potentially harmful chemicals in new products. In foam toys, PBDEs had a DF of 100%, but the levels were lower (median=1 μg/g), as were the DBDPE levels (DF=89%, median=0.7 μg/g). BTBPE and PBBs were not found in foam toys (Chen and Mai (2009)). In rubber/soft plastic toys, the DFs and levels of BFRs were lower than in the other two materials, but the median level of BDE-209 was lower than of DBDPE.

The aim of this study was, therefore, to investigate the occurrence of harmful organic chemicals, such as flame retardants and plasticisers, in current use children's toys to which children may be exposed during play. Also we aimed to determine the extent to which chemicals which have

been phased out are still present in toys. Additionally, we have checked if the thresholds of the REACH directive are being upheld by toy manufacturers, in order to assess the safety of current-use children's toys.

4.3.2. Materials and methods

4.3.2.1. Samples

A number of 106 toys were kindly donated by parents of children of different ages, collected from a recycling park in Antwerp (Belgium) during a period of two months or bought from a flea market and a major toy store. The toys were selected as to be representative for the main exposure pathways to contaminants, such as inhalation, dermal contact, mouthing and oral ingestion. Of special significance is the exposure through mouthing, because children are directly exposed to toxic chemicals at a young age when their organisms are very vulnerable to external stimuli. During sample collection, information about the year and country of production was recorded, to assess if the use of chemicals has changed after the enforcement of the REACH directive and if there are any patterns of use of these additives according to the origin of the toy. Similarly, toys were divided according to the age groups for which they were intended, to the estimated exposure time and to the material type (Table 4.3.1).

Table 4.3.1: Overview of collected toy samples and criteria of sample selection

Criteria of Selection	**Categories of toys considered for each selection criteria**	**Number of toys sampled for each category**
Children's age group (years) for which toys are addressed[a]	0 – 1 *(infants)*	36
	1 – 3 *(toddlers)*	60
	3 – 9	77
	> 9	32
Production date of the sampled toy[b]	< 2007	56
	2007	1
	> 2007	47
	Unknown	2
Production region/Country[a]	China	63
	Other countries	13
	Not specified	30
Estimated pathway of exposure for children[c]	Mouthing and oral	40
	Dermal	94
	Inhalation	20
Estimated children's exposure time to chemicals contained by toys[d]	High	9
	Intermediate	24
	Low	73
Material from which sampled toys were made[e]	Hard plastic	54
	Soft plastic	34
	Wooden	8
	Foam and textile	27

[a] as specified by the manufacturer
[b] 2007 is the year when the REACH regulation went into force

[c] assumed by authors according to the activities of children using the selected toys
[d] estimated by authors as follows: high: 1-4 h daily, intermediary: up to 1 h daily, low: toy not used daily
[e] classified by authors based on the main observed material contained in the sampled toys
Some toys qualified in multiple categories of the selection criteria (age group, pathway of exposure) or contained two of the main matrices studied

4.3.2.2. Materials

All solvents were of analytical or pesticide grade. *n*-Hexane was purchased from Acros Organics (Geel, Belgium). Acetone, ethanol, methanol, toluene and *iso*-octane were purchased from Merck (Darmstadt, Germany). Modified nylon centrifugal filters with 0.2 µm and 0.45 µm pore size were bought from VWR (Leuven, Belgium). Empty polypropylene filtration tubes (3 mL) SPE cartridges were purchased from Supelco, sulphuric acid (95-97%) and Silica gel 60 from Merck.

4.3.2.3. Target compounds

Standards of BDE 28, 47, 66, 85, 99, 100, 153, 154, 183 and 209, together with ^{13}C-BDE 209 were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 was obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of TnBP, TPhP, TCEP, EHDPhP, TCP (mixture of 4 isomers) and TDCPP (mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). TBOEP was purchased from Sigma Aldrich. TCPP (mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBOEP (>94%). Standards of DMP, DEP, DBPht, BBzP, DnOP, DEHP, DPPht, DINP, DIDP and d4-dibenzyl phthalate (d4-DBzP) were purchased from Sigma Aldrich (Schnelldorf, Germany). DINP and DIDP were not included in the study due to the difficulties in accurately and reproducibly quantifying all of the isomers from the complex technical mixture. A certified reference material (BFRs in polypropylene - ERM-EC591, IRMM, Geel, Belgium) contained 30 individual PBDE congeners and BB-209). Another reference material of medium density polyethylene (SPEX Certiprep, Metuchen, New Jersey, USA) was certified for 8 phthalate esters (DMP, DEP, DBPht, BBzP, DEHP, DnOP, DINP and DIDP) and for Bisphenol A. More details about the quantification of target compounds are available in Table 4.3.2.

Phthalate esters are the main type of plasticisers that are added to plastic materials. But similarly to the PBDEs, they were restricted by the REACH directive to 0.1% by mass, due to the rising number of studies about their adverse health effects, such as endocrine disruption (Colon et al., 2000), obesogenicity (Desvergne et al., 2009), allergenicity and even asthma (Bornehag et al., 2004) and protentially reproductive malformations (Howdeshell et al., 2007). Given this and the fact that some of the PFRs also find use as plasticisers, the phthalates were also included in the study to monitor their levels and compare their patterns of use to the ones of PFRs.

Table 4.3.2: The identification and quantification ions (bold values), and respective internal standards (IS) used for quantification of targeted PBDEs, PFRs and phthalates.

Compound	Identification - **Quantification Ions**	**Internal Standard (IS)**	**Instrument for analysis**
BDE 28	81, **79**	BDE 77	GC-ECNI/MS
BDE 47	81, **79**	BDE 77	GC-ECNI/MS
BDE 66	81, **79**	BDE 77	GC-ECNI/MS
BDE 85	81, **79**	BDE 77	GC-ECNI/MS
BDE 99	81, **79**	BDE 77	GC-ECNI/MS
BDE 100	81, **79**	BDE 77	GC-ECNI/MS
BDE 153	81, **79**	BDE 77	GC-ECNI/MS
BDE 154	81, **79**	BDE 77	GC-ECNI/MS
BDE 183	81, **79**	BDE 77	GC-ECNI/MS
BDE 209	485**, 487**	^{13}C-BDE 209	GC-ECNI/MS
BDE 77	*81, **79***	*n.a.*	*n.a.*
^{13}C-BDE 209	*497, **495***	*n.a.*	*n.a.*
TnBP	155, **211**	TAP	GC-EI/MS
TBOEP	199, **299**	TAP	GC-EI/MS
TEHP	99, **211**	TAP	GC-EI/MS
TCEP	251, **249**	TCEP-d12	GC-EI/MS
TCPP	279, **277**	TCEP-d12	GC-EI/MS
TDCPP	379, **381**	TDCPP-d15	GC-EI/MS
TPhP	325, **326**	TPhP -dl5	GC-EI/MS
EHDPhP	250, **251**	TPhP - dl5	GC-EI/MS
TCP	367, **368**	TPhP - dl5	GC-EI/MS
TAP	*169, **239***	*n.a.*	*n.a.*
TCEP-d12	*263, **261***	*n.a.*	*n.a.*
TDCPP-d15	*396, **394***	*n.a.*	*n.a.*
TPhP-d15	*339, **341***	*n.a.*	*n.a.*
DMP	**194**	DBzP-d4	GC-EI/MS
DEP	**222**	DBzP-d4	GC-EI/MS
DBPht	**223**	DBzP-d4	GC-EI/MS
BBzP	**206**	DBzP-d4	GC-EI/MS
DEHP	167**, 279**	DBzP-d4	GC-EI/MS
DPPht	**225**	DBzP-d4	GC-EI/MS
DnOP	261**, 279**	DBzP-d4	GC-EI/MS
DBzP-d4	*91, **153***	*n.a.*	*n.a.*

n.a. – not applicable

4.3.2.4. Extraction procedure

A combination of ultrasound assisted extraction (UAE) and vortexing was employed. As the matrix was not the same for all samples (foam and textile, hard plastic, soft plastic/rubber and wood), different sample preparation procedures and extraction solvents were optimised.

The sample preparation consisted of two main steps: the samples were pre-cleaned with water-impregnated tissue and dried at room temperature, to remove external contamination and were then submitted to one or two size reduction steps. The soft plastics, foam and textiles, wood and rubber toys were easily cut with pliers and scissors to pieces with a size of 2 mm or less. The hard

plastics could not be reduced in size; pieces with a size >3 mm were obtained due to the rigidity of the matrix. These samples underwent an additional size reduction step using a Mixer Mill MM400 from RETSCH (Haan, North Rhine-Westphalia, Germany). The samples along with a 25 mm diameter metal ball were added to the metal grinding jars (50 mL volume), submerged in liquid nitrogen and then ground at 30 Hz for 4 min. Some samples required multiple grinding cycles until a fine powder was obtained, to increase the contact surface with the solvent and thus to improve the extraction efficiency.

A single extraction method was insufficient due to the wide array of materials contained in toys and therefore different combinations of solvents were tested for extraction for each group of toys. The criteria for the solvent choice were to provide an efficient extraction and at the same time, not dissolve the matrix, as to avoid damaging the GC columns. The extraction process consisted of consecutive steps of vortexing (1 min) and ultrasonication (15 min) with 4 to 6 mL of the chosen solvent mixture. The vortexing and ultrasonication steps were repeated 3 times, after which the samples were left in solvent overnight. The extracts were divided in two and for one part a clean-up step on acid silica (44% H_2SO_4, *w/w*) was employed.

4.3.2.5. Instrumental analysis

For the analysis of PBDEs, extracts were injected on an Agilent 6890 GC coupled to an Agilent 5973 MS system operated in electron capture negative ionization (ECNI) mode. The GC system was equipped with a programmable-temperature vaporizer inlet (PTV), run in the pulsed splitless mode. One µL of extract was injected on an Agilent J&W DB-5 column (15m×0.25mm×0.10µm). The GC temperature program was 90 °C, hold 1.25 min, ramp 10 °C/min to 310 °C, hold 12 min. Helium was used as a carrier gas at an initial flow of 1 mL/min (for 20 min), then ramp with 20 mL/min² to 2 mL/min. Methane was used as moderating gas. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

For the analysis of phthalates and PFRs, extracts were injected in an identical GC-MS system operated in the electron ionisation (EI) mode. The PTV was run in the pulsed splitless mode. One µL of extract was injected on a SGE HT-8 column (25m×0.22mm×0.25µm). The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 310 °C, hold 20 min. Helium (purity 5.9) was used as a carrier gas with a constant flow (1 mL/min). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

4.3.2.6. QA/QC

Prior to application to samples, the analytical method was validated. The accepted recovery and accuracy values ranged between 70-130% (ICH Q2 R1, 2005) and as precision, values up to 20% (RSD of the measured spike values) were considered as acceptable. The residual recoveries from the exhaustive extraction of the spiked samples were below 10%. For QA/QC measures, a procedural blank was run every 5-6 samples. The analyte levels in the blanks were low and generally constant (RSD<30%); they were subtracted from those in the sample extracts. The repeatability of the analysis was assessed by analysing six replicates of the ERM-EC591 and CRM-PE002 certified reference materials for PBDEs and phthalate esters, respectively, in a plastic matrices. The RSDs ranged between 4-11% for the PBDE CRM (mean accuracy=91%) and from 2-8% for the phthalate ester CRM (mean accuracy=82%). Mixtures of standards were injected every 5th injection to check the stability of the calibration. The quantification of the analytes was carried out by internal standard procedure.

4.3.2.7. Statistical analysis

The data was analysed by categories (see Table 4.3.1). For this purpose, the XLSTAT 2013 (Addinsoft) software was used in combination with Microsoft Excel. The normality of the data-sets was tested using the Shapiro-Wilk test and the outliers were determined using the outlier labelling rule, as described in Hoaglin et al. (1986) and Hoaglin et al. (1987). Since most data-sets were not normally distributed and to avoid the elimination of many outliers to achieve normality, non-parametric tests were employed. To investigate the possible correlations between different analytes, per category, Spearman's rank correlation was employed for analytes with a detection frequency >50% in the respective category. The significance level was set at α=0.05 throughout the study.

4.3.3. Results and discussion

4.3.3.1. Optimisation of sample preparation

To assess the analytical efficiency, we used the sample preparation method for hard plastics to test the concentrations of BFRs in ERM-EC591 (polypropylene) and the phthalates in CRM-PE002 (medium density polyethylene). Initially, the ERM-EC591 CRM was extracted without any pre-treatment (plastic beads, diameter 0.3 mm). The initial recoveries were low (4-5% for the lower PBDEs and 2% for BDE-209). To improve extraction, a reduction step was added: the beads were frozen with liquid nitrogen and then ground for 4 min (one cycle). Since polypropylene is not very brittle, we obtained pellets rather than a fine powder. This size reduction step resulted in a 17-fold recovery increase for the lower PBDEs and 26-fold for BDE-209. To further improve on this, the CRM beads were submitted to repeated cycles of grinding until they were successfully ground to a

fine powder. The grinding process is more efficient with a larger sized metal ball (25 mm diameter) than with several smaller ones (5 mm diameter). Getting the CRM beads in a fine powder increased the mean recovery of lower PBDEs to 92% and for BDE-209 to 82%.

For the optimization of the extraction solvent, toluene alone or in a mixture with other solvents has been tested, as is often used for non-polar aromatic compounds (Linsinger et al., 2009). For wooden toy samples, several solvent mixtures were tested: *n*-hexane/ethanol, *n*-hexane/methanol, *n*-hexane/ethanol/water and *n*-hexane/ethanol/*iso*-propanol in different ratios. The best combination as extraction efficiency and throughput was *n*-hexane/ethanol (1:4, *v/v*). Wood is comprised out of three major constituents: cellulose, hemicellulose and lignin. Polar solvents (e.g. water) are efficient at distancing the biopolymeric chains, thus allowing (polar) compounds with low molecular weight to be extracted. Yet, water is not a suitable extraction solvent, because of its high boiling point and therefore ethanol was chosen. Hexane was added to elute non-polar compounds. At the aforementioned volume ratio, ethanol and hexane (high purity) form a positive azeotropic mixture with a boiling point of about 59 °C (as opposite to 78.4 °C for ethanol), allowing a shorter evaporation time. For the rubber samples, a mixture of acetone and *n*-hexane was used (3:2, *v/v*), as they form a positive azeotropic mixture with a boiling point of around 50 °C. For the soft and hard plastic samples, a mixture of dichloromethane and acetone was used (1:1, *v/v*).

4.3.3.2 Flame retardants

PBDEs are restricted by the REACH regulation no. 1907/2006 to a maximum mass percentage of 0.1%. However, the overall concentrations for the FRs analysed were low (Table 4.3.3), indicating a low exposure potential for the children using these toys. The predominant congener was BDE 209 (Figure 4.3.1), accounting for 99% of the total PBDE amounts detected. Three samples had concentrations noticeably higher than the others, of which two originated from China and one had an unknown production country. Two of them were produced before 2007 (with concentrations of 143 and 16 μg/g, respectively) and one after this date (concentration: 19 μg/g sample). The highest concentration of BDE 209 encountered (143 μg/g) was measured in a toy that contained a foam inner core and a textile cover. This concentration is one order of magnitude below the REACH threshold and insufficient to impart flame retardancy.

Table 4.3.3. Overview of the detection frequencies (%) and concentrations of PBDEs (ng/g), PFRs (µg/g) and phthalates (µg/g) measured in toy samples.

Class	Analyte	Detection frequency (%)	Analyte concentration		
			Median	90th percentile	Maximum
PBDEs (ng/g) *(n=114)**	BDE 28	5	<LOQ	5	10
	BDE 47	9	10	55	60
	BDE 66	2	10	15	15
	BDE 85	6	1	25	35
	BDE 99	6	20	95	160
	BDE 100	5	1	15	15
	BDE 153	11	10	70	170
	BDE 154	16	15	30	35
	BDE 183	9	20	260	270
	BDE 209	19	75	14500	143000
PFRs (µg/g) *(n=114)**	TnBP	10	45	230	660
	TBOEP	11	40	290	530
	TEHP	52	55	240	890
	TCEP	28	4	25	65
	Σ TCPP	42	1	40	80
	Σ TDCPP	33	5	5	5
	TPhP	52	2	55	12800
	EHDPhP	48	5	35	90
	Σ TCP	14	2	10	14000
Phthalates (µg/g) *(n=50)***	DMP	44	4	25	30
	DEP	90	25	100	250
	DBPht	94	10	480	**6200**
	BBzP	68	5	55	**1900**
	DEHP	98	25	410	**686000**
	DPPht	20	<LOQ	1	2
	DnOP	2	4	4	4

Values in **bold** *are over the REACH threshold (0.1 % by mass or 1000 µg/g); the median and 90th percentiles were calculated for the values over LOQ*

**Some toys contained two of the main matrices studied and were analysed as separate samples (number of samples collected = 106)*

*** Phthalate esters were quantified in part of the samples only, due to lack of analytical standards and methods at the time of the analysis of the first sample batch*

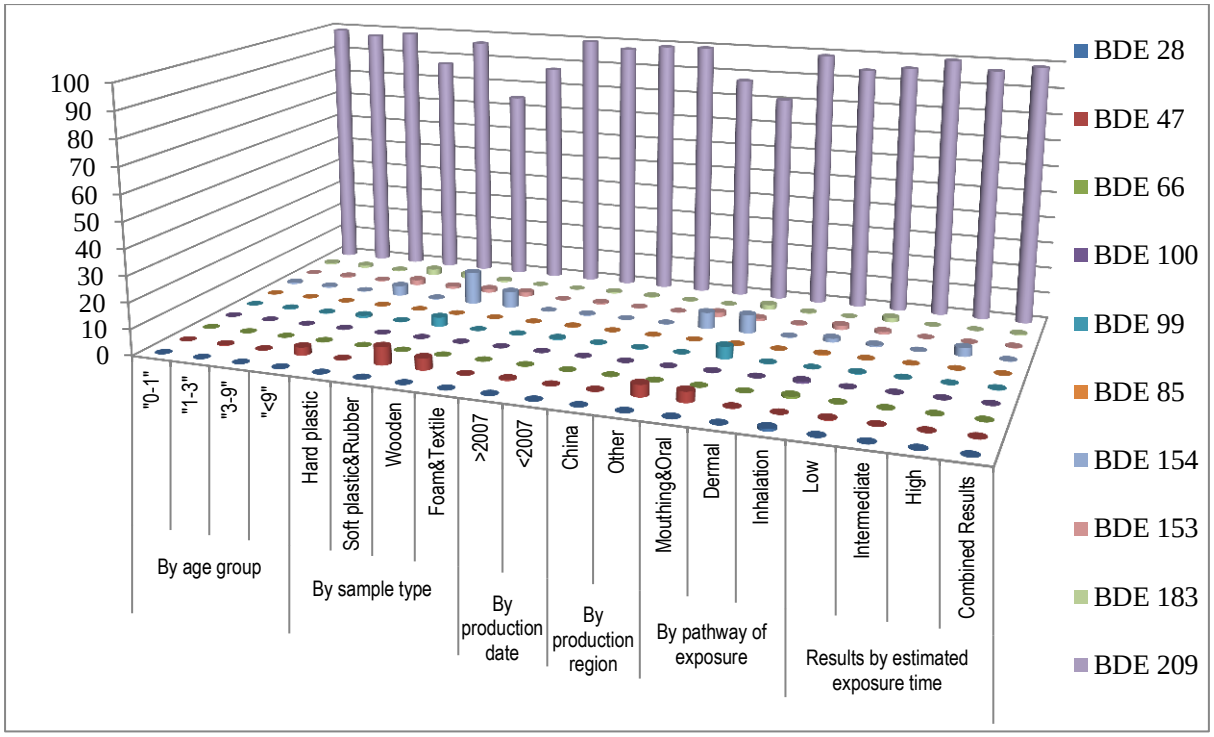

Figure 4.3.1: 3D Column plot of the profiles (% of the total) of PBDEs in each category

Surprisingly, most of the BDE 209 was in the textile material rather than in the foam, most likely because it was added to the back coating of the textile material in order to protect it against flames (Posner, 2004). A possible explanation for the presence of PBDEs at levels insufficient to impart flame retardancy, is that recycled materials containing PBDEs were used in the production of the toys (Kajiwara et al., 2011). Due to their low DFs and concentrations, PBDEs were not included in any further statistical data analysis. The congener with the highest detection frequency in all categories was BDE 209 (DF=19% overall). The most abundant source of exposure to BDE 209 were found to be the foam and textile toys (DF=20%, median=120 ng/g). As a positive finding, the flame retardant levels in toys for children up to 3 years old were lower than in toys for children older than 3 years.

Interestingly, although the levels of the PBDEs were higher before 2007, the DFs were higher after 2007. This further supports the hypothesis that these low levels of PBDEs are a result of the inclusion of flame-retarded materials in the recycling process and the resulted plastics were further used to manufacture toys.

As for the PFRs, the REACH directive only restricts tris (2,3-dibromopropyl)phosphate (TDBPP) (not detected in this study) and TCEP, which follows the same trend as the PBDEs. The TCEP concentrations were 4 orders of magnitude lower than the REACH threshold concentration. In average, PFR levels were 3 orders of magnitude higher than of PBDEs and the detection frequencies were higher (10-52% as compared to 2-19% for PBDEs) (Table 4.3.4). The most frequently detected PFRs were TEHP and TPhP (DF=52% for both). TEHP is used as flame retardant, but also plasticiser

and solvent in some industrial processes. TEP (triethyl phosphate) was also quantified in this study (median=3 μg/g, 90th percentile=17 μg/g, max=20 μg/g), but its recoveries were low and fluctuating as it was the most volatile compound targeted in the study. TiBP (tris-*i*-butyl-phosphate) results were excluded due to high and fluctuating blanks.

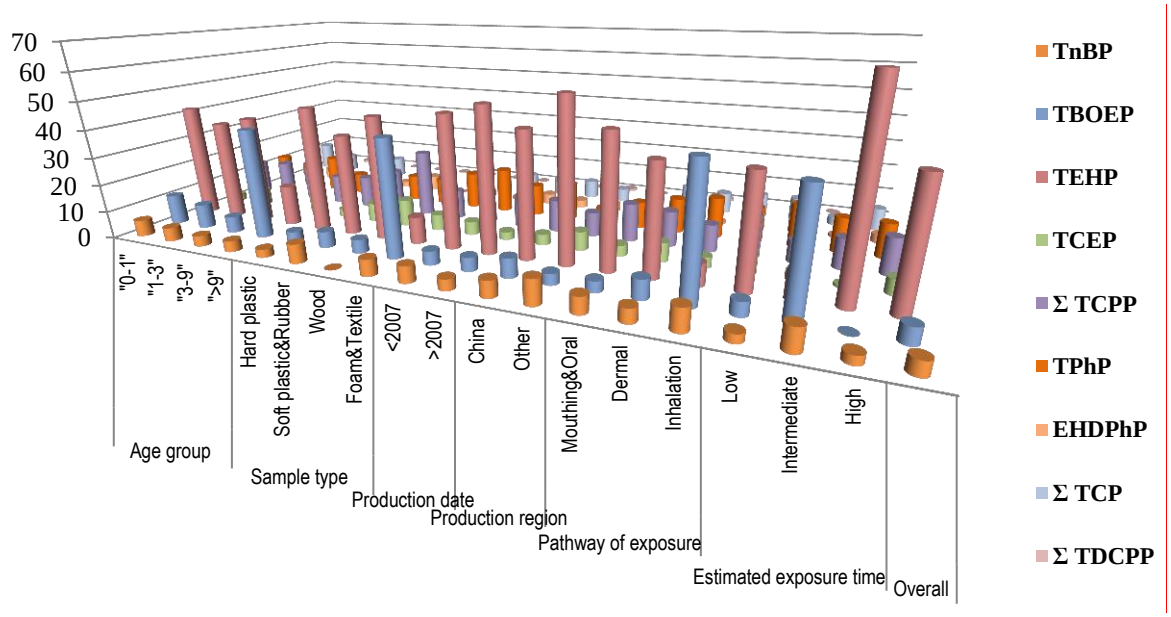

Figure 4.3.2: 3D Column plot of the profiles (% of the total) of PFRs in each category

Table 4.3.4: Concentrations of PBDEs (ng/g), PFRs (µg/g) and phthalates (µg/g) in toys classified according to sample type.

	Hard plastic (n=50)				Soft plastic & rubber (n=31)				Wood (n=8)				Foam and Textile (n=25)			
PBDEs (ng/g)	**DF (%)**	**Median**	**Mean**	**Max**	**DF (%)**	**Median**	**Mean**	**Max**	**DF (%)**	**Median**	**Mean**	**Max**	**DF (%)**	**Median**	**Mean**	**Max**
BDE 28	4	5	5	10	10	<LOQ	<LOQ	<LOQ	0	ND	ND	ND	0	ND	ND	ND
BDE 47	6	3	20	55	13	10	10	15	13	20	20	20	8	35	35	60
BDE 66	4	10	10	15	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND
BDE 85	10	0	1	2	0	ND	ND	ND	0	ND	ND	ND	8	25	10	20
BDE 99	8	5	15	50	3	20	20	20	0	ND	ND	ND	8	95	95	160
BDE 100	8	1	1	3	0	ND	ND	ND	0	ND	ND	ND	8	15	35	50
BDE 153	14	35	50	170	6	4	4	4	13	10	10	10	8	30	35	35
BDE 154	22	2	10	35	13	20	15	25	13	30	30	30	8	35	30	50
BDE 183	14	20	90	270	3	3	3	3	0	ND	ND	ND	8	20	20	25
BDE 209	16	260	4300	19100	19	60	65	95	13	390	390	390	20	120	28700	143000
PFRs (µg/g)	**Hard plastic (n=50)**				**Soft plastic & rubber (n=31)**				**Wood (n=8)**				**Foam and Textile (n=25)**			
TnBP	4	200	200	230	13	180	270	660	0	ND	ND	ND	20	1	1	2
TBOEP	6	110	170	320	16	80	170	530	13	30	30	30	20	5	5	10
TEHP	48	60	110	480	52	30	70	300	38	2	300	890	64	35	40	100
TCEP	14	2	10	25	42	5	10	65	25	4	4	5	36	3	10	45
Σ TCPP	32	1	3	20	48	10	25	75	38	<LOQ	5	20	52	<LOQ	5	80
Σ TDCPP	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND	4	5	5	5
TPhP	44	2	600	12800	52	2	65	940	25	35	35	70	56	1	10	120
EHDPhP	10	15	20	45	23	10	20	90	0	ND	ND	ND	20	2	4	15
Σ TCP	26	2	5	35	39	2	1200	14000	13	2	2	2	48	<LOQ	5	55
Phthalates (µg/g)	**Hard plastic (n=25)**				**Soft plastic & rubber (n=16)**				**Wood (n=1)**				**Foam and Textile (n=8)**			
DMP	28	2	3	10	63	5	10	25	0	ND	ND	ND	63	3	4	10
DEP	96	25	40	250	94	45	60	240	100	20	20	20	63	2	25	110
DBPht	96	4	90	**1700**	**94**	55	660	**6200**	100	150	150	150	88	10	50	230
BBzP	52	5	15	100	75	10	30	260	100	65	65	65	88	15	280	**1900**
DEHP	100	15	17300	**432000**	100	150	43200	**686000**	100	50	50	50	88	70	180	840
DPPht	8	<LOQ	<LOQ	1	19	<LOQ	<LOQ	1	0	ND	ND	ND	38	<LOQ	<LOQ	<LOQ
DnOP	0	ND	ND	ND	0	ND	ND	ND	0	ND	ND	ND	13	4	4	4

Values in **bold** *are above the REACH threshold (0.1 % by mass or 1000 µg/g); the median and 90th percentiles were calculated for the values over LOQ.*
For some analytes median = mean = max, it was detected with levels >LOQ in just one sample
Some toys contained two of the main matrices studied and were analysed as separate samples (number of samples collected = 106)

4.3.3.3. Phthalate esters

Due to possible adverse effects, several phthalates (e.g DBPht, BBzP, and DEHP) were restricted in all toys to a mass percentage of 0.1% by the European Commission through the 2005/84/EC directive followed by the REACH regulation (no. 1907/2006). DnOP, DINP and DIDP were only restricted in toys designed for mouthing. Due to analytical issues, DINP and DIDP were not investigated in this study.

Overall, DEHP, DBPht and BBzP were detected in 98%, 94% and respectively 68% of the samples, while DnOP was only detected in one sample. DEHP was detected in all toys made of hard and soft plastic, rubber and wood (Table 4.3.4), but not in all foam and textile toys (DF=88%). DEP was also frequently detected (DF=90%). The concentration profiles agree with the main uses of these compounds: BBzP and DEHP are commonly added as plasticisers in PVC plastic and BBzP is added to vinyl foam, as well.

Five samples contained more than 0.1% of phthalates and only one of them (a foam toy ball) was produced after 2007, when the REACH directive went into effect. Most samples do not contain sufficient levels of phthalates to significantly improve the properties of the material. This suggests that they have not been added with a specific purpose to the materials, but instead they are originating from the use of recycled plastics or cross-contamination during the manufacturing process in facilities where phthalates are produced or used (Kajiwara et al., 2011).

4.3.3.4. Age group

In toys for infants (0-1 years), the important analytes were TEHP (DF=50%), TCPP (DF=36%), TPhP (DF=39%), TCP (DF=33%), DEP (DF=94%), DBPht (DF=100%), BBzP (DF=47%), and DEHP (DF=100%). Few samples in this group had high enough levels of DEHP to significantly enhance the properties of the polymer. Its mean concentration in this age group was lower than that of TnBP, TBOEP and TEHP. The total PFRs accounted for 68% of total analyte amount and over twice as much as the total phthalates (Figure 4.3.3). Thus, the PFRs may have a similar contribution to exposure, even though the phthalates are detected more often in infant toys.

In the toys for toddlers (1-3 years), the following analytes had the highest detection frequencies: TEHP (46%), TCPP (46%), TPhP (42%), TCP (31%), DEP (91%), DBPht (100%), BBzP (47%) and DEHP (100%). In this group, DBPht (1,700 µg/g), BBzP (1,900 µg/g), and DEHP (3,700 µg/g) had maximum concentrations 1-2 orders of magnitude higher than for the 0-1 years age group and the maximum concentrations were above the 0.1% limit enforced by REACH.

TEHP was the most important contributor to the total PFRs in the first three age categories: 42% of total PFRs for 0-1 years, 37% for 1-3 years and 40% for 3-9 years (Figure 4.3.2). The >9 years category was dominated by TBOEP (40% of total PFRs).

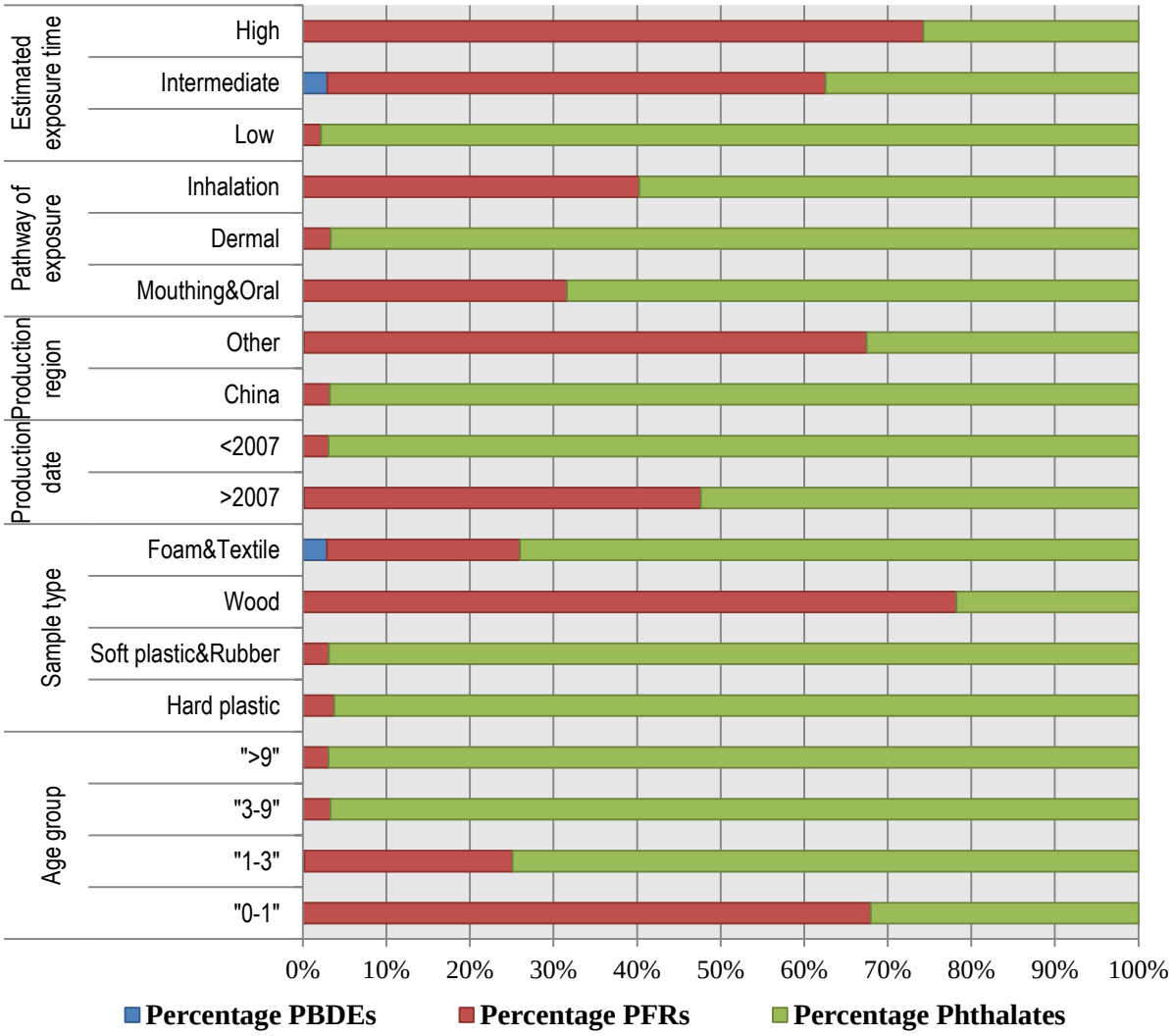

Figure 4.3.3: Stacked bar plot of the profiles (% of the total) of the sums of PBDEs, PFRs and phthalates in each category

4.3.3.5. Production region

Most of the analysed toys which were purchased on the Belgium market or donated for the purpose of this study by volunteers living in Belgium, were produced in China (n=63) and only 13 samples came from other countries. For the rest of the samples (n=30), the country of production was not specified. The toys made in China had higher levels (typically one to two orders of magnitude) for all analytes, except for TDCPP. The profiles of PFRs differed substantially: in China, TCP accounted for 47% and TPhP for 37% of the total PFRs, while in other countries, TEHP accounted for 72% (Figure 4.3.2). Profiles of phthalates in toys from China were dominated by DEHP (99% of the total phthalates), while in other countries, DEP was the major phthalate (55%).

TPhP exhibited a moderate positive correlation with DEHP (r=0.591, p<0.001). TPhP is used as plasticiser in lacquers and varnishes (Windholz, 1983), among others, explaining thus the correlation with DEHP, used as plasticiser in PVC. Many of the plastic toys are made of PVC coated

with paint and varnish. TPhP has however multiple sources – it is an impurity in various triaryl phosphate technical mixtures (Daft, 1982) and this factor can thus lead to the decrease in the correlation with DEHP.

4.3.3.6. Sample type

The highest detection frequency for PBDEs was in foam and textile toys (20%), but the concentrations were lower than in hard plastic toys (median lower by a factor of 2 and the 75th percentile by an order of magnitude). The most frequently detected PFR was TEHP (DF=38-64%) followed closely by TPhP (DF=38-56%) and TCPP (DF=32-52%, prevalent alongside TEHP in the wooden toys). DEHP was the most detected phthalate (DF=88-100%). A foam toy ball produced after 2007 contained more than the BBzP concentration permitted in the REACH regulation (0.1% by mass) and four samples produced before 2007 contained more than 0.1% by mass of phthalates. The older toys are also relevant for exposure, as they are transferred from one family to another, and thus toys produced before 2007 may still be in use.

In hard plastic, soft plastic, rubber and wooden toys, TEHP accounted for 46% of the total PFRs and in foam and textile toys, TBOEP accounted for 42% (Figure 4.3.4). In foam and textile toys, TEHP is strongly correlated with TPhP (r=0.887, p<0.0001), as they are both used as flame retardants, TEHP mostly in textiles and TPhP mostly in the foam.

In soft plastics and rubber toys, TPhP has strong positive correlations with DEHP (r= 0.735, p<0.02) and DBPht (r=0.806, p<0.001). The correlation of TPhP with DBPht is stronger and more significant than with DEHP, indicating a common source for these two chemicals (WHO EHC 1991), most likely dyes, paints, lacquers or varnishes.

4.3.3.7. Production date

With few exceptions, the levels of most analytes were lower in the toys made after 2007. BDE 209, BDE 153 and BDE 99 had levels 2-10 times lower in toys produced after 2007 (Table 4.3.5).

TPhP had lower mean levels after 2007, and its contribution to total PFRs has dropped from 17 to 12% (Table 4.3.5, Figure 4.3.4). The TCP profiles were similar in both categories, but high levels were detected in a few samples produced before 2007 (14,000 μg/g), two orders of magnitude higher than the highest value in toys produced after 2007. TCPP had a similar increase (7 to 12%), and the levels (median=2 μg/g) were three orders of magnitude lower than the levels required to impart flame retardancy (Stapleton et al., 2009). The preferred use for TCPP is in rigid polyurethane foam (EU risk assessment TCPP), while more flexible foams are needed in toys. Such low levels, also encountered in other studies (Stapleton et al., 2011), can thus be the result of the cross-contamination during the recycling process or on the production/assembly lines.

Table 4.3.5: Concentrations of PBDEs (ng/g), PFRs (µg/g) and phthalates (µg/g), by time period of manufacture (before and after the REACH regulation went into force) and country of production (a great percentage of the toys on the Belgian market are made in China)

	< 2007 (n=63)				> 2007 (n=51)			
PBDEs (ng/g)	**DF (%)**	**Median**	**Mean**	**Max**	**DF (%)**	**Median**	**Mean**	**Max**
BDE 28	5	<LOQ	1	1	6	<LOQ	4	10
BDE 47	6	15	25	60	12	5	15	55
BDE 66	2	1	1	1	2	15	15	15
BDE 85	3	10	10	15	10	1	10	35
BDE 99	3	90	90	160	10	10	20	50
BDE 100	5	1	10	25	6	1	1	3
BDE 153	11	10	45	170	10	10	20	55
BDE 154	14	25	20	35	18	1	10	35
BDE 183	6	140	140	270	12	15	20	45
BDE 209	16	110	15800	143000	22	60	1900	19100
PFRs (µg/g)	**< 2007 (n=63)**				**> 2007 (n=51)**			
TnBP	16	25	110	660	4	440	440	660
TBOEP	13	10	35	140	10	110	210	530
TEHP	54	3	60	480	51	85	130	890
TCEP	32	3	10	65	22	5	10	45
Σ TCPP	44	<LOQ	5	60	37	2	15	80
Σ TDCPP	2	5	5	5	4	5	5	5
TPhP	52	1	420	12800	43	3	25	150
EHDPhP	13	4	20	90	18	10	10	45
Σ TCP	32	<LOQ	700	14,000	37	2	5	55
Phthalates (µg/g)	**< 2007 (n=22)**				**> 2007 (n=28)**			
DMP	59	4	10	40	32	4	10	40
DEP	86	30	55	250	93	20	35	160
DBPht	95	25	520	**6200**	93	5	60	850
BBzP	64	4	30	260	68	15	110	**1900**
DEHP	95	35	53500	**686000**	100	20	85	840
DPPht	23	<LOQ	<LOQ	1	18	<LOQ	1	2
DnOP	0	ND	ND	ND	4	4	4	4

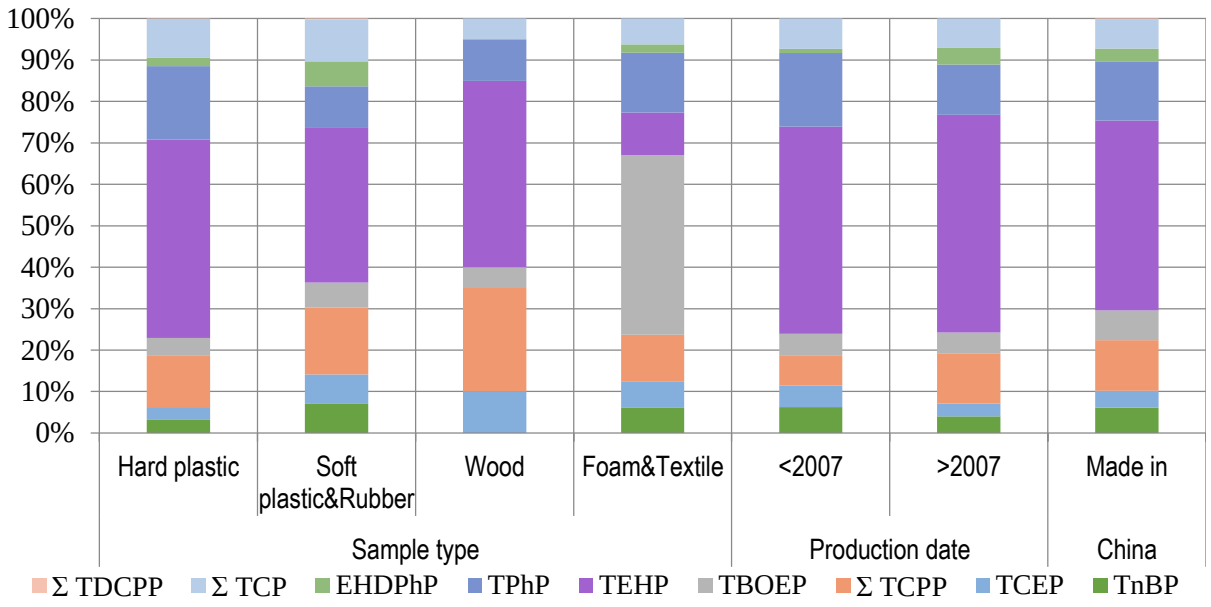

Figure 4.3.4: Profiles of PFRs in different sample materials and by production date. The profiles of toys produced in China were added for comparison.

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Chapter 5

Implications for human exposure: case study for children's exposure to flame retardants through toys

5.1. Considerations about children's exposure to FRs through toys

Based on the following publication:

Ionas, A.C., Dirtu, A.C., Anthonissen, T., Neels, H., Covaci, A., 2014. Downsides of the recycling process: Harmful organic chemicals in children's toys. Environ. Int. 65C, 54–62.

The levels of FRs detected in children's toys are used to estimate possible exposure occurring via this exposure pathway. Phthalates are also included in the analysis of this data, for comparison, as main type of plasticisers.

5.1.2. Exposure time

The compounds with the highest DFs in toys with which the children would play 1-4 h daily (high exposure) were: TEHP (67%), TPhP (42%), TCPP (33%), TCP (25%), DBPht (100%), DEHP (100%), DEP (88%) and DPPht (50%). Interestingly, although their detection frequencies are lower than for the phthalates, the PFRs are in higher amounts: ΣPFRs account for 74% of total analytes in this category (Figure 4.3.3). TEHP accounted for 39% of the total PFRs in the low and 68% in the high exposure category, while the intermediate exposure category was dominated by TBOEP (41%) (Figure 5.1.1).

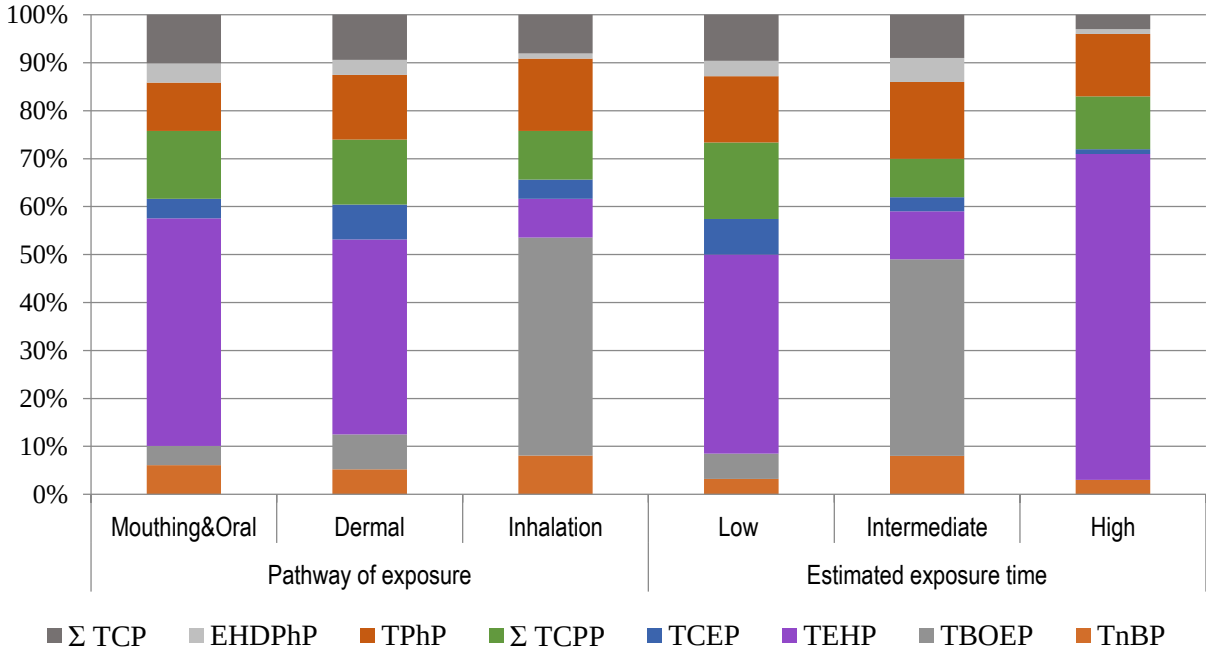

Figure 5.1.1: Profiles of PFRs relevant to exposure. Note that TDCPP was excluded from this graph for simplicity, as its values were below 1% in all categories

5.1.3. Exposure pathways

Samples representative of dermal exposure had the highest concentrations of target analytes, followed by the toys representative for mouthing and inhalation. In the dermal exposure category, a few toys contained high concentrations of TPhP (up to 12,800 μg/g), TCP (up to 14,000 μg/g) and DEHP (up to 686,000 μg/g, the highest concentration encountered in this study). Both in the mouthing and oral and dermal exposure categories, TEHP was the predominant PFR (47% and 39%, respectively), while TBOEP was for inhalation (45%) (Figure 5.1.1).

In the inhalation category, TEHP has a moderate positive correlation to TPhP (r=0.565, p=0.01), both being flame retardants and plasticisers.

5.1.4. Exposure potential

We have determined that 26% of the analysed toys contained at least one PBDE congener, 67% of them contained PFRs and 98% phthalates. The lower DF and levels of PBDEs is a positive find, as it results in lower potential exposure from toys.

For the PFRs, the data and the models in the literature are insufficient to assess the children's exposure from playing with toys. However, the presence of these chemicals in toys suggests that children are being exposed to these compounds from the toys, during playing.

In order to be able to compare the levels detected in this study with the existing literature, potential daily exposure levels were calculated employing the above mentioned exposure scenarios (Table 5.1.1).

The hand-to-mouth behaviour when playing with toys can be compared to the same type of behaviour leading to dust ingestion. The resulting exposure from this type of behaviour is two orders of magnitude lower than for dust. Similarly, we have compared PBDE exposure from indoor air to the inhalation exposure arising from playing with toys. The latter proved to be three to eleven times lower. All types of exposure related to oral intake were lower than the US EPA defined reference dose (RfD) for PBDEs. However, as so many parameters were based on uncertain estimates of parameters like area-specific emission rates and inhalation rates, it is very difficult to estimate the uncertainty associated with such exposure scenarios (Benasik et al. 2010).

Table 5.1.1: Human exposure to PBDEs in pg/kg body weight day, calculated using the model employed by Chen et al. (2009)

	Chen et al. (2009)	*This study*
Age group	**Σ PBDEs**	
	Inhalation	
3-12 months	32.7	5.2
1-3 years	122	19.5
3-5 years	89.3	14.3
5-9 years	64.9	10.4
9-14 years	52.1	8.3
	Mouthing	
3-18 months	8916	1429
19-36 months	782	125
	Dermal contact	
3-12 months	43.3	6.9
1-3 years	39.1	6.3
3-5 years	42	6.7
5-9 years	32.2	5.2
9-14 years	30.5	4.9
	Oral ingestion (hand-to-mouth)	
3-12 months	31	5.0
1-3 years	14.7	2.4
3-5 years	9.0	1.4
5-9 years	2.8	0.5
9-14 years	1.8	0.3

For other pathways of exposure to PBDEs assessed previously for Belgian toddlers (Roosens et al. 2010) (Table 5.1.2), the exposure through mouthing behaviour is an order of magnitude lower than exposure through breast milk. Also for older children, the contribution of mouthing exposure is similar.

Table 5.1.2: Average exposure to PBDEs in pg/kg body weight per day, from human milk, food, dust and air (Roosens et al. 2010)

Matrix	Human milk	Food	Dust			Air (Homes)
			0-1 years	1-3 years	3-6 years	
Σ PBDEs	20700*	2100**	1110*	1020*	280*	54.6

**Estimates based on 95th percentile levels*
***Estimates based on 50th percentile levels*

Approximately 5% of the samples (or 1 out of 20 toys) contained very high concentrations of the chemicals investigated in this study. The maximum values of the summed concentrations, per sample, for the three classes of analytes are two orders of magnitude higher than the 95th percentile values for these sums.

5.2. Child Exposure to Polybrominated Diphenyl Ethers (PBDEs) through Mouthing Toys

Based on the following manuscript:

Ionas AC, Ulevicus J, Ballesteros Gómez A, Brandsma SH, Leonards PEG, van de Bor M, Covaci A. 2016. Child Exposure to Polybrominated Diphenyl Ethers (PBDEs) through Mouthing Toys. Environ. Int. 87, 101–107.

5.2.1. Introduction

There is growing concern that some toys may contain harmful additives which, if absorbed into the body, may adversely impact long-term health (Greenpeace, 2005; Stapleton et al., 2011). It is well-documented that flame retardants (FRs) are present in indoor dust, and therefore available for human absorption through both dietary and non-dietary means, such that young children who spend a high amount of time playing with toys, on the ground in indoor areas, as well as using their hands and mouths to negotiate their environment, have a potentially high risk of exposure (Moya et al., 2004; Cohen Hubal et al., 2000; Xue et al., 2007). Thus, the mouthing of toys and other plastic items likely also contributes to FR exposure, especially as infants express a higher propensity for object mouthing than children of older ages, and may be in contact with the dust and chemicals present in toys (Xue et al., 2007; EPA, 2008). However, the data available on the process through which PBDEs migrate into children's saliva and body is scarce at best.

In a previous study (Ionas et al., 2014), a wide array of toys was examined for potentially harmful additives, including PBDEs. One of the main outcomes was that the most significant pathway of exposure was through mouthing, and the age group most at risk for this exposure was infants. These FRs, which are not covalently bonded to the matrix, have been proven to pose a risk of neurotoxicity and endocrine disruption (Lyche et al., 2015) and display a high potential for environmental leaching during their use, storage, disposal, as well as upcycle into newly manufactured goods (Ionas et al., 2014; Abbasi et al., 2015). This finding is of particular concern because early-life is critical in the physiological development of children and exposure to toxic chemicals at this stage can have lasting health effects.

To our knowledge, similar studies have been published mostly on phthalate esters, such as the ones of Niino et al. (Niino et al., 2002, 2001) and Könemann (Könemann, 1998). Only one publication (Chen et al., 2009) contained a brief, tentative *in vivo* study was done with 5 volunteers, which found highly variable migration rates among the volunteers and congeners. In the same study,

polybrominated biphenyls (PBBs) were detected in 63% of the toy samples, with median values of 30 ng/g. These chemicals, which are very similar to the PBDEs in terms of physico-chemical properties and use, are restricted under the Restriction of Hazardous Substances Directive (RoHS) since 2003, but their use has started to be phased out since the 1970s. Therefore, it is rather likely that even if PBDEs have started to be phased out in many parts of the world, they will still be present in items made with recycled flame retarded materials for decades to come.

The aim of this study was to provide an early exposure assessment by determining the leaching potential of PBDEs from children's toys into saliva in simulated conditions, taking into consideration the infant mouthing behaviour as well.

5.2.2. Experimental

5.2.2.1. Materials

n-Hexane, acetone, toluene, tetrahydrofuran and *iso*-octane were purchased from J.T. Baker (Deventer, the Netherlands) and dichloromethane (DCM) for residue analysis from Promochem (Wesel, Germany). Anhydrous sodium sulphate was obtained from Merck (Darmstadt, Germany). All solvents and reagents were at least of analytical grade. Isotopically labelled (^{13}C) BDE 209 and PBDE congeners were supplied by Wellington Laboratories. Fluorinated PBDE congeners (F-BDE 47 and F-BDE 183) were bought from Chiron (Trondheim, Norway). The ERM-EC591 CRM of BFRs in polypropylene (PBDEs: 17, 28, 47, 49, 66, 74, 75, 85, 97, 99, 100, 101, 118, 119, 138, 139, 153, 154, 155, 173, 180, 181, 182, 183, 190, 197, 204, 207, 208, 209 and BB-209) was purchased from IRMM (Geel, Belgium). Glass beads (G9268-100G, 425-600 µm) were acquired from Sigma Aldrich (St. Louis, MO, USA). Falcon™ conical centrifuge tubes (50 mL) were bought from Fisher Scientific (Waltham, MA, USA).

5.2.2.2. Simulation of mouthing/leaching into saliva

The procedure used was adapted from similar studies on phthalate esters (Könemann, 1998; Niino et al., 2001) which also included a comparison between the *in vitro* methodology and an *in vivo* assay (Niino et al., 2002), to serve as control. An Incubating Orbital shaker from VWR (Radnor, Pennsylvania, USA) was employed at a rotation speed of 250 rpm, and a temperature of 37 °C, to mimic *in vivo* conditions as much as possible.

The saliva simulant solution used was the one described in the British Standard BS 6684 (1987), with the following composition: 4.5 g NaCl, 0.3 g KCl, 0.3 g Na_2SO_4, 0.4 g NH_4Cl, 0.2 g urea, 3.0 g lactic acid, dissolved in 1000 mL MilliQ water (resistivity 18.2 MΩ·cm) and adjusted to pH 6.8. The chosen pH is derived from literature data (Könemann, 1998), to match the pH of infant and toddler saliva. A volume of 30 mL of saliva simulant was used per assay, and was added to a 50 mL Falcon™ conical centrifuge tube, along with the sample. Since the migration of chemicals in a

solution occurs from the surface, the FR concentrations were normalised to surface area rather than weight. A 10 cm^2 total surface area was chosen, to correspond to the surface area of a child's open mouth, as this is the surface area typically available for mouthing at any one time (Earls et al., 2003). The samples were cut in pieces shaped as rectangular parallelepipeds, to facilitate the calculation of their surface.

5.2.2.3. Samples

Real toy samples as well as CRM samples were run in triplicate, for two exposure times (15 and 60 min, low and high exposure scenario, respectively). The concentrations of PBDE congeners in the ERM-EC591 CRM are one order of magnitude lower than what is required to impart flame retardancy. These levels are representative of the threshold defined by the REACH directive (e.g. 0.1%). The two toy samples considered were analysed in a previous study (Ionas et al., 2014). They contained levels one order of magnitude lower than the CRM, and are representative for the scenario in which PBDEs are present only as a result of contamination during the manufacturing or recycling process. The constituent polymers were not specified on the samples. One toy was made of hard (brittle) plastic (toy car, made in China) and the other of softer (bendable) plastic (toy figurines, unknown country of production). Other plastic toy samples from the aforementioned study had similar concentrations as the ones chosen for this study or lower. A toy sample with a very high concentration of PBDEs (percentage amounts) was not available for testing. The toy samples were included in the study as a proof-of-concept of the migration procedure, and to monitor how well the used CRM mimics the real toy samples. To study the scenario in which a child would mouth on an item flame retarded exclusively with PBDEs (e.g. remote control, mobile phone, small toys with electronic components, etc.), a sample (TV-back panel) from another study (Gallen et al., 2014) containing 7% BDE 209 was tested with the same conditions.

5.2.2.4. Extraction

Saliva simulant (30 mL) underwent liquid-liquid extraction using 2 × 5 mL of a 1:1 *v/v* mixture of *n*-hexane and DCM. The organic phase was spiked with internal standards after phase separation, to ensure optimal solubility for the standards. Then the extract was passed over a cartridge containing anhydrous sodium sulfate, to retain all traces of water. The extract was then evaporated, reconstituted in 200 µL mixture of *iso*-octane and toluene (1:1, *v/v*) and transferred to injection vials.

5.2.2.5. Instrumental analysis

The identification and quantification of PBDEs was done on an Agilent GC 6890N (Agilent Technologies Netherlands BV, Amstelveen, the Netherlands) coupled to a 5975XL MS with a chemical ionization source and equipped with a pulsed splitless inlet and an Agilent 7683 auto-

sampler. F-BDE 47, F-BDE 183 and 13C-BDE 209 were used as internal standards. Analyte separation was carried out on an Agilent J&W DB-5HT (15 m × 0.25 mm × 0.1 μm film thickness). One microliter was injected at 275 °C in the pulsed splitless mode (pulse pressure 15 psi kept for 1.5 min). The oven temperature was programmed from 90 °C, for 1.5 min, then raised with 25 °C/min to 190 °C, then raised with 6.75 °C/min to 310 °C which was kept for 4 min. Methane was used as moderating gas (purity 4.5). Helium (purity 5.9) was used as a carrier gas with a ramped flow. The initial flow was 1 mL/min (for 20 min), then ramp 20 mL/min to 2 mL/min. The following mass fragments were monitored: m/z 484.4/486.4 and m/z 494.4/496.4 for BDE 209 and of 13C-BDE 209, respectively and m/z 79/81 for all other PBDEs. The temperatures of the interface, quadrupole and ion source were 300, 150 and 250 °C, respectively and the electron multiplier voltage was set at 1812 V.

5.2.2.6. QA/QC

Controls with the chosen migration media spiked with PBDE congeners were run for every step of the process to assess possible contamination or loss of analytes. Recoveries ranged between 78-86 %. Two procedural blanks were run with every batch of 8 samples analysed. PBDE amounts detected in the blanks varied between non-detected to 300 pg. The data was blank corrected accordingly. The limits of quantification (LOQs), expressed in pg/cm^2 sample, were calculated by dividing the analyte levels detected in the blanks by the sample surface (10 cm^2) considered for the experiments.

5.2.2.7. Exposure scenarios

A number of observational studies were taken as a reference for this study (Moya et al., 2004; Cohen Hubal et al., 2000; Xue et al., 2007; EPA, 2008; Könemann, 1998). Additionally, five parents were interviewed about the mouthing behaviour of their children. The focus was on mouthing of toys of different materials in addition to non-toy items. Both hard and soft plastic toys were most frequently targeted for mouthing. Foam and textile toys, as well as non-toy (e.g. remote control, pens, key chains, cell phones) items were only occasionally mouthed. Based on this information and the existing observational studies, two exposure scenarios were considered for plastic toys: a low exposure scenario (15 min mouthing time/day) and a high exposure scenario (or "favourite toy" scenario of 60 min mouthing time/day).

Children typically display mouthing behaviour all throughout their infancy and partly during their toddler age (up to 24 months). In rare cases, the child can still display mouthing behaviour even after the age of 24 months. This is an indicator of the development of a rare condition called "oral fixation" (Angelo, 2013). Children with this condition will mouth on items also at older ages, and if the items mouthed contain PBDEs, it can lead to increased exposure to these chemicals.

5.2.2.8. Method development

The mouthing behaviour of children was simulated through a step of incubation/shaking. First, a 24 h preliminary test was conducted using the ERM-EC591 CRM, which was chosen for this purpose because it contains PBDEs (0.17 % *w/w*) in amounts similar to the REACH threshold (0.1 % *w/w*). From this test it was determined that the PBDE levels in the solution were very close to the limit of solubility in aqueous media. Three methods of incubation/shaking were then tested: uninterrupted shaking for 1 h (Niino et al., 2002), replenishing the artificial saliva solution after 30 min (Earls et al., 2003) (60 min total shaking time) and uninterrupted shaking for 60 min, with glass beads added (Könemann, 1998), meant to mimic chewing behaviour. The control against which these experimental conditions were tested was an incubation experiment using the same set-up and the same volume of real human saliva, collected by direct discharge, from nine volunteers of different age and gender and pooled together. Since a person can donate 2-4 mL saliva at one time and because 30 mL was required per replicate, this experiment was ran only once, to assess which set of conditions generates values closest to real saliva.

5.2.2.9. Kinetics and magnitude of the migration process

Additional experiments with different migration times (15 and 30 min, followed by 1, 2, 4, 8 and 16 h, all in triplicate) were done, in order to investigate the behaviour and migration rate of the analytes from the plastic matrix (Figure 5.2.1). PBDEs were detected in the low nanogram range even in the incubations with the CRM for the low exposure scenario (15 min).

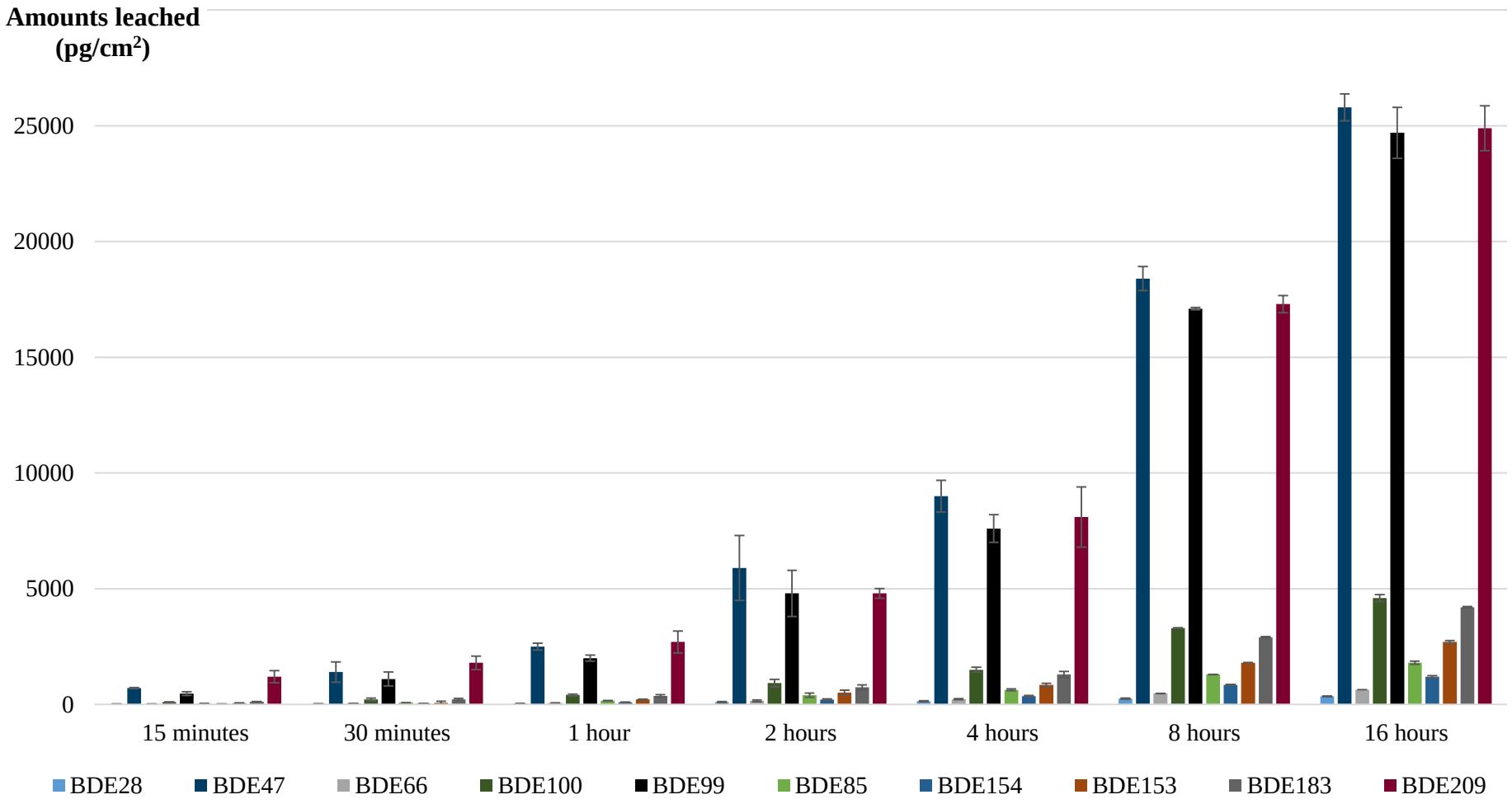

Figure 5.2.1: Mean aounts leached from EC-591 CRM at different time points (results in pg/cm^2) (n=3 replicates / time point; errorbars represent standard deviation)

5.2.3. Results and discussion

The method development showed that the addition of glass beads to the artificial saliva, to simulate chewing behaviour or its replenishment to better mimic the start of the digestive process, did not considerably increase the observed migration rates for PBDEs (Figure 5.2.2). All tested methods generated similar results. The real saliva control displayed a slightly higher migration rate for BDE 209, as compared to the other conditions, while adding the glass beads to the artificial saliva solution seemed to marginally favour the release of the lighter PBDE congeners from the matrix (Figure 5.2.2).

The differences in the amounts of PBDEs migrated out of the CRM were low (Figure 5.2.2) and the procedure described by Niino et al. (Niino et al., 2002) produced data which correlated well with the data of the real saliva migration experiment (R^2=0.96). Therefore, this procedure was chosen for the purpose of our study.

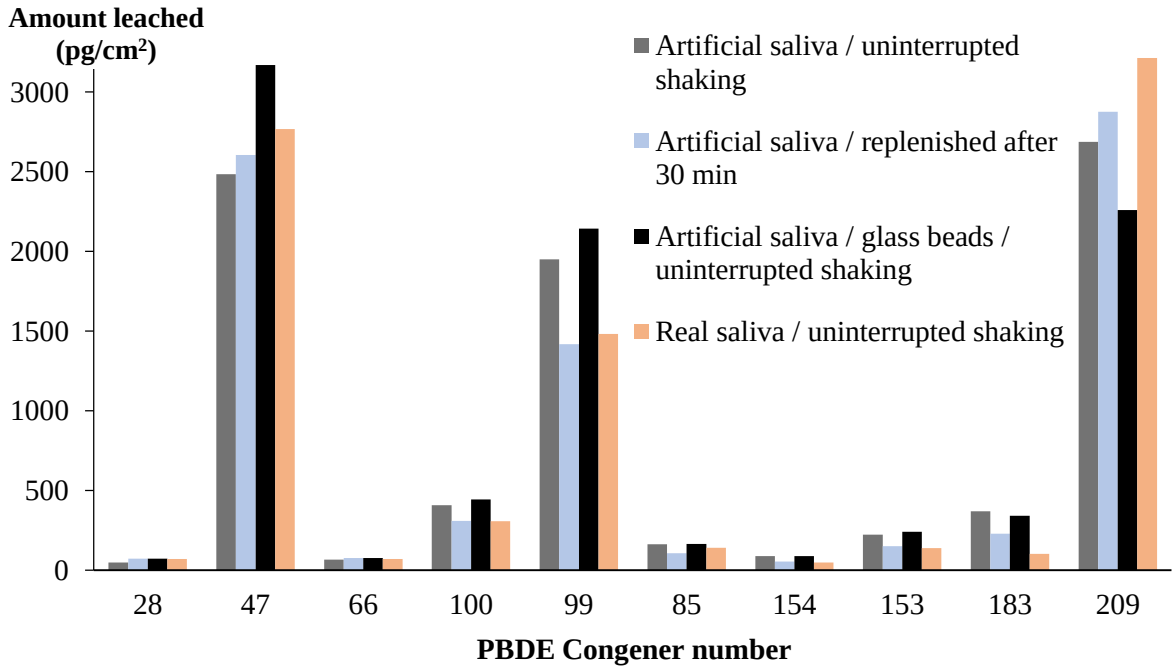

Figure 5.2.2: Test of different experimental conditions employed to simulate mouthing behaviour (incubation time: one hour, CRM used: ERM EC-591 containing 0.17% PBDEs; average sample mass corresponding to 10 cm^2 was 0.27 g)

5.2.3.1. Factors influencing the migration of PBDEs

Variables involved in the migration of PBDEs from the polymer are the sample surface loading, the replacement frequency of the saliva, and the physical-chemical properties of the compounds.

The kinetic leaching experiment showed that the migration rate is the greatest (198 $pg/cm^2 \times min$) during the first 15 min and the detected PBDEs levels were up to 1200 pg/cm^2 even for

this time point which was the low exposure scenario. After 4 h, the migration rate is slowing down and reaching equilibrium with the water phase (Figure 5.2.3).

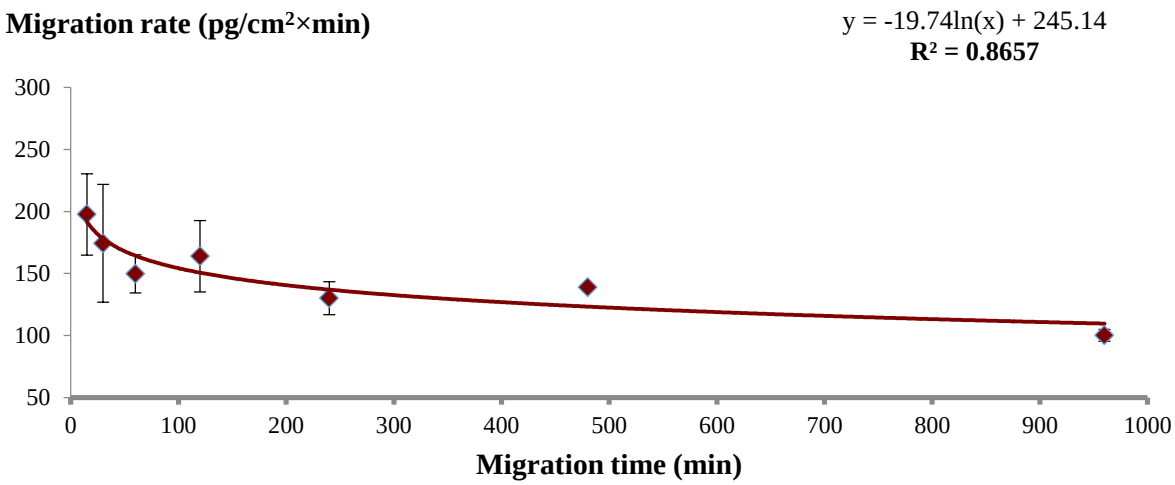

Figure 5.2.3: Migration rate of (total) PBDEs from the ERM EC-591 CRM (polypropylene matrix). Each point is an average of three experiments.

However, considering the amounts which have migrated into the artificial saliva, signs of saturation start to show at the last time point (16 h) (Figure 5.2.4). So the surface loading seems to be an important exposure factor than solution saturation in the first hour of exposure.

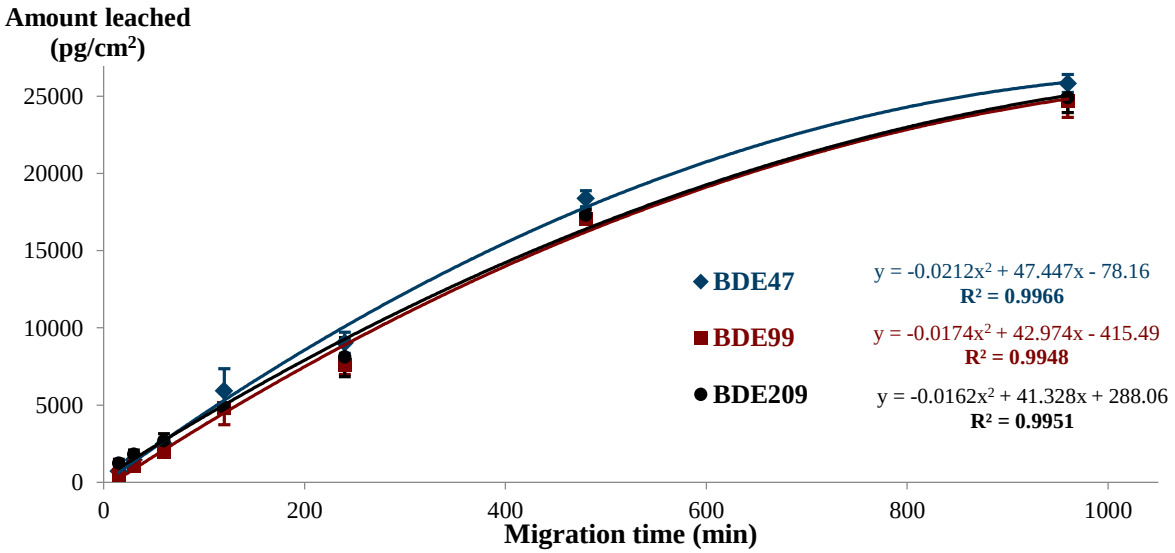

Figure 5.2.4: Profile of PBDE migration from the ERM EC-591 CRM (polynomial regression, n=7). Each point is an average of three experiments. For the migration profiles of the other congeners, see Figure 5.2.1.

In our closed system, the limit of solubility in aqueous media is a limitation to the migration process. However, in real life, the saliva from the mouth is continuously being swallowed into the

digestive tract and is replaced by fresh saliva. As a consequence, the values generated by our model are likely slightly underestimated. But considering that the migration rate is only starting to considerably slow down after 4-8 h (at the PBDE concentrations of the considered CRM), this does not significantly influence the two exposure scenarios of 15 and 60 min.

Another important migration factor is the physical-chemical properties of the PBDE congeners. The surface to saliva leaching was congener-dependent, since the congeners with a lower molecular mass and lower octanol-water partition coefficient leached more readily than the heavier and more lipophilic congeners (Figure 5.2.5).

The lower congeners leached up to 2% from the CRM pellets, while ~0.5% of BDE 209 migrated into the aqueous solution. This indicates that the exposure to the lower congeners, which have been proven to cause adverse health effects (Lyche et al., 2015), is higher via this pathway than for BDE 209. And the higher brominated PBDEs may undergo biotransformation into the body to lower brominated PBDEs (Noyes et al., 2011; Roberts et al., 2012) or other PBDE metabolites (Erratico et al., 2012; Meerts et al., 2001).

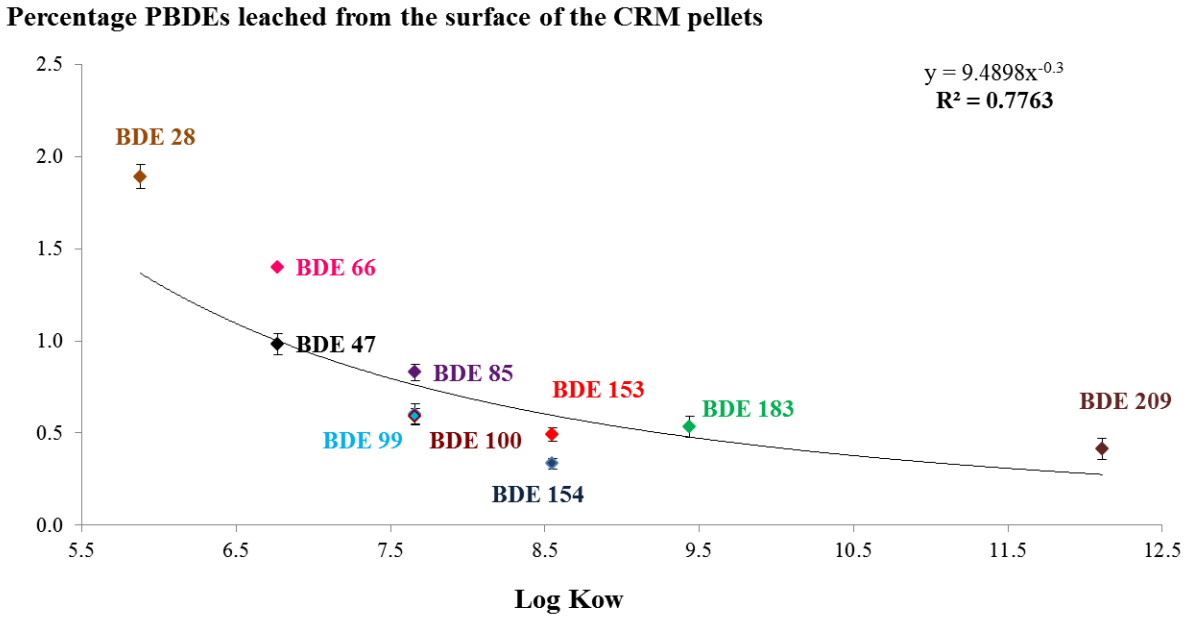

Figure 5.2.5: Influence of Log K_{ow} (calculated using the Syracuse Research Corporation EPI estimation program)(European Chemicals Bureau, 2002) on leaching (one hour shaking time with artificial saliva) (power regression, n=10). Each point is an average between three experiments

5.2.3.2. Influence of PBDE concentration on migration

By comparing the percentages of BDE 209 leached from the surface of the materials determined for the same time point, large differences between the materials can be observed (Figure 6). While both the CRM and the toy car sample leach around 0.3% during the 60 min leaching experiment, the toy figurines sample (made of softer plastic) leaches almost 3%, almost 10 times more.

The PBDE concentrations in the toy samples have been determined in a previous study (Ionas et al., 2014) to be similar (toy figurines 15 µg/g BDE 209, 19 µg/g in the toy car). However, the leaching rates varied between 410 and 50 pg/cm^2, respectively. The toy made out of a harder plastic (toy car) leached lower amounts of PBDEs than the one made out of a softer plastic (toy figurines), although the harder toy had higher levels of BDE 209.

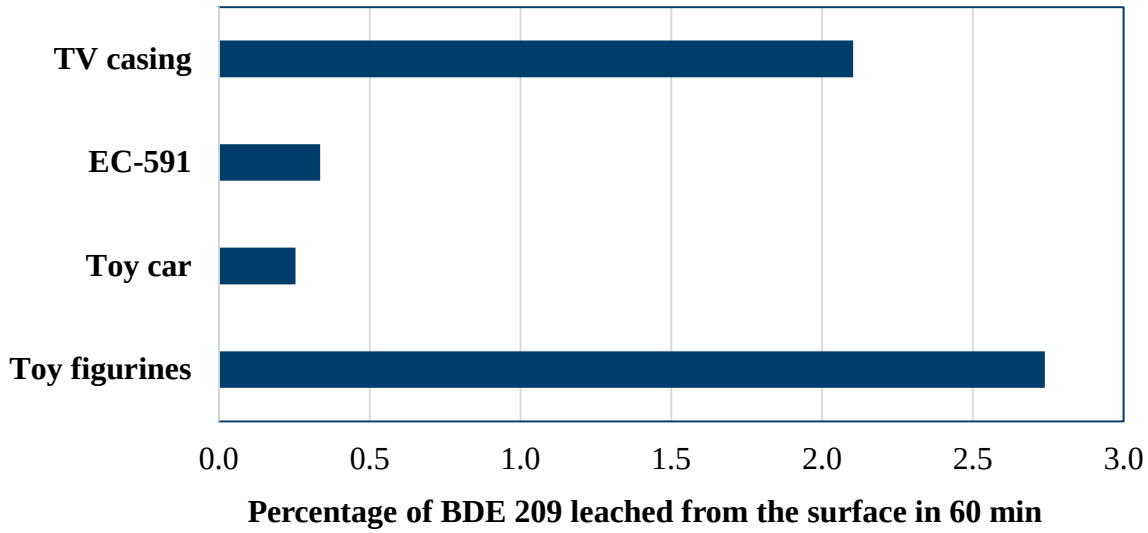

Figure 5.2.6: The percentage of BDE 209 leached after 60 min of simulated mouthing, from the surface of the samples. The amount of BDE 209 on the surface was estimated by dividing the amounts contained in the whole body of the sample by the surface-area-to-volume ratio

The same phenomenon was noted during the preliminary tests with the EC-590 (polyethylene) and EC-591 (polypropylene). This could indicate that the type of material may also be factor in the migration process. The absolute amounts migrated from these toy samples were, as expected, lower than for the CRM (Table 5.2.1). The TV casing with BDE 209 levels one order of magnitude higher than the CRM (7% *w/w*), leached out higher amounts of BDE 209 in only 15 min than the CRM after 16 h incubation. Thus, if a child mouths on an item flame retarded with PBDEs, even in a low exposure scenario, he will get a much higher dose (one order of magnitude), as compared to a toy manufactured under the REACH directive.

5.2.3.3. Evaluation of children's exposure through mouthing

The safety of children's toys has been called into question as a number of (toxic) additives have been reported (Stapleton et al., 2011; Ionas et al., 2014; Chen et al., 2009). Currently, there is insufficient information in the literature about mouthing of toys as a route of infant exposure to FRs. The topic of this study is of special concern for exposure research, and should provide a springboard for future studies since young children are at risk of unnecessary PBDE exposure resulting from activity-driven, indoor contact with contaminated media and sources.

Table 5.2.1: Leaching levels (results in pg/cm^2) from toy samples) (n=3 replicates / time point) and from plastic from an item flame retarded with BDE-209 (n=1, due to low material availability). EC-591 CRM added for comparison.

Sample	Migration time	Analyte	BDE 28	BDE 47	BDE 66	BDE 100	BDE 99	BDE 85	BDE 154	BDE 153	BDE 183	BDE 209
Toy figurines (softer plastic)	15 min	*Mean*	<4	30	<0.5	<0.5	<1	<0.5	<1	<4	<0.5	<29
		SD	-	4	-	-	-	-	-	-	-	-
	60 min	*Mean*	<4	40	<0.5	<0.5	<1	<0.5	<1	<4	10	410
		SD	-	10	-	-	-	-	-	-	2	170
Toy car (hard plastic)	15 min	*Mean*	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	<0.5	50
		SD	-	-	-	-	-	-	-	-	-	15
	60 min	*Mean*	<4	<1	<0.5	<0.5	5	<0.5	<1	30	5	50
		SD	-	-	-	-	0*	-	-	10	1	25
EC-591 CRM (hard plastic, 0.17% PBDEs)	15 min	*Mean*	20	710	20	100	470	35	20	55	120	1200
		SD	2	25	4	10	80	5	4	15	5	260
	60 min	*Mean*	50	2500	65	410	2000	160	90	220	370	2700
		SD	2	150	0*	40	130	10	10	15	50	470
TV Casing (7% BDE 209)	15 min	Amount	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	170	46600
	60 min	Amount	<4	<1	<0.5	<0.5	<1	<0.5	<1	<4	470	152000

*Value lower than 0.5

Early-life is as a particularly sensitive period for exposure to FRs, since the physiological characteristics of a young child are rapidly defining and therefore highly susceptible to influence. Exposure to certain FRs during this time can possibly promote adverse endocrine-related activity (Jugan et al., 2010; Rudel and Perovich, 2009). Therefore, elucidating key sources, transport mechanisms, and exposure-dose relationships during critical stages of development is essential to promote long term health (Lyche et al., 2015).

Infant children are more likely to mouth toys than children of older ages, and receive a higher exposure to PBDEs via this pathway (Figure 5.2.7). However, infants also display mouthing behaviour with other items, including electronics, such as remote controls and mobile phones which fit easily into their mouths. When viewed from the perspective of an expanded exposure model, the mouthing of toys in combination with breast-feeding and the incidental ingestion of dust likely account for the overall daily dose. Although the simulated model presented herein theoretically examines exposure over typical time periods, the mouthing frequency of infants is intermittent and saliva actively reproducing, such that each new contact with a toy may lead to further uptake.

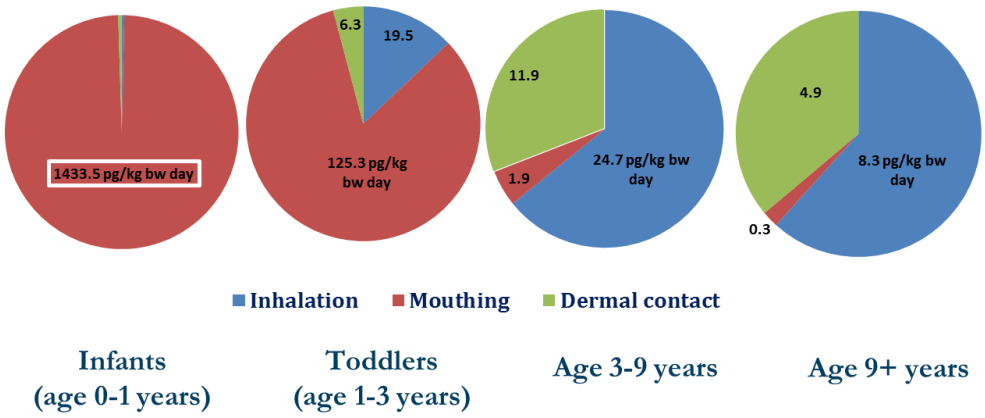

Figure 5.2.7: The estimated ΣPBDE exposure according to the pathways considered, for all age groups (data from Ionas et al., 2014)

Of additional consideration is that each time a toy (or object) is mouthed, and then either put on the floor, table, or elsewhere, the now saliva-saturated surface of the toy may absorb dust or other contaminated media from the child's environment. Apart from pacifiers, it is unlikely that a child will mouth any one object for long periods of time without intermittent opportunities for surface re-contamination. The qualification and quantification of this potential pathway can thus only lend itself useful toward elucidating the overall causal chain of exposure risk.

The current study has aimed to more accurately assess the exposure of infants to PBDEs through mouthing activities by clarifying the potential and rate of source to saliva migration in an experimental model. Our findings suggest that the migration/leaching of PBDEs into saliva is highly dependent on the physicochemical properties of the individual congener, and the type of material. The

surface loading is a very significant factor to consider when elucidating exposure risk between a low-exposure (15 min mouthing time/day) and a high exposure scenario (or "favourite toy" scenario of one hour mouthing time/day), since the migration rate for the low exposure scenario is the highest. And if the item mouthed is flame retarded with PBDEs, it can lead to high exposure levels even in a short time. It is thus important for parents to be informed about what items typically contain these chemicals or other harmful additives, especially if the items are small enough so that they can be mouthed on by their children.

In order to put the obtained values in perspective with other exposure pathways, the experimental values were tentatively compared to data available for other exposure pathways (Table 5.2.2). For the Belgian population (Roosens et al., 2010), the exposure from mouthing on toys containing PBDEs in amounts similar to the REACH threshold (0.1% product) is lower than the exposure from mother's milk, but higher than the exposure through diet or even dust.

PBDE levels from Belgium, in the matrices listed in Table 5.2.2, are typically lower than in other parts of the world, such as the USA (Dodson et al., 2012). This is reflected in the average exposure levels from a similar study focusing on the American population (Johnson-Restrepo and Kannan, 2009), where the mouthing exposure is in the same range as the exposure through ingestion of dust, but still considerably lower than the exposure through maternal nursing.

Table 5.2.2: Exposure levels obtained in this study, (n=3 replicates) and average exposure levels derived from other literature studies, for infants (0-1 years)

Source	Exposure pathway	Σ PBDEs (ng/day)*
(Roosens et al., 2010)	Human milk	152.1
	Food	15.4
	Dust	8.2
	Indoor (house) air	0.4
(Johnson-Restrepo and Kannan, 2009)	Human milk	385.5
	Food	5.5
	Dust	33.5
	Indoor (house) air	3
Experimental data	**Mouthing on toys: Low exposure**	**24 ± 5**
	Mouthing on toys: High exposure	**67 ± 7**

**Because during this stage of development, children experience the fastest increase in body weight (up to 3 fold), it was preferred not to express the exposure depending on average weight.*

Even higher contact times than our “favourite toy” scenario are reported in the literature, such as observed mouthing times of up to 171.5 min per day (Könemann, 1998).

Another important factor determining the leaching of PBDEs from plastic items during mouthing is the intensity of chewing behaviour. In the tentative *in vivo* study by Chen et al. (Chen et al., 2009), this is likely the cause of the considerable variability of the PBDE levels leached from toys. Differences in physicochemical properties of different matrices and different FRs have an impact on surface loadings, dust adherence and the whole set of processes leading to oral transfer (Ruby and Lowney, 2012; Stapleton et al., 2008; Stapleton et al., 2014; Webster et al., 2011; Weschler and Nazaroff, 2012). Chewing behaviour facilitates the leaching of chemicals from toys to saliva, in addition to potential ingestion of material microparticles.

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Chapter 6

General discussion and research gaps

FRs are more extensively used than we realise, they are added to a multitude of items intended for use mostly in the indoor environment. An obvious example is electronics, followed by everything from TVs, laptops, LCD monitors, keyboards, mice, sounds systems, and even extension cords. Various home furnishings, e.g. furniture containing polymeric foam (couches and chairs), textile products (sun screen curtains or theatre-type drapes) or carpets, can also contain FRs to meet strict fire safety regulations. Protective garments, e.g. those worn by firefighters, also contain these chemicals.

In the past, FRs have even been added to general-use clothes for children, e.g., TDBPP has been banned in 1977 from children pajamas. Home insulation materials also typically contain FRs. However, the exposure to FRs does not occur only in homes and offices, these chemicals are also added to materials in car and airplane cabins. Between the homes, cars and offices, humans are exposed to FR chemicals every moment that they spend in these indoor micro-environments, which nowadays is most of time.

FR chemicals are either bonded into the polymeric chains (reactive FRs) or mixed/blended with the material used in the manufacture of consumer goods (additive FRs). This latter category of FRs finds its way out of the materials, either through blooming (migration of solid or liquid compounding materials which have a limited solubility in the polymer matrix), volatilisation from the surface or abrasion/weathering. FR chemicals have been detected close to their sources, in the indoor media, such as air and dust, but also in the outdoor environment, in outdoor air, sediment, river and lake waters, etc. These chemicals also undergo long-range transport and have been found in polar regions, where there are no significant sources of FR emissions, such as big industrial zones or large urban areas.

Although legal restrictions on the use, production, import and sales of some FRs have been imposed in different parts of the world, FRs can still be detected in the environment, decades after they have reached their peak use or after they have been phased out.

The FR industry is thriving, since the demand for consumer products which require FRs to meet fire safety regulations, such as electronics, is ever increasing. While some of the newly developed FR solutions are non-halogenated or are designed to pose a lower health risk than the chemicals they are replacing, many new FRs maintain a similar structure and percentage of halogens, meaning that they are not safer alternatives. To assess the effects on the human health and the environment, new studies need to be undertaken for each of these new / emerging FRs.

In this work, dust and air were studied (Chapter 2) as indicators of indoor contamination with FR chemicals. If FRs present in consumer products or building materials from an indoor environment are migrating out, the less volatile compounds will typically be associated with indoor dust, while the more volatile FRs will be present in the indoor air. A new method was developed to simplify the analysis of dust and the presence and occurrence of FRs was determined in samples of dust from the

USA and Norway. From the latter, paired air samples were also analysed to more accurately assess the levels of FR contamination in the sampled environments. Both classical and emerging FRs were analysed. The dust samples from the USA (California) were collected from the same houses at an interval of five years, allowing to investigate possible time trends in the use of FRs. Concentrations of Firemaster® 550 components (EH-TBB, BEH-TEBP, and TPHP) were higher in 2011 than 2006, consistent with its use as a PentaBDE replacement, after 2004. Chlorinated PFRs (such as TCEP and TDCPP, which some studies have shown to be carcinogenic) were detected in most samples, with levels of up to 0.01% and TDBPP, a known carcinogen, was also detected in 75% of the samples. The change in the FR market is not immediately reflected in (dust from) households. In the same household, there can be products like couches or curtains which are 2-3 decades old and the latest electronics available on the market. Therefore, the occupants of such households are being exposed to a cocktail of chemicals, some of which are proven to be harmful to humans, while some might have not been fully characterised toxicologically.

In the Norway dust samples, BDE-209 and TBOEP had the highest median concentrations. Consequently, the main exposure route for these analytes (among those studied) is dust ingestion. Young children typically get a higher exposure to these FRs because of their increased frequency of hand-to-mouth behaviour. BDE-209 is used as FR in plastic materials and certain textile items (backcoating), so the possible sources are many. As for TBOEP, one of its main uses is in floor polish, which is a likely source for the TBOEP levels detected in both dust and air. In the paired air samples, BDE-47 and TCPP were present at the highest levels. The more volatile FRs are representative of the exposure through inhalation. The PFR levels in air were two orders of magnitude higher than the BFR levels. TBECH, an emerging BFR, was detected at the highest indoor air concentrations reported in the literature (households, 78 pg/m^3 and schools, 47 pg/m^3). This compound has been identified as a potential endocrine disruptor.

Around 80% of the targeted FRs were detected in air and dust from the sampling sites. By correlating this data with inhabitant activity data, it was determined that frequent vacuum cleaning resulted in lower FR concentrations in dust and that, for both children and mothers, dermal contact with dust was as important exposure pathway to PFRs as dust ingestion.

These results highlight the evolving nature of FR exposures and suggest that manufacturers continue to use hazardous chemicals and replace chemicals of concern with chemicals having insufficiently characterised toxicity.

In Chapter 3, new analytical methods have been developed and optimized for the non-target screening of FRs hitherto either un- or rarely –detected and for the quantification of new / emerging PFRs in indoor dust, electronics and textile furnishings, the most likely PFR sources. This latter method was developed on an UPLC-MS/MS system, to be simple as sample preparation, instrumentally very fast (all analytes resolved in a 4.5 min injection), allowing for high sample

throughput and low consumable use and flexible in easily allowing for other analytes to be added to the method. The focus was on V6, which is used mainly in foams and car interiors and on RDP and BDP, which are used in plastic components of electronics. All these FRs migrate into dust, as mentioned in Chapter 2, so this matrix was also studied as an indicator of indoor contamination with these chemicals. Other PFRs relevant to this study (with main application as FR and not plasticiser) were also added to the method: TCEP, TCPP, TDCPP, TDBPP, TPhP and the less studied iDPhP and TXP.

The non-target screening procedure, is a systematic approach employing LC-(Q)TOF-MS and spectra-less databases based only on mono-isotopic accurate mass, which facilitates the challenging process of unambiguously identifying a true unknown compound via its accurate mass MS and MS/MS spectra alone. Considering that most FRs of higher concern are halogenated, strategies to highlight the halogenated FRs were also developed. For this purpose, parameters for Isotope Cluster Analysis were optimised to extract in one chromatogram just the halogenated compounds containing more than three chlorine and/or bromine atoms. Another similar approach employs mass defect filtering. Halogenated chemicals have a negative mass defect ranging from approximately -0.045 Da to about -1.155 Da, for the heaviest known FRs. The use of a value for the mass defect filtering that is somewhere in the middle of this range and an appropriately chosen mass defect tolerance will help highlight the halogenated compounds. Additionally, an in-house database was built and the input parameters used in the data analysis process were optimised for halogenated and organophosphate FR chemicals, so that it can be easily transferred to other laboratories.

In Chapter 4, levels and profiles of classical and emerging FRs were scrutinised in different types of consumer products, such as textile furnishings, electronics and children's toys. For general-use carpets and curtains from the Belgian market, inherently flame-retarded fabrics and alternative FRs are preferred to the more conventional FRs, which have been associated with adverse health effects. This is most likely because Belgium does not have very stringent fire safety regulations for such products. In the plastics from electronics, chemical elements commonly contained in FRs have been monitored (such as Br, Cl and P), along with a number of elements associated with inorganic FRs (Al, Mg, Sb and Zn). Some electronics contained high amounts of Br, which could not be associated with conventional BFRs, and are most likely due to the use of reactive BFRs or brominated polymers, such as brominated polystyrene. Other electronics contained high levels of P, derived from emerging PFRs, such as BDP and RDP (see Chapter 3), while in one sample, the high P levels could not be associated with any conventional PFR and may be due to the use of inorganic (red) P as a FR. Some samples also contained high levels of metallic elements associated with inorganic FRs, sometimes even in samples which also contained conventional organic FRs.

Children's toys were also investigated for the presence of BFRs, PFRs and even phthalate esters. Regular toys are not considered in fire hazard scenarios, so they typically do not contain FRs in

levels encountered in other consumer products, such as electronics. An exception here are toys which contain electronic components which can get hot during use. Overall, the levels of FRs detected were not high enough to impart flame retardancy. The presence of these low levels is likely due to improper recycling practices. The main BFR detected was BDE-209, with levels of up to 0.014% in a foam and textile toy. The levels and detection frequencies of PFRs were higher than the BFRs, most likely because PFRs are also used as plasticisers for certain applications. TPhP was the most often detected PFR (in 52% of the samples) and had the highest levels up to 12800 µg/g. Although the PFR levels were still lower than the phthalate levels, an increasing trend in the PFR concentrations was seen in toys manufactured after 2007, when the REACH directive came into effect, restricting PBDEs and some of the phthalate esters to 0.1% by mass in such products. With levels of PFRs increasing to meet fire safety standards and to impart plasticity to the materials they are added to, and a whole range of possible adverse health effects associated with them, it is important to monitor the levels of these chemicals in items which children come in contact often with, such as toys.

In Chapter 5, a previously unexplored pathway of FR exposure in children, such as playing with toys, was studied. The FR levels described in Chapter 4 were used to estimate the FR exposure from playing with toys. For PFRs, the data and the models in the literature were insufficient to assess the children's exposure via this pathway. For PBDEs, however, similar studies were available and the exposure levels were thus estimated using the described models and compared with other exposure pathways. The exposure arising from hand-to-mouth behaviour when playing with toys was found to be two orders of magnitude lower compared to the same type of behaviour leading to dust ingestion at the FR levels in the analysed toy samples. These exposure levels were also lower than the US EPA defined reference doses (RfD) for PBDEs. The inhalation exposure from playing with toys was found to be three to eleven times lower than the same type of exposure from indoor air, thus negligible.

However, the highest levels of exposure to PBDEs were due to children's mouthing behaviour on toys. This particular case was then explored in more detail by establishing a leaching model for determining the amount of PBDEs that can leach from toys into saliva in simulated conditions. The PBDE migration rate was found to be highest in the first few minutes of the mouthing (198 $pg/cm^2 \times min$ after a migration time of 15 min). The lower brominated PBDE congeners leached out more extensively in the aqueous media than the heavier and more lipophilic compounds. At PBDE levels around 0.1 % by mass (REACH threshold), the exposure levels from mouthing on toys are lower than the exposure from mother's milk, but higher than exposure through other dietary sources and dust (based on levels from Belgium). Children can thus get a considerable amount of exposure to FRs via mouthing, if such chemicals are contained in the corresponding toys. Luckily, it is uncommon to have high levels of BFRs in toys. A more hazardous scenario is when children mouth on small electronic items, which are likely to contain high amounts of FRs, such as a remote control or even a smartphone.

Overall, as a conclusion, house dust and air can offer a wealth of information about the presence, levels and profiles of FR contamination of indoor environments. Gradually, FRs used in previous years are being replaced by new FR chemicals, with improved properties and (in theory at least) less harmful to human health. New and improved non-targeted screening procedures are required to identify the replacement FRs and new robust and sensitive analysis methods are required to monitor these chemicals in the environment. Since the vast majority of these chemicals are used in consumer products (used in the indoor environment), the levels and migration potential of FRs from these sources should continuously be monitored. These measures are meant to minimise human exposure to FRs. To achieve this, comprehensive information about exposure pathways and their magnitude is important. Alongside the classical pathways for human exposure to FRs (diet, dust ingestion, inhalation), children have an additional pathway: through playing with toys. This particular pathway is less significant as the classical ones, as long as the child does not mouth any flame retarded item.

Considering the ever-increasing diversity of chemicals that we are continuously being exposed to, it is of the utmost importance to monitor these chemicals and to elucidate their effects on humans and the environment.

Research gaps in the knowledge about FRs and their impact on the environment

The list of FRs which are deemed of concern for human exposure is not exhaustive. Following the phase-out of PBDEs due to health concerns, several FRs with considerable evidence of toxicity appear to remain at high or increasing levels of use. Some FRs are apparently replaced by less-studied chemicals whose health implications are unknown and for which there is much less information known than for the classical FRs which have been investigated during the past decade.

One aspect that could be significantly improved is the interaction with the companies producing FRs. More transparency from the industry's side about their products would allow the scientific community to help select the products which are best for the environment and humans. Since this is not happening at the moment, the scientific community needs to invest more and more time and resources into non-target screening studies to unravel the identity of the newest FRs on the market.

In the past few years, the tendency of the FR market was to shift from older FRs, which studies have found that they might have adverse health effects, to non-halogenated or organophosphate alternatives. Inorganic FRs and condensed-type organophosphate esters, such as BDP and RDP, have seen an increase in use. For certain applications though, halogenated FRs are still the gold standard. The newer halogenated FRs often have some similarity in their structures with legacy FRs, such as TCEP and V6 or TPhP and RDP. The main difference is that the newer FRs are designed to be heavier and less volatile, in order to minimise the migration of these chemicals from the products they are added to and ultimately to minimise human exposure and migration into the environment. Another example is TTBP-TAZ, a triazine FR with 9 bromine atoms, which is designed to prevent blooming and offer high flame-retarding efficacy, good thermal and UV stability.

Another way of tackling the problem of FRs migrating out the items they are added to, is to employ halogenated polymers, such as dibromostyrene derivatives or brominate polystyrenes. This is a very good idea, in theory, but it is very difficult to predict the behaviour of this material after prologued use or after disposal.

For these new and emerging FRs, it is very important to develop new analytical methodologies and make them available to the scientific community, so that these chemicals can be monitored in indoor and outdoor environment. The biomonitoring of the exposure in the general population to these FRs is also of the outmost importance. Given that the collected volumes of blood, serum and tissue samples are typically low, the new analytical methodologies need to have increased sensitivity and lower detection limits. Since the amount and type of metabolites detected in human matrices allow for a better characterisation of the exposure, it is very important that metabolisation studies are conducted on the new and emerging FRs as soon as they are detected in the indoor environment, as a first step towards exposure assessment.

For the new and emerging FRs, further studies should unravel the relative importance of various exposure pathways, but also the possibility of reducing this exposure. One such gap in the knowledge base about FR exposure pathways that needs to be filled is dermal exposure. From the perspective of exposure pathways, there is a lack of information regarding their relative importance and the multiple factors which influence the magnitude of these exposures.

More studies are needed to evaluate the significance of measured body burdens for each FR in relation with the existing toxicological data. This is necessary to quickly ascertain the toxicological relevance of given levels of exposure to FRs.

There is also a lack of standardised testing of endpoint toxicity (each company might use or request a different method to test a certain type of toxicity) for different groups of chemicals which makes the comparison of toxic effects and health issues for various chemicals very difficult, if not impossible. More coordination of the epidemiological studies in investigating key FRs together with important health endpoints (including clinical parameters) is required.

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Ionas AC, Xu F, Neels H, Covaci A. Development of a liquid chromatography electrospray ionization tandem mass spectrometry method for the analysis of emerging organophosphate flame retardants in dust, plastics and textiles *(in preparation).*

Ionas AC, Uchida N, Suzuki G, Takigami H, Neels H, Covaci A. Characterisation of flame retardants in plastics from electronics: levels, elemental and material analysis *(in preparation).*

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Curriculum Vitae

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Oral presentations:

- **Ionas AC**, Ballesteros Gómez AM, Uchida N, Kajiwara N, Suzuki G, Leonards PEG and Covaci A. Comprehensive characterisation of flame retardants in textile home furnishings by gas chromatography, direct probe coupled to time-of-flight high resolution mass spectrometry and environmental forensic microscopy. Dioxin 2015: 35th International Symposium on Halogenated Persistent Organic Pollutants, Sao Paulo (Brazil); 23-28 August 2015.

- **Ionas AC**, Ballesteros Gómez AM, Brandsma SH, Leonards PEG and Covaci A. Assessment of PBDE exposure through mouthing on toys - *in vitro* migration into saliva simulant. Dioxin 2014: 34th International Symposium on Halogenated Persistent Organic Pollutants, Madrid (Spain); 31 August - 5 September 2014.

- **Ionas AC**, Ballesteros Gómez AM, Leonards PEG and Covaci A. Identification of novel halogenated flame retardants using spectra-less databases, Dioxin 2013 Symposium, Daegu (South Korea); 25-30 August 2013.

- Invited lecture at the periodical Cluster Meeting of the Chemistry and Biology department of the IVM (VU, Amsterdam, Netherlands); 26 February 2013 on the same topic as below.

- **Ionas AC**, Anthonissen T, Dirtu AC and Covaci A. Analysis of harmful organic chemicals in children's toys, Dioxin 2012 Symposium, Cairns (Australia); 26-31 August 2012.

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Poster contributions:

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- **Ionas AC**, Ballesteros Gómez AM, Leonards PEG and Covaci A. Determination of flame retardants in textiles by Gas Chromatography and Direct Probe coupled to Time-of-Flight high resolution Mass Spectrometry, Dioxin 2014: 34th International Symposium on Halogenated Persistent Organic Pollutants, Madrid (Spain); 31 August - 5 September 2014.

- **Ionas AC**, Dirtu AC, Anthonissen T, Neels H and Covaci A. Harmful organic chemicals in children's toys due to use of recycled materials, SETAC Europe 2014 Conference, Basel (Switzerland), 11-15 May 2014. *Poster spotlight presented for this contribution as well.*

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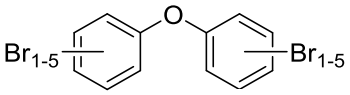
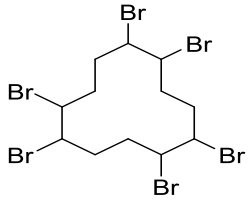
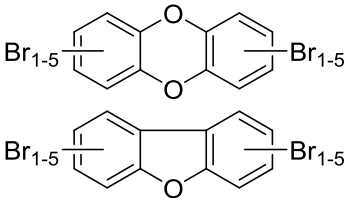
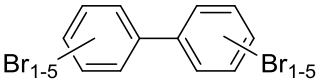
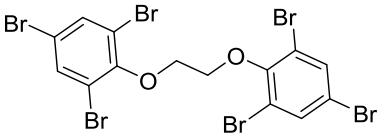
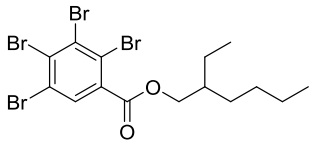
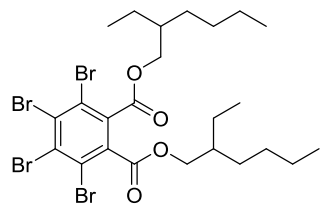
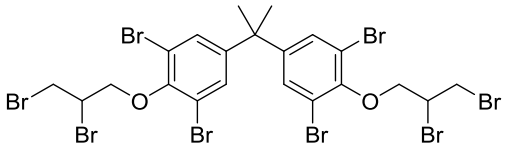
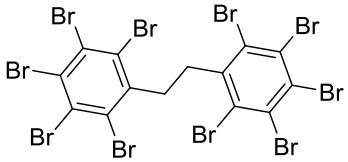
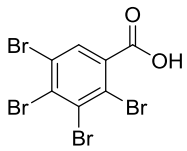
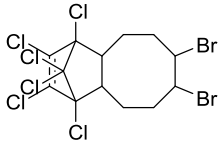
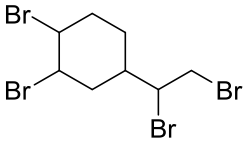
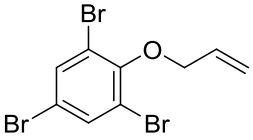
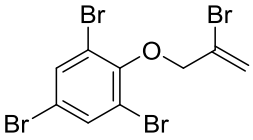
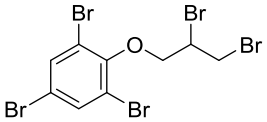
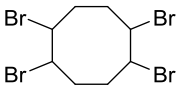
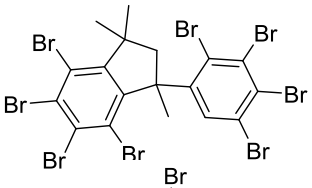
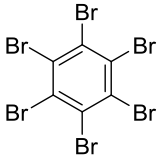
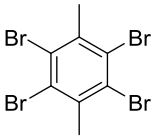
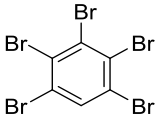
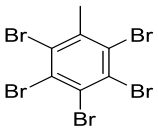
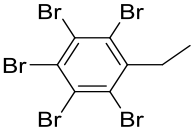
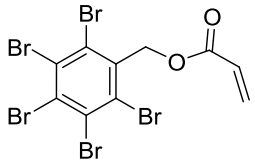
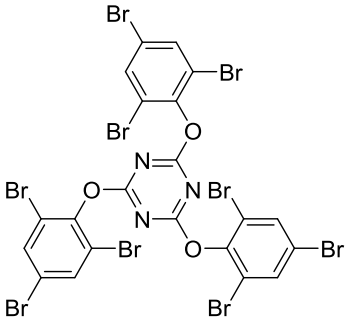
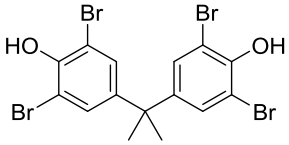
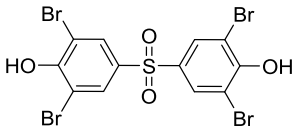
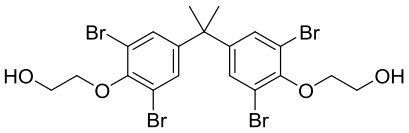
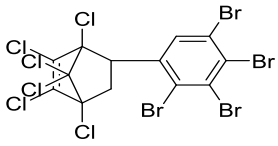
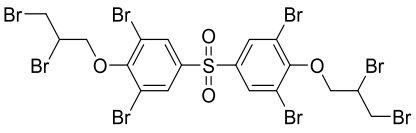
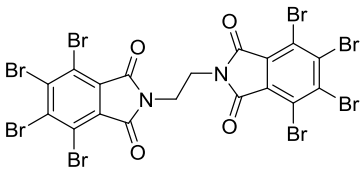
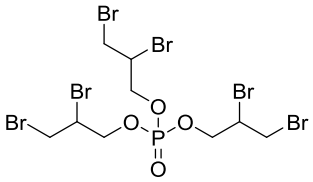
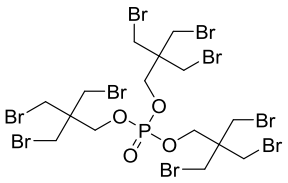
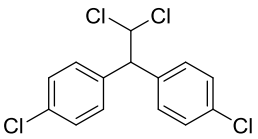
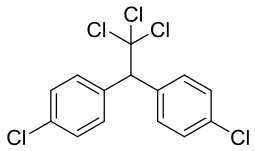
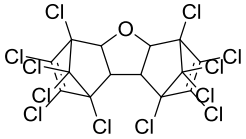
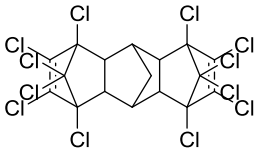
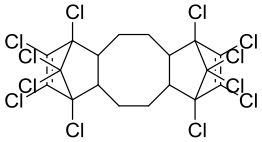
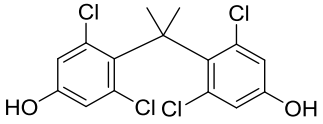
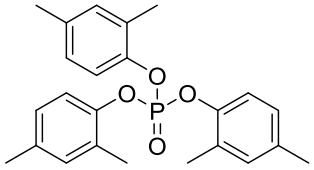
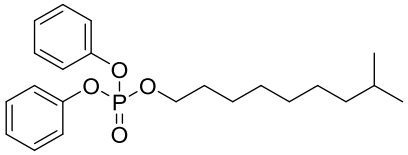
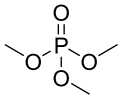
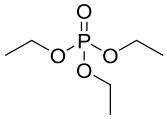
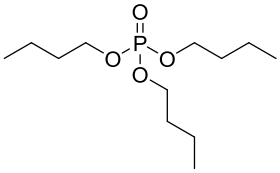
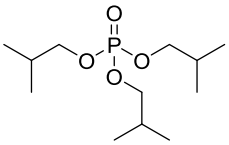
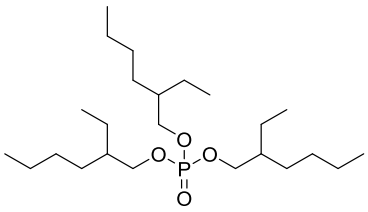
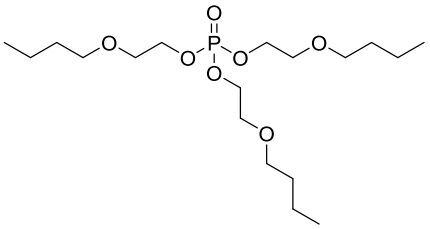
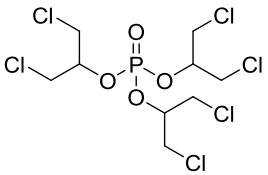
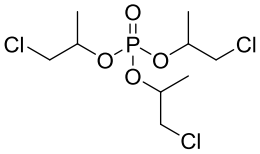
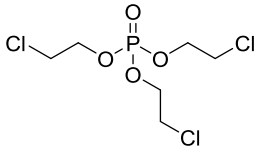
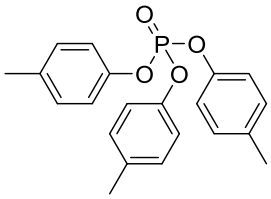
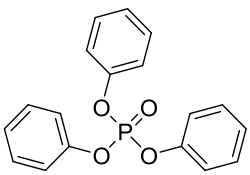
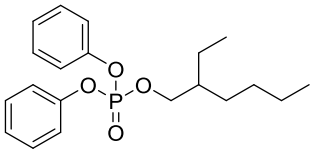
Appendix 1: Additional information about the analytes studied in this work: names, abbreviations, CAS numbers and structures

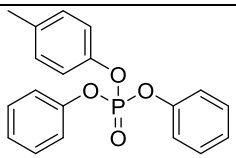
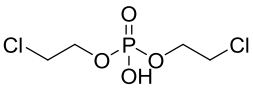
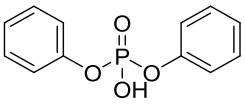
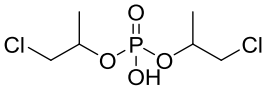
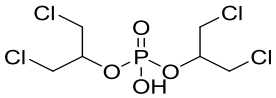
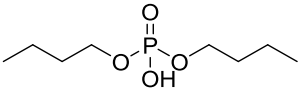
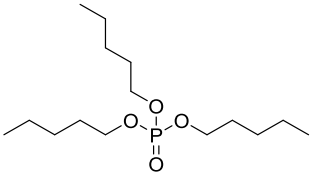
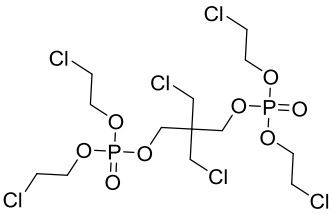
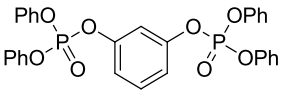
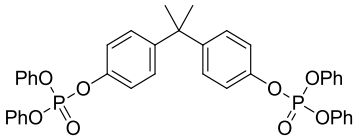
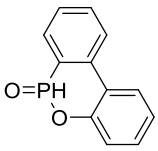
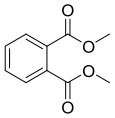
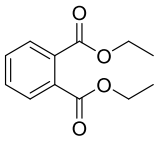
Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
PBDEs	-	-	polybrominated diphenyl ethers	
HBCDDs*	HBCDs	3194-55-6	hexabromocyclododecanes	
PBDD/Fs	-	-	polybrominated dibenzo-p-dioxins, polybrominated dibenzofurans	
PBBs	-	-	polybrominated biphenyls	
BTBPE	-	37853-59-1	1,2-Bis(2,4,6-tribromophenoxy)ethane	
EH-TBB*	TBB	183658-27-7	2-Ethylhexyl 2,3,4,5-tetrabromobenzoate	
BEHTBP*	TBPH	26040-51-7	Bis(2-ethylhexyl) tetrabromophthalate	
TBBPA-BDBPE*	TBBPA-dbpe	21850-44-2	Tetrabromobisphenol A bis(2,3-dibromopropyl) ether	
DBDPE	-	84852-53-9	Decabromodiphenyl ethane	
TBBA	-	183658-27-7	2,3,4,5-tetrabromobenzoic acid	
HCDBCO	DBHCTD*	51936-55-1	5,6-Dibromo-1,10,11,12,13,13-hexachloro-11-tricyclo[8.2.1.02,9]tridecene	

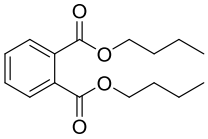
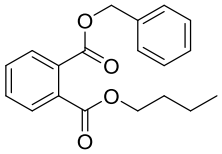
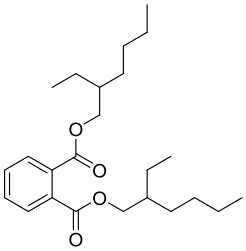
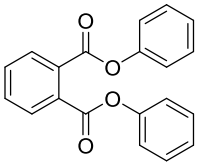
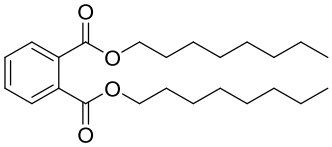
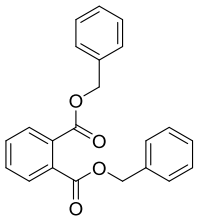
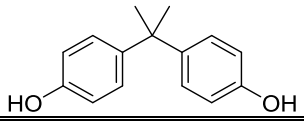
Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
TBECH	DBE-DBCH*	3322-93-8	1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane	
ATE	TBP-AE*	3278-89-5	2,4,6-Tribromophenyl allyl ether	
BATE	TBP-BAE	-	2-bromoallyl-2,4,6-tribromophenyl ether	
DPTE	TBP-DBPE*	35109-60-5	2,4,6-Tribromophenyl 2,3-dibromopropyl ether	
TBCO	-	3194-57-8	1,2,5,6-Tetrabromocyclooctane	
OBIND	Octa-BTMPI*	1084889-51-9	Octabromotrimethylphenyl indane	
HBB	-	87-82-1	Hexabromobenzene	
TBX	-	23488-38-2	2,3,5,6-Tetrabromo-p-xylene	
PBBz	-	608-90-2	pentabromobenzene	
PBT	-	87-83-2	pentabromotoluene	
PBEB	-	85-22-3	pentabromoethylbenzene	

Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
PBBzA	PBB-Acr*	59447-55-1	Pentabromobenzyl acrylate	
TTBP-TAZ	TBP-TAZ*	25713-60-4	Tris(2,4,6-tribromophenoxy)-s-triazine	
TBBPA	-	79-94-7	Tetrabromobisphenol A	
TBBPS	-	39635-79-5	Tetrabromobisphenol S	
TBBPA-BHEE	-	4162-45-2	Tetrabromobisphenol A bis(2-hydroxyethyl) ether	
Dec 604	HCTBPH*	34571-16-9	1,2,3,4,7,7-hexachloro-5-(2,3,4,5-tetrabromophenyl)-Bicyclo[2.2.1]hept-2-ene	
TBBPS-BDBPE	-	42757-55-1	Tetrabromobisphenol S bis(2,3-dibromopropyl ether)	
EBTBPE	-	32588-76-4	N,N'-Ethylenebis(tetrabromophthalimide)	
TDBPP	-	126-72-7	Tris(2,3-dibromopropyl) phosphate	

Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
TTBNPP	-	19186-97-1	Tris[3-bromo-2,2-bis(bromomethyl)propyl] phosphate	
DDD	-	72-54-8	Dichlorodiphenyldichloroethane	
DDT	-	50-29-3	dichlorodiphenyltrichloroethane	
Dec 602	DDC-DBF*	31107-44-5	1,2,3,4,6,7,8,9,10,10,11,11-Dodecachloro-1,4,4a,5a,6,9,9a,9b-octahydro-1,4:6,9-dimethanodibenzofuran	
Dec 603	DDC-Ant*	13560-92-4	1,2,3,4,5,6,7,8,12,12,13,13-dodecachloro-1,4,4a,5,8,8a,9,9a,10,10a-decahydro-1,4:5,8:9,10-Trimethanoanthracene	
DP	DDC-CO	13560-89-9	Dechlorane Plus	
TCBPA	-	79-95-8	Tetrachlorobisphenol A	
TXP	-	25155-23-1	trixylenyl phosphate	
iDPhP	iDPP	29761-21-5	isodecyl diphenyl phosphate	
TMP	TMP	512-56-1	trimethyl phosphate	
TEP	TEP	78-40-0	triethyl phosphate	

Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
TnBP	TNBP*	126-73-8	Tri-n-butyl phosphate	
TiBP	TIBP*	126-71-6	Tris(isobutyl) phosphate	
TEHP	-	78-42-2	Tris(2-ethylhexyl) phosphate	
TBOEP*	TBEP	78-51-3	Tris(2-butoxyethyl) phosphate	
TDCiPP	TDCiPP / TDCIPP*	13674-87-8	Tris(1,3-dichloroisopropyl) phosphate	
TCiPP	TCiPP / TCIPP	13674-84-5	Tris(2-chloroisopropyl) phosphate	
TCEP	-	115-96-8	Tris(chloroethyl) phosphate	
TCP	TMPP*	1330-78-5	Tricresyl phosphate (mixture of ortho, meta, para)	
TPhP	TPP / TPHP*	115-86-6	Triphenyl phosphate	
EHDPhP	EHDPP	1241-94-7	2-ethylhexyl-di-phenylphosphate	

Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
CDP	-	26444-49-5	Cresyl diphenyl phosphate	
BCEP	-	3040-56-0	Bis(2-chloroethyl) hydrogen phosphate	
DPhP	DPP	838-85-7	Diphenyl hydrogen phosphate	
BCiPP	BCPP	3040-56-0	Bis(2-chloropropyl) hydrogen phosphate	
BDCiPP	BDCPP	72236-72-7	Bis(dichloroisopropyl) hydrogen phosphate	
DBP	-	107-66-4	Dibutyl hydrogen phosphate	
TAP	-	2528-38-3	Triamyl phosphate	
V6	BCMP-BBCP*	38051-10-4	2,2-Bis(chloromethyl)-1,3-propanediol bis[bis(2-chloroethyl) phosphate]	
RDP	PBDPP*	57583-54-7	Resorcinol bis(diphenyl phosphate)	
BDP	BPA-BDPP*	5945-33-5	Bisphenol A bis(diphenyl phosphate)	
DOPO	-	35948-25-5	3,4:5,6-Dibenzo-2H-1,2-oxaphosphorin-2-oxide	
DMP	-	131-11-3	Dimethyl phthalate	
DEP	-	84-66-2	Diethyl phthalate	

Abbreviation	Other abbreviation	CAS	Compound and/or compounds class name	Structure
DBPht	-	84-74-2	Dibutyl phthalate	
BBzP	-	85-68-7	Butyl benzyl phthalate	
DEHP	-	117-81-7	Bis(2-ethylhexyl) phthalate	
DPPht	-	84-62-8	Diphenyl phthalate	
DnOP	-	85-68-7	Di-n-octyl phthalate	
DBzP	-	523-31-9	Dibenzyl phthalate	
BPA	-	80-05-7	Bisphenol A	

*As suggested in Bergman et al. (2012)