HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS

Enrique Cequier Manciñeiras

Dipòsit Legal: T 1518-2014

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

IDENTIFYING HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS

Enrique Cequier Manciñeiras

Doctoral Thesis

Supervised by

Dr. Rosa Maria Marcé

Department of Analytical Chemistry and Organic Chemistry

and

Dr. Georg Becher and Dr. Cathrine Thomsen

Norwegian Institute of Public Health

UNIVERSITAT ROVIRA I VIRGILI

Tarragona 2014

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

UNIVERSITAT ROVIRA I VIRGILI

DEPARTAMENT DE QUÍMICA ANALÍTICA I QUÍMICA ORGÀNICA

Campus Sescelades
C/ Marcel.lí Domingo s/n
43007 Tarragona
Tel. 34 977 55 97 69
Fax 34 977 55 84 46
e-mail: secqaqo@urv.net

FEM CONSTAR que el treball "Identifying human exposure pathways to flame retardants", que presenta l'Enrique Cequier Manciñeiras per a l'obtenció del títol de Doctor, ha estat realitzat sota la direcció conjunta de la Dra. Rosa Maria Marcé i Recasens del Departament de Química Analítica i Química Orgànica d'aquesta Universitat i del Dr. Georg Becher i la Dra. Cathrine Thomsen de l'Institut de Salut Pública Noruec, i que acompleix els requeriments per poder optar a Menció Europea.

I, per a que consti, expedim aquest certificat a Tarragona / Oslo, 18 de Juny de 2014

Dra. Rosa Maria Marcè Dr. Georg Becher Dra. Cathrine Thomsen

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

I acknowledge the Coordinator and all Researchers involved in the splendid project funded by the European Commission and entitled “Indoor Contamination with Flame Retardant Chemicals: Causes and Impacts” (INFLAME) because thanks to all of them I had the opportunity to meet beautiful people, to further develop my career in the environmental research field, to travel around the World, and to experience the Norwegian life style (Jeg likte å vare her og jobbe sammen med dere! Tusen takk!).

This work has also been carried out thanks to the support of my supervisors, co-workers and colleagues. Definitely, this Thesis has been much easier to write with their help. Thank you all!

In the following lines, I would like to express my gratitude to the closest persons who have had an enormous impact on this work, but their names do not appear along the pages of this Thesis:

A mis Abuelos, tanto para los que están como para los que ya nos dejaron, debo agradecerles todo su cariño y apoyo durante todas las etapas de mi vida. Me han enseñado, entre muchas otras cosas, que envejecer merece la pena.

Papá, Mamá, es difícil expresar con palabras certeras el profundo agradecimiento y amor que os profeso porque sois las únicas personas del mundo que siempre habéis estado, estáis y estaréis a mi lado. Gracias por todo.

A mi hermano, gracias por tener esa constancia para, a pesar de la distancia, hacerme sentir como uno más de la familia tan maravillosa que has formado.

Para Elisa, la mujer que ha compartido mis buenos y malos momentos durante esta travesía, me ha mejorado como investigador, me complementa diariamente y me ha hecho pensar en un futuro diferente, gracias por estar ahí.

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Table of Contents

1. Introduction

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

The modern world evolves towards the production of a wide variety of new engineered materials. Most of these materials are treated with chemicals in order to modify the properties of the raw substances. Among these chemicals, we find the flame retardants (FRs), which are mainly incorporated into consumer goods present in indoor environments. There is strong evidence that FRs migrate into the environment, and through different routes these chemicals end up in humans [1,2]. Several FRs have been proven as endocrine disruptors in *in vitro* and *in vivo* assays [3-6]. The definition of an endocrine disruptor according to the International Programme on Chemical Safety is: "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations" [7]. A failed communication between the hormones produced in the endocrine glands and the cell receptors might lead to a serious health problem. World Health Organisation (WHO) reported an increasing cancer incidence among children in the U.S.A. between the years 1975 and 2005, an increasing testicular cancer rate between 1960 and 2000 in Denmark, Sweden, Norway and Finland, and an increasing female breast cancer incidence across Europe [8]. All these diseases can be caused by the intervention of endocrine disruptors like FRs. Endocrine disruptors can act at any stage of life, but identified sensitive periods are gestation and early childhood, where the development of muscles, organs, brain, etc. occurs. The observed proliferation of diseases related to hormone disruptors may be also a consequence of transgenerational effects, which would imply that today's effects might correspond to the exposure of a progenitor in the past.

Therefore, studies surveying the levels of FRs in food, indoor environments and in humans are very important to evaluate the risk associated to exposure to FRs in particular for infants, toddlers, and women of childbearing age. With this objective, the European Commission under the 7th Framework Programme funded the E.U. Marie Curie Initial Training Network entitled: "Indoor Contamination with Flame Retardant Chemicals: Causes and Impacts" (INFLAME; GA No. 264600). INFLAME general objectives are to: (1) Study the mechanisms via which FRs migrate from the materials where they have been incorporated, (2) Identify human exposure pathways to FRs and (3) Assess the effects of FRs in human health.

Further knowledge on these main objectives will give a better understanding of the risk of using FRs in household products and/or indoor materials for the human health, and ultimately will lead to more sustainable approaches to meeting fire safety regulations. To do so, nine Universities and Research Institutions (University of Birmingham, Toxicological Centre, University of Antwerp, Free University of Amsterdam, Flemish Institute for Technological

Research (VITO), Swedish Environmental Research Institute, University of Stockholm, Norwegian Institute of Public Health, University of Amsterdam, and University of Reading) have undertaken 14 projects from which six, five and three are dedicated to objectives (1), (2), and (3), respectively. This thesis has been financed by the European Commission and by the Norwegian Research Council and is connected to the objective number (2) "Identify the human exposure pathways to FRs". The experimental part has been carried out at the Norwegian Institute of Public Health with the collaboration of the department of Analytical Chemistry and Organic Chemistry from the Universitat Rovira i Virgili (Tarragona, Spain).

1.1. Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Flame retardants are chemicals used to reduce or prevent the spread of fire in many combustible materials, mainly consisting of polymers that surround us in our daily life (e.g., electric and electronic appliances, synthetic foams, furniture, textiles and toys, among others). The use of FRs in the consumer products is basically a matter of protection of our lives, properties and environment imposed by different legislations worldwide since polymers are derived from petroleum and are highly flammable. The fire regulations differ from country to country, but in essence all demand the action of chemicals reducing or eliminating the flammability of the materials vulnerable to fire. According to the statistics supplied by the European Flame Retardant Association (EFRA), 94% of the fires causing death take place in indoor environments and 5% in transports [9]. Fire is caused by the combination of three elements: heat, fuel and oxygen. The lack of one of these elements causes the extinction of fire and this is exactly the function of the FRs. Therefore, FRs are incorporated into the polymers either as a bounded chemical, like tretrabromobisphenol A, or as an additive blended into the material, like polybrominated biphenyls and diphenyl ethers or hexabromocyclododecane (HBCD) [10]. The description of a precise mechanism of the action of FRs is difficult to summarise [11] as many reactions take place during combustion, and depending on the nature and number of FRs used, their effect can be additive. In general, FRs provide fire resistance in the polymers by two mechanisms: reactions in the gas phase, by eliminating radicals necessary for the fire propagation, and in the solid phase, by denaturation of the polymer creating a char layer and thereby preventing oxygen to feed the fire. FRs can be classified in two main groups depending on their chemical composition (inorganic and organic) (Figure 1) [12]. Inorganic FRs, mainly aluminium and magnesium hydroxides, are less effective than the organic FRs, hence high amounts are needed in the materials to achieve acceptable resistance to fire. In 2006, their production covered around 50% of the total production of FRs in Europe [13]. However, in consumer goods, inorganic FRs are commonly used as a synergist in combination with the more efficient organic FRs [9]. Production of organic FRs from 2006 was almost the other 50% from which phosphorous-based FRs and halogenated FRs were around 20% each.

Chemicals containing halogen atoms show excellent flame retardant properties because they have the capacity to release halogenated molecules, such as HBr, before the ignition of the material occurs. These halogenated molecules quench efficiently the free radicals in the gas phase (e.g., $OH^{\cdot}$ and $H^{\cdot}$) responsible for propagating the fire [14]. Alaee *et al*. [10] reported that the larger the halogen atom, the better is the flame inhibition (i.e., I > Br > Cl > F).

However, iodinated compounds decomposed at low temperatures, making them unsuitable for FRs. On the other hand, fluorinated compounds are very stable and do not decompose to release the halogenated fragments when the material burns and thus, they are not used as FRs. Therefore, the most suitable halogen atoms for flame retardant purposes are bromine and chlorine, bromine being the most efficient.

Organophosphorous-based FRs (PFRs) also provide good flame retardant efficiency through a combined mechanism in the solid and the gas phase. When heated, these PFRs form a char layer that prevents the pyrolysis of the material, avoiding the generation of fumes that would propagate the fire [9]. The mechanism by which the PFRs act in the gas phase is very similar to that from halogenated FRs. To some extent PFRs also releases radical species capable to quench the free radicals in the gas phase (e.g., $PO^{\cdot}$, HPO, $HPO_2^{\cdot}$) [15]. In addition, some PFRs contain chlorinated atoms, which increase the inhibition of fire by radical quenching.

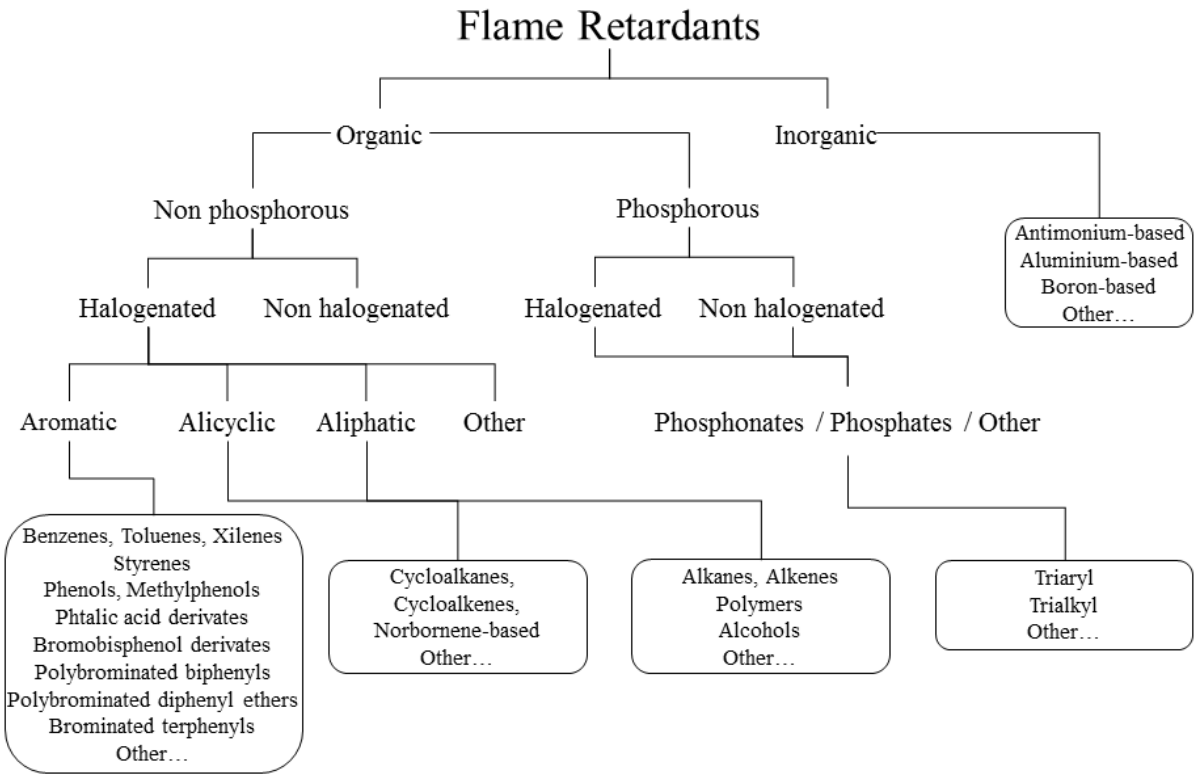


Figure 1. Scheme of flame retardants based on the classification by Fisk *et al.* [12].

1.1.1. Persistent flame retardants

Polybrominated diphenyl ethers (PBDEs) are a group of chemicals comprising up to 209 different congeners. The congeners may be divided in 10 groups depending on the number of bromine substituents in the two phenyl groups linked through an ether bond (from MonoBDE to DecaBDE) [16]. There are three known commercial mixtures of PBDEs that have been used: PentaBDE, OctaBDE and DecaBDE. The major components of the three PBDEs formulations according to La Guardia *et al*. [17] are summarised in Table 1 and the general structure of PBDEs is depicted in Table 2.

Table 1. Congeners used for the PBDEs formulations.

#	**PentaBDE** formulation	Abbreviation	Proportions in formulation
1	2,2',4,4'-tetrabromodiphenyl ether	BDE-47	8
2	2,2',4,4',5-pentabromodiphenyl ether	BDE-99	10
3	2,2',4,4',6-pentabromodiphenyl ether	BDE-100	3
4	2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	1
5	2,2',4,4',5,6'-hexabromo diphenyl ether	BDE-154	1
	OctaBDE formulation		
1	2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	1
2	2,2',3,4,4',5',6-heptabromodiphenyl ether	BDE-183	5
3	2,2',3,3',4,4',6,6'-octabromodiphenyl ether	BDE-197	2
4	2,2',3,3',4,4',5,6'-octabromodiphenyl ether	BDE-196	1
5	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether	BDE-207	1
	DecaBDE formulation		
1	decabromodiphenyl ether	BDE-209	> 90%

The proportions of the congeners in the different formulations presented are very dependent on the manufacturer and some variations can be found from product to product. Due to the proven persistency, toxicity and bioaccumulative properties of the components from Penta- and OctaBDE formulations, both were worldwide banned in 2009 and therefore they are currently considered legacy FRs [18]. DecaBDE is presently phased-out in the U.S.A., added to the Candidate List for Authorisation in Europe in 2010 under the E.U. regulatory framework for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), and banned in Norway since 2008 [19]. The main use of the PentaBDE formulation was in polyurethane foams (PUF), and the OctaBDE was for plastic housing and

office equipment. DecaBDE is still used in electric and electronic appliances, textiles and fabric backings [20]. From all congeners composing the different formulations, the most abundant PBDEs found in many environmental compartments are seven: BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183 and BDE-209 [21].

Table 2. CAS numbers, chemical names, abbreviations (previously used abbreviations also provided in parentheses) and structures of the persistent and similar FRs.

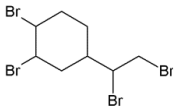
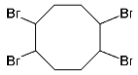
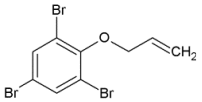
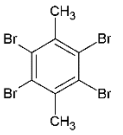
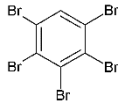
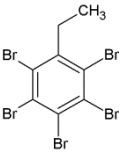
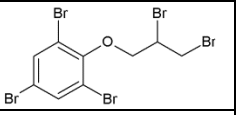
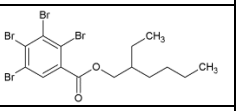
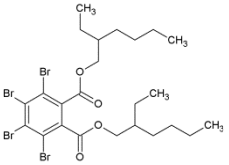
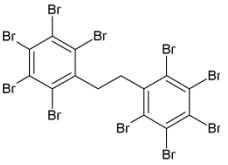
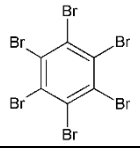
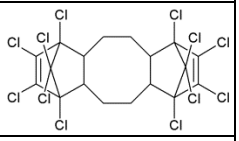
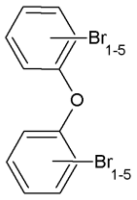
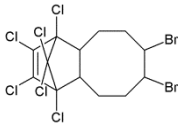
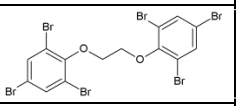
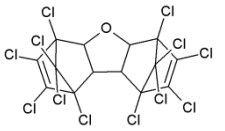
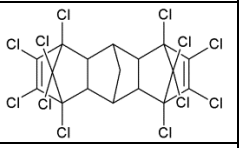
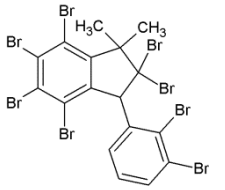
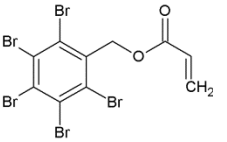
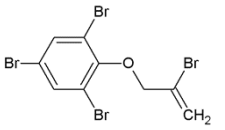
CAS number	Chemical name	Abbreviation	Structure
3322-93-8	[a]4-(1,2-dibromoethyl)-1,2-dibromocyclo-hexane	DBE-DBCH (TBECH)	
3194-57-8	[a]1,2,5,6-tetrabromocyclooctane	TBCO	
3278-89-5	2,4,6-tribromophenyl allyl ether	TBP-AE (ATE)	
23488-38-2	1,2,4,5-tetrabromo-3,6-dimethylbenzene	TBX	
608-90-2	pentabromobenzene	PBB	
87-83-2	pentabromotoluene	PBT	
85-22-3	pentabromoethylbenzene	PBEB	
35109-60-5	2,4,6-tribromophenyl 2,3-dibromopropyl ether	TBP-DBPE (DPTE)	
183658-27-7	2-ethylhexyl 2,3,4,5-tetrabromobenzoate	EH-TBB (TBB)	
26040-51-7	bis(2-ethylhexyl) tetrabromophthalate	BEH-TEBP (TBPH)	
84852-53-9	decabromodiphenyl ethane	DBDPE	

Table 2. continued

CAS number	Chemical name	Abbreviation	Structure
87-82-1	hexabromobenzene	HBB	
13560-89-9	[a]bis(hexachlorocyclopentadieno) cyclooctane	DDC-CO (DP)	
	Polybrominated diphenyl ethers	PBDEs	
41318-75-6	2,4,4'-tribromodiphenyl ether	BDE-28	
5436-43-1	2,2',4,4'-tetrabromodiphenyl ether	BDE-47	
182346-21-0	2,2',3,4,4'-pentabromodiphenyl ether	BDE-85	
60348-60-9	2,2',4,4',5-pentabromodiphenyl ether	BDE-99	
189084-64-8	2,2',4,4',6-pentabromodiphenyl ether	BDE-100	
68631-49-2	2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	
207122-15-4	2,2',4,4',5,6'-hexabromodiphenyl ether	BDE-154	
207122-16-5	2,2',3,4,4',5,6-heptabromodiphenyl ether	BDE-183	
1163-19-5	decabromodiphenyl ether	BDE-209	
51936-55-1	Hexachlorocyclopentadienyl-dibromocyclo–octane	DBHCTD (HCDBCO)	
37853-59-1	1,2-bis(2,4,6-tribromophenoxy)ethane	BTBPE	
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	DDC-DBF (Dec602)	
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	DDC-Ant (Dec603)	
155613-93-7	2,2,4,5,6,7-hexabromo-1-(2,3-dibromophenyl)-1,3,3-trimethylindane	Octa-BTMPI (OBIND)	
59447-55-1	pentabromobenzyl acrylate	PBB-Acr (PBBA)	
N/A	2-bromoallyl-2,4,6-tribromophenyl ether	(BATE)	

[a]mixture of two isomers.

The estimated worldwide production of brominated flame retardants (BFRs), including PBDEs, HBCD, tetrabromobisphenol A, among others, was 310000 tonnes in 2000, from

which 67000 tonnes were of Penta-, Octa- and DecaBDE [10]. Due to the ban of the Penta- and OctaBDE formulations, there is a need for alternatives to cover the market demand resulting from fire regulations in different countries. These alternatives are often called "new", "novel" or "emerging" FRs, and they must meet the fire regulations and needs of the industry. This implies that the replacements should possess similar flame retardant efficiencies as their predecessors and also be economically viable and environmentally friendly. However, there is not a strict control in the production and use of these replacements and their environmental fate and toxicity is unknown. There is the concern that the replacements of the banned FRs might also be hazardous for the environment and humans due to the similarity with the PBDEs. The emerging FRs have been defined by Bergman *et al*. [22] as: "chemicals which are documented regarding production and use as FRs that have been shown to occur/distribute to the environment and/or wildlife, humans or other biological matrices". Following the definition by Bergman emerging FRs have indeed been detected in indoor environments and eventually in biota and humans. The total production volumes of these emerging FRs are difficult to estimate, but many are manufactured as high production volume (HPV) chemicals. E.U. legislation defines HPV chemical as a substance produced above 1000 tonnes/year. It has been estimated that the sum of the worldwide production of emerging FRs is around 100000 tonnes/year [24]. Examples for current use of emerging HPV FRs are DBDPE, which is a substitute for BDE-209 and BTBPE, which is a replacement for the OctaBDE formulation. TBPH and TBB are alternatives to the PentaBDE formulation, but without a reported annual production after 2006 [2]. Pentabromotoluene (PBT) is another emerging BFR with HPV, mostly used in unsaturated polyesters, polyethylene, polypropylene, etc., and some other emerging FRs with low production volumes are hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), 1,2-dibromo-4-(1,2-dibromoethyl) cyclohexane (TBECH) and 2,4,6-tribromophenyl allyl ether (ATE) [2].

Within the halogenated emerging FRs, the family of the norbornene FRs i.e., Dechlorane Plus® (DP), dechlorane 602, dechlorane 603 and dechlorane 604, are chemicals with six chlorine atoms in each of their two norbornene moieties, except dechlorane 604 in which the hexachloronorbornene is linked to a tetrabromophenyl group. DP is composed of two isomers *syn* (30%) and *anti* (70%) and is the most used dechlorane, e.g., in electronics [25]. The worldwide production of DP has been estimated in 5000 tonnes/year in 2008 [26], but there is a lack of information for the production of dechlorane 602, 603 and 604. The environmental

levels of DP are present in indoor and outdoor environments [27], while dechlorane 602, 603 and 604 have been reported only outdoors [28,29].

For this study, the selection of the persistent FRs was based on their production volumes and occurrence in biotic and abiotic environmental matrices. In Table 2, the persistent FRs considered in this thesis are summarised.

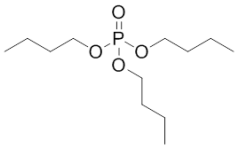
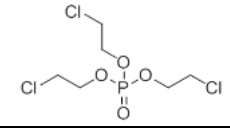
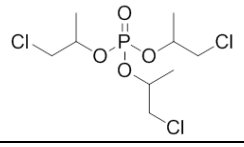
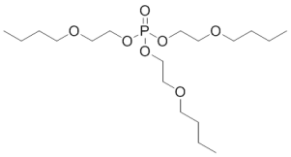
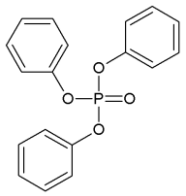
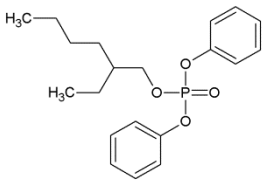
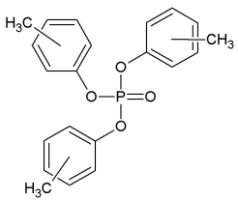
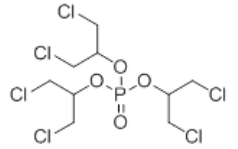
1.1.2. Organophosphate flame retardants

PFRs are chemicals used mainly as additives, stabilisers and plasticisers in polymers, textiles, hydraulic fluids, paints, lacquers and varnishes [13,30]. Their general structure is based on alkyl and/or aryl phosphate triesters (Table 3).

The use of these chemicals dates back in the 1970s [31]. However, the phase-out of PBDEs may cause the increment in production of PFRs in order to cover the demands of the market. Actually, the use of PFRs has increased in the recent years. In 2006, the estimation of the total production of organophosphate triesters in Europe was 465000 tonnes, of which PFRs accounted for 20% [13]. Moreover, the production of PFRs in Western Europe increased by almost 10% between 2001 and 2006 [32]. Chlorinated alkyl and aryl organophosphates are mainly used as PFRs, while the uses of non-chlorinated alkyl phosphates are more related to plasticisers. Moreover, alternative uses for such compounds have been described, for example, tris(2-butoxyethyl) phosphate (TBOEP) has been used as a floor polishing chemical [30] and tri-*n*-butyl phosphate (TNBP) as an extractant in nuclear fuel processing [33]. Within the PFRs, tris(1-chloro-2-propyl) phosphate (TCIPP) is the most produced and represents 80% of the chlorinated PFRs [34]. Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is used for the same applications as TCIPP, although its higher cost makes it only affordable when higher flame retardant efficiency is required. TDCIPP contains two more chlorine atoms than TCIPP in the structure and this feature makes it more efficient as FR. These PFRs together with tris(2-chloroethyl) phosphate (TCEP), which is no longer produced in Europe [35], and triphenyl phosphates (TPHP) have been used as replacements for PentaBDE [36,37] because PFRs are considered less persistent and bioaccumulative than the PBDEs [38]. The half-lives for elimination of TDCIPP and TCEP in fish were reported to be less than two hours [39] and in rats the half-life of TDCIPP was between 1.5 and 5 hours [40]. In general, bioaccumulation and biomagnification of PFRs are limited [13], although chlorinated PFRs have demonstrated some persistency in the environment. TDCIPP has shown reduced environmental degradation in water [13] and TCEP in sewage sludge [39]. The occurrence of PFRs is widespread since

they have been found in many environmental compartments [13]. Table 3 summarises the PFRs considered in this thesis based on the production volumes and the occurrence reported in the environment.

Table 3. CAS numbers, chemical names, abbreviations (previously used abbreviations also provided in parentheses) and structures of the PFRs and plasticisers assessed in this study.

CAS number	Chemical name	Abbreviation	Structure
126-73-8	[a]tri-*n*-butyl phosphate	TNBP (TnBP; TBP)	
115-96-8	tris(2-chloroethyl) phosphate	TCEP	
13674-84-5	[b]tris(2-chloro-2-propyl) phosphate	TCIPP (TCPP)	
78-51-3	[a]tris(2-butoxyethyl) phosphate	TBOEP (TBEP)	
115-86-6	triphenyl phosphate	TPHP (TPP)	
1241-94-7	2-ethylhexyl diphenyl phosphate	(EHDPP)	
1330-78-5	[c]tricresyl phosphate (*ortho*, *meta*, *para*)	(TCP)	
13674-87-8	[d]tris(1,3-dichloro-2-propyl) phosphate	TDICPP (TDCPP)	

[a]used as a plasticiser; [b]mixture of three isomers; [c]mixture of four isomers [d]mixture of two isomers.

1.2. Occurrence of Flame Retardants and Human Exposure Pathways

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

The FRs from Table 2 and Table 3 are used as additives not chemically bounded in consumer goods and materials, and therefore they are susceptible to leach out polluting the indoor environment. As a result, humans are exposed to these chemicals in households, offices, stores, cars, etc. However, further pollution of the outdoor environment might be expected for some FRs since production sites and households act as emitters of these chemicals. Different environmental compartments may be polluted mainly through the distribution of FRs in water, air and particulate matter [41]. Table 4 gives a glance of the occurrence of some PBDEs, emerging FRs and PFRs in different environmental compartments.

Table 4. Maximum concentrations of FRs reported in different matrices.

Matrix	Country	FR	Concentration	Reference
		PBDEs		
Outdoor air	Canada	Σ_5PBDEs	0.30 ng/m^3	[42]
Surface water	Spain	BDE-99	0.05 ng/L	[43]
Sediment	Sweden	Σ_4PBDEs	7190 ng/g d.w.	[44]
Biota (fish)	Sweden	Σ_3PBDEs	515 ng/g l.w.	[45]
		Emerging		
Outdoor air	U.S.A	TBPH	0.08 ng/m^3	[46]
	U.S.A	TBB	0.02 ng/m^3	[46]
Wastewater (Influent)	Norway	HBB	1.8 ng/L	[47]
Sediment	Canada	*anti*-DP	260 ng/g d.w.	[48]
	Canada	BTBPE	6.7 ng/g d.w.	[48]
Biota (eggs)	Canada	HBB	0.43 ng/g w.w.	[49]
	Canada	DBDPE	44 ng/g w.w.	[49]
	Spain	*anti*-DP	1.0 ng/g w.w.	[50]
		PFRs		
Outdoor air	Spain	TPHP	0.19 ng/m^3	[51]
Surface water	Italy	TPHP	165 ng/L	[52]
	Germany	TCPP	379 ng/L	[53]
	Germany	TCEP	184 ng/L	[53]
	Germany	TDCPP	50 ng/L	[54]
Sediment	Austria	TPHP	160 ng/g d.w.	[55]
	Norway	TCPP	24000 ng/g d.w.	[35]
	Norway	TCEP	5500 ng/g d.w.	[35]
	Norway	TDCPP	8800 ng/g d.w.	[35]
Biota (fish)	Sweden	TPHP	810 ng/g l.w.	[56]
	Norway	TCPP	17 ng/g l.w.	[57]
	Sweden	TCEP	160 ng/g l.w.	[56]
	Sweden	TDCPP	140 ng/g l.w.	[56]

Σ_5PBDEs=BDE-47, 99, 100, 153, 154; Σ_4PBDEs=BDE-49, 99, 100, 209; Σ_3PBDEs=BDE-49, 99, 100.

As shown in Table 4, the pollution of persistent FRs and PFRs is spread beyond the boundaries of the indoor environment. Due to the persistency and bioaccumulation properties

of the PBDEs and emerging FRs, and in less degree the PFRs [38], the fate of these FRs is to bioaccumulate and biomagnify in food chains. Consequently, diet appears as an additional route of exposure to humans. Therefore, the two major routes of exposure to FRs for humans are indoor environments and diet [58,59].

Regarding indoor environments, indoor dust and air have been extensively surveyed for persistent FRs and PFRs (except emerging FRs). Probably due to the higher production and volatility of PFRs and related plasticisers, their concentrations in indoor environments have been reported higher than the persistent FRs in air and dust. Median concentration of TBEP detected in household dust from U.S.A. was 12 μg/g [60] and TCPP detected in Swedish [61] and Japanese [62] residences was 1.6 and 19.7 μg/g, respectively, while median concentrations of household dust for BDE-209 (the most abundant PBDE in dust) were 1.2 μg/g in U.S.A. [60], 0.47 μg/g (mean) in Sweden [63] and 0.16-0.62 μg/g in Japan [64]. In some indoor environments from U.S.A., the most abundant BFR in dust seemed to be TBPH (2.8 μg/g) [60]. Microenvironments where humans spend few hours every day, such as private cars, could also be a significant source of exposure since one study performed by Harrad *et al*. [65] showed median concentration of BDE-209 in dust from cars as high as 100 μg/g with a maximum concentration of 0.26% (w/w).

In air, the concentration ratio between PFRs and PBDEs can reach even two orders of magnitude due to the low vapour pressure of the latter. Median indoor air concentrations of TCEP in studies conducted in Japan [62] and Sweden [61] were 89.2 ng/m^3 and 5.6 ng/m^3, respectively, while BDE-47, the most volatile congener among the seven most abundant PBDEs, showed median concentrations of 0.63 ng/m^3, 0.02 ng/m^3 and < 0.01 ng/m^3 in studies conducted in U.S.A. [66], Sweden [61] and Japan [64], respectively. In general, indoor air concentrations of FRs are considerably higher than the outdoor concentrations [67]. This fact supports the role of indoor environments as sources of pollution for the outdoor environment.

Some studies point out that indoor dust is a major source for human exposure to PBDEs [59,60,68,69] and PFRs [70]. Therefore, this might be valid also for the emerging FRs used in consumer products. Geometric means of DBDPE, BTBPE, TBB and TBPH found in dust from living rooms in the U.S.A. were 138 ng/g, 48.1 ng/g, 322 ng/g and 234 ng/g [71], respectively, in U.K. classrooms were 95 ng/g, 8 ng/g, 22 ng/g and 83 ng/g, respectively, and in Belgian offices 612 ng/g, 18 ng/g, 6 ng/g and 67 ng/g, respectively [72]. The concentrations of FRs in dust from U.S.A. are systematically higher than in Europe [73]. Although dust is identified as a significant contributor to body burden of PBDEs, the estimated daily intakes

are still lower than the oral reference dose (RfD) established by the U.S. Environmental Protection Agency (USEPA) [65,74]. Likewise, PFRs and plasticisers present the same tendency in different risk assessment studies [75,76]. As a result, the concentrations of PBDEs and PFRs in dust from most of the indoor environments seem that they do not pose a serious risk for human health. However, in specific scenarios where environments are heavily polluted, and for small children who might have a higher dust intake rates, this exposure can potentially be hazardous [60].

The occurrence of FRs in food has been widely investigated both, in a duplicate diet studies and in a market basket surveys. For example, analysis of duplicate diet for PBDEs in the U.K. showed median concentrations of BDE-47 and BDE-99 of 66.8 and 63.9 pg/g dry weight (d.w.), respectively [77] and in Canada Σ_6PBDEs was 1170 pg/g wet weight (w.w.) [78]. A study performed in Sweden showed mean concentrations of Σ_9PBDEs in different food items from four cities: fish (422 pg/g w.w.), meat (41 pg/g w.w.), dairy products (33 pg/g w.w.) and eggs (26 pg/g w.w.) [79]. A similar study conducted in Belgium showed mean levels of Σ_7PBDEs in fish (460 pg/g w.w.), meat (70 pg/g w.w.), eggs and dairy products (260 pg/g w.w.) and fast food (86 pg/g w.w.) [80]. In the U.S.A. the concentration of Σ_{24}PBDEs in milk, canned sardines and butter were 12 pg/g w.w., 1545 pg/g w.w. and 6211 pg/g w.w. [81], respectively. Some publications state that diet, and especially the consumption of meat [58] and fish [82], contributes significantly more to the body burden of PBDEs in the general population than other pathways like dust ingestion or air inhalation.

Regarding the emerging FRs, there is a lack of studies surveying occurrence in food products. Also for the occurrence of PFRs in food few data are available. A Japanese study identified TNBP and diphenyl 2-ethylhexyl phosphate in food in the range < 0.01-11 μg/g, although these two chemicals were also detected in the packaging material [83]. There are no levels reported of TPHP in specific food items, but the World Health Organisation (WHO) has reported an estimate average intake for adults between 0.5 and 1.6 ng/kg body weight/ day and for infants between 0.3 and 4.4 ng/kg body weight/ day [84].

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

1.3. Human Biomonitoring of Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Humans are continuously exposed to chemicals and human biomonitoring is the technique that permits to assess the degree of interaction between the chemicals and humans [85]. In addition, after the exposure to those chemicals, human biomonitoring determines how the concentrations of these pollutants change in the body across time. Human biomonitoring cannot be considered a modern analytical technique since more than 100 years ago doctors already followed up amounts of salicyluric acid in the patients' urine after application of high doses of salicylic acid for the treatment of the rheumatism [86]. Human biomonitoring applied to environmental science assesses all routes of exposure for a specific chemical or group of chemicals in humans. Therefore, the content of a specific pollutants in the body might be difficult to relate to a particular source of exposure e.g., indoor versus outdoor, or diet versus dust. By analysing matrices such as blood (plasma or serum), breast milk, tissues, saliva, urine, nails, hair, etc., biomonitoring can establish the exposure to certain substances and follow this exposure over time. The choice of these matrices in biomonitoring studies may pose a problem for the recruitment of participants when the sampling is invasive (e.g., blood and adipose tissue), whereas non-invasive sampling is performed when hair, saliva, urine, etc. are collected, and the willingness of participants to join the study is probably higher for obvious reasons. The persistent FRs accumulate in the fatty matter of the human body, therefore many biomonitoring studies in the general population have used primarily the adipose tissue [87,88] and blood [82,89-91] (invasive sampling), and in women in the breastfeeding stage is frequent to use breast milk (non-invasive sampling) [92-95]. Recently, in 2013, emerging FRs such as dechloranes, have been monitored for the first time in serum [96-99] and milk [96,99]. Less commonly non-invasive methods have been developed to monitor the exposure to persistent FRs in matrices like hair [100-103], although due to the limited amount of sample containing low concentrations, the methods are less sensitive than the methods developed for invasive samples.

PFRs are metabolised in the human body to dialkyl or diaryl phosphates (DAPs) that are rapidly excreted [104]. As a consequence, the matrices suitable to monitor the exposure to persistent FRs are not appropriate to monitor PFRs, and vice versa. The most extended human matrix to monitor the exposure to PFRs through their metabolites is the urine [23,105-107].

Table 5 presents examples of concentrations detected in humans for persistent FRs and organophosphate metabolites.

Table 5. Concentrations of several persistent FRs and DAPs in humans.

Matrix	Country	Analyte	Concentration	Year	REF
		BFRs and dechloranes			
Serum	Norway	Σ_5PBDEs	0.93 ng/g l.w.	1981	[108]
	Norway	Σ_5PBDEs	1.76 ng/g l.w.	1990	[108]
	Norway	Σ_8PBDEs	3.8 ng/g l.w.	2003	[109]
	U.S.A	Σ_4PBDEs	1.47 ng/g l.w.	1988	[110]
	U.S.A	Σ_5PBDEs	41.1 ng/g l.w.	2001	[111]
	China	ΣDP	42.6 ng/g l.w.	2005	[98]
	France	ΣDP	1.2 ng/g l.w.	2003/2005	[97]
Breast milk	Sweden	Σ_5PBDEs	0.45 ng/g l.w.	1980	[112]
	Sweden	Σ_5PBDEs	1.84 ng/g l.w.	2000	[113]
	U.S.A	Σ_5PBDEs	29.2 ng/g l.w.	2002	[114]
Tissue	Spain	Σ_5PBDEs	4.12 ng/g l.w.	1998	[115]
Hair	China	Σ_{17}PBDEs	9.95 ng/g l.w.	[a]2010	[102]
	China	ΣDP	1.03 ng/g l.w.	[a]2010	[103]
		DAPs			
Urine	Belgium	BDCPP	< 0.52 ng/mL	[a]2013	[116]
		DPP	1 ng/mL	[a]2013	[116]
	U.S.A.	BDCPP	0.083 ng/mL	[a]2011	[23]
		DPP	0.80 ng/mL	[a]2011	[23]

[a]Year published; Σ_5PBDEs=BDE-47, 99, 100, 153, 154; Σ_8PBDEs=BDE-28, 47, 99, 100, 153, 154, 183, 209; Σ_4PBDEs= BDE47, 99, 100, 153; ΣDP=*syn*+*anti*; Σ_{17}PBDEs=BDE-47, 66, 85, 99, 100, 153, 154, 183, 196, 197, 201, 202, 203, 206, 207, 208, 209; BDCPP= bis(1,3-dichloro-2-propyl) phosphate; DPP=diphenyl phosphate.

In Table 5, the concentration of Σ_5PBDEs in serum from Norway and U.S.A. increases with time until the production of PBDEs ceased in 2004. In the same way, levels of PBDEs in breast milk from Sweden follow a similar tendency. In Norway and Sweden, PBDEs increased from the 1980s until 2000 by a factor of 4, whereas this increment in the U.S.A. was by a factor of 40. This difference in concentrations between Europe and U.S.A. indicates a clearly different use of FRs. Furthermore, the increased concentration of FRs through years, confirms that these chemicals accumulate in humans. FRs have also been detected in other human matrices such as adipose tissue (Σ_5PBDEs 4.12 ng/g l.w.) [115] and hair (Σ_{17}PBDEs 9.95 ng/g l.w.) [102]. In addition, the emerging FR, Dechlorane Plus®, was also detected in serum from China and Europe, with much higher levels in the study from China (42.6 versus 1.2 ng/g l.w.) [97,98]. The literature on human exposure assessment for PFRs is not abundant and few studies have determined urinary metabolites [104]. In Table 5, the Belgian study shows relatively higher levels than the American study, however comparisons between concentrations of metabolites in urine must be treated with care since the levels are very dependent on several factors such as time of exposure, half-life of the PFR, time of collection and dilution of urine, etc.

1.4. Analytical Methods for Determination of Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

1.4.1. Sample collection and treatment

The determination of environmental levels of FRs is challenging because the concentrations in the samples usually are low. Since the occurrence of the FRs is widespread in all environments, special attention must be paid to protect samples and laboratory equipment from contamination. Therefore, when analysing persistent and organophosphate triester FRs, it is convenient to soak glassware in an alkaline solution, subsequently burn it for several hours at high temperatures (e.g., 450°C) and store all material wrapped in aluminium foil to prevent eventual photodegradation.

It is of high interest to maximise the sensitivity of the analytical method in order to achieve the lowest limits of detection. To do so, extraction techniques are of great importance to succeed in the trace determinations. The extraction is a physical process in which the analyte is ideally separated from the matrix. Typically, solid sorbents are used for extraction and/or preconcentration of the pollutants from liquid and gaseous environmental samples. For example, PUF is used to trap the FRs from air [117] and solid-phase extraction (SPE) is used to extract FRs from liquid samples [104], although liquid-liquid extraction (LLE) is also widely used [27]. Several extraction techniques, intended for solid samples, have been used to explore the extraction of FRs from liquid samples [118]. This is the case of cavity-dispersed microwave-assisted extraction (MAE) and focused microwaved assisted extraction (FME). Both techniques heat the medium and generate molecular rotations through microwaves to disrupt the interaction of the analyte with the matrix and thus favour the transfer to the organic solvent. Another example is pressurised liquid extraction (PLE) that enhances the solubility of the analytes in the organic solvent by increasing the temperature and pressure of the extracting medium.

The extraction of FRs from environmental solid samples (including the PUF from the air samples) is commonly carried out by liquid-solid extraction. Soxhlet extraction is performed in a Soxhlet apparatus where the sample is placed in an extraction thimble and distilled organic solvent is continuously dripping down through the sample in a reflux system [119]. Ultrasound extraction is based on the greater penetration of the solvent into the solid samples as a result of the generation of micro-cavitations [67]. PLE is also a preferred extraction technique and has become more popular than Soxhlet because the extraction of the analytes from solid samples is faster and consumes less solvent [120].

1.4.1.1. Indoor air samples

Volatile FRs are mainly found in the gas phase where the distribution is relatively homogeneous, but semi volatile FRs are distributed between the gas and the different solid phases of the environment (e.g., dust, fabrics, horizontal and vertical surfaces, etc.) [121]. Semi volatile FRs are partially adsorbed on the airborne particles. When sampling FRs in air, a filter is typically placed in front of the PUF or an aminopropyl modified silica cartridge to trap PFRs efficiently [61]. The use of a quartz or glass filter is applied to physically separate particulate matter from gaseous FRs. In this way, it is possible to obtain a partition coefficient between these two phases. The concentrations of persistent FRs in indoor air are low, in the range of pg/m^3 [66], while the PFRs are in the range of ng/m^3 [32]. In order to increase the sensitivity of the analytical method, air from the indoor environment is actively sampled with pumps for 8 to 24 hours at flow rates ranging from 1 to 15 L/min [67]. Another, less usual, approach is to deploy passive samplers, consisting of a PUF disk in a protective housing, for longer periods (days or months) [122]. Passive sampling is based on the diffusion of the analytes from the gas phase to the PUF [123] and has normally been used outdoors to overcome the limited duration of the batteries of the active samplers. Extraction of FRs from the PUF used in active or passive sampling is carried out using organic solvents (e.g., hexane, toluene, dichloromethane, acetone or mixtures) in combination with ultrasonic [124-126], PLE [127] or Soxhlet extraction [74,128]. Alternatively, if the sampling method has used cartridges of aminopropyl modified silica, the elution of the PFRs is carried out with more polar solvents like acetone. Recoveries of FRs from both, active and passive samplers, have been reported to be close to 100% for the mentioned extraction techniques [69,70], although Muenhor *et al*. [123] reported recoveries only around 50% for PBDEs from a passive sampler using Soxhlet extraction. Clean-up of air samples is not required since the air is a rather clean matrix for the determination of FRs and is not likely to contain substances such as lipids that can damage the analytical column and affect the stability of the analytes in the liner of the gas chromatograph.

1.4.1.2. Dust samples

Indoor dust has been defined as a complex mixture of solid particles, i.e., combustion products, fragments of fibres and hair, insect remains, human skill cells, sand, pollen grains and fungal spores from 1 μm to 1 mm in diameter [121]. Among these constituents, we also

find persistent FRs at levels of ng/g and PFRs at µg/g [60]. There are three plausible mechanisms from which FRs end up in the dust: volatilisation from products, diffusion from the flame retarded product to dust and through the abrasion or weathering [129]. The contamination of indoor environments may be surveyed by the collection and analysis of dust. Different approaches have been used in order to collect representative samples using vacuum cleaners from various microenvironments such as cars, offices, households, etc. Some studies have collected dust from domestic vacuum cleaner bags [30,69], collected dust from a defined square meters [66,76,123], or the whole floor [70,130], and settled dust in elevated surfaces [61] using nylon socks [65] or forensic filters [67]. After the collection of dust, some researchers sieve the dust to < 500 µm, remove coarse particles and ensure a better sample homogeneity [65,131]. To date, there is no consensus of which type of dust and collection method might be the most representative to evaluate the indoor exposure.

Soxhlet extraction has been a robust and affordable technique to extract the FRs from dust. The recoveries for PBDEs obtained using non-polar organic solvents are high (> 81%) [132], but the main drawbacks are the long time for extraction (6-24 hours) and the larger solvent volumes needed compared to other techniques such as PLE and ultrasonic extraction. PLE resulted in excellent recoveries for PBDEs (90-109%) [133] and also good recoveries for persistent FRs and PFRs (> 70%, except TBPH 46%) [71,134]. A disadvantage of PLE is that the extraction requires specific equipment, and therefore, this technique is not much used for the extraction of FRs from dust. Ultrasonication of dust with organic non-polar solvents is nowadays the mostly applied method for the extraction of persistent FRs and PFRs [30,60,76,135-137]. The extraction performance is satisfactory obtaining recoveries for FRs in the range 80-125%, except for some PFRs (TBOEP 235% and TCEP 142%) [131]. FRs are extracted from dust together with any other lipophilic matter, such as lipids, hence a clean-up of the extract is required.

The removal of lipids can be carried out using destructive or non-destructive methods. The non-destructive lipid removal is based on the separation of the fat from the FRs. To do so, the use of gel permeation chromatography (GPC) [96,138] or adsorption chromatography (Florisil®, alumina and silica) [139-141] are good examples for the clean-up of environmental samples. The destructive lipid removal is based on the decomposition of the organic matter by action of a concentrated strong acid (H_2SO_4). The simplest way to perform the destructive clean-up is by adding the acid to the organic extract, and through consecutives liquid-liquid extractions recover the FRs [66,142]. This process has the inherent problems associated to

LLE, such as the formation of emulsions, larger volumes of solvent used and time consumption. Silica impregnated with sulphuric acid (30-50%; w/v), or several layers comprising acid and base (33% 1 M NaOH), overcome the disadvantages of the LLE providing a faster clean-up with satisfactory recoveries for FRs [70,128]. As a result, the destructive methods are more efficient (99-101% lipid removal) than the non-destructive (60-90%) for samples with a large amount of fat [143,144]. However, for the common determination of persistent FRs and PFRs in dust, the destructive fat removal cannot be applied since PFRs are sensitive to acid. Fractionation of the FRs using adsorption chromatography prior to clean-up overcomes this problem. Fractionation of the extract is carried out to separate groups of compounds usually on Florisil®, silica or alumina cartridges [118]. The fractionation of PBDEs and PFRs has commonly been performed on Florisil® [76] or silica cartridges [141]. The lipids and lipophilic FRs elute with hexane without interacting with the sorbent, whereas the more polar PFRs are retained in the column. To elute the PFRs, a more polar solvent is needed and ethyl acetate has typically been used. Once the acid resistant FRs have been separated from the PFRs, the destructive clean-up is applied to this fraction.

1.4.1.3. Blood samples

Persistent FRs entering the human body tend to accumulate in fatty tissues, while the non-persistent are rapidly metabolised [39,145,146]. Serum or plasma are good invasive matrices, both are suitable to assess the concentration of FRs in the body and the lipid content in blood is typically in the range 0.5-0.8% [109]. Plasma requires anti-coagulants in the vacutainer tube and is obtained after centrifugation, while serum is the supernatant after centrifugation of the coagulated blood, i.e., it is plasma without clotting factors. The reported concentrations of FRs in serum/plasma are normalised to the lipid content for comparison with other serum/plasma samples or other matrices. For the determination of persistent and emerging FRs in blood, 1-5 mL of serum/plasma are necessary to achieve method limits of detection (MLD) in the range 0.2-25 pg/mL by means of gas chromatography-mass spectrometry (GC-MS) [109,147]. Prior to extraction of FRs, a protein denaturation is required to ensure the release of the FRs from their interaction with proteins. This is performed by using either strong inorganic acids (e.g., HCl or H_2SO_4) [98,148] or a weak organic acid (mainly formic acid) in combination with 2-propanol and water [63,147,149]. BFRs and dechloranes have been extracted from serum using LLE with methyl-*tert*-butyl ether (MTBE) and hexane (1:1;

v/v) [97,98,144,148]. Reported recoveries of low brominated diphenyl ethers are close to 100% [144]. However, these extractions are not efficient for highly lipophilic FRs. For example, recoveries of BDE-183 (log K_{ow}=9.4) and ^{13}C-*anti*-DP (log K_{ow}=11.3) using ethanol and diethyl ether yielded 78% and 44%, respectively [96], although recoveries for ^{13}C-*anti*-DP were between 58% and 90% using hexane/MTBE (1:1; v/v) [97]. The same mixture (hexane/MTBE) has been used with PLE to extract lower PBDEs and HBCD obtaining recoveries between 79 and 109% [150]. An interesting approach to extract lower PBDEs and at the same time to pre-concentrate them is the use of stir bar sorptive extraction followed by thermal desorption, although the recoveries obtained were much lower compared to other techniques (< 50%) [151], most probably due to the slow equilibration of FRs between the aqueous phase and the coating of the magnetic rod. The most widespread extraction technique for the extraction of FRs from serum is SPE. The wide range of stationary phases makes this technique useful for different groups of compounds, and the main advantages are the low volume required, the potential for automation, good reproducibility and time saved. Over the last years, comprehensive studies have been performed to find out which of the different phases are most suitable for the extraction of the FRs. Commercial sorbents such as Isolute 101, Isolute C_{18}, Isolute Phenyl, ENV+ (International Sorbent Technology), Strata-X, Strata-SI-1 Silica, Strata-NH_2, Strata-CN (Phenomenex), Oasis®HLB (Waters), C_{18} Empore® (3M Company), SampliQ C_{18} and SampliQ OPT (Agilent Technologies) have been thoroughly tested in numerous studies [63,97,147,149]. Recoveries of PBDEs in all silica based stationary phases performed worse (< 50%) than the co-polymeric divinylbenzene based stationary phases (ENV+ and Oasis®HLB). Oasis®HLB has been reported as the preferred sorbent for extraction of the seven most abundant PBDEs using mainly dichloromethane for their elution. The recoveries obtained for PBDEs are in the range 64-95% [147], 43-148% [149], 39-104% [63], 64-89% [109]. The wide range in percentage obtained in the recoveries can be explained due to the extreme difficulty to extract highly lipophilic FRs from serum or plasma. Several arguments have been presented justifying the poor recoveries for compounds with high log K_{ow} (e.g., BDE-209, DBDPE, Dechlorane Plus®, etc.) like irreversible adsorptions to the sorbent [147] and/or other surfaces [152] as well as incomplete protein denaturation [118]. Nonetheless, these arguments are merely speculations, since the losses in the recovery have not been quantified and the cause of the low extraction efficiency is still unclear.

As in the case of the dust samples, serum and plasma contain lipids and a clean-up of the samples is required. Lipids interact in the same way as the FRs with the non-polar stationary phases or solvents, and they are therefore co-extracted with the target analytes. Destructive and non-destructive clean-up methods have also been employed with serum/plasma samples, but most often silica impregnated with sulphuric acid has been used. For the serum/plasma application, it is highly recommended to use a top layer of activated silica. This additional layer retains cholesterol, avoiding the formation of cholestene, product from the dehydration of cholesterol. Cholestene is a less polar molecule, which is not retain in the impregnated silica with sulphuric acid and therefore it would end up in the final extract and interfere in the GC-MS analysis [153]. In contrast to dust samples, fractionation of serum or plasma extracts is not performed because PFRs are not expected to be found at high levels in blood due to their short half-lives shown in animal experiments [23]. The determination of PFRs in blood has not been investigated thoroughly because these chemicals are excreted from the bodies. However, some studies applied extraction techniques not frequently used in the analysis of plasma. Shah *et al*. [154] used solid-phase microextraction (65 μm polydimethyl-siloxane/divinylbenzene fiber) to extract and pre-concentrate PFRs. The recoveries obtained were 35-66%. Jonsson *et al*. [155] applied stir-bar assisted microporous membrane liquid-liquid extraction for the determination of 8 PFRs in plasma. The recoveries were within the range 30-84%. Amini and Crescenzi [156] used on-line SPE where the PFRs were retained on a LiChrospher® RP-18 column and back flushed into the C_{18} analytical column. The recoveries obtained were between 60% and 92%.

1.4.1.4. Urine samples

Urine is an important route of xenobiotic excretion and a non-invasive matrix suitable to monitor a diverse number of metabolites. Dialkyl or diaryl phosphates (DAPs) are the main metabolites derived from PFR found in urine (Figure 2).

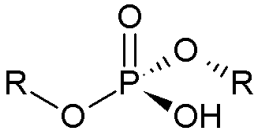


Figure 2. General structure of DAPs.

Urine is a suitable matrix for the determination of DAPs because it is easy to obtain and abundant. The levels of DAPs in urine are very variable depending on the exposure and half-life of the different PFRs. In general the amount of urine required for the determination of DAPs is typically between 1 and 5 mL and the MLD range from 8 to 14000 pg/mL, depending on the method used [104]. The concentrations of DAPs are expressed as ng/mL and commonly corrected for creatinine content or specific gravity. This normalisation of the concentrations is intended to make concentrations of metabolites comparable by compensating different degrees of dilution [157]. Due to the acidic nature of the DAPs (pKa < 2) [158], these compounds are not protonated in urine and therefore new methods to determine organic anions have been developed. For example, Schindler *et al*. [106,159,160] developed a method for the determination of DAPs by GC-MS. Urine was acidified with HCl to maximise the protonation of the DAPs, and thereby enhance the interaction with the ENV+ sorbent of the SPE cartridge. The elution of DAPs was carried out with acetonitrile and after a derivatisation with 2,3,4,5,6-pentafluorobenzylbromide, further clean-up on Bond Elut PSA (top) connected to a Florisil® cartridge (bottom) was performed. The recoveries were between 79% and 113%. This method improved the recoveries of DAPs on polymeric sorbents, which tolerate low and high pH due to the polymeric nature of the sorbent. When the solution was not strongly acidified, the expected recoveries were in the range 50-70% [116]. Yoshida *et al*. [161] used solid-phase dispersive extraction by adding the solid sorbent ENV+ directly on urine to extract a wide range of phosphorous metabolites with the subsequent determination by liquid chromatography-mass spectrometry (LC-MS). The recoveries were between 50% and 104%.

Sorbents based on anion exchange interactions, such as Oasis®WAX (Waters), Bond Elut DEA, PSA, and NH_2 (Agilent) and StrataX-AW (Phenomenex), have been tested to determine DAPs by means of LC-MS [23,116]. The sorbent contains groups positively charged and therefore interaction with anions occurs. To remove this interaction and recover the DAPs, an organic solvent with base is required to exchange the anions. However, depending on the pKa of the sorbent, recoveries may differ between sorbents when using the same solvent for elution. Cooper e*t al*. [162] reported that strong anion exchange columns (SAX) retained completely the DAPs (DPHP and BDCIPP) even after elution with 5% of pyrrolidine in acetonitrile. Using the same solvent to elute DAPs, Bond Elut PSA and DEA showed poor recoveries ranging from 20% to 48%, while Bond Elut NH_2 and Oasis®WAX gave good recoveries (~75%) and StrataX-AW showed the best performance (recoveries around 90%)

[23,163]. Nonetheless, Hoffman *et al*. [105] obtained recoveries between 62% and 72% using StrataX-AW following the method described by Cooper *et al*. Van den Eede *et al*. [116] reported that recoveries from Oasis®WAX were between 78 and 115% using 5% NH_4OH in methanol for a wider number of DAPs (DPHP, BDCIPP, DNBP, BCEP and BBOEP), except for BCIPP (> 150%). Further, Oasis®WAX resulted in less ion suppression than StrataX-AW when analysing the extracts by LC-MS. Möller *et al*. [164] filled a SPE cartridge with a synthesised molecular imprinted polymer being highly specific for the extraction of the target compound. Elution of DAPs was carried out with methanol/triethylamine (1:1; v/v) and the recovery obtained for DPHP was 102%.

Other techniques have been investigated for the extraction of DAPs from urine. Jonsson *et al*. [165] explored the utility of LLE assisted by using of a hollow fibre-based XT tube extractor, but the recoveries were below 50%. Reemtsma *et al*. [107] carried out a clean-up based on the addition of acetonitrile to the urine. The salts precipitate and the result is an increased sensitivity by a factor of 1.5 with recoveries around 100%.

1.4.2. Determination techniques

The complexity of the environmental samples and the low concentrations of FRs require a chromatographic separation to isolate the target compounds from the matrix and very sensitive and selective detectors for a proper identification and quantification, i.e., mass spectrometers. The final goal is to obtain the lowest limit of detection for the determination of the environmental pollutants. Persistent and organophosphate triester FRs are semi volatile compounds that can be determined by GC-MS [166]. The preferred technique for the determination of PFR metabolites is liquid chromatography coupled to mass spectrometry (LC-MS) [104] due to their ionic nature. Nonetheless, as commented above, the polar metabolites of the PFRs have also been analysed by GC-MS after a derivatisation step to make them volatile.

1.4.2.1. Gas chromatography coupled to mass spectrometry

GC is a suitable technique for thermally stable, volatile and semi volatile compounds since the sample is introduced in injection ports typically between 250°C and 300°C. The most frequently used injector in environmental analyses is the splitless injector where 1 to 2 µL are normally injected. In the determination of PBDEs, the molecular weights differ largely

between the lower and the higher brominated congeners. This implies a wide range of boiling points, which can result in discrimination when transferring the analytes from the liner to the column. To circumvent this situation, a pressure pulsed injection is recommended to efficiently transfer the analytes into the chromatographic column and thus, avoiding discrimination of the analytes in the injector, e.g., 150 kPa kept for 1.5 min [120]. Alternatively, on-column injections (1-2 μL) have been used to reduce thermal degradation and discrimination experienced in the splitless injector [167], and the programmed temperature vaporiser has been used for large volume injections (LVI; up to 125 μL) [168], although the increased sensitivity by LVI requires thorough sample preparation and clean-up [166]. In this respect, a dirty injector can affect the stability of some compounds such as BDE-209, causing its degradation into lower brominated congeners, HBCD [166] and tetrakis(2-chloroethyl)dichloroisopentyl diphosphate, known as V6. A retention gap is commonly used between the injector and the column for any of the different injection techniques to preserve the performance of the column from the generated grime.

The most widely used stationary phase in the GC columns for separation of FRs is polydimethylsiloxane with 5% phenyl substitution (e.g., DB-5 from Agilent Technologies, ZB-5 from Phenomenex) [166]. An alternative stationary phase for PFRs is 8% phenyl 92% polycarborane-siloxane (e.g., HT-8 from SGE Analytical Science) [131]**.** The dimensions of the column for the determination of wide range of FRs usually are length of 25-60 m, internal diameter of 0.25 mm and film thickness of 0.25 μm, and for PFRs the column dimensions are 30 m x 0.25 mm x 0.25 μm. For the determination of thermal sensitive FRs, shorter columns with smaller film thickness (15 m x 0.25 mm x 0.1μm) provide sufficient chromatographic resolution between peaks and reduce the residence time in the column, which is important in particular for BDE-209 and DBDPE. The elution of these compounds above oven temperature of 300°C is to be avoided because severe on-column degradation takes place [169]. Therefore, the simplest approach for a faster elution is to increase the flow-rate [170]. Alternatively, to reduce the analysis time, low pressure GC has been used. This technique is based on conducting the gas chromatography at sub-ambient pressure conditions. To do so, a short narrow restriction column is placed between the injector and a megabore column (typically 10 m x 0.53 mm x 0.25 μm) connected to the mass spectrometer. This approach has successfully been applied for the determination of PBDEs in fish with MLD in the range 0.5-10 ng/g w.w [171] and in dust with MLD between 0.2 and 4.1 ng/g [169].

After applying the extraction techniques to the samples, the extract contains interferences from the matrix that can co-elute with the target analytes and generate problems in the detection. For the situations where the resolution power of a conventional GC system is not satisfactory in the simultaneous determination of several groups of pollutants in complex matrices (e.g., organochlorine pesticides, phthalates, polychlorinated biphenyls and polybrominated diphenyl ethers), two dimensional gas chromatography (GCxGC) is normally employed to overcome these drawbacks and provide good MLDs [172,173]. The clear advantage of multidimensional GC over the conventional GC is the increased peak capacity, which is up to ten times higher by using a typical orthogonal separation between the long and non-polar column (1^{st} dimension with temperature gradient) connected to a modulator responsible to refocus the peaks and, release them to a shorter and narrower polar column (2^{nd} dimension with isothermal separation) [174,175].

The detector employed for the identification and quantification of the FRs in complex matrices must be selective and sensitive for our target compounds. Formerly, electron capture detectors (ECD) were used due to the low cost and maintenance and relatively good sensitivity. However, the major drawback of ECD is that it is not a selective detector because any polyhalogenated compound produces a strong signal e.g., polychlorinated and polybrominated biphenyls, PBDEs, polychlorinated paraffins, etc., making a congener specific determination highly challenging. Currently, mass spectrometers are the most accepted detectors for the determination of FRs in environmental analysis. Gas chromatograph coupled to low resolution mass spectrometer equipped with single quadrupole (GC-MS) have been the preferred choice due to its high sensitivity for halogenated compounds when using electron capture negative ionisation (ECNI) and relatively lower cost compared to triple quadrupole analysers (GC-MS-MS) or high resolution mass spectrometers (GC-HRMS). For the analysis of BFRs, the main advantage of the GC-MS resides on the great sensitivity for brominated compounds obtained under the ECNI mode. ECNI is a soft ionisation technique that uses a reagent or buffer gas (e.g., methane, ammonia, nitrogen, etc.) to form negative ions of the analytes in the ionisation source [176,177]. The high energy electrons produced in the ion source, collide primarily with the reagent gas since this is in clear excess with respect the analytes. The reagent gas forms positive ions and generates low energy electrons which are captured by the analytes with enough electron affinity. The fact that the reagent gas is not negatively charged, makes this soft ionisation technique free of background interferences, and therefore very sensitive. Brominated compounds have a high

electron affinity and bromide ions $[Br]^-$ (m/z 79 and 81) are the most abundant ion in their mass spectra. This is a clear advantage compared to ECD because only compounds containing bromine in their structure will be detected. In contrast, the use of isotopically labelled ^{13}C internal standards is not possible for the determination of the majority of the BFRs. There are few exceptions such as some highly brominated congeners, BDE-205 and BDE-209, and the chlorinated dechloranes, which give abundant fragments higher than $[Br]^-$ or $[Cl]^-$ in their mass spectra. The formation of $[Br]^-$ using ECNI is much higher than the typical $[M\text{-}yBr]^+$ obtained by electron impact (EI) ionisation. In EI ionisation, ions are generated when the analytes go through a beam of high energy electrons (70 eV). This beam fragments and ionises the molecule in a predictable pattern irrespectively of the instrument. The main advantage of the EI ionisation is the use of ^{13}C internal standards that allows a more reliable quantification than ECNI. The limits of detection of the mean of 39 PBDEs congeners using ECNI were one order of magnitude lower than using EI (0.19 pg versus 2.01 pg, respectively) [178]. In fact, the method limits of detection of PBDEs using GC-(ECNI)MS in breast milk were comparable to GC-(EI)HRMS (0.3-0.6 pg/g milk versus 0.4-0.7 pg/g milk, respectively) [179].

Contrarily, for PFRs, the sensitivity obtained using EI is clearly superior to ECNI or positive chemical ionisation (PCI; methane reagent gas). The instrument limits of detection for PFRs using GC-(EI)MS range between 20 and 60 pg, while they are not detectable in this range when working with ECNI and PCI [180]. The most abundant ion generated in EI for aryl substituted PFRs is $[M]^+$, whereas for alkyl substituted PFRs the none specific ion $H_4PO_4^+$ is usually the most abundant, although the ion $[M\text{-}R+2H]^+$ is also present in the mass spectra to a minor degree and this ion offers information about the chain composition. For chlorinated PFRs, the most abundant ions produced in EI are $[M\text{-}Cl]^+$ and $[M\text{-}CH_2Cl]^+$ [180].

Determination of persistent FRs by GC-(ECNI)MS has been used in matrices so diverse like serum [63], milk [92], eggs [181], dust [182] sediments [120], sewage sludge [183], children toys [184], pieces of PUF and casing from electronic appliances [74], whereas GC-(EI)MS has seldom been used due to its lower sensitivity. Nonetheless, an interesting application was the determination of BFRs in adipose tissue using LVI obtaining limits of detection comparable to GC-(ECNI)MS [132]. Table 6 summarises the MLD reported in the different matrices. The use of GC-(EI)MS for the determination of PFRs is very common in many abiotic matrices such as outdoor air [51], surface water [54] and dust [76], but rarely PFRs have been determined in biotic matrices like plasma [155,165] and milk [56] because of their

short half-lives in the bodies. In addition, GC connected to inductively coupled plasma (ICP) and MS has also been used for phosphorous specific detection in waste water samples [185] and plasma [154]. Table 6 summarises the MLD reported in the different matrices.

GC-HRMS is the most powerful hyphenated technique in terms of selectivity and sensitivity, although its cost makes it not affordable in many laboratories and, in addition, expert hands are required for its manipulation. HRMS provides exact masses of the ions of the analytes and excellent limits of detection. For FRs analyses, the most used analyser in HRMS is the magnetic sector and rarely time-of-flight (TOF). Some applications using the magnetic sector are the determination of polychlorinated biphenyls and PBDEs in serum [186], dechloranes and PBDEs in milk [97], PBDEs in fish [187] and emerging FRs in sediments, soils and biotic samples [188], and for the TOF, the determination of PBDEs in human serum and milk [153,172]. For the determination of PFRs, spectrometers equipped with magnetic sector have been used to quantify [189] and with TOF to identify [185] target compounds in water waste samples. Table 6 summarises the MLD reported in the different matrices.

A compromised solution between the cost and the performance of GC-MS and GC-HRMS is the GC-MS-MS (triple quadrupole (QqQ) or ion trap), which offers the advantage to provide collision induced ion transitions improving the selectivity with respect to GC-MS, and the sensitivity is even superior to GC-HRMS. The GC-MS-MS and GC-HRMS instrument limits of detection for PBDEs were between 0.04-41 pg and 5-85 pg, respectively [190]. However, their applications are not so frequent in the determination of FRs in environmental samples. Bergh *et al*. [191,192] used GC-(PCI)MS-MS for the determination of PFRs in dust. The MLD were higher than in GC-(ECNI)MS, but sufficient for the analysis of the samples due to the elevated concentrations of FRs in dust. Schindler *et al*. [106] used GC-(EI)QqQ for the determination of metabolites of PFRs in biotic samples after a derivatisation process and Law *et al*. [193] also used GC-(EI)QqQ for the determination of BFRs in blubber of harbour porpoises. Both studies presented high detection frequencies of the analytes in the samples that suggest acceptable MLDs. Carro *et al*. [194] used GC-(EI)MS-MS in an Ion Trap MS for the determination of PBDEs in aquaculture samples, but the method applicability was performed only in four samples with very low detection frequencies for PBDEs. Table 6 summarises the MLDs reported in the different matrices.

Table 6. GC-MS method limits of detection, extraction techniques and clean-ups for FRs in matrices from different countries.

Instrument	FR	Matrix	Extraction / clean-up	MLD	Country	REF
GC-(ECNI)MS	Tri to DecaBDE	Plasma	SPE/ H_2SO_4-SiO_2	0.3-5.5 ng/g l.w.	Sweden	[63]
	DBDPE			1.03 ng/g l.w.		
	Tri to DecaBDE	Milk	LLE/H_2SO_4	1.8-26.7 ng/g l.w.	China	[92]
	Tri to DecaBDE	Eggs	[a]SLE /GPC + SiO_2	0.01-0.1 ng/g w.w.	Canada	[181]
	DP, PBEB, HBB, HBCD			0.01 ng/g w.w.		
	BTBPE, DBDPE, TBB, TBPH	Dust	Ultrasound/ H_2SO_4-SiO_2 + Florisil®	1-20 ng/g	Belgium	[182]
	BDE-209, DBDPE, BTBPE	Sediments	PLE/GPC+ Oasis®HLB+SiO_2	0.02-0.06 ng/g d.w.	Netherlands	[120]
	Tri to DecaBDE	Sewage sludge	Soxhlet/ H_2SO_4 and KOH in SiO_2	0.07-3.33 ng/g d.w.	Korea	[183]
	BTBPE, DBDPE			0.12-11.9 ng/g d.w.		
	Tri to DecaBDE, DBDPE	Pieces of material	Soxhlet/SiO_2-Al_2O_3	0.5-25 ng/g	China	[74]
LVI-GC-(EI)MS	tri to hexaBDEs	Adipose tissue	Soxhlet/ H_2SO_4.SiO_2-Al_2O_3	0.05-0.30 ng/g l.w.	Belgium	[132]
GC-(EI)MS	TNBP/TPHP	Outdoor air	PLE	0.04/0.004 ng/m^3	Spain	[51]
	Σ_6PFRs	Surface water	LLE/freezing	1.5-4 ng/L	Germany	[54]
	Σ_6PFRs	Dust	Ultrasound/ H_2SO_4-SiO_2 + Florisil®	6-24 ng/g	Belgium	[76]
	Σ_5PFRs	Plasma	[b]Stir-bar MMLLE/SiO_2	0.2-0.5 ng/g w.w.	Sweden	[155]
GC-IPC-MS	TNBP/TBEP	Waste water	[c]MAE-SPME	29/45 ng/L	U.S.A	[185]
	TNBP/TPP/TCEP	Plasma	SPME	17/24/240 ng/L	U.S.A	[154]

Table 6. Continued.

Instrument	FR	Matrix	Extraction / clean-up	MLD	Country	REF
GC-(PCI)MS-MS (QqQ)	Σ_6PFRs	Indoor air	SPE	0.2-13 ng/m^3	Sweden	[192]
	Σ_6PFRs	Dust	Ultrasound/Florisil®	2-690 ng/g	Sweden	[191]
GC-(EI)MS-MS (QqQ)	Σ_4DAPs	Urine	SPE(derivatisation)/ Bond Elut PSA and Florisil®	0.5-1 ng/mL	Germany	[159,160]
	Σ_7Emerging FRs	Porpoise blubber	PLE/GPC + Florisil®	0.12-1.2 ng/g w.w.	U.K.	[193]
GC-(EI)MS-MS (ion trap)	BDE-47, 99, 100	Aquaculture samples	MAE/H_2SO_4-SiO_2	0.84-1.4 ng/g w.w.	Spain	[194]
GC-(EI)HRMS (magnetic sector)	Σ_5PBDEs	Serum	SPE/H_2SO_4-SiO_2	0.02-0.12	Greenland Poland Ukraine	[186]
	Σ_5PBDEs, DP, Dec602,603	Serum	SPE/H_2SO_4-SiO_2	0.02-0.75 ng/g l.w.	France	[97]
	Tri to HeptaBDE	Fish	SPE/GPC + SiO_2	0.005-0.093 ng/g	Canada	[187]
	Σ_{12}Emerging FRs	Soil (biota)	Soxhlet/H_2SO_4-KOH-SiO_2+Al_2O_3 (PLE/SPE)	0.01-0.5 ng/g	Canada	[188]
	Σ_6PFRs	Waste water	LLE/freezing	~1 ng/L	[d]Europe	[189]
GC-(EI)HRMS (TOF)	Tri to DecaBDE	Serum	SPE/H_2SO_4-SiO_2	1-6 pg/g w.w.	U.S.A	[153]
GCxGC(EI)HRMS (TOF)	Σ_7PBDEs	Serum/Milk	SPE/H_2SO_4-SiO_2	1-15 ng/mL	U.S.A	[172]

[a] Solid-liquid extraction; Σ_6PFRs=TNBP, TPHP, TCEP, TBEP, TDCPP and TCPP; Σ_5PFRs=TNBP, TPHP, TCEP, TBEP and TCPP; [b] Stir-bar assisted microporous membrane liquid-liquid extractor; [c] microwave assisted extraction/solid-phase microextraction; Σ_4DAPs=DNBP, BCPP, BCEP and DPP; Σ_7Emerging FRs=TBX, TBCT, PBT, PBEB, TBP-DBPE, TBB and DP; Σ_5PBDEs=BDE-47, 99, 100, 153 and 154; Σ_{12}Emerging FRs=HBB, PBEB, TBECH, TBCO, BATE, ATE, DPTE, DBDPE, BTBPE, DP, TBB and TBPH; [d] 90 water waste treatment plants from Europe were evaluated; Σ_7PBDEs=BDE-28, 47, 85, 99, 100, 153 and 154.

1.4.2.2. Liquid chromatography coupled to mass spectrometry

Liquid chromatography (LC) is the technique most extensively used for the determination of polar substances. Persistent FRs are not polar and they are highly immiscible in water (log K_{ow} > 5), hence few studies use LC-MS for their determination. The most relevant applications related to persistent FRs are the separation of the three isomers of the HBCD (α, β and γ) [195] and the determination of tetrabromobisphenol A from serum and milk [150]. Also hydroxylated PBDEs have been determined by means of LC-MS-MS [196,197]. Some studies have investigated the determination of native BFRs and dechloranes with MLDs comparable to GC-MS. Zhou *et al*. [198] determined 36 halogenated FRs (emerging FRs and PBDEs) by LC-MS using two different ionisation techniques for fish and waste water samples. Atmospheric pressure photoionisation (APPI) was used for samples of fish with a MLQ ranging from 0.003 ng/g w.w. (BDE-153) to 0.37 ng/g w.w. (*syn*-DP) and atmospheric pressure chemical ionisation (APCI) was used for waste water samples with MLQ ranging from 0.1 ng/L (BDE-153) to 3.8 ng/L (BDE-28) [199]. Al-Odaini *et al*. [195] determined DP, DBDPE and BTBPE from marine sediments also using LC-(APCI)MS-MS. The MLDs were in the range 0.01-0.1 ng/g d.w. Likewise, Fang *et al*. [134] used LC-(APCI)MS-MS for the determination of a thermally labile PFR known as V6 from dust.

PFRs are less lipophilic ($1.5 < K_{ow} < 6.6$) and more water soluble than the persistent FRs and consequently their analysis by LC-MS should be feasible. Nevertheless, for environmental samples the preferred technique is GC-MS and for biological samples, like serum, few studies have reported their occurrence because of the rapid metabolisation in the human body [156,200]. Therefore, the main use of LC-MS regarding the FRs is the human biomonitoring of PFRs through their metabolites in urine [104], although the determination of DAPs can also be performed by GC-MS after derivatisation, as mentioned above. The most frequently investigated metabolites derived from the PFRs are di-*n*-butyl phosphate (DNBP), diphenyl phosphate (DPHP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCPP) and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP).

During liquid chromatography, DAPs are not fully protonated under reverse phase conditions because of their low pKa ≤ 2 [158]. Acid mobile phases containing buffers (2-5 mmol/L acetic acid / ammonium acetate) or modifiers (1 mmol/L formic acid) in combination with non-polar stationary phases, such as C_{18} and phenyl modified silica, provide satisfactory retention for the separation of the DAPs. Alternatively, a mobile phase with an alkaline

modifier (10 mmol/L of NH_3) and porous graphitic carbon as stationary phase has also shown good retention of DAPs, but this method was intended to prove the suitability of molecular imprinted polymers for the extraction of DAPs in urine, and the reported MLDs are high [164]. The elution of DAPs from modified silica columns is carried out using water and modest amounts of organic phase (usually below 40%). This might affect the ionisation of the analytes due to the high percentage of water in the mobile phase and probably high degree of solvation of the anions [201]. The addition of an ion pairing chemical (e.g., tri-*n*-butylamine) in the mobile phase improves the interaction with the stationary phase. This is translated in higher percentages of organic solvent in the mobile phase to elute the analytes, but on the other hand, ion pairing can also decrease the ionisation response. In fact, the reported MLD of methods without ion pairing are lower than the MLD reported by Reemtsma *et al*. [104] using ion pairing. Two organic solvents, as components of the mobile phase, have been mainly reported for the determination of DAPs, methanol for the silica based columns and THF for porous graphitic carbon. The usual dimensions of the columns for the determination of DAPs range from 50-100 mm of column length, 4.6-2.0 mm of internal diameter and 5-2.1 µm of particle size. Liquid chromatography using fused-core or core shell columns have been commonly employed, but the use of columns with particle diameter < 2µm has not been explored yet [104].

There is only one work assessing the use of hydrophilic interaction liquid chromatography (HILIC) to separate DAPs in urine. Cooper *et al*. [23] reported an acceptable separation between BDCIPP and DPHP, but the reproducibility was unsatisfactory. HILIC retains hydrophilic and charged compounds more efficiently than more neutral hydrophobic compounds, i.e., HILIC provides reverse elution order compared to reverse phase chromatography. The stationary phase in HILIC is silica or functionalised silica with groups such as diol, amino, amide, etc. The mobile phase is initially rich in organic solvent (e.g., 90% MeCN) and when increasing the percentage of water, the elution strength is also increased. The pH control in HILIC is of high importance for the separation of the analytes, especially when the selected stationary phase contains zwitterionic functional groups (e.g., SeQuant®HILIC columns from Merck KGaA, Darmstadt, Germany). HILIC is typically applied in the determination of metabolites of organophosphate pesticides, which contain diethyl or dimethyl chains that do not retain in reverse phase columns [202].

Mass spectrometry is the most sensitive technique to detect trace amounts of DAPs from urine. However, mass spectrometers equipped with a single quadrupole analyser and using

ESI(-) leads to high MLD (e.g., 14-25 ng/mL in urine) [164]. Therefore, the preferred analyser for the determination of DAPs is the QqQ using ESI(-). This combination gives satisfactory MLD for the determination of DAPs in urine (between < 1 and 3.7 ng/mL) [116]. The main drawback to use ESI source is the strong matrix effects experienced by the analytes in the ion source. This fact obligates the analyst to quantify the DAPs by using the internal standard addition method [107] or by spiking with isotopically labelled internal standards and quantifying the DAPs with an internal standard calibration [23,116]. Cooper *et al*. [23] reported very little matrix effects when an APCI source was used leading to excellent MLD for BDCIPP and DPHP in urine (0.08-0.8 ng/mL, respectively). However, Van den Eede e*t al*. [116] reported that APCI performed worse than ESI in their system. In general, the MLD for DNBP, DPHP, BDCIPP and BBOEP are comparable for LC-(ESI)MS-MS and GC-(EI)MS. Nevertheless, the determination using LC-(ESI)MS-MS for the most polar DAPs, such as bis(2-chloroethyl) phosphate (BCEP) and bis(1-chloro-2-propyl) phosphate (BCPP), in urine is challenging because of their low MS response (MLQ of 1.2 and 3.7 ng/mL, respectively) [116]. Currently, the most sensitive technique to monitor these DAPs, after derivatisation, is GC-(EI)MS-MS (MLQ of 0.30 and 0.75 ng mL^{-1}, respectively) [160,203].

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

1.5. References

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

[1] C.A. de Wit, D. Herzke, K. Vorkamp, Sci. Total Environ. 408 (2010) 2885-2918.
[2] A. Covaci, S. Harrad, M.A.E. Abdallah, N. Ali, R.J. Law, D. Herzke, C.A. de Wit, Environ. Int. 37 (2011) 532-556.
[3] C.M. Butt, H.M. Stapleton, Chem. Res. Toxicol. 26 (2013) 1692-1702.
[4] P.I. Johnson, H.M. Stapleton, B. Mukherjee, R. Hauser, J.D. Meeker, Sci. Total Environ. 445-446 (2013) 177-184.
[5] B. Eskenazi, J. Chevrier, S.A. Rauch, K. Kogut, K.G. Harley, C. Johnson, C. Trujillo, A. Sjödin, A. Bradman, Environ. Health Persp. 121 (2013) 257-262.
[6] S. De Coster, N. Van Larebeke, J. Environ. Public Health 2012 (2012) doi:10.1155/-2012/713696.
[7] WHO/IPCS. Switzerland, Geneva. http://www.who.int/ipcs/publications/new_issues/-endocrine_disruptors/en/ (accessed June 2014).
[8] Å. Bergman, J.J. Heindel, S. Jobling, K.A. Kidd, R.T. Zoeller, ISBN: 978 92 4 150503 1. 2012.
[9] EFRA. http://www.cefic-efra.com (accessed June 2014).
[10] M. Alaee, P. Arias, A. Sjödin, Å. Bergman, Environ. Int. 29 (2003) 683-689.
[11] E. Schmitt, Plast. Additit. Compound. 9 (2007) 26-30.
[12] P.R. Fisk, A.E. Girling, R.J. Wildey, http://ec.europa.eu/environment/waste/stake-holders/industry_assoc/ebfrip/annex2.pdf (accessed June 2014).
[13] I. Van der Veen, J. de Boer, Chemosphere 88 (2012) 1119-1153.
[14] V. Babushok, W. Tsang, Combust. Flame 123 (2000) 488-506.
[15] B. Schartel, Materials 3 (2010) 4710-4745.
[16] K. Ballschmiter, M. Zell, Z. Anal. Chem. 302 (1980) 20-31.
[17] M.J. La Guardia, R.C. Hale, E. Harvey, Environ. Sci. Technol. 40 (2006) 6247-6254.
[18] UNEP. http://chm.pops.int/default.aspx (accessed June 204).
[19] BSEF. http://www.bsef.com/ (accessed June 2014).
[20] L.S. Birnbaum, D.F. Staskal, Environ Health Perspect. 112 (2004) 9-17.
[21] R.A. Hites, Environ. Sci. Technol. 38 (2004) 945-956.
[22] Å. Bergman, A. Rydén, R.J. Law, J. de Boer, A. Covaci, M. Alaee, L. Birnbaum, M. Petreas, M. Rose, S. Sakai, N. Van den Eede, I. van der Veen, Environ. Int. 49 (2012) 57-82.
[23] E.M. Cooper, A. Covaci, A.L. van Nuijs, T.F. Webster, H.M. Stapleton, Anal. Bioanal.Chem. 401 (2011) 2123-2132.
[24] M. Harju, E.S. Heimstad, D. Herzke, T. Sandanger, S. Posner, F. Wania. http://www.miljodirektoratet.no/old/klif/publikasjoner/2462/ta2462.pdf (accessed June 2014).
[25] E. Sverko, G.T. Tomy, E.J. Reiner, Y.F. Li, B.E. McCarry, J.A. Arnot, R.J. Law, R.A. Hites, Environ. Sci. Technol. 45 (2011) 5088-5098.
[26] N. Ren, E. Sverko, Y.F. Li, Z. Zhang, T. Harner, D. Wang, X. Wan, B.E. McCarry, Environ. Sci. Technol. 42 (2008) 6476-6480.
[27] Q. Xian, S. Siddique, T. Li, Y.l. Feng, L. Takser, J. Zhu, Environ. Int. 37 (2011) 1273-1284.
[28] L. Shen, E.J. Reiner, K.A. MacPherson, T.M. Kolic, P.A. Helm, L.A. Richman, C.H. Marvin, D.A. Burniston, B. Hill, I.D. Brindle, R. McCrindle, B.G. Chittim, Environ. Sci. Technol. 45 (2011) 693-699.
[29] E. Barón, G. Santín, E. Eljarrat, D. Barceló, J. Hazard. Mater. 265 (2014) 288-295.
[30] A. Marklund, B. Andersson, P. Haglund, Chemosphere 53 (2003) 1137-1146.
[31] L.S. Sheldon, R.A. Hites, Environ. Sci. Technol. 12 (1978) 1188-1194.

[32] T. Reemtsma, J.B. Quintana, R. Rodil, M. García-López, I. Rodríguez, TrAC-Trends Anal. Chem. 27 (2008) 727-737.
[33] C. Lamouroux, H. Virelizier, C. Moulin, J.C. Tabet, C.K. Jankowski, Anal. Chem. 72 (2000) 1186-1191.
[34] A. Leisewitz, H. Kruse, E. Schramm, Research Report 204 08 542 UBA-FB 000171/1e. http://www.oekorecherche.de/english/berichte/volltext/Flame%20Retardants.pdf (accessed June 2014).
[35] N. Green, M. Schlabach, T. Bakke, E.M. Brevik, C. Dye, D. Herke, S. Huber, B. Plosz, M. Remberger, M. Schøyen, H.T. Uggerud, C. Vogelsang. ISBN: 978-82-577-5304-7, 2008.
[36] H.M. Stapleton, S. Klosterhaus, S. Eagle, J. Fuh, J.D. Meeker, A. Blum, T.F. Webster, Environ. Sci. Technol. 43 (2009) 7490-7495.
[37] S.P. McGee, A. Konstantinov, H.M. Stapleton, D.C. Volz, Toxicol. Sci. 133 (2013) 144-156.
[38] S. Pakalin, T. Cole, J. Steinkellner, R. Nicolas, C. Tissier, S. Munn, S. Eisenreich. http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/5259/1/EUR%2022693.pdf (accessed June 2014).
[39] WHO. Environmental Health Criteria 209. Geneva. Switzerland, 1998. http://www.inchem.org/documents/ehc/ehc/ehc209.htm (accessed June 2014).
[40] A.A. Nomeir, S. Kato, H.B. Matthews, Toxicol. Appl. Pharmacol. 57 (1981) 401-413.
[41] R.J. Law, M. Alaee, C.R. Allchin, J.P. Boon, M. Lebeuf, P. Lepom, G.A. Stern, Environ. Int. 29 (2003) 757-770.
[42] T. Gouin, G.O. Thomas, I. Cousins, J. Barber, D. Mackay, K.C. Jones, Environ. Sci. Technol. 36 (2002) 1426-1434.
[43] A. González-Gago, S. Brandsma, P. Leonards, J. de Boer, J. Marchante-Gayón, J. García Alonso, Anal. Bioanal. Chem. 401 (2011) 2639-2649.
[44] U. Sellström, A. Kierkegaard, C.A. de Wit, B.O. Jansson, Environ. Toxicol. Chem. 17 (1998) 1065-1072.
[45] U. Sellström, B. Jansson, A. Kierkegaard, C.A. de Wit, T. Odsjo, M. Olsson, Chemosphere 26 (1993) 1703-1718.
[46] Y. Ma, M. Venier, R.A. Hites, Environ. Sci. Technol. 46 (2012) 204-208.
[47] H.P. Arp, T. Moskeland, P.L. Andersson, J.R. Nyholm, J. Environ. Monit. 13 (2011) 505-513.
[48] X. Qiu, C.H. Marvin, R.A. Hites, Environ. Sci. Technol. 41 (2007) 6014-6019.
[49] L.T. Gauthier, D. Potter, C.E. Hebert, R.J. Letcher, Environ. Sci. Technol. 43 (2009) 312-317.
[50] J. Muñoz-Arnanz, M. Sáez, F. Hiraldo, R. Baos, G. Pacepavicius, M. Alaee, B. Jiménez, Environ. Int. 37 (2011) 1164-1168.
[51] M. Aragón, R.M. Marcé, F. Borrull, Talanta 101 (2012) 473-478.
[52] A. Bacaloni, C. Cavaliere, P. Foglia, M. Nazzari, R. Samperi, A. Laganà, Rapid Commun. Mass Spectrom. 21 (2007) 1123-1130.
[53] J. Regnery, W. Püttmann, Water Res. 44 (2010) 4097-4104.
[54] J.A. Andresen, A. Grundmann, K. Bester, Sci. Total Environ. 332 (2004) 155-166.
[55] E. Martínez-Carballo, C. González-Barreiro, A. Sitka, S. Scharf, O. Gans, Sci. Total Environ. 388 (2007) 290-299.
[56] A.M. Sundkvist, U. Olofsson, P. Haglund, J. Environ. Monit. 12 (2010) 943-951.
[57] P. Leonards, E.H. Steindal, I. van der Veen, V. Berg, J.O. Bustnes, S. van Leeuwen. http://www.miljodirektoratet.no/old/klif/publikasjoner/2786/ta2786.pdf (accessed June 2014).

[58] A.J. Fraser, T.F. Webster, M.D. McClean, Environ. Health Perspect. 117 (2009) 1520-1525.
[59] S. Harrad, E. Goosey, J. Desborough, M.A.E. Abdallah, L. Roosens, A. Covaci, Environ. Sci. Technol. 44 (2010) 4198-4202.
[60] R.E. Dodson, L.J. Perovich, A. Covaci, N. Van den Eede, A.C. Ionas, A.C. Dirtu, J.G. Brody, R.A. Rudel, Environ. Sci. Technol. 46 (2012) 13056-13066.
[61] C. Bergh, R. Torgrip, G. Emenius, C. Östman, Indoor Air 21 (2011) 67-76.
[62] A. Kanazawa, I. Saito, A. Araki, M. Takeda, M. Ma, Y. Saijo, R. Kishi, Indoor Air 20 (2010) 72-84.
[63] M. Karlsson, A. Julander, B. van Bavel, L. Hardell, Environ. Int. 33 (2007) 62-69.
[64] H. Takigami, G. Suzuki, Y. Hirai, S.I. Sakai, Chemosphere 76 (2009) 270-277.
[65] S. Harrad, C. Ibarra, M.A.E. Abdallah, R. Boon, H. Neels, A. Covaci, Environ. Int. 34 (2008) 1170-1175.
[66] S. Batterman, C. Godwin, S. Chernyak, C. Jia, S. Charles, Environ. Int. 36 (2010) 548-556.
[67] J.A. Björklund, K. Thuresson, A.P. Cousins, U. Sellström, G. Emenius, C.A. de Wit, Environ. Sci. Technol. 46 (2012) 5876-5884.
[68] J.G. Allen, M.D. McClean, H.M. Stapleton, J.W. Nelson, T.F. Webster, Environ. Sci. Technol. 41 (2007) 4574-4579.
[69] B. Johnson-Restrepo, K. Kannan, Chemosphere 76 (2009) 542-548.
[70] H. Takigami, G. Suzuki, Y. Hirai, Y. Ishikawa, M. Sunami, S.I. Sakai, Environ. Int. 35 (2009) 688-693.
[71] H.M. Stapleton, J.G. Allen, S.M. Kelly, A. Konstantinov, S. Klosterhaus, D. Watkins, M.D. McClean, T.F. Webster, Environ. Sci. Technol. 42 (2008) 6910-6916.
[72] N. Ali, S. Harrad, E. Goosey, H. Neels, A. Covaci, Chemosphere 83 (2011) 1360-1365.
[73] A. Sjödin, O. Päpke, E. McGahee, J.F. Focant, R.S. Jones, T. Pless-Mulloli, L.M.L. Toms, T. Herrmann, J. Müller, L.L. Needham, D.G. Patterson Jr, Chemosphere 73 (2008) S131-S136.
[74] S.J. Chen, Y.J. Ma, J. Wang, M. Tian, X.J. Luo, D. Chen, B.X. Mai, J. Hazard. Mater. 176 (2010) 979-984.
[75] P.C. Hartmann, D. Bürgi, W. Giger, Chemosphere 57 (2004) 781-787.
[76] N. Van den Eede, A.C. Dirtu, H. Neels, A. Covaci, Environ. Int. 37 (2011) 454-461.
[77] S. Harrad, R. Wijesekera, S. Hunter, C. Halliwell, R. Baker, Environ. Sci. Technol. 38 (2004) 2345-2350.
[78] B.H. Wilford, M. Shoeib, T. Harner, J. Zhu, K.C. Jones, Environ. Sci. Technol., 39 (2005) 7027-7035.
[79] A. Törnkvist, A. Glynn, M. Aune, P.O. Darnerud, E.H. Ankarberg, Chemosphere 83 (2011) 193-199.
[80] S. Voorspoels, A. Covaci, H. Neels, P. Schepens, Environ. Int. 33 (2007) 93-97.
[81] A. Schecter, D. Haffner, J. Colacino, K. Patel, O. Päpke, M. Opel, L. Birnbaum, Environ. Health Perspect. 118 (2010) 357-362.
[82] H.K. Knutsen, H.E. Kvalem, C. Thomsen, M. Frøshaug, M. Haugen, G. Becher, J. Alexander, H.M. Meltzer, Mol. Nutr. Food Res. 52 (2008) 217-227.
[83] S. Tomizawa, I. Takano, M. Kobayashi, Y. Tamura, Y. Tateishi, N. Sakai, K. Kitayama, T. Nagayama, K. Kamata, K. Saito, J. Food Hyg. Soc. Japan 45 (2004) 259-263.
[84] WHO. Environmental Health Criteria 111. (1991). http://www.inchem.org/documents-/ehc/ehc/ehc111.htm (accessed June 2014).
[85] COPHES. http://www.eu-hbm.info/cophes/human-biomonitoring (accesses June 2014).

[86] D.R. Juberg, J. Bus, D.S. Katz, ISBN: 1-890624-73-X. 2008. http://www.acs.org/-content/dam/acsorg/policy/acsonthehill/briefings/biomonitoring/biomonitoring-policy-brief.pdf (accessed June 2014).
[87] J.W. Choi, S. Fujimaki, K. Kitamura, S. Hashimoto, H. Ito, N. Suzuki, S.I. Sakai, M. Morita, Environ. Sci. Technol. 37 (2003) 817-821.
[88] A. Covaci, J. de Boer, J.J. Ryan, S. Voorspoels, P. Schepens, Environ. Res. 88 (2002) 210-218.
[89] L. Roosens, M.A.E. Abdallah, S. Harrad, H. Neels, A. Covaci, Environ. Sci. Technol. 43 (2009) 3535-3541.
[90] C. Thomsen, H.K. Knutsen, V.H. Liane, M. Frøshaug, H.E. Kvalem, M. Haugen, H.M. Meltzer, J. Alexander, G. Becher, Mol. Nutr. Food Res. 52 (2008) 228-237.
[91] H. Fromme, W. Körner, N. Shahin, A. Wanner, M. Albrecht, S. Boehmer, H. Parlar, R. Mayer, B. Liebl, G. Bolte, Environ. Int. 35 (2009) 1125-1135.
[92] S. Ma, Z. Yu, X. Zhang, G. Ren, P. Peng, G. Sheng, J. Fu, Environ. Int. 42 (2012) 72-77.
[93] C. Thomsen, H. Stigum, M. Frøshaug, S.L. Broadwell, G. Becher, M. Eggesbø, Environ. Int. 36 (2010) 68-74.
[94] J.S. Park, J. She, A. Holden, M. Sharp, R. Gephart, G. Souders-Mason, V. Zhang, J. Chow, B. Leslie, K. Hooper, Environ. Sci. Technol. 45 (2011) 4579-4585.
[95] J.D. Coakley, S.J. Harrad, E. Goosey, N. Ali, A.C. Dirtu, N. Van den Eede, A. Covaci, J. Douwes, A. Mannetje, Environ. Int. 59 (2013) 255-261.
[96] S.N. Zhou, S. Siddique, L. Lavoie, L. Takser, N. Abdelouahab, J. Zhu, Environ. Int. 66 (2014) 11-17.
[97] C. Brasseur, C. Pirard, G. Scholl, E. De Pauw, J.F. Viel, L. Shen, E.J. Reiner, J.F. Focant, Environ. Int. 65 (2014) 33-40.
[98] G. Ren, Z. Yu, S. Ma, H. Li, P. Peng, G. Sheng, J. Fu, Environ. Sci. Technol. 43 (2009) 9453-9457.
[99] Y.J. Ben, X.H. Li, Y.L. Yang, L. Li, J.P. Di, W.Y. Wang, R.F. Zhou, K. Xiao, M.Y. Zheng, Y. Tian, X.B. Xu, Environ. Pollut. 173 (2013) 176-181.
[100] Z. Tang, Q. Huang, J. Cheng, Y. Yang, J. Yang, W. Guo, Z. Nie, N. Zeng, L. Jin, Environ. Sci. Technol. 48 (2014) 1508-1516.
[101] G. Malarvannan, T. Isobe, A. Covaci, M. Prudente, S. Tanabe, Sci. Total Environ. 442 (2013) 366-379.
[102] J. Zheng, X.J. Luo, J.G. Yuan, J. Wang, Y.T. Wang, S.J. Chen, B.X. Mai, Z.Y. Yang, Environ. Pollut. 159 (2011) 3706-3713.
[103] J. Zheng, J. Wang, X.J. Luo, M. Tian, L.Y. He, J.G. Yuan, B.X. Mai, Z.Y. Yang, Environ. Sci. Technol. 44 (2010) 9298-9303.
[104] A.C. Dirtu, N. Van den Eede, G. Malarvannan, A.C. Ionas, A. Covaci, Anal. Bioanal. Chem. 404 (2012) 2555-2581.
[105] K. Hoffman, J.L. Daniels, H.M. Stapleton, Environ. Int. 63 (2014) 169-172.
[106] B.K. Schindler, S. Koslitz, T. Weiss, H.C. Broding, T. Brüning, J. Bünger, Int. J. Hyg. Environ. Health 217 (2014) 34-37.
[107] T. Reemtsma, J. Lingott, S. Roegler, Sci. Total Environ. 409 (2011) 1990-1993.
[108] C. Thomsen, E. Lundanes, G. Becher, Environ. Sci. Technol. 36 (2002) 1414-1418.
[109] C. Thomsen, V.H. Liane, G. Becher, J. Chromatogr. B 846 (2007) 252-263.
[110] A. Sjödin, D.G. Patterson Jr, Å. Bergman, Environ. Sci. Technol. 35 (2001) 3830-3833.
[111] A. Mazdai, N.G. Dodder, M.P. Abernathy, R.A. Hites, R.M. Bigsby, Environ. Health Perspect. 111 (2003) 1249-1252.

[112] D. Meironyté, K. Norén, Å. Bergman, J. Toxicol. Environ. Health - Part A, 58 (1999) 329-341.
[113] D. Meironyté Guvenius, A. Aronsson, G. Ekman-Ordeberg, Å. Bergman, K. Norén, Environ. Health Perspect. 111 (2003) 1235-1241.
[114] A. Schecter, M. Pavuk, O. Päpke, J.J. Ryan, L. Birnbaum, R. Rosen, Environ. Health Perspect. 111 (2003) 1723-1729.
[115] M. Meneses, H. Wingfors, M. Schuhmacher, J.L. Domingo, G. Lindström, B. van Bavel, Chemosphere 39 (1999) 2271-2278.
[116] N. Van den Eede, H. Neels, P.G. Jorens, A. Covaci, J. Chromatogr. A 1303 (2013) 48-53.
[117] K. Thuresson, J.A. Björklund, C.A. de Wit, Sci. Total Environ. 414 (2012) 713-721.
[118] J.M. Keller, R.F. Swarthout, B.K. Carlson, J. Yordy, A. Guichard, M.M. Schantz, J.R. Kucklick, Anal. Bioanal.Chem. 393 (2009) 747-760.
[119] S.J. Chen, Y.J. Ma, J. Wang, D. Chen, X.J. Luo, B.X. Mai, Environ. Sci. Technol. 43 (2009) 4200-4206.
[120] P. López, S.A. Brandsma, P.E.G. Leonards, J. De Boer, Anal. Bioanal. Chem. 400 (2011) 871-883.
[121] S. Król, B. Zabiegala, J. Namiesnik, Anal. Bioanal. Chem. 400 (2011) 1751-1769.
[122] S. Harrad, S. Hazrati, C. Ibarra, Environ. Sci. Technol. 40 (2006) 4633-4638.
[123] D. Muenhor, S. Harrad, N. Ali, A. Covaci, Environ. Int. 36 (2010) 690-698.
[124] H.M. Stapleton, S. Klosterhaus, A. Keller, P.L. Ferguson, S. van Bergen, E. Cooper, T.F. Webster, A. Blum, Environ. Sci. Technol. 45 (2011) 5323-5331.
[125] J. Zhu, Y. Hou, Y.L. Feng, M. Shoeib, T. Harner, Environ. Sci. Technol. 42 (2008) 386-391.
[126] W.L. Ma, L.Y. Liu, Q.A. Hong, D.Z. Sun, J.M. Shen, D.G. Wang, Y.F. Li, Environ. Int. 37 (2011) 66-70.
[127] K. Vorkamp, M. Thomsen, M. Frederiksen, M. Pedersen, L.E. Knudsen, Environ. Int. 37 (2011) 1-10.
[128] A. Pettersson-Julander, B. van Bavel, M. Engwall, H. Westberg, J. Environ. Monit. 6 (2004) 874-880.
[129] T.F. Webster, S. Harrad, J.R. Millette, R.D. Holbrook, J.M. Davis, H.M. Stapleton, J.G. Allen, M.D. McClean, C. Ibarra, M.A.E. Abdallah, A. Covaci, Environ. Sci. Technol. 43 (2009) 3067-3072.
[130] J.G. Allen, M.D. McClean, H.M. Stapleton, T.F. Webster, Environ. Int. 34 (2008) 1085-1091.
[131] N. Van den Eede, A.C. Dirtu, N. Ali, H. Neels, A. Covaci, Talanta 89 (2012) 292-300.
[132] A. Covaci, J. de Boer, J.J. Ryan, S. Voorspoels, P. Schepens, Anal. Chem. 74 (2002) 790-798.
[133] S.C. Cunha, K. Kalachova, J. Pulkrabova, J.O. Fernandes, M.B.P.P. Oliveira, A. Alves, J. Hajslova, Chemosphere 78 (2010) 1263-1271.
[134] M. Fang, T.F. Webster, D. Gooden, E.M. Cooper, M.D. McClean, C. Carignan, C. Makey, H.M. Stapleton, Environ. Sci. Technol. 47 (2013) 4449-4454.
[135] A.C. Ionas, A. Covaci, Int. J. Environ. Anal. Chem. 93 (2013) 1074-1083.
[136] N. Ali, S. Harrad, E. Goosey, H. Neels, A. Covaci, Chemosphere 83 (2011) 1360-1365.
[137] S. Brommer, S. Harrad, N. Van Den Eede, A. Covaci, J. Environ. Monit. 14 (2012) 2482-2487.
[138] V. Yusà, A. Pastor, M. de la Guardia, Anal. Chim. Acta 565 (2006) 103-111.
[139] J. de Boer, C. Allchin, R. Law, B. Zegers, J.P. Boon, TrAC-Trends Anal. Chem. 20 (2001) 591-599.

[140] B. Gevao, M. Al-Bahloul, A.N. Al-Ghadban, A. Al-Omair, L. Ali, J. Zafar, M. Helaleh, Chemosphere 64 (2006) 603-608.
[141] L. Sahlström, U. Sellström, C.A. de Wit, Anal. Bioanal. Chem. 404 (2012) 459-466.
[142] A. Kierkegaard, J. Björklund, U. Fridén, Environ. Sci. Technol. 38 (2004) 3247-3253.
[143] B. Muijs, M.T.O. Jonker, J. Chromatogr. A 1216 (2009) 5182-5189.
[144] L. Hovander, M. Athanasiadou, L. Asplund, S. Jensen, E.K. Wehler, J. Anal. Toxicol. 24 (2000) 696-703.
[145] WHO. Environmental health criteria 112. 1991. http://www.inchem.org/documents-/ehc/ehc/ehc112.htm (accessed June 2014).
[146] IPCS. OECD screening information data set: triphenyl phosphate. http://-www.inchem.org/documents/sids/sids/115866.pdf (accessed June 2014).
[147] A. Covaci, S. Voorspoels, J. Chromatogr. B 827 (2005) 216-223.
[148] M. Venier, R.A. Hites, Environ. Sci. Technol. 45 (2011) 4602-4608.
[149] Z. Zhang, S.M. Rhind, Talanta 84 (2011) 487-493.
[150] Z. Shi, Y. Wang, P. Niu, J. Wang, Z. Sun, S. Zhang, Y. Wu, J. Sep. Sci. 36 (2013) 3402-3410.
[151] P.R. Loconto, J. Chromatogr. Sci. 47 (2009) 656-669.
[152] M. Frederiksen, C. Thomsen, M. Frøshaug, K. Vorkamp, M. Thomsen, G. Becher, L.E. Knudsen, Int. J. Hyg. Environ. Health 213 (2010) 233-242.
[153] A. Sjödin, E.E. McGahee 3rd, J.F. Focant, R.S. Jones, C.R. Lapeza, Y. Zhang, D.G. Patterson Jr, Anal. Chem. 76 (2004) 4508-4514.
[154] M. Shah, J. Meija, B. Cabovska, J.A. Caruso, J. Chromatogr. A 1103 (2006) 329-336.
[155] O.B. Jonsson, U.L. Nilsson, J. Sep. Sci. 26 (2003) 886-892.
[156] N. Amini, C. Crescenzi, J. Chromatogr. B 795 (2003) 245-256.
[157] E.J. Cone, Y.H. Caplan, F. Moser, T. Robert, M.K. Shelby, D.L. Black, J. Anal. Toxicol. 33 (2009) 1-7.
[158] W.P. Jencks, F.H. Westheimer, pKa Data Compiled by R. Williams. http://nsdl.org/-resource/2200/20061121124513540T (accessed June 2014).
[159] B.K. Schindler, K. Förster, J. Angerer, J. Chromatogr. B 877 (2009) 375-381.
[160] B.K. Schindler, K. Forster, J. Angerer, Anal. Bioanal.Chem. 395 (2009) 1167-1171.
[161] T. Yoshida, J. Yoshida, J. Chromatogr. B 880 (2012) 66-73.
[162] E.M. Cooper, A. Covaci, A.L.N. van Nuijs, T.F. Webster, H.M. Stapleton, Anal. Bioanal. Chem. 401 (2011) 2123-2132.
[163] J.D. Meeker, E.M. Cooper, H.M. Stapleton, R. Hauser, Environ. Health Perspect. 121 (2013) 580-585.
[164] K. Möller, C. Crescenzi, U. Nilsson, Anal. Bioanal. Chem. 378 (2004) 197-204.
[165] O.B. Jonsson, U.L. Nilsson, Anal. Bioanal. Chem. 377 (2003) 182-188.
[166] A. Covaci, S. Voorspoels, J. de Boer, Environ. Int. 29 (2003) 735-756.
[167] A. Sjödin, L. Hagmar, E. Klasson-Wehler, K. Kronholm-Dlab, E. Jakobsson, Å. Bergman, Environ. Health Perspect. 107 (1999) 643-648.
[168] H.M. Stapleton, Anal. Bioanal.Chem. 386 (2006) 807-817.
[169] A.C. Dirtu, K. Ravindra, L. Roosens, R. van Grieken, H. Neels, R. Blust, A. Covaci, J. Chromatogr. A, 1186 (2008) 295-301.
[170] K. Mastovska, S.J. Lehotay, J. Hajslova, J. Chromatogr. A 926 (2001) 291-308.
[171] Y. Sapozhnikova, S.J. Lehotay, Anal. Chim. Acta 758 (2013) 80-92.
[172] J.F. Focant, A. Sjödin, W.I. Turner, D.G. Patterson Jr, Anal. Chem. 76 (2004) 6313-6320.
[173] M.S. Beldean-Galea, J. Vial, D. Thiebaut, Central Eur. J. Chem. 11 (2013) 1563-1574.
[174] J. Dallüge, J. Beens, U.A.T. Brinkman, J. Chromatogr. A 1000 (2003) 69-108.

[175] A. Ballesteros-Gómez, J. de Boer, P.E.G. Leonards, Anal. Chem. 85 (2013) 9572-9580.
[176] E. Eljarrat, S. Lacorte, D. Barceló, J. Mass Spectrom. 37 (2002) 76-84.
[177] N. Rosenfelder, W. Vetter, Rapid Commun. Mass Spectrom. 23 (2009) 3807-3812.
[178] L.K. Ackerman, G.R. Wilson, S.L. Simonich, Anal. Chem. 77 (2005) 1979-1987.
[179] C. Thomsen, L. Småstuen Haug, H. Leknes, E. Lundanes, G. Becher, G. Lindström, Chemosphere 46 (2002) 641-648.
[180] Y. Ma, R.A. Hites, J. Mass Spectrom. 48 (2013) 931-936.
[181] L.T. Gauthier, C.E. Hebert, D.V. Weseloh, R.J. Letcher, Environ. Sci. Technol. 41 (2007) 4561-4567.
[182] N. Ali, S. Harrad, D. Muenhor, H. Neels, A. Covaci, Anal. Bioanal. Chem. 400 (2011) 3073-3083.
[183] S. Lee, G.J. Song, K. Kannan, H.B. Moon, Sci. Total Environ. 470-471 (2014) 1422-1429.
[184] H.M. Stapleton, S. Klosterhaus, A. Keller, P.L. Ferguson, S. van Bergen, E. Cooper, T.F. Webster, A. Blum, Environ. Sci. Technol. 43 (2011) 7490-7495.
[185] J. Ellis, M. Shah, K.M. Kubachka, J.A. Caruso, J. Environ. Monit. 9 (2007) 1329-1336.
[186] V. Lenters, C. Thomsen, L.A.M. Smit, B.A.G. Jönsson, H.S. Pedersen, J.K. Ludwicki, V. Zviezdai, A.H. Piersma, G. Toft, J.P. Bonde, G. Becher, R. Vermeulen, D. Heederik, Environ. Int. 61 (2013) 8-16.
[187] M. Alaee, D.B. Sergeant, M.G. Ikonomou, J.M. Luross, Chemosphere 44 (2001) 1489-1495.
[188] T.M. Kolic, L. Shen, K. MacPherson, L. Fayez, T. Gobran, P.A. Helm, C.H. Marvin, G. Arsenault, E.J. Reiner, J. Chromatogr. Sci. 47 (2009) 83-91.
[189] R. Loos, R. Carvalho, D.C. Antonio, S. Comero, G. Locoro, S. Tavazzi, B. Paracchini, M. Ghiani, T. Lettieri, L. Blaha, B. Jarosova, S. Voorspoels, K. Servaes, P. Haglund, J. Fick, R.H. Lindberg, D. Schwesig, B.M. Gawlik, Water Res. 47 (2013) 6475-6487.
[190] S.A. Mackintosh, A. Pérez-Fuentetaja, L.R. Zimmerman, G. Pacepavicius, M. Clapsadl, M. Alaee, D.S. Aga, Anal. Chim. Acta 747 (2012) 67-75.
[191] C. Bergh, G. Luongo, S. Wise, C. Östman, Anal. Bioanal. Chem. 402 (2012) 51-59.
[192] C. Bergh, R. Torgrip, C. Östman, Rapid Commun. Mass Spectrom. 24 (2010) 2859-2867.
[193] R.J. Law, S. Losada, J.L. Barber, P. Bersuder, R. Deaville, A. Brownlow, R. Penrose, P.D. Jepson, Environ. Int. 60 (2013) 81-88.
[194] A.M. Carro, R.A. Lorenzo, F. Fernández, R. Phan-Tan-Luu, R. Cela, Anal. Bioanal. Chem. 388 (2007) 1021-1029.
[195] N.A. Al-Odaini, U.H. Yim, N.S. Kim, W.J. Shim, S.H. Hong, Anal. Methods 5 (2013) 1771-1778.
[196] Y. Kato, S. Okada, K. Atobe, T. Endo, K. Haraguchi, Anal. Bioanal. Chem. 404 (2012) 197-206.
[197] H. Chang, Y. Wan, J. Naile, X. Zhang, S. Wiseman, M. Hecker, M.H.W. Lam, J.P. Giesy, P.D. Jones, J. Chromatogr. A 1217 (2010) 506-513.
[198] S.N. Zhou, E.J. Reiner, C. Marvin, T. Kolic, N. Riddell, P. Helm, F. Dorman, M. Misselwitz, I.D. Brindle, J. Chromatogr. A 1217 (2010) 633-641.
[199] S.N. Zhou, E.J. Reiner, C. Marvin, P. Helm, N. Riddell, F. Dorman, M. Misselwitz, L. Shen, P. Crozier, K. MacPherson, I.D. Brindle, Anal. Bioanal. Chem. 396 (2010) 1311-1320.
[200] S. Inoue, T. Saito, H. Mase, Y. Suzuki, K. Takazawa, I. Yamamoto, S. Inokuchi, J. Pharm. Biomed. Anal. 44 (2007) 258-264.

[201] C.R. Mitchell, Y. Bao, N.J. Benz, S. Zhang, J. Chromatogr. B 877 (2009) 4133-4139.
[202] A.L.N. van Nuijs, I. Tarcomnicu, A. Covaci, J. Chromatogr. A 1218 (2011) 5964–5974.
[203] B.K. Schindler, K. Forster, J. Angerer, J. Chromatogr. B 877 (2009) 375-381.

2. Scope of the Thesis

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Human exposure to PBDEs has extensively been studied, and several works have described their occurrence in indoor environments and in humans in Norway. However, for emerging FRs, the occurrence and exposure pathways are totally unknown in Norway and in the majority of the European countries. Regarding the occurrence of PFRs, there are no previous data from Norway and again, few studies have been conducted in Europe. The lack of data for both emerging and organophosphate FRs requires the development of new analysis methods for their reliable quantification. The main objectives of the Thesis were to:

1) Survey the levels of persistent FRs and PFRs in air and dust from the living rooms of the participants' households by means of GC-MS.

2) Develop a fast and sensitive GC-MS method for simultaneous determination of legacy and emerging FRs in human serum, and also develop a sensitive UPLC-HRMS method for the determination of PFR metabolites in urine.

3) Characterise the levels of persistent FRs in the mothers' serum from the mother-child cohort recruited in the Greater area of Oslo and also characterise the levels of "metabolisable" FRs in urine from the mothers and their children.

4) Identify the indoor sources of exposure to FRs by correlating concentrations in air and dust with the number of consumer goods and/or type of materials in the households.

5) Identify which food items might contribute to the body burden of persistent and "metabolisable" FRs by correlating their intake with the serum and urine levels of the participants, respectively.

6) Elucidate the contribution of different exposure pathways (e.g., via diet, inhalation of air, ingestion and/or dermal contact with dust) to the internal dose of FRs in mothers and children.

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

3. Experimental, Results and Discussion

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

This chapter has been structured in 4 sections. In each section, there is a brief introduction to give the reader an overview of the research, and at the end, there is a discussion of the results that summarises the most important findings.

The first section describes how the recruitment of the participants was performed and what methodologies were used for the collection of the samples. Biological samples were collected from the participants, and indoor air and dust (floor and settled) were also collected in their respective households.

The second section describes the human exposure to FRs through dust and air from households and schools. In this section, we basically characterise the levels of persistent, emerging and organophosphate FRs in air and dust from the living rooms and classrooms. In addition, a rough risk assessment of the indoor environment was performed by calculating the intakes of FRs via inhalation of air, dermal contact and ingestion of dust for children and mothers.

The third and fourth sections describe the human biomonitoring of persistent and organophosphate FRs in serum and urine, respectively. In both sections, an analytical method was developed due to the lack of specific methods for the determination of emerging FRs or metabolites of the organophosphate FRs. Moreover, relationships between the levels of FRs in serum and the metabolites in urine were correlated with the levels in the air and dust from the living rooms in order to find out the most significant contributors to the body burden of FRs.

In the third section, a GC-(ECNI)MS method was developed for the characterization of the levels of persistent FRs in serum. The method was comprehensively validated by spiking FRs in horse serum, and to test its applicability to human serum, a pilot study comparing the serum from fish consumers from Lake Mjøsa with serum from the general population was carried out. Subsequently, the serum samples collected during the sampling campaign were analysed using the same method. Furthermore, through a food frequency questionnaire, we assessed the amount of food ingested during the last year by the participants. This gave us the opportunity to explore the relationship between the concentrations of FRs in serum and the amount of different food products consumed.

In the fourth section, a sensitive UPLC-HRMS method was developed for the determination of PFR metabolites in urine with no sample preparation other than centrifugation. The collection of urine of the mothers was performed during 24 hours, which allowed the investigation of the diurnal variability of the metabolites.

The experimental data of the second, third and fourth sections of this chapter are presented in the format of the publications of the international scientific peer-reviewed journals where the articles have been published or submitted. The complete list of articles can be found in the Appendix II.

3.1. Recruitment of the Cohort and Sampling Campaign

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

The proposal of the INFLAME project aimed to undertake an experimental approach to examining correlation between external exposure and human body burdens. The recruitment of 40 households with the participation of the mothers and children (6-12 years) was assumed to be statistically acceptable. The study was approved by the Regional Committee for Medical Research Ethics. Hence, a mother-child cohort comprising finally 48 mothers and their offspring (56 children in total) was recruited through primary schools in the Greater Oslo area. In addition, we sampled 6 classrooms from the two schools hosting most students in the cohort. The response rate to participate was about 2%. The mothers age spanned from 32 to 56 years (median 41 years) and the children's age, both genders, spanned from 6 to 12 years old (median 10 years).

The sampling campaign was performed in 2012 from January to mid-May, a period when ventilation of living rooms and classrooms was expected to be low and therefore exposure to be high. Forty-eight households and 2 schools were visited to collect the samples. Each house and school was visited twice in consecutive days. The set up for the collection of samples was carefully chosen in collaboration with the University of Stockholm. This means that the equipment used was tested to avoid background levels in the samples since inefficient sampling procedures might affect the quality of the results. First day, the pumps were deployed in the living rooms of the two households (or two classrooms) to collect air during 24 hours. For the air collection, we used a Leland legacy pump (SKC Limited, Dorset, U.K.), kindly provided by NILU (Norwegian Institute for Air Research), at a flow-rate of 12 L/min with 4 "train" holders hanging from a tripod and pointing downward at a height of 1.2 meters (Figure 3).

Figure 3. Picture of the "train" holders for the air sampling.

Each "train" holder was connected to the same pump with Tygon® R3603 tubing (VWR International, Vienna, Austria) and composed by a filter to retain particulate matter and two PUF plugs to trap target analytes in the gas phase (Figure 4). The "train" holders, filters and PUFs were kindly provided by the Department of Applied Environmental Sciences (University of Stockholm).

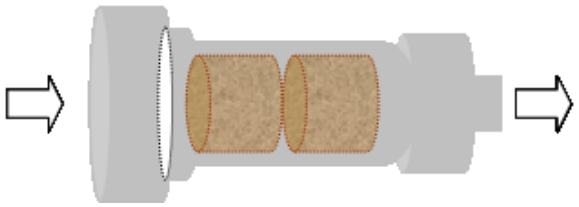


Figure 4. "Train" holder with the glass filter (white) and two PUFs (brown) for air sampling.

In order to avoid noise nuisance from the pumps, they were enclosed in a hard plastic box with the tubing and electrical wires passing through a hole on the top. After sampling, the four filters and eight PUFs were pooled and wrapped in aluminium foil, kept inside a low density polyethylene (LD-PE) sealing bag and stored at -20°C.

Cleaning of the train holders was carried out with dichloromethane in an ultrasonic bath for 10-15 minutes. PUFs were pre-cleaned in a Soxhlet system at the University of Stockholm with toluene for 24 hours 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.

During the second day visit, dust was collected using a GM80 vacuum cleaner (Nilfisk, Morgantown, WV, U.S.A.). Figure 5 depicts the different components employed for the collection of dust: a forensic filter (KTM Krim Teknisk Materiel AB, Bålsta, Sweden), a nozzle and a plastic grid (1-3 mm pore size) to collect floor dust, and without the grid for settled dust. Fifty to 500 mg of settled dust were vacuumed from elevated surfaces, sofas and couches. Hundred to 300 mg of floor dust were vacuumed from the living room floor (4 - 50 m^2). After sampling, coarse particles and hair were removed from the floor dust samples with stainless steel tweezers, and both types of dust were kept on the forensic filters, wrapped in aluminium foil, put into a LD-PE sealing bag and stored at -20°C.

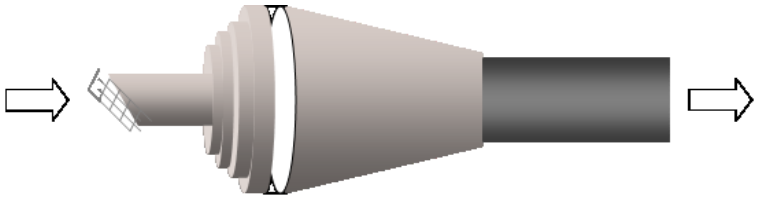


Figure 5. Grid, nozzle, forensic filter (white) and holder for dust sampling.

The biological samples, i.e., saliva, hair, urine and blood, were collected from mother and children (6-12 years), although blood was not drawn from the children. Saliva collection was carried out by asking the mothers and children to spit five times into a Nalgene® polypropylene 60 mL bottles (Thermo Scientific, MA, U.S.A.) obtaining around 2 - 3 mL of saliva. Hair collection was performed on mothers and children by cutting 2 - 3 cm horizontal straight thin line in the right and left side of the nape of the neck. The hair was placed in a regular post envelope and put it into a sealing bag. Both types of samples were stored at -20°C and lately shipped to one of the Research Institutes collaborating in the INFLAME project (the Flemish Institute for Technological Research (VITO)), where the suitability of these non-invasive samples for biomonitoring will be explored.

During the first visit, we also provided the participants with thirteen high density polyethylene (HD-PE) bottles of 500 mL, ten for the mother and three for the child. We asked mothers to collect urine for 24 hours, when possible, or at least twice for both, mother and child. Blood sampling was performed in medical centres. Once the blood sample arrived at the Institute, it was centrifuged at 3000 rpm for 2 minutes and the supernatant was transferred to Nalgene® HD-PE 8 mL bottles (Thermo Scientific). Around 10 mL of serum were obtained and stored at -20°C.

In addition, the participants filled out a questionnaire regarding indoor parameters, a 24 hours food recall and a food frequency questionnaire. At the end of the sampling campaign we had collected almost 700 samples (including field blanks) and more than 200 questionnaires.

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

3.2. Occurrence of Flame Retardants in Indoor Air and Dust

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

FRs are mainly used to reduce the flammability of consumer goods and household materials used in the indoor environments. These FRs can be chemically bounded or simply added to the product material. As mentioned previously, additives can be released over time from their original products and pollute the environment that surrounds us. Human exposure to PBDEs and PFRs has been studied by surveying the FRs in indoor environments [1,2]. As a consequence of previous bans, e.g., PBDE formulations in 2004 [3] and before that, tris(2,3-dibromopropyl) phosphate [4,5], the environmental occurrence of these legacy FRs, and also their replacements, needs to be investigated in households and schools where humans spend much of their time. For this reason, we collected floor dust and air from indoor environments (households and classrooms) in order to survey the levels of persistent and organophosphate FRs in Norway. Due to the comprehensive sampling performed of both dust and air, we had the possibility to investigate whether steady state conditions occurred in the sampling sites.

This section does not contain the development of analytical methodologies since we collaborated with the Toxicological Center located at the University of Antwerp that developed a method for the determination of legacy, emerging and phosphorous FRs in 2012 [1]. The method was successfully applied in the analysis of dust samples from Belgium [1] and U.S.A [2] showing the ubiquity of all targeted FRs, but especially the emerging FRs, which have been detected in both countries.

In order to evaluate the potential hazard of the indoor environment for human health, a risk assessment was performed for the FRs found in air and dust following the guidelines of the U.S. Environmental Protection Agency (USEPA) [6]. Previous European risk assessment studies concluded that dust concentrations in households do not pose a risk to human health [7,8]. However, very few studies take into consideration all three major pathways of exposure in the indoor environment: inhalation of air, dermal absorption and ingestion of dust. We attempted to figure out which of those human exposure pathways are more significant for the human body burden of FRs in children and mothers.

The results of this comprehensive study, and the extensive supplementary information, have been published in collaboration with the Toxicological Center (Antwerp) in Environmental Science and Technology.

References

[1] N. Van den Eede, A.C. Dirtu, N. Ali, H. Neels, A. Covaci, Talanta 89 (2012) 292-300.

[2] R.E. Dodson, L.J. Perovich, A. Covaci, N. Van den Eede, A.C. Ionas, A.C. Dirtu, J.G. Brody, R.A. Rudel, Environ. Sci. Technol. 46 (2012) 13056-13066.

[3] UNEP. http://chm.pops.int/default.aspx (accessed June 2014).

[4] A. Blum, M.D. Gold, B.N. Ames, F.R. Jones, E.A. Hett, R.C. Dougherty, E.C. Horning, I. Dzidic, D.I. Carroll, R.N. Stillwell, J.P. Thenot, Science 201 (1978) 1020-1023.

[5] U.S. CPSC. https://www.cpsc.gov/en/Newsroom/News-Releases/1977/CPSC-Bans-TRIS-Treated-Childrens-Garments/ (accessed June 2014).

[6] USEPA. http://www.epa.gov/oswer/riskassessment/ragsa/ (accessed June 2014).

[7] P.C. Hartmann, D. Bürgi, W. Giger, Chemosphere 57 (2004) 781-787.

[8] S. Harrad, C. Ibarra, M.A.E. Abdallah, R. Boon, H. Neels, A. Covaci, Environ. Int. 34 (2008) 1170-1175.

3.2.1. Occurrence of a Broad Range of Legacy and Emerging Flame Retardants in Indoor Environments in Norway

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Environ. Sci. Technol. 48 (2014) 6827-6835

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

Enrique Cequier,[†,‡,*] Alin C. Ionas,[§] Adrian Covaci,[§] Rosa Maria Marcé,[‡] Georg Becher,[†,||] and Cathrine Thomsen[†]

[†] *Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Norway, P.O. Box 4404, Nydalen, 0403 Oslo, Norway*

[‡] *Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Marcel·lí Domingo s/n, 43007 Tarragona, Spain*

[§] *Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk-Antwerp, Belgium*

[||] *Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo, Norway*

Abstract

This study investigates the occurrence of 37 organohalogen and organophosphate flame retardants (FRs) from Norwegian households (n=48) and classrooms from two primary schools (n=6). Around 80% of the targeted FRs were detected in air and dust from the sampling sites. The comparison of settled dust with floor dust revealed no statistical differences between median concentrations of the FRs (n=12). Decabromodiphenyl ether and tris(2-butoxyethyl) phosphate showed the highest median floor dust concentrations in both environments. In the air samples, the highest concentrations were observed for 2,2´,4,4´-tetrabromodiphenyl ether and tris(1-chloro-2-propyl) phosphate. Remarkably, the emerging FR, 4-(1,2-dibromoethyl)-1,2-dibromocyclohexane, abbreviated as TBECH or DBE-DBCH, showed the highest indoor air concentrations reported in the literature (households, 77.9 pg/m^3 and schools, 46.6 pg/m^3). Good Spearman correlations between the FR concentrations in dust and air (0.36<R<0.76) showed that is possible to estimate the concentrations in air from analyzed dust, or vice versa. Sources and pathways of exposure to FRs were assessed for the households. The main findings were that frequent vacuum cleaning resulted in lower FR concentrations in dust and that dermal contact with dust, for both children and mothers, was as important for the intake of organophosphate FRs as dust ingestion.

INTRODUCTION

Brominated flame retardants (BFRs) and organophosphate flame retardants (PFRs) are chemicals used in a wide range of households and consumer products to reduce their flammability in order to meet the fire regulations. Many of these flame retardants (FRs) are manufactured in high production volumes (>1000 tons/year)[1,2] and their content in the materials can reach percentage amounts by weight.[3,4] Of the main BFRs

produced are the polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane and tetrabromobisphenol-A.[4] Except tetrabromobisphenol-A, these BFRs have now been classified as persistent organic pollutants under the Stockholm Convention due to their persistence, long-range air transport, bioaccumulation and toxicity to the environment and human health.[5] The production of two commercial formulations of PBDEs (PentaBDE and OctaBDE) has ceased and DecaBDE is presently phased-out in the U.S.A., listed in Europe for authorization under the EU regulatory framework for Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) in 2010, and banned in Norway since 2008.[6] Replacements for the banned BFRs comprise both halogenated FRs and PFRs and are often termed "emerging FRs". Despite their phase-out, PBDEs are still present in consumer products and may leach into indoor environments.[1] Contamination of the indoor environments is commonly surveyed by collection and analysis of air and dust. Different strategies have been assessed in order to collect representative samples from various microenvironments: dust from vacuum cleaner bags,[7,8] collected dust from a delimited floor area[9-11] or the entire floor area[12,13], and dust from elevated surfaces[14] using nylon socks[15] or forensic filters[16] in front of 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[10,13] although most studies use pumps for active sampling. Typically, polyurethane foam (PUF) has been used to trap volatile PBDEs[17] and PFRs[18] from air, while polytetrafluoroethylene[9] or glass fiber[16] filters have been used to separate the FRs adsorbed onto suspended particles.

Most emerging FRs have been put in use because of their similarity in properties and structure to existing FRs. For example, decabromodiphenyl ethane (DBDPE) replaced decabromodiphenyl ether (BDE-209),[1] 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE), replaced the OctaBDE formulation,[1] and formulations containing 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) and bis(2-ethylhexyl)-3,4,5,6-tetra-bromophthalate (BEH-TEBP) are used as replacement for the PentaBDE formulation in the U.S.A.[19] These latter chemicals have been reported in dust from indoor environments at ng/g levels in U.S.A., Belgium and the U.K.[20,21] Other emerging FRs, such as hexabromobenzene (HBB), Dechlorane Plus® (DDC-CO), Dechlorane 602 (DDC-DBF) and Dechlorane 603 (DDC-Ant) have been detected in human serum from Norway at pg/g lipid weight levels.[22] Hence, these replacements pose a potential risk to the environment and human health.[23]

To further compensate for the ban of the major BFRs, the use of PFRs has increased in recent years. In 2006, 20% of the world consumption of FRs was based on PFRs, while BFRs only contributed around 10%.[2] Moreover, the production of PFRs in Western Europe increased almost 10% between 2001 and 2006.[24] Organo-phosphates are added to a wide range of materials both as FRs and plasticizers. Chlorinated alkyl organophosphates, such as tris(2-chloroethyl) phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCIPP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), are commonly added to PUFs as replacement for the PentaBDE formulation.[25] Aryl phosphates, such as triphenyl (TPHP) or tricresyl (TCP)

phosphates, are mainly used as FRs and non-chlorinated alkyl phosphates are primarily used as plasticizers (e.g., tri-*n*-butyl phosphate (TNBP)).[2] In general, PFRs are less persistent and bioaccumulative than BFRs,[26] although, there are indications that chlorinated alkyl PFRs, such as TCEP, might be carcinogenic.[27] PFRs and related plasticizers have been detected at higher levels than BFRs in air and dust. The median dust concentration of tris(2-butoxyethyl) phosphate (TBOEP) detected in households from the U.S.A. was 12 μg/g[21] and TCIPP was detected in Swedish[14] and Japanese[28] residences at 1.6 and 19.7 μg/g, respectively.

Some studies point out that indoor dust is a major source for human exposure to PBDEs[7,21,29,30] and PFRs.[13] 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)[15,31] and PFRs and plasticizers present the same tendency in different risk assessment studies.[11,18] However, in highly contaminated environments, and in particular for small children, for whom the dust intake rates can be high, this exposure poses a potential health risk.[21]

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 in Norwegian households and 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. Further, we wanted to compare the contents of FRs in settled dust from elevated surfaces with floor dust collected from the same residences and to investigate the feasibility of estimating FR concentrations in air from measured concentrations in dust.

MATERIALS AND METHODS

Sample Collection. Through two primary schools in the greater Oslo area (Norway), 48 households were recruited for sample collection. In addition, sampling was performed in six classrooms of these schools.

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 (see Supporting Information (SI) for further details about questionnaires and samples).

Indoor Air. Air sampling was carried out during winter and partially in spring in order to have minimal ventilation of 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 downward 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.

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).

Selection of Flame Retardants. Organic brominated, chlorinated and phosphorus-based flame retardants were chosen for the study of indoor environments due to their proven ubiquity and potential hazard for human health.[1,2] SI Table S1 summarizes the FRs and their abbreviations, following the standard proposed by Bergman *et al.*,[32] as well as the method limits of detection (MLD) and the octanol-air partition coefficients (K_{oa}).

Analytical Method and Quality Control. The analytical method for the analysis of the dust has been published elsewhere,[33] and the same method, with slight modifications, was also applied to the air samples. Briefly, dust or the air sample consisting of 8 PUFs and 4 quartz filters were extracted 3 times using hexane/acetone (3:1; v/v) in an ultrasonic bath. Fractionation of FRs was performed on Florisil® (Supelco, Bellefonte, PA, U.S.A.). BFRs and dechloranes from fraction 1 and PFRs from fraction 2 were determined on a GC-ECNI/MS (Agilent Technologies, Santa Clara, CA, U.S.A.) and GC-EI/MS (Agilent Technologies), respectively (see SI for further details).

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[11] from SRM2585 ranged from 78 to 107% (mean 95%; RSD 12%; excluding TCP) (SI Table S2). Recoveries of the internal standards in air and dust ranged from 43% to 111% (except TBOEP 172% and 268%, respectively) (SI Table S3).

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 (SI Table S4).

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). Nondetects were replaced by half of the MLD. 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.

Environ. Sci. Technol. 48 (2014) 6827-6835

Finally, only those multiple linear regression models having two or more significant parameters were discussed.

RESULTS AND DISCUSSION

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

Air Samples. Twenty nine out of the 37 measured FRs were detected in indoor air samples from households and schools (Figure 1), of which 13 and 12, respectively were detected in at least 90% of the samples (SI Table S5).

BFRs. In residential living rooms and primary school classrooms, 4-(1,2-dibromoethyl)-1,2-dibromocyclohexane (DBE-DBCH), 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 DBE-DBCH (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 TBP-AE were detected frequently (>50%), whereas in the classrooms both medians were below MLD. 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 the median of BDE-209 was below MLD with a maximum of 101 pg/m^3. The reason for the detection of a compound with such a low vapor pressure in air samples is because BDE-209 is adsorbed to the fine particles suspended in the air.[34] Those particles are retained on the glass fiber filter which was extracted together with the PUF plug.

DBE-DBCH was the second most abundant FR in both indoor environments. There is no production of DBE-DBCH in Norway and, to the best of our knowledge; these are so far the highest concentrations of DBE-DBCH reported in indoor environments. As DBE-DBCH has been identified as a potential endocrine disruptor,[35] the relatively high abundance in indoor air raises concern about this emerging FR. Another interesting finding is the abundance of HBB, detected at median concentrations of around 4 pg/m^3 in households and schools. HBB is used in indoor materials and consumer goods (wood, textiles, electronics and plastics),[1] and it has previously been detected in outdoor air in Norway as well.[36]

In general, levels of non-PBDE 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),[9] comparable to Denmark[37] and higher than in Swedish households[16] (SI Figure S1).

PFRs. Overall concentrations of PFRs in air were around 2 orders of magnitude higher than BFRs (Figure 1), as expected due to the typically higher vapor pressures of PFRs (SI Table S1) as well as higher production volumes. TBOEP was the most abundant PFR in classrooms with a median

Environ. Sci. Technol. 48 (2014) 6827-6835

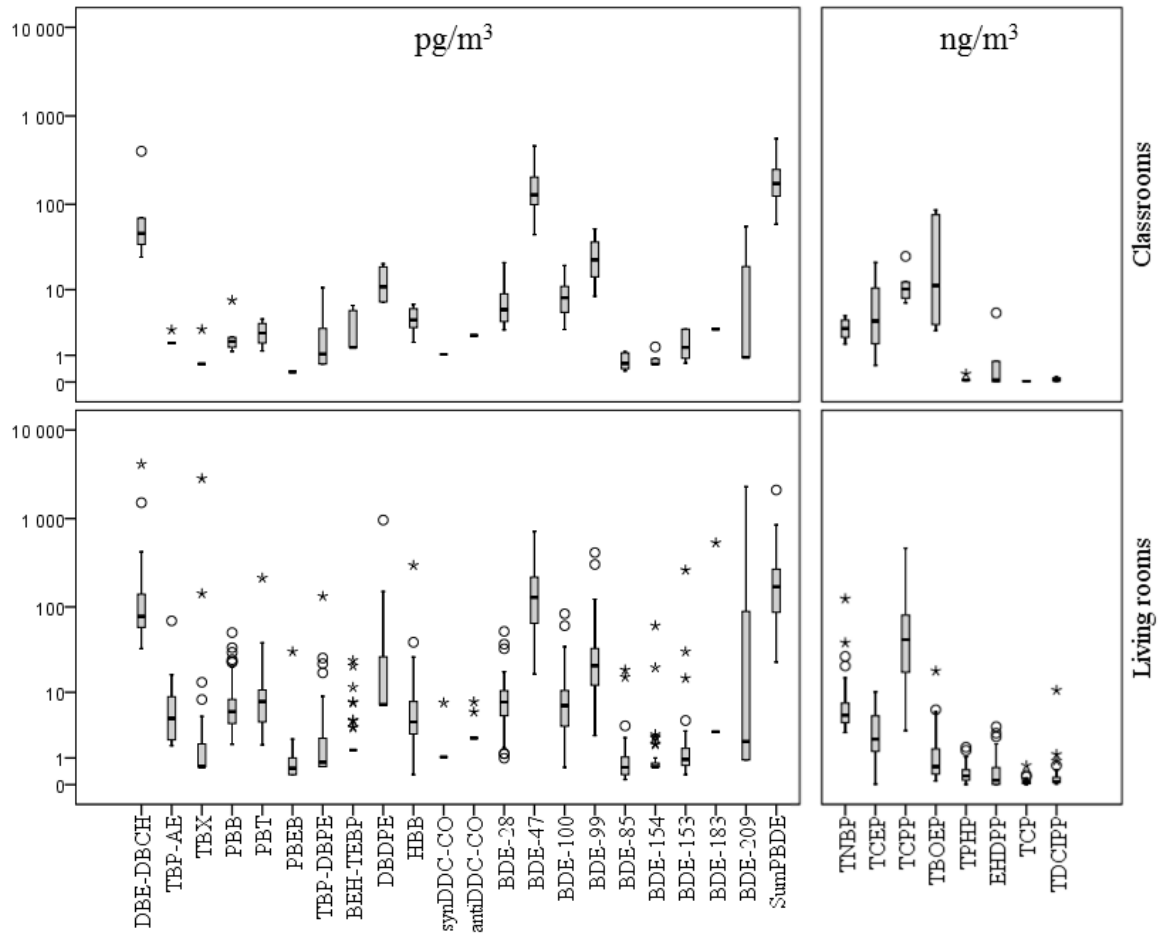


Figure 1. Box plots of air concentrations of the BFRs, dechloranes and PFRs in living rooms (n=47) and classrooms (n=6).

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.[2,8] In living rooms, TCIPP had the highest median concentration (42.3 ng/m^3), whereas in classrooms the median was 10.2 ng/m^3. Although median concentrations of TNBP, TPHP, EHDPP and TDCIPP were higher in living rooms than in classrooms (SI Table S5), PFRs levels 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,[14] except TCIPP for which they were almost 1 order of magnitude higher in Norway. Nevertheless, the PFR concentrations were much lower than the levels detected in residential dwellings in Japan[28] (TCIPP 89.2, TNBP 27.1 and TCEP 15.5 ng/m^3) (SI Figure S2).

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.[1,38] Several other correlations were

found between FRs, but it was difficult to assess whether the association was causal or accidental (SI Table S6).

Dust Samples. Thirty-one out of the 37 target FRs were found in floor dust samples (Figure 2). In households and schools, 23 and 19 analytes, respectively, were detected at least in 90% of the dust samples (SI Table S7).

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 BEH-TEBP (78.5 ng/g). BDE-209 was also the most abundant BFR in classrooms, (507 ng/g), followed by DBDPE (156 ng/g) and BEH-TEBP (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 ($t(40)=0.067$; $p=0.947$).

PBDE concentrations in dust from Norwegian households were much lower than in dust from U.S.A. (California),[21] but higher than those reported in urban areas from China,[39] Belgian homes, and classrooms from the U.K.[20] 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) (SI Figure S3a). The ratio of BDE-209 to DBDPE might give an indication of the level of replacement of BDE-209 by 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).

BEH-TEBP levels in dust from this study was comparable to dust from other studies conducted in U.K. classrooms, higher than in households from Belgium[20] and much lower than in California[21] (SI Figure S3b). The high levels of EH-TBB and BEH-TEBP seen in the study from California are suggested to be 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 BEH-TEBP.[21] In contrast, Norwegian dust contained a substantial excess of BEH-TEBP. Therefore, assuming similar persistence of EH-TBB and BEH-TEBP in the indoor environment, the elevated levels of BEH-TEBP found in our study originated probably from other sources than commercial Firemaster mixtures, reflecting a different usage pattern of BEH-TEBP compared to the U.S.A. Considerable amounts of BTBPE (~5 ng/g), DBE-DBCH (~2 ng/g), EH-TBB (~3 ng/g), and DDC-CO (*anti*, ~3 ng/g and *syn*, ~1 ng/g) were detected in living rooms and classrooms. For DDC-CO, the fraction of the *anti* isomer was within the range reported for the technical mixture (0.65-0.75),[40,41] confirming the presence of Dechlorane Plus® in consumer products in Norway.

Environ. Sci. Technol. 48 (2014) 6827-6835

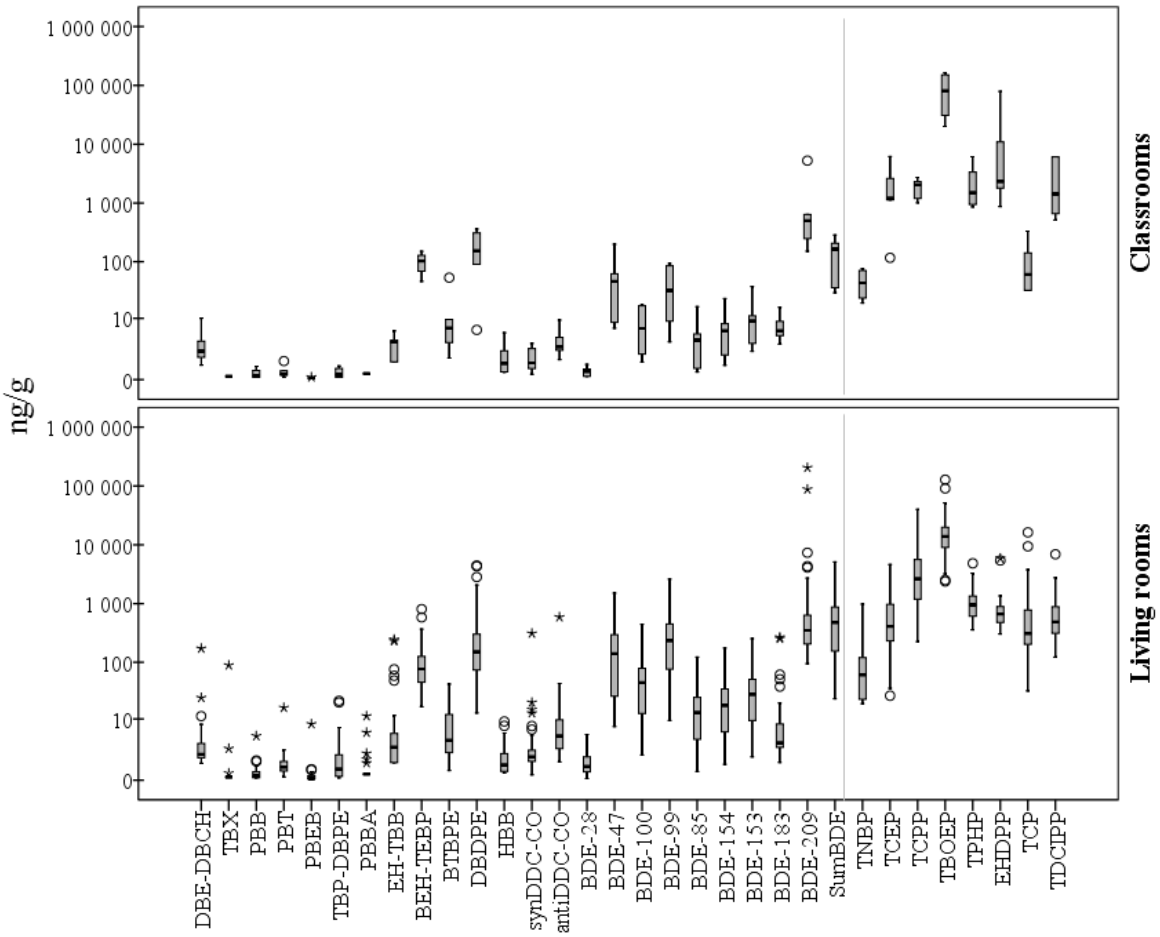


Figure 2. Box plots of dust concentrations (ng/g) of the BFRs, dechloranes and PFRs in living rooms (n=48) and classrooms (n=6). Y-axis is in log scale.

PFRs. The overall concentrations of PFRs in dust were approximately 1 order of magnitude higher than BFRs (Figure 2). TBOEP was detected at highest median concentrations in classrooms (87 200 ng/g) and living rooms (13 400 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 common use in floor polishing.[2,8] TCIPP was the second most abundant PFRs in both environments with similar median concentrations (2680 and 2040 ng/g in households and classrooms, respectively). The median concentrations of EHDPP, TDICPP, TPHP, and TCEP were significantly higher in classrooms than in living rooms (SI Table S7) suggesting higher use of these FRs in materials in 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, TCIPP, TPHP and TDCIPP in dust of this study were similar to those from other studies conducted in Belgium,[11] Sweden[14] or Spain[42] and only TBOEP and EHDPP were slightly above the overall mean values. However, all PFRs were found at much lower concentrations than in dust from a Japanese study (TBOEP and TCIPP were 1 570 000 ng/g and 18 700 ng/g, respectively)[28] (SI Figure S4).

Correlations between FRs in Dust. As already observed in air samples, high

Environ. Sci. Technol. 48 (2014) 6827-6835

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) (SI Table S8). 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-DDC-CO. BEH-TEBP and EH-TBB were moderately 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/BEH-TEBP in the dust samples. Interesting correlations (0.38<R<0.44; p<0.01) were obtained for HBB with DDC-CO, PBB, DBDPE, BDE-183 and TNBP. In addition, HBB was less strongly correlated with TCEP, TCIPP, TCP and TDCIPP (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 any differences between floor and settled dust (Figure 3).

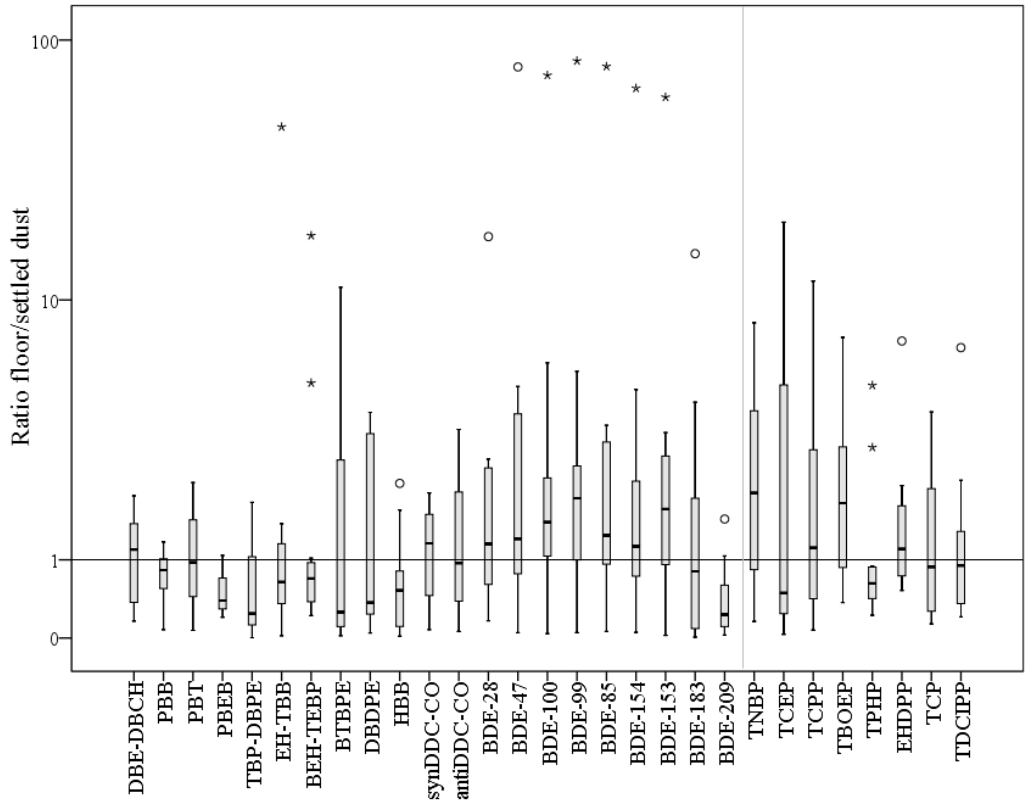


Figure 3. Box plot of concentration ratios of BFRs, dechloranes, and PFRs in floor and settled dust (n=12). Note Y-axis is in log scale.

Environ. Sci. Technol. 48 (2014) 6827-6835

The concentration ratios of PFRs in floor and settled dust 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[15] 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 uncontaminated outdoor particles as well as 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.

Correlations between FR Concentrations in Air and Dust. Spearman rank analyses gave good correlations between concentrations in air and dust ($0.36<R<0.76$) (Table S9) and highly significant linear regression correlations were obtained for more volatile FR, e.g., DBE-DBCH, 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 (Table S10). Similar relationships were observed by Fromme *et al*.[17] Levels in air and dust of the PFRs TCIPP, TNBP, TBOEP, and TCEP were also significantly correlated in the linear regression models (Table S10). Again, similar relationships were observed by Bergh *et al*. for TNBP, TCEP and TCIPP.[14] 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 ($K_{dust-air}$) is expected to be proportional to the K_{oa}, which has been used to describe the sorption of FRs to organic matter in dust.[43] In Figure 4, the average of the $K_{dust-air}$ for the FRs are plotted against their K_{oa}.

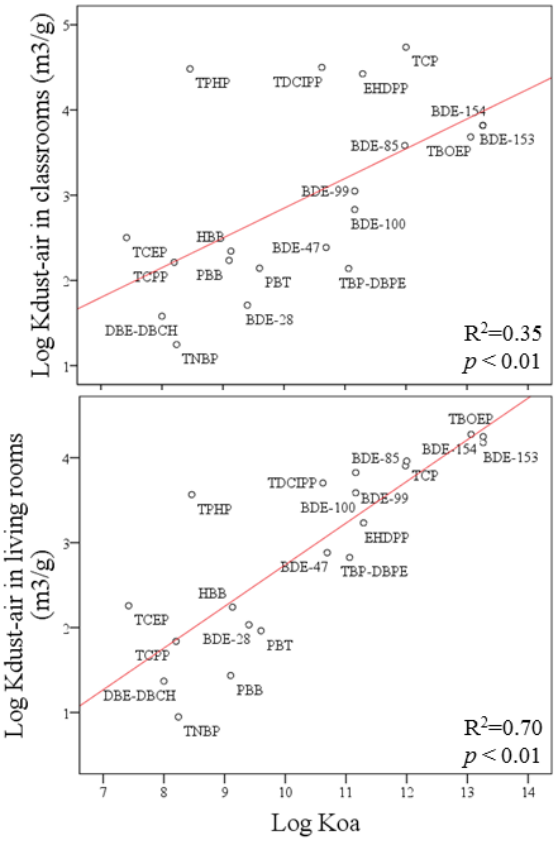


Figure 4. Log $K_{dust-air}$ plotted against log K_{oa} of the FRs in living rooms (below) and classrooms (above).

The significantly 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.

Environ. Sci. Technol. 48 (2014) 6827-6835

Consequently, by analyzing 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 (see example in SI).[43] 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.

Sources of Exposure in Indoor Environments. Chemicals in consumer goods and household materials may pollute the indoor environment. Most FRs are additives and not chemically bounded in the different products.[44] Consequently, they can leach out of the products and contaminate air and 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.

A bivariate analysis (Spearman rank correlation) was performed to identify factors that were relevant for more detailed analysis (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) (some examples in the SI; Figure S5). Only occasional associations were seen for other parameters in the multiple linear models (data not shown).

Positive correlations between tri- to heptaBDE (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 (Table S11). However, negative correlations were obtained in dust for DBE-DBCH, PBB, 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 that 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 (Table S11), 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 (Table S11), suggesting that households without this heating system contain higher levels of these FRs. Size of living rooms also correlated positively in dust with TCIPP and negatively with BDE-28 and BDE-47 in dust and TBP-DBPE in air.

Since only few consistent correlations were seen, the dispersal of FRs is probably highly affected by the spatial variability within the home[12] 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.

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" exposure 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 USEPA risk assessment guidance[45] (SI Table S12).

BFRs. As expected, the main route of exposure for less volatile FRs (vapor pressures $>10^{-7}$ Torr) was dust ingestion for both, women and children, followed by dermal contact and to a small degree by inhalation (Figure 5 and Table S13). In contrast, for the more volatile compounds, e.g., DBE-DBCH and pentabromobenzene (PBB), 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 (SI Table S14). Children have less skin uptake and smaller inhalation volumes than women,[45] but since averaged residence time in the households for children is assumed higher and body weight lower, these factors drive their intake. If estimated oral intakes (dust ingestion) of the most exposed group (children) are compared with oral RfDs (SI Table S13) for BDE-47,[46] BDE-99,[47] BDE-153,[48] and BDE-209,[49] then the intake is 344, 267, 3390, and 6151 times lower than the RfDs, respectively. Even when assuming a high-end scenario using maximum concentrations (SI Table S7) and double intake factors, the values are still around 1 order of magnitude lower than the RfDs.

PFRs. Air contributes significantly to human intakes of organophosphates with vapor pressures $>10^{-5}$ Torr. For example, for children and women, the intake in households through inhalation of TNBP, TCIPP, and TCEP is approximately 85, 49 and 25%, respectively (SI Table S14). For PFRs with lower vapor pressures (e.g., TDCIPP), dermal absorption contributes 45% to the intake for children and 58% for women, whereas ingestion of dust contributes 54 and 41%, respectively. This higher contribution of dermal uptake of PFRs compared to BFRs is notable in Figure 5 and is attributed to the higher absorption factor used in the calculation[50,51]. 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*.[52] (SI Table S13), the PFR daily intakes are some orders of magnitude lower than the RfD values, TBOEP being the highest with a high-end scenario of only 10 times lower intake than its RfD.

Environ. Sci. Technol. 48 (2014) 6827-6835

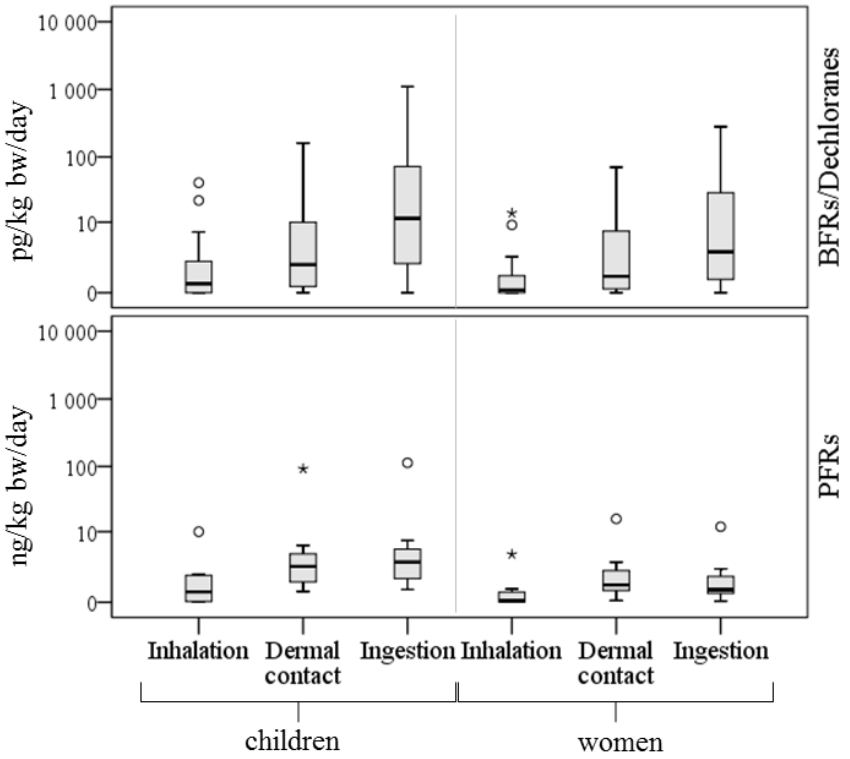


Figure 5. Comparison of the intake of PFRs (ng/kg bw/day) and BFRs and dechloranes (pg/kg bw/day) for children and women through the inhalation of air, dermal contact, and ingestion of dust from indoor environments. Note Y-axis is in log scale.

ASSOCIATED CONTENT

Supporting information available

Supplementary tables, figures and equations are comprised in the supporting information. This information is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding author

*E-mail: enrique.cequier@fhi.no

Tel.: +4721076604; fax: +4721076686

ACKNOWLEDGMENTS

This study was funded by the European Commission, 7th Framework Programme under the E.U. Marie Curie Initial Training Network INFLAME (GA No. 264600) and the Research Council of Norway.
The Department of Applied Environmental Science (ITM) from the University of Stockholm and the Norwegian Institute for Air Research (NILU) are gratefully acknowledged for supplying equipment for air sampling. Dr. Hidetoshi Kuramochi is kindly acknowledged for fruitful discussions and Dr. Amrit Sakhi is deeply acknowledged for her splendid organization of the sampling campaign. Finally, participants are greatly acknowledged for their willingness to participate in the study.

REFERENCES

(1) Covaci, A.; Harrad, S.; Abdallah, M.A.E.; Ali, N.; Law, R.J.; Herzke, D.; de Wit, C.A. *Environ. Int*. **2011**, 37 (2), 532-556.

(2) van der Veen, I.; de Boer, J., *Chemosphere* **2012,** *88* (10), 1119-1153.

(3) Kajiwara, N.; Noma, Y.; Takigami, H. *J. Hazard. Mater.* **2011,** *192* (3), 1250-1259.

(4) Alaee, M.; Arias, P.; Sjödin, A.; Bergman, Å. *Environ. Int.* **2003,** *29* (6), 683-689.

(5) UNEP. Stockholm Convention on POPs. http://chm.pops.int/default.aspx. 2004 (accessed May 2014).

(6) BSEF, http://www.bsef.com/ (accessed May 2014).

(7) Johnson-Restrepo, B.; Kannan, K. *Chemosphere* **2009,** *76* (4), 542-548.

(8) Marklund, A.; Andersson, B.; Haglund, P. *Chemosphere* **2003,** *53* (9), 1137-1146.

(9) Batterman, S.; Godwin, C.; Chernyak, S.; Jia, C.; Charles, S. *Environ. Int.* **2010,** *36* (6), 548-556.

(10) Muenhor, D.; Harrad, S.; Ali, N.; Covaci, A. *Environ. Int.* **2010,** *36* (7), 690-698.

(11) Van den Eede, N.; Dirtu, A. C.; Neels, H.; Covaci, A. *Environ. Int.* **2011,** *37* (2), 454-461.

(12) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Webster, T. F. *Environ. Int.* **2008,** *34* (8), 1085-1091.

(13) Takigami, H.; Suzuki, G.; Hirai, Y.; Ishikawa, Y.; Sunami, M.; Sakai, S. *Environ. Int.* **2009,** *35* (4), 688-693.

(14) Bergh, C.; Torgrip, R.; Emenius, G.; Östman, C. *Indoor Air* **2011,** *21* (1), 67-76.

(15) Harrad, S.; Ibarra, C.; Abdallah, M. A.; Boon, R.; Neels, H.; Covaci, A. *Environ. Int.* **2008,** *34* (8), 1170-1175.

(16) Björklund, J. A.; Thuresson, K.; Cousins, A. P.; Sellström, U.; Emenius, G.; de Wit, C. A. *Environ. Sci. Technol.* **2012,** *46* (11), 5876-5884.

(17) Fromme, H.; Körner, W.; Shahin, N.; Wanner, A.; Albrecht, M.; Boehmer, S.; Parlar, H.; Mayer, R.; Liebl, B.; Bolte, G. *Environ. Int.* **2009,** *35* (8), 1125-1135.

(18) Hartmann, P. C.; Bürgi, D.; Giger, W. *Chemosphere* **2004,** *57* (8), 781-787.

(19) USEPA. http://www.epa.gov/dfe/-pubs/flameret/ffr-alt.htm (accessed May 2014).

(20) Ali, N.; Harrad, S.; Goosey, E.; Neels, H.; Covaci, A. *Chemosphere* **2011,** *83* (10), 1360-1365.

(21) Dodson, R. E.; Perovich, L. J.; Covaci, A.; Van den Eede, N.; Ionas, A. C.; Dirtu, A. C.; Brody, J. G.; Rudel, R. A. *Environ. Sci. Technol.* **2012,** *46* (24), 13056-13066.

(22) Cequier, E.; Marcé, R. M.; Becher, G.; Thomsen, C. *J. Chromatogr. A* **2013,** *1310* (0), 126-132.

(23) UNEP/WHO http://www.unep.org/-annualreport/2012/ (accessed May 2014).

(24) Reemtsma, T.; Quintana, J. B.; Rodil, R.; García-López, M.; Rodríguez, I. *TrAC-Trends Anal. Chem.* **2008,** *27* (9), 727-737.

(25) Stapleton, H. M.; Klosterhaus, S.; Eagle, S.; Fuh, J.; Meeker, J. D.; Blum, A.; Webster, T. F. *Environ. Sci. Technol.* **2009,** *43* (19), 7490-7495.

(26) Pakalin, S.; Cole, T.; Steinkellner, J.; Nicolas, R.; Tissier, C.; Munn, S.; Eisenreich, S. European Commission, 2007, http://publications.jrc.ec.europa.eu/repository/handle/111111111/5259 (accessed May 2014).

(27) European Commission. http://echa.-europa.eu/documents/10162/f42be21b-33a3-4063-ad4d-2b0f937e41b4 (accessed May 2014).

(28) Kanazawa, A.; Saito, I.; Araki, A.; Takeda, M.; Ma, M.; Saijo, Y.; Kishi, R. *Indoor Air* **2010,** *20* (1), 72-84.

(29) Allen, J. G.; McClean, M. D.; Stapleton, H. M.; Nelson, J. W.; Webster, T. F. *Environ. Sci. Technol.* **2007,** *41* (13), 4574-4579.

(30) Harrad, S.; Goosey, E.; Desborough, J.; Abdallah, M. A. E.; Roosens, L.; Covaci, A. *Environ. Sci. Technol.* **2010,** *44* (11), 4198-4202.

(31) Chen, S. J.; Ma, Y. J.; Wang, J.; Tian, M.; Luo, X. J.; Chen, D.; Mai, B. X. *J. Hazard. Mater.* **2010,** *176* (1-3), 979-984.
(32) Bergman, Å; Rydén, A.; Law, R. J.; de Boer, J.; Covaci, A.; Alaee, M.; Birnbaum, L.; Petreas, M.; Rose, M.; Sakai, S.; Van den Eede, N.; van der Veen, I. *Environ. Int.* **2012,** *49*, 57-82.
(33) Van den Eede, N.; Dirtu, A. C.; Ali, N.; Neels, H.; Covaci, A. *Talanta* **2012,** *89* (0), 292-300.
(34) Webster, T. F.; Harrad, S.; Millette, J. R.; Holbrook, R. D.; Davis, J. M.; Stapleton, H. M.; Allen, J. G.; McClean, M. D.; Ibarra, C.; Abdallah, M. A. E.; Covaci, A. *Environ. Sci. Technol.* **2009,** *43* (9), 3067-3072.
(35) Khalaf, H.; Larsson, A.; Berg, H.; McCrindle, R.; Arsenault, G.; Olsson, P. *Environ. Health Perspect.* **2009,** *117* (12), 1853-1859.
(36) Arp, H. P.; Moskeland, T.; Andersson, P. L.; Nyholm, J. R. *J. Environ. Monit.* **2011,** *13* (3), 505-513.
(37) Vorkamp, K.; Thomsen, M.; Frederiksen, M.; Pedersen, M.; Knudsen, L. E. *Environ. Int.* **2011,** *37* (1), 1-10.
(38) WHO, Environmental Health Criteria 162. Geneva, Switzerland. 1994.
(39) Zheng, J.; Luo, X. J.; Yuan, J. G.; Wang, J.; Wang, Y. T.; Chen, S. J.; Mai, B. X.; Yang, Z. Y. *Environ. Pollut.* **2011,** *159* (12), 3706-3713.
(40) Sverko, E.; Tomy, G. T.; Reiner, E. J.; Li, Y. F.; McCarry, B. E.; Arnot, J. A.; Law, R. J.; Hites, R. A. *Environ. Sci. Technol.* **2011,** *45* (12), 5088-5098.
(41) Tomy, G. T.; Pleskach, K.; Ismail, N.; Whittle, D. M.; Helm, P. A.; Sverko, E.; Zaruk, D.; Marvin, C. H. *Environ. Sci. Technol.* **2007,** *41* (7), 2249-2254.
(42) García, M.; Rodríguez, I.; Cela, R. *J. Chromatogr. A* **2007,** *1152* (1-2), 280-286.
(43) Weschler, C. J.; Nazaroff, W. W. *Atmos. Environ.* **2010,** *44* (30), 3609-3620.
(44) EFRA. http://www.cefic-efra.com (accessed May 2014).
(45) USEPA. http://www.epa.gov/oswer-/riskassessment/ragsa/ (accessed May 2014).
(46) USEPA. http://www.epa.gov/iris-/toxreviews/1010tr.pdf (accessed May 2014).
(47) USEPA. http://www.epa.gov/iris/-toxreviews/1008tr.pdf (accessed May 2014).
(48) USEPA. http://www.epa.gov/iris/-toxreviews/1009tr.pdf (accessed May 2014).
(49) USEPA. http://www.epa.gov/iris/-toxreviews/0035tr.pdf (accessed May 2014).
(50) USEPA. http://cfpub.epa.gov/ncea/-cfm/recordisplay.cfm?deid=85843 (accessed May 2014).
(51) Ulsamer, A. G.; Porter, W. K.; Osterberg, R. E. *J. Environ. Pathol. Toxicol.* **1978,** *1* (5), 543-549.
(52) Ali, N.; Dirtu, A. C.; Van den Eede, N.; Goosey, E.; Harrad, S.; Neels, H.; Mannetje, A.; Coakley, J.; Douwes, J.; Covaci, A. *Chemosphere* **2012,** *88* (11), 1276-1282.

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Supporting Information

MATERIALS AND METHODS

Chemicals and reagents. The organophosphate standards TNBP, TPHP, TDCIPP (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.). TCIPP (3 isomers) and TAP were purchased from Pfaltz & Bauer (Waterbury, CT, U.S.A.) and TCI Europe (Zwijndrecht, Belgium), respectively. TCEP-d12, TDCIPP-d15 and TBOEP-d6 were synthesized by Dr. Vladimir Belov (KAdemCustomChem GmbH, Göttingen, Germany). 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® (DDC-CO; *syn* and *anti* isomers) were purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.), Dechlorane 602 (DDC-DBF) and Dechlorane 603 (DDC-Ant) (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, BEH-TEBP, DBDPE, DBE-DBCH, TBP-AE, BATE, TBP-DBPE, BTBPE, DBHCTD, TBCO, OBTMPI, TBX, PBT, PBB, PBEB and PBBA 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 purchase from Acros Organics (Geel, Belgium). *Iso*-octane, toluene, dichloromethane, ethyl acetate and acetone were purchased from Merck Chemicals (Darmstadt, Germany).

Sample collection. Considered demographic information from the participants was age, height and weight. Parameters of the households considered in the questionnaires were 27: [1] Age of the women (year), [2] Building construction year (year), [3] Years lived in the household, [4] Number of inhabitants in the household, [5] Size of the apartment in m^2, [6] Size of the living room in m^2, [7] Number of picture tube TVs in the household, [8] Number of flat screen type TVs, [9] Number of DVD and Video players, [10] Number of consoles, [11] Number of CD and MP3 players, [12] Number of PCs, [13] Number of laptops, [14] Number of radios, [15] Number of telephones, [16] Total electronic devices in the household, [17] Number of vacuum cleaning in the living room per week, [18] Distance of the sampling equipment from TV in the living room (m), [19] Humidity in the living room (%), [20] Temperature in the living room (°C), [21] Location of the house (Rural/Urban), [22] Type of

household (non-attached/attached), [23] Electric heating system (no/yes), [24] Fireplace in the living room (no/yes), [25] Carpets in the living room (no/yes), [26] Chairs made of PUF in the living room (no/yes), [27] 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. One sample of air was not collected due to malfunctioning of the pump. 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. 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 aluminium foil after cooling.

Indoor dust. Large particles and hair were removed with stainless steel tweezers. The dust was kept on the forensic filters, wrapped in aluminium foil, put into a sealed low-density polyethylene bag and stored in the freezer at -20°C.

Table S1. Chemical names, abbreviations according Bergman *et al.*[1] (previously used abbreviations also provided in parenthesis), [a]physico-chemical properties and method limits of detection (MLD) of flame retardants in this study

	Brominated flame retardants and dechloranes	Abbreviation (previously used)	Vapour pressure (Torr)	Log $^{b}K_{oa}$	^{c}Air MLD (pg/m^3)	^{c}Dust MLD (ng/g)
1	^{d}4-(1,2-Dibromoethyl)-1,2-dibromocyclohexane	^{d}DBE-DBCH (TBECH)	2.2 E-5	8.0	0.88	0.20
2	^{d}1,2,5,6-Tetrabromocyclooctane	^{d}TBCO	3.6E-5	8.0	nd	nd
3	2,4,6-Tribromophenyl allyl ether	TBP-AE (ATE)	1.8 E-4	8.6	3.5	nd
4	1,2,4,5-Tetrabromo-3,6-dimethylbenzene	TBX	4.4E-5	8.8	1.2	0.27
5	Pentabromobenzene	(PBB)	9.3E-6	9.1	1.5	0.20
6	Pentabromotoluene	PBT	4.5E-6	9.6	0.59	0.20
7	Pentabromoethylbenzene	PBEB	1.2E-6	10.0	0.59	0.13
8	2,4,6-Tribromophenyl 2,3-dibromopropyl ether	TBP-DBPE (DPTE)	9.5E-8	11.1	1.2	0.20
9	2-Ethylhexyl 2,3,4,5-tetrabromobenzoate	EH-TBB (TBB)	2.8E-9	12.3	7.4	2.0

Table S1. Continued

10	Bis(2-ethylhexyl) tetrabromophthalate	BEH-TEBP (TBPH)	1.2E-13	16.9	2.9	4.0
11	Decabromodiphenyl ethane	DBDPE	6.0E-15	19.2	14	12
12	Hexabromobenzene	HBB	8.5E-7	9.1	2.9	0.67
13	[d]Bis(hexachlorocyclopentadieno)cyclooctane	[d]DDC-CO (DP)	1.0E-13	14.8	2.1 syn 4.7 anti	0.47syn 1.1anti
14-22	Polybrominated diphenyl ethers (BDE-28, 47, 85, 99, 100, 153,154, 183, 209) and [e]BDE-77 and 128	PBDE	6.7E-7 4.7E-12	9.4 18.4	0.59-1.8	0.13-0.47
23	Hexachlorocyclopentadienyl-dibromocyclooctane	DBHCTD (HCDBCO)	6.2E-9	11.0	nd	nd
24	1,2-Bis(2,4,6-tribromophenoxy)ethane	BTBPE	2.9E-12	15.7	nd	0.93
25	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	DDC-DBF (Dec602)	1.1E-11	15.0	nd	nd
26	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	DDC-Ant (Dec603)	6.9E-12	15.2	nd	nd
27	Octabromo-1,1,3-trimethyl-1-phenylindan	OBTMPI (OBIND)	1.3E-14	nd	nd	nd
28	Pentabromobenzyl acrylate	PBB-Acr (PBBA)	1.3E-7	12.4	nd	0.53
29	2-bromoallyl-2,4,6-tribromophenyl ether	(BATE)	nd	9.6	nd	nd
	Organophosphate esters	**Abbreviation**	**Vapour pressure (Torr)**	**Log $^{b}K_{oa}$**	**Air MLD (pg/m3)**	**Dust MLD (ng/g)**
30	[f]Tri-*n*-butyl phosphate	[f]TNBP (TnBP; TBP)	1.13E-03	8.2	29	37
31	Tris(chloroethyl) phosphate	TCEP	1.08E-4	7.4	29	32
32	[g]Tris(2-chloroisopropyl) phosphate	[g]TCIPP	2.02E-05	8.2	29	5.3
33		[f]TBOEP	2.50E-08	13.1	44	22

	[f]Tris(2-butoxyethyl) phosphate	(TBEP)				

Table S1. Continued

34	Triphenyl phosphate	TPHP (TPP)	6.28E-06	8.5	18	2.7
35	2-Ethylhexyl diphenyl phosphate	(EHDPP)	2.55E-6	11.3	18	3.7
36	[h]Tricresyl phosphate (*ortho*, *meta*, *para*)	([h]TCP)	6.00E-07	12.0	35	64
37	[d]Tris(1,3-dichloroisopropyl) phosphate	[d]TDICPP	4.07E-8	10.6	18	7.6
	[e]Tri-*iso*-amyl phosphate	([e]TAP)				

[a] Collected from different sources (EPISuite® and Chemdraw software, material safety data sheets, etc); [b] K_{oa} = octanol air partition coefficient; [c]MLD 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 plasticizer; [g] Mixture of 3 isomers; [h] Mixture of 4 isomers; nd, not detected

Analytical method and quality control . Forty 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 of internal standards, ranging from 1.25 to 150 ng, were added (^{13}C-HBB, ^{13}C-syn-DDC-CO, ^{13}C-anti-DDC-CO, ^{13}C-BDE-209, BDE-77, BDE-128, TCEP-d12, TPHP-d15, TBOEP-d6, TDCIPP-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, BEH-TEBP, PBBA 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 analyzed 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, from SRM2585 ranged from 78 to 107% (mean 95%; RSD 12%; excluding TCP) (Table S2). 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. 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) (Table S3).

The MLD was defined as S/N≥3 and determined experimentally. MLD for BFRs in air and dust samples ranged from 0.59 to 14 pg/m^3 and from 0.13 to 12 ng/g. MLD for PFRs in air and dust samples ranged from 18 to 44 pg/m^3 and from 2.7 to 64 ng/g (Table S1).

Table S2. 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
TCIPP	1063	11	820	94
[b]TBOEP	45795	7	49000	107
TPHP	1052	13	990	94
TDCIPP	1933	14	2020	105
TCP	nd		1070	

[a] Van den Eede *et al.* Environ. Int. 37 (2011) 454-461; [b]Used as plasticizer

Table S3. Amount of FRs (ng) and RSD (%) in parentheses in the air and dust field and procedural blanks.

	Air blanks			Dust blanks		
	Field (n=13) (ng)	[a]Conc. (ng/m^3)	Procedural (n=10)/(ng)	Field (n=8) (ng)	[a]Conc. (ng/g)	Procedural (n=10)/(ng)
BFRs						
PBB	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)
TCIPP	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	
EHDPP	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.

BFRs concentration in air and dust procedural blanks

- Mean air blank concentrations represented <13% of the mean concentrations, with blank subtraction performed, from Table S5, except for HBB (23%).
- Mean dust blank concentrations represented <3% of the mean concentrations, with blank subtraction performed, from Table S7, except for HBB (57%) and PBB (58%).

PFRs concentration in air and dust procedural blanks

- Mean air blank concentrations represented <7% of the mean concentrations, with blank subtraction performed, from Table S5, except for TPHP (22%), TCP (30%) and EHDPP (76%).
- Mean dust blank concentrations represented <15% of the mean concentrations, with blank subtraction performed, from Table S7, except for TNBP (34%).

Table S4. Recoveries of internal standards in dust and air samples calculated employing CB-207 as recovery standard

Internal standard	[a]Air sample		Dust sample	
	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-DDC-CO (1.25 ng)	72	25	75	13
^{13}C-anti-DDC-CO (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
TDCIPP-d15 (75 ng)	95	8	92	25

[a]Extraction of analytes from spiked PUF

RESULTS AND DISCUSSION

Air samples.

Table S5. 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)										
DBE-DBCH	77.9	222	4120	100	28	46.6	104	399	100	19
[a]TBP-AE	4.59	6.69	69.3	70	2	<MLD	<MLD	2.88	0	<1
[b]TBX	<MLD	64.5	2830	38	<1	<MLD	<MLD	2.92	17	<1
[c]PBB	5.66	9.30	50.8	100	2	1.87	2.66	7.35	83	1
[d]PBT	7.64	14.3	213	100	3	2.57	2.64	4.14	100	1
[e]PBEB	0.531	1.29	30.6	45	<1	<MLD	<MLD		0	0
[f]TBP-DBPE	0.789	5.49	132	40	<1	0.841	2.54	10.6	50	<1
BEH-TEBP	<MLD	1.85	24.2	19	<1	<MLD	1.95	6.32	33	<1
DBDPE	<MLD	38.2	963	47	<1	8.30	9.34	20.6	50	3
HBB	4.11	12.4	297	70	3	4.03	4.20	6.52	83	4
synDDC-CO	<MLD	0.176	7.39	2	<1	<MLD	<MLD		0	0
antiDDC-CO	<MLD	0.281	7.61	4	<1	<MLD	<MLD		0	0
BDE-28	7.53	9.53	52.5	98	3	5.63	7.99	21.1	100	2
BDE-47	128	178	718	100	46	131	178	459	100	55
BDE-100	6.78	10.9	83.6	96	2	8.02	9.11	19.6	100	3
BDE-99	21.0	40.5	413	100	8	23.0	26.3	52.2	100	10
BDE-85	0.565	1.40	18.7	49	<1	0.628	0.719	1.20	50	<1
BDE-154	0.383	2.25	61.0	15	<1	0.501	0.607	1.49	17	<1
BDE-153	0.927	7.57	262	81	<1	0.746	0.742	1.89	67	<1
BDE-183	<MLD	11.4	534	6	<1	<MLD	<MLD		0	0
BDE-209	3.76	323	4150	51	1	<MLD	22.7	101	33	1
$\sum_8$PBDEs	170	261	2100			177	223	555		
PFRs (ng/m^3)										
[g]TNBP	5.09	9.66	124	100	10	3.06	3.09	4.57	100	13
TCEP	2.25	3.23	10.1	98	4	3.95	6.99	21.3	100	16
TCIPP	42.3	83.1	462	100	83	10.2	12.1	25.1	100	42
[g]TBOEP	0.598	1.45	18.2	100	1	12.9	18.6	50.1	100	29
TPHP	0.258	0.375	1.65	89	1	0.052	0.079	0.234	100	<1
[h]EHDPP	0.119	0.489	3.51	62	<1	0.064	0.98	5.03	67	<1
[i]TCP	<MLD	0.080	0.644	57	<1	<MLD	<MLD		0	0
TDCIPP	0.084	0.394	10.6	98	<1	0.046	0.067	0.140	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 plasticizer; [h]2-ethylhexyl diphenyl phosphate; [i]tricresyl phosphate.

Indoor air levels of PBDEs from different countries are compared in Figure S1 (households, except offices from U.S.A.).[2-4] Lower brominated PBDEs are more ubiquitous in air due to their volatility. BDE-47 is the predominant congener in all countries followed by BDE-99. The presence of BDE-209 in air is most likely because it adsorbs to the particulate matter.

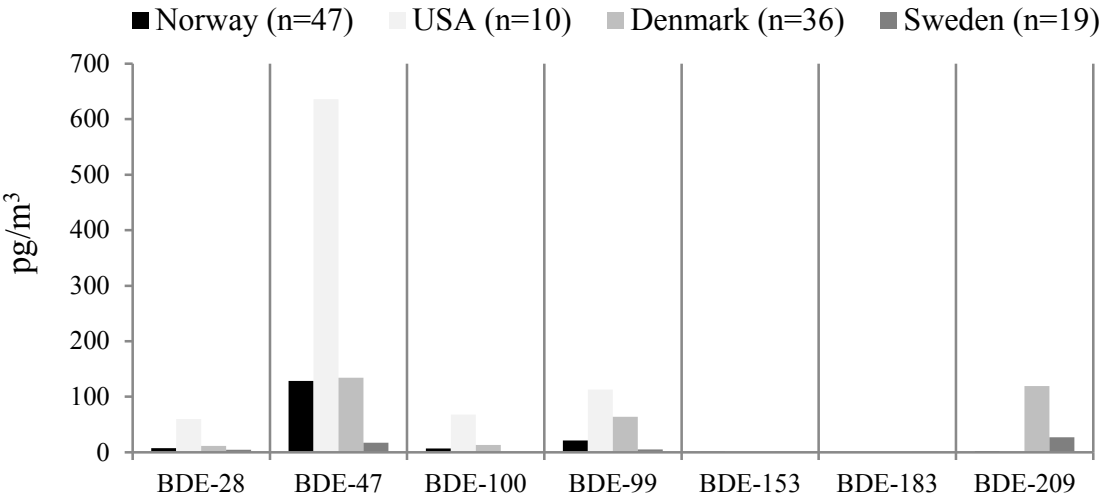


Figure S1. Comparison of levels of PBDEs in indoor air from studies conducted in Norway, U.S.A. (Michigan; offices), Denmark and Sweden.

Figure S2 shows concentrations of PFRs in four studies in indoor environments (households).[5-7] TCIPP is the most abundant PFR in the current study and in the study from Japan, while the studies from Sweden and Switzerland show different profiles.

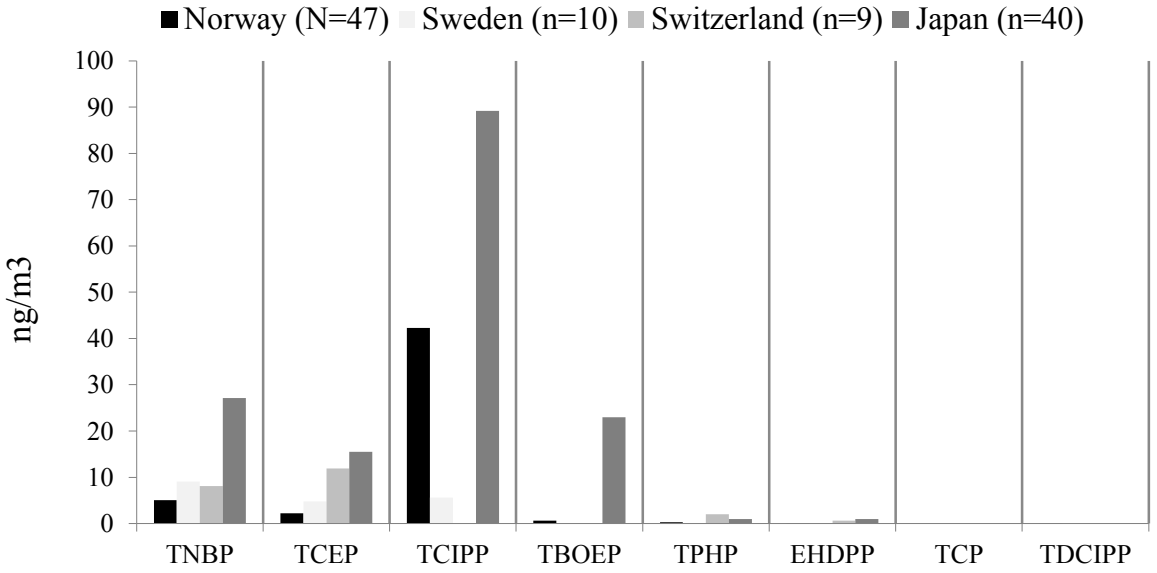


Figure S2. Comparison of concentrations of PFRs in indoor air from studies conducted in Norway, Sweden, Switzerland and Japan.

Table S6. Spearman rank correlation for the concentrations of FRs in air

	DBE-DBCH	TBP-AE	PBB	PBT	PBEB	TBP-DBPE	DBDPE	HBB	BDE28	BDE47	BDE100	BDE99	BDE85	BDE153	BDE209	Σ_8PBDE	TNBP	TCEP	TCPP	TBOEP	TPP	EHDPP	TCP
TBP-AE	0.005																						
PBB	.295*	0.022																					
PBT	0.238	0.142	.505**																				
PBEB	0.024	0.075	0.206	0.034																			
TBP-DBPE	0.165	-0.007	-0.177	-0.059	0.100																		
DBDPE	0.099	-0.104	0.051	-0.019	-0.066	-0.102																	
HBB	0.058	0.158	.397**	-0.063	0.245	-0.149	0.015																
BDE28	0.059	.344*	-0.065	.373**	0.133	0.174	0.030	-0.088															
BDE47	0.059	.290*	-0.031	.375**	0.156	0.190	0.027	-0.011	.947**														
BDE100	0.103	0.229	0.034	.405**	0.131	0.163	0.071	0.005	.922**	.983**													
BDE99	0.096	0.249	0.025	.435**	0.157	0.167	0.051	-0.029	.916**	.963**	.983**												
BDE85	-0.058	0.274	0.117	0.225	.408**	0.131	0.093	0.078	.567**	.610**	.648**	.644**											
BDE153	-0.009	0.144	-0.106	0.172	0.268	0.130	0.084	-0.117	.500**	.503**	.536**	.599**	.526**										
BDE209	-0.035	0.016	0.079	0.125	0.223	-0.128	0.207	.285*	-0.021	0.025	0.041	0.018	0.076	0.188									
Σ_8PBDE	0.062	.287*	-0.012	.401**	0.150	0.185	0.040	-0.020	.946**	.998**	.989**	.973**	.626**	.524**	0.026								
TNBP	.299*	0.104	-0.001	-0.099	0.006	0.087	0.204	.400**	0.069	0.053	0.074	0.047	0.005	0.067	0.140	0.053							
TCEP	0.224	0.049	0.145	0.080	0.003	0.149	0.196	0.047	-0.006	-0.024	-0.004	0.027	-0.130	-0.075	-0.271	-0.012	0.072						
TCPP	0.254	0.139	0.248	0.269	-0.014	-0.113	0.139	0.271	0.237	0.241	0.277	0.256	0.081	-0.022	0.193	0.252	0.214	0.249					
TBEP	-0.055	-0.235	-0.121	0.206	0.033	0.184	0.143	-.296*	0.030	0.093	0.100	0.137	0.080	0.190	0.080	0.098	-0.201	0.032	-0.227				
TPP	0.179	0.126	-0.118	0.177	0.016	0.093	0.184	-0.092	0.141	0.174	0.198	0.227	0.161	.342*	0.103	0.186	-0.083	.315*	0.097	0.205			
EHDPP	-0.038	-0.039	0.175	0.224	-0.143	-.303*	-0.126	-0.029	-0.099	-0.089	-0.078	-0.068	-0.202	-0.115	-0.061	-0.087	-0.070	0.243	0.123	-0.082	.408**		
TCP	0.000	.345*	-0.015	0.161	0.084	-0.210	0.037	0.063	0.057	0.005	0.022	0.053	0.233	.293*	.314*	0.015	-0.029	-0.121	0.111	-0.054	.484**	0.265	
TDCPP	-0.085	0.196	-0.098	0.063	0.050	-0.192	0.095	0.072	0.028	0.013	0.031	0.053	0.197	.379**	.307*	0.019	-0.129	-0.055	0.132	0.238	.501**	.311*	.750**

*Correlation was significant at the level 0.05 (two tailed; light red); **Correlation was significant at the level 0.01 (two tailed; light green)

Dust samples.

Table S7. 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										
DBE-DBCH	1.72	6.45	172	96	<1	2.10	3.31	10.0	100	<1
[a]TBX	<MLD	1.91	88.8	6	<1	<MLD	<MLD		0	0
[b]PBB	0.177	0.343	4.64	40	<1	0.114	0.222	0.682	50	<1
[c]PBT	0.633	1.07	16.1	94	<1	0.232	0.355	1.06	67	<1
[d]PBEB	<MLD	0.254	8.00	33	<1	<MLD	0.017	0.103	0	<1
[e]TBP-DBPE	0.505	1.93	21.4	69	<1	0.178	0.269	0.707	50	<1
[f]PBBA	<MLD	0.447	11.3	13	<1	<MLD	<MLD		0	0
EH-TBB	2.54	16.1	245	58	<1	3.32	2.67	5.72	67	<1
BEH-TEBP	78.5	132	809	100	8	103	99.9	151	100	11
BTBPE	3.76	8.73	41.9	92	<1	6.55	13.4	53.0	100	<1
DBDPE	147	512	4460	96	16	156	179	360	83	17
HBB	0.671	1.48	8.94	50	<1	0.901	1.61	5.27	67	<1
synDDC-CO	1.45	9.07	311	92	<1	0.921	1.31	3.13	83	<1
antiDDC-CO	4.16	18.9	590	92	<1	2.63	3.68	9.25	100	<1
BDE-28	0.688	1.01	4.99	94	<1	0.371	0.389	0.807	100	<1
BDE-47	126	199	1510	100	13	46.9	61.6	199	100	5
BDE-100	33.1	61.1	443	98	4	7.95	8.86	17.6	100	<1
BDE-99	171	348	2610	98	18	42.4	45.8	92.8	100	5
BDE-85	9.32	18.1	123	98	1	3.69	4.94	16.4	100	<1
BDE-154	12.7	24.7	175	98	1	5.76	7.44	22.8	100	<1
BDE-153	26.0	39.7	254	98	3	8.93	11.9	37.2	100	1
BDE-183	3.22	18.2	267	94	<1	5.80	7.31	15.9	100	<1
BDE-209	325	6840	204000	98	34	507	1220	5270	100	56
$\sum_8$PBDEs	426	711	5125			166	148	286		
PFRs										
[g]TNBP	55.0	119	979	58	<1	43.5	42.7	754	50	<1
TCEP	414	796	4630	98	2	1210	2080	6160	100	1
TCIPP	2680	5740	40100	100	14	2040	1890	2740	100	2
[g]TBOEP	13400	18700	128000	100	71	87200	89800	163000	100	91
TPHP	981	1240	4850	100	5	1540	2400	6150	100	2
[h]EHDPP	617	876	5900	100	3	2340	16200	79000	100	2
[i]TCP	307	1120	16200	92	2	56.4	97.8	333	50	<1
TDICPP	501	797	6920	100	3	1490	2730	6140	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 plasticizer; [h]2-ethylhexyl diphenyl phosphate; [i]tricresyl phosphate.

Concentrations of BDE-47, 99, 209, DBDPE, EH-TBB, BEH-TEBP and BTBPE in dust from households were compared with reported concentrations from other studies: U.S.A. (California),[8] China,[9] Belgium and U.K. (classrooms).[10]

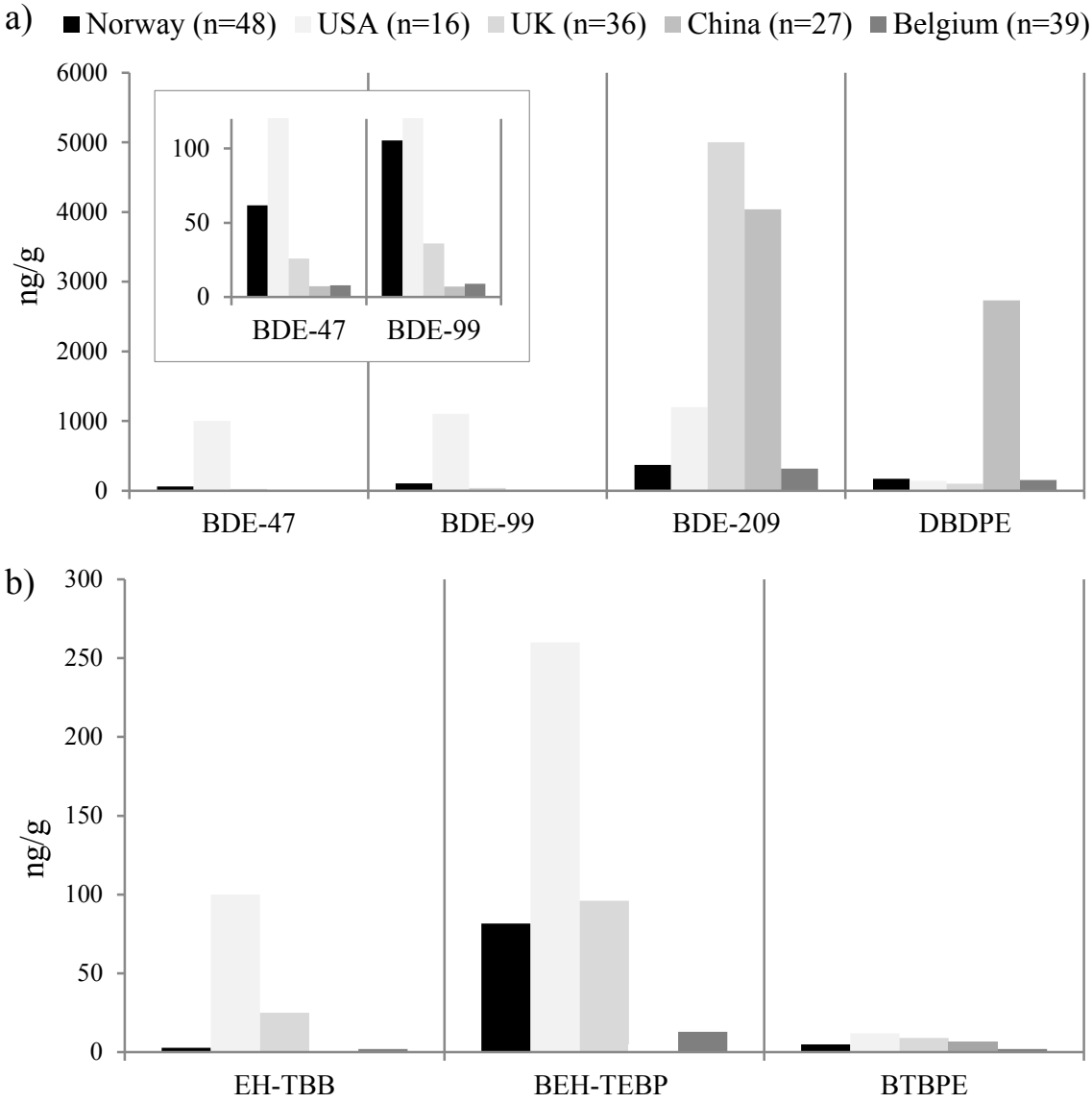


Figure S3. Comparison of levels of BFRs in indoor dust from studies conducted in Norway, U.S.A. (California), U.K., China and Belgium.

Levels of PFRs in indoor dust from Norway are compared in Figure S4 with reported levels in households from Japan[7], U.S.A. (California)[8], Spain[11], Sweden[5] and Belgium[12]. Japan reported the highest levels in dust for all the analyzed PFRs. TBOEP and TCIPP are the PFRs most detected in indoor dust.

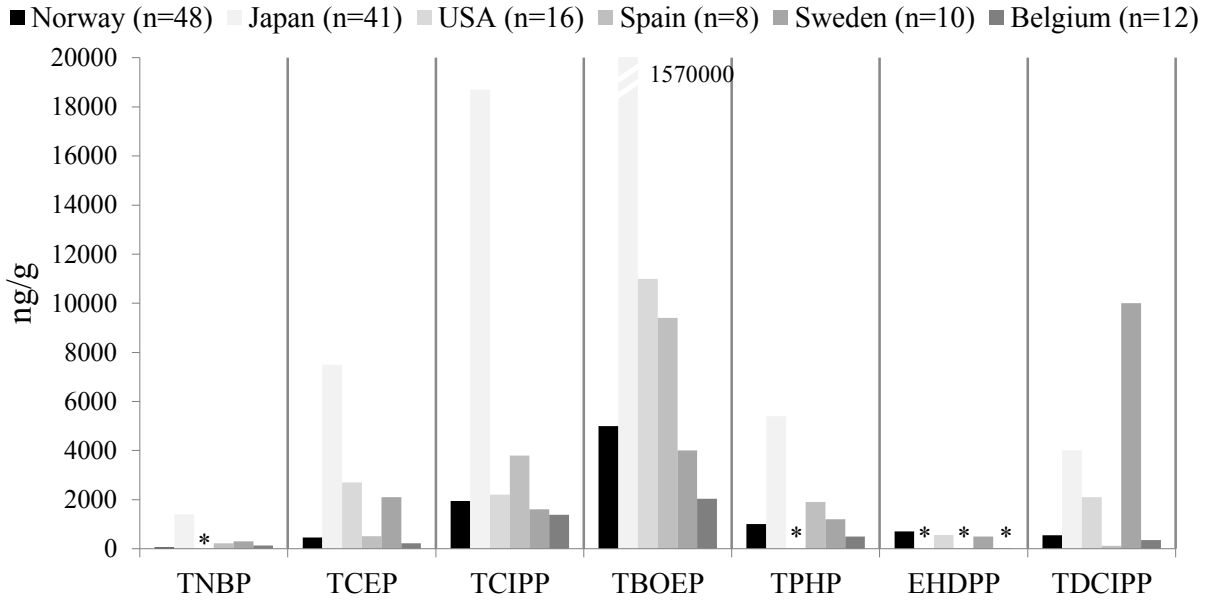


Figure S4. Comparison of levels of PFRs in indoor dust from studies conducted in Norway, Japan, U.S.A (California), Spain, Sweden and Belgium. (*) indicates no analysis performed.

Table S8. Spearman rank correlation for the concentrations of FRs in dust

	DBE-DBCH	PBB	PBT	TBP-DBPE	EH-TBB	BEH-TEBP	BTBPE	DBDPE	HBB	synDDC-CO	antiDDC-CO	BDE28	BDE47	BDE100	BDE99	BDE85	BDE154	BDE153	BDE183	BDE209	Σ_8PBDE	TNBP	TCEP	TCPP	TBOEP	TPHP	EHDPP	TCP
PBB	0.203																											
PBT	-0.083	0.262																										
TBP-DBPE	0.101	0.019	0.234																									
EH-TBB	.342*	0.255	0.014	0.146																								
BEH-TEBP	0.026	0.240	-0.122	0.014	.511**																							
BTBPE	0.026	0.088	.321*	0.147	-0.052	-0.061																						
DBDPE	0.009	0.221	0.159	0.220	0.131	0.257	0.174																					
HBB	0.200	.391**	0.060	0.222	.361*	0.183	0.231	.435**																				
synDDC-CO	-0.067	.350*	0.271	0.210	-0.018	0.079	0.222	.295*	.392**																			
antiDDC-CO	-0.089	0.276	.293*	0.267	-0.021	0.002	0.189	.353*	.378**	.839**																		
BDE28	-0.024	0.064	.340*	0.148	0.218	-0.017	0.117	-0.031	-0.016	0.060	0.055																	
BDE47	-0.026	-0.080	.288*	0.221	0.042	-0.137	0.170	-0.045	-0.024	0.025	0.077	.789**																
BDE100	-0.037	-0.068	.293*	0.191	0.059	-0.143	0.175	-0.012	0.039	0.091	0.128	.718**	.972**															
BDE99	-0.033	-0.073	.304*	0.192	0.065	-0.135	0.214	0.023	0.061	0.099	0.139	.704**	.962**	.993**														
BDE85	0.003	-0.077	.329*	0.182	0.050	-0.179	0.234	0.014	0.070	0.102	0.132	.671**	.949**	.988**	.992**													
BDE154	-0.040	-0.045	.323*	0.191	0.042	-0.127	0.225	0.047	0.072	0.129	0.160	.687**	.950**	.989**	.992**	.990**												
BDE153	-0.052	-0.056	.328*	0.203	0.063	-0.119	0.257	0.090	0.126	0.114	0.155	.661**	.926**	.967**	.983**	.979**	.985**											
BDE183	-0.023	0.090	0.205	.322*	0.199	-0.064	0.148	.305*	.427**	0.130	0.223	.353*	.330*	.347*	.346*	.339*	.359*	.413**										
BDE209	0.278	.469**	-0.011	0.185	0.252	0.184	0.170	0.238	0.256	0.153	0.150	-0.096	-0.013	0.040	0.039	0.052	0.039	0.039	0.182									
Σ_8PBDE	-0.038	-0.079	.319*	0.208	0.070	-0.153	0.217	0.017	0.045	0.075	0.118	.737**	.977**	.990**	.993**	.986**	.987**	.979**	.391**	0.026								
TNBP	0.248	0.192	-0.178	0.169	.298*	.378**	-0.047	.320*	.408**	.345*	0.239	-0.053	-0.267	-0.268	-0.258	-0.274	-0.247	-0.239	0.206	.311*	-0.264							
TCEP	0.203	0.135	0.162	0.145	-0.028	-0.064	0.163	0.014	.316*	-0.116	0.025	0.104	0.199	0.174	0.163	0.177	0.161	0.158	0.074	-0.033	0.158	-0.147						
TCPP	-0.014	0.014	0.036	-0.145	0.163	0.070	0.138	0.122	.363*	0.004	-0.001	-0.038	-0.061	-0.083	-0.061	-0.064	-0.105	-0.046	-0.120	-0.166	-0.070	0.036	0.242					
TBOEP	-0.149	-0.192	-0.203	0.010	-.348*	-0.132	-0.082	-0.037	-0.121	-0.005	0.115	0.013	0.114	0.139	0.128	0.113	0.139	0.119	0.170	-0.053	0.114	0.000	-0.043	-0.190				
TPHP	-0.082	-0.039	-.308*	0.058	-0.030	0.157	.322*	0.069	0.181	-0.014	0.013	-0.005	0.164	0.215	0.237	0.232	0.252	0.269	0.014	-0.012	0.213	-0.176	0.081	-0.021	0.269			
EHDPP	-0.087	.423**	0.209	-0.029	0.229	.370**	0.150	0.173	0.274	.372**	0.227	0.099	-0.089	-0.015	0.020	-0.003	0.043	0.069	0.088	0.144	-0.008	0.230	-0.094	-0.030	-0.175	0.154		
TCP	0.097	.427**	0.235	-0.001	0.252	.407**	0.183	0.093	.338*	0.121	-0.035	0.083	-0.101	-0.107	-0.084	-0.087	-0.050	-0.036	0.077	0.145	-0.073	0.284	-0.009	0.075	-0.033	0.143	.370**	
TDCIPP	0.211	0.265	-0.021	0.029	0.126	0.080	0.014	0.069	.296*	0.182	0.153	-0.077	-0.244	-0.240	-0.233	-0.234	-0.242	-0.219	-0.032	-0.083	-0.248	0.226	0.142	.316*	0.040	-0.086	0.177	0.216

*Correlation was significant at the level 0.05 (two tailed; light red); **Correlation was significant at the level 0.01 (two tailed; light green)

Table S9. Spearman rank correlation for the concentrations of FRs in dust (brown) and air (blue)

	DBE-DBCH	TBP-AE	PBB	PBT	PBEB	TBP-DBPE	DBDPE	HBB	BDE28	BDE47	BDE100	BDE99	BDE85	BDE153	BDE209	Σ_8PBDE	TNBP	TCEP	TCPP	TBOEP	TPHP	EHDPP	TCP	TDCIPP
DBE-DBCH	.719**	-0.096	0.048	-0.186	0.136	0.232	0.139	0.021	-0.256	-0.230	-0.196	-0.221	-0.005	-0.063	0.102	-0.238	0.162	0.026	-0.137	-0.039	0.128	-0.145	0.071	-0.009
PBB	0.218	0.183	.568**	0.102	.314*	-0.030	0.142	.348*	-0.190	-0.185	-0.149	-0.118	0.073	0.026	0.058	-0.175	0.103	0.032	0.044	-0.204	-0.052	-0.052	0.005	-0.110
PBT	0.237	0.251	.296*	.757**	-0.042	0.043	0.065	-0.127	0.266	0.271	.303*	.335*	0.081	0.239	0.102	.295*	0.048	0.032	0.194	0.138	0.188	0.131	0.187	0.016
TBP-DBPE	0.122	0.169	-0.216	-0.124	0.054	.405**	-0.079	-0.070	0.163	0.180	0.121	0.108	-0.193	0.220	-0.135	0.165	0.135	0.047	-0.127	0.018	0.083	-0.189	-0.189	-0.102
EH-TBB	0.150	0.057	-0.006	-0.196	0.030	0.055	-0.105	0.051	0.151	0.155	0.191	0.178	0.279	.322*	0.036	0.148	-0.154	-0.142	0.088	-.305*	0.056	-0.132	0.177	0.086
BEH-TEBP	-0.127	-0.111	0.130	-0.121	0.150	-0.188	-0.069	0.152	0.033	-0.010	0.028	0.045	0.245	.413**	-0.076	-0.005	-0.082	-0.196	-0.147	-0.075	-0.033	-0.085	0.136	0.212
BTBPE	0.126	0.231	0.004	0.139	0.056	0.105	.367*	0.067	0.081	0.065	0.092	0.098	0.105	.300*	0.159	0.079	0.260	0.213	0.249	-0.056	.389**	0.075	.345*	.307*
DBDPE	-0.090	-0.132	0.138	-0.098	0.132	-0.073	0.114	0.229	-0.163	-0.114	-0.079	-0.110	0.066	0.057	-0.104	-0.115	0.230	-0.071	-0.008	-0.274	-.286*	-0.148	-0.144	-0.146
HBB	0.153	0.044	0.141	-0.222	.307*	0.007	0.159	.677**	0.013	0.078	0.127	0.081	0.165	0.131	0.224	0.079	.461**	0.087	.296*	-.343*	0.061	-0.189	0.030	-0.010
synDDC-CO	-0.082	0.272	0.136	0.000	0.254	0.137	0.104	0.262	0.076	0.079	0.074	0.049	0.183	0.203	0.068	0.083	.433**	-0.218	0.046	-0.192	-0.007	0.073	0.071	0.048
antiDDC-CO	-0.114	0.158	0.028	0.008	.347*	.311*	0.027	0.144	0.098	0.097	0.095	0.089	0.206	0.167	0.055	0.099	.360*	-0.091	0.037	-0.067	-0.058	-0.025	-0.005	0.026
BDE28	0.035	0.068	-0.077	0.173	-0.076	.326*	0.127	-0.216	.423**	.344*	.354*	.338*	0.206	0.210	-0.067	.347*	0.003	0.027	-0.026	0.166	0.101	0.023	0.114	0.012
BDE47	0.143	0.004	-0.093	0.177	-0.038	.431**	-0.011	-0.231	.368*	.375**	.392**	.371**	0.178	0.207	-0.125	.380**	0.013	0.188	0.074	0.274	0.274	0.127	-0.038	-0.002
BDE100	0.116	-0.017	-0.070	0.163	-0.062	.435**	-0.029	-0.157	.331*	.370**	.399**	.379**	0.200	0.228	-0.077	.378**	0.028	0.145	0.093	0.252	.313*	0.198	-0.029	0.032
BDE99	0.114	-0.006	-0.083	0.155	-0.066	.449**	-0.033	-0.141	.309*	.353*	.383**	.364*	0.204	0.241	-0.071	.360*	0.047	0.148	0.112	0.258	.308*	0.176	-0.003	0.046
BDE85	0.167	-0.029	-0.072	0.181	-0.074	.432**	-0.031	-0.144	.294*	.345*	.378**	.359*	0.182	0.238	-0.056	.353*	0.068	0.155	0.128	0.238	.343*	0.217	-0.001	0.046
BDE154	0.120	-0.027	-0.063	0.170	-0.053	.413**	-0.009	-0.148	.312*	.359*	.391**	.373**	0.212	0.260	-0.080	.367*	0.062	0.130	0.083	0.261	.325*	0.201	-0.009	0.044
BDE153	0.127	-0.037	-0.068	0.159	-0.037	.413**	-0.024	-0.101	.296*	.354*	.386**	.366*	0.205	0.256	-0.061	.360*	0.094	0.151	0.166	0.242	.313*	0.181	0.006	0.046
BDE183	-0.009	-0.209	-0.162	-0.027	.312*	0.217	0.041	0.084	0.167	0.267	0.276	0.242	0.141	0.172	0.131	0.262	0.139	-0.103	0.062	-0.046	0.013	-0.128	-0.158	-0.189
BDE209	0.205	-0.037	0.059	-0.203	0.071	0.234	-0.154	0.137	-0.275	-0.245	-0.221	-0.206	-0.158	0.017	0.024	-0.241	0.016	-0.113	-0.096	-0.237	0.038	-0.104	-0.127	-0.055
Σ_8PBDE	0.132	-0.008	-0.089	0.176	-0.046	.440**	-0.029	-0.178	.347*	.386**	.412**	.390**	0.216	0.241	-0.085	.392**	0.033	0.137	0.120	0.251	.302*	0.152	-0.010	0.023
TNBP	-0.026	-0.048	-0.226	-.362*	0.097	0.069	0.139	0.125	-0.073	-0.081	-0.074	-0.105	0.036	0.220	0.026	-0.082	0.266	-0.222	-0.156	-0.242	-0.041	-.361*	0.003	-0.063
TCEP	.365*	-0.032	0.198	0.120	0.107	0.075	0.104	0.189	0.054	0.030	0.106	0.133	-0.047	-0.001	-0.079	0.045	0.240	.679**	0.242	0.030	0.215	0.146	-0.147	-0.083
TCPP	0.109	0.150	0.159	0.048	-0.091	-0.261	0.143	.310*	0.049	0.006	0.042	0.004	0.011	-0.190	0.110	0.011	0.238	0.202	.759**	-.331*	-0.072	0.089	0.202	0.141
TBOEP	-.323*	-.442**	-.290*	-0.232	0.050	0.209	0.179	-0.218	-0.099	-0.034	-0.035	-0.038	-0.048	-0.016	0.035	-0.036	-0.053	-0.123	-0.159	.645**	-0.093	-0.131	-.364*	0.000
TPHP	-0.126	-.293*	-0.013	-0.225	0.079	0.174	0.170	0.195	-0.064	-0.031	-0.015	-0.027	0.131	0.174	0.020	-0.031	0.066	0.081	0.119	0.169	0.194	-0.019	0.036	.293*
EHDPP	-0.123	0.095	0.216	0.059	-0.120	0.001	0.028	.309*	-0.098	-0.080	-0.052	-0.041	0.076	0.148	0.089	-0.078	0.121	-0.167	0.047	-0.103	0.035	0.074	0.069	0.091
TCP	-0.005	-0.021	0.154	0.090	0.116	-0.260	0.188	0.204	-0.153	-0.108	-0.074	-0.046	0.081	0.217	0.084	-0.101	-0.107	-0.171	-0.044	0.150	0.233	-0.064	.358*	.285*
TDCIPP	0.068	0.275	0.088	-0.086	.313*	-0.135	0.153	.362*	-0.076	-0.050	-0.053	-0.033	0.120	0.039	0.186	-0.062	0.024	0.024	0.194	0.048	0.147	0.047	0.265	.386**

*Correlation was significant at the level 0.05 (two tailed; light red); **Correlation was significant at the level 0.01 (two tailed; light green)

Correlations between FR concentrations in air and dust.

Table S10. Linear correlations (R^2) and significances between concentrations of FRs in air and dust and their partition coefficients ($K_{dust-air}$).

	R^2	[a]Sign.	[b]log $K_{dust-air}$ (m^3/g)
DBE-DBCH	0.73	0.000	1.47
PBB	0.29	0.002	1.83
PBT	0.39	0.000	2.05
TBP-DBPE	0.40	0.000	2.48
DBDPE	0.06	0.112	3.73
HBB	0.49	0.000	2.29
BDE-28	0.22	0.001	1.87
BDE-47	0.23	0.001	2.63
BDE-100	0.22	0.001	3.21
BDE-99	0.08	0.051	3.44
BDE-85	0.04	0.198	3.74
BDE-153	0.02	0.353	3.99
BDE-209	0.01	0.543	4.45
TNBP	0.21	0.001	1.10
TCEP	0.40	0.000	2.38
TCIPP	0.64	0.000	2.02
TBOEP	0.52	0.000	3.98
TPHP	0.01	0.563	4.02
EHDPP	0.05	0.123	3.83
TCP	0.15	0.007	4.35
TDCIPP	0.14	0.009	4.10

[a]values below 0.05 were considered significant; [b]average from living rooms and classrooms.

Estimation of concentrations in air from dust

Households

When using median dust concentration to estimate the concentration in air, the error in the prediction is ±35% for 7 FRs (HBB, BDE-47, 153,154, TCPP, TBEP and TCP) and ±80% for 6 FRs (DBE-DBCH, PBT, TBP-DBPE, BDE-28, TNBP and PBB). The other FRs showed a variation lower than 8 times, except TPHP for which the estimation was 48 times too high.

Classrooms

The estimation of the concentrations of FRs in the air was ±35% for 5 FRs (BDE-99, 154, 153, TCPP and TBEP) and ±80% for 5 FRs (DBE-DBCH, BDE-47, 85, 100 and HBB). The other FRs showed a variation lower than 10 times, except TNBP (x14), EHDPP (x15), TDCIPP (x24) and TPHP (x129). These estimations are not as accurate as in households, but the concentrations obtained give a satisfactory overall estimation of the contamination in the air.

Sources of exposure in indoor environments. Spearman rank correlation was used to evaluate the relationship between the FRs and the indoor parameters. Only correlations with significances $p < 0.15$ were considered to be included in the multiple linear regression models.

Table S11. Significant multiple linear correlations of FRs with relevant household parameters

		Age of the woman [1]	Building constr. year [2]	Size of apartment (m2) [5]	Living room (m2) [6]	# Picture tube TVs [7]	# Video DVD players [9]	Vacuum Cleaning / week [17]	TV distance from sampling site [18]	Humidity (%) [19]	Temp. (ºC) [20]	Location of the house (Rural/ Urban) [21]	Type of house. non-detached /detached [22]	Electric panel heaters (no/yes) [23]	Fireplace (no/yes) [24]	Carpets (no/yes) [25]	Chairs of PUF (no/yes) [26]	Renovation of house (no/yes) [27]
Air																		
TBP-AE	R			0.536											0.536			
	β			0.002											0.295			
	p			0.043											0.002			
PBT	R									0.716	0.716			0.716				
	β									0.021	0.107			-0.287				
	p									0.019	0.004			0.041				
TBP-DBPE	R				0.508												0.508	
	β				-0.017												-0.466	
	p				0.023												0.013	
HBB	R							0.627					0.627					
	β							-0.086					-0.513					
	p							0.049					0.000					
BDE-47	R							0.484	0.484									
	β							0.093	0.104									
	p							0.040	0.010									
BDE-100	R							0.493	0.493									
	β							0.093	0.113									
	p							0.046	0.007									

Table S11. Continued

		Age of the woman [1]	Building constr. year [2]	Size of apartment (m2) [5]	Living room (m2) [6]	# Picture tube TVs [7]	# Video DVD players [9]	Vacuum Cleaning / week [17]	TV distance from sampling site [18]	Humidity (%) [19]	Temp. (ºC) [20]	Location of the house (Rural/ Urban) [21]	Type of house. non-detached /detached [22]	Electric panel heaters (no/yes) [23]	Fireplace (no/yes) [24]	Carpets (no/yes) [25]	Chairs of PUF (no/yes) [26]	Renovation of house (no/yes) [27]
BDE-99	R							0.521	0.521									
	β							0.091	0.122									
	p							0.044	0.003									
BDE-85	R						0.652							0.652				0.652
	β						0.282							-0.371				0.236
	p						0.000							0.003				0.029
Σ_8PBDE	R							0.498	0.498									
	β							0.092	0.114									
	p							0.045	0.006									
TCIPP	R										0.622						0.622	0.622
	β										0.088						0.283	-0.350
	p										0.034						0.082	0.028
Dust																		
TBECH	R							0.574				0.574						
	β							-0.175				-0.397						
	p							0.002				0.005						
PBB	R							0.568								0.568		
	β							-0.097								0.251		
	p							0.007								0.010		
HBB	R							0.541					0.541					
	β							-0.120					-0.359					
	p							0.012					0.010					
syn-DDC-CO	R	0.587				0.587												
	β	0.065				-0.179												
	p	0.000				0.018												

Table S11. Continued

		Age of the woman [1]	Building constr. year [2]	Size of apartment (m2) [5]	Living room (m2) [6]	# Picture tube TVs [7]	# Video DVD players [9]	Vacuum Cleaning / week [17]	TV distance from sampling site [18]	Humidity (%) [19]	Temp. (°C) [20]	Location of the house (Rural/ Urban) [21]	Type of house. non-detached /detached [22]	Electric panel heaters (no/yes) [23]	Fireplace (no/yes) [24]	Carpets (no/yes) [25]	Chairs of PUF (no/yes) [26]	Renovation of house (no/yes) [27]
anti-DDC-CO	R	0.580				0.580												
	β	0.066				-0.153												
	p	0.000				0.040												
BDE-28	R		0.645		0.645									0.645				
	β		-0.008		-0.010									-0.456				
	p		0.008		0.032									0.001				
BDE-47	R				0.465									0.465				
	β				-0.016									-0.551				
	p				0.037									0.013				
TNBP	R						0.514	0.514			0.514							
	β						0.273	-0.133			-0.102							
	p						0.150	0.043			0.013							
TCEP	R					0.535												0.535
	β					0.257												-0.408
	p					0.003												0.010
TCIPP	R				0.538												0.538	
	β				0.012												0.439	
	p				0.041												0.004	
TCP	R		0.513					0.513										
	β		-0.007					-0.154										
	p		0.044					0.014										

[1] Age of the women; [2] Building construction year; [5] Size of the apartment in m2; [6] Size of the living room in m2; [7] Number of picture tube TVs in the household; [9] Number of DVD and Video players; [17] Number of vacuum cleaning in the living room per week; [18] Distance (m) of the sampling equipment from TV in the living room; [19] Humidity in the living room; [20] Temperature in the living room; [21] Location of the house (Rural/Urban); [22] Type of household (non-detached/detached); [23] Electric panel heaters (no/yes); [24] Fireplace in the living room (no/yes); [25] Carpets in the living room (no/yes); [26] Chairs made of PUF in the living room (no/yes); [27] Renovation of areas of the house in the last 5 years (no/yes).

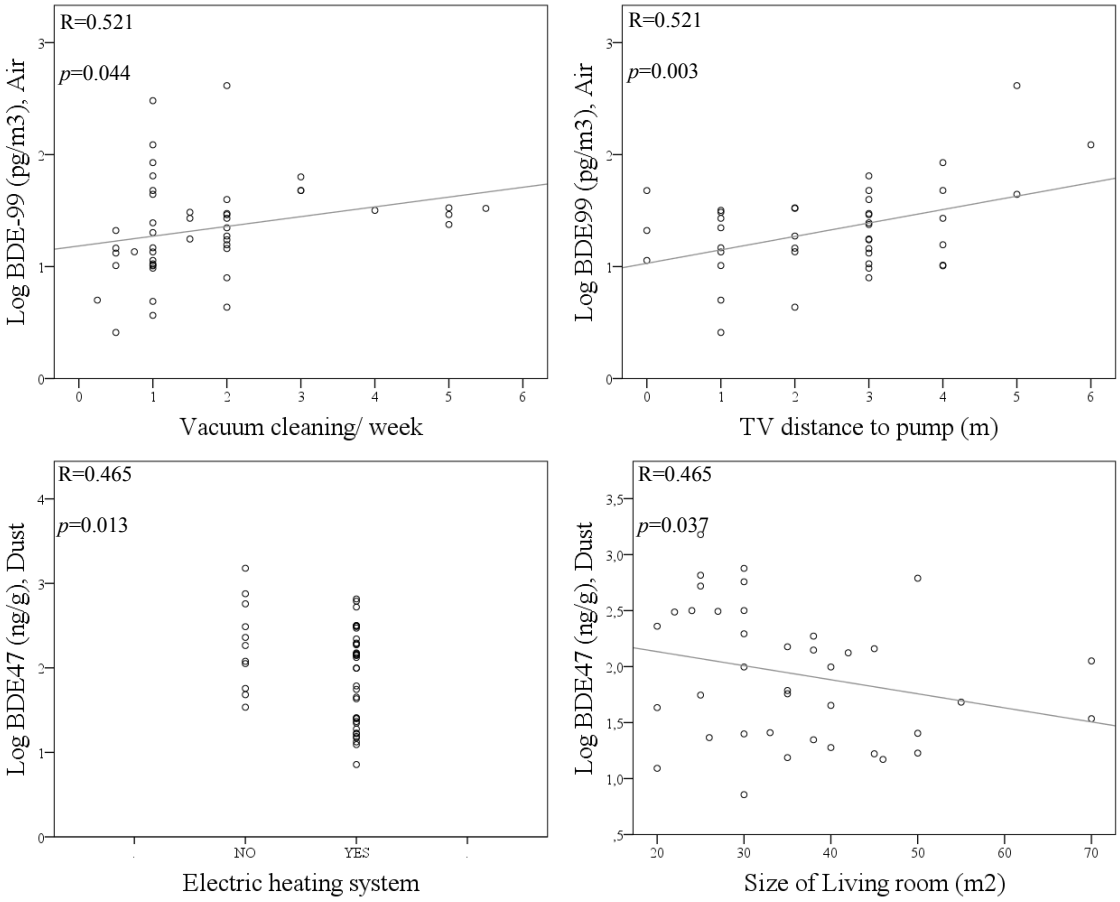


Figure S5. Examples of multiple linear correlation of BDE-99 concentrations with vacuum cleaning/week and TV distance to sampling site and also BDE-47 with electric electric panel heaters and size of the living room.

Estimated intakes of FRs for children and adults.

Table S12. Equations and parameters used for the calculations of the daily exposure dose (DED)

Daily exposure dose (DED)	
Air inhalation	[a] $DED = \frac{C \times IR \times ED}{BW}$
Dust ingestion	[a,b] $DED = \frac{C \times DI \times ED}{BW}$
Dermal absorption	[a,c] $DED = \frac{C \times SA \times DA \times AF \times ED}{BW}$

[a]C = concentration of FR in air [pg/m^3]; IR = inhalation rate children/adult was 10.9/13.3 [m^3/day];[13] ED = exposure duration [h/day] and BW = body weight [Kg], assessed by the questionnaires.
[b]DI = dust ingestion was 0.1 [g/day] for groups older than 6 years old.[13]
[c]SA = skin surface area (arms, hands and legs) children/adult was 4970/8620 [cm^2/day][13]; DA = dust adherence was 0.096 [g/cm^2];[14] AF = absorption factor was 0.03 and 0.17 [%] for HFRs and PFRs, respectively.[14,15]

Table S13. 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	
[a,b]RfD		[c]Inhalation	Dermal Contact	Ingestion	[c]Inhalation	Dermal Contact	Ingestion
BFRs and dechloranes (pg/kg bw/day)							
	DBE-DBCH	22	0.78	5.4	9.0	0.37	1.5
	TBP-AE	1.0	0	0	0.53	0	0
	PBB	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
	TBP-DBPE	0.26	0.17	1.2	0.09	0.11	0.43
	EH-TBB	0	1.2	8.2	0	0.54	2.2
	BEH-TEBP	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
	synDDC-CO	0	0.53	3.7	0	0.31	1.3
	antiDDC-CO	0	1.5	11	0	0.89	3.6
	BDE-28	2.2	0.24	1.7	0.86	0.15	0.59
$1.0x10^5$	BDE-47	41	42	$2.9x10^2$	14	27	$1.1x10^2$
	BDE-100	2.3	10	72	0.78	7.1	29
$1.0x10^5$	BDE-99	6.9	53	$3.7x10^2$	2.4	36	$1.5x10^2$
	BDE-85	0.19	3.1	22	0.07	2.0	8
	BDE-154	0.14	4.4	30	0.04	2.7	11
$2.0x10^5$	BDE-153	0.28	8.5	59	0.11	5.6	22
	BDE-183	0	1.7	12	0	0.69	2.8
$7.0x10^6$	BDE-209	0.44	$1.6x10^2$	$1.1x10^3$	0.24	70	$2.8x10^2$
	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)							
$2.4x10^4$	[d]TNBP	1.4	0.44	0.54	0.57	0.06	0.04
$2.2x10^4$	TCEP	0.91	1.6	2.0	0.27	0.59	0.42
$8.0x10^4$	TCIPP	10	5.9	7.2	4.1	2.9	2.1
$1.5x10^4$	[d]TBOEP	1.6	92	113	0.07	16	12
$7.0x10^4$	TPHP	0.06	2.8	3.4	0.03	1.2	0.87
	EHDPP	0.03	2.9	3.5	0.01	0.93	0.59
$1.3x10^4$	TCP	0	0.53	0.65	0	0.40	0.29
$1.5x10^4$	TDCIPP	0.02	2.0	2.5	0.01	0.68	0.48
	TOTAL	14	108	133	5.1	23	16

[a]Oral RfD values of BFRs extracted from USEPA[16-19]; [b]RfD values of PFRs extracted from Ali *et al.*[20]; [c]FRs from suspended particles are also taken into account; [d]used mainly as a plasticizer.

Table S14. 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 b.w./day)												
TBECH	17	82	0.48	2	3.3	16	9.0	83	0.37	3	1.48	14
TBP-AE	0.97	100	0	0	0	0	0.53	100	0	0	0	0
PBB	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
TBP-DBPE	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
BEH-TEBP	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
synDDC-CO	0	0	0.40	13	2.8	88	0	0	0.31	19	1.3	81
antiDDC-CO	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 b.w./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
TCIPP	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
EHDPP	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
TDCIPP	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.

REFERENCES

(1) Bergman, Å.; Rydén, A.; Law, R. J.; de Boer, J.; Covaci, A.; Alaee, M.; Birnbaum, L.; Petreas, M.; Rose, M.; Sakai, S.; Van den Eede, N.; van der Veen, I. *Environ. Int.* **2012,** *49*, 57-82.

(2) Batterman, S.; Godwin, C.; Chernyak, S.; Jia, C.; Charles, S. *Environ. Int.* **2010,** *36* (6), 548-556.

(3) Björklund, J. A.; Thuresson, K.; Cousins, A. P.; Sellström, U.; Emenius, G.; de Wit, C. A. *Environ. Sci. Technol.* **2012,** *46* (11), 5876-5884.

(4) Vorkamp, K.; Thomsen, M.; Frederiksen, M.; Pedersen, M.; Knudsen, L. E. *Environ. Int.* **2011,** *37* (1), 1-10.

(5) Bergh, C.; Torgrip, R.; Emenius, G.; Östman, C. *Indoor Air* **2011,** *21* (1), 67-76.

(6) Hartmann, P. C.; Bürgi, D.; Giger, W. *Chemosphere* **2004,** *57* (8), 781-787.

(7) Kanazawa, A.; Saito, I.; Araki, A.; Takeda, M.; Ma, M.; Saijo, Y.; Kishi, R. *Indoor Air* **2010,** *20* (1), 72-84.

(8) Dodson, R. E.; Perovich, L. J.; Covaci, A.; Van den Eede, N.; Ionas, A. C.; Dirtu, A. C.; Brody, J. G.; Rudel, R. A. *Environ. Sci. Technol.* **2012,** *46* (24), 13056-13066.

(9) Zheng, J.; Luo, X. J.; Yuan, J. G.; Wang, J.; Wang, Y. T.; Chen, S. J.; Mai, B. X.; Yang, Z. Y. *Environ. Pollut.* **2011,** *159* (12), 3706-3713.

(10) Ali, N.; Harrad, S.; Goosey, E.; Neels, H.; Covaci, A. *Chemosphere* **2011,** *83* (10), 1360-1365.

(11) García, M.; Rodríguez, I.; Cela, R. *J. Chromatogr. A* **2007,** *1152* (1-2), 280-286.

(12) Van den Eede, N.; Dirtu, A. C.; Neels, H.; Covaci, A. *Environ. Int.* **2011,** *37* (2), 454-461.

(13) USEPA Risk assessment guidance for superfund volume I human health evaluation manual (Part A). http://www.epa.gov/oswer/riskassessment/ragsa/ (accessed May 2014).

(14) USEPA Example Exposure Scenarios Assessment Tool. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=85843. 1997. (accessed May 2014).

(15) Ulsamer, A. G.; Porter, W. K.; Osterberg, R. E. *J. Environ. Pathol. Toxicol.* **1978,** *1* (5), 543-549.

(16) USEPA. Toxicological review of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) http://www.epa.gov/iris/toxreviews/1010tr.pdf (accessed May 2014).

(17) USEPA. Toxicological review of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) http://www.epa.gov/iris/toxreviews/1008tr.pdf (accessed May 2014).

(18) USEPA. Toxicological review of 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153) http://www.epa.gov/iris/toxreviews/1009tr.pdf (accessed May 2014).

(19) USEPA. Toxicological review of decabromodiphenyl ether (BDE-209). http://www.epa.gov/iris/toxreviews/0035tr.pdf (accessed May 2014).

(20) Ali, N.; Dirtu, A. C.; Van den Eede, N.; Goosey, E.; Harrad, S.; Neels, H.; Mannetje, A.; Coakley, J.; Douwes, J.; Covaci, A. *Chemosphere* **2012,** *88* (11), 1276-1282.

3.2.2. Discussion of the Results

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

This is the first study conducted in indoor environments from Norway investigating the occurrence of 37 legacy, emerging and phosphorous FRs in air and dust from households (48 living rooms) and schools (6 classrooms). The performance of the method developed at the Toxicological Centre (University of Antwerp) [1] was checked by measuring the content of PBDEs and some PFRs in a certified reference material of dust (SRM2585). The accuracies obtained were satisfactory and ranged between 78 and 113%. Moreover, the method was sensitive enough to allow the determination of the FRs at low concentrations in the Norwegian samples. The study confirmed the widespread occurrence of all FRs since around 80% of the targeted FRs were detected in both environments and revealed a highly similar distribution patterns of FRs between households and schools.

Regarding BFRs in air samples, highest median concentrations in households and schools were observed 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). TBECH was the second most abundant FR in households and schools and, to the best of our knowledge, these are the highest concentrations of TBECH ever reported in indoor environments. TBECH has been identified as a potential endocrine disruptor and the high abundance found in Norwegian indoor air raises concerns about this emerging FR [2]. Regarding the levels of PFRs, their concentrations were 100 times higher than those of BFRs because of their higher vapour pressures and production volumes. TBOEP was the most abundant PFR in classrooms and with a median concentration of 12.9 ng/m^3, approximately 20 times higher than in the households. In living rooms, TCIPP had the highest median concentration (42.3 ng/m^3), whereas in classrooms the median was 10.2 ng/m^3. A risk assessment based on the children's daily intake via inhalation resulted in 22 pg/kg bw/day for TBECH, which is much higher than the intake via dust ingestion (5.4 pg/kg bw/day). There is no published RfD for TBECH, and health effects of such exposure are difficult to assess. The estimated daily intakes of PBDEs, or any BFRs, were still below the established RfD for PBDEs [3-6] after using the maximum concentrations detected in the sampling sites and double intakes. Consequently, the indoor air of the studied Norwegian households and schools does not seem to be hazardous for children's health.

In dust samples, the median concentrations of the FRs in floor and settled dust were not statistically different. The most abundant BFRs in floor dust from the households were BDE-209 (325 ng/g) followed by BDE-99 (171 ng/g) and DBDPE (147 ng/g), while BDE-209 was the highest median detected in schools (507 ng/g), followed by DBDPE (156 ng/g) and BEH-TEBP (103 ng/g). Regarding the PFRs, TBOEP showed the highest median floor dust

concentrations in both environments (13 400 ng/g and 87 200 ng/g in households and schools, respectively). The high concentration found in schools is most probably due to the use of TBOEP as a floor polish additive [7,8]. A risk assessment, conducted in the same manner as for air samples, was performed resulting in concentrations below the RfD for all the PFRs [9], even after assuming worst case scenarios. However, for both children and mothers, dermal contact contributed as much as dust ingestion to the intake of PFRs. In addition, sources of exposure to FRs were assessed for the households. The main finding was that frequent vacuum cleaning resulted in lower FR concentrations in dust.

Good Spearman rank correlations between the FR concentrations in dust and air ($0.36<R<0.76$) showed that the estimation of the concentrations in air from analysed dust was feasible and that conditions close to steady state between phases was reached. Following the accepted approximation by Weschler and Nazaroff [10] of using the K_{oa} to represent the organic matter of dust used, we calculated dust-air partition coefficients for the FRs and plotted them against their log K_{oa}. The obtained linearity was very satisfactory in living rooms ($R^2=0.7$; $p<0.01$) and classrooms ($R^2=0.35$; $p<0.01$) with acceptable deviations in the prediction of the air concentrations.

This is one of the most comprehensive studies performed in indoor environments, which clearly shows the extended use and ubiquity of emerging FRs in households and schools.

References

[1] N. Van den Eede, A.C. Dirtu, N. Ali, H. Neels, A. Covaci, Talanta 89 (2012) 292-300.
[2] H. Khalaf, A. Larsson, H. Berg, R. McCrindle, G. Arsenault, P. Olsson, Environ. Health Perspect. 117 (2009) 1853-1859.
[3] USEPA. Toxicol. review of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) http://www.epa.gov/iris/toxreviews/1010tr.pdf, 2008 (accessed June 2014).
[4] USEPA, Toxicol. review of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) http://www.epa.gov/iris/toxreviews/1008tr.pdf, 2008 (accessed June 2014).
[5] USEPA, Toxicol. review of 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153) http://www.epa.gov/iris/toxreviews/1009tr.pdf, 2008 (accessed June 2014).
[6] USEPA, Toxicol. review of decabromodiphenyl ether (BDE-209) http://www.epa.gov/iris/toxreviews/0035tr.pdf, 2008 (accessed June 2014).
[7] A. Marklund, B. Andersson, P. Haglund, Chemosphere 53 (2003) 1137-1146.
[8] I. van der Veen, J. de Boer, Chemosphere 88 (2012) 1119-1153.
[9] N. Ali, A.C. Dirtu, N. Van den Eede, E. Goosey, S. Harrad, H. Neels, A. Mannetje, J. Coakley, J. Douwes, A. Covaci, Chemosphere 88 (2012) 1276-1282.
[10] C.J. Weschler, W.W. Nazaroff, Atmos. Environ. 44 (2010) 3609-3620.

3.3. Human Exposure to Persistent Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Nowadays, people spend many hours per day in indoor environments e.g., houses, offices, cars, stores, public transport, etc. It has previously been discussed that FRs are applied in materials and products used in the indoor environment, and that these chemicals leach out becoming accessible to humans through several routes of exposure, i.e., dietary exposure, inhalation of air, ingestion and dermal contact with dust. Once the persistent FRs enter the human body, they accumulate in fatty tissues, which justifies a serious concern because some of the persistent FRs have been already proven as endocrine disruptors, for example, PBDEs [1]. Human biomonitoring of persistent FRs has been performed by analysing blood, adipose tissue, and hair, although the most common matrix is the serum or plasma from blood. The volume or weight of biological samples is usually very limited and therefore the total amount of FR to detect is very low. In consequence, extraction processes are essential to determine the sensitivity of the overall analytical method, and therefore all analytical methodologies try to maximise the recoveries of the analytes. The extraction of FRs from complex matrices like serum or plasma is challenging especially for the most lipophilic compounds, irrespectively of the extraction technique. For example, low recoveries of BDE-209 from serum using SPE have commonly been reported, 64% being one of the highest recoveries obtained [2]. Recoveries of the emerging FRs from serum are unknown, but it is suspected that the methodologies applied to PBDEs can also be valid, since PBDE congeners cover a wide range of polarities.

The first study of this section explores the extraction efficiencies of several SPE cartridges, as well as LLE using non-polar solvents, and mixtures of them in order to assess the recoveries of the emerging FRs and to find out which conditions maximise the extraction of the highly lipophilic FRs. Furthermore, the causes for the low recoveries are discussed pointing the content of lipids in serum as a critical factor in the extraction efficiencies. The quantification of the FRs was performed using GC-(ECNI)MS, and further optimisation of the chromatographic parameters and MS conditions resulted in the method presented in the second study for the characterisation of the levels of persistent FRs in Norwegian women from our cohort. The GC-(ECNI)MS method was specifically optimised for the determination of the emerging FRs, in particular, HBB, HCDBCO, DBDPE, Dec602, DP (*syn* and *anti*), Dec603 and BTBPE. GC-(ECNI)MS is a highly sensitive technique for the analysis of halogenated compounds that provides instrument limits of detection in the low picogram range for PBDEs [3]. Emerging FRs and PBDEs were analysed simultaneously using a DB5-MS short column. The GC-(ECNI)MS method was comprehensively validated spiking three batches of horse serum with all standards under study at different levels. The validation of the

method was performed twice. The applicability of the method was tested on human serum samples, five from general population and five from fish consumers from Lake Mjøsa, which in 2008 was a contaminated lake in Norway [4].

Furthermore, in the third study, we attempted to elucidate to what extent dust and air, from residential living rooms, and diet contribute to the concentrations of emerging and legacy FRs in the serum of the Norwegian women. The major pathway of exposure to PBDEs in U.S.A. is dust [5], but no associations between concentration in dust and levels in serum have been found in Europe [6,7]. Nonetheless, diet has been reported as an important source of exposure to PBDEs for some European population [7,8], and there are no published studies exploring ingestion of food as an exposure pathway for emerging FRs. Therefore, we looked at correlations between the serum concentrations of the participants and measured concentrations of FRs in air and dust, and with amount of food ingested. To assess the amount of food consumed, the participants answered a comprehensive food frequency questionnaire, which covered 42 food items categorised in meat, fish, vegetables, daily products and fruit and vegetables.

The first study was published in Environmental Science and Technology Letters, the second study was published in Journal of Chromatography A, and the third one has been submitted to Environment International.

References

[1] Å. Bergman, J.J. Heindel, S. Jobling, K.A. Kidd, R.T. Zoeller, ISBN: 978 92 4 150503 1, 2012.
[2] A. Covaci, S. Voorspoels, J. Chromatogr. B 827 (2005) 216-223.
[3] L. K. Ackerman, G. R. Wilson, S. L. Simonich, Anal. Chem. 77 (2005) 1979-1987.
[4] C. Thomsen, H. K. Knutsen, V.H. Liane, M. Frøshaug, H.E. Kvalem, M. Haugen, H.M. Meltzer, J. Alexander, G. Becher, Mol. Nutr. Food Res. 52 (2008) 228-237.
[5] M. Lorber, J. Expo. Sci. Environ. Epid. 18 (2008) 2-19.
[6] L. Roosens, M. A. E. Abdallah, S. Harrad, H. Neels, A. Covaci, Environ. Sci. Technol. 43 (2009) 3535-3541.
[7] H. Fromme, W. Körner, N. Shahin, A. Wanner, M. Albrecht, S. Boehmer, H. Parlar, R. Mayer, B. Liebl, G. Bolte, Environ. Int. 35 (2009) 1125-1135.
[8] H. K. Knutsen, H.E. Kvalem, C. Thomsen, M. Frøshaug, M. Haugen, G. Becher, J. Alexander, H.M. Meltzer, Mol. Nutr. Food Res. 52 (2008) 217-227.

3.3.1. The Lipid Content in Serum Affects the Extraction Efficiencies of Highly Lipophilic Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

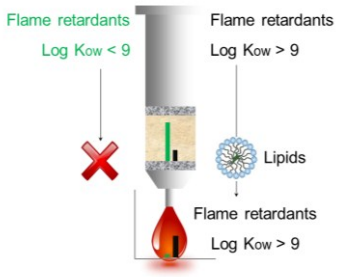
Environ. Sci. Technol. Lett. 1 (2014) 82-86

Lipid content in serum affects the extraction efficiencies of highly lipophilic flame retardants

Enrique Cequier[†,‡,*], Rosa Maria Marcé[‡], Georg Becher[†,§] and Cathrine Thomsen[†]

† Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway
‡ Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona, Spain
§ Department of Chemistry, University of Oslo, Norway

Abstract



This work investigates the recoveries from human serum of 8 halogenated flame retardants of emerging concern: hexabromobenzene, hexachlorocyclopentenyl-dibromocyclooctane, 1,2-bis[2,4,6-tribromophenoxy]ethane, Dechlorane 602, Dechlorane 603, Dechlorane Plus®, decabromodiphenyl ether and decabromodiphenyl ethane. Extraction efficiencies were assessed using solid phase extraction (Oasis® HLB) at two spiking levels with recoveries ranging from 18 to 84% (RSDs 4 - 25%; n=8). Recoveries for DBDPE, BDE-209 and Dechlorane Plus® averaged 24 (RSD=18%), 38 (RSD=20%) and 49% (RSD=12%), respectively. These low recoveries were negatively associated with the lipid content of the serum and Pearson correlations ranged from -0.798 to -0.839 (p-values <0.002). This fact indicates that interactions between highly lipophilic flame retardants and lipids affect the extraction efficiencies. Therefore, even with thoroughly optimised SPE procedures, studies carried out without a proper internal standard (similar recovery) might result in erroneous calculated concentrations of the highly lipophilic halogenated flame retardants in serum.

INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in a wide range of consumer products, but have been restricted or banned due to environmental and health concerns.[1] This has increased the use of a number of replacements often termed emerging flame retardants.[2,3] Biomonitoring using serum samples has frequently been used to assess human exposure to halogenated flame retardants (HFRs) such as PBDEs. However, data on emerging HFRs in human serum are scarce in the literature. Techniques such as solid phase extraction (SPE) and liquid-liquid extraction (LLE) have been widely used in sensitive methods for analysis of common HFRs in human body fluids,[4,5] although for some HFRs recoveries are insufficient regardless of the extraction technique.[6,7]

When using SPE, highly lipophilic compounds are not always satisfactorily extracted from serum. For instance, in the analysis of the most abundant poly-brominated diphenyl ethers, the majority is well extracted, but recoveries for BDE-183 and also BDE-209 are usually lower than for the less brominated PBDEs. For BDE-183, recoveries reported from sheep[8] and human[9,10] serum ranged from 43 to 89%, while recoveries for BDE-209 from human

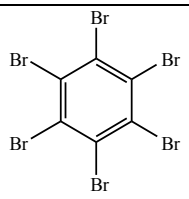
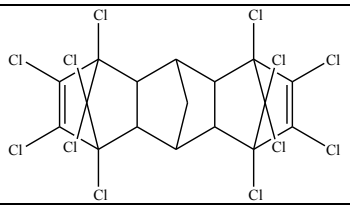
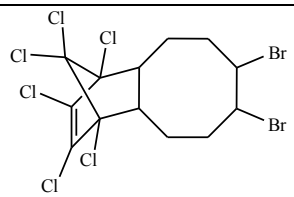
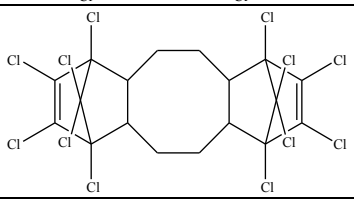
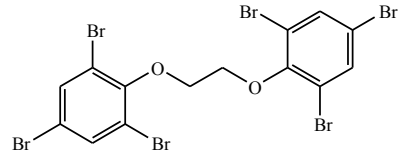
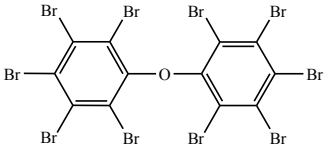
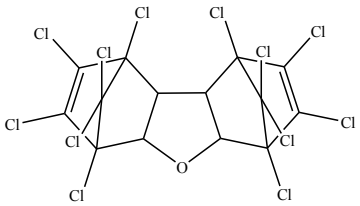
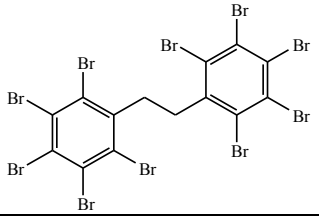
Environ. Sci. Technol. Lett. 1 (2014) 82-86

serum ranged from 13 to 64%.[10-13] It has been suggested that these poor recoveries are due to irreversible adsorption to the stationary phase[10] and/or other surfaces[13] as well as incomplete protein denaturation,[14] but this has not been experimentally proven. Therefore, this study focuses on the use of SPE for the extraction of 8 HFRs of emerging concern from human serum: hexabromobenzene (HBB), hexachloro-cyclopentenyl-dibromocyclo-octane (HCDBCO), 1,2-bis[2,4,6-tribromophen-oxy]ethane (BTBPE), decabromodiphenyl ethane (DBDPE), Dechlorane 602 (Dec602), Dechlorane 603 (Dec603), Dechlorane Plus® (DPs, *syn* and *anti*) and decabromodiphenyl ether (BDE-209) (Table 1). The objectives are to investigate the extraction efficiencies of these HFRs and sources of losses during the extraction process.

MATERIALS AND METHODS

Spiking Solutions of Flame Retardants. Spiking solution (A) containing 7 emerging HFRs (^{13}C-HBB, HCDBCO, Dec602, BTBPE, Dec603, ^{13}C-DPs, DBDPE) and ^{13}C-BDE-209 were prepared in toluene at two concentrations: low level (LL) at 4 pg/µL, except for ^{13}C-BDE-209 and DBDPE that were 18 pg/µL and 89 pg/µL, respectively, and high level (HL) at 81 pg/µL, except for [^{13}C]BDE-209 and DBDPE, that were 161 pg/µL and 806 pg/µL, respectively. A second spiking solution (B) containing BDE-18, 103, 181, [^{13}C]HBB, [^{13}C]DPs and [^{13}C]BDE-209 at 10 pg/µL was also prepared in toluene. Recovery standards (RS; [^{13}C]BDE-205 and [^{13}C]BDE-139) were diluted to100 pg/µL in toluene. See the Supporting Information for further information on standards/solvents.

Table 1. Chemical structures, CAS numbers and octanol water partition coefficients (^{a}K$_{ow}$) for the emerging HFRs and BDE-209

molecular structure	compound	molecular structure	compound
	HBB CAS 87-82-1 Log K$_{ow}$ 7.3		Dec603 CAS 13560-92-4 Log K$_{ow}$ 11.2
	HCDBCO CAS 51936-55-1 Log K$_{ow}$ 7.9		DPs CAS 13560-89-9 Log K$_{ow}$ 11.3
	BTBPE CAS 37853-59-1 Log K$_{ow}$ 9.1		BDE-209 CAS 1163-19-5 Log K$_{ow}$ 12.1
	Dec602 CAS 31107-44-5 Log K$_{ow}$ 8.1		DBDPE CAS 84852-53-9 Log K$_{ow}$ 13.6

^{a}Log K$_{ow}$ data were obtained from EPI Suite™ 4.1 software (see electronic supporting information for further details).

Sample Pretreatment. Horse serum (Sigma Aldrich, St. Louis, MO, USA; further details see the Supporting Information) and an in-house quality control sample of pooled human sera were used to develop the analytical method and assess recoveries of emerging HFRs and [^{13}C]BDE-209. Ten individual serum samples from non-occupationally exposed persons which participated in studies at the Norwegian Institute of Public Health were used to assess extractions on samples with different lipid content. Informed consent was obtained from all participants, and the Regional Committee for Medical Research Ethics approved the study. All sera were stored at -20°C. Serum concentrations of triglycerides, phospholipids and total cholesterol were determined enzymatically at the Oslo University Hospital and the total lipid content was calculated according to the summation method described by Grimvall *et al*.[15]

Horse and human serum were thawed overnight. Two mL of serum were spiked with 30 µL of solution A (LL or HL) or B. After brief manual whirl mixing (Heidolph REAX top, Schwabach, Germany) and 10 min of sonication (Branson 2510, Sigma Aldrich), samples were placed in the refrigerator overnight. Denaturation of proteins was carried out in 20 mL glass tubes at room temperature using 2 mL of formic acid followed by whirl mixing and 10 min sonication. Prior to SPE extraction, samples were diluted with water to a final volume of 6 mL.

Extraction and Cleanup. Labelled compounds, if available, were used in the recovery experiments to avoid the possible contamination of native HFRs from the environment. SPE was performed on Oasis® HLB columns (500 mg; Waters, Milford, MA, USA) applying the following method in four steps: (1) Conditioning of the column using consecutively 4 mL each of dichloromethane (DCM), methanol and water. (2) Loading denaturised serum. (3) Washing of column in two steps: 4 mL of water and 1 mL of methanol. (4) Before eluting target analytes with 8 mL of DCM, the column was dried by suction (30 min). The eluate was concentrated to approximately 1 mL using a Rapidvap® (Labconco, Kansas, MO, USA). Subsequently, further clean-up was conducted using SPE cartridges packed with (bottom to top): silica (~150 mg), sulphuric acid/silica (33%, v/w) (~1 g), silica (~150 mg) and sodium sulphate (~600 mg). The clean-up cartridges were conditioned by 4 mL of heptane/DCM (3:1, v/v). After loading the extract from the SPE, elution was carried out by gravity with 8 mL of heptane/DCM (3:1, v/v). The eluate was evaporated to approximately 0.5 mL in the Rapidvap®. This volume was transferred to inserts and reduced to a few microliters with a gentle stream of nitrogen. Finally, 20 µL of RS were added and the volume adjusted to 50 µL with toluene.

For comparison, 2 mL of serum, denaturised with 2 mL of formic acid and spiked with solution A at LL, were liquid-liquid extracted (1 min whirl mixing) using 3 x 7 mL of either toluene or toluene/heptane (1:1, v/v) or heptane/methyl *tert*-butyl ether (1:1, v/v). A mixture of heptane/DCM (6:1, v/v) was used for LLE of the eluted aqueous phase from the SPE to look for non-retained HFRs. After whirl mixing aqueous phase (serum) and organic phase for 1 min and following centrifugation at room temperature (g-force = 2163 RCF for 2 min) (Rotina 46, Hettich Lab Technology, Tuttlingen, Germany), organic phases were collected and reduced to approximately 1 mL. Clean-up was performed using the

procedure described above. In addition, an experiment to assess the efficiency of the former LLE for the highly lipophilic HFRs was carried out using an ultrasonic bath (Transonic TS 540, Elma, Singen, Germany). First, 1 mL of DCM was added to a 2 mL of denaturised horse serum and then 4 mL of a mixture of heptane/DCM/2-propanol (85:5:10; v/v) were added to obtain three separated phases. The rest of the process followed the previous description of LLE except that ultrasonication was applied for 45 min.

To check for losses due to adsorption of HFRs onto the walls of the original sample tubes, these were dried by a flow of nitrogen and rinsed repeatedly with DCM. The combined volumes were reduced and subjected to GC-MS analysis (see the Supporting Information for instrumental analysis, Tables S1 and S2, and chromatograms in Figures S1-S3).

RESULTS AND DISCUSSION

Extraction Efficiency of SPE. For method development, horse serum was used as it was unlikely to contain HFRs and was expected to result in similar matrix effects as human serum. Procedural blanks (5% 2-propanol in water) contained BTBPE, Dec603 and BDE-209 (Figure S3 of the Supporting Information). Since [^{13}C]labelled standards were not available for BTBPE and Dec603, blank subtraction was performed on serum samples.

Emerging HFRs and [^{13}C]BDE-209 were extracted from horse and human serum using Oasis® HLB as previous studies have demonstrated a better performance for halogenated compounds such as PBDEs compared to other sorbents (C_{18} Empore™ from 3M Company; Strata-X from Phenomenex and Isolute-phenyl, 101 and ENV+ from International Sorbent Technology).[10,11] A tendency of decreasing recovery with increasing K_{ow} was observed (Figure 1; see Table S3 of the Supporting Information for numerical data).

In general, the SPE recoveries were lower for human than for horse serum (e.g., 35% for DPs) which might be ascribed to different percentage in lipid content (0.21 and 0.57% for horse and human serum, respectively) and/or composition. Ren *et al.* also reported lower recoveries for DPs from human and bovine serum using LLE (around 20%).[16] Recoveries obtained by LLE of horse and human serum were in the same range as those obtained by SPE (Table S4 of the Supporting Information) indicating similar extraction efficiencies for the two methods. Consequently, either SPE or LLE have been demonstrated to be suitable techniques to extract HFRs with log K_{ow} < 8-9 from human serum. For the SPE extraction of the more lipophilic HFRs, it was possible to increase the recoveries of dechloranes by optimizing the SPE procedure, e.g., by thorough drying of the SPE sorbent prior to elution.[17]

Breakthrough of HFRs during SPE. To date, obtaining good extraction efficiencies for compounds with log K_{ow} > 9 is still a challenge. To investigate the loss during SPE extraction, the aqueous eluent from the SPE column was subjected to LLE. Only HFRs with very high K_{ow} were found in the extract: Dec602, Dec603, [^{13}C]DPs, [^{13}C]BDE-209 and DBDPE. Their recoveries ranged from 1 to 6% (RSD 27-67%; n=3) and from 1 to 8% (RSD 18-42%; n=3) at LL and HL spiking, respectively (Figure S4 of the Supporting Information). Strikingly, the most lipophilic flame retardants ([^{13}C]BDE-209 and DBDPE) had the highest percentages in the aqueous eluent representing around 20% of their total SPE recoveries. A possible explanation for

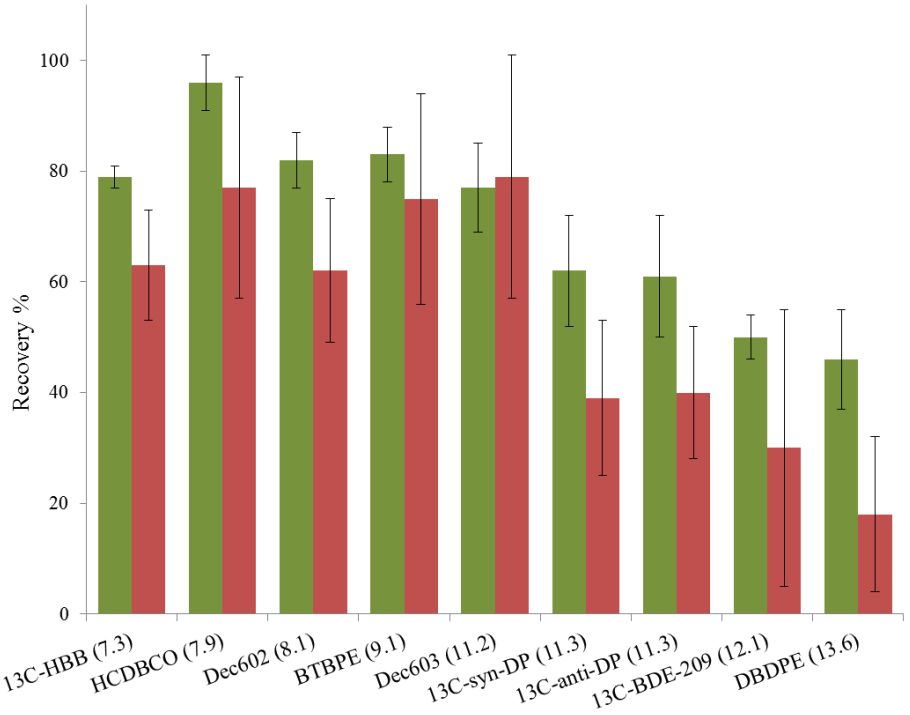


Figure 1. SPE recoveries of emerging HFRs and ^{13}C-BDE-209 from human (red; n=4) and horse (green; n=3) serum spiked with solution A at LL. Error bars show% RSD. The log K_{ow} is given in parentheses.

the breakthrough might be that the highly lipophilic HFRs are trapped in micellar type structures formed by surface active serum constituents such as phospholipids. Measured concentrations of phospholipids in serum were within the range of 2.0-3.3 mM. Since the critical micellar concentration for typical phospholipids is between 0.05 and 0.77 mM,[18] this phenomenon is therefore likely to occur both during SPE and LLE. Then, HFRs would be trapped in the inner lipophilic core of the amphipathic substance, while the outer polar surface would have little interaction with the hydrophobic stationary phase from SPE or organic liquid phase from LLE, and thereby reducing the extraction efficiency. Such encapsulation or trapping is well-known in analytical chemistry e.g., in investigations of macromolecules by nuclear magnetic resonance[19] and separation of persistent pollutants by cloud point extraction.[20] Figure 2 visualises the increasing breakthrough of emerging HFRs and [^{13}C]BDE-209 with increasing K_{ow} at both spiking levels.

The pattern in Figure 2 was tested and confirmed by repeating the extraction experiments of human and horse serum after 10 and 30 days, respectively (Figure S5 of the Supporting Information). However, the detected breakthrough does not explain the total loss of highly lipophilic HFRs in the SPE recoveries. An exhaustive extraction of the sample tubes and SPE sorbent did not reveal any residues adsorbed to the surfaces. Consequently, the loss of analytes might still remain in the aqueous phase as suggested by the breakthrough. Horse serum was spiked with solution B + DBDPE (100 pg/µL) and HFRs extracted in the 3 phases system using ultrasonication. Significantly higher recoveries for [^{13}C]BDE-209 (83%; RSD

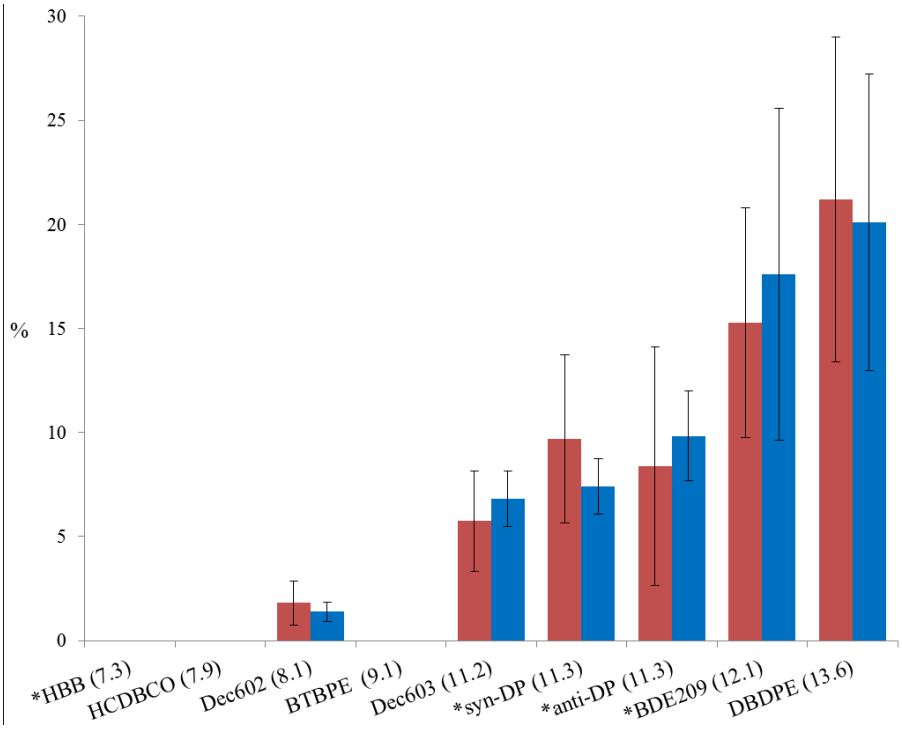


Figure 2. Amount of emerging HFRs and ^{13}C-BDE-209 in aqueous phase expressed as a percentage of SPE recovery. Human serum spiked with solution A at LL (red) and HL (blue). The log K_{ow} is given in parentheses. Error bars show % RSD.

22%; n=4) and DBDPE (65%, RSD 23%, n=4) compared to SPE recoveries of [^{13}C]BDE-209 (50%; RSD 4%; n=3) and DBDPE (46%; RSD 9%; n=3) were obtained (Figure S6 of the Supporting Information). The ultrasonic treatment in LLE seems to extract the highly HFRs more efficiently than SPE, possibly by disrupting their interactions with the active serum constituents. This supports the hypothesis that micelle formation of highly lipophilic HFRs with serum constituents is responsible for their low recoveries in SPE.

Association between SPE Recoveries of HFRs and Lipid Content in Serum. Recoveries from matrices such as dust are considerably higher for BDE-209 and DBDPE than from serum.[21,22] Although dust also may contain lipids, these cannot form micelles because HFRs are extracted using non-polar organic solvents, i.e., there is no aqueous phase where the lipids can form micelles with lipophilic HFRs. Since lipids are suspected to take part in the SPE breakthrough and therefore in the low recoveries, a new set of extractions of samples with differing lipid content i.e., procedural blanks, horse and 10 human sera was carried out. Sera and procedural blanks were spiked with 30 µL of solution B and solid phase extracted as described above. Table 2 shows that compounds with log K_{ow} > 9.4 have in general lower recoveries (red) than the rest of analytes (green), indicating the expected different behaviour in their extraction efficiencies. Recoveries obtained from BDE-18 were somewhat lower than expected, possibly due to partial evaporation during sample preparation.

To increase the statistical power, procedural blank recoveries were included in the statistics (see calculation without procedural blank in Table S5 of the Supporting Information) SPE recoveries of [^{13}C]-

Table 2. SPE recoveries (%) and statistical associations with the total lipid content (%) in serum spiked with solution B

Matrix	Total lipid	BDE-18	^{13}C-HBB	BDE-103	BDE-181	^{13}C-syn-DP	^{13}C-anti-DP	^{13}C-BDE-209
[a]procedural blank	0.00	70	82	85	73	81	79	86
[b]horse serum	0.21	59	75	75	67	65	61	55
human A	0.44	53	68	68	59	46	44	39
human B	0.45	63	78	74	59	38	39	34
human C	0.49	64	79	64	64	44	42	51
human D	0.49	66	75	80	70	45	43	42
human E	0.53	79	89	93	74	53	51	45
human F	0.59	50	62	67	62	35	33	38
human G	0.60	76	87	85	63	37	37	33
human H	0.62	64	74	79	71	57	54	57
human I	0.72	49	56	59	53	38	36	37
human J	0.79	65	82	77	60	38	37	31
	Log K_{ow}	5.9	7.3	7.7	9.4	11.3	11.3	12.1
	[c]Pearson (r)	-0.162	-0.224	-0.273	-0.495	-0.823	-0.839	-0.798
	p-value	0.615	0.484	0.391	0.102	0.001	0.001	0.002

[a] Procedural blank (5% 2-propanol in water); n=4; RSD 2-6%

[b] n=4; RSD 3-10%

[c] Performed using SPSS® v.20

labelled *anti*-DP, *syn*-DP and BDE-209 were significantly and highly correlated with the total lipid content of the sample (*p*-values <0.01). Strong correlations were also seen with triglycerides, cholesterol and phospholipids individually (Table S6 of the Supporting Information). These tendencies suggest that the lipid content negatively influences the recoveries of HFRs, but especially those with high K_{ow} values.

This fact could affect the determination of the emerging HFRs and ^{13}C-BDE-209 in serum samples if the internal standards (IS) used for their quantification have different K_{ow}. This is, for example, the case in some published studies which do not use [^{13}C]labelled IS for the determination of DPs[23,24] and for DBDPE, which is usually determined against [^{13}C]BDE-209.[12,17] As an example from our study, if BDE-181 (GC retention time close to *syn*-DP) is used as IS for quantification of DPs, the reported concentrations would be underestimated by around 30% (based on the recoveries) when an additional correction factor is not applied. The choice of an IS based merely on GC retention times can lead to errors in quantification for compounds with different K_{ow}. Therefore, use of isotopically labelled analogues as IS, whenever available or possible, is strongly recommended.

AUTHOR INFORMATION

Corresponding author

*E-mail address: enrique.cequier@fhi.no

ACKNOWLEDGEMENTS

This study is funded by the European Commission, 7th Framework Programme under the EU Marie Curie Initial Training Network INFLAME (GA No. 264600).

ASSOCIATED CONTENT

Supporting information. Technical details regarding GC-MS, chromatograms and extraction efficiencies using LLE and SPE are provided in the supporting information.

Environ. Sci. Technol. Lett. 1 (2014) 82-86

This material is available free of charge via Internet at http://pubs.acs.org.

REFERENCES

(1) Stockholm Convention on Persistent Organic Pollutants. http://chm.pops.int/ (accessed May 2014).

(2) NORMAN Network of reference laboratories for monitoring emerging environmental pollutants, http://www.-normannetwork.net/ (accessed May 2014).

(3) Scientific opinion on emerging and novel brominated flame retardants in food. European food safety authority **(**EFSA) Journal 9:2156. 2011.

(4) Covaci, A.; Harrad, S.; Abdallah, M.A.E.; Ali, N.; Law, R.J.; Herzke, D.; de Wit, C.A. *Environ. Int*. **2011**, 37 (2), 532-556.

(5) Xian, Q.; Siddique, S.; Li, T.; Feng, Y.; Takser, L. ; Zhu, J. *Environ. Int*. **2011**, 37 (7), 1273-1278.

(6) Park, J.; She, J.; Holden, A.; Sharp, M.; Gephart, R.; Sounders-Mason, G.; Zhang, V.; Chow, J.; Leslie, B.; Hooper, K. *Environ. Sci. Technol*. **2011**, 45 (10), 4579-4585.

(7) Thomsen, C.; Stigum, H.; Frøshaug, M.; Broadwell, S.L.; Becher, G.; Eggesbø, M. *Environ. Int*. **2010**, 36 (1), 68-74.

(8) Zhang, Z.; Rhind, S.M. *Talanta* **2011**, 84 (2), 487-493.

(9) Karlsson, M.; Julander, A.; van Bavel, B.; Lindström, G. *Chromatographia* **2005**, 61 (1-2), 67-73.

(10) Covaci, A.; Voorspoels, S. *J. Chromatogr. B* **2005**, 827 (2), 216-223.

(11) Thomsen, C.; Liane, V.H.; Becher, G. *J. Chromatogr. B* **2007**, 846 (1-2), 252-263.

(12) Karlsson, M.; Julander, A.; van Bavel, B.; Hardell, L. *Environ. Int*. **2007**, 33 (1), 62-69.

(13) Frederiksen, M.; Thomsen, C.; Frøshaug, M.; Vorkamp, K.; Thomsen, M.; Becher, G.; Knudsen, L.E. *Int. J. Hyg. Environ. Health* **2010**, 213 (4), 233-242.

(14) Keller, J.M.; Swarthout, R.; Carlson, B.K.; Yordy, J.; Guichard, A.; Schantz, M.M.; Kucklick, J.R. *Anal. Bioanal. Chem*. **2009**, 393 (2), 747-760.

(15) Grimvall, E.; Rylander, L.; Nilsson-Ehle, P.; Nilsson, U.; Stromberg, U.; Hagmar, L.; Östman, C. *Arch. Environ. Contam. Toxicol*. **1997**, 32 (3), 329-336.

(16) Ren, G.; Luo, X.F.; Ma, S.; Sun, Y.F.; Yu, Z.; Sheng, G.; Fu, J. *Chin. J. Anal. Chem*. **2011**, 39 (2), 235-238.

(17) Cequier, E.; Marcé, R.M.; Becher, G.; Thomsen, C. *J. Chromatogr. A* **2013,** 1310 (4), 126-132.

(18) King, M.D.; Marsh, D. *Biochemistry* **1987**, 26 (5), 1224-1231.

(19) van Horn, W.D.; Ogilvie, M.E.; Flynn, P.F. *J. Am. Chem. Soc*. **2009**, 131 (23), 8030-8039.

(20) Xie, S.; Paau, M.C.; Li, C.F.; Xiao, D.; Choi, M.M.F. *J. Chromatogr. A* **2010,** 1217 (16), 2306-2317.

(21) Van den Eede, N.; Dirtu, A.C.; Ali, N.; Neels, H.; Covaci, A. *Talanta* **2012**, 89 (30), 292-300.

(22) Sahlström, L.; Sellström, U.; de Wit, C.A. *Anal. Bioanal. Chem*. **2012**, 404 (2), 459-466.

(23) Venier, M.; Hites, R.A. *Environ. Sci. Technol*. **2011**, 45 (10), 4602-4608.

(24) Yan, X.; Zheng, J.; Chen, K.; Yang, J.; Luo, X.; Yu, L.; Chen, S.; Mai, B.; Yang, Z. *Environ. Int*. **2012**, 49 (15), 31-37.

Supporting Information

Supporting Information / Environ. Sci. Technol. Lett. 1 (2014) 82-86

INTRODUCTION

(EPI)Suite™ 4.1 software was employed to obtain octanol-water partition coefficients of all emerging HFRs and BDE-209 from the same source.

The EPI (Estimation Programs Interface) Suite™ is a Windows®-based suite of physical/chemical property and environmental fate estimation programs developed by the U.S. Environmental Protection Agency's (USEPA) Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).

http://www.epa.gov/opptintr/exposure/pubs/episuite.htm (accessed May 2014)

MATERIALS AND METHODS

BTBPE, DBDPE, HCDBCO, BDE-18, BDE-181 and the [^{13}C]labelled BDE-139, 205 and 209 were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). HBB and DPs (both, native and [^{13}C]labelled) were purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.), BDE-103 was purchased from AccuStandard Inc. (New Haven, CT, USA), Dec602 and Dec603 (both powder) were purchased from Toronto Research Chemical Inc. (North York, Ontario, Canada). All purities were ≥ 98%, except Dec602 (95%). Heptane, toluene, dichloromethane (DCM), methyl *tert*-butyl ether and methanol were of pesticide grade and purchased from LabScan (Dublin, Ireland). Sulphuric and formic acid 98 % were purchased from VWR International (Radnor, PA, U.S.A.), silica gel 60 (0.063 - 0.200 mm) and sodium sulphate were obtained from Merck (Darmstadt, Germany). Water was purified using an Elga Option 4 Water Purifier device (Elga, Bucks, U.K.). All non-volumetric glassware was carefully cleaned and heated to 450 ºC prior to use.

Sample Pretreatment. Horse serum composition: Total lipids 0.21% (cholesterol 44%, triglycerides 7% and phospholipids 49%)

Instrumental Analysis. The determination was performed on a HP 6890 series gas chromatograph equipped with a 7683 autosampler coupled to a 5973 mass selective detector (Avondale, PA, U.S.A.). A DB5-MS column from Agilent was used. Detection was performed using electron capture negative ionization (ECNI) with methane as reagent gas and selected ion monitoring. Table S1 and S2 show technical details regarding the GC-MS determination and Figure S1-S3 show chromatograms of procedural blank, non-spiked and spiked human serum.

Supporting Information / Environ. Sci. Technol. Lett. 1 (2014) 82-86

Table S1. Chromatographic and MS conditions

Injection volume	1 µL
Injector temperature	300°C
Pulsed splitless	55 psi kept for 2min
Interface temperature	300°C
Carrier gas	Helium
Flow	1.5 mL/min (hold 11 min) up to 3 mL/min at 50 mL/min[2]
Column	Deactivated retention gap (0.7 m x 0.32 mm) + DB5-MS (15 m x 0.25 mm x 0.1 µm)
Oven temperature	100°C up to 280°C (hold 3 min) at 35°C/min 280°C up to 300°C (hold 7 min) at 25°C/min
Interface temperature	300°C
[a]Ion source temperature	200°C
Methane pressure	$2.2x10^{-4}$ Torr
Quadrupole temperature	106°C

[a] 200 °C was found optimal to obtain larger fragments than Br^-, enabling the use of ^{13}C labelled standards for HBB, DPs, BDE-209 and RS.

Table S2. Ions (m/z) monitored in GC-MS for each compound

Compound	Quantifier	Qualifier
HBB	551.5	471.6
[^{13}C]HBB	559.6	479.6
[a]HCDBCO	541.7	537.5
[a]Dec602	613.7	611.6
[^{13}C]BDE-139 (RS)	573.8	414.9
[a]Dec603	637.7	635.8
[b] BTBPE	79.0	251.8
DP (*syn* & *anti*)	653.8	651.8
[^{13}C]DP (*syn* & *anti*)	663.8	661.8
[^{13}C]BDE-205 (RS)	573.5	653.7
BDE-209	486.7	484.6
[^{13}C]BDE-209	494.6	496.6
[b] DBDPE	79.0	81.0

[(a)] No ^{13}C-labelled standards commercially available; [(b)] [^{13}C]labelled standards did not produce abundant ions significantly higher than Br^-

Supporting Information / Environ. Sci. Technol. Lett. 1 (2014) 82-86

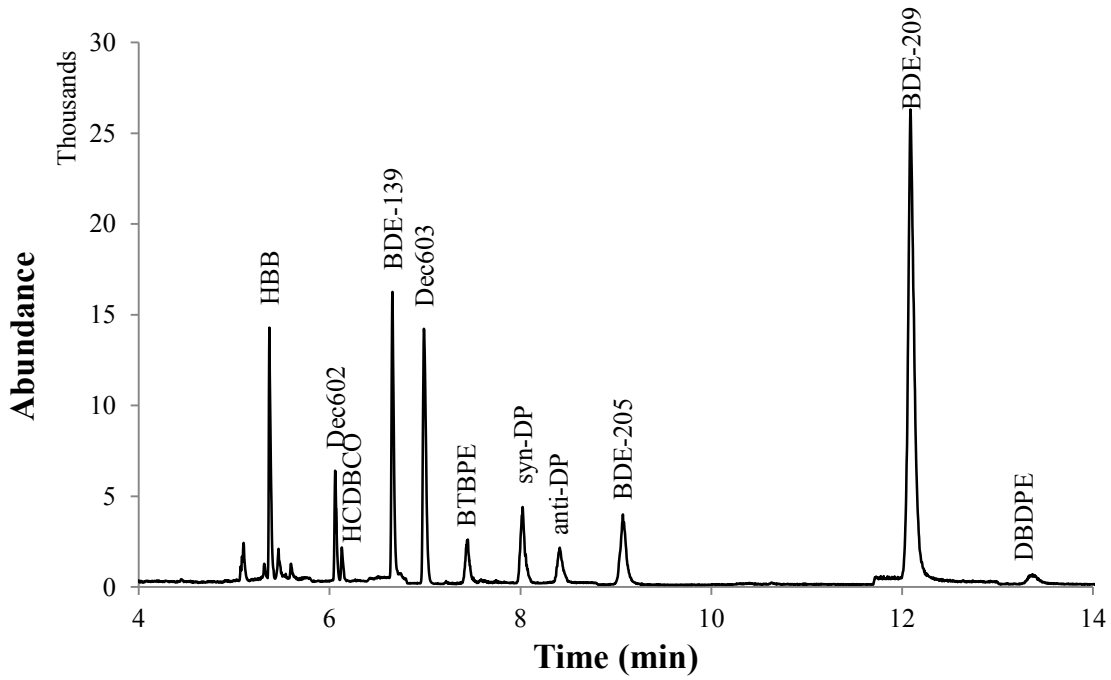


Figure S1. Chromatogram of emerging HFRs and BDE-209 from human serum spiked with solution A at LL and recovery standard ([^{13}C]BDE-205).

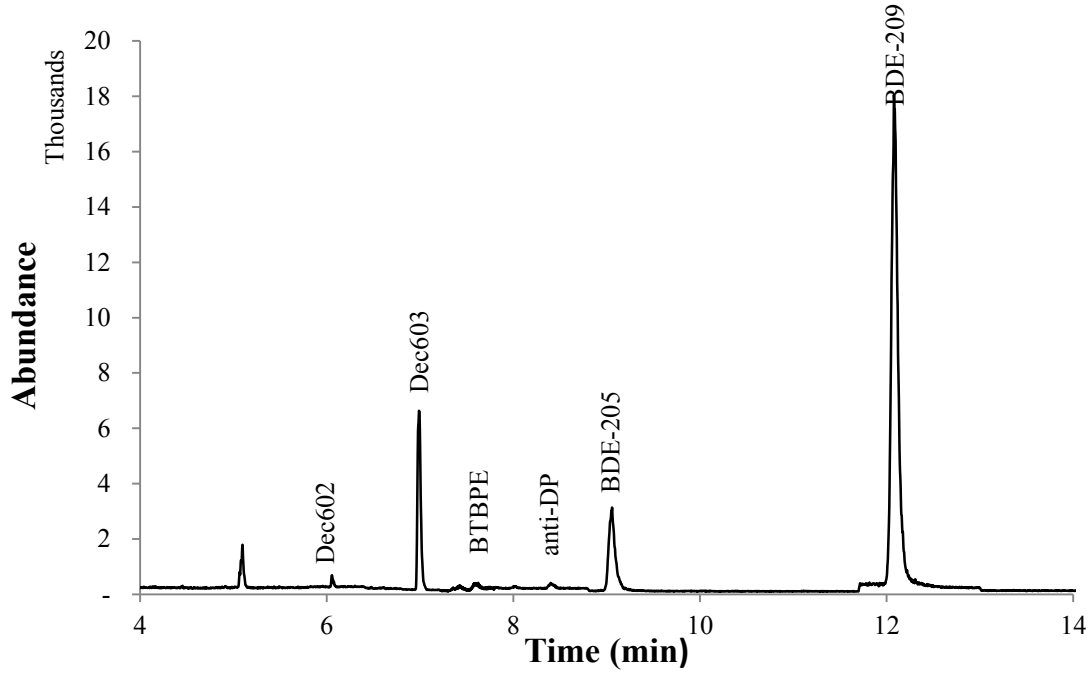


Figure S2. Chromatogram of a non-spiked human serum with RS ([^{13}C]BDE-205).

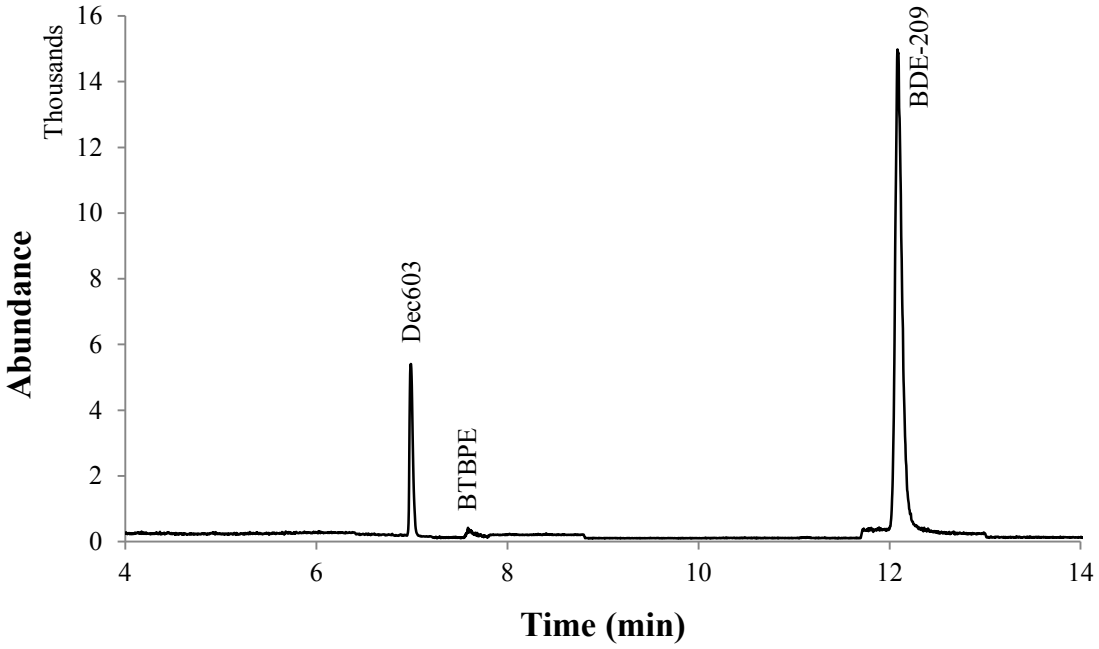


Figure S3. Chromatogram of a procedural blank (5 % 2-propanol in water).

The amount of Dec603 found in the blanks (~68 pg) was high when compared to the LL spiking. Nevertheless, the relatively low RSD (8-22 %, see Table S3) in the recoveries for Dec603 indicates the constant amount in the blanks. We have a relative high background of BDE-209 in all of our tests. This is why the experiments in this study have been carried out using [^{13}C]BDE-209.

RESULTS AND DISCUSSION

Extraction efficiency of SPE

Table S3. SPE recoveries of emerging HFRs and [^{13}C]BDE-209 obtained from horse and human serum

		[a]Horse serum (n=3)	[a]Human serum (n=4)	
Log K_{ow}		300 pg	LL	HL
7.3	[^{13}C]HBB	79 (2)	63 (10)	72 (13)
7.9	HCDBCO	96 (5)	77 (20)	81 (10)
8.1	Dec602	82 (5)	62 (13)	66 (10)
9.1	BTBPE	83 (5)	75 (19)	84 (4)
11.2	Dec603	77 (8)	79 (22)	65 (7)
11.3	[^{13}C]*syn*-DP	62 (10)	39 (14)	58 (10)
11.3	[^{13}C]*anti*-DP	61 (11)	40 (12)	57 (11)
12.1	[^{13}C]BDE-209	50 (4)	30 (25)	45 (14)
13.6	DBDPE	46 (9)	18 (14)	29 (22)

[a] Recoveries calculated by external calibration using recovery standard; % RSDs are given in parentheses

To test possible matrix effects on the quantification after extractions, analytes were added to non-spiked horse serum extracts just prior to injection. The concentrations determined ranged from 92 to 111 % of the added amount (data not shown), excluding any severe matrix effect.

Table S4. LLE recoveries of emerging HFRs and [^{13}C]BDE-209 from horse and human serum spiked at LL with solution A

	[(a)]Horse serum (n=3)			[(a)]Human serum (n=4)
	Hep/tol 1:1	Toluene	Hep/MTBE 1:1	Hep/tol 1:1
[^{13}C]HBB	78 (7)	83 (14)	154 (6)	70 (15)
HCDBCO	73 (9)	81 (11)	106 (14)	75 (14)
Dec602	-	-	-	64 (10)
BTBPE	84 (8)	52 (15)	60 (9)	64 (13)
Dec603	-	-	-	76 (6)
[(b)] [^{13}C]*syn*-DP	74 (11)	33 (22)	58 (23)	58 (5)
[(b)] [^{13}C]*anti*-DP	63 (15)	49 (17)	58 (29)	58 (9)
[^{13}C]BDE-209	58 (10)	25 (27)	50 (24)	48 (7)
DBDPE	54 (20)	25 (27)	45 (51)	45 (7)

[(a)] Recoveries calculated by external calibration using recovery standards; [(b)] [^{13}C]labelled DPs not used in horse serum; % RSDs are given in parentheses; (-) not determined

Breakthrough of emerging HFRs

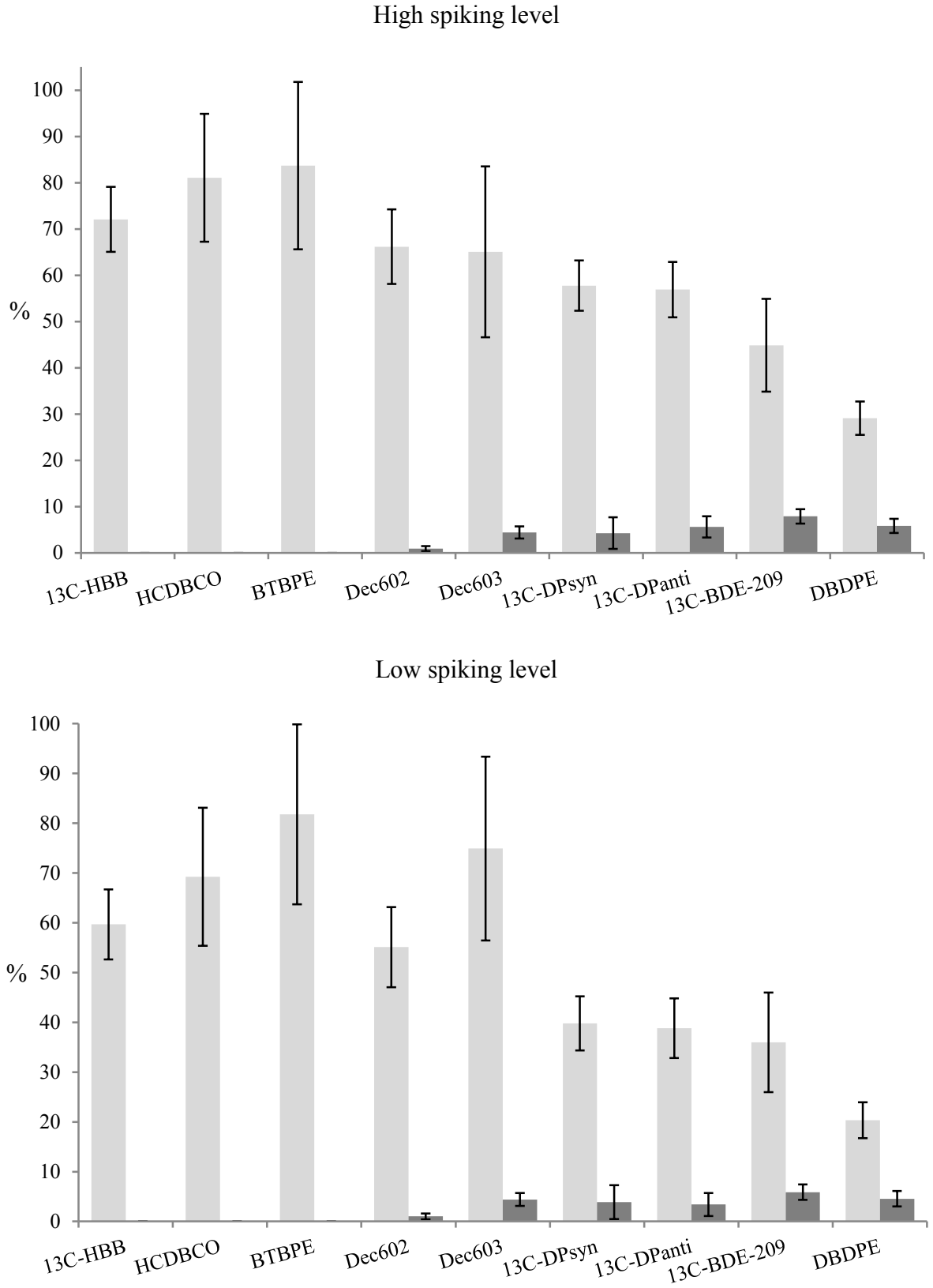


Figure S4. Recoveries of emerging HFRs and [^{13}C]BDE-209 from human serum in SPE (grey light) and from the aqueous eluted phase (grey dark) spiked with solution A at low and high levels. Error bars show % RSD.

Supporting Information / Environ. Sci. Technol. Lett. 1 (2014) 82-86

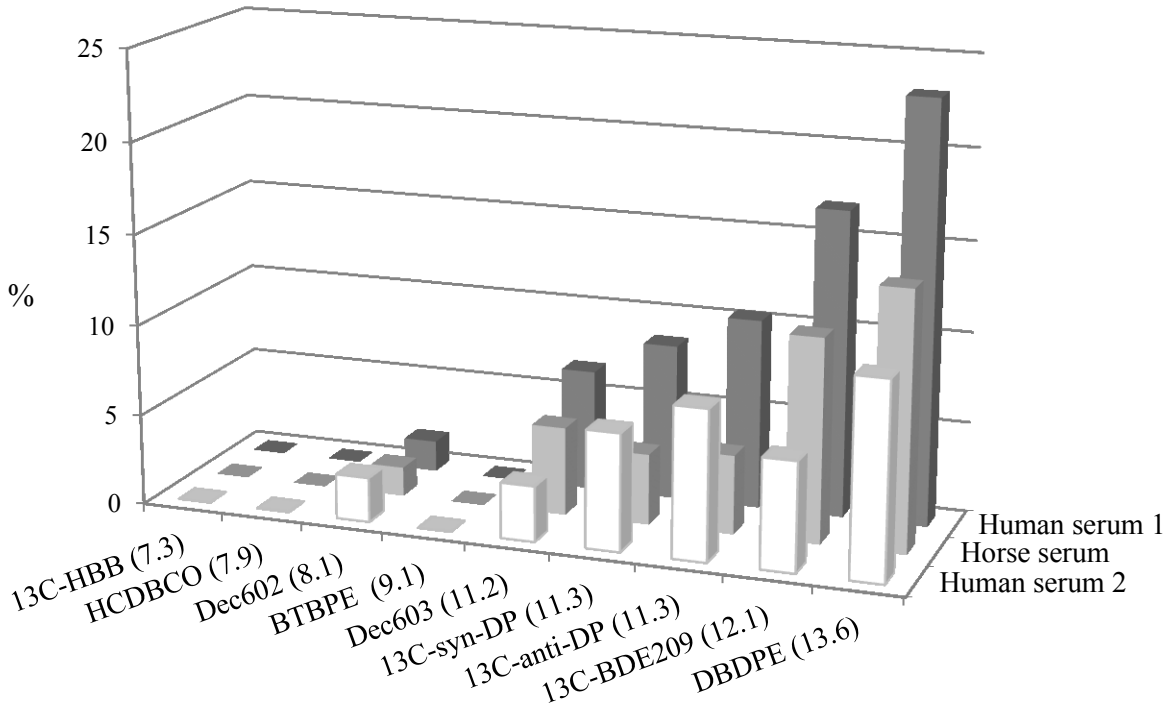


Figure S5. Amount of emerging HFRs and [^{13}C]BDE-209 in aqueous eluent expressed as percentage of SPE recovery. All sera spiked with solution A at HL. Human serum 1 (n=4) and horse serum (n=3) extracted with heptane/DCM (6:1, v/v) and human serum 2 (n=4) extracted with heptane/toluene (1:1, v/v). The log K_{ow} is given in parentheses.

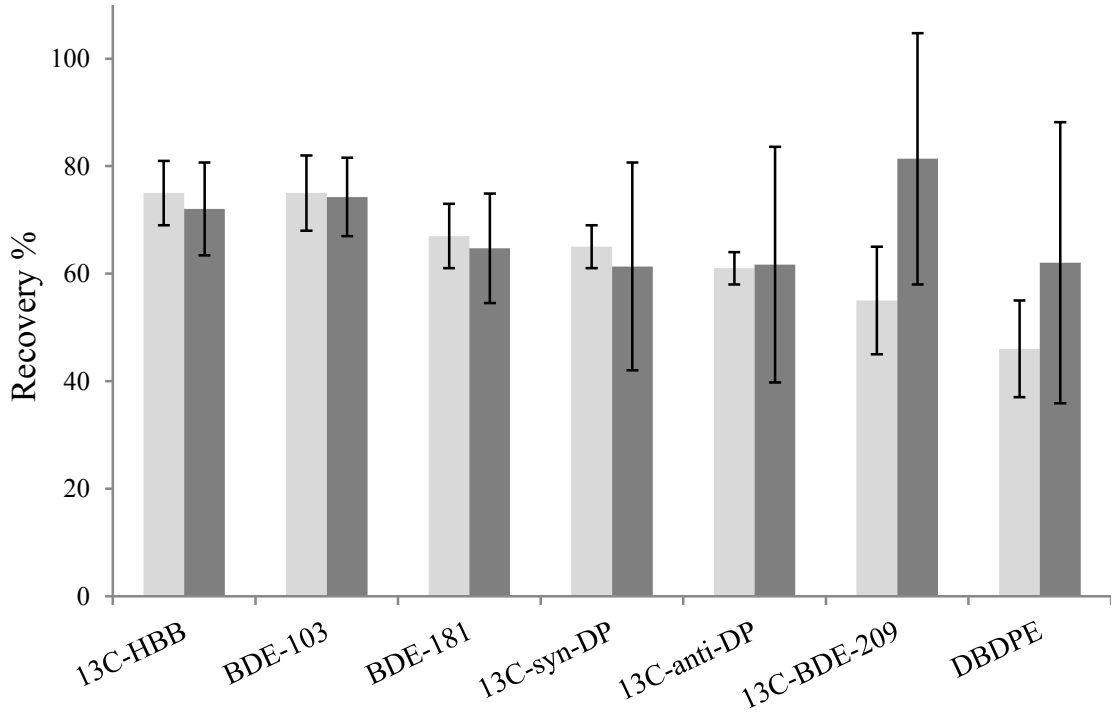


Figure S6. SPE recoveries (light grey) and ultrasonic LLE (dark grey) of HFRs in horse serum spiked with solution B + DBDPE. The log K_{ow} is given in parentheses. Error bars show % RSD.

For the ultrasonic LLE, the contact surface of horse serum with organic solvents was maximised by using DCM (bottom), serum (middle) and heptane mixture (top). The heptane phase contained 10% of 2-propanol to favour interaction with polar components from serum and reduce emulsion formation. The ultrasonic treatment seemed to disrupt the interactions between the HFRs and the active serum constituents. Nevertheless, the average % RSD of [^{13}C]BDE-209 and DBDPE from ultrasonic LLE is considerably higher than SPE (mean: 22% vs 7%).

Association between recoveries of HFRs and lipid content in serum. After excluding blank from calculations, we still have a clear trend in the Table S5 (the higher is K_{ow}, the higher the negatively correlation with lipid content). However, [^{13}C]BDE-209 is no longer statistically significant ($p = 0.11$).

Table S5. Recoveries (%) and statistical associations with the total lipid content (%) in serum spiked with solution B

Matrix	Lipid content	BDE-18	^{13}C-HBB	BDE-103	BDE-181	^{13}C-syn-DP	^{13}C-anti-DP	^{13}C-BDE-209
[a]procedural blank	0.00	70	82	85	73	81	79	86
[b]Horse serum	0.21	59	75	75	67	65	61	55
Human A	0,44	53	68	68	59	46	44	39
Human B	0.45	63	78	74	59	38	39	34
Human C	0.49	64	79	64	64	44	42	51
Human D	0.49	66	75	80	70	45	43	42
Human E	0.53	79	89	93	74	53	51	45
Human F	0,59	50	62	67	62	35	33	38
Human G	0.60	76	87	85	63	37	37	33
Human H	0,62	64	74	79	71	57	54	57
Human I	0,72	49	56	59	53	38	36	37
Human J	0.79	65	82	77	60	38	37	31
	Log K_{ow}	5.9	7.3	7.7	9.4	11.3	11.3	12.1
	[c]Pearson (r)	0.009	-0.103	-0.090	-0.320	**-0.612**	**-0.631**	-0.509
	p-value	0.978	0.763	0.792	0.337	0.045	0.037	0.110

[a] Procedural blank (5 % 2-propanol in water); n=4; RSD 2-6 %

[b] n=4; RSD 3-10 %

[c] Performed using SPSS® v.20; Procedural blank excluded

Table S6. Pearson correlations between recoveries of flame retardants (blank and serum) and total lipid content (%), phospholipids (mg/L), cholesterol (mg/L) and triglycerides (mg/L)

		BDE-18	HBB	BDE-103	BDE-181	syn-DP	anti-DP	BDE-209
Total lipid content	Correlation	-.162	-.224	-.273	-.495	**-.823****	**-.839****	**-.798****
	Sig.	.615	.484	.391	.102	.001	.001	.002
Phospholipid	Correlation	-.145	-.191	-.243	-.469	**-.768****	**-.787****	**-.776****
	Sig.	.652	.551	.447	.124	.004	.002	.003
Cholesterol	Correlation	-.156	-.283	-.328	**-.583***	**-.728****	**-.725****	**-.648***
	Sig.	.629	.373	.298	.047	.007	.008	.023
Triglyceride	Correlation	-.204	-.216	-.265	-.397	**-.811****	**-.841****	**-.802****
	Sig.	.524	.500	.404	.201	.001	.001	.002

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

3.3.2. Determination of Emerging Halogenated Flame Retardants and Polybrominated Diphenyl Ethers in Serum by Gas Chromatography-Mass Spectrometry

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

J. Chromatogr. A 1310 (2013) 126-132

Determination of emerging halogenated flame retardants and polybrominated diphenyl ethers in serum by gas chromatography mass spectrometry

Enrique Cequier [a,b,*], Rosa Maria Marcé [b], Georg Becher [a,c], Cathrine Thomsen [a]

[a] *Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway;*
[b] *Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Tarragona, Spain,*
[c] *Department of Chemistry, University of Oslo, Norway*

Abstract

Emerging flame retardants are used in a great variety of household goods and thus have the potential to pollute our indoor environment. Health concerns regarding exposure to these flame retardants demand new methods to survey their occurrence in humans. This work describes development and optimization of an analytical method comprising solid phase extraction and gas chromatography coupled to mass spectrometry for the determination of besides 15 polybrominated diphenyl ethers, 7 emerging halogenated flame retardants in human serum (1,2-bis[2,4,6-tribromophenoxy] ethane, decabromodiphenyl ethane, hexabromobenzene, Dechlorane Plus®, hexachlorocyclopentenyl-dibromocyclooctane, dechlorane 602 and 603). The method was thoroughly validated at three spiking levels obtaining averaged recoveries > 80 % with a RSD of 5 % (n=12). Accuracies ranged from 88 to 125 % except for DBDPE which averaged 66 % with overall RSD of 11 % (n=12). Method limits of detection (MLD) ranged from 0.3 to 5.4 pg/mL serum, except for decabromodiphenyl ether and decabromodiphenyl ethane for which MLDs were 14 and 20 pg/mL serum, respectively. In human serum samples from Norway, we were able to detect and quantify hexabromobenzene, 1,2-bis[2,4,6-tribromophenoxy] ethane, Dechlorane Plus®, Dechlorane 602 and 603.

Keywords: emerging flame retardant, PBDE, blood, GC-MS, dechlorane.

* Corresponding author: Tel.: +4721076604; fax: +4722042686.
E-mail address: enrique.cequier@fhi.no

1. Introduction

Halogenated flame retardants (HFRs) are chemicals used to reduce or prevent the spread of fire in many consumer goods that surround us in our daily life (electric and electronic equipment, synthetic foams, furniture, textiles and toys, among others). Their content reaches percentage amounts by weight [1] and in most cases, these HFRs are not chemically bonded to the products allowing them to leach out and pollute the indoor environment.

Several HFRs (e.g. polybrominated diphenyl ethers, PBDEs) have been totally or partially banned as they are toxic, highly persistent, lipophilic, and accumulate in food chains, thus being a risk for human health [2]. As a result of this ban, industry is increasingly using novel or unregulated flame retardants

in products, often named emerging HFRs [3,4]. For instance, decabromodiphenyl ethane (DBDPE) and 1,2-bis[2,4,6-tribromophenoxy]ethane (BTBPE) have been proposed as replacements for two PBDEs formulations [5,6]. Many of the emerging HFRs are manufactured in high production volumes (> 1000 tons/year) and have been detected in biotic and abiotic matrices worldwide [7].

To study human exposure to emerging HFRs, development of sensitive methods for their determination is needed. Persistent HFRs are lipophilic, and thus, accumulate in the body fat of humans. For assessment of general populations, blood and serum in particular, are commonly analysed. Analysis of environmental pollutants in serum is challenging because the matrix is very complex, the concentrations are low and the amount of sample is often limited. For that reason, yields of extraction and instrument sensitivity must be maximised. Two approaches have mainly been used to extract emerging HFRs from serum, liquid-liquid extraction (LLE) and solid phase extraction (SPE) [7,8]. For quantification of HFRs by GC-low resolution MS using electron capture negative ionization (ECNI) [7,8] has demonstrated comparable sensitivity as GC-high resolution MS using electron impact ionization [9].

The main objective of this study was to develop a SPE GC-MS (ECNI) method for determination of 7 emerging HFRs in serum: hexabromobenzene (HBB), Dechlorane Plus® (DPs, *syn* and *anti* isomers), DBDPE, BTBPE, hexachlorocyclopentenyl-dibromocyclooctane (HCDBCO), dechlorane 602 (Dec602) and dechlorane 603 (Dec603). The method was further extended to comprise 15 PBDEs (from tribromo to decabromodiphenyl ether) for simultaneous determination with the emerging HFRs. The method has been thoroughly validated and applied to 10 individual human serum samples from Norway.

2. Experimental

2.1 Chemicals and materials

Native PBDEs (28, 47, 99, 153, 154, 181, 183, 190, 196, 197, 203, 206, 207, 208 and 209), native and ^{13}C-HBB and ^{13}C-DPs (*syn* and *anti* isomers) and a predominant congener mixture of PBDEs (EO-5103-1/100) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). BTBPE, DBDPE, HCDBCO, BDE-100, ^{13}C labelled BDE-209 and BDE-205 (recovery standard, RS) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). BDE-103 was purchased from AccuStandard Inc. (New Haven, CT, USA) and Dec602 and Dec603 (both powder) were purchased from Toronto Research Chemical Inc. (North York, Ontario, Canada). All purities were ≥ 98 %, except for Dec602 (95 %).

A standard stock solution of HFRs was prepared in toluene at a concentration of 1 ng/µL. Internal standards (IS) (BDE-18, ^{13}C-HBB, BDE-103, BDE-181, ^{13}C-BDE-209, ^{13}C-*syn* and *anti*-DP) and RS were diluted with toluene to 10 pg/µL.

A mixture of PBDE congeners was purchased from Cambridge Isotope Laboratories having a concentration of 25 pg/µL, except BDE-209 which was 100 pg/µL. The mixture was diluted to two levels: low and high. The low level was 0.325 pg/µL for BDE-28, 47, 99, 100, 153, 154, 183 and 1.25 pg/µL for BDE-209 and the high level was 12.5 pg/µL for BDE-28, 47, 99, 100, 153, 154, 183 and 50 pg/µL for BDE-209. All solvents were of pesticide grade and were purchased from LabScan

(Dublin, Ireland). Sulphuric and formic acid 98 % were purchased from VWR International (Radnor, PA, USA), silica gel 60 (0.063–0.200 mm) and sodium sulphate were obtained from Merck (Darmstadt, Germany). Water was purified using an Elga Option 4 Water Purifier device (Elga, Bucks, U.K.). All glassware was washed in 2 % Extran® MA 01 from Merck and rinsed with distilled water. Non volumetric glassware was also heated at 450°C for 4 h and wrapped in aluminium foil after cooling down.

SPE was performed on a 12-port Visiprep™ vacuum manifold (Sigma Aldrich, St. Louis, MO, USA) using Oasis® HLB columns (12 c.c.; 500 mg) (Waters, Milford, MA, USA). Evaporation of solvents was carried out on a Rapidvap® vacuum evaporation system (Labconco, Kansas, MO, USA).

2.2 Serum samples and lipid content

Horse serum (Sigma Aldrich, St. Louis, MO, USA) was used to develop and validate the analytical method and 10 Norwegian human serum samples were analysed to assess its applicability. The samples were from non-occupationally exposed persons participating in on-going studies at the Norwegian Institute of Public Health. Venous blood from non-fasting subjects was drawn into BD Vacutainer tubes (Becton Dickinson, Plymouth, U.K.) and serum was obtained after centrifugation at 2000 rpm for 10-15 min. Sera were stored at -20°C and thawed overnight and let stand till room temperature before analysis.

To normalise final concentrations of HFRs in serum, the lipid content was determined in each sample. Two hundred µL of serum were analysed for triglycerides, phospholipids and total cholesterol at the Oslo University Hospital and the total lipid content calculated according to the method described by Grimvall et al. [10].

2.3 Solid phase extraction and clean-up

During method development, both SPE and LLE (data not shown) were tested. Comparable recoveries were obtained (paired T test, p=0.826). SPE was chosen to avoid practical problems caused by emulsions and higher analysis throughput.

To a 20 mL glass tube, 30 µL of IS were added and toluene gently reduced with nitrogen (5.0, Yara Praxair, Oslo, Norway). Two mL of serum were added and the solution was whirl mixed (Heidolph, Schwabach, Germany) and homogenised by sonication for 10 min (Branson 2510, Sigma Aldrich). Samples were left in the refrigerator (+2°C) overnight. Denaturation of proteins was carried out at room temperature using 2 mL of formic acid followed by whirl mixing and 10 min sonication. The samples were diluted with 5 % isopropanol in distilled water (5 % IPA) to a final volume of 6 mL.

The SPE process was performed in a 12 port Visiprep™ vacuum manifold by gravity. SPE consisted on five steps performed under nitrogen atmosphere during solvent flowing and column drying: 1) The column was conditioned with 8 mL of dichloromethane (DCM), 4 mL of methanol and 4 mL of 5 % IPA. 2) Denatured serum was loaded onto the SPE column. 3) SPE column was washed, first with 4 mL of 5 % IPA and secondly with 500 µL of methanol which aided to eliminate water and will ensure an efficient elution with DCM. 4) The column was dried by suction for approximately 1.5 hours. Visual inspection of the cartridge (without sample) showed that about 60 min was needed to dry the SPE column. Weighing of the cartridge showed that

approximately 75 µL of water was left. 5) Elution of analytes was carried out using 8 mL of DCM and subsequently 1 mL of heptane. Thorough drying of the cartridge increased the recovery for some of the highly halogenated flame retardants (e.g., DPs).

The DCM was evaporated in a Rapidvap® (40°C; 600 mbar) and the remaining heptane extract was loaded for lipid removal on to pre-washed (4 mL heptane/DCM (3:1, v/v)) clean-up cartridge (6 c.c.) of 4 layers (bottom - top): silica (150 mg) // 30 % (v/w) sulphuric acid / silica (1 g) // silica (150 mg) // sodium sulphate (600 mg). Acidic silica was chosen as it is highly efficient for lipid removal and well suited for high throughput analysis [11]. A top layer of silica was necessary to retain polar serum constituents (e.g., cholesterol) before reaching the acid-silica layer where they can dehydrate and pass through the clean-up column [12]. The bottom layer of silica was included to prevent elution of acid to the final extract.

Elution was carried out with 2 x 4 mL of heptane/DCM (3:1, v/v). The DCM was evaporated, 500 µL of toluene added and solvent reduced (40°C; 105 mbar) until approximately 200 µL. Solvent was transferred to a glass insert and finally 40 µL of RS were added. The final extract was evaporated under a gentle stream of nitrogen to about 50 µL.

2.4 GC-MS conditions

The chromatographic separation of the HFRs was carried out on an HP 6890 series GC (Avondale, PA, USA) using a DB5-MS column of 15 m length, 0.25 mm I.D. and 0.1 µm film thickness (Agilent Technologies Inc. CA, USA). A deactivated retention gap from Agilent (0.7 m x 0.32 mm ID) was used in front of the column. The injection parameters were optimised and the final conditions were 1 µL pulsed splitless injection (55 psi kept for 2 min) at 300°C. Helium (6.0, Yara Praxair, Oslo, Norway) was used as carrier gas. The initial flow rate of 1.5 mL/min was increased to 3 mL/min after 11 min. The temperature was increased from 50°C up to 300°C in 10 min and was held at 300°C for 5 min.

The detection was performed with an HP 5973 mass selective detector using selected ion monitoring in ECNI mode with methane (3.5, AGA, Oslo, Norway) as a reagent gas. Optimization of the MS parameters was performed to achieve maximum sensitivity. The final MS parameters were: reagent gas pressure, $2.2x10^4$ Torr; emission current, 133 µA; electron energy, 210 eV; temperatures of interface 300°C; source, 200°C; and quadrupole, 106°C. The HFRs were monitored and quantified as described in Table 1. A chromatogram of a spiked horse serum is presented in Fig. 1.

2.5 Quality control

Quality control of the results was performed by including 2 procedural blanks (2 mL 5 % IPA) and 4 horse sera spiked with IS in each batch of 6 serum samples. The procedural blanks contained HBB, BTBPE, DPs, BDE-47, 99, 153, 183, 206, 207 and 208. When these HFRs were present in ≥ 50 % of the procedural blanks, the concentration of the serum samples were corrected by subtracting the mean content of the procedural blanks. For values below method limit of detection (MLD), half of the MLD value was assigned. In some batches BDE-209 was present at high concentrations making the determination impossible [12].

Identification and quantification of the

Table 1
Retention times and ions (m/z) for detection of HFRs and their internal standards [(a)]

	Retention time (min)	Quantifier ion (m/z)	Qualifier ion (m/z)
BDE-18 (IS)	6.39	79.0 / 81.0	-
^{13}C-HBB (IS)	7.24	559.6	479.6
BDE-28	6.65	79.0 / 81.0	-
HBB	7.24	551.5	471.6
BDE-47	7.48	79.0 / 81.0	-
BDE-103 (IS)	7.84	79.0 / 81.0	-
BDE-100	8.06	79.0 / 81.0	-
Dec602	8.21	613.7	611.6
BDE-99	8.25	79.0 / 81.0	-
HCDBCO	8.29	537.5	541.7
BDE-154	8.71	79.0 / 81.0	-
BDE-153	8.96	79.0 / 81.0	-
Dec603	9.42	637.7	635.8
BDE-181 (IS)	9.94	79.0 / 81.0	-
BDE-183	9.60	79.0 / 81.0	-
BTBPE	9.79	79.0 / 81.0	251.8
BDE-190	10.01	79.0 / 81.0	
^{13}C-*syn*-DP (IS)	10.18	663.8	661.8
syn-DP	10.18	653.8	651.8
^{13}C-*anti*-DP (IS)	10.41	663.8	661.8
anti-DP	10.41	653.8	651.8
^{13}C-BDE-209 (IS)	13.41	494.7	496.6
BDE-197	10.37	79.0 / 81.0	-
BDE-203	10.51	79.0 / 81.0	-
BDE-196	10.57	79.0 / 81.0	-
BDE-208	11.49	79.0 / 81.0	-
BDE-207	11.59	79.0 / 81.0	-
BDE-206	11.85	79.0 / 81.0	-
BDE-209	13.81	486.7	484.6
DBDPE	14.77	79.0 / 81.0	-
^{13}C-BDE-205 (RS)	10.78	573.5	653.7

[(a)] IS = Internal standard for quantification; RS = Recovery standard

compounds in the samples followed three criteria: (1) retention time of the analyte should be within ± 0.1 min of the standards, (2) the signal considered for integration should have a S/N ≥ 3 and (3) the ratio of the qualifier ion to the target ion should not deviate more than 20 % from the theoretical isotopic ratios.

No standard reference material for emerging HFRs in serum was available. Therefore, an external mixture of predominant PBDE congeners was used to assess the GC-MS performance from batch to batch.

3. Results and discussion

3.1 Optimization of GC-MS

3.1.1 Injection parameters

The aim to optimise the injector variables was to ensure efficient evaporation while keeping thermal degradation at a minimum, in particular for compounds with more than 8 bromine atoms [13]. The injector variables were optimised for HBB, HCDBCO, BTBPE, DPs and DBDPE since these compounds cover the whole chromatographic range. A higher injection temperature and splitless time usually resulted in increased responses. However

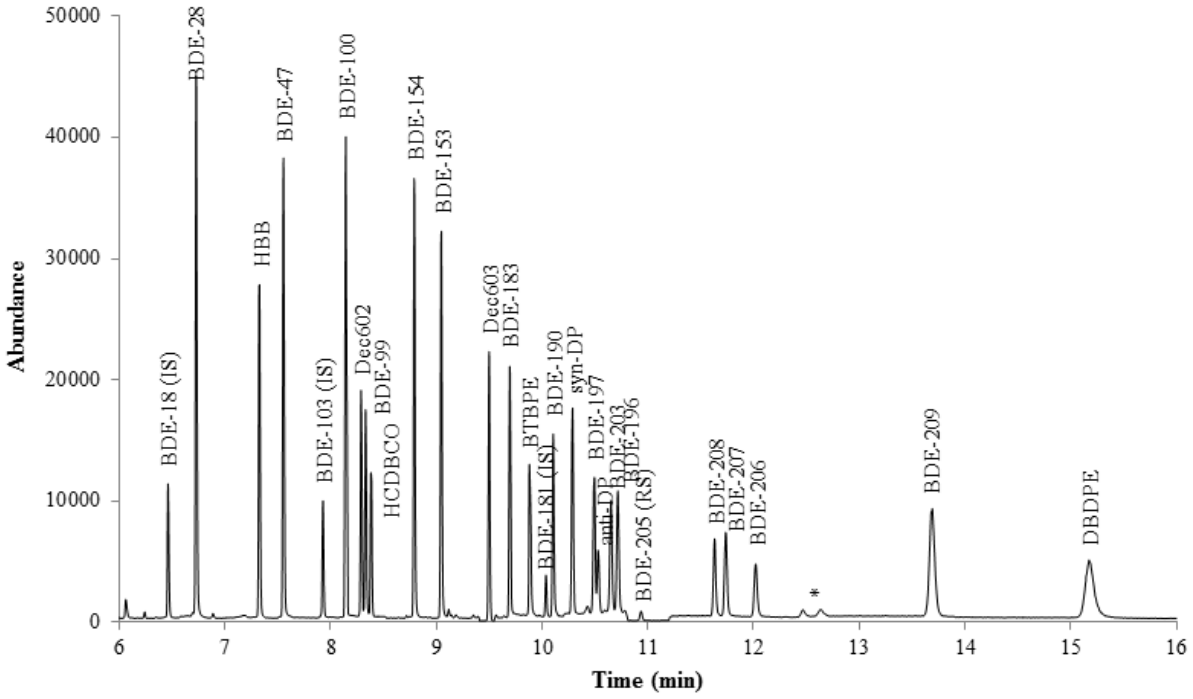


Fig. 1. Chromatogram of a spiked horse serum containing approximately 20 pg/µL of emerging HFRs and BDEs (28-183), 4 pg/µL of IS, 15-30 pg/µL of BDEs (190-209) and 165 pg/µL of DBDPE. (*) Most likely debromination products of DBDPE.

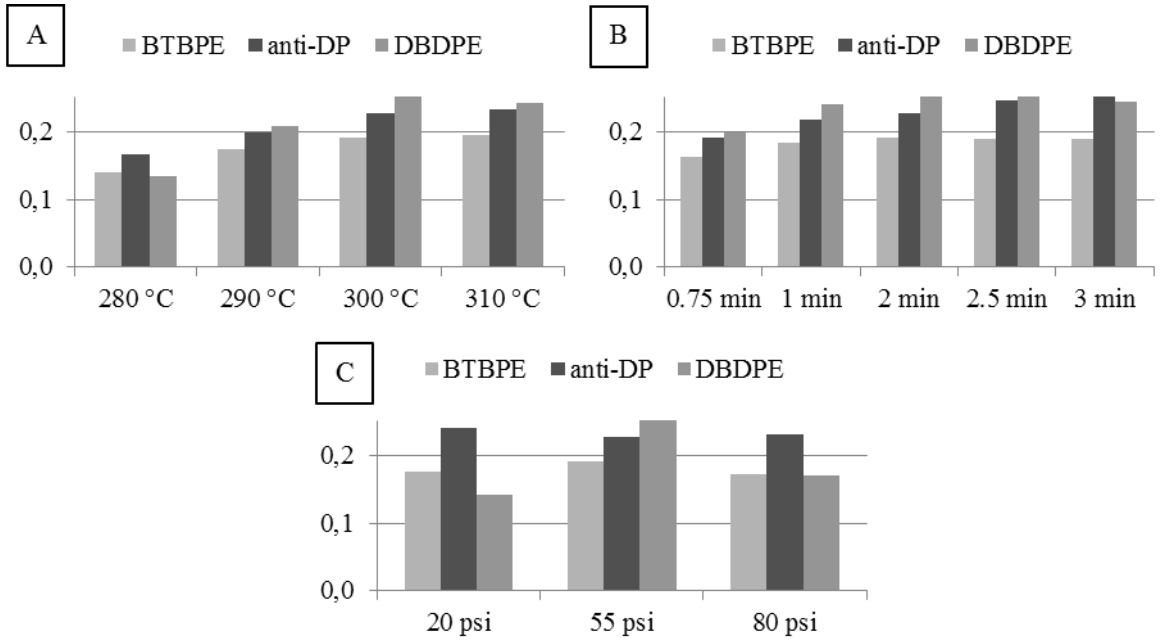


Fig. 2. Variation of ratio A_{STD}/A_{HBB} when modifying [A] injector temperature (55 psi and 2 min), [B] Splitless time (300 °C; 2 min) and [C] splitless pressure (300 °C and 2 min).

some compounds behaved differently as shown in Fig. 2, where the areas presented are relative to HBB in order to correct fluctuations in the injections. DBDPE was most sensitive to changes in the injector preferring high temperature (300°C), moderate residence time (2 min) and a splitless pressure of 55 psi. These injector parameters were chosen to obtain maximum sensitivity for DBDPE.

3.1.2 Oven and column parameters

A short non-polar column (15 m) with thin film (0.1 μm) was selected in order to minimise on-column degradation for BDE-209 and DBDPE [14]. The initial temperature was increased from 50°C to 300°C in 10 min. Oven temperatures higher than 300°C lead to severe degradation of DBDPE, visible as a long and broad band in front of the peak. This is similar to the on-column degradation observed for BDE-209 [11,15]. A linear temperature ramp was found to be effective to obtain separation of PBDEs and emerging HFRs, achieving almost baseline separation for all compounds (Fig. 1). The less resolved compounds (e.g., BDE-197 / *anti*-DP) were separated by mass (Table 1).

Further increase of the oven temperature to achieve faster elution of labile compounds was not feasible due to on-column degradation, thus an increment in flow rate was investigated. The initial flow rate was set to 1.5 mL/min resulting in a chromatogram of approximately 17 min. Flow rate of 1.5 mL/min was optimal for separation of the earlier eluting compounds, therefore it was increased after 11 min to 3 and 5 mL/min for the elution of octa-, nona-, decaBDEs and DBDPE. The chromatogram was shortened by almost 2 and 3 min, respectively (Fig. 3). Flow increment did not affect the performance of the detector and the peak areas obtained for DBDPE were even higher compared to the initial flow rate [16]. A flow rate of 3 mL/min was selected because 5 mL/min was considered too extreme for regular analyses.

The combination of a short column, thin film thickness, high temperature ramp and

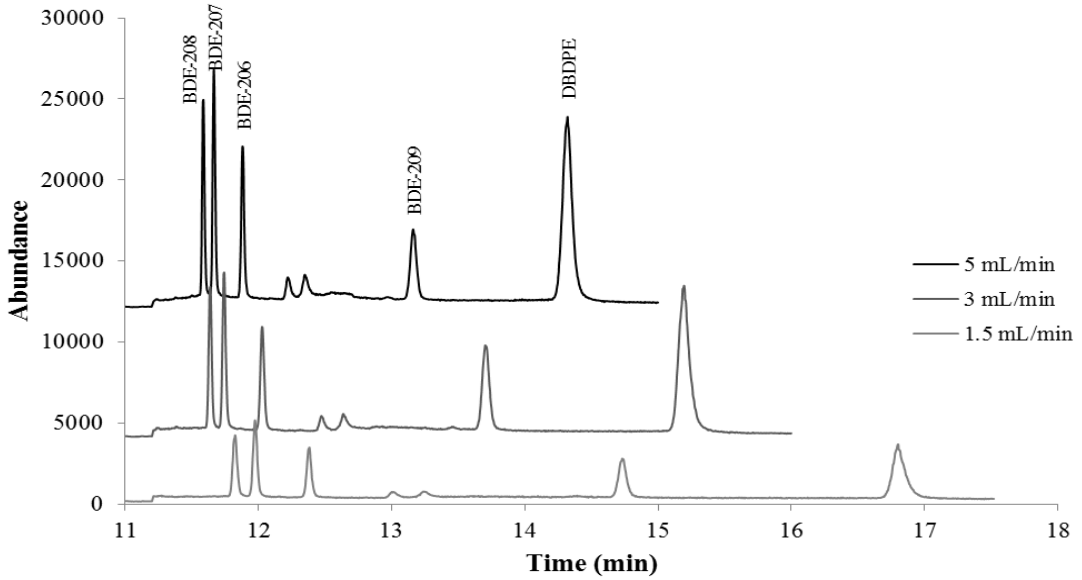


Fig. 3. Chromatograms of a spiked horse serum containing approximately 15-30 pg/μL of highly brominated PBDEs and 165 pg/μL of DBDPE at different flow rates (5, 3 and 1.5 mL/min).

J. Chromatogr. A 1310 (2013) 126-132

the increment in the flow rate lead to a determination of the emerging HFRs and PBDEs in 15 min. This chromatographic method, in terms of analysis time, is comparable to those based on low-pressure GC, which are intended to provide an adequate analysis of thermally labile compounds [15].

Two analyses are often performed for the determination of PBDEs using long columns for separation of non-thermo labile compounds (30-60 m), and a second analysis with shorter columns (10-15 m) mainly for determination of BDE-209 [11,17,18]. The presented optimised method makes possible the simultaneous analyses of selected emerging HFRs and PBDEs.

3.1.3 MS parameters

Bromide is the most abundant ion produced under ECNI for many brominated compounds. By modifying the ion source temperature, it is possible to obtain ion fragments of higher mass for certain molecules. This enables use of ^{13}C-labelled IS (listed above). Three temperatures were investigated: 150, 200 and 250°C (initial), where 200°C was found to be most adequate for identification and quantification of emerging HFRs. In the same manner, three methane pressures were also explored: $1.8x10^{-4}$ (initial), $2.2x10^{-4}$, $2.7x10^{-4}$ Torr, with $2.2x10^{-4}$ Torr resulting in higher response than the others [19]. The emission current was not particularly optimised and the default value was used (133 μA). Electron energy was tested only at 100 and 200 eV since Ackerman *et al*. reported better sensitivity at high voltages [20]. Sensitivity did not change much in that range, therefore we used the default value from the mass tuning (210 eV).

3.2 Validation results

The method was validated according to the International Conference of Harmonization guidelines [21]. Recovery refers to the percentage quantitated with respect to the amount of analyte added to the original sample and accuracy is the recovery relative to the IS. MLD and quantification (MLQ) are defined as the amount resulting in S/N ≥ 3 and S/N ≥ 10 in 2 mL of spiked serum, respectively. MLD and MLQ were established by spiking 2 mL of horse serum (4 replicates) with 3 pg of standards, except for ^{13}C-BDE-209 and DBDPE where 300 pg were added. The S/N was provided by the Chemstation software. When the S/N was higher or lower than 3, an extrapolation was carried out to obtain MLD/Q.

Assessment of the remaining validation parameters was carried out by analysing two sets of samples with 3 months difference. Two mL of horse serum were spiked with HFRs at three levels i.e, 30, 300 and 3000 pg, as well as 300 pg of IS. Each level was studied in quadruplicate. Intermediate precision of the method was obtained averaging residual standard deviations from the two validation experiments. Determination of BDE-190, octa- and nona-BDEs was included at a later stage, and recoveries, accuracies and limits of detection were not assessed.

3.2.1 Linearity and MLD

The HFRs were quantified using a 6 level inverse concentration-weighed linear regression (except for DBDPE where a quadratic fit was more suitable) covering the range 0.1-1000 pg/μL, although more accurate concentrations were obtained using a calibration curve of smaller range (0.1-100 pg/μL, 5 levels). For BDE-190, octa-, nona-

and deca-BDEs 4 calibration levels were used in the range 0.2-400 pg/µL. Correlation coefficients (R^2) were all higher than 0.993 and homoscedasticity was checked plotting the calibration residuals against concentration. Their distribution was random across axis and within limits ±2 SD (results not shown).

For 2 mL of serum, the MLD ranged from 0.6 to 11 pg for emerging HFRs and PBDEs, except for ^{13}C-BDE-209 and DBDPE, which MLDs were 28 and 40 pg, respectively (Table 2). These values are in the same range as reported in other methods for determination of PBDEs using 5 mL of serum (0.3 to 42 pg [22,23]).

3.2.2 Recovery, accuracy and precision

Recoveries relative to the RS ranged from 67-142 % (mean 100 %), 36-105 % (mean 90 %), 34-93 % (mean 80 %) at the 30, 300 and 3000 pg level, respectively with overall RSD between 1-27 % (mean 7 %) (n=12) (Table 2). Lower recoveries were observed for the highly lipophilic HFRs. This has also been observed by other researchers and could be explained by irreversible adsorptions onto surfaces throughout the whole analytical process [11,22].

Accuracies relative to the IS ranged from 88-122 % (mean 103 %), 69-125 % (mean 105 %), 62-117 % (mean 102 %) at the 30, 300 and 3000 pg level, respectively with an overall RSD between 1-28% (mean 5 %) (n=12) (Table 2).

The horse serum contained a significant amount of BDE-28, which affected recovery, accuracy and precision at the lowest spiking level for this compound. Recoveries for native BDE-209 could not be determined due to high and variable contamination of procedural blank samples, making blank correction impossible. However, its recovery was calculated using the labelled IS at 300 pg spiking level.

The accuracy was close to 100 % for all compounds, except for DBDPE which had

Table 2
Recoveries (%) and accuracies (%) with RSD in parentheses, and method limits of detection (pg/mL serum) for the HFRs

	30 pg (n = 4)		300 pg (n = 4)		3000 pg (n = 4)		MLD
	Rec.	Acc.	Rec.	Acc.	Rec.	Acc.	
BDE-28	142 (27)	110 (28)	96 (2)	96 (2)	83 (10)	93 (3)	0.69
HBB	102 (5)	118 (4)	99 (5)	104 (2)	93 (8)	107 (2)	0.30
BDE-47	122 (8)	90 (4)	96 (3)	98 (2)	85 (8)	97 (4)	1.2
BDE-100	104 (3)	104 (7)	94 (4)	107 (2)	84 (7)	107 (4)	1.6
Dec602	99 (9)	105 (6)	95 (4)	110 (2)	88 (7)	111 (3)	1.7
BDE-99	106 (9)	95 (10)	105 (3)	112 (5)	89 (6)	113 (4)	1.5
HCDBCO	113 (8)	122 (7)	97 (5)	125 (6)	89 (7)	117 (6)	5.4
BDE-154	104 (11)	102 (7)	95 (5)	108 (2)	87 (6)	109 (5)	0.82
BDE-153	102 (5)	107 (8)	94 (6)	110 (2)	87 (5)	109 (5)	0.54
Dec603	87 (11)	102 (4)	90 (7)	99 (2)	78 (6)	95 (6)	0.64
BDE-183	95 (6)	88 (7)	90 (4)	101 (1)	84 (4)	100 (3)	1.1
BTBPE	67 (1)	108 (9)	94 (5)	120 (2)	91 (3)	111 (4)	2.3
syn-DP	82 (6)	104 (7)	84 (3)	110 (2)	68 (13)	102 (3)	1.1
anti-DP	82 (12)	91 (15)	79 (6)	108 (2)	65 (12)	102 (3)	2.3
^{13}C-BDE-209	-	-	53 (5)	-	-	-	14
DBDPE	nd	nd	36 (8)	69 (6)	34 (13)	62 (16)	20

(-), not determined; nd, not detected

J. Chromatogr. A 1310 (2013) 126-132

an average of 63 % when quantified using labelled BDE-209 as IS. Despite the low accuracy for DBDPE, the use of labelled BDE-209 as internal standard is still acceptable when applying a correction factor of 1.6 to reach 100 % of accuracy since its RSD was relatively low (6-16 %).

The overall intermediate precision (calculated as the mean of the RSD from the two validation experiments (n=4 in each validation)) and accuracy difference ([% of accuracy in validation 1] – [% of accuracy in validation 2]) are shown in Table 3. Intermediate precision ranged from 2-44 % (mean 9 %) and was < 19 % for all compounds at all spiking levels (excluding level 30 pg for BDE-28). These results show the satisfactory robustness of the method. Accuracy difference ranged from -25 to 29 % with mean values of -0.2, 4.4 % and 8.8 % in the spiking levels at 30, 300 and 3000 pg respectively, indicating a satisfactory reproducibility.

BDE-190, octa- and nonaBDEs were not assessed in the validation experiment, and their determination thus has to be considered semi-quantitative even though they are expected to behave similar to the related congeners during analyses.

In order to survey the performance of the GC-MS system, low and high level solutions of the predominant congener mixture of PBDEs were injected before and after a sequence of the samples in the GC-MS. Averaged precision of the PBDE concentrations was > 90% with RSD < 15%.

3.3 Application to human serum

The method was used to analyse 10 Norwegian serum samples to assess its applicability. Five serum samples were collected from residents in Oslo (Group A) and the other 5 serum samples were collected from residents around Lake Mjøsa (Group B), a lake previously known to be contaminated with PBDEs [24]. Table 4 summarises the results. Group B had higher concentrations of Sum 7 PBDEs than Group A, which is likely to be related to consumption of fish from the polluted lake. The PBDE concentrations in Group A were

Table 3
Intermediate precision (%) and accuracy difference (%) for the HFRs

	30 pg (n = 4)		300 pg (n = 4)		3000 pg (n = 4)	
	Int. precision	Accuracy	Int. precision	Accuracy	Int. precision	Accuracy
BDE-28	44	-7	5	-3	5	-6
HBB	11	21	4	7	6	11
BDE-47	15	-25	8	-14	6	-9
BDE-100	7	7	10	18	17	29
Dec602	12	19	16	28	16	28
BDE-99	10	-11	4	3	9	15
HCDBCO	12	15	8	13	7	12
BDE-154	7	-3	2	1	10	16
BDE-153	7	-8	4	-6	7	10
Dec603	5	3	5	5	9	12
BDE-183	11	-14	2	2	4	3
BTBPE	19	21	4	7	5	1
syn-DP	7	3	7	4	5	7
anti-DP	17	-22	14	-3	4	4
DBDPE	nd	nd	11	2	13	0

nd, not detected

however, in the same range as seen in other Scandinavian countries [25,26]. Octa- to nonaBDEs were not detected above MLD, except BDE-197, 206 and 207.

Regarding the emerging HFRs, DPs were detected in all samples at similar levels in both groups, except subject B4 which had a concentration slightly higher than average levels found in people living nearby an e-waste dismantling area in China [27]. The fraction of anti DP (*f-anti* = *anti*-DP/(*syn*+*anti*-DP)) in the technical mixture ranges from 0.65 to 0.75 [28,29]. In our study, *f-anti* averaged 0.79 and 0.77 in group A and B, respectively. These values are somewhat higher than the technical mixture, suggesting higher bioaccumulation or less biotransformation, of the *anti* isomer in humans. Interestingly, values reported for *f-anti* in humans have varied from 0.57 to 0.78, *syn*-DP often being the more persistent isomer [27]. The range of *f-anti* is even wider (ranging from 0.35 to 0.95) in other biotic and abiotic matrices [8].

HBB, BTBPE, Dec602 and 603 were also found, but at lower detection frequencies. Levels of Dec602 and 603 have so far only been reported for environmental samples [30,31], and to our knowledge, this is the first time that Dec602 and Dec603 are reported in humans. HCDBCO and DBDPE could not be detected in this limited number of samples.

4. Conclusions

This study shows that after a thorough optimisation of the analytical method, determination of 7 emerging HFRs and 15 PBDEs can be performed fast and simultaneously by GC/MS (ECNI). The validation parameters show that the method is accurate and robust with satisfactory MLDs. For the first time, emerging HFRs have been detected in human serum from the Norwegian population, hence biomonitoring studies are warranted to further characterise human exposure to emerging HFRs and assess population groups at risk of higher exposure.

Acknowledgements

This study is funded by the European Commission, 7th Framework Programme under the EU Marie Curie Initial Training Network INFLAME (GA No. 264600).

References

[1] WHO. Environmental Health Criteria 162. Geneva, Switzerland. 1994.
[2] Stockholm Convention on Persistent Organic Pollutants. http://chm.pops.int/ (accessed May 2014).
[3] NORMAN Network of reference laboratories for monitoring emerging environmental pollutants. http://www.-norman-network.net/ (accessed May 2014).
[4] EFSA. Journal 10 (2012) 2908. http://www.efsa.europa.eu/en/efsajournal/pub/2908.htm (accessed May 2014)
[5] A. Kierkegaard, J. Björklund, U. Friden, Environ. Sci. Technol. 38 (2004) 3247.
[6] R. Renner, Environ. Sci. Technol. 38 (2004) 945.
[7] A. Covaci, S. Harrad, M.A.E. Abdallah, N. Ali, R.J. Law, D. Herzke, C.A. de Wit, Environ. Int. 37 (2011) 532.
[8] Q. Xian, S. Siddique, T. Li, Y. Feng, L. Takser, J. Zhu, Environ. Int. 37 (2011) 1273.
[9] C. Thomsen, L.S. Haug, H. Leknes, E. Lundanes, G. Becher, G. Lindström, Chemosphere 46 (2002) 641.
[10] E. Grimvall, L. Rylander, P. Nilsson-Ehle, U. Nilsson, U. Stromberg, L. Hagmar, C. Östman, Arch. Environ. Contam. Toxicol. 32 (1997) 329.
[11] A. Covaci, S. Voorspoels, J. de Boer, Environ. Int. 29 (2003) 735.

J. Chromatogr. A 1310 (2013) 126-132

[12] A. Sjödin, R.S. Jones, C.R. Lapeza, J.F. Focant, E.E. McGahee 3rd, D.G. Patterson Jr., Anal. Chem. 76 (2004) 1921.
[13] P. Korytár, A. Covaci, P.E.G. Leonards, J. de Boer, U.A.T. Brinkman, J. Chromatogr. A 1100 (2005) 200.
[14] A. Kierkegaard, U. Sellström, M.S. Maclachlan, J. Chromatogr. A 1216 (2009) 364.
[15] A.C. Dirtu, K. Ravindra, L. Roosens, R. van Grieken, H. Neels, R. Blust, A. Covaci, J. Chromatogr. A 1186 (2008) 295.
[16] K. Mastovská, S.J. Lehotay, J. Hajslová, J. Chromatogr. A 926 (2001) 291.
[17] P. López, S.A. Brandsma, P.E.G. Leonards, J. de Boer, Anal. Bioanal. Chem. 400 (2011) 871.
[18] K. Vorkamp, M. Thomsen, M. Frederiksen, M. Pedersen, L.E. Knudsen, Environ. Int. 37 (2011) 1.
[19] E. Eljarrat, S. Lacorte, D. Barceló, J. Mass Spectrom. 37 (2002) 76.
[20] L. K. Ackerman, G.R. Wilson, S.L. Simonich, Anal. Chem. 77 (2005) 1979.
[21] ICH Topic Q2B, Validation of analytical methods: methodology, Pharmeuropa 8 (1996) 108.
[22] A. Covaci, S. Voorspoels, J. Chromatogr. B 827 (2005) 216.
[23] C. Thomsen, V.H. Liane, G. Becher, J. Chromatogr. B 846 (2007) 252.
[24] C. Thomsen, H.K. Knutsen, V.H. Liane, M. Frøshaug, H.E. Kvalem, M. Haugen, H.M. Meltzer, J. Alexander, G. Becher, Mol. Nutr. Food Res. 52 (2008) 228.
[25] K. Jakobsson, J. Fang, M. Athanasiadou, A. Rignell-Hydbom, Å. Bergman, Environ. Int. 47 (2012) 121.
[26] M. Karlsson, A. Julander, B. van Bavel, L. Hardell, Environ. Int. 33 (2007) 62.
[27] G. Ren, Z. Yu,S. Ma, H. Li, P. Peng, G. Sheng, J. Fu, Environ. Sci. Technol. 43 (2009) 9453.
[28] G.T. Tomy, K. Pleskach, N. Ismail, D.M. Whittle, P.A. Helm, E. Sverko, D. Zaruk, C.H. Marvin, Environ. Sci. Technol. 41 (2007) 2249.
[29] E. Sverko, G.T. Tomy, E.J. Reiner, Y. Li, B.E. McCarry, J.A. Arnot, R.J. Law, R.A. Hites, Environ. Sci. Technol. 45 (2011) 5088.
[30] M. L. Feo, E. Barón, E. Eljarrat, D. Barceló, Anal. Bioanal. Chem. 404 (2012) 2625.
[31] J. Sun, A. Zhang, L. Fang, J. Wang, W. Liu, Sci. Total Environ. 443 (2013) 194

3.3.3. Comparing Human Exposure to Emerging and Legacy Flame Retardants from the Indoor Environment and Diet with Concentrations Measured in Serum

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Environ. Int. (submitted 2014)

Comparing human exposure to emerging and legacy flame retardants from the indoor environment and diet with concentrations measured in serum

Enrique Cequier[1,2], Rosa Maria Marcé[2], Georg Becher[1,3] and Cathrine Thomsen[1]

[1]*Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway;*
[2]*Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili, Tarragona, Spain,*
[3]*Department of Chemistry, University of Oslo, Norway.*

Abstract

This study investigates associations between serum concentrations of emerging and legacy halogenated flame retardants (HFRs) in 46 Norwegian women and measured indoor air and dust concentrations of the HFRs as well as detailed dietary information. Hexabromobenzene (median 0.03 ng/g lipid) and Dechlorane 602 (0.18 ng/g lipid) were detected in around 50% of the samples and Dechlorane Plus *syn* (0.45 ng/g lipid) and *anti* (0.85 ng/g lipid) in more than 78%. The most abundant polybrominated diphenyl ethers were 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153; 0.82 ng/g lipid) and 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47; 0.49 ng/g lipid) detected in more than 70% of the samples. No consistent associations were observed between the biomonitoring data and measured concentrations in indoor air and dust. On the other hand, significant bivariate correlations were found between concentrations of PBDEs with specific food items (mainly lamb/mutton and margarine). Some of these associations were confirmed by multivariate linear regression analyses.

Key words: dietary exposure, emerging flame retardants, dust, blood

1. Introduction

Halogenated flame retardants (HFRs) are a group of chemicals used to provide fire resistance to a wide variety of consumer goods. The occurrence of the most frequently used HFRs, such as polybrominated diphenyl ethers (PBDEs) and 1,2,5,6,9,10-hexabromocyclododecane (HBCDD), has been well documented in humans (Jakobsson *et al.* 2012; Rawn *et al.* 2014) even after they have been phased-out due to their proven hazardous effects on animals and humans (Darnerud 2008; Marvin *et al.* 2011; Turyk *et al.* 2008). After the ban of these chemicals, other HFRs, such as hexabromobenzene (HBB) and dechloranes, have been increasingly detected in humans (Brasseur *et al.* 2014; Cequier *et al.* 2013; Ren *et al.* 2009). These new or "emerging" HFRs have similar physico-chemical properties as the former flame retardants (FRs) and are thus also potentially hazardous for the environment and human health. The most studied sources of human exposure to these compounds are house dust and diet. The content of HFRs in dust may vary geographically due to different fire regulations and uses in different countries. In Europe, some studies suggest that exposure from house dust is lower than from the diet (Harrad *et al.* 2004; Roosens *et al.* 2009). However, countries where indoor environments are higher contaminated, like the U.S.A., dust ingestion accounted for 82% of the overall PBDE intakes (Lorber 2008). Special attention should be paid to sensitive groups of the population, like toddlers, which

can have a higher dust intake per kg body weight due to their closer contact with dust. The contamination of indoor environments has been surveyed to evaluate exposure to emerging and legacy HFRs, like PBDEs, through ingestion of dust (Cequier *et al.* 2014; Dodson *et al.* 2012). However, most studies have not found any significant associations between exposure to PBDEs from indoor dust and levels in human serum (Fromme *et al.* 2009; Imm *et al.* 2009; Roosens *et al.* 2009; Zheng *et al.* 2011), with the exception of a few studies from the U.S.A. (Johnson *et al.* 2010; Watkins *et al.* 2011) where indoor contamination with PBDEs is considerably higher than in Europe (Sjödin *et al.* 2008). There is increasing awareness of short exposures in micro-environments with high contamination of HFRs like in cars (Harrad *et al.* 2008). Although the exposure time is generally low, such exposures could explain to the lack of associations between human biomarkers and house dust.

On the other hand, the exposure to HFRs through diet is more likely because HFRs are lipophilic and tend to bioaccumulate in the food web. Diet has been reported as a significant source of human exposure to PBDEs (Fraser *et al.* 2009; Knutsen *et al.* 2008). Two studies from Scandinavia, assessing the impact of fish consumption (Sjödin *et al.* 2000; Thomsen *et al.* 2008), and one study from U.S.A. (Anderson *et al.* 2008) reported fish as the food item that contributed most to the intake of PBDEs. In contrast, some studies reported significant contribution of poultry and red meat to PBDE intake in the U.S.A. (Fraser *et al.* 2009; Rose *et al.* 2010). To the best of our knowledge, there are no studies assessing the relationship between dietary exposure and blood levels of emerging HFRs.

This work undertakes an experimental approach to elucidate the extent to which diet, as well as dust and air from residential living rooms, contribute to the concentrations of emerging and legacy HFRs in the serum of 46 Norwegian women.

2. Methods and materials

2.1. Recruitment of participants, sampling and questionnaires

A Norwegian mother-child cohort comprising 48 women and 56 children was established in 2012 to measure concentrations of halogenated and organo-phosphate FRs in air and dust from the participants' living rooms as well as biomarkers of exposure in serum and urine. Detailed information about the cohort has been published elsewhere (Cequier *et al.* 2014). This paper covers the occurrence of persistent flame retardants in blood from the women in the study group (no blood was drawn from the children). Informed consent was received from all participants, and the study was approved by the Regional Committee for Medical Research Ethics.

The participants were asked to donate blood and to answer a food frequency questionnaire (FFQ). A total of 46 blood samples were obtained from medical centers where venous blood was drawn into 10 mL BD Vacutainers® (Sarstedt, Nümbrecht, Germany), and serum was separated. The serum samples were shipped to the Norwegian Institute of Public Health for storage at -20°C. Lipid analysis of sera was carried out at the Oslo University Hospital and total lipid content calculated according to Grimvall *et al*. method based on the enzymatically determined concentrations of three lipid groups (Grimvall *et al.* 1997).

Samples of air and dust were collected from the living room of the participants' residences. A comprehensive description of the collection of the samples and levels of HFRs in air and dust has been published previously (Cequier *et al.* 2014).

Dietary exposure to HFR was assessed through the FFQ consisting on 340 questions organized in 42 food categories according to Norwegian dietary habits (Meltzer *et al.* 2008). The FFQs categorized consumption per day, week and month, and food intake expressed as g/day over the last year, were calculated. The FFQ has been subjected to a comprehensive validation in a pregnancy sub-cohort study (Brantsæter *et al.* 2008).

2.2. Analytical method

Regarding the abbreviation of the HFRs, this study follows the system proposed recently by Bergman *et al*. (2012). Detailed description of the analytical method and information of the standards, i.e., PBDEs, HBB, Dechlorane Plus® (DDC-CO or DP), decabromodiphenyl ethane (DBDPE), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE), hexachlorocyclopentadienyl dibromocyclo-octane (DBHCTD or HCDBCO), Dechlorane 602 (DDC-DBF or Dec602) and Dechlorane 603 (DDC-Ant or Dec603), has been published elsewhere (Cequier *et al.* 2013). Briefly, two mL of serum were spiked with 300 pg of isotopically labelled internal standards and denatured with formic acid. Samples were applied on SPE columns (Oasis® HLB, Waters, Milford, MA, U.S.A.) followed by washing with 5% 2-propanol in water. Columns were dried and analytes eluted with DCM. Clean-up was performed on 33% sulphuric acid-silica (v/w) to remove lipids. Extracts were analysed on a GC HP 6890 series (Avondale, PA, U.S.A.) using a DB5-MS column of 15 m length, 0.25 mm I.D. and 0.1 µm film thickness (Agilent Technologies Inc. CA, U.S.A.). Quantification was performed with an HP 5973 mass selective detector working in electron capture negative ionisation mode. The method was linear in the range of 0.1 to 1000 pg/µL, recoveries ranged from 36 to 122% with RSDs from 1 to 13%, and the method limit of detection (MLD) ranged from 0.3 to 20.2 pg/g serum. As part of the quality control, twelve procedural blanks were included. When FRs were present in at least 50% of the procedural blanks, the content in the samples was corrected by subtracting the mean blank value. A quality control solution with known concentrations of predominant PBDE congeners was used to assure good performance of the GC-MS from batch to batch. The average accuracy of the determination of PBDE concentrations was 90% with RSD <15%.

2.3. Statistical analysis

Statistical analyses were performed using SPSS v.20 (Chicago, IL, U.S.A.). Concentrations of HFRs in serum are presented as median, mean, minimum, maximum and number of samples above MLD for each compound. Detection of HFRs <48 were not subjected to statistical analysis. For HFRs with detection frequencies ≥48%, non-detects were replaced by half the MLD, and Spearman's rank correlation test was employed to calculate correlations among concentrations of different HFRs in serum, and between HFRs in serum and in dust and air. Correlations with $p<0.05$ were considered statistically significant. In order to assess the relationship between concentrations of HFRs in serum and diet, concentrations were log transformed to approach a normal distribution. A Spearman's rank correlation test was used to

identify relevant categories of food items that were correlated with the HFRs serum concentrations. Correlations with $p<0.2$ were further assessed in a multivariate linear regression model. Lifestyle factors and demographic information were evaluated, but only BMI was found to influence the HFR levels and it was included in the regression models. Significances in the model with $p<0.05$ were considered statistically relevant. Finally, the models were checked for concentrations of HFRs with high influence and for co-linearity. Multiple co-linearity was rejected when the variance inflation factor was <3 for all the variables in the model.

3. Results and discussions

3.1. Occurrence of HFRs in serum

Emerging HFRs. Five emerging HFRs were detected in the serum samples: HBB, DDC-DBF, DDC-CO, BTBPE and DDC-Ant (Table 1). HBB and DDC-DBF were detected in approximately half of the samples and DDC-CO showed frequencies of detection higher than 78%. The highest median concentrations found in serum were for *anti*-DDC-CO (0.85 ng/g lipid) followed by *syn*-DDC-CO (0.45 ng/g lipid), BTBPE and DDC-DBF (0.19 and 0.18 ng/g lipid, respectively) and DDC-Ant and HBB (0.06 and 0.03 ng/g lipid, respectively). DDC-CO accounted for approximately 70% of the total emerging HFRs on both weight and molar basis. The *f*-anti ratio, defined as the concentration of the *anti* isomer divided by the sum of the *syn* and *anti* isomer, had a median and mean of 0.67. This ratio is within the values reported for the technical mixtures, which suggests that the bioaccumulation in humans is equal for both isomers (Yang *et al.* 2013). Nonetheless, the *f*-anti ratio spanned from 0.44 to 0.94, hence in certain individuals there must be a selective enrichment of one of the isomers. (Zhou *et al.* 2014). In Europe, there are only two studies reporting concentrations of dechloranes in humans. The first work was a pilot study (n=10) performed to demonstrate the applicability of our analytical method for the simultaneous determination of emerging FRs and PBDEs in serum samples (Cequier *et al.* 2013), and the second was a more comprehensive study conducted in France by Brasseur *et al*. (Brasseur *et al.* 2014). Their median concentrations and frequency of detection of *anti*-DDC-CO (0.89 ng/g lipid) and *syn*-DDC-CO (0.22 ng/g lipid) did not differ much from the values in this study. However, the median concentration of DDC-Ant (2.01 ng/g lipid) and DDC-DBF (0.44 ng/g lipid) was 30 and two times higher, respectively, than the median concentrations in our study (Table 1). There is no report on the use of these chemicals in Europe and we have no explanation for these differences between Norway and France. The source of exposure to these two compounds has not yet been clarified. Neither DDC-Ant nor DDC-DBF were detected in indoor air or dust in our previously reported study on the indoor environments of this Norwegian cohort (Cequier *et al.* 2014).

The dechlorane pattern in our study, DDC-CO > DDC-DBF > DDC-Ant, follows the same order as seen in one Canadian study (Zhou *et al.* 2014), but is different from that of the study conducted in France (DDC-Ant > DDC-CO > DDC-DBF). There is a switch of DDC-Ant from the least abundant in the Norwegian and Canadian serum to the most abundant in the French serum. Sverko *et al.* (2011) reported clearly higher levels of DDC-DBF in wastewater, biota, sediment and soils compared to DDC-Ant. Therefore, assuming similar biological half-lives, the

Table 1
Concentrations (ng/g lipid) and detection frequencies (%) of emerging HFRs and PBDEs in serum from Norway.

	Median	Mean	Max	% detect
Emerging HFRs				
HBB	0.03	0.07	0.41	48
DDC-DBF	0.18	0.29	0.75	50
DBHCTD	nd	nd	nd	0
DDC-Ant	0.06	0.08	0.38	22
BTBPE	0.19	0.21	0.65	9
DBDPE	nd	nd	nd	0
syn-DDC-CO	0.45	0.77	6.7	78
anti-DDC-CO	0.85	1.8	25	89
ΣDDC-CO	1.3	2.6	31	
Σ_8EmergingHFRs	2.0	3.2	32	
PBDEs				
BDE-28	0.33	0.71	6.4	63
BDE-47	0.49	1.0	11	74
BDE-100	0.14	0.24	2.0	20
BDE-99	0.13	0.25	2.6	17
BDE-154	0.07	0.12	0.57	22
BDE-153	0.82	1.1	5.1	100
BDE-183	0.09	0.15	1.5	17
Σ_7PBDEs	2.3	3.6	23	

nd, not detected.

pattern in humans is expected to be DDC-DBF > DDC-Ant. As previously reported, we found only DDC-CO in dust from the households of the Norwegian cohort (Cequier *et al.* 2014). This fact might explain that DDC-CO was the most abundant dechlorane in serum samples from Norway because indoor dust contributed significantly to the intake of DDC-CO. The median concentration of ΣDDC-CO (Table 1) was lower than the median of 2.37 ng/g lipid presented by Zhou *et al*. (2014), but in the same range as observed in populations from China and France (~1 ng/g lipid) (Brasseur *et al.* 2014; Yang *et al.* 2013).

With respect to other measured emerging HFRs, HBB and BTBPE have been detected in eggs of herring gulls from the Great Lakes (Gauthier *et al.* 2009) and in mammals from the arctic environment (de Wit *et al.* 2010). However, information on their occurrence in humans is very limited. The median concentration of HBB (0.03 ng/g lipid) observed in the present study was far below the concentration reported in the general population from China (0.26 ng/g lipid) (Zhu *et al.* 2009). BTBPE levels (0.19 ng/g lipid) could only be compared with a study reporting levels in cat and dog hair below 1 ng/g lipid (Ali *et al.* 2013). Some other studies attempted to determine BTBPE in human serum, but the levels were below MLD (Karlsson *et al.* 2007; Zhu *et al.* 2009). An investigation of men in the U.S.A. found a strong association between levels of BTBPE in dust and the total T3 (thyroid hormone) indicating an endocrine disrupting potential, However, BTBPE blood concentrations were not determined (Johnson *et al.* 2013).

PBDEs. The sum of PBDEs in Table 1 is similar to the sum of the emerging HFRs in serum. Brasseur *et al*. reported even higher levels of dechloranes compared to PBDEs

(Brasseur *et al.* 2014). In our study, BDE-153 was detected in all sera with a median concentration of 0.82 ng/g lipid. The second most abundant PBDE was BDE-47 (0.49 ng/g lipid), followed by co-eluting pair BDE-28/BDE-33 (0.33 ng/g lipid) and BDE-100 and BDE-99 (~0.1 ng/g lipid) (Table 1). Such high levels of BDE-28/BDE-33 are rarely detected, however, a study from China in breast milk also reported the same PBDE pattern (BDE-153 > BDE47 > BDE-28) (Ma *et al.* 2012) at similar levels as in this study. Interestingly, when comparing median serum concentrations of PBDE congeners from studies conducted in different countries, the pattern is quite variable. For example, in one study from U.S.A. the most abundant PBDE was BDE-47 (23.2 ng/g lipid), being three to four times higher than BDE-153 (6.1 ng/g lipid) (Fraser *et al.* 2009). The same pattern was obtained in contemporary serum samples from Californian residents (Petreas *et al.* 2012) and also in a study conducted on the general population from China (Zhu *et al.* 2009). In Europe, one study from Denmark followed the mentioned pattern (BDE-47 > BDE-153) (Vorkamp *et al.* 2014), but this trend was less evident in the French and Slovakian studies showing only slightly higher levels of BDE-47 than BDE-153 (1.56 and 1.14 ng/g lipid, and 0.24 and 0.23 ng/ g lipid, respectively) (Brasseur *et al.* 2014; Chovancová *et al.* 2012). A Korean study obtained BDE-153 (2.07 ng/g lipid) as most abundant PBDE followed by BDE-47 (1.69 ng/g lipid) (Kim *et al.* 2012). This distribution is similar to the pattern seen in this study, as well as in other studies conducted around Europe (Fromme *et al.* 2009; Kalantzi *et al.* 2011).

For Norway, a change of the ratio BDE-47/BDE-153 in serum with time is evident, i.e., from 3:1 in 1988 and 1:1 in 2003 (Thomsen *et al.* 2007) to 1:2 in 2012 (this study) showing a continuous increase in the abundance of BDE-153 over BDE-47. This can most probably be explained by the longer half-life of the hexabrominated BDE compared to the tetrabrominated BDE as uses of both the Penta- and OctaBDE formulations containing these congeners, were phased out in the early 2000s. The longer half-life of BDE-153 compared to BDE-47 has been demonstrated in animal experiments (Sanders *et al.* 2006). It is therefore expected, that in countries still observing higher concentrations of BDE-47 than BDE-153, such as U.S.A. and China, the ratio will gradually switch to the one already seen in several European countries.

The inter-correlation between individual HFRs in serum is shown in Table 2. High correlations would indicate which HFRs are used in the same applications or even in the same formulation. There was a moderate to high correlation between all PBDEs ($0.30<R<0.85$), except for BDE-28/BDE-33 correlating only with Σ_7PBDEs. These correlations of PBDEs in serum show the persistence and ubiquity of the banned Penta and OctaBDE formulations. Regarding the emerging HFRs detected in serum, HBB did not correlate with any PBDE showing only a correlation with DDC-DBF ($R=0.36$). DDC-DBF was correlated to some PBDEs ($0.31<R<0.37$) suggesting that DDC-DBF is a compound used, to some extent, in similar products as the PBDEs. However, DDC-DBF was not found in house dust and therefore other sources of exposure might drive this relationship. Contrarily, DDC-CO correlations were found only between the two isomers, *syn* and *anti* ($R=0.75$), suggesting that DDC-CO is not used for common applications with PBDEs. This is supported by the lack of correlation between DDC-CO and PBDEs in the dust from Norwegian households (Cequier *et al.* 2014).

Table 2
Spearman rank correlations between HFRs in serum.

	BDE-28	BDE-47	BDE-153	Σ_7PBDE	HBB	DDC-DBF	*syn*-DDC-CO
BDE-47	0.120						
BDE-153	-0.170	**0.544****					
Σ_7PBDE	**0.427****	**0.788****	**0.615****				
HBB	0.178	0.116	0.075	0.124			
DDC-DBF	0.118	0.110	**0.335***	0.222	**0.363***		
syn-DDC-CO	-0.154	0.079	-0.032	-0.039	0.162	0.131	
anti-DDC-CO	-0.262	-0.151	0.053	-0.107	-0.031	0.168	**0.746****

3.2. Indoor exposure to HFRs

Since indoor environments are suspected to be major contributors to HFR exposure, the living rooms in the participants' houses were sampled for dust and air. Concentrations of PBDEs and emerging HFRs in these samples were determined and their relationship with the corresponding levels in serum from the mother in the house was assessed (Table 3). None of the HFRs detected in indoor air was correlated with serum concentrations, suggesting inhalation to be a minor pathway of exposure to these chemicals. Neither did we find any association between HFRs in dust and serum, except a negative correlation for Σ_7PBDE (R= -0.33). This indicates that dust from the living rooms is not a primary source of HFRs for this study group or that dust intake from the living rooms does not reflect the overall exposure.

There are a few publications which reported positive relationships between concentrations of PBDEs in human serum (Johnson *et al.* 2010; Watkins *et al.* 2012) or human milk (Wu *et al.* 2007) and levels in indoor dust. For example, Johnson *et al*. reported good correlations between PBDEs in serum and content in dust in North America (Boston). Watkins *et al*. (2012) also observed a positive correlation between ΣPentaBDEs in dust from living rooms and bedrooms and in serum. Both studies stated that the main source of exposure to PBDEs was dust, and Watkins *et al*. concluded that diet was no predictor of the PBDE body burdens for the participants in the study conducted in Boston. Contrarily, the study of Wu *et al*. (2007) reported good correlations of the sum of PBDEs in both dust and food with levels in human milk from residents of the Greater Boston area. The authors stated that both routes of exposure were important in the exposure of adults to PBDEs.

The absence of relationships between concentrations of HFRs in dust and human biomarkers in the European studies, including this one, might be due to the much lower HFR content in house dust compared to levels found in the studies conducted in the U.S.A. (Sjödin *et al.* 2008), i.e., the associations are no longer dominated by dust exposure.

3.3. Dietary exposure to HFRs

Information on the consumption of 42 food items from the questionnaires covering meat, fish, vegetables, fruit, dairy and other products, were used to investigate whether they contribute to the exposure of individual PBDEs and emerging HFRs. Spearman's rank correlation was used to evaluate associations between food consumption (g/day) and the serum concentrations of the HFRs. Many individual associations were

Table 3
Spearman rank correlations between HFRs in serum and dust.

Serum \ Dust	BDE-28	BDE-47	BDE-153	Σ_7PBDE	HBB	*syn*-DDC-CO	*anti*-DDC-CO
BDE-28	-0.194	-0.257	-0.252	-0.278	0.125	0.071	0.035
BDE-47	-0.013	-0.225	-0.269	-0.274	-0.023	-0.045	-0.035
BDE-153	-0.117	-0.176	-0.183	-0.176	0.024	-0.181	-0.055
Σ_7PBDE	-0.194	-0.303*	-0.302*	-0.329*	0.126	-0.036	0.013
HBB	-0.346*	-0.417**	-0.428**	-0.440**	-0.188	-0.149	-0.179
DDC-DBF	-0.291	-0.378**	-0.377**	-0.372*	-0.019	-0.099	-0.046
syn-DDC-CO	-0.117	-0.060	-0.053	-0.026	-0.388**	-0.143	-0.027
anti-DDC-CO	-0.181	-0.056	0.009	0.015	-0.381**	-0.331*	-0.238

found in the bivariate test (Table S1), but the only food items where several likely correlations with HFRs were observed (0.30<R<0.42), were margarine (BDE-47, 153, ΣPBDEs and *anti*-DDC-CO (R=-0.30)) and lamb/mutton (BDE-47, 153 and ΣPBDEs). The significant influence of meat on the body burden of PBDEs has been previously reported in a study conducted in the U.S.A. (n>1500), where poultry and red meat showed significant associations with PBDE levels in the general U.S. population (Fraser *et al.* 2009). Further, we performed multivariate linear regression analyses to assess the variation in the serum concentrations related to food consumption, but only significant models for BDE-47 and 153 were obtained with adjusted R^2 value of 0.15 and 0.36, being lamb/mutton and margarine the major contributors, respectively (Table S2 and S3). The lack of significant associations in the other models might be attributed to the relatively low sample size of our study.

Seafood did not contribute significantly to body burdens of HFR in the women. This is not totally unexpected since a previous work, which targeted a selected group of Norwegians (n=126) with a wide range of seafood consumption could only find weak correlations between dietary exposure and levels of PBDEs in serum from men, but not in women (Knutsen *et al.* 2008).

Although our study is limited in size, the extensive and detailed information on food consumption obtained from the FFQs and the high quality of the HFR measurements point out food as an important source of HFR exposure.

4. Conclusions

DDC-CO (*syn* and *anti*) was found in serum from Norwegian women in a similar concentration range as Σ_7PBDEs. This highlights the need to expand biomonitoring of emerging HFRs to different and larger populations. Further, toxicological studies are needed to be able to assess the risk the exposure to the emerging HFRs pose to human health. In this study group, serum concentrations of HFRs were not associated with levels in dust or air collected in the living rooms of the participants. Significant Spearman's rank correlations were found between serum levels of some PBDEs and consumption of lamb/mutton and margarine. Based on these results, it seems likely that food plays a more important role in the exposure to HFRs than indoor air and dust.

Acknowledgements

This study is funded by the European Commission, 7th Framework Programme under the EU Marie Curie Initial Training Network INFLAME (GA No. 264600) and the Norwegian research council. Participants in the study are greatly acknowledged.

References

Ali N, Malik RN, Mehdi T, Eqani SA, Javeed A, Neels H, Covaci A. Sci Total Environ 2013; 449: 29-36

Anderson HA, Imm P, Knobeloch L, Turyk M, Mathew J, Buelow C, Persky V. Chemosphere 2008; 73: 187-194

Bergman Å, Rydén A, Law RJ, de Boer J, Covaci A, Alaee M, Birnbaum L, Petreas M, Rose M, Sakai S, Van den Eede N, van der Veen I. Environ Int 2012; 49: 57-82

Brantsæter A, Haugen M, Alexander J, Meltzer HM. Matern Child Nutr 2008; 4: 28-43

Brasseur C, Pirard C, Scholl G, De Pauw E, Viel JF, Shen L, Reiner EJ, Focant JF. Environ Int 2014; 65: 33-40

Cequier E, Ionas AC, Covaci A, Marcé RM, Becher G, Thomsen C. Environ Sci Technol 2014 doi:10.1021/es500516u

Cequier E, Marcé RM, Becher G, Thomsen C. J Chromatogr A 2013; 1310: 126-132

Chovancová J, Čonka K, Fabišiková A, Sejáková ZS, Dömötörová M, Drobná B, Wimmerová S. Chemosphere 2012; 88: 1383-1389

Darnerud PO. Int J Androl 2008; 31: 152-160

de Wit CA, Herzke D, Vorkamp K. Sci Total Environ 2010; 408: 2885-2918

Dodson RE, Perovich LJ, Covaci A, Van den Eede N, Ionas AC, Dirtu AC, Brody JG, Rudel RA. Environ Sci Technol 2012; 46: 13056-13066

Fraser AJ, Webster TF, McClean MD. Environ Health Perspect 2009; 117: 1520-1525

Fromme H, Körner W, Shahin N, Wanner A, Albrecht M, Boehmer S, Parlar H, Mayer R, Liebl B, Bolte G. Environ Int 2009; 35: 1125-1135

Gauthier LT, Potter D, Hebert CE, Letcher RJ. Environ Sci Technol 2009; 43: 312-317

Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Stromberg U, Östman C. Arch Environ Contam Toxicol 1997; 32: 329-336

Harrad S, Ibarra C, Abdallah MA, Boon R, Neels H, Covaci A. Environ Int 2008; 34: 1170-1175

Harrad S, Wijesekera R, Hunter S, Halliwell C, Baker R. Environ Sci Technol 2004; 38: 2345-2350

Imm P, Knobeloch L, Buelow C, Anderson HA. Environ Health Perspect 2009; 117: 1890-1895

Jakobsson KF, Fång J, Athanasiadou M, Rignell-Hydbom A, Bergman Å. Environ Int 2012; 47: 121-130

Johnson PI, Stapleton HM, Mukherjee B, Hauser R, Meeker JD. Sci Total Environ 2013; 445-446: 177-184

Johnson PI, Stapleton HM, Sjödin A, Meeker JD. Environ Sci Technol 2010; 44: 5627-5632

Kalantzi OI, Geens T, Covaci A, Siskos PA. Environ Int 2011; 37: 349-353

Karlsson M, Julander A, van Bavel B, Hardell L. Environ Int 2007; 33: 62-69

Kim J, Kang JH, Park H, Baek SY, Kim YH, Chang YS. Environ Pollut 2012; 164: 46-52

Knutsen HK, Kvalem HE, Thomsen C, Frøshaug M, Haugen M, Becher G, Alexander J, Meltzer HM. Mol Nutr Food Res 2008; 52: 217-227

Lorber M. J Expo Sci Environ Epidemiol 2008; 18: 2-19

Ma S,Yu Z, Zhang X, Ren G, Peng P, Sheng G, Fu J. Environ Int 2012; 42: 72-77

Marvin CH, Tomy GT, Armitage JM, Arnot JA, McCarty L, Covaci A, Palace V. Environ Sci Technol 2011; 45: 8613-8623

Meltzer HM, Brantsæter AL, Ydersbond TA, Alexander J, Haugen M, Hareide B, Hovengen R, Lie KK, Magnus P, Nordhagen R, Nystad W, Rønningen KS, Vollset SE. Mater Child Nutr 2008; 4: 14-27

Petreas M, Park JS, Wang M, Wang Y, Guo W, Tarrant D, Rhee A, Harwani S. Global Nest J 2012; 14: 80-85

Rawn DFK, Ryan JJ, Sadler AR, Sun WF, Weber D, Laffey P, Haines D, Macey K, van Oostdam J. Environ Int 2014; 63: 26-34

Ren G,Yu Z, Ma S, Li H, Peng P, Sheng G, Fu J. Environ Sci Technol 2009; 43: 9453-9457

Roosens L, Abdallah MAE, Harrad S, Neels H, Covaci A. Environ Sci Technol 2009; 43: 3535-3541

Rose M, Bennett DH, Bergman Å, Fängström B, Pessah IN, Hertz-Picciotto I. Environ Sci Technol 2010; 44: 2648-2653

Sanders JM, Lebetkin EH, Chen LJ, Burka LT. Xenobiotica 2006; 36: 824-837

Sjödin A, Hagmar L, Klasson-Wehler E, Björk J, Bergman Å. Environ Health Perspect 2000; 108: 1035-1041

Sjödin A, Päpke O, McGahee E, Focant JF, Jones RS, Pless-Mulloli T, Toms LML, Herrmann T, Müller J, Needham LL, Patterson Jr DG. Chemosphere 2008; 73: S131-S136

Sverko E, Tomy GT, Reiner EJ, Li YF, McCarry BE, Arnot JA, Law RJ, Hites RA. Environ Sci Technol 2011; 45: 5088-5098

Thomsen C, Knutsen HK, Liane VH, Frøshaug M, Kvalem HE, Haugen M, Meltzer HM, Alexander J, Becher G. Mol Nutr Food Res 2008; 52: 228-237

Thomsen C, Liane VH, Becher G. J Chromatogr B 2007; 846: 252-263

Turyk ME, Persky VW, Imm A, Knobeloch L, Chatterton RJ, Anderson HA. Environ Health Persp 2008; 116: 1635-1641

Watkins DJ, McClean MD, Fraser AJ, Weinberg J, Stapleton HM, Sjödin A, Webster TF. Environ Health Perspect 2011; 119: 1247-1252

Watkins DJ, McClean MD, Fraser AJ, Weinberg J, Stapleton HM, Sjödin A, Webster TF. Environ Sci Technol 2012; 46: 1192-1200

Wu N, Herrmann T, Päpke O, Tickner J, Hale R, Harvey E, La Guardia M, McClean MD, Webster TF. Environ Sci Technol 2007; 41: 1584-1589

Yang Q, Qiu X, Li R, Liu S, Li K, Wang F, Zhu P, Li G, Zhu T. Chemosphere. 2013; 91: 205-211

Zheng J, Luo XJ, Yuan JG, Wang J, Wang YT, Chen SJ, Mai BX, Yang ZY. Environ Pollut 2011; 159: 3706-3713

Zhou SN, Siddique S, Lavoie L, Takser L, Abdelouahab N, Zhu J. Environ Int 2014; 66: 11-17

Zhu L, Ma B, Hites RA. Environ Sci Technol 2009; 43: 6963-6968

Supporting Information

3.3.Dietary exposure to HFRs

Table S1
Spearman's rank correlations between serum concentrations of HFRs and associated food consumption.

n=46		BDE-28	HBB	BDE-47	DDC-DBF	BDE-153	syn-DDC-CO	anti-DDC-CO	SumPBDE
water	R	.206	.204	.030	-.127	-.109	-.146	-.067	.004
	p	.170	.173	.845	.399	.472	.333	.657	.979
milk	R	-.104	-.062	.169	-.028	.187	-.043	.076	.179
	p	.493	.684	.260	.851	.212	.777	.615	.234
cheese	R	.185	.267	.051	.060	.096	-.089	-.082	.094
	p	.218	.073	.738	.691	.524	.556	.588	.534
beef	R	.106	-.007	.032	**-.356***	-.111	-.033	-.114	.114
	p	.483	.963	.835	.015	.464	.828	.452	.449
lamb/mutton	R	.001	-.048	**.355***	.204	**.391****	.042	-.018	**.299***
	p	.997	.753	.016	.174	.007	.783	.906	.044
elg	R	-.113	-.106	.279	-.142	.191	.052	-.017	.243
	p	.456	.485	.061	.347	.203	.730	.910	.103
lean fish	R	.015	.127	.101	**.340***	.253	-.154	.017	**.305***
	p	.919	.401	.504	.021	.090	.307	.909	.039
semi-oily fish	R	.036	.205	.064	.223	.169	-.066	-.019	.073
	p	.810	.171	.672	.136	.261	.664	.899	.628
fish roe	R	-.064	**-.381****	-.187	-.096	-.013	.005	.237	-.092
	p	.675	.009	.214	.527	.930	.976	.113	.543
fish roe/liver spread	R	-.041	**-.297***	-.098	-.198	-.042	-.026	.240	-.088
	p	.788	.045	.516	.188	.781	.865	.108	.561
fruit and berries	R	-.060	**.299***	-.021	.234	-.041	-.220	-.033	-.016
	p	.692	.043	.890	.118	.787	.142	.830	.914
nuts and seeds	R	-.024	.018	-.181	-.111	**-.381****	.015	-.025	-.185
	p	.875	.904	.230	.464	.009	.923	.871	.219
margarines	R	.115	.154	**.352***	.276	**.378****	-.193	**-.320***	**.420****
	p	.446	.308	.017	.063	.010	.199	.030	.004
butter	R	-.218	.049	-.029	-.023	.184	-.100	.057	-.020
	p	.146	.747	.850	.879	.222	.510	.709	.895
cooking oil	R	**-.370***	-.073	.139	.283	.229	-.131	-.068	-.061
	p	.011	.630	.358	.057	.126	.386	.655	.687
sugar, honey, syrup	R	**-.348***	-.032	-.085	.030	.247	-.260	-.187	-.141
	p	.018	.834	.574	.843	.098	.081	.214	.351
chocolate and cocoa	R	-.009	.148	-.073	-.048	-.122	-.156	-.288	.009
	p	.952	.328	.630	.751	.418	.302	.052	.954
sweets	R	-.079	-.105	.233	-.004	.248	-.046	-.078	**.316***
	p	.602	.487	.119	.981	.097	.761	.608	.032

Table S1 (continued)

n=46		BDE-28	HBB	BDE-47	DDC-DBF	BDE-153	syn-DDC-CO	anti-DDC-CO	SumPBDE
salty snacks	R	-.081	-.030	.229	-.211	**.336***	-.053	-.188	.160
	p	.593	.843	.126	.160	.022	.726	.210	.288
coffee, tea, soft drinks	R	.274	.165	.087	-.173	-.099	-.101	-.106	.074
	p	.066	.272	.564	.250	.514	.504	.482	.627
age	R	.257	-.173	.267	.097	.222	-.064	-.148	**.360***
	p	.085	.251	.073	.520	.139	.674	.326	.014
BMI	R	.252	-.144	-.033	**-.494****	**-.412****	.103	-.019	-.073
	p	.092	.341	.828	.000	.004	.497	.901	.630

Shaded cells correspond to p-values <0.2; */**Correlation was significant at the level 0.05/0.01 (two tailed).

Table S2
Multiple linear regression model of serum concentrations of BDE-47 with associated food consumption

Model (R^2=0.15)	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-.489	.378		-1.294	.203
BMI	-.009	.015	-.088	-.566	.575
lamb/mutton	.065	.029	.316	2.243	**.031**
elk (elg)	.057	.032	.248	1.789	.081
sweets	.003	.003	.153	1.078	.288
salty snacks	.006	.004	.221	1.473	.149

Table S3
Multiple linear regression model of serum concentrations of BDE-153 with associated food consumption

Model (R^2=0.36)	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	.359	.156		2.308	.026
BMI	-.021	.006	-.442	-3.253	**.002**
nuts and seeds	-.005	.002	-.356	-2.883	**.006**
margarines	.014	.006	.288	2.354	**.024**
sweets	.002	.001	.215	1.732	.091
salty snacks	.004	.002	.270	2.080	**.044**

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

3.3.4. Discussion of the Results

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

In the previous section we have seen that in samples of air and dust from indoor environments the majority of the FRs are found above the MLD in households and schools. In order to identify the human exposure pathways to these persistent FRs, we investigated the correlations of concentrations in serum with both, the dietary intake and concentrations of FRs in the indoor environment (dust and air). The concentrations of persistent FRs in serum are much lower than in air or dust, in consequence the methods must be much more sensitive to increase the detection frequency of FRs. Since our study is relatively limited in number of blood donors (n=46), obtaining high detection frequencies aids to increase the statistical power when correlating concentrations in humans with the sources of exposure.

When determining highly lipophilic FRs in serum, the main challenge of the analytical method is to obtain quantitative extractions. Extraction efficiencies of SPE and LLE were similar for the extraction of FRs from serum. Both techniques are suitable for the extraction of the majority of the compounds, but for those highly lipophilic (DP, DBDPE and BDE-209), the recovery dropped significantly. Breakthrough was observed for highly lipophilic FRs during the loading of the sample on the hydrophobic SPE polymeric sorbent. Moreover, the breakthrough increased with increasing octanol-water partition coefficient, i.e., the higher the K_{ow} of the FRs, the higher the amount detected in the SPE waste. We suggest that amphiphilic compounds in serum play a major role in this previously unreported phenomenon. We hypothesise that the lipid content in serum traps or encapsulates the highly lipophilic FRs hindering their extraction. As a result, when extracting highly lipophilic pollutants (log K_{ow} > 9), quantitative recoveries are not achievable. To diminish this issue, we used isotopically labelled internal standards (e.g., ^{13}C-DP and ^{13}C-BDE-209), when possible, for a reliable quantification of these compounds in humans. The isotopically internal standard of DBDPE is not adequate when using ECNI because the main ions obtained correspond to the non-specific bromide (m/z=79/81). In any case, DBDPE was not detected in the serum of the participants and therefore its quantification was not necessary. Due to the low recoveries for some of our targeted FRs, the optimisation of all chromatographic and spectrometric parameters to achieve maximum sensitivity became essential to achieve good MLD and thus high frequencies of detection. The optimised GC-MS method was fast (less than 15 min), robust and sensitive. The MLD ranged from 0.30 pg/mL serum (HBB) to 20 pg/mL serum (DBDPE). These method limits of detection are better than, or comparable to, other publications [1,2]. In the pilot study based on ten serum samples, we detected for the first time Dec602 and Dec603 in humans, and confirmed the ubiquity of DP that had only been reported in the Chinese population [3]. This finding is remarkable since DP occurrence in Norway and Europe was unknown. The research

was extended to the serum samples from the 48 women in the study group. The median concentrations of the most detected FRs were: *anti*-DP (0.85 ng/g l.w.) > BDE-153 (0.82 ng/g l.w.) > BDE-47 (0.49 ng/g l.w.) > *syn*-DP (0.45 ng/g l.w.). Median concentrations for Dec602 and Dec603 were 0.18 ng/g l.w. and 0.06 ng/g l.w. The concentrations of DPs are very similar to those recently reported by Brasseur *et al*. [4] and Zhou *et al*. [5] in a French and Canadian population, respectively. However, concentration of Dec603 was much higher in the French study (2.01 ng/g l.w.), this emerging FRs being the most abundant, while the Canadian study presented concentrations for Dec602 (0.53 ng/g l.w.) and Dec603 (0.11 ng/ g l.w.) more similar to our results. In summary, the sum of emerging FRs was in the proportion 1:1 with respect the sum of PBDEs in Norwegian serum and therefore, upcoming biomonitoring studies should include dechloranes among other important emerging FRs.

After characterising the levels of FRs in indoor environments (previous section) and in humans, we were able to assess the exposure pathways using statistical tests. As in previous studies, we did not find any significant correlation between the levels in serum and the concentrations of FRs in dust and air from the respective houses of the participants [6]. The dietary exposure was assessed through the food frequency questionnaire. The most remarkable findings were the associations of lamb and mutton with BDE-47, 153 and Σ_7PBDEs, and margarines with Dec602, BDE-47, BDE-153, Σ_7PBDEs, and *anti*-DP (negative correlation). In conclusion, we found more numerous and consistent correlations between FRs in serum and the amount of food ingested (g/day) than with air or dust, which means that the dietary exposure better explains the variations in the concentrations of FRs in serum than the exposure from the indoor environment in this study group. One reason why dietary exposure is more correlated with the levels of FRs in serum might be because the food frequency questionnaire assesses more reliably the past exposure to FRs with long half-lives in the human body than the mere intake of dust and/or air from the living rooms, which only represents a partial exposure.

References

[1] A. Covaci, S. Voorspoels, J. Chromatogr. B 827 (2005) 216-223.
[2] C. Thomsen, V. H. Liane, G. Becher, J. Chromatogr. B 846 (2007) 252-263.
[3] G. Ren, Z. Yu, S. Ma, H. Li, P. Peng, G. Sheng; J. Fu, Environ. Sci. Technol. 43 (2009) 9453-9457
[4] C. Brasseur, C. Pirard, G. Scholl, E. De Pauw, J. F. Viel, L. Shen, E.J. Reiner, J.F. Focant, Environ. Int. 65 (2014) 33-40.
[5] S. N. Zhou, S. Siddique, L. Lavoie, L. Takser, N. Abdelouahab, J. Zhu, Environ. Int. 66 (2014) 11-17.
[6] L. Roosens, M. A. E. Abdallah, S. Harrad, H. Neels, A. Covaci, Environ. Sci. Technol. 43 (2009) 3535-3541.

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

3.4. Human Exposure to Organophosphate Flame Retardants

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

In the same way as for the persistent FRs discussed in the previous section, humans are also exposed to PFRs in the indoor environment. The production volumes of PFRs are commonly higher than the BFRs [1]. After the prohibition of PBDE formulations in 2004 by the Stockholm Convention [2] due to the toxic, persistent, and bioaccumulative nature of the congeners, the use of PFRs has increased. According to Reemtsma *et al*. [3], the production of PFRs in Western Europe increased between 2005 and 2006 by 7.1%. Pakalin *et al.* [4], reported that PFRs might be good alternatives to replace BFRs because they are less persistent and bioaccumulative, although there are indications that chlorinated alkyl PFRs, such as TCEP might be carcinogenic [5] and TCDIPP mutagenic [6]. Indeed, late in the 1970's, the brominated analogue to TDCIPP was proved to be an animal mutagen and therefore was banned from use in clothing [7,8]. Because of the large and increasing production volumes of PFRs and the lack of information on toxicity relevant for the human health, there is a justified concern to examine to what extent humans are exposed to the PFRs. When uptake of PFRs occurs in humans, the PFRs are readily metabolised and they have low half-lives in biological systems [9]. PFRs are excreted as dialkyl and diaryl phosphates (DAPs) in urine, and it has been demonstrated that these metabolites are suitable for biomonitoring exposure to PFR [10-12]. However, the high polarity of the metabolites makes their determination challenging and new methods have to be developed in order to achieve more sensitive and faster analytical methods. Therefore, in the first study of the section, we developed a fast and sensitive UPLC-HRMS method for the determination of DNBP, BDCIPP, BBOEP and DPHP. Contrarily to the former published and widely used LC-QqQ methods, we did not use any preconcentration or off-line sample preparation, just direct injection of centrifuged urine. Furthermore, we explored several pH in the mobile phase to obtain the most suitable conditions for the determination of the DAPs.

So far, no studies have been conducted in Norway assessing the levels of DAPs in urine. The application of the UPLC-HRMS method to the urine samples from mothers and their offspring provided the first insight of the concentrations of DAPs in urine from Norway. The collection of mothers' urine was carried out for 24 hours to investigate the diurnal variability of the concentrations of DAPs in mothers. In order to investigate the human exposure pathways to PFRs, we used statistical tests to correlate DAP concentrations with PFR concentrations in dust and air collected from the living rooms of the participants' residences. This type of analysis has already been performed by Meeker *et al*. who obtained a positive correlation between BDCIPP levels in urine from adults and its parent compound in house

dust from the U.S.A. [13]. However, so far only one study reports concentrations of DAPs in children's urine [14]. Therefore our study significantly contributed to the knowledge of the PFR exposure in children. Further, we considered food as a possible exposure pathway. The studies of FRs in foodstuff are scarce [15-17] and in general the reported intakes from food are low [18]. For this study, food intake of mothers and children were assessed through a detailed 24 hours food recall to identify if any foodstuffs contribute significantly to the human body burden of PFRs.

The results of the first study have been published in Analytica Chimica Acta, and the second study has been submitted for publication to Environment International.

References

[1] I. van der Veen, J. de Boer, Chemosphere 88 (2012) 1119-1153.
[2] UNEP. http://chm.pops.int/default.aspx. (accessed June 2014).
[3] T. Reemtsma, J.B. Quintana, R. Rodil, M. García-López, I. Rodríguez, TrAC-Trends Anal. Chem. 27 (2008) 727-737.
[4] S. Pakalin, T. Cole, J. Steinkellner, R. Nicolas, C. Tissier, S. Munn, S. Eisenreich, European Commission. http://publications.jrc.ec.europa.eu/repository/handle/-111111111/5259. 2007 (accessed June 2014).
[5] European Comission. http://echa.europa.eu/documents/10162/f42be21b-33a3-4063-ad4d-2b0f937e41b4 (accessed June 2014).
[6] M. Gold, A. Blum, B. N. Ames, Science 200 (1978) 785-787.
[7] A. Blum, M. D. Gold, B.N. Ames, F.R. Jones, E.A. Hett, R.C. Dougherty, E.C. Horning, I. Dzidic, D.I. Carroll, R.N. Stillwell, J.P. Thenot, Science 201 (1978) 1020-1023.
[8] U.S. CPSC. https://www.cpsc.gov/en/Newsroom/News-Releases/1977/CPSC-Bans-TRIS-Treated-Childrens-Garments/. 1977 (accessed June 2014).
[9] A. A. Nomeir, S. Kato, H.B. Matthews, Toxicol. Appl. Pharm. 57 (1981) 401-413.
[10] E.M. Cooper, A. Covaci, A.L.N. van Nuijs, T.F. Webster, H.M. Stapleton, Anal. Bioanal. Chem. 401 (2011) 2123-2132.
[11] N. Van den Eede, H. Neels, P.G Jorens, A. Covaci, J. Chromatogr. A 1303 (2013) 48-53.
[12] T. Reemtsma, J. Lingott, S. Roegler, Sci. Total Environ. 409 (2011) 1990-1993.
[13] J.D. Meeker, E.M. Cooper, H.M. Stapleton, R. Hauser, Environ. Health Perspect. 121 (2013) 580-585.
[14] H. Fromme, T. Lahrz, M. Kraft, L. Fembacher, C. Machs, S. Dietrich, R. Burkardt, W. Völkel, T. Göen, Environ. Int. 2014 (in press).
[15] J.W. Kim, T. Isobe, K.H. Chang, A. Amano, R.H. Maneja, P.B. Zamora, F.P. Siringan, S. Tanabe, Environ. Pollut. 159 (2011) 3653-3659.
[16] L. Campone, A.L. Piccinelli, C. Östman, L. Rastrelli, Anal. Bioanal. Chem. 397 (2010) 799-806.
[17] T. Eggen, E.S. Heimstad, A.O. Stuanes, H.R. Norli, Environ. Sci. Pollut. Res. 20 (2013) 4520-4531.
[18] WHO. Environmental Health Criteria 111. Triphenyl phosphate. Geneve, Switzerland. 1991.

3.4.1. A high-throughput Method for Determination of Metabolites of Organophosphate Flame Retardants in Urine by Ultra Performance Liquid Chromatography-High Resolution Mass Spectrometry

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry

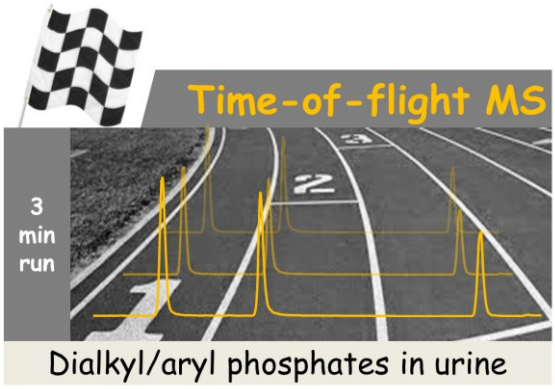
Enrique Cequier [a,b,*], Rosa Maria Marcé [b], Georg Becher [a,c], Cathrine Thomsen [a]

[a] *Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, P.O. Box 4404, Nydalen, 0403 Oslo, Norway*

[b] *Departament of Analytical Chemistry and Organic Chemistry, University of Rovira i Virgili, Carrer Marcel·lí Domingo s/n, 43007 Tarragona, Spain*

[c] *Department of Chemistry, University of Oslo, P.O. Box 1033, Blindern, 0315 Oslo, Norway*

Abstract



Organophosphate triesters are common flame retardants used in a wide variety of consumer products from which they can migrate and pollute the indoor environment. Humans may thus be continuously exposed to several organophosphate triesters which might be a risk for human health. An analytical method based on direct injection of 5 µL urine into an ultra performance liquid chromatography system coupled to a time-of-flight mass spectrometry has been developed and validated to monitor exposure to organophosphate triesters through their respective dialkyl and diaryl phosphate metabolites (DAPs). The targeted analytes were: di-*n*-butyl phosphate (DNBP), diphenyl phosphate (DPHP), bis(2-butoxyethyl) phosphate (BBOEP), bis(2-chloroethyl) phosphate (BCEP), bis(1-chloro-2-propyl) phosphate (BCPP) and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP). Separation was achieved in less than 3 minutes on a short column with narrow diameter and small particle size (50 mm x 2.1 mm x 1.7 µm). Different mobile phases were explored to obtain optimal sensitivity. Acetonitrile /water buffered with 5 mM of ammonium hydroxide/ ammonium formate (pH 9.2) was the preferred mobile phase. Quantification of DAPs was performed using deuterated analogues as internal standards in synthetic urine (averaged DAP accuracy was 101 %; RSD 3 %). Low method limits of quantification (MLQ) were obtained for DNBP (0.40 ng mL^{-1}), DPHP (0.10 ng mL^{-1}), BDCIPP (0.40 ng mL^{-1}) and BBOEP (0.60 ng mL^{-1}), but not for the most polar DAPs, BCEP (~12 ng mL^{-1}) and BCPP (~25 ng mL^{-1}). The feasibility of the method was tested on 84 morning urine samples from 42 mother and child pairs. Only DPHP was found above the MLQ in the urine samples with geometric mean (GM) concentrations of 1.1 ng mL^{-1} and 0.57 ng mL^{-1} for mothers and children respectively. BDCIPP was however, detected above the method limit of detection (MLD) with GM of 0.13 ng mL^{-1} and 0.20 ng mL^{-1}. While occasionally detected, the GM of DNBP and BBOEP were below MLD in both groups.

Keywords: human biomonitoring, urinary metabolites, time-of-flight mass spectrometry

* Corresponding author. Tel.: +4721076604; fax: +4721076686
E-mail address: enrique.cequier@fhi.no

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

1. Introduction

Organophosphate triesters (PFRs) are manufactured in high production volumes and are used primarily as flame retardants and plasticisers [1]. Many PFRs are additives in a wide variety of consumer products and may be emitted to the indoor environment. Tris-2-chloroethyl phosphate (TCEP), tris(1-chloro-2-propyl) phosphate (TCPP), tris-1,3-dichloro-2-propyl phosphate (TDCIPP), triphenyl phosphate (TPHP), tris(2-butoxyethyl) phosphate (TBOEP) and tri-*n*-butyl phosphate (TNBP) have been reported at μg g^{-1} levels in indoor dust [2-4] and at ng m^{-3} in indoor air [4-6] in many countries worldwide. *In vitro* studies have shown TDCIPP to be mutagenic [7] and suggested TPHP to be a possible endocrine disruptor [8], thus exposure to organophosphate triesters comprises a potential risk for human health. Several animal studies have reported rapid metabolism of the parent triesters to dialkyl or diaryl phosphates (DAPs), which are readily excreted in urine [9-11]. Reemtsma *et al*. reported in their study of human urine (n=19) that further hydrolysis to monoalkyl and monoaryl phosphates might occur, but nevertheless, DAPs were the most abundant metabolites with the exception of di-n-butyl phosphate which was considerably lower than its mono-alkylated analogue [12]. It is therefore generally accepted that human exposure to organophosphate triesters can be monitored by measuring DAPs in urine [13]. Due to the high polarity and ionic nature of the metabolites (pKa ≤2) [14], the preferred analytical methods are based on liquid chromatography coupled to a triple quadrupole (QqQ) mass spectrometer (LC-MS/MS), although GC-MS/MS has also been used [15]. One of the main advantages of GC analysis is the absence of strong matrix effects often experienced in LC-MS, but on the other hand, derivatisation of the metabolites is required. Matrix effect is a significant drawback for the determination of DAPs by LC-MS. However, recent synthesis of isotopically labelled internal standards has led to more reliable determinations of DAPs in urine by LC-MS [16,17]. Determination of DAPs such as di-*n*-butyl phosphate (DNBP), diphenyl phosphate (DPHP), bis(1,2-dichloro-2-propyl) phosphate (BDCIPP) and bis(2-butoxyethyl) phosphate (BBOEP) has been carried out, without any pre-treatment with glucuronidase/sulphatase enzymes for deconjugation, by means of SPE in combination with LC-MS-MS using hydrophobic stationary phases (e.g., C18 or phenyl modified silica or porous graphitic carbon), acid mobile phases, with or without buffer, and using MS in the negative electrospray ionisation (ESI(-)) mode [15]. However, one study reported better method limits of quantification (MLQ) for BDCIPP and DPHP using a negative atmospheric pressure chemical ionisation source (APCI(-)) [17]. Comparable MLQs of DNBP, DPHP, BDCIPP and BBOEP are obtained using LC-MS and GC-MS [16]. Determination of bis(2-chloroethyl) phosphate (BCEP) and bis(1-chloro-2-propyl) phosphate (BCPP) in urine is challenging using LC-MS-MS because of their generally low MS response (MLQ of 1.2 and 3.7 ng mL^{-1}, respectively) [16]. Currently, the most sensitive technique for the determination of these compounds is off-line SPE and GC-MS-MS after derivatisation (MLQ of 0.30 and 0.75 ng mL^{-1}, respectively) [18,19].

High-resolution mass spectrometers (HR-MS), e.g., time-of-flight (TOF) or tandem

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

quadrupole-TOF (Q-TOF), has so far not been explored for determination of DAPs in urine. The reason might be the good sensitivity and robustness of the QqQ experienced for targeted analysis. However, the spectral information obtained using HRMS is a significant advantage that allows retrospective searches for non-target compounds.

Human biomonitoring in epidemiologic studies demand high-throughput methods to be able to analyse a large number of samples in reasonable time. Therefore, the aim of this study was to develop a simple, rapid and sensitive method using ultra-performance liquid chromatography coupled TOF mass spectrometry to monitor free DAPs in human urine as biomarkers of organo-phosphate triester exposure. The final method was thoroughly validated and applied to 84 pair spot morning urine samples collected from 42 mother-child pairs.

2. Experimental

2.1. Materials

2.1.1. Chemicals and consumables

DNBP and DPHP (purity ≥97 %) were purchased from Sigma–Aldrich (St. Louis, MO, U.S.A.). BBOEP, BCEP, BCPP, BDCIPP, BBOEP-d4, BCEP-d8, BDCIPP-d10 and DPHP-d10 were synthesised by KAdemCustomChem GmbH (Göttingen, Germany) (Table S1). Purities of the synthesised standards were >97 % as shown by ^{1}H-NMR, except for BCPP and DPHP-d10 where the purity was 91 and 85 %, respectively. Water, acetonitrile (MeCN) and methanol (MeOH) were of LC-MaScan grade (>99.9% for organic solvents) and purchased from Labscan (Dublin, Ireland). Synthetic urine (UriSub®) was purchased from CST Technologies, Inc. (New York, NY, U.S.A.). Its composition is not reported, but according to the manufacturer, this synthetic urine is equivalent to human urine with respect to specific gravity, osmolality, viscosity and pH [20]. Ammonium hydroxide (NH_4OH, 29 %) and formic acid (HCOOH, 98 %) were purchased from Sigma–Aldrich. Acquity UPLC® BEH C18 column (50 mm x 2.1 mm x 1.7 µm; pH range 2-12) was purchased from Waters Corp. (Milford, MA, U.S.A.).

2.1.2. Urine samples

DAPs have been determined in 84 morning urine samples from 42 mother-child pairs living in the Greater Oslo area. The study was approved by the Regional Committee for Medical Research Ethics, and consent was obtained from all participants. Samples were stored in the freezer at -20 °C. After thawing and equilibration to room temperature, 1.5 mL of urine was centrifuged for 4 min at g-force of 18210 g (Eppendorf centrifuge 5430, Hamburg, Germany). The supernatant (980 µL) was spiked with 20 µL of internal standards (IS) (i.e., 246 pg of DPHP-d10, 1158 pg of BCEP-d8, 604 pg of BDCIPP-d10 and 136 pg of BBOEP-d4) and transferred to an amber glass vial.

2.2. Instrumentation

An ultra performance liquid chromatography (UPLC) instrument from Waters was equipped with a 2777C sample manager that incorporated a temperature regulated compartment for vials (10 °C), a binary and a quaternary Acquity^{TM} solvent managers

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

and a thermostated column chamber (40 °C). The initial mobile phase was 98 % eluent A (H_2O) and 2 % eluent B (MeCN/H_2O 90:10), both buffered with 5 mM NH_4OH/ammonium formate ($HCOONH_4$) (pH 9.2 in the aqueous phase). The flow rate was 0.5 mL min^{-1}. Five microliters of the centrifuged urine were injected. Separation and elution of the DAPs was achieved by increasing eluent B from 2 to 40 % in 3 min. Subsequently, the column was washed (1 min) increasing the percentage of eluent B to 95 %. The presented method is the result of optimisation of the chromatography by varying the organic solvent, concentration of buffer and pH.

A tandem mass spectrometer Xevo® G2-S QTOF from Waters was equipped with an electrospray ion source working in sensitivity mode (FWHM 22.5 k) and negative ionisation. Capillary, cone and offset voltage were 3000 V, 40 V and 80 V, respectively, and source and desolvation temperatures were 120 and 650 °C, respectively. Nitrogen was supplied by a nitrogen generator Genius3020 from Peak Scientific (Billerica, MA, U.S.A.). Cone gas and desolvation gas were set to 100 and 1000 L h^{-1}, respectively. Argon 5.0 from Yara Praxair (Oslo, Norway) was used as collision gas. Calibration of the high resolution mass spectrometer was performed with sodium formate in the range 50-1200 Da and scan time was 0.1 s. To obtain exact masses, leucine enkephalin was co-introduced with the analyte to correct changes in experimental conditions over the course of the MS analysis. Table 1 shows the exact masses monitored for each analyte. Data were processed using Masslynx® v4.1 software from Waters.

2.3. Procedures

Internal standard calibration with deuterated analogues was used for quantification of DAPs. Three calibration curves (inverse concentration weighed) with 8 levels each ranging from 0.054 to 70 ng mL^{-1} (final urinary concentrations prior to injection) were prepared by spiking the DAPs in 1 mL of water, human urine and synthetic urine. All the levels of the calibration were injected before and after the urine samples, and in addition, individual standards were injected every 10 samples. The calibration curves of DAPs were built up averaging all the A_{STD}/A_{IS} ratios of each level from the sequence. Slopes for all quantified DAPs were 1±0.1, except for DNBP (slope=0.49), intercepts were in the range -0.04 and 0.12, correlation coefficients (R^2) were all ≥0.993 and residuals were distributed randomly. MLQs were determined assessing the signal-to-noise (S/N) ratios from the urinary concentrations of DAPs. The S/N ratio was given by the data processing software Masslynx®.

Procedural blanks, consisted in 1 mL of water, MeCN, MeOH, and synthetic urine, were analysed in the same way as the samples. Solvents were free of DAPs, hence blank subtraction in the samples was not necessary and synthetic urine showed residual levels of DPHP and BBOEP not quantifiable as the areas were below the lowest calibration level.

In order to assess the matrix effects, the slopes of the three external calibration curves (without internal standards) in water, synthetic urine and human urine were compared, and accuracy and precision of the method were assessed comparing the calibration slopes (with internal standards)

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

Table 1
Elemental composition, base peaks, collision energy (V) and ion fragments (theoretical mass; m/z) used for the determination of DAPs.

	Elemental composition	[a]Base peak	[b]Collision energy	Ion fragments
BCEP	$C_4H_9O_4PCl_2$	220.9537		
BCEP-d8	$C_4HD_8O_4PCl_2$	229.0039		
BCPP	$C_6H_{13}O_4PCl_2$	248.9850		
DNBP	$C_8H_{19}O_4P$	209.0943	0 / 5-27	78.9585
DPHP-d10	$C_{12}HD_{10}O_4P$	259.0944		
DPHP	$C_{12}H_{11}O_4P$	249.0317	0 / 5-27	93.0340
BDCIPP-d10	$C_6HD_{10}O_4PCl_4$	328.9669		
BDCIPP	$C_6H_{11}O_4PCl_4$	318.9071	0 / 5-27	318.9071
BBOEP-d4	$C_{12}H_{23}D_4O_6P$	301.1718		
BBOEP	$C_{12}H_{27}O_6P$	297.1467	0 / 5-27	197.0579

[a]Base peak in the mass spectrum did not correspond to the exact mass $[M-H]^-$ for BCEP, BCEP-d8 and BCPP; [b]Two channels in the collision cell: low energy set to 0 V (mass of the parent ion) and high energy applying a gradient from 5 to 27 V (mass of the fragmented ions).

with respect to water, as well as, the areas from the extremes of the calibration curve. Intermediate repeatability of the method was evaluated injecting the previously prepared standards in human urine (n=7) after three months and comparing the slope with the one obtained in the first validation.

3. Results and discussion

3.1. Chromatography

Rapid determination of DAPs in urine was obtained using a short column (50 mm) and small particle size (1.7 μm). Separation of DAPs on reversed phase columns has typically been carried out at acidic pH [16,21,22]. In this study, three mobile phases at different pH were explored to find the optimal conditions for the separation and detection of DAPs, i.e., aqueous phase at pH 2.6 with 0.1 % formic, 5 mM buffer with $HCOOH/HCOONH_4$ at pH 4.5 and 5 mM buffer with $NH_4OH/HCOONH_4$ at pH 9.2.

The chromatography of DAPs using a mobile phase containing 0.1 % formic acid resulted in excessive peak tailing. The use of a buffer at pH 4.5 reduced this peak tailing, and using a buffer at pH 9.2 led to even better peak symmetry of the DAPs resulting in a significant increase in sensitivity (S/N ratio). This effect is illustrated in Fig. 1 with two DAPs (DNBP and DPHP).

Repeated injections of DAPs (n=3) were carried out to confirm the higher sensitivity achieved using the alkaline mobile phase compared to a buffer at pH 4.5. The peak height for all DAPs was enhanced by an average of 3, except for BBOEP which did not change (see electronic supplementary material (ESM); Fig. S1. This general increment in peak height using the buffer at pH 9.2 was probably achieved because the organophosphate anions were forming ion pairs with the ammonium cation in the buffer equilibrium. In addition, MeCN was preferred over MeOH as eluent B since repeated injections (n=4) showed a large increment in the peak height of DAPs (from 3 to 30 times higher), except for BBOEP for which the area was invariable (ESM; Fig. S2). The MS response of the analytes was also optimised by preparing mobile phases (H_2O/MeCN) buffered at 0.5, 5, 10 and

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

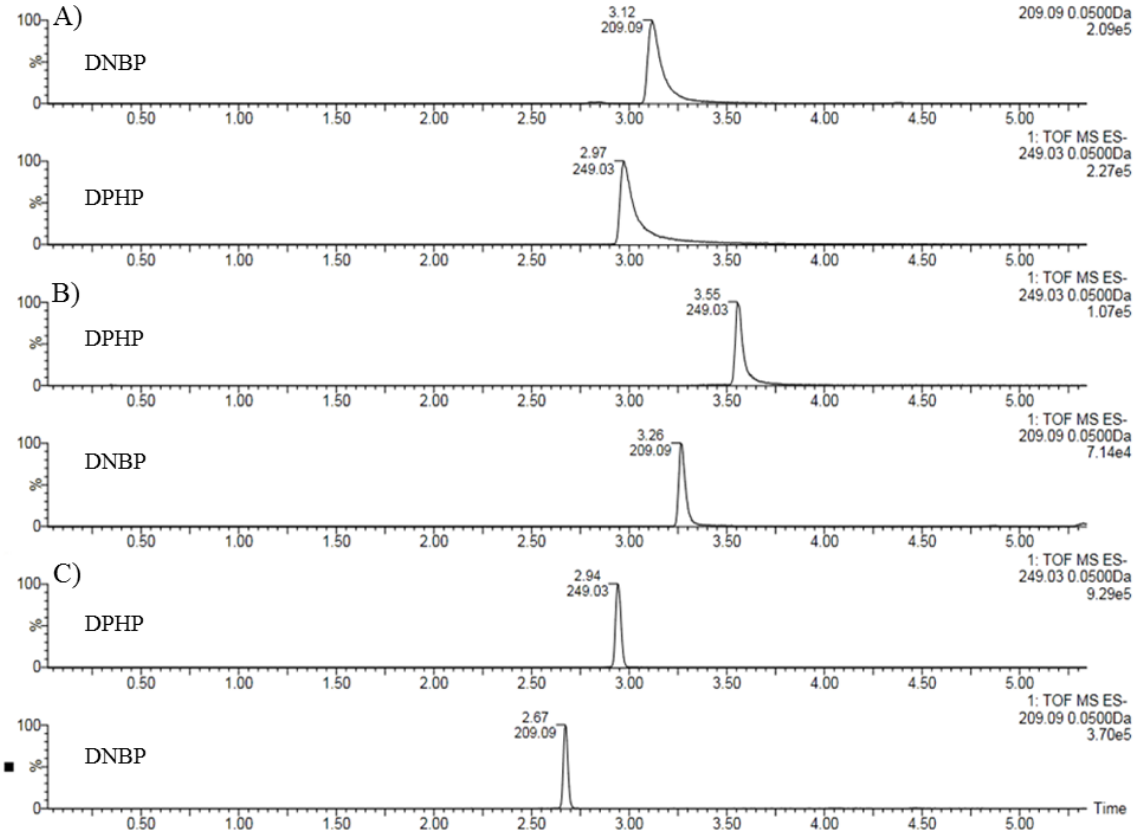


Fig. 1. Extracted ion chromatograms of DNBP and DPHP in three mobile phases. A) H_2O/MeCN with 0.1 % formic acid, B) Eluents A/B buffered with 5 mM HCOOH/$HCOONH_4$ (pH 4.5 in the aqueous phase) and C) Eluents A/B buffered with 5 mM NH_4OH/$HCOONH_4$ (pH 9.2 in the aqueous phase). Note that elution order of DNBP and DPHP is reversed in A). Flow rate was 0.4 mL min^{-1}.

20 mM. A mobile phase of 5 mM was the optimal concentration for chromatography and MS response of DAPs (ESM; Fig. S3). The chromatographic performance remained unchanged even after analysis of more than hundred urine samples without increase of backpressure or clogging of the LC system.

3.2. Mass spectrometry

Only the TOF was used for identification and quantification of the DAPs and the first quadrupole was not employed because the sensitivity of MS/MS was slightly lower than single high-resolution mass acquisitions (Fig. S4). Nevertheless, a "pseudo MS-MS" acquisition (MS^E, Waters Inc.) [23] was used when sensitivity was not compromised for reliable identification of DNBP, DPHP and BBOEP (Table 2). MS^E is based on the principle to rapidly alternate between two channels in the collision cell: low energy set at 0 V (exact mass of the parent ion) and high energy applying a gradient voltage (exact mass of the fragment ions). The criteria followed to confirm the presence of the metabolite in urine were: (1) difference in retention time relative to the adjacent peak must be within 0.01 min for standards and samples and (2) mass accuracy must be <5 ppm. In the case of high analyte concentrations, we have used MS^E for confirmation. Here, the mass accuracy criterion must be fulfilled for both

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

Table 2
MS identification of target DAPs detected in a typical spot urine of a child.

Rt (min)	Metabolite	Elemental composition	Parent mass				Fragment mass			
			Theoretical	Measured	Mass error		Theoretical	Measured	Mass error	
					mDa	ppm			mDa	ppm
1.92	DNBP	$C_8H_{18}O_4P$	209.0943	209.0944	0.5	0.5	78.9585	nd		
2.09	DPHP	$C_{12}H_{10}O_4P$	249.0317	249.0323	0.6	2.4	93.0340	93.0343	0.3	3.2
2.31	BDCIPP	$C_6H_{11}O_4PCl_4$	316.9061	316.9061	0.0	0.0	318.9042	318.9058	1.6	5.0
2.54	BBOEP	$C_{12}H_{27}O_6P$	297.1467	297.1467	1.2	4.0	197.0579	nd		

nd, not detected

the parent and fragment ions.

The mobile phase was diverted to waste the first 0.7 min of the MS acquisition in order to prevent salt deposition in the ion source. We also tested APCI(-) because Cooper *et al*. [17] suggested that despite the lower ionisation of DPHP and BDCIPP using this technique, it resulted in a much lower background signal giving higher S/N ratios compared to ESI(-). However in our system, the sensitivity obtained with the APCI source was far lower than that obtained with the ESI source. The parameter affecting the ionisation of the DAPs in ESI(-) most was the capillary voltage. Optimal ionisation was achieved with 3000 V. Base peaks in the mass spectra corresponded to the ions $[M-H]^-$ of the molecules (Table 1), except for BCEP and BCPP for which abundances of $[M-H]^-$ were very low. This was probably caused by formation of various adducts (e.g., oligomers) in the ion source, and was observed irrespectively of a buffer used in the mobile phase (Fig. S5). In addition, the intensities of BCEP and BCPP varied largely depending on the matrix. As a result, the method was not further optimised and validated for these two DAPs.

3.3. Method performance

Linearity was studied in the range 0.054-525 pg μL^{-1}, although the linear response began to fail for DNBP, DPHP, BDCIPP and BBOEP at 45, 68, 45 and 79 pg μL^{-1}, respectively, due to the low dynamic linear range of TOF instruments [24]. Strong matrix effects were detected for the DAPs when comparing the slopes of the three calibration curves (in water, in synthetic urine and in human urine) [25] (Fig. S6). The standards were injected three times in the course of one week to study time variability in the calibration slopes without ISs. Fig. 2 shows the enhancement of the signal for all the analytes in human and synthetic urine compared to water assessed by the slopes of the calibration curves. The reason for the very high enhancement of the BBOEP signal in the synthetic urine is not known.

Van den Eede *et al*. also experienced ion enhancement for BCPP (143 %) and DPHP (166 %), but ion suppression for BDCIPP (40 %) and BBOEP (53 %) [16]. Cooper *et al*. experienced a weak ion enhancement for BDCIPP (107-117 %) and DPHP (98-128 %) using APCI(-) [17]. These differences might be attributed to the use of different ion source configurations and different chromatographic conditions. To quantify the

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

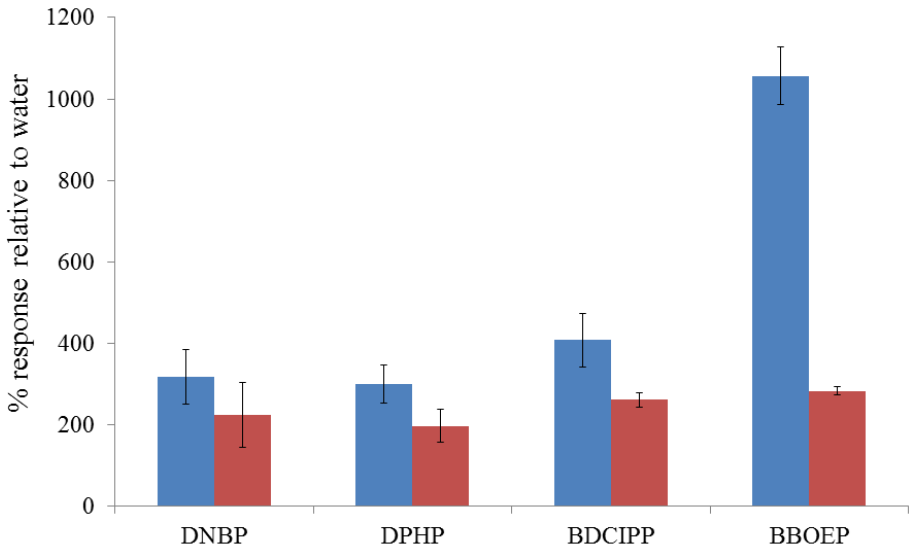


Fig. 2. Ion enhancement of MS DAP response in calibrations using synthetic urine (blue) and urine (red) compared to water. Error bars indicate standard deviations (n=3).

DAPs, synthetic urine spiked with the standards was chosen over human urine because it is free of organophosphate metabolites, whereas human urine will always contain some DAPs which must be corrected for.

To overcome the challenging issue of the strong matrix effects obtained in both synthetic and human urine, we used isotopically labelled DAPs for their quantification. The exception was DNBP for which a deuterated analogue was not available and which was quantitated using DPHP-d10. Table 3 presents the accuracy and precision of the method for DAPs. These were obtained from comparison of the slopes of the calibration curves prepared in urine (n=7) and synthetic urine (n=7) with respect to water (n=7). All standards were injected in triplicate during the course of a week. Accuracies of DNBP, DPHP, BDCIPP and BBOEP were within the range 85-125 % and RSD between 1 and 8 % showing excellent performance of the IS in the three matrices and therefore, compensating satisfactorily the matrix effects discussed above. This was even true for DNBP for which a deuterated analogue could not be used. The intercept of the calibration curves of DAPs in synthetic urine was between -0.04 and 0.12 which confirms the negligible amounts of DAPs in the matrix. Moreover, we investigated the individual accuracies and precision of the DAPs in the extremes of the calibration curve and also the precision of a standard injected five times throughout the sequence. The results obtained were similar than those from Table 3, except for the low extreme of the calibration curve where the precision was higher than in Table 3 (RSD between 14 and 30%, mean 19%) (see Table S2 and S3). The reproducibility of the method was confirmed after three months by the injection of the calibration curve in human urine (n=7). The accuracy of the slope obtained for DNBP, DPHP, BDCIPP and BBOEP was 112, 117, 112 and 104 %, respectively. In addition, the reproducibility was tested re-analysing three human urine samples for each DAP after several days and after the injection of all the urine samples.

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

Table 3
Internal standards used for the quantification, accuracies (in percent) and precision (relative standard deviation) of DAPs. MLQ (ng mL^{-1}) were obtained from real urine samples.

DAP	IS	Calibration slopes (n=3 each level)				MLQ
		Synthetic urine		Urine		
		Accuracy	RSD (%)	Accuracy	RSD (%)	
DNBP	DPHP-d10	93	5	86	8	0.40
DPHP	DPHP-d10	98	2	85	7	0.10
BDCIPP	BDCIPP-d10	107	2	124	3	0.40
BBOEP	BBOEP-d4	104	2	99	1	0.60

The concentrations of the DAPs in urine ranged from 0.15 to 37 ng mL^{-1} and the accuracy in the two determinations averaged between 80 % and 104 % with RSDs lower than 13 % (see Table S4).

We further evaluated the effect on the MS response after the direct injection of approximately 100 samples of urine (including calibration levels and two injections of MeOH and synthetic urine as procedural blanks). The areas of the internal standards increased 24 %, 5 % and 1 % for DPHP, BDCIPP and BBEP, respectively. However, after 200 injections of urine, the sensitivity of BBOEP was still unaffected, but decreased almost 30 % for DPHP and BDCIPP (Table S5). Therefore, a quick routine cleaning of the ion source is recommended before reaching 200 injections to recover the initial sensitivity. The MLQ was determined from the lowest level of the calibration curve prepared in synthetic urine producing a S/N≥10. The obtained MLQ of DAPs ranged from 0.08 to 0.45 ng mL^{-1}. However, in real urine MLQs were different, which can be attributed to the higher ion enhancement of DAPs and lower baseline background in synthetic urine compared to the real samples. Therefore, the final method limits of detection (MLDs) were obtained from human urine samples (Table 3). The MLQ for DPHP (0.1 ng mL^{-1}) and DNBP (0.4 ng mL^{-1}) were lower than other methods published using off-line SPE or large volume injections and LC-MS-MS (QqQ) (MLQ were in the range 0.2-0.9 ng mL^{-1} for DPHP and 3.4 ng mL^{-1} for DNBP) [12,16,17], and also lower than methods based on SPE and GC-MS-MS (MLQ were 1.5 ng mL^{-1} for DPHP and 0.75 ng mL^{-1} for DNBP) [18,19]. The MLQ for BDCIPP (0.4 ng mL^{-1}) is in the range of the method developed by Van den Eede *et al*. (0.52 ng mL^{-1}) [16] and much higher than the MLQ published by Cooper *et al*. (0.008 ng mL^{-1}) [17]. The MLQ for BBOEP (0.6 ng mL^{-1}) was also higher than that obtained by Van den Eede *et al*. (0.15 ng mL^{-1}).

3.4. Application of the method to real samples.

Morning urine samples from 42 mother-child pairs were analysed for the selected DAPs. Fig. 3 shows good S/N ratios of the chromatogram of a urine sample from one child. In the urine of the children, DNBP and BBOEP were below the MLQ, but the detection frequencies (S/N≥3) were 14 % and 33 %, respectively. BDCIPP was detected in 79 % of the urine samples and DPHP in all of them. Geometric mean (GM)

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

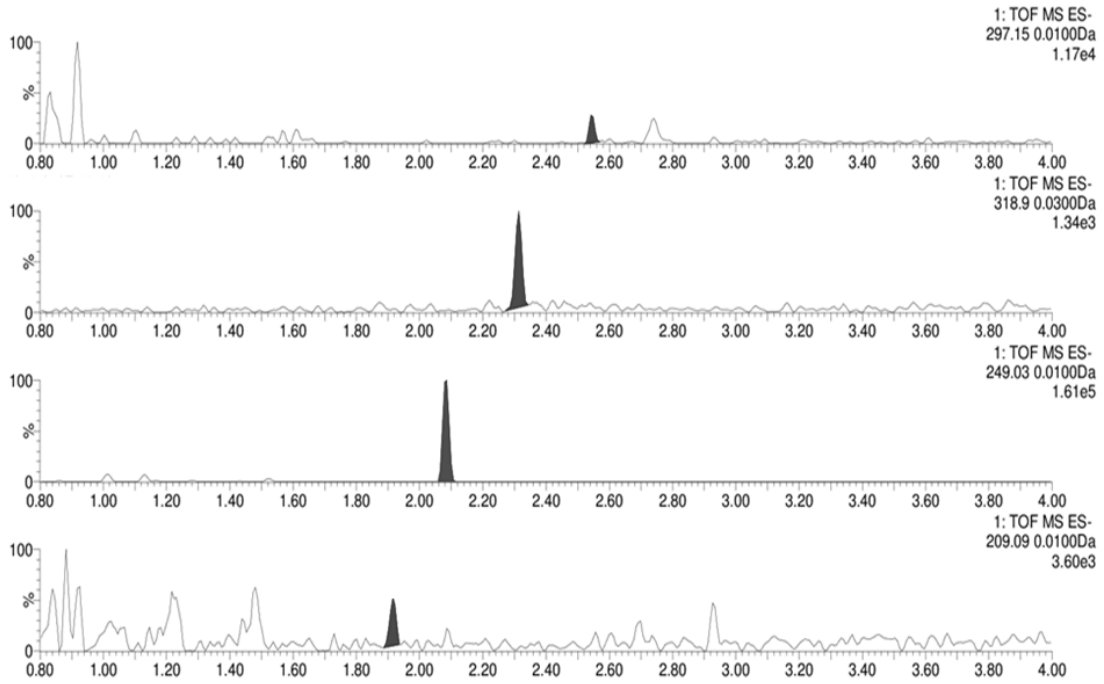


Fig. 3. Extracted ion chromatograms of DAPs from the typical spot urine sample of a child. DNBP at 1.92 min (<0.4 ng mL^{-1}); DPHP at 2.09 min (14 ng mL^{-1}); BDCIPP at 2.31 min (0.57 ng mL^{-1}) and BBOEP at 2.54 min (0.24 ng mL^{-1}). Flow rate was 0.5 mL min^{-1}.

concentrations of BDCIPP and DPHP were 0.20 and 1.1 ng mL^{-1}, respectively. In children's urine there were two extreme outlier concentrations of DPHP (14 and 37 ng mL^{-1}) (SPSS® v.20, Chicago, IL, U.S.A.). The highest concentration is comparable to the maximum concentrations reported by Hoffman *et al*. in a similar study (n=39) [26]. Also the corresponding mothers showed high urine levels of DPHP (2.2 and 1.5 ng mL^{-1}, respectively being the third and second highest) suggesting similar sources of exposure. In the urine of the mothers, BBOEP and DNBP concentrations were all below the MLQ, and frequencies of detection (S/N≥3) were 0 % and 5 %. Also BDCIPP levels were below MLQ (0.13 ng mL^{-1}), although its detection frequency was quite high (57 %).DPHP was detected in all urine samples with a GM concentration of 0.57 ng mL^{-1}. In general, the urine of the children contained higher concentrations of all target DAPs than the urine from the mothers (Fig. 4).For statistical purposes, the concentrations of DAPs below MLD were replaced by half MLD. The Mann-Whitney test showed that concentrations of BDCIPP (p=0.010) and DPHP (p=0.000) in urine from mothers and children were significantly different. The Wilcoxon test also identified concentrations significantly different in the pairs mother-child for DPHP (p=0.001), BDCIPP (p=0.015) and BBOEP (p=0.001). This suggests that the exposure of children to organophosphate triesters is higher than the exposure of their mothers when assuming that there are no major differences in the toxicokinetics of PFRs between both groups.

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

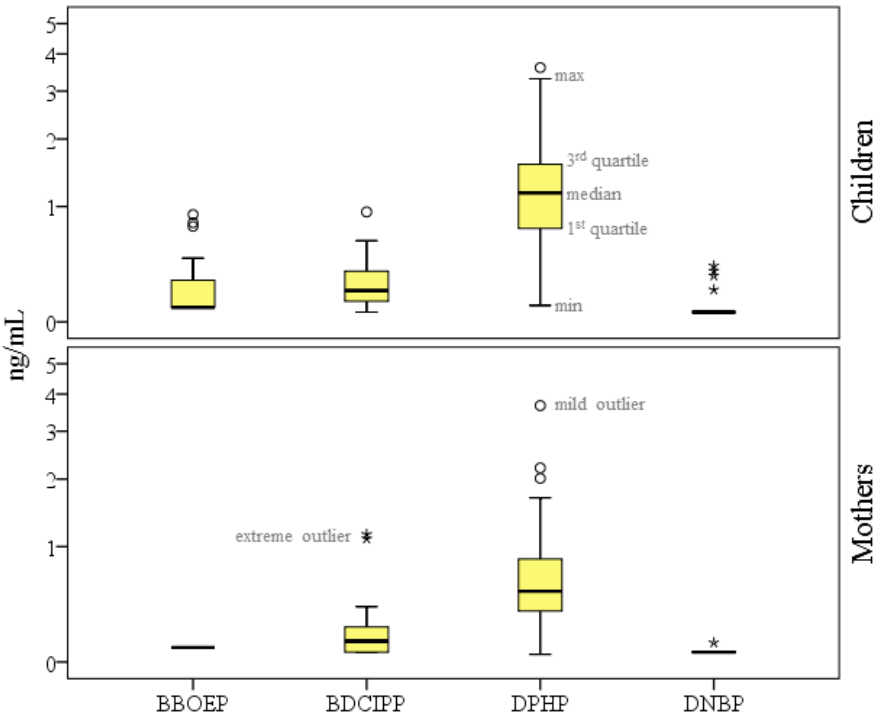


Fig. 4. Box plot of the concentrations of DAPs (ng mL^{-1}) in urine from the mothers and children. Median of BBOEP and DNBP are below MLD for children and mothers. Two extreme outlier concentrations for DPHP in children's urine are outside the chosen Y axis log scale (14.0 and 36.8 ng mL^{-1}).

4. Conclusions

A novel, rapid and sensitive UPLC-QTOF method has been developed to monitor four organophosphate triester metabolites by direct injection of urine. DNBP, DPHP, BDCIPP and BBOEP were determined in less than three minutes by using a short column with small particle size.

The validation proved high accuracy and precision of the method and the robustness was demonstrated in a run of up to 200 samples of urine. Omitting sample preparation reduced the risk for background contamination. The MLQs for the DAPs were better or comparable to MLQs in previously published methods using pre-concentration of urine and multiple reaction monitoring. The satisfactory determination of DNBP, DPHP, BDCIPP and BBOEP has been confirmed in a study of urine samples from mother-child pairs. DPHP was the most abundant DAP in both mothers and children, and levels of DPHP and BDCIPP were statistically higher in children.

Acknowledgements

This study was funded by the European Commission, 7th Framework Programme under the EU Marie Curie Initial Training Network INFLAME (GA No. 264600) and by the Research Council of Norway. Dr. Adrian Covaci and co-workers from the Toxicological Centre at the University of Antwerp (Belgium) are greatly acknowledged for providing the standards and their deuterated analogues.

Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2014.06.026.

References

[1] I. van der Veen, J. de Boer, Chemosphere 88 (2012) 1119-1153.
[2] N. Van den Eede, A.C. Dirtu, H. Neels, A. Covaci, Environ. Int. 37 (2011) 454-461.
[3] R.E. Dodson, L.J. Perovich, A. Covaci, N. Van den Eede, A.C. Ionas, A.C. Dirtu, J.G. Brody, R.A. Rudel, Environ. Sci. Technol. 46 (2012) 13056-13066.
[4] A. Kanazawa, I. Saito, A. Araki, M. Takeda, M. Ma, Y. Saijo, R. Kishi, Indoor Air 20 (2010) 72-84.
[5] C. Bergh, R. Torgrip, C. Östman, Rapid Commun. Mass Spectrom. 24 (2010) 2859-2867.
[6] S. Brommer, S. Harrad, N. Van Den Eede, A. Covaci, J. Environ. Monitor. 14 (2012) 2482-2487.
[7] M. Gold, A. Blum, B.N. Ames, Science 200 (1978) 785-787.
[8] P. Honkakoski, J.J. Palvimo, L. Penttilä, J. Vepsäläinen, S. Auriola, Biochem. Pharm. 67 (2004) 97-106.
[9] WHO. Environmental health criteria 112. Switzerland, Geneva. 1991.
[10] WHO. Environmental health criteria 209. Switzerland, Geneva, 1991.
[11] IPCS/OECD http://www.inchem.org-/documents/sids/sids/115866.pdf (accessed June 2014).
[12] T. Reemtsma, J. Lingott, S. Roegler, Sci. Total Environ. 409 (2011) 1990-1993.
[13] C.C. Carignan, M.D. McClean, E.M. Cooper, D.J. Watkins, A.J. Fraser, W. Heiger-Bernays, H.M. Stapleton, T.F. Webster, Environ. Int. 55 (2013) 56-61.
[14] W.P. Jencks, F.H. Westheimer, http://nsdl.org/resource/2200/2006112 1124513540T; pKa Data Compiled by R. Williams. (accessed April 2014).
[15] A.C. Dirtu, N. Van den Eede, G. Malarvannan, A.C. Ionas, A. Covaci, Anal. Bioanal. Chem. 404 (2012) 2555-2581.
[16] N. Van den Eede, H. Neels, P.G. Jorens, A. Covaci, J. Chromatogr. A 1303 (2013) 48-53.
[17] E.M. Cooper, A. Covaci, A.L.N. van Nuijs, T.F. Webster, H.M. Stapleton, Anal. Bioanal. Chem. 401 (2011) 2123-2132.
[18] B.K. Schindler, K. Förster, J. Angerer, J. Chromatogr. B 877 (2009) 375-381.
[19] B.K. Schindler, K. Förster, J. Angerer, Anal. Bioanal.Chem. 395 (2009) 1167-1171.
[20] CST Technologies. http://www.cstti.-com/index.php/products/urisub.html (accessed April 2014).
[21] J.B. Quintana, R. Rodil, T. Reemtsma, Anal. Chem. 78 (2006) 1644-1650.
[22] S. Dulaurent, F. Saint-Marcoux, P. Marquet, G. Lachâtre, J. Chromatogr. B 831 (2006) 223-229.
[23] Waters. An overview of the principles of MSE. http://waters.com/webassets-/cms/library/docs/720004036en.pdf (accessed April 2014).
[24] T. Reemtsma, TrAC-Trend Anal. Chem. 20 (2001) 533-542.
[25] B.K. Matuszewski, J. Chromatogr. B 830 (2006) 293-300.
[26] K. Hoffman, J.L. Daniels, H.M. Stapleton, Environ. Int. 63 (2014) 169-172.

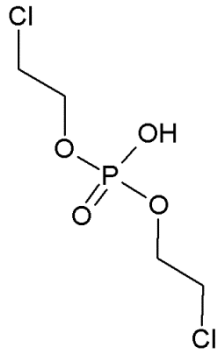
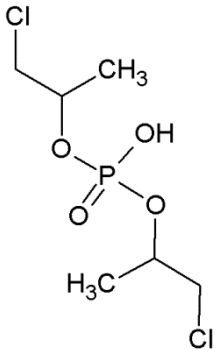
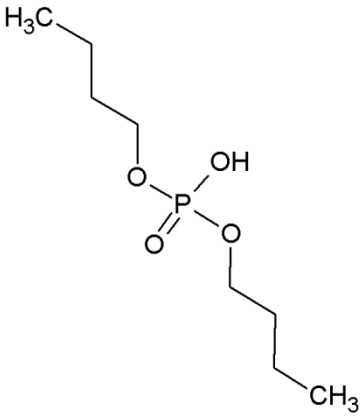
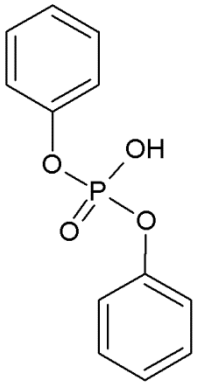
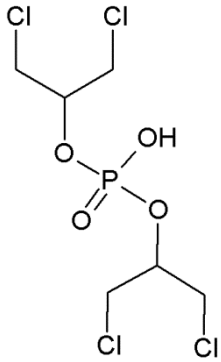
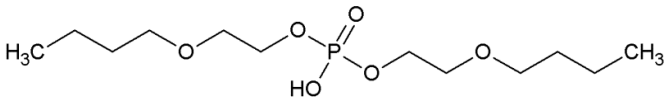
Supporting Information

Supporting Information / Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

2. Experimental

2.1 Materials

Table S1
Structures of the targeted dialkyl and aryl phosphates. Abbreviations used according to Bergman *et al.*[1]

BCEP  bis(2-chloroethyl) phosphate	**BCPP**  bis(1-chloro-2-propyl) phosphate
DNBP  di-*n*-butyl phosphate	**DPHP**  diphenyl phosphate
BDCIPP  bis(1,3-dichloro-2-propyl) phosphate	**BBOEP**  bis(2-butoxyethyl) phosphate

3. Results and discussion

3.1. Chromatography

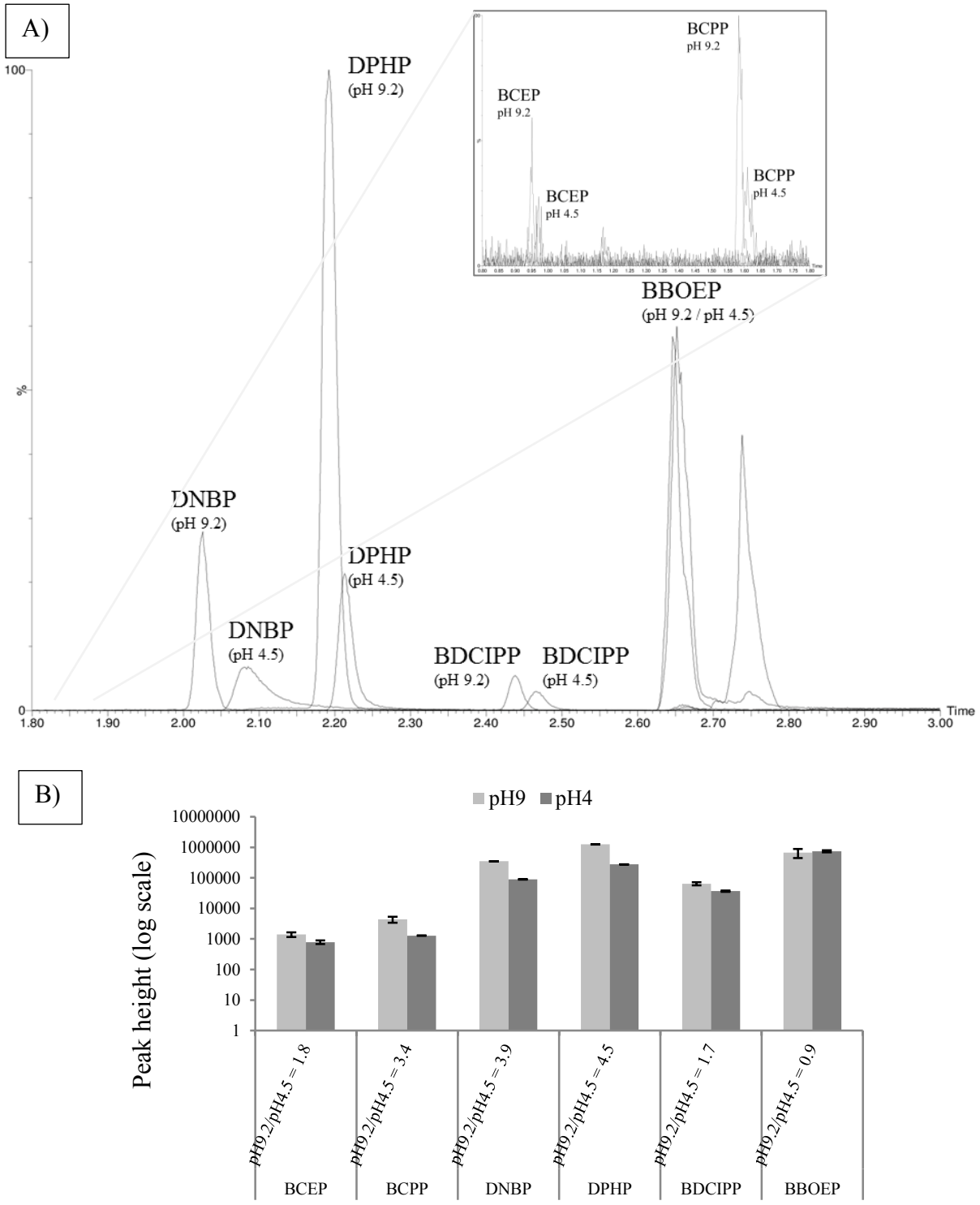


Fig. S1. A) Chromatograms of DAPs (~90 ng/µL in synthetic urine) using mobile phase buffered at pH 9.2 and 4.5. B) Peak height ratios (pH9.2/pH4.5; n=3) are shown in X-axis, error bars display standard deviations and note Y-axis is in log-scale.

Supporting Information / Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

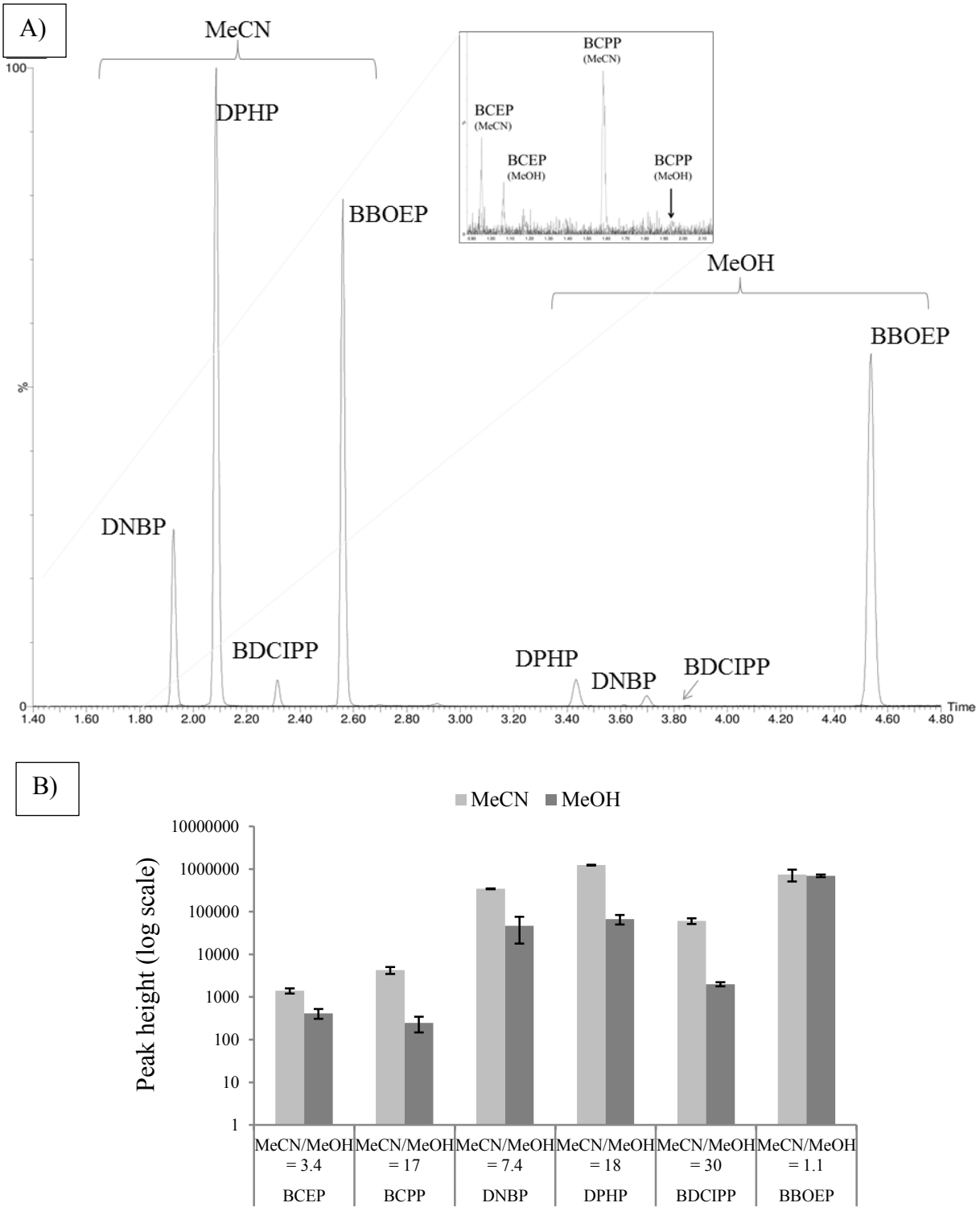


Fig. S2. A) Chromatograms of DAPs (~90 ng/µL in synthetic urine; n=4) using MeCN or MeOH in the mobile phase buffered at pH 9.2. B) Peak height comparison of DAPs using MeCN or MeOH. Peak height ratios (MeCN/MeOH) are shown in X-axis, error bars display standard deviations and note Y-axis is in log-scale.

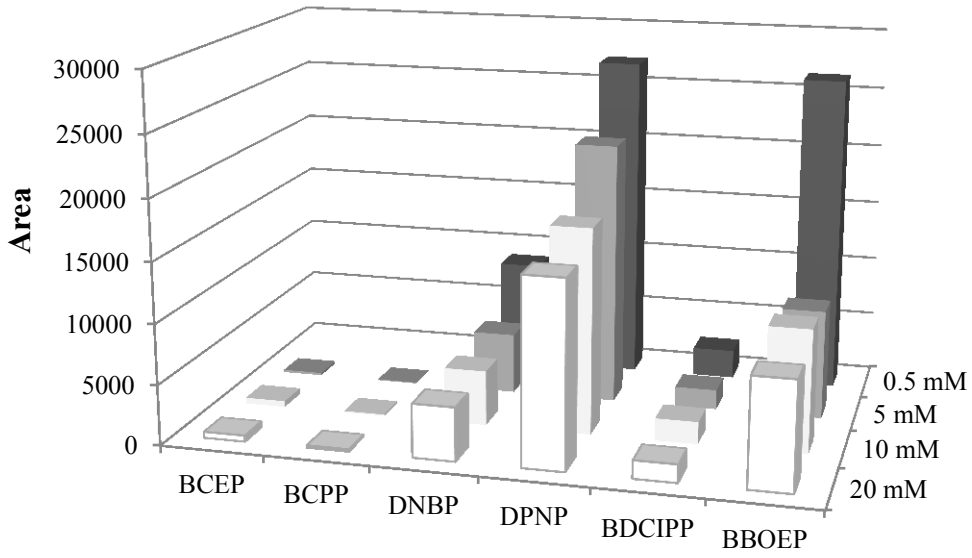


Fig. S3. Effect of different concentrations of buffer (NH_4OH/NH_4HCOO at pH 9.2) on areas of DAPs (amount injected of DAPs was between 400 and 750 pg).

Fig. S3 shows that peak area increases with decreasing concentration of the buffer, except for BCEP and BCPP for which the highest areas were obtained at 20 mM. For the other DAPs, highest areas were achieved with buffer at 0.5 mM. However, due to the peak tailing observed at 0.5 mM, a buffer concentration of 5 mM was chosen.

3.2. Mass spectrometry

Sensitivity acquiring in HRMS (TOF) and MS/MS (Q-TOF) was evaluated in single injections at two different concentrations. S/N ratios in spiked urines were higher when acquiring in HRMS than in MS/MS mode, except for BDCIPP, because no fragment was produced in the collision cell for this DAP (Fig. S4). Acquisition in HRMS mode turned out to be more suitable, because of higher sensitivity compared to MS/MS and the advantage of having complete mass spectra information.

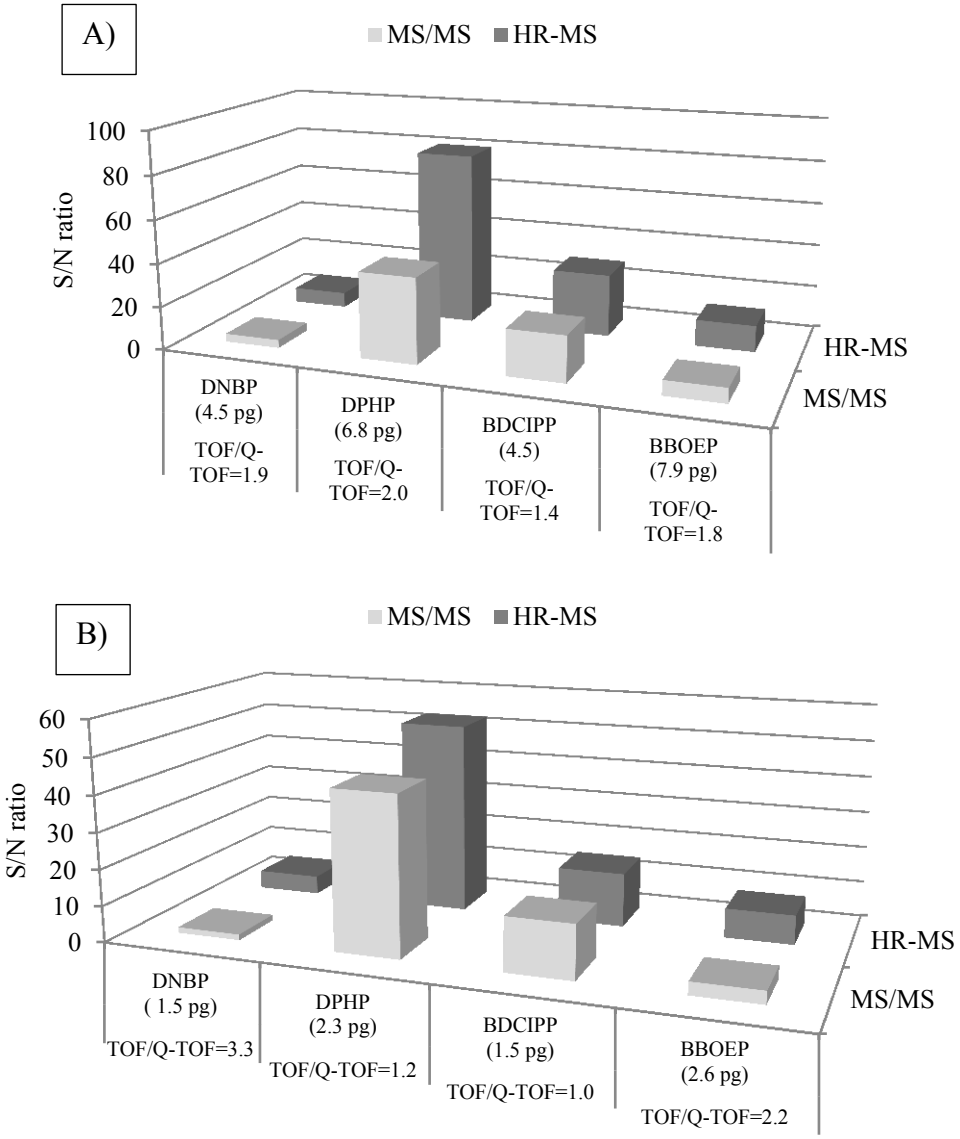


Fig. S4. S/N ratios of DAPs acquiring in HR-MS (TOF) or MS/MS (QTOF) in two different spiked urines. In X-axis the ratio [(signal-to-noise from TOF) / (signal-to-noise from QTOF)] is also displayed. A) Spiked urine at 22.3-39.4 ng/mL. B) Spiked urine at 7.4-13.1 ng/mL.

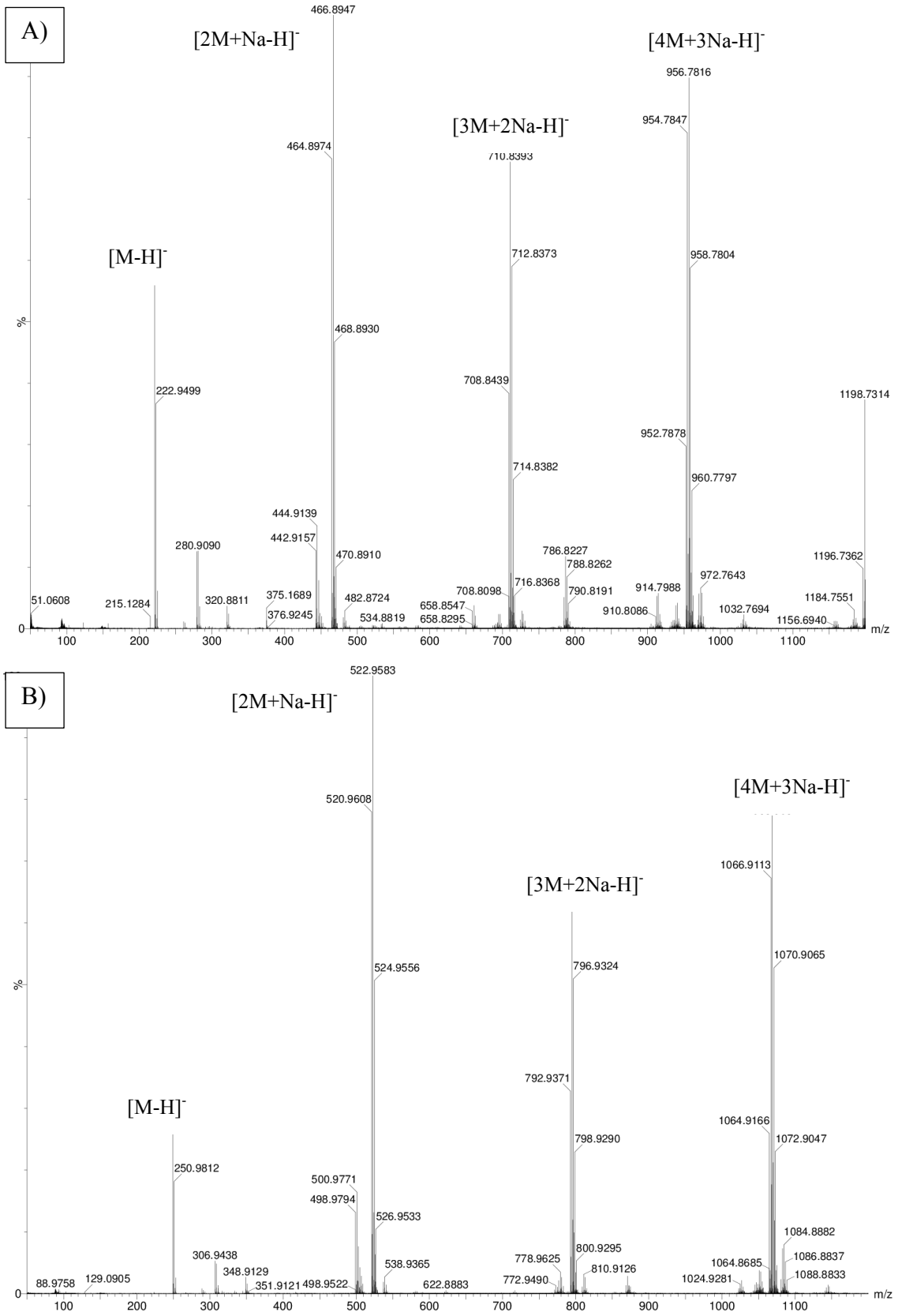


Fig. S5. A) Mass spectra of 1.4 ng of BCEP and B) Mass spectra of 0.95 ng of BCPP. Clusters from mass spectra are tentatively identified as oligomers.

Supporting Information / Anal. Chim. Acta (2014) doi: 10.1016/j.aca.2014.06.026

Several attempts were performed to avoid formation of dimers and oligomers of BCEP and BCPP from the mass spectra (modification of cone voltage, desolvation temperature and flow, etc.), but in any case the ion $[M\text{-}H]^-$ increased in intensity. Draper *et al*. also observed the dimer formation in the case of dimethyl dithiophosphate using APCI(-) [2].

3.3. Method performance

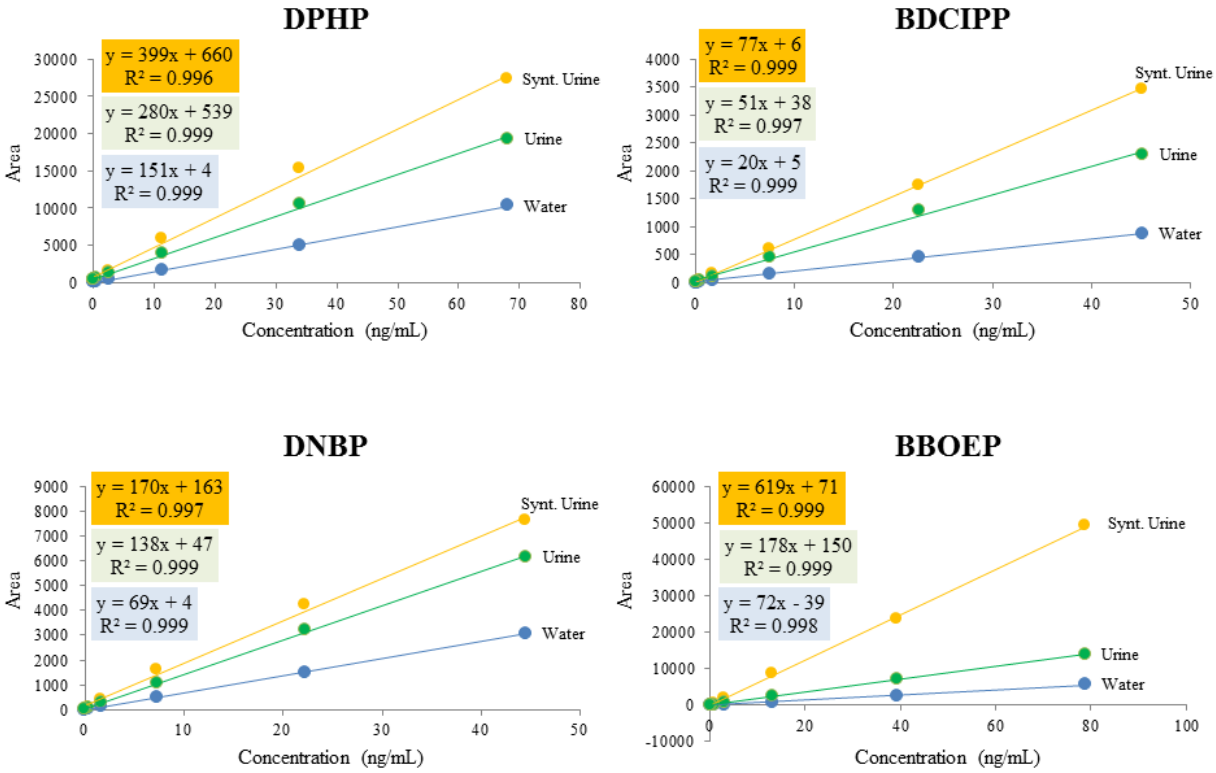


Fig. S6. Calibration curves of the four DAPs (without internal standards) prepared in water, synthetic urine and human urine.

-Accuracies and precision of the DAPs in the sequence with urine samples:

Table S2
Accuracy and precision of the standard prepared in synthetic urine injected between urine samples

	DNBP 0.45 ng mL^{-1}	DPHP 0.68 ng mL^{-1}	BDCIPP 0.45 ng mL^{-1}	BBOEP 0.79 ng mL^{-1}
1	0.36	0.59	0.37	0.71
2	0.38	0.62	0.38	0.7
3	0.4	0.59	0.41	0.63
4	0.3	0.65	0.35	0.69
5	0.35	0.66	0.41	0.66
Mean	0.36	0.62	0.38	0.68
RSD (%)	11	5	7	5
Accuracy	80	91	85	86

-Accuracies and precision of the DAPs in the extremes of the calibration curve:

Concentrations ranging from 45 to 78 ng mL^{-1} (STD_{high}) and from 0.15 to 0.26 ng mL^{-1} (STD_{low}) were tested, except for BDCIPP, which lowest concentration was 0.45 ng mL^{-1} since concentration of 0.15 ng mL^{-1} was undetectable in water (Table S3).

Table S3
Individual accuracies of DAPs with respect the water response at high (45 and 78 ng mL^{-1}) and low (0.15-0.45 ng mL^{-1}) concentrations

STD_{low}					STD_{High}			
DNBP	DPHP	BDCIPP	BBOEP		DNBP	DPHP	BDCIPP	BBOEP
109	77	97	124		93	100	113	109
102	87	118	94		103	89	131	112
82	133	128	128		103	98	131	111
98	99	114	115	Mean	100	96	125	111
14	30	14	16	RSD (%)	6	6	9	1

As could be expected, the RSD in STD_{low} is higher than in STD_{high}, mainly due to the error incorporated in the integration of the small peak and the interpolation of the area in the calibration curve.

Table S4
Accuracy (%) between the first and the second analysis of three different urines for each DAP.

DNBP		DPHP		BDCIPP		BBOEP	
ng mL^{-1}	%	ng mL^{-1}	%	ng mL^{-1}	%	ng mL^{-1}	%
0.88	89	37	105	3.7	103	0.36	94
0.77	78	14	94	1.2	97	0.15	74
0.40	74	23	112	1.2	97	0.17	78
Mean	80	Mean	104	Mean	99	Mean	82
RSD	10	RSD	9	RSD	3	RSD	13

Table S5
Area variation in the MS response of the deuterated IS after the injection of approximately 100 and 200 urine samples

	Initial Areas			Area after 100 samples			% Difference		
	DPHP	BDCIPP	BBOEP	DPHP	BDCIPP	BBOEP	DPHP	BDCIPP	BBOEP
	4160	3258	4925	4878	3047	3993	117	94	81
	4647	3115	4059	5928	3391	4697	128	109	116
	4593	3154	4065	5797	3537	4367	126	112	107
Mean	4467	3176	4350	5534	3325	4352	124	105	101
RSD (%)	6	2	11	10	8	8	5	9	18

	Area after 200 samples			% Difference		
	DPHP	BDCIPP	BBOEP	DPHP	BDCIPP	BBOEP
	3205	2277	4440	77	70	90
	3120	2106	4232	67	68	104
	3318	2378	4723	72	75	116
Mean	3214	2254	4465	72	71	104
RSD (%)	3	6	6	7	6	13

References

[1] Å. Bergman, A. Rydén, R.J. Law, J. de Boer, A. Covaci, M. Alaee, L. Birnbaum, M. Petreas, M. Rose, S. Sakai, N. Van den Eede, I. van der Veen, Environ. Int. 49 (2012) 57-82.

[2] W.M. Draper, P. Behniwal, D. Wijekoon, Rapid Commun. Mass Spectrom. 22 (2008) 2613-2620.

3.4.2. Human Exposure Pathways to Organophosphate Triesters – A Biomonitoring Study of Mother-Child Pairs

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Environ. Int. (submitted 2014)

Human exposure pathways to organophosphate triesters – A biomonitoring study of mother-child pairs

Enrique Cequier[a,b,*], Amrit Sakhi[a], Rosa Maria Marcé[b], Georg Becher[a,c] and Cathrine Thomsen[a]

[a] *Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway*

[b] *Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona, Spain*

[c] *Department of Chemistry, University of Oslo, Norway*

Abstract

The worldwide ban of several flame retardant formulations has caused an increase in the production of organophosphorous flame retardants (PFRs) to meet the existing fire regulations for a wide range of household products. This biomonitoring study surveys the occurrence of the metabolites from PFRs and related plasticisers (dialkyl and diaryl phosphates; DAPs) in urine from a Norwegian mother-child cohort (n=104). Detection frequencies of DAPs were higher in the children than in their mothers. Median concentrations of diphenyl phosphate (DPHP) were 1.0 and 0.91 ng/mL (normalised to specific gravity) in children and mothers, respectively, followed by bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) with medians of 0.23 and <0.22 ng/mL, respectively. Bis(2-butoxyethyl) phosphate (BBOEP) was only detected in children's urine with a frequency of detection of 32% and a median <0.17 ng/mL. Di-*n*-butyl phosphate (DNBP) was not observed above the method limit of detection in any sample (0.11 ng/mL and 0.22 ng/mL for children and women respectively). The concentrations of DPHP, BBOEP and BDCIPP in urine from children were significantly correlated with those found for their parent compounds in air and dust from the households (Spearman rank correlations 0.289<R<0.491). The concentration of BDCIPP in the mothers' urine was also highly correlated to its precursor in dust from the households (R=0.53). The moderate to strong correlations obtained between biomonitoring and measurements of PFRs in the indoor air and dust samples suggest that the residential environment is an important exposure pathway to PFRs. However, no significant associations were found between organophosphate metabolites in urine and food consumption.

Key words: biomonitoring, exposure, dust, organophosphates, metabolites, flame retardants, variability, food

[*]E-mail: enrique.cequier@fhi.no / Tel.: +4721076604 / fax: +4721076686

1. Introduction

Triaryl and trialkyl phosphates (TAPs) are chemicals used as plasticisers and flame retardants (FRs) in a large variety of consumer goods (van der Veen *et al.* 2012), from which they may leach out and contaminate the environment (Marklund *et al.* 2003; Stapleton *et al.* 2009). Concentrations of TAPs, e.g., tri-*n*-butyl

phosphate (TNBP), triphenyl phosphate (TPHP), tris(2-butoxyethyl) phosphate (TBOEP) and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), have been found at µg/g levels in household dust (Bergh *et al.* 2011; Cequier *et al.* 2014a; Dodson *et al.* 2012). Therefore, the indoor environment may play an important role for human exposure to these chemicals. So far, there is little knowledge on the occurrence of TAPs in foodstuffs and on their importance as sources for human exposure to TAPs (Campone *et al.* 2010; Eggen *et al.* 2013; Kim *et al.* 2011).

Exposure to some organophosphorous FRs (PFRs) may pose a potential threat to human health (Van der Veen *et al.* 2012), e.g., TCEP is a suspected human carcinogen (European Comission 2006) and TDCIPP is identified as a mutagenic (Gold *et al.* 1978). For risk assessment purposes, it is important to quantitatively assess human exposure. Experiments on rodents have shown that PFRs, such as TPHP and TDCIPP, are readily hydrolysed and excreted largely as dialkyl or diaryl phosphate metabolites (DAPs) (Lynn *et al.* 1981; Sasaki *et al.* 1984). Consequently, urinary DAP concentrations can be used as biomarkers of human exposure to TAPs. However, DAPs determined in urine reflects an integrated exposure comprising different sources and pathways, and knowledge on concentrations of TAPs in air, dust and food is necessary for identifying major contributors to exposure.

House dust has been reported as an important source of exposure to other FRs, such as PBDEs (Allen *et al.* 2007; Harrad *et al.* 2010), and it is likely that this also applies for TAPs (Hoffman *et al.* 2014). A common approach to assess exposure from the indoor environment is to estimate ingestion, dermal absorption and inhalation of chemicals based on measurements in dust and/or air (Cequier *et al.* 2014a; Van den Eede *et al.* 2011). Although the World Human Organisation has given estimated daily dietary intakes for some TAPs based on studies from 1990s and earlier (WHO 1991a; WHO 1991b; WHO 1998). Data on exposure to TAPs through the diet are limited.

In the present study, a previously developed method based on ultra-performance liquid chromatography coupled to a time-of-flight mass spectrometer (UPLC-TOF-MS) (Cequier *et al.* 2014b) was used to determine di-*n*-butyl phosphate (DNBP), diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and bis(2-butoxyethyl) phosphate (BBOEP) in urine from a Norwegian mother-child cohort. Urine collected from mothers covering a period of 24 hours was analysed to assess day variability of DAPs. To investigate the importance of the indoor environment for exposure to TAPs, urinary DAP concentrations for mothers and children were compared to the TAP levels measured in dust and air collected from the living rooms of the participants' residences. Likewise, food intake of mothers and children was assessed through a detailed 24 hours recall to identify foodstuffs that contribute significantly to exposure to TAPs.

2. Materials and methods

2.1. Recruitment of participants and sample collection

A mother-child cohort comprising 48 mothers and their offspring (56 children in total) was recruited through primary schools in the greater Oslo area. Invitations were

sent to 3500 families and 80 positively replied. Of these, 50 mother-child pairs were randomly selected, however two participants dropped out after the study had started. All parents provided a written informed consent and the study was approved by the Regional Committee for Medical Research Ethics. The mothers were between 32 and 56 years old (median 41 years) and the children (both genders) were between 6 and 12 years old (median 10 years).

The sampling campaign started in January and lasted until mid May 2012. Winter and early spring were chosen because exposure from the indoor environment was expected to be higher when doors and windows are not frequently opened. Four houses were visited twice during one week. The first day was dedicated to explain in detail the aims of the project, provide bottles (500 mL) of high density polyethylene for urine collection (ten for each mother and three for each child), deploy pumps in the living rooms for 24 hours of air collection and deliver questionnaires related to indoor parameters (n=48). Detailed information on the indoor questionnaires has been published elsewhere (Cequier *et al.* 2014a). The mothers collected 2 to 8 urine samples over a period of 24 hours, and the children collected 2 to 3 samples (at least morning and afternoon). Collection times between minutes 01-30 were assigned to the previous full hour and between minutes 31-59 to the following one. The mothers and children were interviewed both days to assess their dietary intakes through a 24-hours food recall. The amount in grams of food or liquid consumed was categorised in the following groups: bread, cereal based products, vegetables, fruit, meat, fish, milk, cheese, butter and oils, drinks and others. Moreover, on the second visit, dust was collected from the living room floors. Details about the sampling procedures have been published elsewhere (Cequier *et al.* 2014a). In brief, air from living rooms was sampled for 24 hours using a Leland Legacy pump (SKC Limited, Dorset, U.K.) at a flow rate of 12 L/min (total volume of air was 17 m^3). A holder containing a quartz filter and a polyurethane foam (PUF) plug were used to collect the airborne TAPs. Dust was collected from the floor in the living rooms using forensic filters (KTM Krim. Teknisk Materiel AB, Bålsta, Sweden) coupled to a GM80 vacuum cleaner (Nilfisk, Morgan-town, WV, U.S.A.). After sampling, dust, air and urine were stored at -20°C until analysis. All together, around 450 samples of air, dust and urine, 48 questionnaires and 104 24-hours food recalls were collected.

2.2. Analysis of urine samples

The analytical method for determination of DAPs has previously been published (Cequier *et al.* 2014b). Briefly, the 356 urine samples were centrifuged and 5 µL analysed directly by UPLC-TOF-MS for DNBP, DPHP, BDCIPP and BBOEP. The chromatography was performed on an Acquity® C18 BEH column (50 mm x 2.1 mm x 1.7 µm) from Waters (Milford, MA, U.S.A.) using a gradient of acetonitrile (5 mM ammonium hydroxide/ammonium formate) from 2% to 40% in three minutes. Identification and quantification of the DAPs was carried out using a Xevo® G2-S QTof from Waters. Levels of DAPs in the blanks were lower than the method limit of detection (MLD). Possible matrix effects were overcome using deuterated internal standards (IS). Accuracies were assessed comparing the slopes of the calibration curves prepared in synthetic and human

urine to the standards of the calibration curve prepared in water. Accuracies ranged from 86 to 124% (RSD 1-8%). As a quality control of the analysis, calibration solutions at several levels were injected every ten samples (n=3-8), the RSDs of the concentrations were <11% for all DAPs except for DNBP (<21%). The non-normalised MLDs were determined from real urine samples and ranged from 0.1 to 0.6 ng/mL. Specific gravity (SG) was measured for all samples and ranged from 1.003 to 1.032 (median = 1.013) in mothers and from 1.009 to 1.032 (median = 1.026) in children and all the individual concentrations of DAPs and MLDs were normalised to SG (Cooper *et al.* 2011).

In order to study whether there were significant differences in DAP concentrations in urine sampled at different time points during the 24 hours period, the data were stratified in several time frames (TF). The grouping was based on visual inspection of the hours with lowest and highest concentrations. The chosen TF for statistical analysis for DPHP were TF1 (17-24h), TF2 (3-8h), TF3 (9-14h) and TF4 (15-18h) and for BDCIPP were TF1 (17-3h), TF2 (6-15h) and TF3 (16-18h) (Fig. S1).

2.3. Analysis of air and dust samples

The TAPs concentrations in air and dust from the households of the cohort have been published elsewhere (Cequier *et al.* 2014a), and an overview is given in Fig. S2. Detailed information about the analytical method has been published elsewhere (Van den Eede *et al.* 2012). Coarse particles and hair in the floor dust samples were removed, and 40 to 75 mg of non-sieved dust were extracted by sonication 3 times using 2 mL of hexane/acetone (3:1; v/v). The same procedure was applied to the air samples (quartz filter and PUFs), but the extraction was carried out using 8 mL of hexane/acetone (3:1; v/v). Clean-up was performed on Florisil® (500 mg, 3 mL, Supelco, Bellefonte, PA, U.S.A.). After eluting non-polar FRs with 10 mL of hexane, TAPs were eluted with 10 mL of ethyl acetate. The eluate was evaporated and the residue reconstituted in 100 µL of *iso*-octane/ethyl acetate (1:1; v/v). TAPs were determined on a GC-EI/MS (Agilent Technologies, Santa Clara, CA, U.S.A.) using a HT-8 column (25 m x 0.22 mm x 0.25 µm; SGE Analytical Science Pty. Ltd., Victoria, Australia). As part of our quality control, we analysed the TAPs in a reference dust sample (SRM2585, National Institute of Standards and Technology, MD, U.S.A.) obtaining accuracies in the range 84 to 128% (mean 103%; RSD 11%) with respect to average results reported by others (Bergh *et al.* 2012; Van den Eede *et al.* 2011). The only exception was TBOEP accuracy of which was 56% of that reported by Bergh *et al*. (2012). Recoveries of IS of TAPs (except TBOEP-d6) from air and dust samples ranged from 75% to 95% (RSD 12%) and from 74% to 100% (RSD 13%), respectively. The recoveries of TBOEP-d6 in air and dust were 172% (RSD 15%) and 268% (RSD 27%), respectively. The enhanced response of TBOEP might explain the large difference in accuracy for SRM2585 dust sample between our result and that of Bergh *et al*. since they used deuterated tributyl phosphate as internal standard for the quantification of TBOEP. MLDs for TAPs in air and dust samples ranged from 18 to 44 pg/m^3 and from 2.7 to 64 ng/g.

2.4. Statistical analysis

Analytes with detection frequencies ≥20% in urine (equivalent to more than 20 children spot urine samples) were subjected to statistical analysis using SPSS v.20 (Chicago, IL, U.S.A.). Non-detects were assigned a concentration of half the MLD. The median urinary concentrations for each participant were not normally distributed, and Spearman rank correlation was employed to calculate associations between metabolites in urine, and between metabolites in urine and parent compounds in food, air and dust. Similarly, only parent compounds with detection frequencies ≥40% (minimum of 19 samples) in air and dust were considered in the statistics. Correlations were regarded statistically significant at $p<0.05$. A nonparametric paired sample test (Wilcoxon signed-rank test) was used to investigate differences in median concentrations of DAPs for different time frames within the 24 hours of urine collection. A stepwise multiple linear regression analysis considering all food categories was performed to explore the associations between the log transformed individual concentrations of DAPs with the amount of food ingested by mothers and children. The stepwise method iterates multiple regressions with the different variables, each time eliminating the weakest correlated. At the end of the process, only the significant variables remain in the model. After log transformation of the DAP concentrations the residuals were randomly distributed, the independent variables were not correlated among them (absence of collinearity) and *p*-values <0.05 were considered statistically significant.

3. Results and discussion

3.1. DAP concentrations in urine

This is the hitherto most comprehensive investigation of the occurrence of DAPs in urine comprising 356 urine samples collected from 96 participants.

All urine DAP concentrations were normalised according to Cooper *et al*., 2011 using SG to compensate for varying dilution of the spot urines. Concentrations of DAPs in urine from the mothers and children are summarised in Table 1.

Table 1
Concentration (ng/mL) and frequency of detection (%) of DAPs in urine from mothers and children.

	DNBP	DPHP	BDCIPP	BBOEP
54 children (n=112)				
Median	< MLD	1.0	0.23	< MLD
Mean	0.11	3.2	0.33	0.18
Maximum	0.87	129	3.2	1.0
[a]MLD	0.11	0.03	0.11	0.17
% detected	21	99	85	32
48 mothers (n=244)				
Median	< MLD	0.91	< MLD	< MLD
Mean	< MLD	1.7	0.36	< MLD
Maximum	0.56	60	3.3	< MLD
[a]MLD	0.22	0.06	0.22	0.33
% detected	3	98	43	0

[a]MLDs differ between mother and children due to the different SG median value of 1.026 and 1.013 used to normalise the concentration for children and mothers, respectively.

The occurrence of DAPs was in general higher in children than in mothers as indicated by the higher frequency of detection. The maximum concentrations in this study for DPHP in one mother and one child (60 and 129 ng/mL, respectively) are considerably higher than those reported from previous studies. Samples with high concentrations were re-analysed using QTOF. Re-analysis confirmed the elevated concentrations measured initially (variation of DPHP from mother and child along the day is illustrated in Fig. S3). A comparison of published data for DPHP and BDCIPP in adults is given in Table 2.

A Belgian study (n=59) reported higher non-normalised median concentration for DPHP (0.82 ng/mL) (Van den Eede *et al.* 2013). Studies conducted in the U.S.A. and published in 2011 (n=9) and 2014 (n=39) showed also higher non-normalised geometric mean (GM) concentrations of BDCIPP (0.15 and 1.3 ng/mL) and DPHP (1.1 and 1.9 ng/mL), respectively (Cooper *et al.* 2011; Hoffman *et al.* 2014). Nevertheless, another study from U.S.A. (n=61) (Meeker *et al.* 2013) reported a SG normalised GM for DPHP (0.31 ng/mL) and BDCIPP (0.13 ng/mL) which is lower than the SG normalised GM concentrations of this work (0.85 and <0.22 ng/mL, respectively). Higher DPHP levels were also reported in a study conducted in Germany (n=19) (non-normalised median was 1.3 ng/mL) (Reemtsma *et al.* 2011), although another study from Germany (n=25) could not report levels above MLD (0.5 ng/mL) (Schindler *et al.* 2009). As can be seen from Table 2, the levels are highly dependent on whether a dilution correction is applied or not. In general, it is recommended that urine concentrations are reported normalised with respect to SG (or creatinine content) to avoid biases from varying dilutions and obtain comparable concentrations (Cone *et al.* 2009).

3.2. Diurnal variation of DAPs in urine

The diurnal excretion of DAPs was studied for the mothers by plotting the median urine concentrations against a rounded collection time. DNBP and BBOEP averaged ½ MLD due to low frequency of detection. Concentrations of BDCIPP were quite stable during the whole collection period while DPHP showed a much greater variability during 24 hours (Fig. 1).

Table 2
Comparison of urinary DAP concentrations (ng/mL) expressed as normalised and non-normalised medians and geometric means in studies conducted in several countries.

Country	DPHP	BDCIPP	REF
Norway (n=244)	[a]0.91/ [b]0.51/ [c]0.85/ [d]0.41	[a,c]<0.22/ [b,d]<0.12	This study
U.S.A. (n=39)	[d]1.9	[d]1.3	(Hoffman *et al.* 2014)
Belgium (n=59)	[b]0.82		(Van den Eede *et al.* 2013)
U.S.A. (n=61)	[a]0.27/ [c]0.31	[a]0.12 /[c]0.13	(Meeker *et al.* 2013)
U.S.A. (n=9)	[a]1.8/ [b]0.80/ [c]3.0/ [d]1.1	[a]0.37/ [b]0.08/ [c]0.41/ [d]0.15	(Cooper *et al.* 2011)
Germany (n=19)	[b]1.3		(Reemtsma *et al.* 2011)
Germany (n=25)	[b]<0.5		(Schindler *et al.* 2009)

[a]Median normalised to SG; [b]Median non-normalised to SG; [c]Geometric mean normalised to SG; [d]Geometric mean non-normalised to SG.

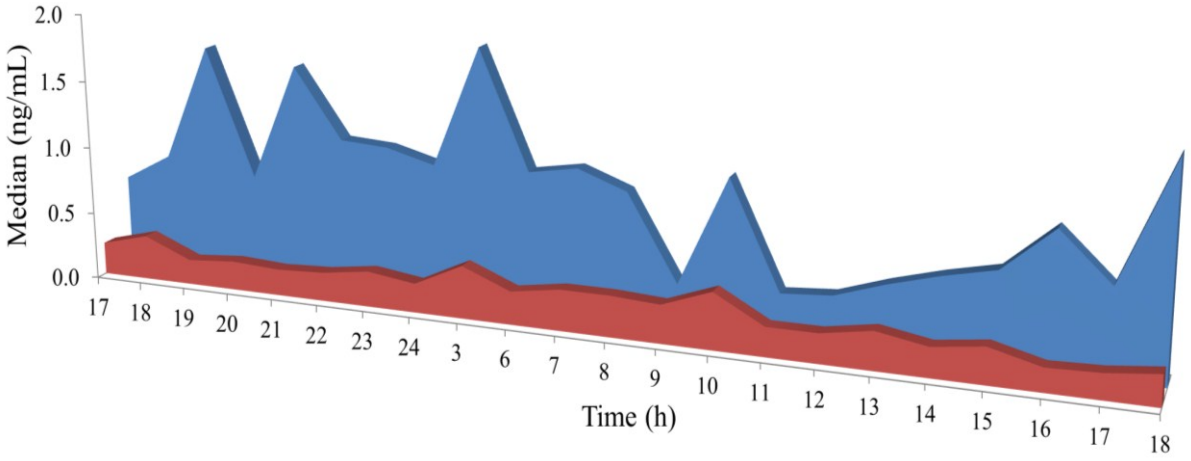


Fig. 1. Diurnal variation of DPHP (blue) and BDCIPP (red) in urine from all mothers.

We choose four and three time frames (TF) for statistical analysis for DPHP and BDCIPP, respectively (Fig. S1). The Wilcoxon signed-rank test indicated that the concentration of DPHP in urine collected in the TF1 (17-24h) and TF2 (3-8h) were the highest and that TF3 (9-14h) was significantly lower than the other periods (p<0.014) (Table S1). However, as there is lack of information on the half-life of DPHP in humans, it is difficult to identify the exposure scenario responsible for this change in urinary metabolite levels. Nevertheless, since DPHP was detected in all TFs above MLD, the dust from the living rooms is likely to be a source of exposure for adults. For BDCIPP, all TFs of were statistically different (p<0.01) with TF2 being significantly higher than the others (Table S2). Interestingly, the period with highest concentrations of BDCIPP (TF2; 6-15h) overlapped with the lowest period of DPHP (TF3; 9-14h). This suggests that the exposures and/or the half-lifes of the precursors are different. Morning void spot samples of urine have often been used to assess the exposure to TAPs (Hoffman *et al.* 2014; Kissel *et al.* 2004). For the mothers, we investigated the linear fit of the mean concentrations in all their 24 hours urine samples plotted against concentrations in their first morning voids. The closer the slope and the correlation coefficient are to 1.0, the better the spot urine reflects the 24 hours exposure.

In our study, the first morning void (6 to 8 a.m.) showed a good linear fit for DPHP (R^2=0.79; slope=0.73) and BDCIPP (R^2=0.91; slope=0.73) (Fig. 2). This demonstrates that the morning void reflects reasonably well the average excretion of DPHP and BDCIPP during 24 hours.

Urine from children was only collected at two time points: morning (6-11) and afternoon (12-19). As half-lives of TAPs longer than 10 hours are unlikely, we expect that the morning urine reflects the exposure from the household environment. DPHP was the only DAP that showed significantly higher concentrations in the morning than in the afternoon (p<0.01), while there was no statistical difference found for DNBP, BDCIPP and BBOEP (p>0.197) (Table S3). This confirms that the living rooms are a likely source of exposure to TPHP for children.

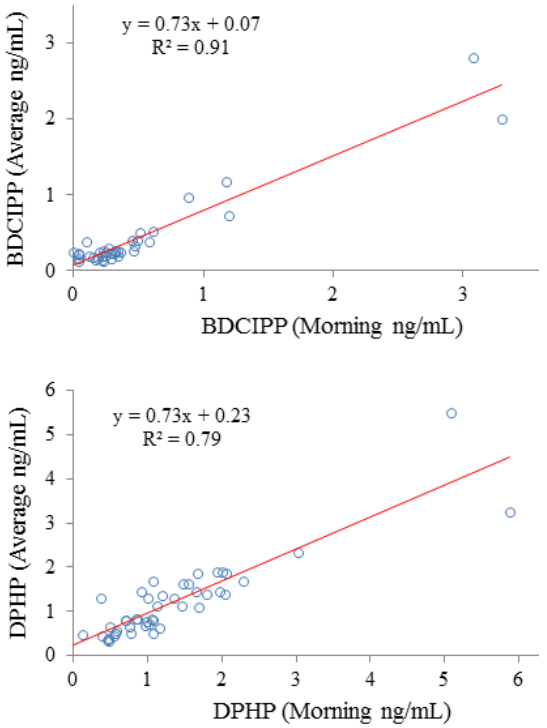


Fig. 2. Linear fit of the morning urine and the average concentrations for BDCIPP and DPHP.

3.3. Correlations between DAPs in urine and the parent TAPs in air, dust and food

A bivariate analysis (Spearman rank correlation) was performed to explore the correlations among the median concentrations of DAPs in urine (Table 3), and the correlations between DAPs in urine and their precursors in air, dust from the households (Table 4) and food ingested during the sampling.

Table 3
Spearman rank correlations between concentrations of DAPs in urine.

	DNBP	DPHP	BDCIPP
54 Children (n = 112)			
DPHP	**0.340***		
BDCIPP	0.120	**0.295***	
BBOEP	0.134	0.268	0.081
[a] 48 Mothers (n = 244)			
BDCIPP		**0.361***	

*Correlation was significant at the 0.05 level (two tailed); [a] Statistics of DNBP and BBOEP in mothers are excluded due to the low frequency of detection.

For both, mothers and children urinary concentrations of DPHP were significantly correlated with BDCIPP (Table 3) and in children also with DNBP. DNBP is mainly used as plasticiser and lubricant (van der Veen *et al.* 2012), hence the association of DPHP with DNBP indicates that both chemicals are used in the same products. The observation of the same correlations for children and mothers suggests the same sources of exposure, i.e., the indoor environment and less likely the food. Food intake during the 24 h sampling period was extracted from the 24-h recall for mothers and children. The bivariate analysis revealed only occasionally correlations between the overall median urinary concentrations of DPHP and BDCIPP for mothers and children with the type and amount of food ingested. Furthermore, none of the correlations were significant in the linear regression model (data not shown). It seems therefore that food does not play a major role in the exposure to TAPs.

This is supported by a rough estimation of the dietary contribution of TPHP exposure in adults using the highest estimated dietary intake of 1.6 ng/kg b.w./day reported by WHO (WHO 1991a), the median body weight from our study (67 kg) and the excretion of 1.6 L of urine per day (Daudon *et al.* 2005). Assuming 100% absorption and metabolism, this would contribute to only 10% of the median urinary DPHP concentration found in our study.

Concentrations of TAPs in air and DAPs in urine from the mothers were not correlated. However, BDCIPP correlated strongly ($R=0.533$; $p<0.01$) with its precursor TDCIPP in dust, pointing at dust from the living rooms as a significant source of exposure. This association was also reported

Table 4
Spearman rank correlations coefficients for the relationship between median and mean concentrations of DAPs in urine for mothers and children, respectively, and the parent compounds in air and dust (n=48).

	Children				Mothers			
				AIR				
	TNBP	TBOEP	TPHP	TDCIPP	TNBP	TBOEP	TPHP	TDCIPP
BBOEP	.006	**.414****	.139	-.060				
BDCIPP	-.032	.062	.164	**.414****	-.048	-.096	-.006	.188
DPHP	.052	.177	**.295***	**.433****	.200	.154	-.033	-.066
DNBP	-.178	-.122	-.070	.113				
				DUST				
	TNBP	TBOEP	TPHP	TDCIPP	TNBP	TBOEP	TPHP	TDCIPP
BBOEP	-.209	**.442****	.185	-.059				
BDCIPP	.103	-.042	.085	**.491****	.154	-.064	-.063	**.533****
DPHP	-.035	-.016	**.289***	.178	.041	.226	.145	.072
DNBP	-.048	-.100	-.030	-.054				

$^{*/**}$Correlation was significant at the level 0.05/0.01 (two tailed)

by Meeker *et al*. in a study conducted on men from the U.S.A. (Meeker *et al.* 2013). In contrast, DPHP the most abundant metabolite in urine from mothers and children, was not correlated to TPHP in dust when median concentrations were used. This is in agreement with earlier observations suggesting that house dust is not a primary source of exposure to TPHP among adults (Meeker *et al.* 2013). However, when median concentration from the urine collected in the TF1 (17-24), was used in the statistics a weak correlation was seen for DPHP in urine and TPHP in dust (R=0.287; p=0.048). A stronger correlation (R=0.339; p=0.019) appeared when we used TF2 (1-8h) corresponding to night time and representing most likely exposure experienced in the household (Table S4). These associations show an influence of the living rooms on the exposure to TPHP, although other significant sources cannot be excluded.

For the children, several significant associations of DAP excretion were observed, both with air and dust. BBOEP and BDCIPP were moderately to highly correlated with their respective parent compounds in the living rooms ($0.41<R<0.49$; $p<0.004$), while weaker though significant correlations were observed for DPHP (R=0.29; $p<0.05$). These findings suggest that the indoor environment, represented by air and dust from the living rooms, has a stronger influence on children than on mothers.

4. Conclusions

This study has shown that exposure of children to TDCIPP, TBOEP and TPHP is associated with the residential environment as demonstrated by the correlations of the concentrations found in indoor dust and air and their respective metabolites in urine. Likewise, metabolites of BDCIPP from mothers' urine were correlated with TDCIPP from indoor dust.

The most abundant DAPs detected in the study were DPHP and BDCIPP, but both metabolites were found more frequently in children than in mothers which suggest a higher exposure of children in the households. To confirm this finding, more

biomonitoring studies are needed including younger children who are expected to have a higher contact with floor dust. This study also showed that morning urine from mothers reflected well enough the mean 24 hours excretion of DPHP and DDCIPP.

Acknowledgments

This study is funded by the European Commission, 7th Framework Programme under the EU Marie Curie Initial Training Network INFLAME (GA No. 264600) and the Research Council of Norway. Participants are greatly acknowledged for their willingness to participate in this biomonitoring study.

Supplementary data

Supplementary data associated with this article can be found in the on-line version.

References

Allen, J.G.; McClean, M.D.; Stapleton, H.M.; Nelson, J.W.; Webster, T.F. Environ Sci Technol 2007; 41: 4574-4579

Bergh, C.; Luongo, G.; Wise, S.; Östman, C. Anal Bioanal Chem 2012; 402: 51-59

Bergh, C.; Torgrip, R.; Emenius, G.; Östman, C. Indoor Air 2011; 21: 67-76

Campone, L.; Piccinelli, A.L.; Östman, C.; Rastrelli, L. Anal Bioanal Chem 2010; 397: 799-806

Cequier, E.; Ionas, A.C.; Covaci, A.; Marcé, R.M.; Becher, G.; Thomsen, C. Environ Sci Technol 2014a; 48: 6827-6835

Cequier, E.; Marcé, R.M.; Becher, G.; Thomsen, C. Anal Chim Acta 2014b; doi: 10.1016/j.aca.2014.06.026

Cone, E.J.; Caplan, Y.H.; Moser, F.; Robert, T.; Shelby, M.K.; Black, D.L. J Anal Toxicol 2009; 33: 1-7

Cooper, E.M.; Covaci, A.; van Nuijs, A.L.N.; Webster, T.F.; Stapleton, H.M. Anal Bioanal Chem 2011; 401: 2123-2132

Daudon, M.; Hennequin, C.; Boujelben, G.; Lacour, B.; Jungers, P. Kidney Int 2005; 67: 1934-1943

Dodson, R.E.; Perovich, L.J.; Covaci, A.; Van den Eede, N.; Ionas, A.C.; Dirtu, A.C.; Brody, J.G.; Rudel, R.A. Environ Sci Technol 2012; 46: 13056-13066

Eggen, T.; Heimstad, E.S.; Stuanes, A.O.; Norli, H.R. Environ Sci Pollut Res 2013; 20: 4520-4531

European Commission. http://echa.europa.-eu/documents/10162/f42be21b-33a3-4063-ad4d-2b0f937e41b4. 2006 (accessed May 2014)

Gold, M.; Blum, A.; Ames, B.N. Science 1978; 200: 785-787

Harrad, S.; Goosey, E.; Desborough, J.; Abdallah, M.A.E.; Roosens, L.; Covaci, A. Environ Sci Technol 2010; 44: 4198-4202

Hoffman, K.; Daniels, J.L.; Stapleton, H.M. Environ Int 2014; 63: 169-172

Kim, J.W.; Isobe, T.; Chang, K.H.; Amano, A.; Maneja, R.H.; Zamora, P.B.; Siringan, F.P.; Tanabe, S. Environ Pollut 2011; 159: 3653-3659

Kissel, J.C.; Curl, C.L.; Kedan, G.; Lu, C.; Griffith, W.; Barr, D.B.; Needham, L.L.; Fenske, R.A. J Expo Anal Environ Epid 2004; 15: 164-171

Lynn, R.K.; Wong, K.; Garvie-Gould, C.; Kennish, J.M. Drug Metab Dispos 1981; 9: 434-441

Marklund, A.; Andersson, B.; Haglund, P. Chemosphere. 2003; 53: 1137-1146

Meeker, J.D.; Cooper, E.M.; Stapleton, H.M.; Hauser, R. Environ Health Perspect 2013; 121: 580-585

Reemtsma, T.; Lingott, J.; Roegler, S. Sci Total Environ 2011; 409: 1990-1993

Sasaki, K.; Suzuki, T.; Takeda, M.; Uchiyama, M. Bul Environ Contam Toxicol 1984; 33: 281-288

Schindler, B.K.; Förster, K.; Angerer, J. J Chromatogr B 2009; 877: 375-381

Stapleton, H.M.; Klosterhaus, S.; Eagle, S.; Fuh, J.; Meeker, J.D.; Blum, A.; Webster, T.F. Environ Sci Technol 2009; 43: 7490-7495

Van den Eede, N.; Dirtu, A.C.; Ali, N.; Neels, H.; Covaci, A. Talanta 2012; 89: 292-300

Van den Eede, N.; Dirtu, A.C.; Neels, H.; Covaci, A. Environ Int 2011; 37: 454-461

Van den Eede, N.; Neels, H.; Jorens, P.G.; Covaci, A. J Chromatogr A 2013; 1303: 48-53

van der Veen, I.; de Boer, J. Chemosphere 2012; 88: 1119-1153

WHO. Environmental Health Criteria 111. Geneve, Switzerland. 1991a

WHO. Environmental Health Criteria 112. Geneva, Switzerland. 1991b

WHO. Environmental Health Criteria 209. Geneva, Switzerland. 1998

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

Supporting Information

2. Materials and methods

2.2. Analysis of air and dust samples

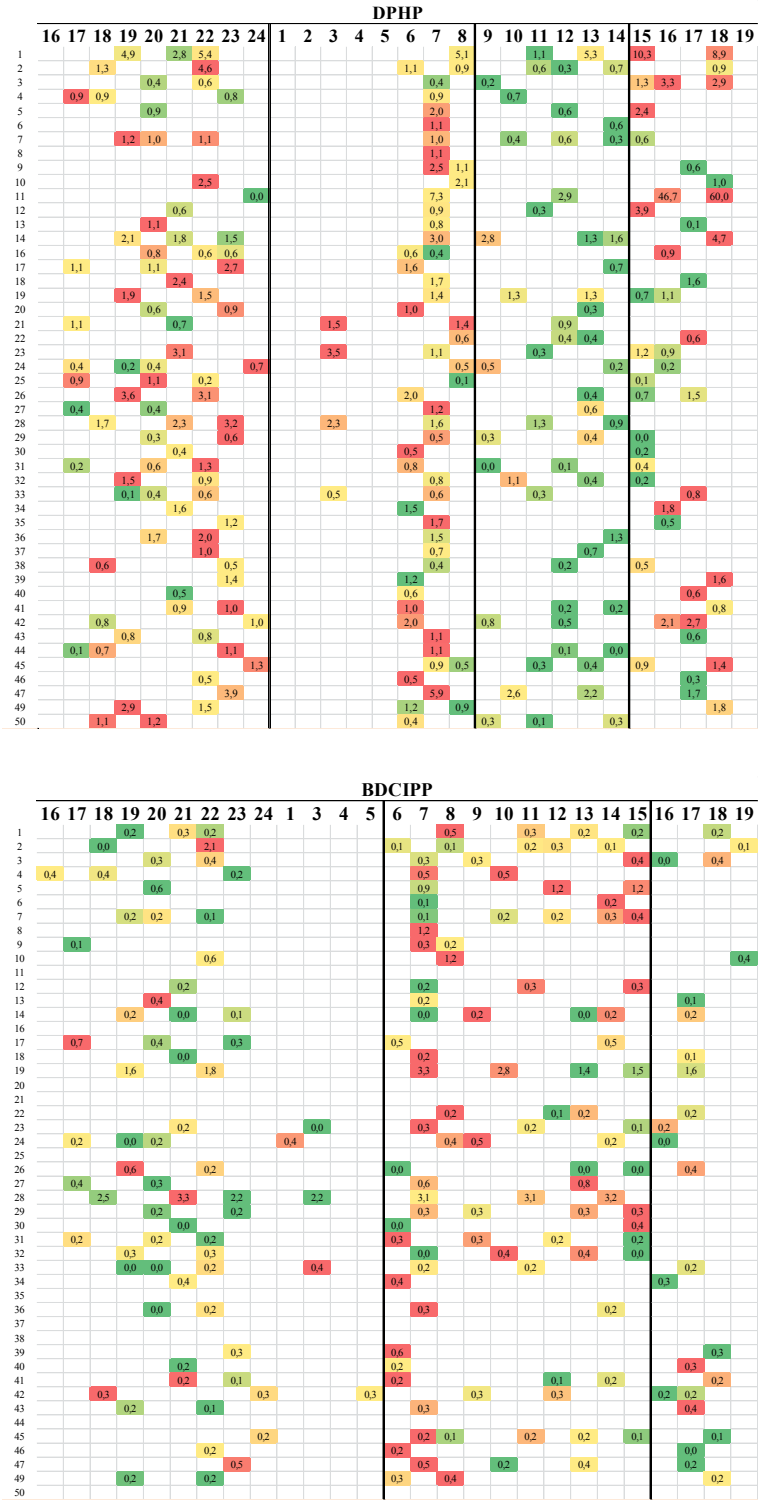


Fig. S1. Collection of 24 hours urine from the mothers (n=48). Red indicates highest urine concentration for each participant and green lowest. Vertical lines show selected time frames. Upper figure is DPHP and lower figure is BDCIPP.

2.3. Analysis of air and dust samples

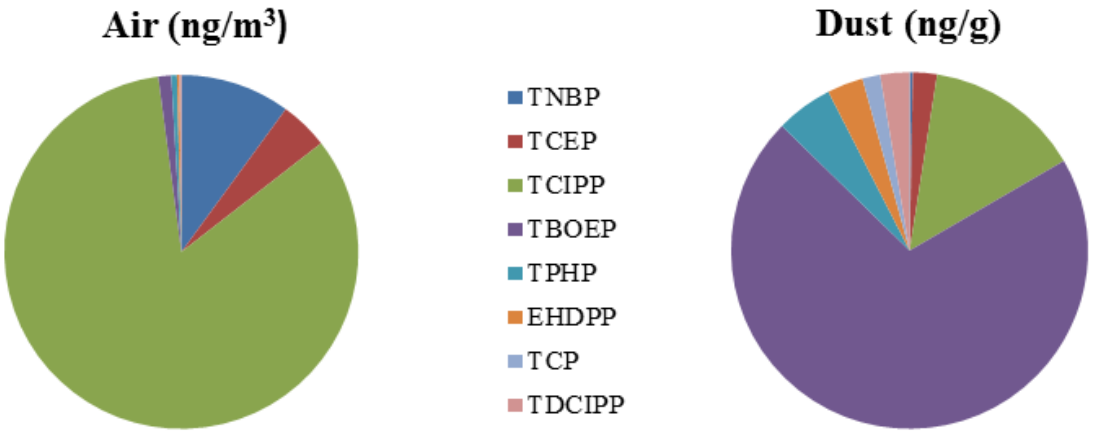


Fig. S2. Pie plot showing the concentration of PFRs in air (ng/m^3) and dust (ng/g) from the living rooms of the participants' households.

3. Results and discussion

3.1. DAP concentrations in urine

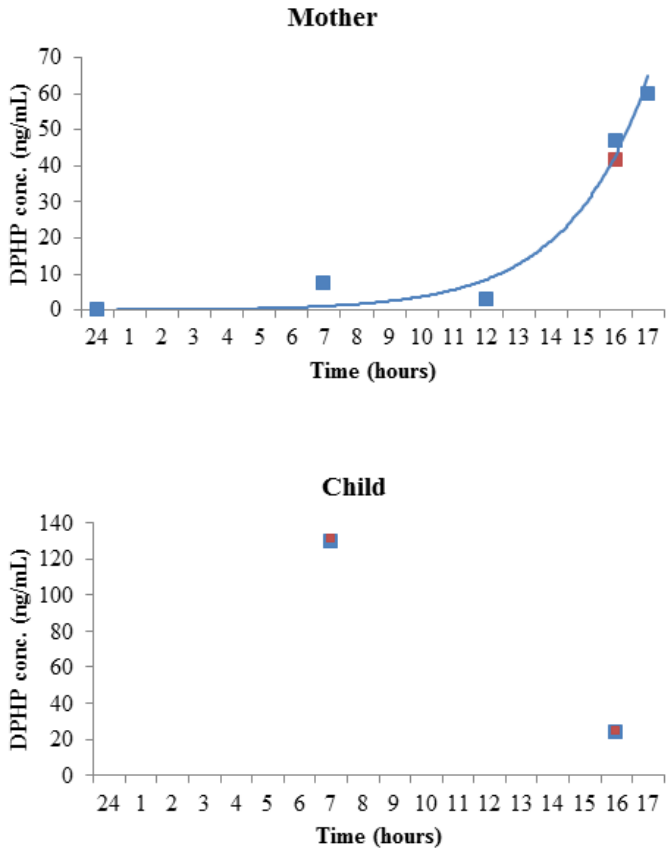


Fig. S3. Day variation of the highest normalised concentrations of DPHP for the mother and the child. Red squares indicate concentrations from the re-analysis of the urine sample.

3.2. Diurnal variation of DAPs in urine

Table S1
Wilcoxon signed-rank test of DPHP samples from mothers collected in different time periods within 24 hours.

Ranks

		N	Mean Rank	Sum of Ranks
TF2 - TF1	Negative Ranks	21[a]	22,45	471,50
	Positive Ranks	25[b]	24,38	609,50
	Ties	2[c]		
	Total	48		
TF3 - TF1	Negative Ranks	40[d]	22,68	907,00
	Positive Ranks	4[e]	20,75	83,00
	Ties	4[f]		
	Total	48		
TF4 - TF1	Negative Ranks	31[g]	21,94	680,00
	Positive Ranks	14[h]	25,36	355,00
	Ties	3[i]		
	Total	48		
TF3 - TF2	Negative Ranks	46[j]	23,50	1081,00
	Positive Ranks	0[k]	,00	,00
	Ties	2[l]		
	Total	48		
TF4 - TF2	Negative Ranks	31[m]	23,18	718,50
	Positive Ranks	14[n]	22,61	316,50
	Ties	3[o]		
	Total	48		
TF4 - TF3	Negative Ranks	15[p]	21,00	315,00
	Positive Ranks	31[q]	24,71	766,00
	Ties	2[r]		
	Total	48		

a. TF2 < TF1
b. TF2 > TF1
c. TF2 = TF1
d. TF3 < TF1
e. TF3 > TF1
f. TF3 = TF1
g. TF4 < TF1
h. TF4 > TF1
i. TF4 = TF1
j. TF3 < TF2
k. TF3 > TF2
l. TF3 = TF2
m. TF4 < TF2
n. TF4 > TF2
o. TF4 = TF2
p. TF4 < TF3
q. TF4 > TF3
r. TF4 = TF3

Test Statistics[a]

	TF2 - TF1	TF3 - TF1	TF4 - TF1	TF3 - TF2	TF4 - TF2	TF4 - TF3
Z	-,755[b]	-4,811[c]	-1,836[c]	-5,909[c]	-2,271[c]	-2,467[b]
Asymp. Sig. (2-tailed)	,450	,000	,066	,000	,023	,014

a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.
c. Based on positive ranks.

Table S2
Wilcoxon signed-rank test of BDCIPP samples from mothers collected in different time frames (TFs) within 24 hours.

Ranks

		N	Mean Rank	Sum of Ranks
TF2 - TF1	Negative Ranks	5[a]	15,10	75,50
	Positive Ranks	23[b]	14,37	330,50
	Ties	19[c]		
	Total	47		
TF3 - TF1	Negative Ranks	23[d]	17,11	393,50
	Positive Ranks	7[e]	10,21	71,50
	Ties	17[f]		
	Total	47		
TF3 - TF2	Negative Ranks	29[g]	17,88	518,50
	Positive Ranks	4[h]	10,63	42,50
	Ties	14[i]		
	Total	47		

a. TF2 < TF1
b. TF2 > TF1
c. TF2 = TF1
d. TF3 < TF1
e. TF3 > TF1
f. TF3 = TF1
g. TF3 < TF2
h. TF3 > TF2
i. TF3 = TF2

Test Statistics[a]

	TF2 - TF1	TF3 - TF1	TF3 - TF2
Z	-2,931[b]	-3,346[c]	-4,278[c]
Asymp. Sig. (2-tailed)	,003	,001	,000

a. Wilcoxon Signed Ranks Test
b. Based on negative ranks.
c. Based on positive ranks.

Table S3
Wilcoxon signed-rank test of DAP samples from children collected in the morning and afternoon within 24 hours.

Ranks

		N	Mean Rank	Sum of Ranks
TF2 - DPHP - TF1 - DPHP	Negative Ranks	34[a]	26,06	886,00
	Positive Ranks	15[b]	22,60	339,00
	Ties	5[c]		
	Total	54		
TF2 - BDCIPP - TF1 - BDCIPP	Negative Ranks	19[d]	19,50	370,50
	Positive Ranks	15[e]	14,97	224,50
	Ties	20[f]		
	Total	54		
TF2 - BBOEP - TF1 - BBOEP	Negative Ranks	18[g]	19,08	343,50
	Positive Ranks	15[h]	14,50	217,50
	Ties	21[i]		
	Total	54		
TF2 - DNBP - TF1 - DNBP	Negative Ranks	12[j]	12,50	150,00
	Positive Ranks	13[k]	13,46	175,00
	Ties	0[l]		
	Total	25		

a. TF2 - DPHP < TF1 - DPHP
b. TF2 - DPHP > TF1 - DPHP
c. TF2 - DPHP = TF1 - DPHP
d. TF2 - BDCIPP < TF1 - BDCIPP
e. TF2 - BDCIPP > TF1 - BDCIPP
f. TF2 - BDCIPP = TF1 - BDCIPP
g. TF2 - BBOEP < TF1 - BBOEP
h. TF2 - BBOEP > TF1 - BBOEP
i. TF2 - BBOEP = TF1 - BBOEP
j. TF2 - DNBP < TF1 - DNBP
k. TF2 - DNBP > TF1 - DNBP
l. TF2 - DNBP = TF1 - DNBP

Test Statistics[a]

	TF2 - DPHP - TF1 - DPHP	TF2 - BDCIPP - TF1 - BDCIPP	TF2 - BBOEP - TF1 - BBOEP	TF2 - DNBP - TF1 - DNBP
Z	-2,723[b]	-1,291[b]	-1,127[b]	-,336[c]
Asymp. Sig. (2-tailed)	,006	,197	,260	,737

a. Wilcoxon Signed Ranks Test
b. Based on positive ranks.
c. Based on negative ranks.

3.3. Correlations between DAPs in urine and the parent TAPs in in air, dust and food

Table S4
Spearman rank correlation between DPHP in urine from women collected in two time frames (TF) and household dust (n=48).

		TNBP	TBOEP	TPHP	TDCIPP
TF1 (17-24h)	R	.150	-.010	**.287***	-.159
	p	.308	.944	.048	.281
TF2 (3-8h)	R	.093	.187	**.339***	.123
	p	.529	.204	.019	.406

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

3.4.3. Discussion of the Results

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

The biomonitoring of PFRs metabolites in urine was successfully performed in samples from mothers and their children. The developed method for the determination of four DAPs (DNBP, DPHP, BDCIPP and BBOEP) provides advantages with respect to previous methods. The direct injection of urine avoids background contamination of the samples during pre-treatment and reduces the analysis time considerably. This is the first study applying columns packed with sub-2 micron particle for the determination of DAPs in human urine [1]. The use of UPLC-TOF allows the determination of DAPs in less than 3 minutes. In terms of speed of analysis, we obtain the fastest and most suitable analytical method for large sample series. The use of high pH (9.2) in the mobile phase, in combination with acetonitrile, instead of methanol, resulted in an increased response for all DAPs, except for BBOEP. The enhancement of the response of the organophosphates using ESI(-) leads to excellent MLQ for DPHP (0.1 ng mL^{-1}) and DNBP (0.4 ng mL^{-1}) (other methods reported MLQ in the range 0.2-1.5 ng mL^{-1} for DPHP and 0.75-3.4 ng mL^{-1} for DNBP) [2-6]. The MLQ for BDCIPP (0.4 ng mL^{-1}) is in the range of the method developed by Van den Eede *et al*. [3] (0.52 ng mL^{-1}) and much higher than the MLQ published by Cooper *et al*. [2] using APCI(-) (0.008 ng mL^{-1}). Finally, the MLQ for BBOEP (0.6 ng mL^{-1}) was also higher than that obtained by Van den Eede *et al*. (0.15 ng mL^{-1}).

The results show that DPHP was the most abundant (~1 ng/mL) DAP followed by BDCIPP (~0.2 ng/mL) in morning urines from mothers and their children. However, the detection frequency of all DAPs was systematically higher in children. The statistical comparison of the sample pairs from mothers and children revealed higher levels of DPHP and BDCIPP in children indicating a higher exposure to PFRs compared to their mothers. When human exposure pathways to PFRs were investigated, we reported for the first time significant correlations between the median urinary DAP concentrations in children and the concentrations of the parent compounds in both air and dust from the living rooms. This finding suggests that the indoor environment from the households is a major contributor to PFR exposure for children, and to a lesser degree for mothers, since the correlations for the mothers were weaker than those from the children. TDCIPP, a mutagenic FR, has been detected in house dust and air, and is correlated with its metabolite in children's urine. Nevertheless, the calculated intake of TDCIPP for children in the worst case scenario was much lower than the RfD cited by Ali *et al*. [7].

The diurnal variability of the two main DAPs was also investigated. In contrast to DPHP, excretion of BDCIPP was practically constant indicating that the exposure to TDCIPP might

take place in several environments, whereas the DPHP exposure is more localised in certain environments. In any case, morning urines correlated well with the total exposure and could therefore be used as spot samples when 24 hours samples are difficult to collect.

References

[1] A. C. Dirtu, N. Van den Eede, G. Malarvannan, A.C. Ionas, A. Covaci, Anal. Bioanal. Chem. 404 (2012) 2555-2581.

[2] E. M. Cooper, A. Covaci, A. L. N. van Nuijs, T. F. Webster, H. M. Stapleton, Anal. Bioanal. Chem. 401 (2011) 2123-2132.

[3] N. Van den Eede, H. Neels, P.G. Jorens, A. Covaci, J. Chromatogr. A 1303 (2013) 48-53.

[4] T. Reemtsma, J. Lingott, S. Roegler, Sci. Total Environ. 409 (2011) 1990-1993.

[5] B. K. Schindler, K. Förster, J. Angerer, J. Chromatogr. B 877 (2009) 375-381.

[6] B. K. Schindler, K. Förster, J. Angerer, Anal. Bioanal. Chem. 395 (2009) 1167-1171.

[7] N. Ali, A.C. Dirtu, N. Van den Eede, E. Goosey, S. Harrad, H. Neels, A. Mannetje, J. Coakley, J. Douwes, A. Covaci, Chemosphere 88 (2012) 1276-1282.

4. Conclusions

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

The main conclusions of the Thesis are summarised below:

1) The content of lipid in the serum affects the SPE and LLE extraction efficiencies of highly lipophilic flame retardants (log K_{ow} > 9), which implies that quantitative recoveries are not achievable.

2) Chromatographic separation of 15 PBDEs (including BDE-209) and 7 emerging FRs can be achieved in less than 15 minutes using short GC columns (15 m x 0.25 mm x 0.15 µm).

3) The ultra-fast analysis of 4 DAPs (<3 minutes), without any sample preparation of the urine, by means of UPLC-TOF is sensitive enough and adequate for high throughput analysis.

4) The characterisation of the levels of 37 FRs in indoor environments allowed us to report the highest concentrations of TBECH in air ever measured before (78 and 47 pg/m^3 in households and schools respectively).

5) Concentrations of FRs in settled and floor dust from the living rooms were not statistically different (n=12).

6) In the pilot study (n=10), dechlorane 602 and Dechlorane 603 were detected in human serum for the first time (mean of 0.69 and 0.29 ng/g l.w., respectively) and Dechlorane Plus was detected for the first time in serum from Europe (mean of 3.2 ng/g l.w.). These emerging FRs were also detected frequently in the serum samples of the study group.

7) Emerging FRs are in the proportion 1:1 with respect to PBDEs in Norwegian serum of the cohort and therefore they should be considered in future biomonitoring studies with especial attention to dechloranes.

8) DPHP and BDCIPP were the most detected PFRs metabolites in children and mothers urine. The children had systematically higher concentrations (medians of 1.0 and 0.23 ng/mL versus 0.91 and <0.22 ng/mL, respectively).

9) DPHP, BBOEP and BDCIPP in children's urine were significantly correlated with their parent compounds in both air and dust from the households (Spearman rank correlations $0.29<R<0.49$).

10) For PFRs, residential environments (air and dust) seem to be important sources of exposure. The major exposure pathways are dermal absorption and ingestion of dust, the diet being a minor contributor in this study.

11) Frequent vacuum cleaning reduces the concentrations of FRs in dust by removing the aged dust, which has been more exposed to FRs.

12) For persistent FRs, diet is postulated as a major source of exposure for the participants of the study.

13) The preliminary risk assessment of the quality of the Norwegian indoor environment for PBDEs points towards indoor air and dust do not pose a threat for human health. However, this assessment cannot be performed for emerging FRs due to the lack of toxicological and epidemiological studies.

Annex I – List of Abbreviations

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

AF	Absorption factor
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photoionisation
BFR	Brominated flame retardant
b.w.	Body weight
COPHES	Consortium to perform human biomonitoring on a European Scale
CPSC	U.S. Consumer Product Safety Commission
DA	Dust adherence
DAP	Dialkyl or diaryl phosphate
DED	Daily exposure dose
DI	Dust ingestion
d.w.	Dry weight
ECD	Electron capture detector
ECNI	Electron capture negative ionisation
ED	Exposure duration
EFRA	European Flame Retardant Association
EFSA	European Food Safety Agency
EI	Electron impact
ESI	Electrospray ionisation
ESM	Electronic supplementary material
FME	Focused microwaved assisted extraction
FR	Flame retardant
GC	Gas chromatography
GCxGC	Comprehensive two-dimensional gas chromatography
GM	Geometric mean
GPC	Gel permeation chromatography
HD-PE	High density polyethylene
HFR	Halogenated flame retardant
HILIC	Hydrophilic liquid interaction chromatography
HL	High level
HPV	High production volume
HR	High resolution
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP	Inductively coupled plasma
IF	Impact factor
IPCS	International Programme on Chemical Safety
IR	Inhalation rate
IS	Internal standard
$K_{dust-air}$	Dust-air partition coefficient
K_{oa}	Octanol-air partition coefficient
K_{ow}	Octanol-water partition coefficient
LC	Liquid chromatography
LD-PE	Low density polyethylene
LL	Low level
LLE	Liquid-liquid extraction
LR	Low resolution
LVI	Large volume injection
l.w.	Lipid weight
MAE	Microwave-assisted extraction

MLD	Method limit of detection
MLQ	Method limit of quantification
MS	Mass spectrometry
MS-MS	Tandem mass spectrometry
MTBE	Methyl-*tert*-butyl ether
NIST	U.S. National Institute of Standards and Technology
PCI	Positive chemical ionisation
PFRs	Organophosphorous-based flame retardants
PLE	Pressurised liquid extraction
PUF	Polyurethane foams
QqQ	Triple quadrupole
REACH	E.U. regulatory framework for Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Oral reference dose
RS	Recovery standard
SA	Skin surface area
SAX	Strong anion exchange
SG	Specific gravity
SI	Supporting Information
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRM	Standard reference material
TOF	Time-of-flight
UNEP	United Nations Environmental Programme
UPLC	Ultra performance liquid chromatography
USEPA	U.S. Environmental Protection Agency
WAX	Weak anion exchange
WHO	World Health Organisation
w.w.	Wet weight

Annex II – List of Publications

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014

1) Determination of emerging halogenated flame retardants and polybrominated diphenyl ethers in serum by gas chromatography mass spectrometry. **Journal of Chromatography A**, 1310 (2013) 126-132, (section 3.3.2).
2) The lipid content in serum affects the extraction efficiencies of highly lipophilic flame retardants. **Environmental Science & Technology Letters**, 1 (2014) 82-86, (section 3.3.1).
3) Occurrence of a broad range of legacy and emerging flame retardants in indoor environments in Norway. **Environmental Science & Technology** 48 (2014) 6827-6835, (section 3.2.1).
4) A high-throughput method for determination of metabolites of organophosphate flame retardants in urine by ultra performance liquid chromatography-high resolution mass spectrometry. **Analytica Chimica Acta** (2014) doi: 10.1016/j.aca.2014.06.026, (section 3.4.1).
5) Human exposure pathways to organophosphate triesters – A biomonitoring study of mother-child pairs. **Environment International** (Submitted 2014), (section 3.4.2).
6) Comparing human exposure to emerging and legacy flame retardants from the indoor environment and diet with concentrations measured in serum. **Environment International** (Submitted 2014), section (3.3.3).

UNIVERSITAT ROVIRA I VIRGILI
HUMAN EXPOSURE PATHWAYS TO FLAME RETARDANTS
Enrique Cequier Manciñeiras
Dipòsit Legal: T 1518-2014