University of Reading

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In vitro bioaccessibility of emerging flame retardants present in indoor dust using simulated human fluids

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Thesis presented for the degree of Doctor in Philosophy

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“Cynics are only idealists with awkwardly high standards”

Friedrich Wilhelm Nietzsche (1844 - 1900)

Declaration

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Aikaterini A. Kademoglou

List of publications

Paper 1
Towards a unified approach for the determination of the bioaccessibility of organic pollutants

Collins*, C.D., Craggs, M., Garcia-Alcega, S., **Kademoglou, K**., Lowe, S., 2015. Environ. Int. 78, 24–31. doi:10.1016/j.envint.2015.02.005

Paper 2
Legacy and alternative flame retardants in Norwegian and UK indoor environment: Implications of human exposure via dust ingestion

Authors: **Katerina Kademoglou*,** Fuchao Xu, Juan Antonio Padilla-Sanchez, Line Småstuen Haug, Adrian Covaci, Chris D. Collins, 2016, Environ. Int., doi: 10.1016/j.envint.2016.12.012

Paper 3
Bioaccessibility of flame retardants present in indoor dust: A novel dialysis membrane method with a Tenax TA® absorption sink *(Submitted; Science of the Total Environment; Manuscript ID: STOTEN-D-17-05899)*

Authors: **Katerina Kademoglou*,** Adrian C. Williams, Chris D. Collins

Paper 4
In vitro inhalation bioaccessibility of plasticisers present in indoor dust using artificial lung fluids *(in prep)*

Authors: **Katerina Kademoglou*,** Georgios Giovanoulis, Anna Palm-Cousins, Cynthia de Wit, Line Småstuen Haug, Adrian C. Williams, Jörgen Magnér, Chris D. Collins

**refers to the corresponding author*

Statement

I, Katerina Kademoglou, made the following contributions to the papers presented in this thesis

Paper 1

I was responsible for conducting and writing the literature review related to flame retardants and indoor dust bioaccessibility and provided major contributions in writing and editing the paper collectively with the co-authors involved.

Paper 2

I wrote the paper proposal, organised and performed sample collection for UK and Norwegian dust samples, performed sample preparation and instrumental analysis together with Fuchao Xu (UA), performed all data and statistical analysis and wrote the paper.

Paper 3

I wrote the paper proposal, organised and performed all method development experiments including sample preparation and instrumental analysis, performed all data and statistical analysis and wrote the paper.

Paper 4

I wrote the paper proposal, organised the lung bioaccessibility method, performed sample preparation and instrumental analysis together with Georgios Giovanoulis (IVL-SU), performed all data and statistical analysis and wrote the paper.

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Cheers!

Katerina A. Kademoglou

Contents

List of abbreviations

ALF	Artificial lysosomal fluid
APPI	Atmospheric pressure photoionisation.
BAF%	Bioaccessible fraction
BARGE	Bioaccessibility Research Group of Europe
BC	Black carbon
BFR	Brominated FR
BW	Body weight.
CE-PBET	Colon Extended Physiologically Based Extraction Test
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DecaBDE	Decabromodiphenyl ether
DIN	Method E DIN 19738, Ruhr-Universität Bochum (RUB)
EBFRIP	European Brominated Flame Retardant Industry Panel
ECNI	Electron capture negative ionisation.
EHFR	Emerging halogenated FR
EI	Electron impact.
ESI	Electrospray ionisation.
EU	European Union
FOREhST	Fed ORganic Estimation Human Simulation Test
FR	Flame retardant
GC	Gas chromatography.
GIT	Gastrointestinal tract
GMB	Gamble's solution
HBCD	Hexabromocyclododecane.
HCH	Hexachlorocyclohexane
HOC	Hydrophobic organic compound
ISTD	Internal standard.
ITQ	Ion trap quadrupole
Kow	octanol/water partition coefficient
L/S ratio	Liquid to solid ratio
LOD	Limit of detection.
LOQ	Limit of quantification.
m/z	Mass to charge ratio.
m-PFR	monomeric PFR
MRM	Multiple reaction monitoring.
MS	Mass spectrometry.
NIST	National Institute of Standards and Technology
OCP	Organochlorine pesticides
o-PFR	oligomeric PFR
OPFR	Organophosphorus Flame Retardants
PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenyl ether
PCB	Polychlorinated biphenyls
PEs	Phthalate esters

POP	Persistent Organic Pollutants
PVC	Polyvinyl chloride
RC membrane	Regenerated Cellulose membrane
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RfD	Reference Dose
SBET	Simplified bioaccessibility extraction test
SHIME	Simulator of the Human Intestinal Microbial Ecosystem
SRM 2585	Standard Reference Material Organic Contaminants in House Dust
TDI	Tolerable daily intake.
TIM	TNO Gastric Intestinal Model
TOC	Total organic carbon
UBM	Unified bioaccessibility method
US EPA	United States Environmental Protection Agency
μm	micrometre

Abstract

Polybrominated diphenyl ethers (PBDEs) are flame retardants (FRs) used as additives against fire ignition accidents, present in everyday consumer products including carpets, electronic appliances, clothing and textiles, thermal insulation and cable coatings. PBDE continuous and excessive use in consumer products, has raised concerns regarding their potential adverse health effects including endocrine and thyroid disruption and neurodevelopmental disorders in children. Hence, legislative restrictions on the production and use of PBDEs in the global market have been imposed by the competent authorities. However, limited data exist on the fate, environmental levels and potential effects on human health of PBDE alternatives such as emerging halogenated FRs (EHFRs), phthalate esters (PEs), non-halogenated phosphorous FRs (PFRs) and alternative plasticisers. Oral bioaccessibility (*i.e.* uptake) studies have been widely used as a research tool to determine the potential human exposure to ingested contaminants via solid matrices such as indoor dust. Colon Extended - Physiologically Based Extraction Test (CE-PBET) is a well-established bioaccessibility protocol specifically developed for the testing of organic compounds, rich in dietary components which act as a "biological sink" for organic pollutants, enhancing thus the sorption capacity of the system. Also, strong adsorbents such as Tenax TA®, silicone-activated contaminant traps, cyclodextrins and silicone rods have also been proposed as "absorption sink" materials. Taken all together, the aim of the PhD studies presented here is two-fold: a) to assess human exposure to legacy and alternatives FRs via indoor dust ingestion and inhalation and b) to develop a robust and unified oral bioaccessibilty method with the inclusion of Tenax TA® as a non-biologically active "infinite sink" to the previously established CE-PBET model. Regarding the *in vitro* gut bioaccessibility, a novel physical separation of the incubated dust with the Tenax TA was successful by employing a regenerated cellulose (RC) dialysis membrane method. The newly developed system was optimised for Tenax TA® bead loading (i.e. 0.25, 0.5 or 0.75g) and allowed sorption to be studied in the stomach, small intestine and colon compartments. Our results show that sorption on Tenax TA® in the stomach was 43.7% and 25.6% for BDE28 and BDE47 respectively, unlike in the colon compartment which was nearly 50% for BDE154 and BDE183. With Tenax TA® inclusion, gut bioaccessibility reached 40% for BDE153 and BDE183, with greater increases seen for less hydrophobic FRs such as BDE28 and BDE47 (60.6%). The combination of Tenax TA® as an infinite sink together with the lipid-rich colon compartment of CE-PBET act as a substantial advance

towards a cost-effective and more realistic estimates of FR uptake via the gut and can liaise regulators to redefine human exposure estimates.

We also investigated the presence of PBDEs and alternative FRs such as emerging halogenated FRs (EHFRs) and organophosphate flame retardants (PFRs) in indoor dust samples from British and Norwegian houses as well as British stores and offices. BDE209 was the most abundant PBDE congener with median concentrations of 4,700 ng g^{-1} and 3,400 ng g^{-1} in UK occupational and house dust, respectively, 30 and 20 fold higher than in Norwegian house dust. Monomeric PFRs (m-PFRs), including triphenyl phosphate (TPHP), tris(chloropropyl) phosphate (TCPP) and tris(2-chloroethyl) phosphate (TCEP) dominated all the studied environments. This is the first report of isodecyldiphenyl phosphate (iDPP) and trixylenyl phosphate (TXP) in indoor environments. iDPP was the most abundant oligomeric PFR (o-PFR) in all dust samples, with median concentrations one order of magnitude higher than TXP and bisphenol A bis(diphenyl phosphate (BDP). iDPP and TXP worst-case scenario exposures for British workers during an 8h exposure in the occupational environment were equal to 34 and 1.4 ng kg bw^{-1} day^{-1}, respectively considerably below the proposed reference values.

With respect to inhalation as an alternative route of exposure, this is the first study assessing the *in vitro* pulmonary uptake of established PEs including dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-(2-ethylhexyl) phthalate (DEHP) and alternative plasticisers used as phthalate substitutes such as bis(2-ethylhexyl) terephthalate (DEHT) and cyclohexane-1,2-dicarboxylic acid diisononyl ester (DINCH) present in indoor dust. Two artificial lung fluids, mimicking two distinctively different interstitial conditions were used, namely artificial lysosomal fluid (ALF, pH=4.5) representing the fluid that inhaled particles would contact after phagocytosis by alveolar and interstitial macrophages within the lung and Gamble's solution (GMB, pH=7.4) as a fluid for deep lung deposition of dust within the interstitial fluid of the lung. Our results suggest that low molecular weight (MW) and short-chained phthalates such as DMP and DEP are highly bioaccessible (>75%) in both artificial pulmonary media tested, whereas high MW compounds such as DEHP, DINCH and DEHT were <5% bioaccessible. Such findings confirm the hypothesis of hydrophobicity and water solubility primarily influencing inhalation bioaccessibility of organic pollutants.

Finally, human exposure to alternative FRs is expected to increase in the future, hence continuous monitoring is required. The *in vitro* bioaccessibility methods presented in this thesis can thus form the foundation upon which an integrated and robust testing strategy for chemicals of emerging concern can be built.

Chapter 1

General introduction

Literature review was based on "*Towards a unified approach for the determination of the bioaccessibility of organic pollutants*"

Collins, C.D., Craggs, M., Garcia-Alcega, S., **Kademoglou, K**., Lowe, S., 2015. Environ. Int. 78, 24–31. doi:10.1016/j.envint.2015.02.005

1. Introduction

1.1 Emerging flame retardants

Flame retardants (FRs) are widely used in everyday consumer products including carpets, electronic appliances, clothing and textiles, thermal insulation and cable coatings. Since the 1970s, polybrominated diphenyl ethers (PBDEs) have been widely used in consumer products as FRs (Alaee et al., 2003). Various human health effects are associated with PBDE exposure such as disruption of the endocrine and thyroid homeostasis (Legler and Brouwer, 2003) and impaired neurodevelopmental growth of children (Costa and Giordano, 2007). The commercial mixtures Penta-BDE and Octa-BDE have been listed as persistent organic pollutants (POPs) for elimination under the Stockholm Convention (Stockholm Convention, 2009a, 2009b), while the Deca-BDE mixture is currently under review. The use of Deca-BDE was banned in Norway in 2008 (EBFRIP, 2008), while it was included by the EU in the amended Annex XVII of REACH (EC No 1907/2006), banning its production, use and marketing in the EU (European Commission, 2016). As a result of the REACH amendment, furniture and fire safety regulations in the UK are currently under review by the national competent authorities (UK Department for Business, Energy & Industrial Strategy, 2016). Due to legislative restrictions on their commercial use, PBDEs have been replaced with alternatives, known as "emerging" halogenated flame retardants (EHFRs) including 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB; Penta-BDE replacement), bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TEBP; Penta-BDE replacement), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE; Octa-BDE replacement), decabromodiphenyl ethane (DBDPE; Deca-BDE replacement) and Dechlorane Plus (DPs; Deca-BDE replacement) (Stapleton et al., 2008; Wang et al., 2011) and organophosphate flame retardants (PFRs) such as tris(2-chloroethyl) phosphate (TCEP) and tris(chloropropyl)phosphate (TCPP) (van der Veen and de Boer, 2012a).

Several studies have indicated that also EHFRs and PFRs may pose potential risks to humans. EH-TBB and BEH-TEBP, major components in the commercial product Firemaster 550®, have been proven to act as endocrine disruptors and obesogens when orally administered to rats (Patisaul et al., 2013) and can bind and activate the transcription of peroxisome proliferator-activated receptor γ (PPAR γ) ligands, while triphenyl phosphate (TPHP)-

induced *in vitro* adipocyte differentiation and diverted osteogenic differentiation towards lipid accumulation has been reported (Pillai et al., 2014). DPs, EH-TBB, BEH-TEBP and PFRs, such as TCEP and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) have been detected in human breast milk and blood in Asian populations (Ben et al., 2013; Kim et al., 2014), as well as in blood, hair and nails in USA residents (Liu et al., 2016). TDCIPP has been linked with reduction in free thyroxine and increase in prolactin secretion in US men, while TPHP was associated with weakening sperm quality (Meeker and Stapleton, 2010). An *in vitro* estrogenic and anti-androgenic potency of TDCIPP, tris(2-butoxyethyl) phosphate (TBOEP), and TPHP on human osteosarcoma (U2OS) cell line exposed to indoor dust extracts has also been reported (Suzuki et al., 2013). In the EU, restrictions on the use of chlorinated PFRs, such as TDCIPP and TCPP, have been issued based on toxicological concerns related to their carcinogenic potency (ECHA, 2008a, 2008b).

Monomeric PFR (m-PFRs), including TDCIPP, TCPP and TCEP, are routinely used as FRs in flexible polyurethane foams (PUFs) and textiles (Ali et al., 2012; Cao et al., 2014b). TPHP can be used as a plasticiser and a FR in PVC, thermoplastics and synthetic polymers, while TBOEP is exclusively used as a plasticiser in floor polish and rubber products (Marklund et al., 2003; Stapleton et al., 2009; van der Veen and de Boer, 2012a). The use of EHFRs and m-PFRs in consumer products has thus increased and this is reflected by their high abundance in indoor dust in the UK (Brommer and Harrad, 2015), China (Cao et al., 2014a), Japan (Tajima et al., 2014), Sweden (Newton et al., 2015) and Norway (Cequier et al., 2014). PFRs such as TCPP, TCEP and TBOEP dominate house, office and hotel environments, with levels in hotel dust six fold higher than office dust from China (Cao et al., 2014b). A few studies have reported oligomeric PFRs (o-PFRs) in considerable amounts in dust, such as tetraekis(2-chlorethyl)-dichloroisopentyl diphosphate (V6), an alternative of Penta-BDE, TCPP and TDCIPP (ECHA, 2008c), along with resorcinol bis(diphenyl phosphate (RDP) and bisphenol A bis(diphenyl phosphate (BDP) as Deca-BDE alternatives in electronic and plastic consumer products (Ballesteros-Gómez et al., 2014; Brandsma et al., 2013; Matsukami et al., 2015). Since house dust acts as a repository sink for EHFRs and PFRs, dust originating from indoor environments (e.g. houses, offices, stores) is considered as a major source of human exposure to FRs (Alves et al., 2014; Jones-Otazo et al., 2005).

In April 2016, the Washington State House Bill 2545 (Toxic-free Kids and Families Act) was approved to ban children's products and residential upholstered furniture from the market containing more than 0.1% of TCEP, TDCIPP, Deca-BDE, hexabromocyclododecane

(HBCD) and tetrabromobisphenol A (TBBPA) with an effective date set for June 2016. Additional six FRs, including TPHP, TCPP, V6, EH-TBB, BEH-TEBP, and isopropylated triphenyl phosphate (IPTPHP) will be evaluated and recommended to the Legislature for possible restriction in consumer products (State of Washington, 2016). The implementation of this bill may potentially trigger the phasing out PBDE alternatives, thus initiate the development and use of newer FRs. Therefore, the continuous and rigorous assessment of legacy and alternative FRs in the indoor environment is essential due to their potential adverse effects on human health.

1.2 Plasticisers

Phthalate esters (PEs) are widely used as plasticiser additives enhancing the durability, elasticity and flexibility of polymeric products (Wilkes et al., 2005). The ubiquitous nature of plasticisers and their lack of migration stability allows them to be distributed throughout the indoor environment, leading to their classification as major indoor organic contaminants (Zhang and Smith, 2003). Due to limited toxicological information available and their "pseudo-persistent" environmental fate and behaviour, alternative plasticisers presented in this thesis are categorised together with PEs into two groups with respect to their molecular weight (MW) and application in consumer products (Bui et al., 2016). Low MW phthalates, such as dimethyl phthalate (DMP) and diethyl phthalate (DEP), are typically added as chemical stabilisers during the manufacture of personal care products, solvents, air refreshers, pharmaceutical coatings and as colouring or fragrance additives (Hauser et al., 2004; Heudorf et al., 2007). High MW phthalates, such as di-(2-ethylhexyl) phthalate (DEHP), benzylbutyl phthalate (BBzP) and di-iso-nonyl phthalate (DiNP), are primarily used in polyvinyl chloride (PVC) production with main applications in floor polishing and wall coatings, PVC tubing, children toys, medical products (e.g. blood preservation flasks) and food packaging materials (Dodson et al., 2015). High phthalate levels have been reported in indoor dust from houses and day-care centres in Denmark (Langer et al., 2010), (Bekö et al., 2013), Sweden (Luongo and Östman, 2016), Germany (Fromme et al., 2013), Kuwait (Gevao et al., 2013) and the USA (Dodson et al., 2015). Within the EU legislative framework, the use of DEHP, diisobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP) and BBzP have been partly restricted in children's toys and cosmetic products to 0.1% by weight (EU Directives 2005/84/EC; 2004/93/EC), leading to increasing use of alternative plasticisers such as bis(2-ethylhexyl) terephthalate (DEHT) and cyclohexane-1,2-dicarboxylic acid diisononyl ester

(DINCH) as substitutes for traditional phthalates in PVC materials (Bui et al., 2016; Correia-Sá et al., 2017).

Apart from indoor dust ingestion acting as an important route of human exposure to PEs (Alves et al., 2014), alternative routes such as inhalation and dermal uptake have may have substantial contribution to human exposure (Bekö et al., 2013; Xie et al., 2016). Due to their physicochemical properties, phthalate esters and alternative plasticisers tend to volatilise and partition from the gas phase as aerosols and following sorption processes, they are absorbed onto dust particles (settled or floor) (Bui et al., 2016; Weschler et al., 2008). Phthalates bound onto the dust particles can be inhaled, following desorption which can occur in the pulmonary environment - analogous to orally ingested dust particles in the gastrointestinal fluids with desorption releasing organic contaminants that can pass to the systemic circulation (Collins et al., 2015). According to (Wormuth et al., 2006) human exposure to phthalates varies with respect to age; DMP inhalation exposure is nearly 100% for infants, toddlers and younger children and 70-90% for teenagers and adults whereas it is almost 30% for DEP and DnBP in toddlers and younger children and nearly 20% for BBzP in children and 20% for DiNP in teenagers. Hence, inhalation of phthalate-contaminated dust may act as an alternative exposure route, probably greater than vapour phase phthalate exposure due to long residence time and deep lung deposition of dust particles. Constant phthalate desorption from indoor dust to the lung environment is a dynamic process, leading to continuous phthalate release and bioavailability, thus lung bioaccessibility contribution as a first tier of chronic human exposure to phthalates may play a substantial role (Jaakkola et al., 1999; Oberdorster, 1995; Oie et al., 1997).

1.3 Bioaccessibility and human exposure

Within this chapter, we address solid matrices, namely soil and indoor dust, as they are known “suspects” of human ingestion of hydrophobic organic compounds (HOCs) (Watanabe & Sakai, 2003; Abb et al., 2010). The contaminants associated with these matrices often differ; in soils, there are concerns from the direct ingestion from inadvertent hand to mouth activity or ‘pica’ of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins from past and present industrial activities, including gasworks, petroleum exploration and refining processes (James et al., 2011). During the past decade, ingestion of dust contaminated with FRs by toddlers and younger children has received

attention as there is the potential to exceed previously established guidance values (Stapleton et al., 2012; US EPA, 2010).

The total pollutant concentration of the ingested matrix (as a mass fraction) is frequently used for the determination of the risk posed by toxic chemicals to public health. This is considered to be a conservative approach, but aligns with the precautionary principle (*i.e*. first-do-no-harm) being adopted in 1998 by the Science and Environmental Health Network as a guideline in environmental policy decision making, tailored for "*when an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically*" (Kriebel et al., 2001; Raffensperger and Tickner, 1999). Hence, under the regime of identifying and controlling potential environmental hazards, the precautionary principle has been extensively used in environmental risk assessments worldwide regarding contaminated land and pesticide registration (Rosner and Markowitz, 2002; Zander, 2010). However, synthetic chemicals are extremely useful to the modern lifestyle and a more scientifically rigorous approach may result in a better quantification of their health impacts. To address this need, a number of bioaccessibility tests have been developed which determine the transfer of pollutants from the contaminated solid matrix into the gut fluid, including physiologically based extraction tests (PBET) in fed or unfed status, meaning with or without the addition of carbohydrates, food components and bile salts, *e.g.* SBET (BGS; UK), SHIME (LabMet; Belgium), DIM (RUB; Germany) and TIM (TNO; the Netherlands) (Ruby et al., 1996; Oomen et al., 2002; Bruce et al., 2007; Cave et al., 2010b; Tilston et al., 2011).

Bioaccessibility tests have proliferated because of their relative simplicity, high throughput, significantly reduced ethical considerations, sustainability, reduced costs and the ability to develop a reproducible standard operating procedure (UK Env Agency, 2005). It is known that the bioaccessible fraction can be considerably less than the total. In soils, the recoveries of lindane, endosulfan 1, endosulfan II, endrin, DDE and DDD following intestinal extraction were between 5.5 to 13.5% (Scott & Dean, 2005). Assessing 20 marine and freshwater fish species, (Wang et al., 2011a) found bioaccessible DDT concentrations were between 5.5 and 17.6%. (Wang et al., 2013b) reported the average bioaccessibility of DDT in a total dust sample to be 24.5%. While these tests do not predict the actual amount of chemical that will be transferred across the gut lining and enter the blood stream, *i.e.* the bioavailable fraction, they do offer the ability to fine tune the risk assessment. While bioaccessibility tests have been used in research for many years they are not widely accepted by the regulatory

authorities; the US test for lead is a notable exception (US EPA, 2008). However, these tests are currently being used in the UK as part of a 'body of evidence' approach when determining the risks of contaminated land. This low level of adoption has two principle reasons; a) the lack of validation by animal trials and b) the wide variability seen both within and between tests regarding statistical parameters such as method reproducibility assessed by coefficient of variation (CV) values and outliers, as well as general test formats mimicking the human gut stream (saliva, stomach, GI, colon), presence of food components in the gastric compartment, mass of sample loaded (g), analytical method etc. (Oomen et al., 2002; Koch et al., 2013)

Within this chapter, we focus on the emerging FRs presented in sections 1.1 and 1.2 regarding gut bioaccessibility, since these chemical classes have received less attention than the toxic elements and there is more uncertainty on which components from a wide spectrum of the current bioaccessibility test formats are influential (Agency, 2005; EPA, 2008). Also, the purpose of this chapter is to bring together the numerous bioaccessibility methods that have been used the past decade for organic pollutants *e.g*. FOREhST (Cave et al., 2010), CE-PBET (Tilston et al., 2011), SHIME (fasting status) (Laird et al., 2007; Yu et al., 2012) and sorptive CE-PBET with the addition of silicone rods (Gouliarmou et al., 2013) and assess if from these, a common set of principles can be proposed. Current configurations vary widely in the total incubation time, composition of the incubating media and the amount of material being introduced. Significantly our review focuses on a range of matrices soil and dust, whereas previous reviews have only addressed soil (Dean & Ma, 2007). We are also reviewing a wider range of HOCs including legacy and alternative FRs, polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PEs) and organochlorine pesticides (OCPs) (Gron et al., 2007; Yu et al., 2009; Wang et al., 2011b; Cheng et al., 2013).

Definition of Bioaccessibility

The assumption that 100% of the ingested toxicant within a matrix being available is unrealistic (Collins et al., 2015). Animal bioavailability studies (*e.g*. rodents or swine) are representative of the *in vivo* situation, but are often hindered due to financial and ethical restrictions (Oomen et al., 2003; Ruby et al., 2002). To avoid risk overestimation, bioaccessibility, *i.e.* the maximal fraction of an organic pollutant released from an ingested matrix (*e.g.* dust) into the gastro-intestinal tract (GIT) fluids of the organism has been proposed as a more realistic but conservative approach in human exposure assessment of

persistent organic pollutants (POPs), serving as a surrogate to bioavailability (Brandon et al., 2006; Dean and Ma, 2007; Oomen et al., 2000). There are a number of definitions of bioaccessibility, these can be particularly confusing because they can relate to both human ingestion and microbial degradation. We use the following definition 'The maximal amount of contaminant released from the test matrix in a synthetic gastrointestinal system' (Semple et al., 2004). This fraction represents the maximum amount of a contaminant that is available for absorption within the human gastrointestinal tract (Oomen et al., 2000). It is also important to separate bioaccessibility from bioavailability, usually the latter requires transfer across a biological membrane, *i.e.* across the intestinal cell wall where it then enters the systemic circulation (blood or lymph). Under this regime, oral bioavailability is the collective effect of ingestion, bioaccessibility and absorption (Oomen et al., 2002) (Fig. 1). Several physiologically-based extraction tests (PBET) have been proposed to assess organic pollutant release and uptake from an ingested matrix via the GIT fluids *in vitro* (Brandon et al., 2006; Cave et al., 2010; Gouliarmou and Mayer, 2012; Tilston et al., 2011; Van de Wiele et al., 2004), as a substitute to *in vivo* studies (James et al., 2011) or for high-throughput estimates of bioaccessibility when animal studies are not feasible (Rodríguez-Navas et al., 2017; Ruby et al., 1996).

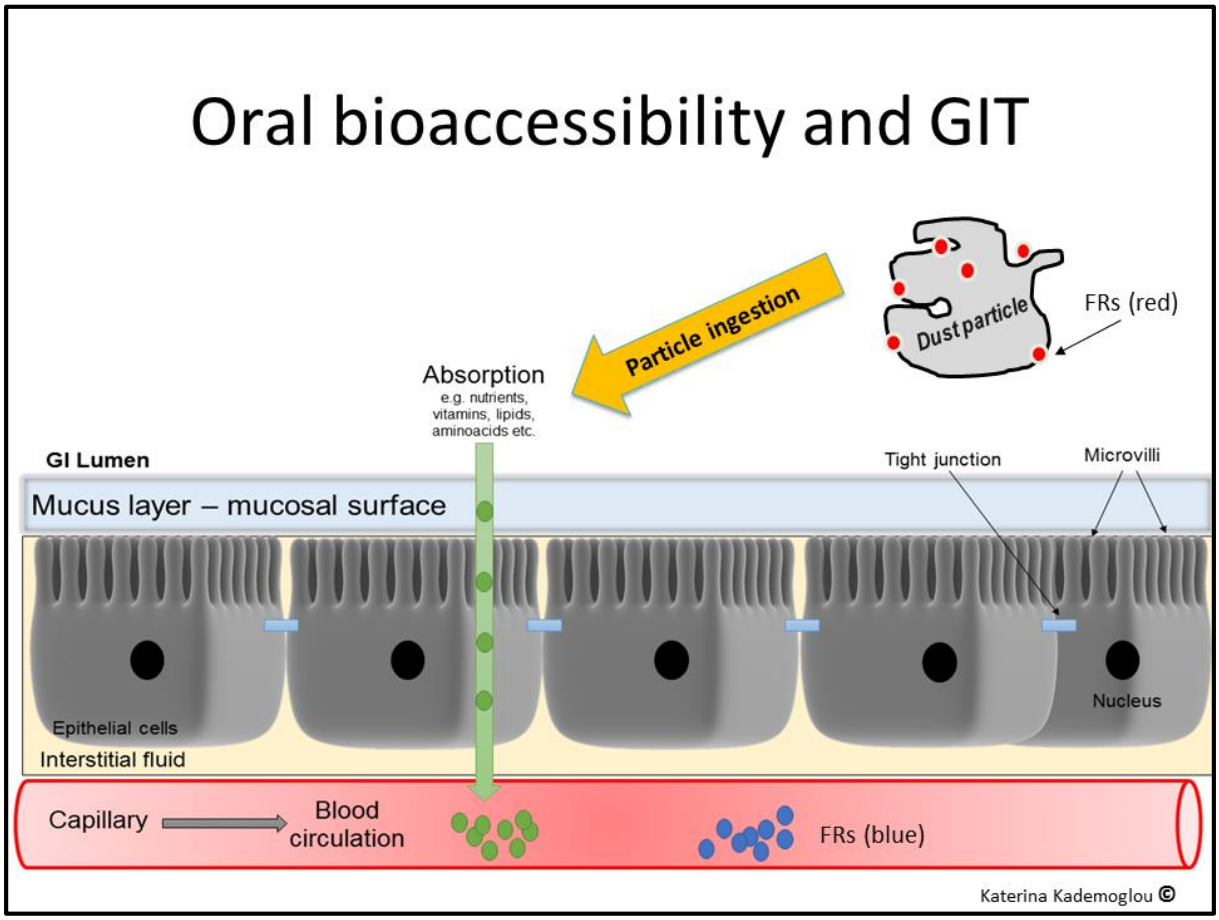


Figure 1 Schematic representation of dust particle ingestion and oral bioaccessibility via the gastrointestinal tract (GIT). Shown in the figure are the most important intestinal compartments and mechanisms related to FR absorption via the GIT, reaching the body's systemic circulation.

In figure 2, we present the basic sorption and desorption processes regarding ingested solid matrices (e.g. indoor dust) and bioaccessibility. When in contact with an aqueous or air matrix, organic contaminants become associated with the solid matrix through physical and chemical processing, sorption processes, surface binding as well as within-pores diffusion. Once the particles are ingested and enter the GI tract, then the sorption processes are reversed leading to release of xenobiotics from the solid matrix to the GI fluids (*i.e.* bioaccessible fraction) with eventual transport into the blood circulation. Human ingestion of a solid matrix (e.g. soil, indoor dust) is partly governed by particle size, particularly in the case of accidental ingestion. This pathway is frequently the primary human exposure route from contaminated solid matrices. In bioaccessibility studies, most tests are concerned with the particle size that is considered adhesive to hands, but a range of values have been recommended < 250 μm (Calabrese et al., 1996; Stanek, 2000), < 50 μm (Ljung et al., 2006) and < 45 μm (Siciliano et al., 2010). In pigs, the bioavailability of the PAH benzo[a]pyrene was correlated with the carbon in in the sand and silt fraction, *i.e* < 50 μm (Duan et al., 2014). Finally, <250 μm was recommended by (Yu et al., 2012) as the most appropriate particle cut-off size of dust particles to be likely ingested by humans, therefore used in their study regarding oral bioaccessibility of PBDEs and indoor dust.

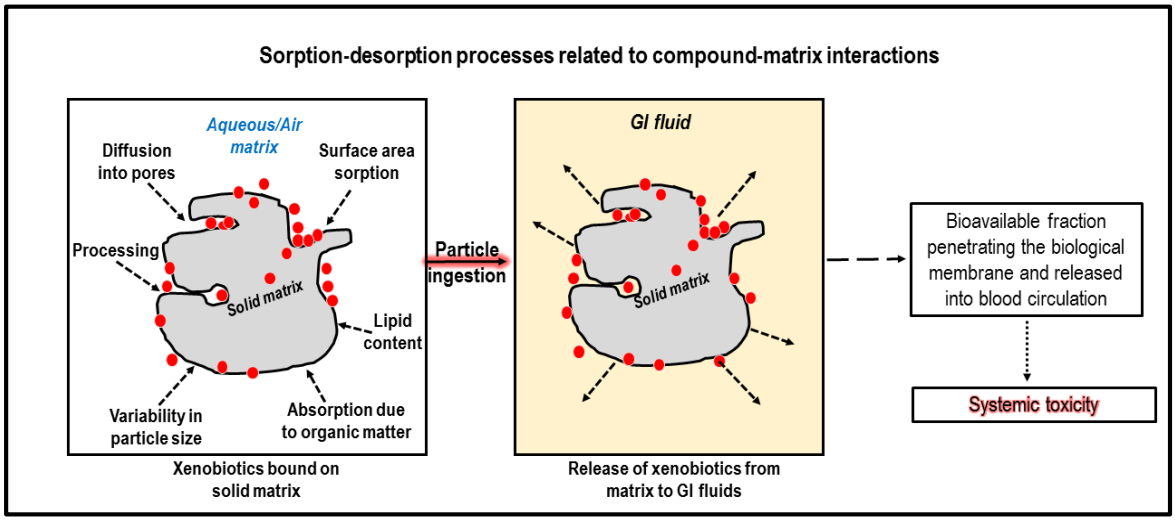


Figure 2 Particle properties affecting the bioaccessibile fractions via solid matrix (food, soil or dust) ingestion. (Solid matrix is shown in grey and the organic contaminants bound to it are highlighted in red).

1.4 Indoor dust and bioaccessibility

Although there is significant geographical and seasonal variation, non-dietary dust ingestion acts as an important source of human exposure to pesticides and FRs which are contained in every-day consumer products (Shalat et al., 2003; Hazrati & Harrad, 2006). These chemicals are non-covalently bound additives for prevention of fire ignition in consumer products and electronic household equipment such as personal computers and television sets, as well as plastics, textiles and flexible polyurethane foam (PUF) (Bakker et al., 2008). Given its colloid and complex character as a heterogeneous mixture of organic compounds and the particle-bound organic matter derived from biological matrices ranging from skin cells, pollen, human and animal hair to fungal spores (Butte & Heinzow, 2002), dust is present in houses, offices, cars many other indoor environments where urban populations spend up to 90% of their time (Brasche & Bischof, 2005).

Oral bioaccessibility studies of dusts have investigated a range of organic contaminants such as PCBs, organochlorine pesticides (OCPs), PBDEs and phthalate esters respectively (Ertl & Butte, 2012; Kang et al., 2012; Kang et al., 2013; Wang et al., 2013d). (Wang et al., 2013b) employed a PBET test (stomach and small intestine compartments only), performing a risk assessment of OCPs under the hypothesis of dust ingestion as a major route to total OCP exposure for children and found that the bioaccessibility of DDT in dust samples (mean 24.5%) was higher than HCH (mean 10.4%), while the intestinal desorption was higher than the gastric phase. The log K_{ow} for DDT is 6.9, whereas for the HCH isomers log K_{ow} values range from 3.7 - 4.14. Bioaccessibility of PCBs in dust was 37.3% and 20.8% and 13.6% for tri-PCB, tetra-PCBs and octa-PCBs respectively, mainly due to the log K_{OW} which increases with respect to the degrees of chlorination (Wang et al., 2013c).

Children spend more than 90% of their time indoors (*e.g*. house, day-care centres) and, compared to adults, their breathing zone tends to be closer to the floor (Kocbach Bølling et al., 2013). Children's higher metabolic and body development rate, rate of oxygen consumption, smaller lung surface area per kg and lower body weight, together with behavioural and activity factors such as floor mobility may result in higher dust inhalation

rates compared to adults, leading to greater internal exposure to environmental contaminants (Moya et al., 2004). Children are thus considered particularly vulnerable to FR exposure and associations between phthalate exposure and respiratory health problems such as asthma and allergies have been reported (Bornehag et al., 2004; Bornehag and Nanberg, 2010; Fromme et al., 2013). Prenatal exposure to PEs has been linked with neurodevelopmental disorders during adult life (Bellinger, 2013), while phthalate-induced oxidative stress and asthma-related pulmonary inflammation have been reported for adults (Franken et al., 2017). In children, allergic reactions and chronic respiratory problems such as asthma, bronchial hyperactivity and inflammation have been linked with high DEHP and BBzP levels in indoor dust and with the use of indoor PVC flooring (Hsu et al., 2012), (Doelman et al., 1990; Jaakkola and Knight, 2008; Mendell, 2007). Asthma occurrence in children has been linked with considerable DEHP concentrations in indoor dust (Bornehag et al., 2004), while inhalation of DEHP-contaminated dust triggered a nasal mucosa immune response in adults previously allergic to dust (Deutschle et al., 2008).

The most abundant PBDE congeners present in dust are BDE47, BDE99, BDE183 and BDE209 (Watanabe & Sakai, 2003; Yu et al., 2012). BDE209 was the least bioaccessible compound (10-20%) compared to the lower brominated PBDE congeners which had values between 40 to 60% (Lepom et al., 2010; Yu et al., 2012). Despite this low bioaccessibility potential, the study of BDE209 is still essential, due to its high levels in dust compared to the rest of PBDE congeners (μg vs ng levels) (ECB, 2002; Harrad et al., 2008; Sjodin et al., 2008); because of the debromination potential of BDE209, lower brominated PBDEs are likely to be generated and released to indoor environment, thus becoming more bioaccessible (Gerberding, 2004; Lee & He, 2010).

There is high variance in the amount of dust incubated in bioaccessibility tests to date, ranging from 9 mg up to 1 g. Additionally, inconsistency exists regarding the ratio between the matrix (solid) and gastric solutions (liquid). (Yu et al., 2009) suggest a liquid-to-solid (L/S) dependent release of PBDEs from food matrix (Grass carp fish) when L/S ratio was lower than 100, resulting in incomplete release of PBDEs to the GI fluids. In the case of contaminant saturation phenomena in the GI fluids, a decrease in the bioaccessibility would be expected as L/S ratio increases. A fixed L/S ratio between 100 to 150 is therefore recommended to achieve successful contaminant release from the matrix to the GI fluids (Abdallah et al., 2012).

Variation in the bioaccessibility values have been reported for dust particle size fractions as was observed for soils. (Wang et al., 2013b) assessed OCP bioaccessibility values in four different indoor dust particle fractions between <63 μm, 63-100, 100-280 and 280-2000 μm respectively. Their findings suggest no significant difference in bioaccessibility between outdoor and indoor dust ($p<0.05$) or between 63-100 μm and 100-280 μm fractions, whereas significantly higher ($p<0.05$) bioaccessibility was reported for the <63 μm fraction compared to the 280-2000 μm suggesting higher accumulation potential of OCP in the <63 μm fraction. For the case of phthalate esters, the highest bioaccessible fraction was reported for dimethyl phthalate (DMP) in dust particles <63 μm (DMP bioaccessibility mean 15.5% in gastric conditions, 23.1% in intestinal conditions and 38.6% for total digestive juice), while significantly lower ($p<0.05$) values were reported in 280-2000 μm size fraction (Wang, Wu, et al., 2013). Finally, and for the case of PBDEs, (Yu et al., 2013) suggest statistically significant negative correlation ($R^2=0.473$; $p=0.028$) between particle size and bioaccessibility for tri- and hepta-BDEs with the highest value of 45% for size fraction <30 μm, but not for the case of BDE209. Taken all together, such findings support evidence for higher health risk for dust particles <63 μm compared to particle fractions higher than 63 μm with respect to volatile and semi-volatile organic compounds such as low MW phthalate esters and PBDEs with low bromine content.

1.5 Basis of the "infinite" sink in bioaccessibility

An organic chemical's tendency towards lipophilicity or hydrophobicity is a central controller in their bioaccessibility (Henry & DeVito, 2003), through their potential to sorb to the soil matrix and desorb from particles within the gastro-intestinal tract (Fig. 2). Two bioaccessibility protocols specifically developed for the testing of HOCs are the Fed ORganic Estimation Human Simulation Test (FOREhST) (Cave et al., 2010a) and the Colon Extended - Physiologically Based Extraction Test (CE-PBET) both operated in the fed state as this is known to enhance the desorption of HOCs, with dietary components such as complex carbohydrates and peptides (*e.g.* starch, xylan, cysteine) acting as a "biologically active sink" for organic pollutants (Hack & Selenka, 1996; Tilston et al., 2011). CE-PBET also introduces an extended colon section as the final stage of the extraction, which is carbohydrate rich and will further enhance bioaccessibility (Fig. 3). Both these tests contain bile salts which are known to increase the bioaccessibility as they have both hydrophobic and hydrophilic regions (Oomen et al., 2000). The bioaccessibility of PAHs is reduced with increasing

hydrophobicity in both FOREhST (Cave et al., 2010a) and CE-PBET (Tilston et al., 2011). (Hack & Selenka, 1996) concluded that salinity, pH value, addition of bile salts and digestive enzymes were identified as key factors bioaccessibility determination. In an attempt to incorporate a biologically active sink in a bioaccessibility test format, (Van de Wiele et al., 2005) employed a modified SHIME method with the addition of *in vitro* cultured human colon microbiota to assess the estrogenic potency of four PAHs (*i.e*. pyrene, phenanthrene, benzo(a)pyrene and naphthalene) and their metabolites after soil ingestion; PAH metabolites were found to induce an estrogenic effect *in vitro* in the colon compartment as GIT biotransformation products due to their high degree of aromaticity. However, such an *in vitro* approach focuses on potential xenobiotic microbial biotransformation primarily, rather than mimicking the *in vivo* colon epithelium environment where enzymatic metabolism is likely to occur (Van de Wiele et al., 2005).

The central role of a sink was also highlighted by (James et al., 2011), where C18 membranes enhanced desorption from soils by 1-2 orders of magnitude depending on the test employed. Both studies agree that the inclusion of a sink avoids under-estimation of the bioaccessible fraction in organic studies. However, (James et al., 2011) found that despite accuracy in the measurement of soil PAH content, *in vitro* models with the sink did not adequately correspond to the results of an *in vivo* bioavailability study performed on juvenile swine (R^2 = 0.45). Using a two-compartment PBET method (*i.e*. stomach and small intestine), (Tao et al., 2009) reported bioaccessibility values between 4% and 97% through *in vitro* testing of organochloride pesticide (OCP) contaminated soils. Differences in soil properties, particularly organic content, and the physicochemical properties of the analytes such as log K_{ow} and water solubility were cited as reasons for variation within the test. When compared with *in vivo* tests, the FOREhST model underestimated the bioavailability of DDT in a mouse model by 3-15 fold (Smith et al., 2012), while it also under predicted the bioavailability of PAHs in soil by 50 fold (Juhasz et al., 2014). The under prediction of *in vivo* bioavailability may be addressed by adding a non-biologically active 'sink' (e.g. silicone, Tenax TA) to the bioaccessibility test, to simulate the uptake from the gastric solution enhancing the physiological relevance of the test by mimicking the large surface area, and sorptive potential, of the human GI tract (Collins et al., 2013).

Sink conditions better mimic the sorption/desorption processes in the human GIT *in vivo* and, coupled with the lipid-rich environment of the GI lumen and a long matrix:fluid contact time, may improve the bioaccessibility estimates of HOCs, such as PBDEs (Collins et al., 2015;

Zhang et al., 2016, 2015). Strong non-biological adsorbents such as silicone-activated contaminant traps, cyclodextrins and silicone rods have also been proposed as "absorption sink" materials in PBET systems, to improve bioaccessibility estimates for PAH-contaminated soil and biochar (Gouliarmou et al., 2013; Mayer et al., 2016; Zhang et al., 2015). In the case of CE-PBET, the inclusion of an activated charcoal as an infinite sink significantly increased the PAH bioaccessibility estimates (1.2 – 2.8 fold), by removing more PAH from a range of field soils of varying total organic carbon (TOC) and black carbon (BC) content (Collins et al., 2013). As part of the International Organization for Standardization (ISO) guideline on bioavailability, an exhaustive (20h) Tenax-based extraction method achieved increased mobilisation (*i.e.* bioaccessibility) of HOCs from soils and sediments onto this infinite sink and has been proposed for standarisation (ISO, 2015; Ortega-Calvo et al., 2015). Tenax TA® is a versatile absorption sink with large surface area and high sorption capacity for HOCs and was thus used as an "infinite" sink in PBET systems, studying the uptake of FRs and PAHs via indoor dust (Fang and Stapleton, 2014) and soil (Li et al., 2015), respectively(Fang and Stapleton, 2014a; Li et al., 2015). Cornelissen et al (1997) employed Tenax TA® studying sorption/desorption kinetics of PAHs, alkylbenzenes and PCBs from dredged sediments; the sink captured the organic pollutants from the solid matrix but the Tenax TA® beads adhered to the glassware with consequent problems for physical separation and recovery of Tenax TA® from the matrix (Cornelissen et al., 1997). The variability in Tenax TA® mass recovery, its separation from the matrix and the design of an appropriate vessel for Tenax TA® inclusion (*e.g.* stainless steel net) during PBET incubation has discouraged further applications of Tenax TA® in environmental exposure studies (C. Li et al., 2016; Mayer et al., 2016). Hence, in order to establish a harmonised *in vitro* gut bioaccessibility method under the influence of the ISO 16751 method on the environmental availability of non-polar compounds being currently approved for registration, Tenax TA® addition was selected as an adsorptive sink in the present study as the most appropriate non-biological adsorbent for assessing *in vitro* bioaccessibility of FRs.

1.6 PhD thesis objectives

The bioaccessibility of a range of compounds sorbed to a variety of matrices is frequently below 100%, proving its potential to fine tune human health risk assessment. For this potential to be realised we must understand the controlling factors in such tests and adopt them to create a single model approach. It has been clearly established above that the

predominant controls on bioaccessibility are a) the matrix the pollutant is attached to, b) the physico-chemical properties of the pollutant and c) the configuration of the test; the first two points cannot be controlled by the experimental operator, whereas the latter can.

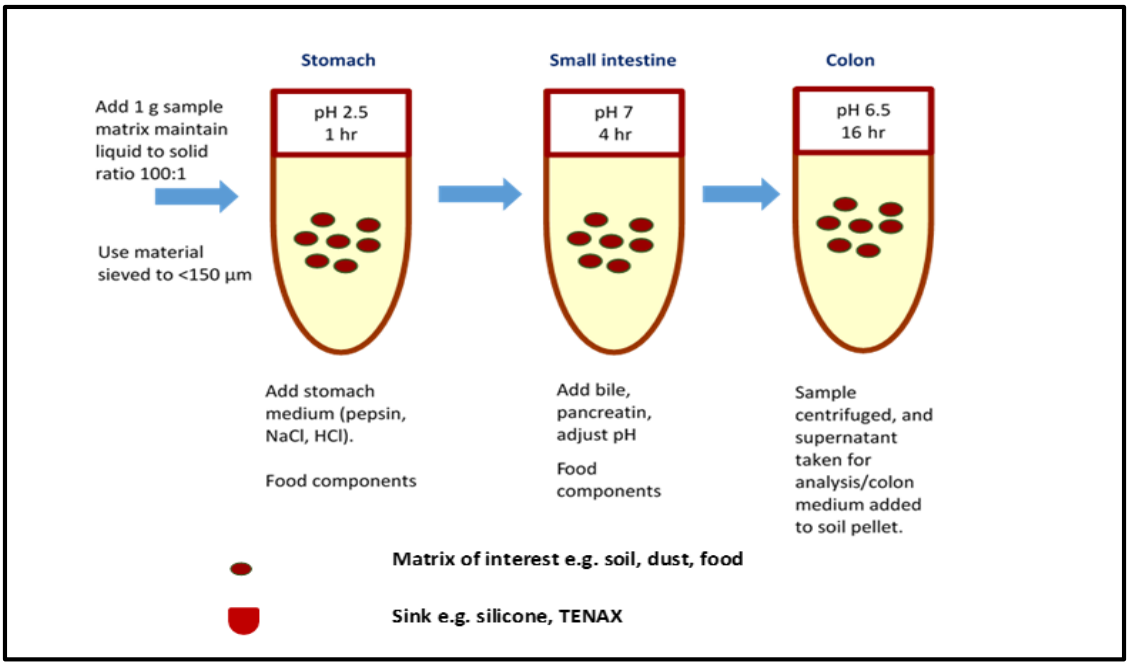


Figure 3 - Schematic representation of a proposed CE-PBET bioaccessibility test format with the inclusion of Tenax TA® as an absorption sink

Good practice has been established by BARGE (the Bioaccessibility Research Group in Europe; http://www.bgs.ac.uk/barge/home.html), which states that a bioaccessibility test should have the following components:

i. It should be physiologically based, mimicking the human GI physico-chemical environment in the stomach and small intestine.

ii. It should represent a conservative case;

iii. There should be one set of conditions for all potentially harmful elements (PHE) being studied;

iv. It must be demonstrated that the test is a good analogue of *in vivo* conditions

v. The test must be able to produce repeatable and reproducible results within and between testing laboratories.

According to Collins et al (2015), a unified *in vitro* bioaccessibility test should address the aforementioned points with respect to the following remarks during method development and validation:

i - It is suggested that the addition of water-based digestive compartments (e.g. saliva) is of limited importance compared to the use of the stomach and small intestine, as contacts times are short (5 min) and compounds of interest are sparingly water soluble. There is potential for the addition of the colon compartment, this increases the potential bioaccessibility. There is also the requirement of a sink to provide a desorption gradient as would be anticipated in the GI tract.

ii + iii - The system should be in the fed state to maximise the desorption of the contaminants. Conservatism would also be ensured by addition of a colon compartment and a 'sink'. These conservative conditions should be applied collectively when conducting any bioaccessibility test.

iv – This has yet to be demonstrated. Results to date appear to under predict bioavailability in real soils. This is possibly a consequence of the tests not being operated in the most conservative condition (see i-iii).

v - No single test design has been agreed upon. Only CE-PBET and FORhEST have been designed specifically for HOCs. Points i-iii need to be agreed in the research community before this an inter laboratory trial can proceed. Then the expensive in-vivo tests can be conducted or soils from previous animal trials used.

Given the fact that human uptake of FRs in exposure studies is generally considered as 100% bioaccessible, leading to potential risk overestimation, a series of *in vitro* tests has to be developed, validated and employed with respect to traditional routes of exposure such as ingestion, as well as formerly unexplored exposure pathways such as inhalation. Apart from a few studies on *in vitro* metabolism of FRs and plasticisers, limited data exist on the uptake and absorption potential of FRs, thus making the practice of *in vitro* bioaccessibility studies a necessity due to their cost-effective and time-efficient nature. The aim of the studies presented in this thesis is thus two-fold; a), to assess human exposure to legacy FRs such as PBDEs as well as alternatives of emerging concern including EHFRs, PFRs and phthalate esters via indoor dust and b) to develop *in vitro* bioaccessibilty methods with the inclusion of Tenax TA® as a non-biologically active "infinite sink", thus providing a cost-effective and

more realistic estimate of FR uptake via the gut and redefine human exposure. The research work presented in the following chapters begins with an extensive human exposure assessment via indoor dust ingestion to a wide range of FRs with diverse physicochemical properties and proceeds in exploring alternative *in vitro* testing strategies for human uptake to FRs, including a modification of a previously established gut bioaccessibility test with the inclusion of Tenax TA as an absorption sink, followed by a novel *in vitro* pulmonary bioaccessibility test for organic pollutants. Both *in vitro* bioaccessibility tests aim to tackle potential risk overestimation, hence provide a simple approach in first-tier human risk assessments for abiotic matrices giving a conservative, yet realistic indication of risk.

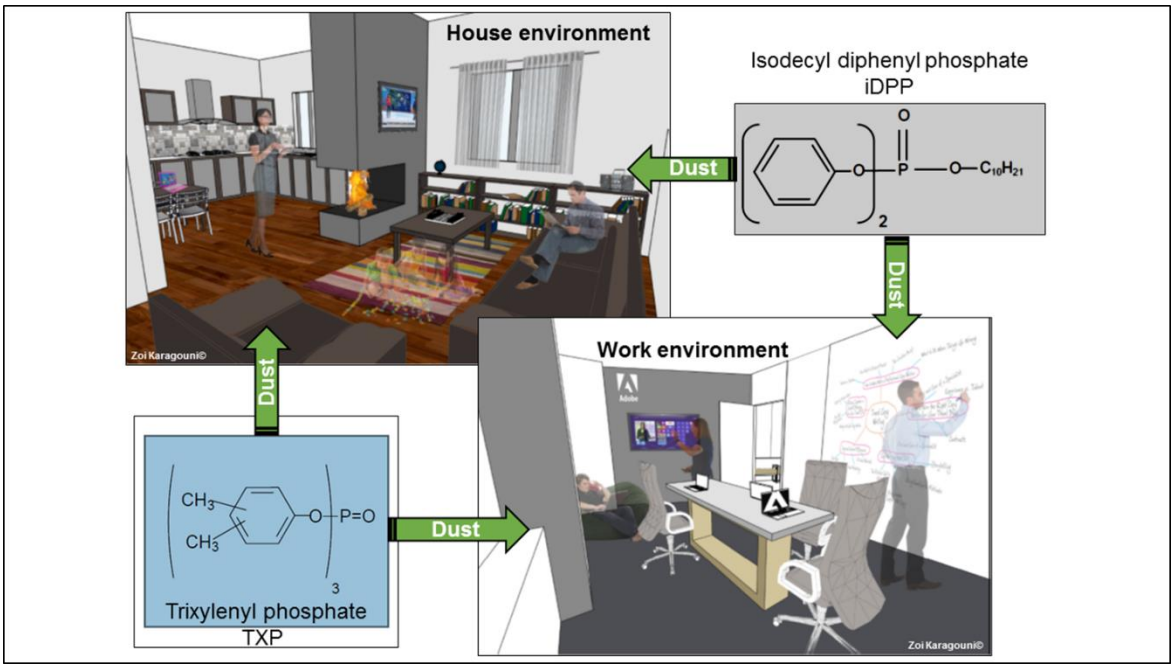
1.7 PhD thesis outline

In chapter 1, a brief overview of the physicochemical characteristics and environmental fate of legacy and alternative FR studied in this thesis are presented. This was followed by and some of the key elements of *in vitro* bioaccessibility and human exposure to HOCs such as flame retardants via indoor dust and soil ingestion have been outlined. Even though soil does not fall within the scope of the present study, it is included in this chapter as it is the most extensively tested matrix in vitro bioaccessibility studies, as well as the current regime in in vitro dust bioaccessibility testing are largely based upon assays developed for soils. In chapter 2, the emerging occurrence and human exposure to legacy and alternative FRs via indoor dust from two diverse geographical regions, the UK and Norway, were presented and human exposure assessment results were critically discussed against existing data. Moving to chapter 3, a novel experimental method assessing the in vitro bioaccessibility of legacy FRs via indoor dust ingestion was developed; Tenax TA® was employed as an absorption "infinite" sink aided by dialysis membrane for successful encapsulation and physical separation of the Tenax TA from the dust as a matrix. This novel experimental approach was targeting towards the development and validation of a unified and exhaustive GIT extraction for the testing of hydrophobic organic compounds (HOCs) such as FRs using artificial GIT fluids as briefly discussed earlier in this chapter. Method development steps involved Tenax TA® mass optimisation and FR sorption capacity evaluation, alongside method validation of the selected CE-PBET parameters using indoor dust standard reference material for organic contaminants in house dust SRM 2585 (NIST, USA) as a well-characterised and homogenous dust sample. In chapter 4, we introduce inhalation as a previously unexplored alternative route of exposure for phthalate esters and non-phthalate alternative plasticisers. A novel *in*

vitro inhalation bioaccessibility method using two artificial lung fluids, namely Gamble's solution and artificial lysosomal fluid was employed with respect to the physiological and inflammatory status of the pulmonary environment. Alongside traditional phthalate esters, phthalate-free alternative plasticisers were included in the list of target analytes tested since they act as substitutes to legacy phthalate esters in consumer products. Finally, chapter 5 provides an overview of the results obtained in this thesis, discussing potential methodological limitations and future perspectives of *in vitro* bioaccessibility studies. Taken all together, we propose two novel *in vitro* bioaccessibility test formats with respect to indoor dust ingestion and inhalation as two substantial exposure routes for FRs. Our main outcome from this thesis is orchestrating the foundation steps in designing and harmonising *in vitro* methods for the testing of organic compounds as an essential tool in risk assessment of chemical of emerging concern within a conservative and realistic human risk assessment framework.

Chapter 2

Legacy and Alternative Flame Retardants in Norwegian and UK Indoor Environment: Implications of Human Exposure via Dust Ingestion



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Abstract

Indoor dust has been acknowledged as a major source of flame retardants (FRs) and dust ingestion is considered a major route of exposure for humans. In the present study, we investigated the presence of PBDEs and alternative FRs such as emerging halogenated FRs (EHFRs) and organophosphate flame retardants (PFRs) in indoor dust samples from British and Norwegian houses as well as British stores and offices. BDE209 was the most abundant PBDE congener with median concentrations of 4,700 ng g^{-1} and 3,400 ng g^{-1} in UK occupational and house dust, respectively, 30 and 20 fold higher than in Norwegian house dust. Monomeric PFRs (m-PFRs), including triphenyl phosphate (TPHP), tris(chloropropyl) phosphate (TCPP) and tris(2-chloroethyl) phosphate (TCEP) dominated all the studied environments. To the best of our knowledge, this is the first report of isodecyldiphenyl phosphate (iDPP) and trixylenyl phosphate (TXP) in indoor environments. iDPP was the most abundant oligomeric PFR (o-PFR) in all dust samples, with median concentrations one order of magnitude higher than TXP and bisphenol A bis(diphenyl phosphate (BDP). iDPP and TXP worst-case scenario exposures for British workers during an 8h exposure in the occupational environment were equal to 34 and 1.4 ng kg bw^{-1} day^{-1}, respectively. The worst-case scenario for BDE209 estimated exposure for British toddlers (820 ng kg bw^{-1} day^{-1}) did not exceed the proposed reference dose *(*RfD) (7,000 ng kg bw^{-1} day^{-1}), while exposures for sum of m-PFRs (Σm-PFRs) in British toddlers and adults (17,900 and 785 ng kg bw^{-1} day^{-1} respectively) were an order of magnitude higher than for Norwegian toddlers and adults (1,600 and 70 ng kg bw^{-1} day^{-1}).

Keywords: PBDEs; alternative flame retardants; UK; Norway; indoor dust; human exposure

Highlights

- PBDEs, EHFRs and PFRs were analysed in Norwegian and UK house, store & office dust
- First report of iDPP and TXP in indoor dust with several o-PFRs also detected
- m-PFRs dominated all indoor environments, followed by EHFRs, PBDEs, and o-PFRs
- BDE209 levels were significantly higher in British than Norwegian house dust
- iDPP is commonly added in toys and culinary products, while TXP is used in IT products

2.1 Introduction

Flame retardants (FRs) are widely used in everyday consumer products including carpets, electronic appliances, clothing and textiles, thermal insulation and cable coatings. Since the 1970s, polybrominated diphenyl ethers (PBDEs) have been widely used in consumer products as FRs (Alaee et al., 2003). Various human health effects are associated with PBDEs exposure such as disruption of the endocrine and thyroid homeostasis (Legler and Brouwer, 2003) and neurodevelopmental growth of children (Costa and Giordano, 2007). The commercial mixtures Penta-BDE and Octa-BDE have been listed as persistent organic pollutants (POPs) for elimination under the Stockholm Convention (Stockholm Convention, 2009a, 2009b), while the Deca-BDE mixture is currently under review. The use of Deca-BDE was banned in Norway in 2008 (EBFRIP, 2008), while it was included by the EU in the amended Annex XVII of REACH (EC No 1907/2006), banning its production, use and marketing in the EU (European Commission, 2016). As a result of the REACH amendment, furniture and fire safety regulations in the UK are currently under review by the national competent authorities (UK Department for Business, Energy & Industrial Strategy, 2016). Due to legislative restrictions on their commercial use, PBDEs have been replaced with alternatives, known as "emerging" halogenated flame retardants (EHFRs) including 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB; Penta-BDE replacement), bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (BEH-TEBP; Penta-BDE replacement), 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE; Octa-BDE replacement), decabromodiphenyl ethane (DBDPE; Deca-BDE replacement) and Dechlorane Plus (DPs; Deca-BDE replacement) (Stapleton et al., 2008; Wang et al., 2011) and organophosphate flame retardants (PFRs) such as tris(2-chloroethyl) phosphate (TCEP) and tris(chloropropyl)phosphate (TCPP) (van der Veen and de Boer, 2012a).

In response to consumer and regulatory pressures worldwide, alongside high production volumes and excessive use of FRs in consumer products, high levels of PBDEs (also known as legacy FRs) and their alternatives including EHFRs and PFRs have been identified in abiotic matrices such as soil, indoor air and dust (Covaci et al., 2011; Dodson et al., 2012; Newton et al., 2015), in aquatic and terrestrial biota such as fish, marine mammals and polar bears (Anh et al., 2016; de Wit et al., 2010) as well as in human biological matrices including breast milk, blood and urine (Kalantzi et al., 2004; Kim et al., 2014; Sjödin et al., 1999;

Venier et al., 2016). Hence, given the considerable FR amounts identified in abiotic matrices (e.g. air and indoor dust), as well as their hydrophobic and potentially bioaccumulative nature, human exposure to FRs as a result of unintentional dust ingestion and inhalation have been identified as substantial routes of exposure (Alves et al., 2014; Jones-Otazo et al., 2005).

To bridge this knowledge gap, the main objectives of the present study are:

a) To assess the presence of legacy and alternative FRs in three different indoor environments from two European countries (the UK and Norway)

b) To estimate and compare human intakes to a wide range of FRs via dust ingestion using the same dust samples for non-working adults and toddlers in Norwegian and British houses, as well as for working adults in British stores and offices.

2.2 Materials and Methods

2.2.1 Sampling

Ten indoor dust samples were collected from pre-existing vacuum cleaner bags (houses) in Norway (Oslo) as a part of the A-TEAM cohort sampling during November 2013 – April 2014 (Papadopoulou et al., 2016). Twenty-two indoor dust samples from pre-existing vacuum cleaner bags (10 houses, 6 stores and 6 offices; Table SI-1) were collected in Reading (UK) during August – December 2013. The UK house dust samples were collected from the houses of University of Reading employees, while UK office and store vacuum cleaner bags were collected in Reading with respect to the participant's approval and willingness to cooperate in the present study. All dust samples were sieved to <250 μm using a methanol-washed metallic sieve; this size fraction of dust is likely to be ingested according to (Yu et al., 2012). Oven-baked Na_2SO_4 (granular) was also sieved as field blank. All dust samples were kept in hexane-washed amber glass bottles and stored at 4°C till analysis.

Table 1 – Sample codes with country of origin, source, collection year, mass used (g) and flooring material

Sample code	Country	Source	Collection year	Mass used (g)	Flooring material	General remarks
H1	UK	House	2013	0.034	wall-to-wall Carpet	
H2	UK	House	2013	0.032	wall-to-wall Carpet	
H3	UK	House	2013	0.031	wall-to-wall Carpet	
H4	UK	House	2013	0.032	wall-to-wall Carpet	
H5	UK	House	2013	0.033	wall-to-wall Carpet	
H6	UK	House	2013	0.032	wall-to-wall Carpet	
H7	UK	House	2011	0.030	wall-to-wall Carpet	
H8	UK	House	2011	0.031	wall-to-wall Carpet	
H9	UK	House	2012	0.032	wall-to-wall Carpet	
H10	UK	House	2011	0.032	wall-to-wall Carpet	
H11	Norway	House	2013	0.030	Laminated floor	
H12	Norway	House	2013	0.030	Wooden floor	
H13	Norway	House	2013	0.032	Laminated floor	
H14	Norway	House	2013	0.032	Other/not-defined	
H15	Norway	House	2013	0.033	Wooden floor	
H16	Norway	House	2013	0.030	No data	
H17	Norway	House	2013	0.032	No data	
H18	Norway	House	2013	0.033	parquet	
H19	Norway	House	2013	0.032	Wooden floor	
H20	Norway	House	2013	0.031	Flooring	
S1	UK	Office	2013	0.031	wall-to-wall Carpet	Library offices
S2	UK	Office	2013	0.030	Laminated floor and carpet flooring	University offices
S3	UK	Store	2013	0.030	Laminated wood flooring	Store with office supplies, printers, office furniture
S4	UK	Store	2013	0.031	wall-to-wall Carpet; PVC floor in repair room only	Computer store
S5	UK	Store	2013	0.032	Laminated wood flooring	Luggage store
S6	UK	Office	2013	0.032	wall-to-wall Carpet	Lettings office

S7	UK	Office	2013	0.031	wall-to-wall Carpet (two floors) and laminated floor (30% 1st floor)	Bank offices
S8	UK	Store	2013	0.032	Laminated wood flooring (two floors)	Kitchenware store
S9	UK	Office	2013	0.032	Laminated flooring (1st floor) wall-to-wall Carpet (2nd floor)	Lettings office
S10	UK	Office	2013	0.032	wall-to-wall Carpet	Construction management office
S11	UK	Store	2013	0.032	wall-to-wall Carpet	Phone store
S12	UK	Store	2012	0.031	Laminated wood flooring	Toys store

2.2.2 Extraction and clean-up

The method was based on a previous study (Van den Eede et al., 2012a) with some modifications. Briefly, 30 mg of dust was extracted with 2.5 mL hexane:acetone (3:1 v/v) using ultra-sonication extraction for 10 min and vortexing for 1 min three times. 50 µL of an ISTD mix (prepared in iso-octane) were added ranging from 5 to 200 ng (^{13}C-EH-TBB-d17, ^{13}C-BTBPE, ^{13}C-BEH-TEBP-d17, ^{13}C-syn-DP, ^{13}C-anti-DP, ^{13}C-BDE-209, BDE-77, BDE-128, TCEP-d12, TPHP-d15, TDCPP-d15, TBEP-d6, and TAP). The supernatant was collected after each extraction cycle and evaporated to near dryness under a gentle stream of N_2. The combined extract was concentrated to one mL in hexane, then was loaded on aminopropyl (NH_2) silica cartridges (500 mg, 3 mL, Agilent, USA) and further fractionated with 10 mL hexane (F1) and 12 mL of ethyl acetate (F2). F1 was further concentrated, following a clean-up on an acidified silica cartridge (5%, 1 g, 6 mL) and elution with 12 mL dichloromethane. F2 was equally aliquoted into two portions, F2a and F2b. Then, F1, F2a and F2b were evaporated, reconstituted with 100 µL of iso-octane (F1 & F2a) and methanol (F2b), respectively, and then filtered. Finally, the extracts were transferred to injection vials and analyzed on GC-ECNI-MS (F1, for PBDEs and EHFRs), GC-EI-MS (F2a, for m-PFRs, except TXP) and LC-QqQ-MS (F2b, for o-PFRs and TXP). All samples were analysed in batches of 20 samples in two consecutive days, along with one SRM 2585 (NIST, USA) as quality control and two field blanks. Oligomeric PFRs were detected in all procedural blanks. The average blank value was calculated in ng/g and then subtracted from the sample o-PFR values.

2.2.3 QA/QC

Overall, 28 and 31 compounds (out of 33) were detected in house and occupational dust samples, respectively (Tables SI-2, SI-3, SI-4, and SI-5). SRM 2585 (n=2, NIST, USA) was used for QC testing and the results were in line with the literature (Table 2). Four Na_2SO_4 samples (30mg) were used as procedural blanks for background checking and results were blank corrected for all analytes by subtraction of the mean field blank values from the raw FR values (expressed in ng/g) according to (Abdallah and Covaci, 2014) (Table 3). Method limits of detection (mLOD) were calculated as three times the standard deviation of the procedural blanks. For non-detected analytes, mLOD was calculated based on signal-to-noise-ratio 3:1. According to (Van den Eede et al., 2012a), analytical method validation by matrix spiking demonstrated good accuracy ranging from 81 to 130%. Typical recoveries

ranged between 80 and 110% at both spiking levels, though occasional deviations were observed at low spiking concentrations. Precision between different days was generally below 24% relative standard deviation (RSD) at low concentrations and below 11% RSD at high concentrations.

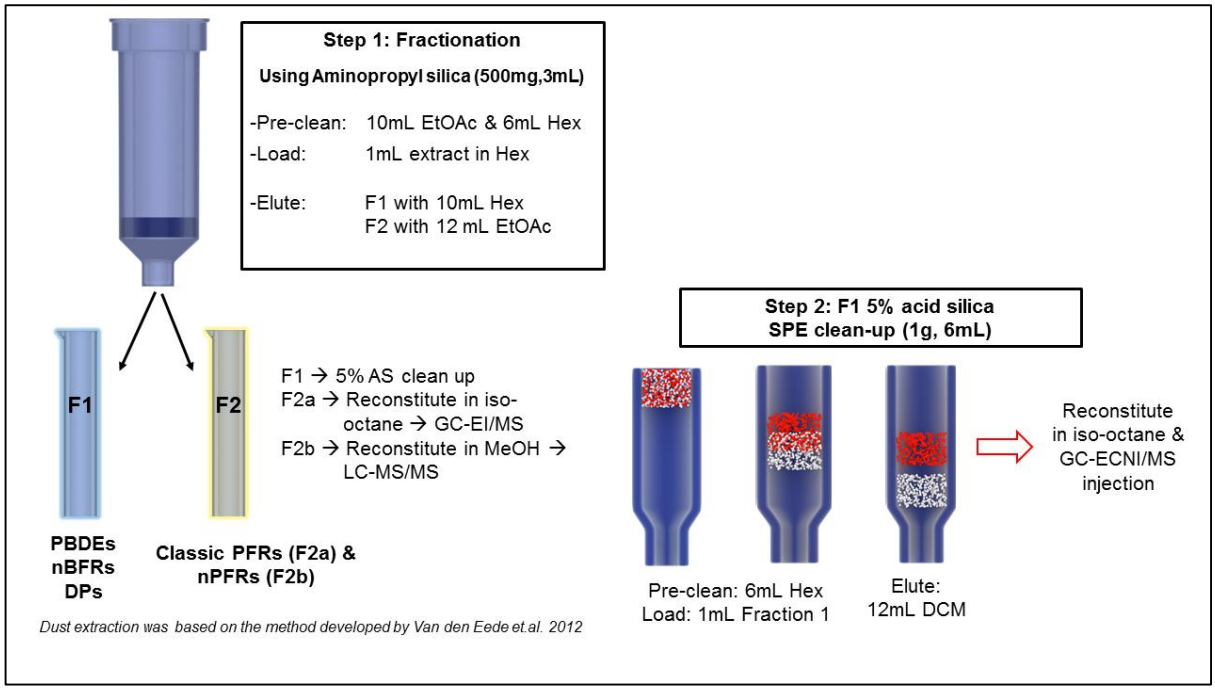


Figure 4 - Schematic representation of aminopropyl silica fractionation (step 1) and 5% acidified silica (AS) clean up (step: 2) based on (Van den Eede et al., 2012b)

2.2.4 Data Analysis

GraphPad Prism® version 7.00 for Windows, (GraphPad Software, La Jolla CA, USA) was used for statistical analysis. Compounds with detection frequencies (DF) lower than 40% were excluded from statistical analysis. Where needed, non-detections were replaced by half of mLOD for statistical analysis. All data were checked for normality using the D'agostino and Pearson tests, data that failed the normality test were log-transformed and checked for normality again. Not all data were normally distributed after log-transformation. Ordinary two-way ANOVA (Uncorrected Fisher's test, $p<0.05$) was performed to assess statistically significant differences of FRs between UK house and occupational concentrations and between UK and Norwegian houses. Due to some data failing to pass normality tests, Spearman's correlation ($p<0.05$) was employed to assess statistical dependence and correlation between FRs in the three different dust categories.

Table 2 - Accuracy of PBDEs and PFRs in SRM 2585 (N=2)

PBDEs	**Mean (ng/g)**	**STDEV**	***RSD%**	**Ref value**	**Accuracy %**
BDE28	15.3	6.8	44.1	46.9	33
BDE47	446.5	26.9	6	497	90
BDE66	22.8	1.7	7.4	29.5	77
BDE85	117.5	10.6	9.1	145	81
BDE100	35.8	3.8	10.5	43.8	82
BDE153	137.5	42.9	31.2	119	116
BDE154	99	33.8	34.2	83.5	119
BDE183	52.5	27.1	51.6	43	122
BDE209	2420.4	362.9	15	2510	96
PFRs	**Mean (ng/g)**	**STDEV**	***RSD%**	**†Ref value**	**Accuracy %**
TEHP	252.7	11.4	4.5	NM**	NM
TnPP	17	6.3	36.8	NM	NM
TnBP	266.3	21.6	8.1	197	135
EHDPHP	1049.2	57.3	5.5	NM	NM
TCEP	962.6	48.6	5	899	107
TBOEP	50460.2	2444.3	4.8	45795	110
TPHP	963	52.9	5.5	1052	92
TMPP	53435.1	652	1.2	NM	NM
TDCIPP	2221.8	69.8	3.1	1933	115
TCPP	1156	98.5	8.5	1063	109
V6	47	24	51.1	NM	NM
TDBPP	18	7	38.9	NM	NM
iDPP	122	15	12.3	NM	NM
RDP	nd	nd	nd	NM	NM
TXP	73	2	2.7	NM	NM
BDP	nd	nd	nd	NM	NM

*RSD= (Stdev/mean)*100, **NM=not measured, †taken from (Cequier et al., 2014)

Our results of PBDEs and PFRs were in compliance with SRM 2585 indicative levels and PFR levels from (Cequier et al., 2014). PBDEs levels ranged from 90 to 118% (median: 95%) with the exception of BDE-28 (33%) and BDE-183 (122%). As for some PFRs, no indicated values were available while drafting of the current manuscript, therefore no comparison could be performed. More details about sample preparation and instrumental analysis are found in SI.

Table 3 - Values of target analytes in field blanks (ng/g) and method limit of detection (mLOD)

PBDEs	**BL1**	**BL2**	**BL3**	**BL4**	**AVG**	**STEDV**	**mLOD* (ng/g)**
BDE28	1.3	1.0	1.3	N.D.	1.2	0.2	0.5
BDE47	1.0	1.0	1.3	N.D.	1.1	0.2	0.5
BDE66	N.D.**	N.D.	N.D.	N.D.	N.D.	N.D.	0.6†
BDE85	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.6
BDE100	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.6
BDE153	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.7
BDE154	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.6
BDE183	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.6
BDE209	N.D.	N.D.	5.7	4.3	5.0	1.0	3.0
EHFRs	**BL1**	**BL2**	**BL3**	**BL4**	**AVG**	**STEDV**	**mLOD**
EH-TBB	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3
BTBPE	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3
BEH-TEBP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.3
syn-DP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.6
anti-DP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.6
αTBECH	0.3	0.7	0.3	0.7	0.5	0.2	0.7
βTBECH	0.3	0.3	0.3	0.3	0.3	0.0	0.6
DBDPE	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	13.3
PFRs	**BL1**	**BL2**	**BL3**	**BL4**	**AVG**	**STEDV**	**mLOD**
TEHP	16.7	13.0	10.3	9.7	12.4	3.2	9.5
TnPP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	26.7
TnBP	12.3	10.0	11.0	12.0	11.3	1.1	3.2
EHDPHP	4.7	N.D.	N.D.	N.D.	4.7	N.D.	2.3
TCEP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	44.1
TBOEP	159.3	158.0	121.7	153.0	148.0	17.7	53.3
TPHP	4.3	N.D.	N.D.	N.D.	4.3	N.D.	2.7
ΣTMPP	3.3	N.D.	N.D.	N.D.	3.3	N.D.	5.4
TDCIPP	61.3	N.D.	71.7	N.D.	66.5	7.4	21.9
ΣTCPP	17.3	*N.D.*	15.0	N.D.	16.2	1.6	4.9
V6	1.0	0.7	0.7	0.6	0.8	0.2	0.5
TDBPP	0.9	0.7	0.6	0.6	0.7	0.2	0.5
iDPP	4.9	4.2	4.7	3.9	4.4	0.4	1.3
RDP	3.9	2.6	3.0	2.6	3.0	0.6	1.8
TXP	2.8	1.9	2.1	2.1	2.2	0.4	1.1
BDP	6.1	3.9	4.7	3.5	4.6	1.1	3.4

* mLOD= 3 x STDEV of blank;

** N.D. = not detected

† For non-detected analytes, mLOD was calculated as signal-to-noise ratio 3:1

2.3 Results and discussion

This study reports concentrations of four groups of FRs in dust from UK stores and offices (n=6 offices and n=6 stores), UK houses (n=10) and Norwegian houses (n=10). Studied chemicals included nine PBDE congeners, eight EHFRs, ten monomeric PFRs, and six oligomeric PFRs (Tables S1-8, SI-9, SI-10, SI-11, SI-12, and SI-13). Overall, the UK occupational dust samples had the highest FR contamination, followed by UK and Norwegian house dust. In an attempt to define newly identified PFRs, this group is divided in monomeric (m-PFRs), including TPHP, TnBP, TCPP, TDCIPP etc., and oligomeric (o-PFRs), including V6, BDP and RDP, using the abbreviation nomenclature as suggested by (Matsukami et al., 2015). In our study, monomeric PFRs presented the highest levels in total, followed by EHFRs, PBDEs and oligomeric PFRs.

2.3.1 PBDEs

Most PBDEs were frequently detected in UK houses and occupational dust with DF>50%, unlike in the Norwegian house dusts (Fig. 1A, B, C; Table SI-8, SI-10, and SI-12). BDE28 to BDE183 levels were relatively lower compared to BDE209, probably because of the global phase out of Penta- and Octa-BDE commercial mixtures (Dodson et al., 2012). Similar to indoor dust samples from Belgium, China and Sweden (Ali et al., 2011; Newton et al., 2015; Zheng et al., 2011) median level of BDE47 was four-fold higher in UK (12 ng g^{-1}) than in Norwegian house dust samples. Median concentrations of BDE47 (9.1 ng g^{-1}) and BDE183 (11 ng g^{-1}) in occupational dust were within the concentration range of studies from Belgium and Germany (Ali et al., 2011; Brommer et al., 2012), but lower than the USA (Michigan) and China (Batterman et al., 2010; Cao et al., 2014b). BDE209 , the most abundant PBDE congener, was detected in all samples, with median concentrations of 4,700 ng g^{-1} and 3,400 ng g^{-1} in UK occupational and house dust, respectively, which is much higher than a recent study of Norwegian classroom dust (507 ng g^{-1}) (Cequier et al., 2014) and also in the Norwegian house dust samples from the present study (160 ng g^{-1}) (Fig 1A). A statistically significant difference of BDE209 concentrations was observed between UK and Norwegian house dust ($p=0.014$). Since DBDPE acts a major replacement of BDE209, the BDE209/DBDPE ratio is indicative of the progress of phasing out Deca-BDE. The median BDE209 /DBDPE ratio was <1 for Norwegian house dust while it was >3 in UK house dust.

Table 4 Descriptive statistics of PBDEs and EHFRs measured in the UK house dust samples (N=10). Concentrations and mLOD in ng/g.

PBDEs - UK Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
BDE28	0.9	1.1	2.1	7.7	10.6	2.5	3.9	3.9	100	0.5
BDE47	2.6	4.9	10.9	38.7	684	15.7	83.8	212.0	100	0.5
BDE66	<0.6	<0.6	<0.6	9.6	9.6	9.6	9.6	0.0	10	0.6
BDE85	<0.6	1.0	3.7	66.3	126	4.7	27.6	55.2	50	0.6
BDE100	<0.6	1.8	3.2	14.3	272	5.4	35.0	89.2	90	0.6
BDE153	1.6	3.3	8.2	15.6	448	9.3	52.0	139	100	0.7
BDE154	0.9	1.9	4.1	9.1	273	5.5	34.3	89.6	90	0.6
BDE183	3.8	4.6	5.8	14.3	133	10.3	25.7	47.6	70	0.6
Σ_8PBDEs	19.4	25.3	49.9	175.6	1958.4	63	272	637		
BDE209	265	1636	3351	13843	50601	3810	11081	17437	100	3.0
Σ_9PBDEs	304	1689	3451	14194	54518	3936	11625	18710		
EHFRs - UK Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
EH-TBB	2.5	3	5.0	20.5	32.0	6.7	10.7	11.2	90	1.3
BTBPE	<1.3	<1.3	<1.3	100	100	36.3	48.7	45.2	30	1.3
BEH-TEBP	18.0	62.3	106	179	234	95	116	67.9	100	1.3
syn-DP	2.1	2.85	4.5	14.3	31.5	5.7	9.1	11.2	60	1.6
anti-DP	1.6	4.48	6.8	10.6	31.6	7.0	9.5	8.9	100	1.6
α-TBECH	<0.7	0.7	1.2	5.4	5.6	2.2	3.1	2.3	60	0.7
β-TBECH	<0.6	<0.6	0.6	1.7	1.7	1.4	1.4	0.4	30	0.6
DBDPE	531	849	1091	4594	39221	1902	5576	11924	100	13.3
Σ_8EHFRs	572	923	1245	4925	39659	2055	5774	12071		

Table 5 Descriptive statistics of PBDEs and EHFRs measured in the Norwegian house dust samples (N=10). Concentrations and mLOD in ng/g.

PBDEs – Norway Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
BDE28	<0.5	1.8	2.6	3.7	3.8	2.5	2.7	1.0	50	0.5
BDE47	<0.5	1.7	2.3	49.0	94.4	4.6	20.7	41.2	50	0.5
BDE66	<0.6	<0.6	1.3	1.3	1.3	1.3	1.3	0.0	10	0.6
BDE85	<0.6	<0.6	5.8	5.8	5.8	5.8	5.8	0.0	10	0.6
BDE100	<0.6	<0.6	23.3	23.3	23.3	23.3	23.3	0.0	10	0.6
BDE153	<0.7	<0.7	8.9	14.7	14.7	3.4	6.2	7.4	30	0.7
BDE154	<0.6	<0.6	9.6	9.6	9.6	2.6	5.2	6.3	20	0.6
BDE183	<0.6	<0.6	3.6	98.2	130	7.5	34.7	63.3	40	0.6
Σ_8PBDEs	35.6	39.0	48.3	196	285.2	50.6	94.0	111.2		
BDE209	26.7	97.3	161	536	3084	203	518	929	100	3.0
Σ_9PBDEs	98	185	258	938	3654	304	706	1151		
EHFRs – Norway Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
EH-TBB	<1.3	3.8	5.4	8.7	9.2	3.7	5.1	3.4	50	1.3
BTBPE	299	299	299	299	299	299	299	0	10	1.3
BEH-TEBP	7.9	12.1	27.1	156	426.0	38.3	89.6	132.0	100	1.3
syn-DP	<1.6	<1.6	2.6	3.4	3.4	2.2	2.4	1.1	30	1.6
anti-DP	1.6	1.8	3.1	4.7	5.1	2.9	3.2	1.5	40	1.6
α-TBECH	<0.7	1.2	1.2	2.3	3.1	1.1	1.4	1.0	40	0.7
β-TBECH	<0.6	<0.6	<0.6	<0.6	<mLOD	0.6	0.6	0.6	0	0.6
DBDPE	81.8	219	686	834	1802	484	689	536	80	13.3
Σ_8EHFRs	389	538	1025	1308	2549	831	1090	675		

Table 6 - Descriptive statistics of PBDEs and EHFRs measured in the UK stores and offices (N=12). Concentrations and mLOD in ng/g.

PBDEs - UK Stores N=12 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
BDE28	0.7	4.9	8.5	15.0	7352	13.6	677	2213	92	0.5
BDE47	<0.5	8.3	9.1	17.2	119	13.5	22	33.9	83	0.5
BDE66	1.0	1.0	3.4	5.8	5.8	2.4	3.4	3.4	17	0.6
BDE85	1.0	1.1	2.1	7.3	8.8	2.4	3.5	3.6	33	0.6
BDE100	1.9	2.1	4.0	5.5	27.9	4.2	6.6	8.7	67	0.6
BDE153	3.0	5.0	5.2	8.1	29.2	6.8	8.3	7.3	92	0.7
BDE154	1.9	2.5	3.4	4.6	14.9	3.7	4.5	3.8	83	0.6
BDE183	2.2	7.9	11.4	27.0	44.4	11.8	16.3	12.8	100	0.6
Σ_8PBDEs	13.3	32.7	48.4	90.5	7602.0	54.2	684.1	2190.5		
BDE209	92.2	2068.0	4654	4654	10874	2937	4529	3098	100	3.0
Σ_9PBDEs	118.8	2133.5	4751	4835.0	26078	3045	5897	7479		
EHFRs - UK Stores N=12 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
EH-TBB	1.6	5.3	18.1	45.2	143	13.9	29.4	41.1	92	1.3
BTBPE	13.3	18.9	27.1	40.8	79.9	28.2	32.9	21.4	67	1.3
BEH-TEBP	25.3	111.0	248	1367.0	2541	310.0	678.0	798.0	100	1.3
syn-DP	3.6	12.1	15.2	80.4	1237	27.9	152	383	83	1.6
anti-DP	<1.6	17.4	43.1	311.0	5547	49.3	555	1579	100	1.6
α-TBECH	2.3	2.9	4.5	11.4	4201	9.3	426	1326	100	0.7
β-TBECH	<0.6	0.9	1.3	4.25	1462	3.1	164	486	100	0.6
DBDPE	110	3049	5387	14259	23977	4759	8322	7646	100	13.3
Σ_8EHFRs	154	3218	5744	16115	39188	5197	10248	12101		

Such findings can be possibly attributed to low Deca-BDE usage in Norway and its unilateral ban since 2008 (EBFRIP, 2008), contrary to the EU where Deca-BDE was added to the candidate list of substances of very high concern for authorisation under the REACH regulation in 2012 with its use in consumer products eventually banned within the REACH framework earlier in 2016 (ECHA, 2012; European Commission, 2016). Unlike the Nordic indoor environment where hard-surfaced wooden flooring is more frequently applied (Roos and Hugosson, 2008), an evident preference towards carpet flooring in UK houses could potentially contribute to the higher BDE209 levels, hence the high BDE209/DBDPE median ratio in UK house dust (Jonsson, 2005). However, the median BDE209 /DBDPE ratio in the UK occupational dust was <1, probably due to the replacement of Deca-BDE in newer products in stores and offices compared to house environment.

2.3.2 Emerging halogenated FRs

Nearly all EHFRs were frequently detected (DF>50%) in all three types of dust (Fig. 1A, B, C, Table SI-8, SI-10, SI-12). DBDPE and BEH-TEBP were the most abundant EHFRs (DF>80%). In house dust, DBDPE median concentration was two-fold higher in UK (1,100 ng g^{-1}) than Norway (686 ng g^{-1}) (Fig 1A&B), which was in agreement with a previous Norwegian study (Cequier et al., 2014) and considerably higher than DBDPE levels in dust from Belgium and Sweden (Ali et al., 2011; Newton et al., 2015). The median concentration of BEH-TEBP in UK house dust **(**110 ng g^{-1}) was equivalent to recent studies from USA and Sweden (Dodson et al., 2012; Newton et al., 2015). However, BEH-TEBP median in our Norwegian dust samples was lower than a previous Norwegian study (Cequier et al., 2014)**.** The small sample size of the Norwegian dust collection analysed in the present study (n=10) may act as a limiting factor. Our dust samples were collected from pre-existing vacuum cleaner bags, whereas in (Cequier et al., 2014) dust samples (n=48) were collected using forensic filters. In UK occupational dust, DBDPE had the highest median concentration (5,400 ng g^{-1}), followed by BEH-TEBP (250 ng g^{-1}), both of which were higher than previous studies on Belgian and German office dust (Ali et al., 2011; Brommer et al., 2012), but lower than a recent Chinese study (Cao et al., 2014b). EH-TBB was several folds lower than BEH-TEBP in all three types of dust. Additional sources of BEH-TEBP in consumer products other than Firemaster 550® (EH-TBB/BEH-TEBP ratio 4:1 in commercial mixture (Stapleton et al., 2008) are suspected to be Great Lakes DP-45™ and Firemaster® BZ-54 (Chemtura Inc., USA), (Bearr et al., 2012; Zheng et al., 2015)**.** A statistically significant difference between UK house and occupational dust concentrations was found for two Deca-BDE

alternatives, DBDPE ($p<0.05$) and *anti*-DP ($p<0.05$) (Stapleton et al., 2008; Zhu et al., 2007). *Anti*-DP (median: UK occupational 43.1 ng g^{-1}; UK house 6.8 ng g^{-1}; Norwegian house 4.5 ng g^{-1}) was the predominant DP isomer compared to *syn*-DP (median: UK occupational 15.2 ng g^{-1}; UK house 4.6 ng g^{-1}; Norway 2.6 ng g^{-1}), in agreement with other studies (Cequier et al., 2014; Newton et al., 2015; Zheng et al., 2015). TBECH isomers were less frequently detected (DF<60%), with concentrations of α-TBECH consistently higher than β-TBECH, although the β-TBECH isomer was not detected in Norwegian house dust samples. This may be attributed to β-TBECH being less volatile compared to α-TBECH, leading to lower β-TBECH levels in indoor dust, unlike the 50:50 α/β TBECH isomer ratio in the commercial mixture (Tao et al., 2016; Wong et al., 2015).

2.3.3 Monomeric PFRs

All m-PFRs were frequently detected (DF>50%) in all three types of samples, apart from TnPP which was found <mLOD in all samples (Fig. 1 D, E, F; Table SI-9, SI-11 & SI-13). The median concentration of sum of 10 m-PFRs (Σ_{10}m-PFRs) (88,000 ng g^{-1}) in UK occupational dust was similar to UK house dust (79,000 ng g^{-1}), but four-fold higher than in Norwegian house dust (23,000 ng g^{-1}). Individual PFR levels in our UK house dust samples were in agreement with a recent study of UK house dust (Brommer and Harrad, 2015). TCPP and TBOEP presented the highest median concentrations in UK houses (65,000 ng g^{-1} and 8,100 ng g^{-1}, respectively) (Fig.1E), with TCPP median in UK houses two-fold higher than house dust from Japan (30,900 ng g^{-1}) and considerably lower from another Japanese house study (1,570,000 ng g^{-1}) (Kanazawa et al., 2010). In Norwegian houses, TBOEP ranked first (18,000 ng g^{-1}), nearly two-fold higher than previously reported data from USA house dust (11,000 ng g^{-1}) (Dodson et al., 2012) and in agreement with (Cequier et al., 2014). TBOEP (median 33,000 ng g^{-1}) and TCPP (median 25,000 ng g^{-1}) were also two predominant m-PFRs in UK occupational dust. Used as a plasticiser in flexible PVC, thermoplastics and food packaging, EHDPHP median concentration (20,000 ng g^{-1}) ranked as the third highest m-PFR in occupational dust, one to two orders of magnitude higher than its median in UK house dust. This may suggest that EHDPHP usage in the UK occupational environment and its application in new consumer products are steadily increasing. EHDPHP median concentration in UK house dust (2,400 ng g^{-1}) was 12-fold higher than in the Norwegian house dust, yet marginally lower than recently reported UK house dust concentrations (Brommer and Harrad, 2015). No statistically significant difference was observed between

UK and Norwegian house dust concentrations (p=0.07) or between UK house and occupational dust samples (p=0.055) for EHDPHP.

TCPP median concentration in UK houses from our study was 30 times higher compared to Norwegian house median concentration, while TCPP in Norwegian house dust was lower than levels from Belgium and another Norwegian house dust study (Cequier et al., 2014; Van den Eede et al., 2011). A statistically significant difference was found for TCPP (p=0.016) when comparing UK and Norwegian house dust concentrations. This may be possibly attributed to higher TCPP usage in the UK where TCPP is a TCEP replacement, while TCPP production and use in Norway have decreased during the past decade (ECHA, 2008a; van der Veen and de Boer, 2012a). Median concentrations of TPHP and TDCIPP in UK house dust were two-fold higher (1,500 and 750 ng g^{-1}, respectively) than in Norwegian houses (830 and 340 ng g^{-1}, respectively), but lower than TPHP and TDCIPP levels reported from the USA (Betts, 2013; Stapleton et al., 2009). TMPP and TEHP were marginally different between house dusts in the two countries, while the median concentration of TnBP was two-fold higher in Norwegian house dust compared to UK house dust. Concentrations of m-PFRs have recently been reported in floor and surface dust, sampled with dust collection filters, from the same Norwegian population group (n=61) (Xu et al., 2016). The range of m-PFRs levels in floor and surface dust (collected from the living room) from Xu et al (2016) is of the same order as the vacuum cleaner dust in the present study (n=10, Norwegian house dust). TBOEP dominated the Norwegian house environment both in our study and in Xu et al (2016). The TBOEP concentration range in the present study (1300-48,000 ng g^{-1}) was within the range of floor (727-311,000 ng g^{-1}) and settled dust (<mLOD-540,000 ng/g) from Xu et al (2016), yet with higher median concentrations (our study: 18,000 ng g^{-1}; Xu floor dust: 8,100 ng g^{-1}; Xu settled dust: 6,800 ng g^{-1}). Such results may be attributed to: a) differences in sample size; b) vacuum cleaner dust was sieved, but floor and settled were not; the large particles, like sand and hair, might dilute the contamination in dust sample; c) vacuum cleaner dust is representative of the entire house, while Xu et al (2016) only studied the living room; d) vacuum cleaner dust represents long term indoor contamination, while floor and settled dust represent short term contamination. This suggests that the sampling strategy factors such as collection season, area, tools and population selection, could potentially influence the study outcome. Therefore, researchers are advised to choose a sampling strategy firmly based on the aim and scope of their study.

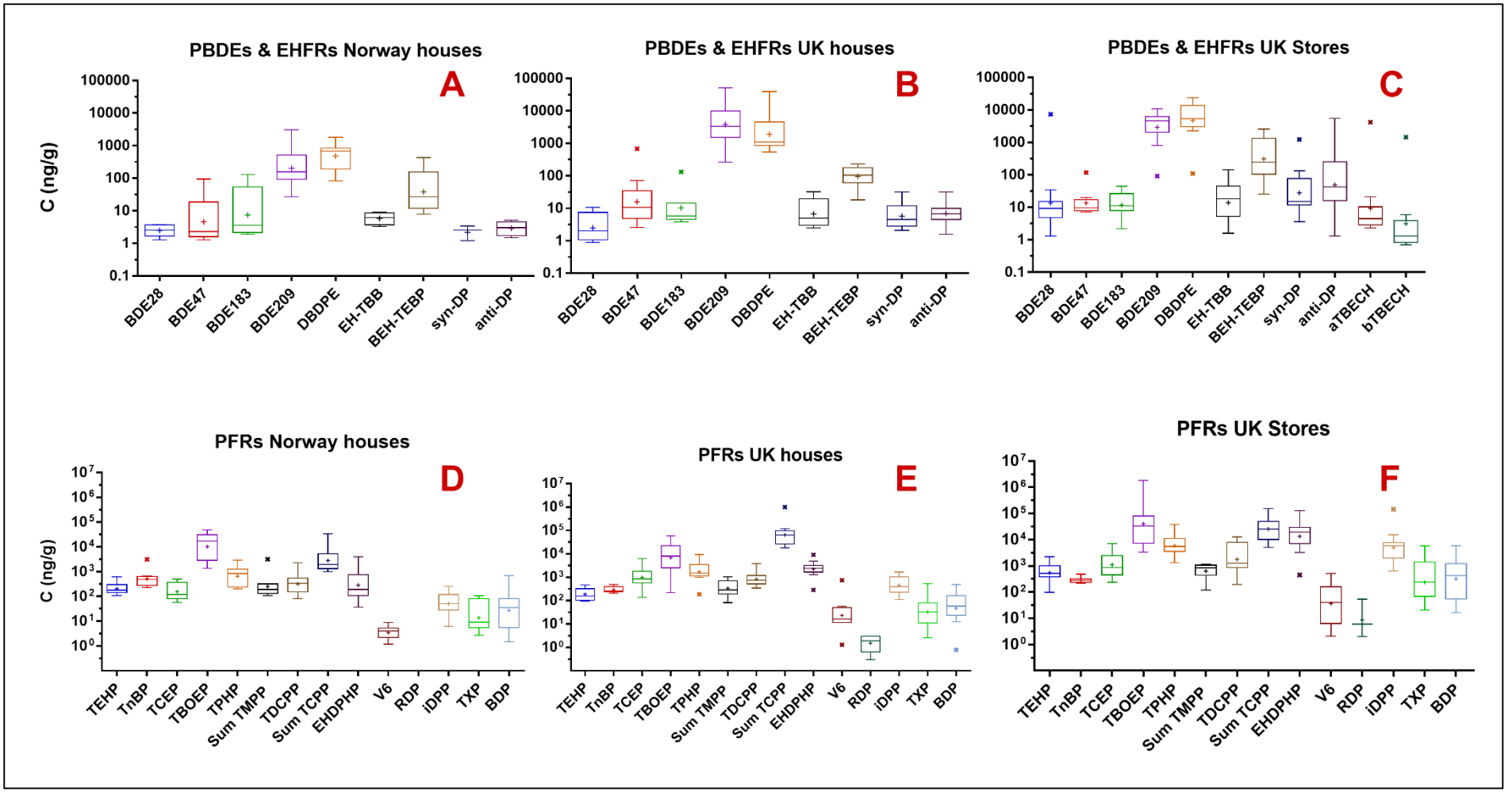


Figure 5 - Boxplots of indoor dust concentrations for selected PBDEs, EHFRs and PFRs from Norwegian houses (A&D) (N=10), UK houses (B&E) (N=10) and UK stores and offices (C&F) (N=12). Shown in the whiskers are 25th and 75th percentiles, median (central line), mean (+ symbol) and outlier (x symbol) values. All data shown are log transformed. Please note the linear scale for concertation (ng g^{-1}) on y axis.

2.3.4 Oligomeric and monomeric PFRs

Although TXP and TDBPP are considered as monomeric PFRs (Table SI-5), we will discuss them together with oligomeric PFRs (o-PFRs) due to the novel character of their environmental emissions and their usage in similar FR products (Matsukami et al., 2015). To the best of our knowledge, this is the first report of iDPP and TXP in the indoor environment. Most o-PFRs were detected in all three types of dust (DF>80%) (Fig.1 D, E, F; Table SI-9, SI-11, and SI-13), apart from RDP (no detection in Norwegian house dust) and TDBPP (DF<50% in UK and Norwegian house dust samples). All o-PFRs were frequently detected in occupational dust samples with substantially higher concentrations compared to the house dust samples. iDPP was the most abundant o-PFR in our dust samples, ranging from 600-145,000 ng g^{-1}, 110-1,700 ng g^{-1} and 6-260 ng g^{-1} in UK occupational dust, UK house dust and Norwegian house dust, respectively. Also, median concentrations of BDP (UK occupational dust 480 ng g^{-1}; UK house dust 66 ng g^{-1}; Norwegian house 35 ng g^{-1}), TXP (UK occupational dust 240 ng g^{-1}; UK house dust 26 ng g^{-1}; Norwegian house dust 9.1 ng g^{-1}) and V6 (UK occupational dust 40 ng g^{-1}; UK house dust 17 ng g^{-1}; Norwegian house dust 4 ng g^{-1}) were relatively higher than RDP and TDBPP, which were in general close to the mLOD. Maximum values for iDPP and BDP were close to 145,000 ng g^{-1} and 6,000 ng g^{-1}, respectively, both found in dust from a toy store. In a personal computer (PC) store, the maximum concentration of TXP was near 6,000 ng g^{-1}. iDPP concentrations of UK house and occupational dusts were statistically significantly different (p=0.019). We can assume that this is a result of the faster replacement rates of consumer products in the occupational environment compared to UK houses. No significant difference was found for TXP and BDP (p=0.07), possibly as a result of the small sample size analysed in the present study (10 UK houses and 12 stores and offices).

A few studies have reported the presence of oligomeric PFRs in indoor dust and SRM 2585. RDP and BDP have been identified in our dust samples, but not in SRM2585 (Table SI-6). (Brandsma et al., 2013) reported higher concentrations of BDP and RDP in house dust when collected on/around electric items than in distance. Although it has been reported in baby products and car dust since 2011, V6 may have been used in consumer products since the early 1990s considering that SRM 2585 was prepared using a pool of samples collected during mid to late 1990s (Fang et al., 2013; Stapleton et al., 2011). An average concentration of 117 ± 6 ng g^{-1} for V6 was reported by (Fang et al., 2013) in SRM2585 with LC-APCI-MS/MS, two-fold higher than our result (47 ± 23 ng g^{-1}) where LC-ESI-MS/MS was

employed for instrumental analysis. Since TDBPP, iDPP, and TXP were also present in SRM 2585 with very low levels (Table SI-6), we can assume their commercial use has been ongoing earlier than has been generally perceived or that such compounds may be impurities of PFRs, such as TPHP, TMPP and EHDPHP (Derouet et al., 1996; UK Environment Agency, 2009a).

3.5 iDPP and TXP commercial mixtures

According to the UK Environment Agency (2009), iDPP, an alkyl diaryl phosphate ester, is manufactured in the UK and distributed by Ferro UK Ltd. and ICL-IP Europe B.V. in unknown amounts so far and is used as a FR plasticiser in flexible PVC, synthetic rubber, textiles and pigment products. The registered trademarks for iDPP available in Europe are Phosflex® 390 and Santicizer® 148 with the commercial mixture composition set as 90% iDPP and <5% TPHP as a technical mixture impurity (UK Environment Agency, 2009b). Newer PFRs such as iDPP have a general impurity due to their manufacture process which potentially causes a diverse contamination profile indoors with similarly structured PFRs, e.g. iDPP with EHDPHP. In the present study, iDPP highest concentrations were found in British-based toys (145,000 ng g^{-1}) and kitchenware stores (15,000 ng g^{-1}). Extensive use of laminated wooden flooring, plasticised vinyl polymer products and displays was observed in the two stores. As legislation on the use of PBDEs and their alternatives in consumer products gets stricter, higher levels of "newer" FR are likely to be observed in the indoor environment, including iDPP. TCEP and TDCIPP will be partly restricted to 0.1% by weight in children's products from 2017 by Washington State (USA) (State of Washington, 2016), which might pave the way for replacement of earlier PBDE alternatives with newer FRs in consumer products. We may also assume that low levels of iDPP in Norwegian house dust could be due to limited commercial availability of iDPP in consumer products in the Norwegian market by comparison with the UK.

Trixylenyl phosphate (TXP) is a triaryl phosphate ester currently manufactured by Chemtura Inc. (formerly Great Lakes Chemical Corp., USA) under the registered trademark Kronitex® TXP (Chemtura Corp, 2013) and by ICL-IP Ltd. (Israel) as Syn-O-Ad® 8475 (ICL IP Inc., 2008) with an estimated usage in Europe between 100 – 1000 tonnes/year (ECHA, 2015). In 2013, the European Chemicals Agency (ECHA) indicated the use of TXP as a tricresyl phosphate (TCP) substitute and formally listed it as a "substance of very high concern" because of its potential reproductive toxicity (ECHA, 2013). Xylenols such as TXP are

naturally derived alcohols with recommended application in wire and cable insulation, fire resistant lubricants and PVC applications where low volatility and high resistance products are essential (Harper, 2003). In our study, TXP maximum concentration (5,800 ng g^{-1}) was reported in a dust sample collected from a computer store. The store's interior design was covered with PVC and carpet flooring, numerous computer displays and repair rooms where cables and wires are frequently found.

Table 7 Descriptive statistics of PFRs measured in the UK house dust samples (N=10). Concentrations and mLOD in ng/g.

PFRs - UK Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
TEHP	96.2	105	157	348	465	188	223	144	90	9.5
TnPP	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	0	26.7
TnBP	210	239	262	403	479	294	306	99	100	3.2
EHDPHP	292	1703	2375	3385	9172	2228	3010	2481	100	2.3
TCEP	138	590	873	1830	6265	991	1566	1793	100	44.1
TBOEP	225	2806	8070	24347	58745	6711	16080	19724	100	53.3
TPHP	190	1129	1509	3724	9549	1716	2737	2915	100	2.7
TMPP	83	198	293	740	1052	340	459	359	100	5.4
TDCIPP	346	523	752	1229	3792	836	1081	1019	100	21.9
TCPP	18331	27054	64546	98080	1010000	64605	152691	303883	100	4.9
Σ_{10}m-PFRs	19911	34347	78837	134086	1099519	77909	178165	332419		
V6	1.3	11.8	16.6	50.8	756	23.5	96.8	232.0	100	0.5
TDBPP	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	n.d.	n.d.	0	0.5
iDPP	114	233.0	401	1053.0	1687	452	617	506	100	1.3
RDP	<1.8	<1.8	1.9	3.1	3.1	1.5	1.9	1.2	50	1.8
TXP	<1.1	6.6	26.5	73.6	537		84.0	162.0	100	1.1
BDP	<3.4	25.8	66.8	167.0	485	47.7	116.0	144.0	100	3.4

Table 8 Descriptive statistics of PFRs measured in the Norwegian house dust samples (N=10). Concentrations and mLOD in ng/g.

PFRs - Norway Houses N=10 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
TEHP	107	144	178	302	618	206	240	161	90	9.5
TnPP	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	0	26.7
TnBP	229	281	485	649	3123	512	713	859	100	3.2
EHDPHP	37.1	108	195	818	4011	285	743	1229	100	2.3
TCEP	56.7	81	120	370	498	158	210	162	100	44.1
TBOEP	1343	2912	18364	30999	48006	10232	19145	17269	100	53.3
TPHP	202	240	830	1273	2922	656	931	826	100	2.7
TMPP	110	131	194	321	3176	252	503	943	100	5.4
TDCIPP	81	159	344	554	2306	319	511	654	100	21.9
TCPP	997	1323	1959	5431	33891	2800	5832	10122	100	4.9
Σ_{10}m-PFRs	3163	5379	22669	40717	98551	15420	28828	32230		
V6	1.2	2.2	4.1	5.3	8.8	3.5	4.1	2.4	80	0.5
TDBPP	<0.5	<0.5	<0.5	0.7	0.7	0.6	0.6	0.1	20	0.5
iDPP	6.3	28.7	51.3	119	262	51.4	80.5	76.7	100	1.3
RDP	<1.8	<1.8	<1.8	<1.8	<1.8	<1.8	n.d.	n.d.	0	1.8
TXP	2.7	5.7	9.1	81.8	105.0	13.7	32.3	41.6	100	1.1
BDP	<3.4	56.5	35.4	1240	697	27.4	118	236	80	3.4

Table 9 - Descriptive statistics of PFRs measured in the UK stores and offices (N=12). Concentrations and mLOD in ng/g.

PFRs - UK Stores N=12 (ng/g)	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%	mLOD
TEHP	97.7	389	529	1008	2259	548	743	640	75	9.5
TnPP	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	<26.7	0	26.7
TnBP	223	235	281	329	488	288	297	80.1	100	3.2
EHDPHP	457	7374	19648	29767	127686	13554	26020	33613	100	2.3
TCEP	237	456	897	2489	7185	1103	1895	2220	100	44.1
TBOEP	3371	8531	32700	82439	1.8E+06	40294	305634	647120	100	53.3
TPHP	1331	3581	5752	11251	38094	5885	8834	9917	100	2.7
TMPP	118	439	850	1068	1163	638	758	359	100	5.4
TDCIPP	195	877	1274	9827	12774	1790	3974	5243	100	21.9
TCPP	5012	10174	25751	51148	155955	25867	44714	53044	100	4.9
Σ_{10}m-PFRs	11042	32068	87682	189341	2165604	89967	392883	752240		
V6	2.1	6.3	40.4	158	511	36.5	132	192	100	0.5
TDBPP	2.6	2.6	3.0	15.1	15.1	4.9	6.9	7.1	100	0.5
iDPP	644	1990	5898	7653	145455	5083	18018	42467	100	1.3
RDP	2.0	2	6.1	53.5	53.5	8.7	20.5	28.6	100	1.8
TXP	20.8	69.2	244	1406	5820	240	935	1733	100	1.1
BDP	16.5	56.5	483	1240	5932	322	1083	1785	100	3.4

2.3.6 Correlation between FRs present in dust from different environments

Spearman's correlation revealed significant and positive correlations among low brominated PBDEs in all environments (Fig.2) in agreement with (Cequier et al., 2014) as they formulate a group of compounds with similar structural and physico-chemical characteristics and are present in the same commercial mixtures. In the occupational environment, where oligomeric PFRs were more abundant than PBDEs and EHFRs, iDPP, RDP and BDP were strongly correlated between each other, EHDPHP and TPHP ($\rho>0.9$; $p<0.01$) and TnBP, TCEP and TBOEP ($0.6<\rho<0.8$; $p<0.01$) also occurred together probably due to their application as plasticisers and FRs in similar consumer products and RDP being used as TCEP substitute due to its low release to the environment (van der Veen and de Boer, 2012a). Also, TPHP is present in the indoor environment either as an individual FR or as an impurity in the BDP and RDP technical mixtures (Mihajlović, 2015; UK Environment Agency, 2009c; van der Veen and de Boer, 2012a). In the UK house environment, V6 was highly correlated with TCEP ($\rho>0.7$; $p<0.01$) probably due to TCEP impurity in V6 formulation, while no significant correlation was achieved for Norway, where TCEP use has significantly decreased since 2003 (van der Veen and de Boer, 2012a). In UK houses, oligomeric PFRs including RDP and BDP, were strongly correlated with each other ($\rho>0.9$; $p<0.01$), while only BDP was correlated with BDE-209 ($\rho>0.79$; $p<0.01$), although they are both proposed as Deca-BDE alternatives in electronics (Ballesteros-Gómez et al., 2014). In Norwegian houses, TXP was moderately to highly correlated with Tetra-BDEs, Hepta-BDEs, and BDE-209 ($0.6>\rho>0.7$; $p<0.01$), with *anti*-DP and TCEP ($\rho>0.7$; $p<0.01$) and with TDCIPP ($\rho>0.7$; $p<0.05$). Alpha- and β-TBECH isomers were highly correlated with each other and BDE-28 ($\rho>0.9$; $p<0.01$) in all environments and with BEH-TEBP in the occupational environment ($\rho>0.7$; $p<0.01$) may be caused by the banned Tri-BDE formulations and parallel Firemaster 550® or Great Lakes DP-45™ and Firemaster® BZ-54 applications in electronic products.

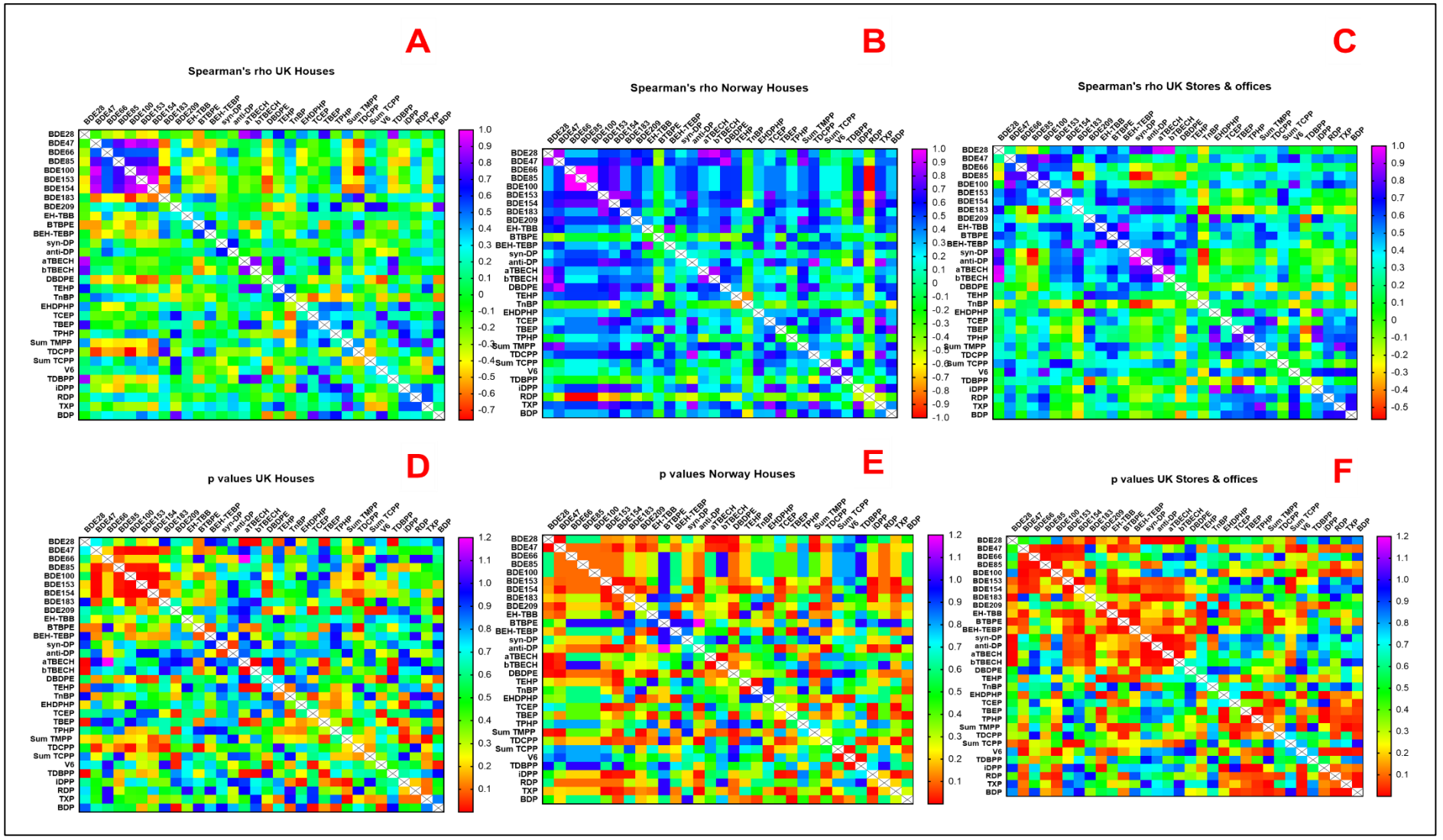


Figure 6 - Heat maps presenting Spearman's rank correlation (ρ) and p-values for all FRs in UK houses (2A&D),) Norwegian houses (2B&E) and UK stores nd offices (2C&F).

2.3.7 Human exposure assessment

Different scenarios of human exposure via dust ingestion have been estimated for oligomeric PFRs in the present study (Table-1, Tables SI-15, SI-16, and SI-17). All the exposure scenario equations and parameters used for adults and toddlers daily estimated intake to FRs were based on Eq.1 (USEPA, 1997) and table 10 (Brandsma et al., 2013). In our study, both average (20 mg/24 h for adult and 50 mg/24 h for toddler) and high (50 mg/24 h for adult and 200 mg/24 h toddler) dust intake situations were calculated. The exposure of home-based adults and toddlers were assessed with 24 h exposure duration. Intakes for adult workers in offices and stores were estimated with 8 and 24h exposure duration, given the assumption of a work day split as 16h at home and 8h at work environment. Body weight of 70 kg and 12.3 kg were used for adults and toddlers, respectively. To the best of our knowledge, this is the first study providing multi-scenario exposure assessment for this wide variety of FRs based on the same samples from two different countries. However, we recognise that the small number and representativeness of samples analysed in the present study represents a major uncertainty in these intake calculations.

Equation used for calculating daily exposure to FRs via dust ingestion (Eq. 1)

$$Daily\ Exposure\ \left(\frac{\text{ng/kg bw}}{\text{day}}\right) = \frac{Concentration\ x\ Dust\ ingestion\ rate\ x\ Exposure\ duration}{Body\ weight}$$

Table 10 – Parameters used for calculating daily exposure to FRs via dust ingestion

	Dust ingestion rate (g/day)		**Body Weight (kg)**
	Average	High	
Adults	0.02	0.05	70
Toddlers	0.05	0.2	12.3

In all scenarios (Tables SI-15, SI-16, and SI-17), much higher intakes from dust ingestion have been calculated for m-PFRs than for PBDEs, EHFRs and o-PFRs. Toddlers were found to have much higher estimated exposure to all FR than adults, due to higher dust ingestion rates (average exposure scenario 20 and 50 mg per 24 h, for adults and toddlers, respectively; high exposure scenario, 50 and 200 mg per 24 h for adults and toddlers, respectively) and lower body weight (12.3 kg for toddlers and 70 kg for adults). Close-to-floor activity and more frequent hand-mouth-contact are rationales behind using higher dust ingestion rates for toddlers. In the worst case scenario, estimated exposure of British toddlers from dust

ingestion were 890, 17,900, and 650 ng kg bw^{-1} day^{-1} for ΣPBDEs, Σm-PFRs and ΣEHFRs, respectively; while the estimated exposures for Norwegian toddlers were equal to 60, 1,600, and 40 ng kg bw^{-1} day^{-1} for ΣPBDEs, Σm-PFRs and ΣEHFRs, respectively. Contrary to the exposure of Norwegian toddlers, the estimated exposure of BDE209 for British toddlers in the worst case scenario was equal to 820 ng kg bw^{-1} day^{-1}, about 12% of the daily reference dose (RfD) (7,000 ng kg bw^{-1} day^{-1}) (Table SI-17). Based on our assessment, Norwegian stay-home adults and toddlers, have one order of magnitude lower exposure of ΣPBDEs and Σm-PFRs from average dust ingestion (50 mg) than British adults and toddlers. For TBOEP exposure with average dust intake rate, Norwegian stay-home adults (median 5.3 ng kg bw^{-1} day^{-1}) and toddlers (median 75 ng kg bw^{-1} day^{-1}) were two-fold higher compared to British counter parts (2.3 and 33 ng kg bw^{-1} day^{-1} respectively). However, Norwegian adults and toddlers were found to have lower exposure for other m-PFRs and o-PFRs, such as TPHP and BDP, set below the proposed RfD values (Table SI-16&17). (Ali et al., 2013) reported slightly higher exposure to ΣPBDEs for both adult and toddler via house dust ingestion in Kuwait compared to Norway, but lower than our British non-workers. For Σm-PFRs and ΣEHFRs, the calculated intake for adults and toddlers from Kuwait and Pakistan were lower compared to our study for Norwegians and British non-workers. In another study from Norway, slightly higher median exposure of ΣPBDEs (female 0.4 ng kg bw^{-1} day^{-1}, children 1 ng kg bw^{-1} day^{-1}) and Σm-PFRs (female 16 ng kg bw^{-1} day^{-1}, children 133 ng kg bw^{-1} day^{-1}) were reported compared to our assessment for Norwegians (Cequier et al., 2014), but lower than our British stay-home adult. With 24h of exposure (8h at work and 16h at home) (50 mg day^{-1} dust intake rate), the estimated exposures of British workers for ΣPBDEs, Σm-PFRs and ΣEHFRs were higher than Norwegian non-workers (Table SI-15&16). Estimated exposure for British workers to ΣEHFRs was two-fold higher (median 0.79 ng kg bw^{-1} day^{-1}) than British stay-home adults (median 0.36 ng kg bw^{-1} day^{-1}), unlike ΣPBDEs and Σm-PFRs exposures in these population groups. In the worst case scenario with high dust intake rate (200 mg day^{-1}), the estimated exposure for British workers to Σm-PFRs (1,040 ng kg bw^{-1} day^{-1}) was comparable to British stay-home adults (785 ng kg bw^{-1} day^{-1}), while nearly 15-fold higher than Norwegians stay-home adults (70 ng kg bw^{-1} day^{-1}).

Table 11 - The estimated daily human intake (median and maximum) to selected PFRs in different scenario (ng kg bw^{-1} day^{-1}).

Human exposure assessment for selected PFRs (ng kg bw^{-1} day^{-1})											
	Compound	Stay-home toddler (t=24h)				Stay-home adult (t=24h)				Adult workers (t=8h work +16h home)	
		UK houses		Norway houses		UK houses		Norway houses		UK Offices and Stores	
		Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum
Normal exposure scenario (dust intake 50 mg/d)	V6	0.067	3.073	0.016	0.036	0.005	0.216	0.001	0.003	0.007	0.193
	TDBPP	0.002	0.002	0.002	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
	iDPP	1.63	6.858	0.209	1.065	0.115	0.482	0.015	0.075	0.638	14.174
	RDP	0.008	0.013	0.007	0.007	0.001	0.001	0.001	0.001	0.001	0.006
	TXP	0.108	2.183	0.037	0.427	0.008	0.153	0.003	0.03	0.028	0.657
	BDP	0.272	1.972	0.144	2.833	0.019	0.139	0.01	0.199	0.059	0.657
High exposure scenario (dust intake 200 mg/d)	V6	0.27	12.293	0.066	0.143	0.012	0.54	0.003	0.006	0.018	0.482
	TDBPP	0.008	0.008	0.01	0.011	<0.001	<0.001	<0.001	0.001	<0.001	0.004
	iDPP	6.52	27.431	0.834	4.26	0.286	1.205	0.037	0.187	1.595	35.435
	RDP	0.031	0.05	0.029	0.029	0.001	0.002	0.001	0.001	0.002	0.014
	TXP	0.431	8.732	0.148	1.707	0.019	0.384	0.007	0.075	0.071	1.641
	BDP	1.086	7.886	0.576	11.333	0.048	0.346	0.025	0.498	0.147	1.643

***** Normal scenario was considered for dust intake of 20 mg and 50 mg per 24 h, for adults and toddlers, respectively; while for high exposure scenario, 50 mg and 200 mg per 24 h, respectively, dust exposures were considered for stay-home adults and toddlers. Daily exposure for working adults in UK stores and offices has been estimated as well using the same parameters (median, maximum, ingestion rate), but for 24h exposure duration (8h work + 16h at home). Body weights, 70 kg for adults and 12.3 kg for toddlers, were applied for the estimation

Given the small sample size in our study from UK stores and offices, we present here FR estimated intakes for the two dust sample groups combined as a general exposure scenario for British workers with 8h and 24h (8h at work and 16h at home) exposure duration (SI Table 15). A more elaborate view on estimated intakes for all FRs for individual offices and stores is available in SI. Briefly, in average and high dust intake rate scenarios, exposure for ΣPBDEs, Σm-PFRs and ΣEHFRs between UK office employees (SI Table 18) and UK stores employees (SI Table 19) were found to be within a comparable range. In the worst case scenario, estimated intakes for British-based toys store workers were 14-fold higher for TBOEP than EHDPHP, reaching 434 and 30 ng kg bw^{-1} day^{-1}, respectively. The second highest estimated intake for TBOEP was found for workers in a British-based store selling office supplies, electronics and furniture equal to 147 and 368 ng kg bw^{-1} day^{-1} in average and high dust intake rates, respectively. The two cases of store employees (in toys store and office supplies store) did not exceed the proposed RfD for TBOEP ($1.5x10^4$ ng kg bw^{-1} day^{-1}) in both dust ingestion rate scenarios and 8h of exposure. Human exposure via dust ingestion has never been estimated for most o-PFRs, except BDP and RDP (Brandsma et al., 2013). Among all o-PFRs, in most scenarios, the highest intakes via dust ingestion were calculated for iDPP, followed by BDP or TXP (Table 1). Considering 8h of exposure during a workday, British employees were found to have higher estimated exposure of individual o-PFRs than British and Norwegian stay-home adults (24 h) (Table 1). The worst-case scenario for iDPP was estimated for employees in a British-based toy store, where the estimated exposure was 335.4 ng kg bw^{-1} day^{-1}, nearly three-fold higher than the average dust intake scenario, set considerably below the proposed LOAEL (Table 1). In the worst case scenario for toddlers, Norwegian toddlers may have an exposure of equal to 11.3 ng kg bw^{-1} day^{-1} for BDP, while British toddlers have TXP exposure equal to 8.7 ng kg bw^{-1} day^{-1}. In contrast, for Dutch and Greek toddlers (worst case scenario), higher BDP exposures were reported equal to 1,100 ng kg bw^{-1} day^{-1} and 750 ng kg bw^{-1} day^{-1}, respectively; while their RDP exposure were also thousand-fold higher than our assessment (Brandsma et al., 2013). Based on findings in this study, exposure to TDBPP does not seem to raise major toxicological concerns for humans, as TDBPP was rarely detected in our dust samples or other environmental samples (Lopez et al., 2011).

Table 12 - Daily exposure of FRs for adults (workers) in UK stores and offices (n=12) with average and high dust ingestion rate

	Adults ng/kg bw/day - Average ingestion rate -UK Workers t=8h at work		**Adults ng/kg bw/day - High ingestion rate - Workers t=8h at work - Worst case scenario**		**Adults ng/kg bw/day - Average ingestion rate -UK Workers t=24h (8h work + 16h home)**		**Adults ng/kg bw/day - High ingestion rate - Workers t=16h (8h work +16h home) - Worst case scenario**		
	UK worker		UK worker		UK worker		UK worker		
FR	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	**RfD (ng/kg bw day)**
BDE28	0.001	0.700	0.002	1.750	0.001	0.702	0.003	1.756	
BDE47	0.001	0.011	0.002	0.028	0.003	0.142	0.008	0.354	1×10^{2}
BDE66	0.000	0.001	0.001	0.001	0.002	0.002	0.005	0.006	
BDE85	0.000	0.001	0.001	0.002	0.001	0.025	0.002	0.062	
BDE100	0.000	0.003	0.001	0.007	0.001	0.054	0.002	0.136	
BDE153	0.000	0.003	0.001	0.007	0.002	0.088	0.005	0.220	2×10^{2}
BDE154	0.000	0.001	0.001	0.004	0.001	0.053	0.003	0.134	
BDE183	0.001	0.004	0.003	0.011	0.002	0.030	0.005	0.074	
Σ_8PBDEs	0.005	0.724	0.012	1.810	0.014	1.097	0.034	2.742	
BDE209	0.443	1.036	1.108	2.589	1.082	10.674	2.704	26.685	7×10^{3}
Σ_9PBDEs	0.452	2.484	1.131	6.209	1.109	12.867	2.772	32.168	
EH-TBB	0.002	0.014	0.004	0.034	0.003	0.020	0.007	0.049	
BTBPE	0.003	0.008	0.006	0.019	0.008	0.027	0.021	0.067	
BEH-TEBP	0.024	0.242	0.059	0.605	0.044	0.287	0.110	0.716	
syn-DP	0.001	0.118	0.004	0.295	0.002	0.124	0.006	0.310	
anti-DP	0.004	0.528	0.010	1.321	0.005	0.534	0.013	1.336	
αTBECH	0.000	0.400	0.001	1.000	0.001	0.401	0.003	1.003	
βTBECH	0.000	0.139	0.000	0.348	0.000	0.140	0.001	0.349	
DBDPE	0.513	2.284	1.283	5.709	0.721	9.754	1.802	24.385	
ΣEHFRs	0.547	3.732	1.368	9.330	0.785	11.286	1.962	28.215	

TEHP	0.050	0.215	0.126	0.538	0.080	0.304	0.201	0.759	
TnBP	0.027	0.046	0.067	0.116	0.077	0.138	0.192	0.344	2.4×10^4
EHDPHP	1.871	12.161	4.678	30.401	2.324	13.908	5.809	34.769	6×10^{6}**
TCEP	0.085	0.684	0.214	1.711	0.252	1.878	0.629	4.694	2.2×10^4
TBOEP	3.114	173.333	7.786	433.333	4.651	184.523	11.629	461.307	1.5×10^4
TPHP	0.548	3.628	1.370	9.070	0.835	5.447	2.088	13.617	7×10^4
TMPP	0.081	0.111	0.202	0.277	0.137	0.311	0.342	0.778	
TDCPP	0.121	1.217	0.303	3.041	0.265	1.939	0.661	4.847	1.5×10^4
TCPP	2.452	14.853	6.131	37.132	14.747	207.234	36.867	518.085	8×10^4
Σ_{10}PFRs	8.351	206.248	20.877	515.620	23.367	415.680	58.418	1039.200	
V6	0.004	0.049	0.010	0.122	0.007	0.193	0.018	0.482	
TDBPP	0.000	0.001	0.001	0.004	0.000	0.001	0.001	0.006	
iDPP	0.562	13.853	1.404	34.632	0.638	14.174	1.595	35.435	3×10^{7}***
RDP	0.001	0.005	0.001	0.013	0.001	0.006	0.002	0.014	
TXP	0.023	0.554	0.058	1.386	0.028	0.657	0.071	1.641	
BDP	0.046	0.565	0.115	1.412	0.059	0.657	0.147	1.643	

*taken from (Cequier et al., 2014), **taken from (UK Environment Agency, 2009c),*** taken from (UK Environment Agency, 2009b)

Table 13 – Daily exposure of FRs from dust for adults (non-workers) from UK and Norway houses with average and high dust ingestion rates

	Adults ng/kg bw/day - Average ingestion rate - Non-workers t=24h				**Adults ng/kg bw/day - High ingestion rate -Non-workers - t=24h - Worst case scenario**				
	UK houses		Norwegian houses		UK houses		Norwegian houses		
FR	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	**RfD (ng/kg bw day)**
BDE28	0.001	0.003	0.001	0.001	0.002	0.008	0.002	0.004	
BDE47	0.003	0.196	0.001	0.027	0.009	0.489	0.002	0.068	1×10^2
BDE66	0.003	0.003	0.000	0.000	0.007	0.007	0.001	0.001	
BDE85	0.001	0.036	0.002	0.002	0.003	0.090	0.004	0.004	
BDE100	0.001	0.078	0.007	0.007	0.002	0.194	0.017	0.017	
BDE153	0.002	0.128	0.001	0.004	0.006	0.320	0.002	0.011	2×10^2
BDE154	0.001	0.078	0.001	0.003	0.003	0.195	0.004	0.007	
BDE183	0.002	0.038	0.001	0.037	0.004	0.095	0.003	0.093	
Σ_8PBDEs	0.014	0.560	0.014	0.081	0.036	1.399	0.035	0.204	
BDE209	0.957	14.457	0.046	0.881	2.394	36.144	0.115	2.203	7×10^3
Σ_9PBDEs	0.986	15.577	0.074	1.044	2.465	38.941	0.184	2.610	
EH-TBB	0.001	0.009	0.002	0.003	0.004	0.023	0.004	0.007	
BTBPE	0.008	0.029	0.085	0.085	0.021	0.071	0.214	0.214	
BEH-TEBP	0.030	0.067	0.008	0.122	0.076	0.167	0.019	0.304	
syn-DP	0.001	0.009	0.001	0.001	0.003	0.023	0.002	0.002	
anti-DP	0.002	0.009	0.001	0.001	0.005	0.023	0.002	0.004	
α-TBECH	0.000	0.002	0.000	0.001	0.001	0.004	0.001	0.002	
β-TBECH	0.000	0.001	0.000	0.000	0.000	0.002	0.000	0.001	
DBDPE	0.312	11.206	0.196	0.515	0.779	28.015	0.490	1.287	
ΣEHFRs	0.356	11.331	0.293	0.728	0.889	28.328	0.732	1.821	
TEHP	0.045	0.133	0.051	0.177	0.112	0.332	0.127	0.441	

TnBP	0.075	0.137	0.139	0.892	0.187	0.342	0.346	2.231	2.4×10^4
EHDPHP	0.679	2.621	0.056	1.146	1.696	6.551	0.139	2.865	6×10^{6}**
TCEP	0.249	1.790	0.034	0.142	0.624	4.475	0.086	0.356	2.2×10^4
TBOEP	2.306	16.784	5.247	13.716	5.764	41.961	13.117	34.290	1.5×10^4
TPHP	0.431	2.728	0.237	0.835	1.078	6.821	0.593	2.087	7×10^4
TMPP	0.084	0.301	0.055	0.907	0.209	0.751	0.139	2.269	
TDCIPP	0.215	1.083	0.098	0.659	0.537	2.709	0.246	1.647	1.5×10^4
TCPP	18.442	288.571	0.560	9.683	46.104	721.429	1.399	24.208	8×10^4
Σ_{10}PFRs	22.525	314.148	6.477	28.157	56.312	785.371	16.192	70.394	
V6	0.005	0.216	0.001	0.003	0.012	0.540	0.003	0.006	
TDBPP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	
iDPP	0.115	0.482	0.015	0.075	0.286	1.205	0.037	0.187	3×10^{7}***
RDP	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	
TXP	0.008	0.153	0.003	0.030	0.019	0.384	0.007	0.075	
BDP	0.019	0.139	0.010	0.199	0.048	0.346	0.025	0.498	

*taken from (Cequier et al., 2014), **taken from (UK Environment Agency, 2009c),*** taken from (UK Environment Agency, 2009b)

Table 14 - Daily exposure of FRs from dust for toddlers from UK and Norway houses with average and high dust ingestion rate

	Toddlers ng/kg bw/day - Average ingestion rate t=24h				**Toddlers ng/kg bw/day - High ingestion rate - t=24h - Worst case scenario**				
	UK houses		Norwegian houses		UK houses		Norwegian houses		
FR	Median	Maximum	Median	Maximum	Median	Maximum	Median	Maximum	**RfD (ng/kg bw day)***
BDE28	0.013	0.048	0.014	0.020	0.053	0.192	0.055	0.081	
BDE47	0.049	2.785	0.011	0.388	0.195	11.138	0.045	1.553	1×10^2
BDE66	0.039	0.039	0.005	0.005	0.156	0.156	0.021	0.021	
BDE85	0.015	0.512	0.024	0.024	0.060	2.049	0.094	0.094	
BDE100	0.013	1.106	0.095	0.095	0.052	4.423	0.379	0.379	
BDE153	0.033	1.821	0.012	0.060	0.133	7.285	0.049	0.239	2×10^2
BDE154	0.017	1.110	0.021	0.039	0.067	4.439	0.084	0.156	
BDE183	0.024	0.541	0.015	0.528	0.094	2.163	0.059	2.114	
Σ_8PBDEs	0.203	7.961	0.196	1.159	0.811	31.844	0.785	4.637	
BDE209	13.622	205.695	0.654	12.537	54.488	822.780	2.618	50.146	7×10^3
Σ_9PBDEs	14.027	221.617	1.047	14.855	56.109	886.468	4.189	59.421	
EH-TBB	0.020	0.130	0.022	0.037	0.081	0.520	0.088	0.150	
BTBPE	0.121	0.407	1.215	1.215	0.483	1.626	4.862	4.862	
BEH-TEBP	0.431	0.951	0.110	1.732	1.724	3.805	0.441	6.927	
syn-DP	0.018	0.128	0.011	0.014	0.073	0.512	0.042	0.055	
anti-DP	0.027	0.128	0.012	0.021	0.110	0.514	0.050	0.083	
α-TBECH	0.005	0.025	0.005	0.013	0.021	0.101	0.020	0.050	
β-TBECH	0.002	0.009	0.002	0.004	0.010	0.037	0.010	0.015	
DBDPE	4.435	159.435	2.789	7.325	17.740	637.740	11.154	29.301	
ΣEHFRs	5.060	161.214	4.166	10.361	20.241	644.855	16.666	41.442	
TEHP	0.638	1.890	0.724	2.512	2.553	7.561	2.894	10.049	

TNBP	1.065	1.947	1.972	12.695	4.260	7.789	7.886	50.780	2.4×10^4
EHDPHP	9.654	37.285	0.793	16.305	38.618	149.138	3.171	65.220	6×10^{6}**
TCEP	3.549	25.467	0.488	2.024	14.195	101.870	1.951	8.098	2.2×10^4
TBOEP	32.805	238.801	74.650	195.146	131.220	955.203	298.602	780.585	1.5×10^4
TPHP	6.134	38.817	3.374	11.878	24.537	155.268	13.496	47.512	7×10^4
TMPP	1.191	4.276	0.789	12.911	4.764	17.106	3.154	51.642	
TDCIPP	3.057	15.415	1.398	9.374	12.228	61.659	5.593	37.496	1.5×10^4
TCPP	262.382	4105.691	7.963	137.768	1049.528	16422.764	31.854	551.073	8×10^4
Σ_{10}m-PFRs	320.476	4469.589	92.150	400.614	1281.902	17878.358	368.602	1602.455	
V6	0.067	3.073	0.016	0.036	0.270	12.293	0.066	0.143	
TDBPP	0.002	0.002	0.002	0.003	0.008	0.008	0.010	0.011	
iDPP	1.630	6.858	0.209	1.065	6.520	27.431	0.834	4.260	3×10^{7}***
RDP	0.008	0.013	0.007	0.007	0.031	0.050	0.029	0.029	
TXP	0.108	2.183	0.037	0.427	0.431	8.732	0.148	1.707	
BDP	0.272	1.972	0.144	2.833	1.086	7.886	0.576	11.333	

*taken from (Cequier et al., 2014), **taken from (UK Environment Agency, 2009c),*** taken from (UK Environment Agency, 2009b)

2.4 Conclusions

Our study reports levels of legacy and alternative FRs in house dust samples from Norway and the UK, as well as from British stores and offices. The median levels of m-PFRs were found to be considerably higher in all environments compared to EHFRs, PBDEs and o-PFRs. Due to higher FR concentrations in British house dust samples, the estimated human intakes for FRs for toddlers in Britain were found to be higher than toddlers in Norway. However, the small number and representativeness of samples analysed in the present study should be carefully considered as it represents a major uncertainty in these intake calculations. In the worst case scenario, BDE209 estimated intake for British toddlers did not exceed the proposed RfD, yet it was considerably higher than for Norwegian toddlers, thus setting British toddlers more prone to potentially adverse health effects related to BDE209 exposure compared to Norwegian ones. This is the first study reporting human exposure via dust ingestion for most o-PFRs. Toddler estimated intakes for o-PFRs were found to be higher than stay-home adults in both countries. In the worst case scenario, iDPP estimated intake for employees in a British-based toy store was considerably higher than for other o-PFRs, together with TDBPP and TXP. This is the first study reporting considerable concentrations of iDPP and TXP in the indoor environment of Norway and the UK. iDPP and TXP together with other halogen-free alternatives such as EHDPHP, are likely to be considered in the future as substances of high toxicological interest for two reasons: a) their potential for human exposure via dust ingestion is considerable and b) their toxicological potency to humans remains unresolved. TXP reproductive toxicity to humans has been reported (ECHA, 2013; Latendresse et al., 1994), while signs of teratogenic alterations have been observed when iDPP and EHDPHP were orally administered in rodents (Robinson et al., 1986) . Also, inhalation has been proposed as a significant route of exposure for several m-PFRs (Cequier et al., 2015; Schreder et al., 2016). Therefore, future research should be considered on the possible adverse health effects of o-PFRs in humans and potential alternative routes of exposure such as inhalation and dermal uptake, as well as measuring their levels in the indoor environment.

Supporting information

Chemicals and Reagents

Standards of BDE 28, 47, 66, 85, 100, 153, 154, 183 and 209, EH-TBB, BTBPE, Dechlorane plus (*syn*- and *anti*-DP isomers), BEH-TEBP, TBECH (alpha and beta isomers) and labelled internal standards (IS) ^{13}C-BDE 209 were purchased from Wellington Laboratories (Guelph, ON, Canada). BDE 77 and 128 IS were obtained from AccuStandard Inc. (New Haven, CT, USA). Standards of tri-n-propyl phosphate (TnPP), tri-isobutyl phosphate (TiBP), tri-n-butyl phosphate (TnBP), triphenyl phosphate (TPHP), tris(2-chloroethyl) phosphate (TCEP) and tris(1,3-dichloropropyl) phosphate (TDCIPP, mixture of 2 isomers) were purchased from Chiron AS (Trondheim, Norway). Triamyl phosphate (TAP; IS) was purchased from TCI Europe (Zwijndrecht, Belgium). Labeled TPP-d15 IS and tris(2-butoxyethyl) phosphate (TBOEP) were purchased from Sigma Aldrich. Tris(1-chloro-2-propyl) phosphate (TCPP, mixture of 3 isomers) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Purity of analytical standards was >98%, except for TBOEP (>94%). Resorcinol bis(diphenyl phosphate) (PBDPP or RDP) and Bisphenol A bis(diphenyl phosphate) (BPA-BDPP or BDP) were purchased from TRC (Toronto, ON, Canada). The purities of the standards were 95.8% for RDP and 98% for BDP, respectively. Standards of isodecyl diphenyl phosphate (iDPP) were purchased from Accustandard (New Haven, CT, USA) and purity was 45% (in a mix with 55% TPHP, marketed as "Santicizer 148"), while Trixylenyl phosphate (TXP) standard was purchased from Chemos (Regenstauf, Germany) and was of technical grade. Standard stock solutions were prepared in iso-octane for PBDEs, EHFRs and m-PFRs, whereas standard stock solutions for o-PFRs were prepared in MeOH.

Indoor dust reference material SRM 2585 was purchased from the US National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Empty, pre-fritted polypropylene filtration tubes (6 mL) for silica SPE cartridge preparation and Amino Propyl (NH_2)/silica-based cartridges (500 mg, 3 mL) were purchased from Agilent. For 5% acidified silica gel preparation, concentrated sulphuric acid (H_2SO_4, >96%) was used and was purchased from Merck. Briefly, 1.9 mL of pure sulphuric acid was added drop-wise to 50 g of hexane-washed, oven-dried silica gel under continuous and vigorous stirring. Glass test tubes were cleaned by soaking for at least 12 h in an alkali solution. After washing, the tubes were rinsed with water and dried at 100 ºC for at least 12 h and burned at 400°C to remove all traces of contamination.

Target analytes and analytical characteristics

Table SI-1 – Nomenclature and analytical characteristics of the internal standards

Abbreviation	Full name	Used as	Quantifier ion (m/z)	Qualifier ion (m/z)	Category	Instrumental analysis
BDE 77	3,3 ,4,4 -Tetrabromodiphenyl ether	IS	79	81	PBDE	GC-ECNI-MS
BDE 128	2,2 ,3,3 ,4,4 -Hexabromodiphenyl ether	IS	79	81	PBDE	GC-ECNI-MS
^{13}C-BDE 209	^{13}C-labeled decabromodiphenyl ether	IS	495	497	PBDE	GC-ECNI-MS
^{13}C-anti-DP	^{13}C-syn-dechlorane plus	IS	664	662	EHFR	GC-ECNI-MS
^{13}C-syn-DP	^{13}C-anti-dechlorane plus	IS	664	662	EHFR	GC-ECNI-MS
^{13}C-EH-TBB	2-ethylhexyl-D17-2,3,4,5-tetrabromo[$^{13}C_6$]benzoate	IS	363	365	EHFR	GC-ECNI-MS
^{13}C-BEH-TEBP	Bis(2-ethylhexyl-D17)-tetrabromo[$^{13}C_6$]phthalate	IS	470	390	EHFR	GC-ECNI-MS
^{13}C-BTBPE	1,2-Bis(2,4,6-tribromo[$^{13}C_6$]phenoxy)ethane	IS	257	259	EHFR	GC-ECNI-MS
TAP	Triamyl phosphate	IS	239	169	m-PFR[1]	GC-EI-MS
TPHP-d15	Triphenyl phosphate-D15	IS	341	339	m-PFR	GC-EI-MS/LC-MS-MS
TDCPP-d15	Tris(1,3-dichloropropyl) phosphate-D15	IS	394	396	m-PFR	GC-EI-MS/LC-MS-MS
TBOEP-d6	tris-(butoxyethyl)-phosphate-D6	IS	303	202	m-PFR	GC-EI-MS
TCEP-d12	Tris(2-chloroethyl) phosphate-D12	IS	341	339	-m-PFR	GC-EI-MS

1: monomeric PFRs

Table SI-2 – Nomenclature and analytical characteristics of PBDEs and EHFRs

Abbreviation	Full name	Used as	Quantifier ion (m/z)	Qualifier ion (m/z)	Quantify against IS	Category	Instrumental analysis
BDE28	2,4,4'-Tribromodiphenyl ether	Target	79	81	BDE 77	PBDE	GC-ECNI-MS
BDE47	2,2',4,4'-Tetrabromodiphenyl ether	Target	79	81	BDE 77	PBDE	GC-ECNI-MS
BDE66	2,3',4,4'-Tetrabromodiphenyl ether	Target	79	81	BDE 77	PBDE	GC-ECNI-MS
BDE85	2,2′,3,4,4′-penta- bromodiphenyl ether	Target	79	81	BDE 77	PBDE	GC-ECNI-MS
BDE100	2,2',4,4',6-Pentabromodiphenyl ether	Target	79	81	BDE 77	PBDE	GC-ECNI-MS
BDE153	2,2',4,4',5,5'-Hexabromodiphenyl ether	Target	79	81	BDE 128	PBDE	GC-ECNI-MS
BDE154	2,2',4,4',5,6'-Hexabromodiphenyl ether	Target	79	81	BDE 128	PBDE	GC-ECNI-MS
BDE183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	Target	79	81	BDE 128	PBDE	GC-ECNI-MS
BDE209	Decabromodiphenyl ether	Target	487	485	^{13}C-BDE 209		GC-ECNI-MS
EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate	Target	357	359	^{13}C-EH-TBB	EHFR	GC-ECNI-MS
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane	Target	251	249	^{13}C-BTBPE	EHFR	GC-ECNI-MS
BEH-TEBP	Bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate	Target	464	384	^{13}C- BEH-TEBP	EHFR	GC-ECNI-MS
syn-DP	*syn*-dechlorane plus isomer	Target	654	652	^{13}C-syn-DP	EHFR	GC-ECNI-MS
anti-DP	*anti*-dechlorane plus isomer	Target	654	652	^{13}C-anti-DP	EHFR	GC-ECNI-MS
α-TBECH	alpha isomer tetrabromoethylcyclohexane	Target	79	81	BDE 77	EHFR	GC-ECNI-MS
β-TBECH	beta isomer tetrabromoethylcyclohexane	Target	79	81	BDE 77	EHFR	GC-ECNI-MS
DBDPE	Decabromodiphenyl ethane	Target	79	81	^{13}C-BDE 209	EHFR	GC-ECNI-MS

Table SI-3 – Nomenclature and analytical characteristics of monomeric PFRs

Abbreviation	Full name	Used as	Quantifier ion (m/z)	Qualifier ion (m/z)	Quantify against ISTD	Category	Instrumental analysis
TEHP	Tris(2-ethylhexyl)phosphate	Target	211	99	TAP	m-PFR	GC-EI-MS
TnPP	Tri-n-propyl phosphate	Target	99	183	TAP	m-PFR	GC-EI-MS
TnBP	tri(n-butyl)phosphate	Target	211	155	TAP	m-PFR	GC-EI-MS
EHDPHP	2-ethylhexyl-di-phenylphosphate	Target	251	250	TAP	m-PFR	GC-EI-MS
TCEP	Tris(2-chloroethyl)phosphate	Target	249	251	TCEP-d12	m-PFR	GC-EI-MS
TBOEP	tris-(butoxyethyl)-phosphate	Target	299	199	TBOEP-d6	m-PFR	GC-EI-MS
TPHP	triphenyl phosphate	Target	326	325	TPHP-d15	m-PFR	GC-EI-MS
TMPP*	tri-4-methoxythphenyl phosphate	Target	368	367	TPHP-d15	m-PFR	GC-EI-MS
TDCIPP	tris(1,3-dichloro-2-propyl) phosphate	Target	381	379	TDCPP-d15	m-PFR	GC-EI-MS
TCPP**	tris(chloropropyl)phosphate	Target	277	279	TDCPP-d15	m-PFR	GC-EI-MS

*in four isomers, ** in two isomers;

Table SI-4 - Nomenclature and analytical characteristics of monomeric PFRs and oligomeric PFRs

Abbreviation	Full name	Used as	Quantifier ion (m/z)	Qualifier ion 1 (m/z)	Qualifier ion 2 (m/z)	Quantify against ISTD	Category	Instrumental analysis
V6	tetrakis(2-chlorethyl) dichloroisopentyldiphosphate	Target	580.9->358.9	582.9 -> 234.9	584.9 -> 360.9	TDCPP-d15	o-PFR*	LC-MS-MS
TDBPP	Tris (2,3-dibromopropyl) phosphate	Target	698.6->99	696.6 -> 99.0	700.6 -> 99.0	TDCPP-d15	m-PFR	LC-MS-MS
iDPP	isodecyldiphenyl phosphate	Target	251.0->77.1	391.2 -> 251.0	391.2 -> 77.1	TPHP-d15	o-PFR	LC-MS-MS
RDP	resorcinol bis(diphenyl phosphate)	Target	575.1->77	575.1 -> 152.0	575.1 -> 419.1	TPHP-d15	o-PFR	LC-MS-MS
TXP	trixylenyl phosphate	Target	411.1 -> 105.0	411.1 -> 77.1	411.1 -> 179.0	TPHP-d15	m-PFR	LC-MS-MS
BDP	bisphenol A bis(diphenyl phosphate)	Target	693.2->367.1	694.1 -> 367.1	694.1 -> 368.1	TPHP-d15	o-PFR	LC-MS-MS
TPHP-d15	Triphenyl phosphate-D15	IS	446.0->101.9	444.0->101.9				LC-MS-MS

TDCPP-d15	Tris(1,3-dichloropropyl) phosphate-D15	IS	342.2->82.1	342.2->223.0	342.2->159.5			LC-MS-MS

*: o-PFR: oligomeric PFR

Instrumental methods

GC/ECNI-MS: Two µL of cleaned extract were injected on a DB-5 column (15 m×0.25 mm×0.10 µm) using solvent vent injection. The injection temperature was set at 92 °C, hold 0.04 min, ramp 700 °C/min to 295 °C. Injection was performed under a pressure of 0.19 bar until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.50 min, ramp 10 °C/min to 300 °C, hold 3 min, ramp 40 °C/min to 310 °C, hold 5 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The mass spectrometer was employed in selected ion monitoring (SIM) mode. Dwell times were set on 35 ms. The ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V. Methane was used as moderating gas.

GC/EI-MS: One µL of purified extract was injected on a HT-8 column (25 m×0.22 mm×0.25 µm) using cold splitless injection. The injection temperature was set at 90 °C, hold 0.03 min, ramp 700 °C/min to 290 °C. Injection was performed using a pressure of 1 bar until 1.25 min and purge flow to split vent of 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.25 min, ramp 10 °C/min to 240 °C, ramp 20 °C/min to 310 °C, hold 16 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The mass spectrometer was run in selected ion monitoring (SIM) mode. Dwell times ranged between 20 and 30 ms in different acquisition windows. The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively and the electron multiplier voltage was at 2200 V.

LC-MS/MS: For the instrumental analysis, an Agilent 1290 Infinity liquid chromatography (LC) system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 6460 Triple Quadrupole mass spectrometer (MS) was employed, equipped with a Jetstream® electrospray ionization (ESI) ion source. The LC parameters were optimised to provide both good chromatographic separation and minimal run tine, in order to maximise sample throughput. A volume of 3 µL of extract was injected on a Phenomenex (Torrance, CA, USA) Kinetex Biphenyl reversed phase column (2.1 x 50 mm, 1.7 µm), at a column oven temperature of 55 °C. The mobile phases were A: ultrapure H_2O and B: MeOH, both containing 5 mM ammonium formate. Separation was achieved using a flow rate of 0.5 mL/min and a gradient from 55 B to 94% B in 3.4 min, followed by 1 min hold before returning to the initial conditions, making the total run time of 4.5 minutes. The column is re-equilibrated for the next run during a 2.5 min post time. The source parameters were initially optimised for all main analytes individually and subsequently a set of values for these parameters were selected to provide the best response for all considered analytes. As such, the drying gas temperature was set at 350 °C, the gas flow at 3 L/min, the nebulizer at 25 psi, sheath gas temperature 400 °C, sheath gas flow 12 L/min, capillary voltage 2700 V and nozzle voltage 0 V. The MS was operated in dynamic multiple-reaction

monitoring (dMRM) mode, with 2-10 ion transitions for each analyte in their specific retention time (RT) window (RT ± 0.5 min). The Agilent MassHunter Workstation Software version B.06.00 was used for all aspects of data analysis.

Table SI 5 – Daily exposure of FRs (ng/kg bw/day) for adult workers (t=8h) in UK offices (n=6) with average and high dust ingestion rate

	S1		S2		S6		S7		S9		S10		
FR	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	**RfD (ng/kg bw day)***
BDE28	0.000	0.001	0.000	0.000	0.003	0.008	0.001	0.002	0.700	1.750	0.000	0.000	
BDE47	0.002	0.004	0.000	0.000	0.001	0.002	0.001	0.002	0.001	0.002	0.000	0.000	1×10^{2}
BDE66	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
BDE85	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
BDE100	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
BDE153	0.001	0.002	0.000	0.001	0.001	0.003	0.000	0.001	0.001	0.002	0.000	0.000	2×10^{2}
BDE154	0.000	0.001	0.000	0.000	0.000	0.001	0.000	0.001	0.001	0.001	0.000	0.000	
BDE183	0.000	0.001	0.001	0.002	0.004	0.011	0.001	0.003	0.003	0.007	0.000	0.001	
Σ_8PBDEs	0.004	0.010	0.001	0.003	0.010	0.025	0.004	0.010	0.705	1.763	0.000	0.001	
BDE209	0.482	1.206	0.076	0.190	0.186	0.465	0.608	1.519	0.545	1.363	0.009	0.022	7×10^{3}
Σ_9PBDEs	0.487	1.217	0.077	0.193	0.196	0.490	0.612	1.529	1.250	3.125	0.009	0.023	
EH-TBB	0.002	0.004	0.000	0.000	0.000	0.001	0.002	0.004	0.004	0.011	0.000	0.000	
BTBPE	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.008	0.004	0.010	0.000	0.000	
BEH-TEBP	0.059	0.147	0.002	0.006	0.008	0.020	0.083	0.208	0.242	0.605	0.005	0.012	
syn-DP	0.000	0.001	0.000	0.000	0.118	0.295	0.006	0.015	0.013	0.031	0.000	0.000	
anti-DP	0.001	0.003	0.000	0.000	0.528	1.321	0.042	0.105	0.037	0.093	0.000	0.000	
aTBECH	0.000	0.001	0.000	0.000	0.002	0.005	0.000	0.001	0.400	1.000	0.000	0.000	
bTBECH	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.000	0.139	0.348	0.000	0.000	
DBDPE	0.297	0.741	0.700	1.749	0.439	1.098	0.587	1.467	1.922	4.805	0.010	0.026	
ΣEHFRs	0.359	0.898	0.703	1.756	1.098	2.744	0.724	1.809	2.762	6.904	0.016	0.040	
TEHP	0.043	0.107	0.009	0.023	0.000	0.001	0.050	0.126	0.051	0.128	0.000	0.001	

TnBP	0.039	0.097	0.033	0.081	0.021	0.053	0.027	0.067	0.027	0.067	0.028	0.069	2.4×10^4
EHDPHP	3.023	7.557	0.580	1.449	0.306	0.766	2.272	5.680	1.070	2.676	0.043	0.109	6×10^6**
TCEP	0.040	0.101	0.023	0.056	0.039	0.097	0.052	0.131	0.199	0.497	0.521	1.304	2.2×10^4
TBOEP	3.312	8.279	0.468	1.171	0.321	0.803	3.421	8.552	2.078	5.194	0.537	1.341	1.5×10^4
TPHP	0.857	2.143	0.127	0.317	0.145	0.364	1.143	2.858	0.310	0.775	0.444	1.109	7×10^4
TMPP	0.042	0.104	0.011	0.028	0.024	0.059	0.086	0.216	0.076	0.190	0.042	0.106	
TDCPP	0.099	0.246	0.019	0.046	0.066	0.166	0.115	0.287	1.214	3.034	0.204	0.511	1.5×10^4
TCPP	14.853	37.132	0.958	2.396	14.511	36.278	5.343	13.357	2.830	7.076	0.477	1.193	8×10^4
Σ_{10}PFRs	22.307	55.769	2.228	5.571	15.436	38.589	12.510	31.275	7.856	19.641	2.299	5.747	
V6	0.003	0.009	0.000	0.001	0.001	0.002	0.004	0.010	0.000	0.000	0.000	0.001	
TDBPP	0.000	0.000	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	
iDPP	0.729	1.822	0.190	0.474	0.086	0.214	0.681	1.703	0.000	0.000	0.061	0.153	3×10^7***
RDP	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
TXP	0.007	0.016	0.002	0.005	0.004	0.009	0.035	0.087	0.000	0.000	0.007	0.018	
BDP	0.002	0.004	0.000	0.000	0.003	0.008	0.087	0.218	0.000	0.000	0.068	0.169	

*taken from (Cequier et al., 2014), **taken from (UK Environment Agency, 2009c),*** taken from (UK Environment Agency, 2009b)

Table SI 6- Daily exposure of FRs (ng/kg bw/day) for adult workers (t=8h) in UK stores (n=6) with average and high dust ingestion rate

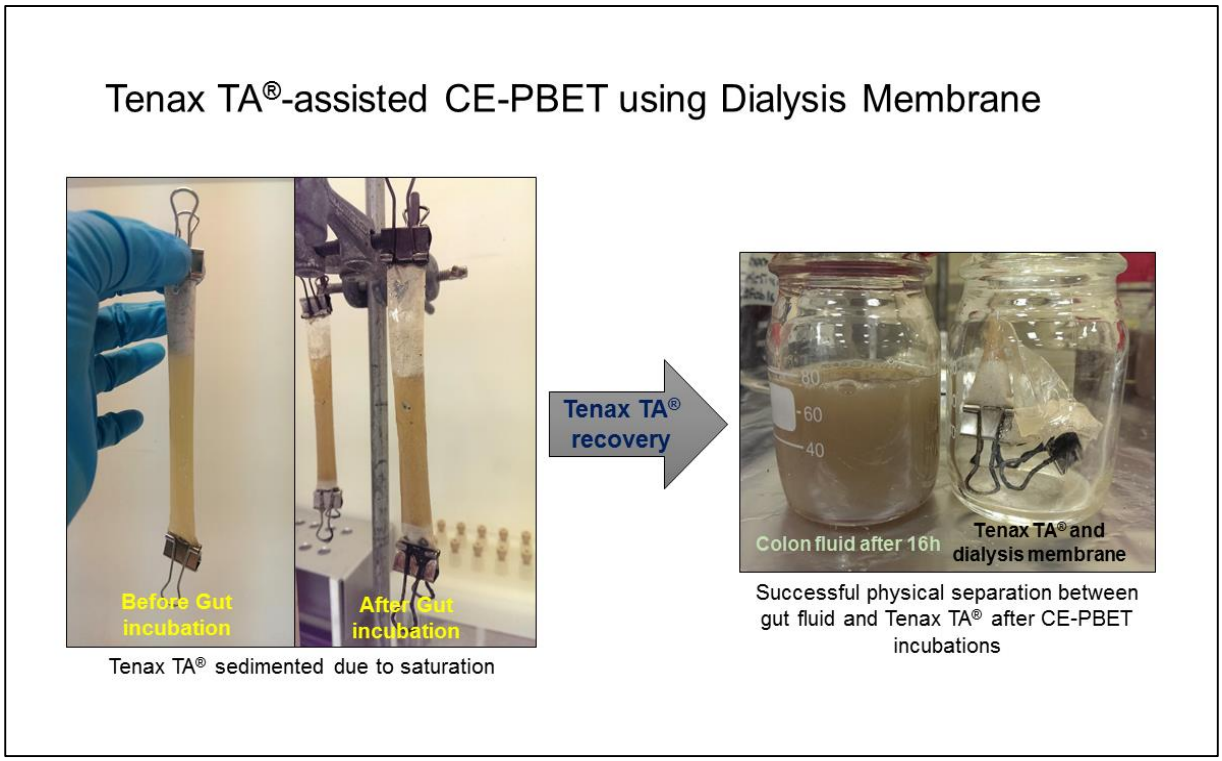
	S3		S4		S5		S8		S11		S12		
FR	Average	High	Average	High	Average	High	Average	High	Average	High	Average	High	**RfD (ng/kg bw day)***
BDE28	0.000	0.001	0.001	0.002	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.004	
BDE47	0.001	0.003	0.011	0.028	0.002	0.004	0.001	0.002	0.001	0.002	0.002	0.005	1×10^{2}
BDE66	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
BDE85	0.000	0.000	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
BDE100	0.000	0.001	0.003	0.007	0.001	0.001	0.000	0.001	0.000	0.000	0.001	0.001	
BDE153	0.000	0.001	0.003	0.007	0.001	0.002	0.000	0.001	0.000	0.001	0.000	0.001	2×10^{2}
BDE154	0.000	0.001	0.001	0.004	0.000	0.001	0.000	0.001	0.000	0.000	0.000	0.001	
BDE183	0.001	0.002	0.002	0.004	0.002	0.005	0.003	0.007	0.001	0.002	0.001	0.002	
Σ_8PBDEs	0.003	0.008	0.022	0.055	0.006	0.014	0.006	0.014	0.003	0.007	0.006	0.014	
BDE209	0.404	1.010	0.230	0.574	0.739	1.848	1.036	2.589	0.281	0.702	0.580	1.450	7×10^{3}
Σ_9PBDEs	0.407	1.018	0.252	0.629	0.745	1.862	1.041	2.603	0.284	0.710	0.586	1.464	
EH-TBB	0.001	0.001	0.005	0.012	0.014	0.034	0.001	0.002	0.001	0.001	0.003	0.006	
BTBPE	0.002	0.005	0.008	0.019	0.002	0.005	0.002	0.004	0.000	0.000	0.003	0.008	
BEH-TEBP	0.018	0.046	0.146	0.364	0.147	0.366	0.018	0.044	0.021	0.053	0.026	0.065	
syn-DP	0.001	0.002	0.001	0.003	0.002	0.005	0.001	0.004	0.001	0.004	0.001	0.003	
anti-DP	0.003	0.007	0.004	0.011	0.006	0.016	0.004	0.010	0.004	0.011	0.003	0.009	
aTBECH	0.000	0.001	0.001	0.001	0.000	0.001	0.000	0.001	0.000	0.001	0.001	0.002	
bTBECH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	
DBDPE	0.288	0.721	0.893	2.232	2.284	5.709	0.217	0.543	1.513	3.783	0.362	0.904	
ΣEHFRs	0.313	0.783	1.057	2.642	2.455	6.136	0.243	0.608	1.541	3.853	0.399	0.998	
TEHP	0.100	0.250	0.032	0.079	0.215	0.538	0.045	0.113	0.000	0.001	0.092	0.230	

TnBP	0.021	0.053	0.025	0.061	0.023	0.058	0.022	0.055	0.028	0.070	0.046	0.116	2.4×10^4
EHDPHP	1.380	3.451	1.751	4.377	2.123	5.309	3.036	7.589	1.992	4.979	12.161	30.401	6×10^{6}**
TCEP	0.099	0.249	0.250	0.624	0.124	0.310	0.071	0.178	0.063	0.157	0.684	1.711	2.2×10^4
TBOEP	147.190	367.976	1.640	4.101	2.917	7.292	8.804	22.011	4.993	12.482	173.616	434.040	1.5×10^4
TPHP	0.446	1.115	0.650	1.624	0.435	1.087	1.215	3.038	0.697	1.743	3.628	9.070	7×10^4
TMPP	0.086	0.214	0.109	0.273	0.102	0.254	0.075	0.188	0.102	0.254	0.111	0.277	
TDCPP	1.180	2.949	0.132	0.329	0.088	0.220	0.128	0.319	0.082	0.205	1.217	3.041	1.5×10^4
TCPP	2.277	5.694	2.627	6.569	1.001	2.502	1.926	4.814	0.841	2.102	3.457	8.642	8×10^4
Σ_{10}PFRs	152.781	381.953	7.216	18.041	7.029	17.573	15.324	38.311	8.798	21.994	195.013	487.532	
V6	0.006	0.016	0.049	0.122	0.048	0.120	0.015	0.038	0.001	0.003	0.011	0.027	
TDBPP	0.000	0.000	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
iDPP	0.650	1.625	0.279	0.698	0.562	1.404	1.448	3.620	0.338	0.845	13.853	34.632	3×10^{7}***
RDP	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.013	
TXP	0.023	0.058	0.554	1.386	0.026	0.064	0.009	0.023	0.179	0.447	0.134	0.335	
BDP	0.150	0.376	0.107	0.268	0.019	0.047	0.024	0.061	0.006	0.015	0.565	1.412	

*taken from (Cequier et al., 2014), ** taken from (UK Environment Agency, 2009c),*** taken from (UK Environment Agency, 2009b)

Chapter 3

Bioaccessibility of flame retardants present in indoor dust: A novel dialysis membrane method with a Tenax TA® absorption sink



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Science of the Total Environment (*submitted)*

Abstract

Human uptake of flame retardants (FRs) such as polybrominated diphenyl ethers (PBDEs) via indoor dust ingestion is commonly considered as 100% bioaccessible, leading to potential risk overestimation. Here, we present a novel *in vitro* colon-extended physiologically-based extraction test (CE-PBET) with Tenax TA® as an absorptive "sink" capable to enhance PBDE gut bioaccessibility. A cellulose-based dialysis membrane (MW cut-off 3.5kDa) with high pH and temperature tolerance was used to encapsulate Tenax TA®, facilitating efficient physical separation between the absorbent and the dust, while minimizing re-absorption of the ingested PBDEs to the dust particles. As a proof of concept, PBDE-spiked indoor dust samples (n=3) were tested under four different conditions; without any Tenax TA® addition (control) and with three different Tenax TA® loadings (*i.e.* 0.25, 0.5 or 0.75 g). Our results show that in order to maintain a constant sorptive gradient for the low MW PBDEs, a larger mass of Tenax TA® is required, hence 0.5 g of Tenax TA® were used below. Tenax TA® inclusion favoured gut bioaccessibility reaching 40% for BDE153 and BDE183, with greater increases seen for less hydrophobic PBDEs such as BDE28 and BDE47 (~60%). When tested against SRM 2585 (n=3), our new Tenax TA® method did not present any statistically significant spiking effect ($p>0.05$) between treatments. Our study describes an efficient method where due to the sophisticated design, the sorption capacity of Tenax TA® is predominantly used for PBDEs rather than media components, thus leading to simplified Tenax TA® recovery and desorption processes.

Keywords: bioaccessibility, Tenax, dialysis membrane, flame retardants, indoor dust

Highlights

- First method employing dialysis membrane for physical separation between Tenax TA® and dust
- Tenax TA® used as an absorption sink trapped in dialysis membrane mimics the situation *in vivo*
- CE-PBET performance was tested under different Tenax TA® loadings (0.25, 0.5 & 0.75 g)
- Two to three-fold bioaccessibility increase with Tenax TA® inclusion for all PBDEs

3.1 Introduction

Due to the non-polar and hydrophobic nature of hydrophobic organic compounds (HOCs) such as PBDEs, sorption to indoor dust is likely to occur via volatilisation (García-Alcega et al., 2016), marking dust ingestion as a potential major route of exposure to FRs for humans (Alves et al., 2014; Jones-Otazo et al., 2005). Hence, *in vitro* bioaccessibility studies have been deployed, assessing human exposure to contaminated indoor dust on a wide spectrum of HOCs including brominated flame retardants (BFRs) (Abdallah et al., 2012), organophosphate FR (OPFRs) (He et al., 2016a; Quintana et al., 2017), pesticides and polychlorinated biphenyls (PCBs) (Ertl and Butte, 2012) and polybrominated diphenyl ethers (PBDEs) (Yu et al., 2012). However, the lack of an adsorption sink in the various test formats may lead to risk underestimation due to the absence of constant concentration gradient (Collins et al., 2015).

To separate aqueous and solid matrices, a regenerated cellulose (RC) dialysis tubing method was employed, studying the sorption and dissolution of perchloroethane and PAHs from clay-rich materials and sewage sludges, respectively (Allen-King et al., 1995; Woolgar and Jones, 1999). RC membranes present high pH and temperature tolerances, carry no fixed charge and are highly resistant to halogenated hydrocarbons, such as PBDEs (Pollard, 1987). Tubing characteristics including length, width, membrane sealing method and molecular weight cut off (MWCO) have been evaluated. For example, 2.5 g of contaminated sewage sludge were introduced into 10 cm of dialysis tubing with a 3.5 kDa MWCO (Woolgar and Jones, 1999). Alternatively, 20 cm of dialysis tubing (29 mm width; 12-14 kDa MWCO) was used to ensure that at least 30% of the analyte mass would remain in the solid phase after equilibration (Allen-King et al., 1995). The solid material in the tubing was then introduced inside glass bottles with synthetic groundwater spiked with the HOCs of interest. During equilibration, all non-settling particles were retained inside the dialysis membrane, while dissolved organic pollutants could permeate through the membrane and equilibrate across the dialysis tubing by passive diffusion (Allen-King et al., 1995).

In the work presented here, we describe a novel *in vitro* method capable to overcome the aforementioned challenges concerning physical separation and recovery of Tenax TA® from the matrix, while facilitating its successful inclusion and performance as an adsorption sink in a previously established bioaccessibility test, namely CE-PBET, for the assessment of oral bioaccessibility of PBDEs from indoor dust. Our study aims are to systematically (a) develop

an efficient method to separate Tenax TA® and indoor dust as a matrix whilst enabling desorption of PBDEs to the Tenax TA® and (b) optimise Tenax TA® as an absorption sink for PBDEs in a colon-extended gastro-intestinal bioaccessibility *in vitro* system (CE-PBET).

3.2 Materials and methods

3.2.1 Target analytes and indoor dust

An indoor dust sample was collected in 2013 from a pre-existing vacuum cleaner bag in an office at Reading (UK) and was used during method development tests. The dust sample was sieved to <250μm, a particle cut off likely to be ingested by humans (Yu et al., 2012), using a hexane-washed, metallic sieve and stored in hexane-washed, amber glass bottles at +4ºC. Concentrations of all target analytes in all dust samples were determined using methods described elsewhere (Kademoglou et al., 2017). Briefly, 30 mg of dust was extracted with 2.5 mL hexane:acetone (3:1) using ultra-sonication extraction for 10 min and vortexing for 1 min three times. The combined extract was concentrated to 1 mL and loaded on aminopropyl (NH_2) silica cartridges (500 mg, 3 mL, Agilent, USA) and further fractionated with 10 mL hexane. The eluate was then further concentrated, following a clean-up on an acidified silica cartridge (5%, 1 g, 6 mL) and elution with 12 mL dichloromethane. The dust extracts were then evaporated, reconstituted with 100 μL of iso-octane and filtered (0.45 μm). Finally, the extracts were transferred to injection vials and analyzed on GC-ECNI-MS. Standard reference material for indoor dust SRM 2585 (organic contaminants in house dust), purchased from the US National Institute of Standards and Technology (NIST, USA), was used for method validation. Both SRM 2585 (used for method validation) and dust samples (0.5g) (used for method development) were spiked at environmentally relevant concentrations (200 ng; 200 μL of PBDEs native standard mix 1 ng/μL prepared in iso-octane and 100 μL nBFRs native standard mix 2 ng/μL prepared in toluene) and the validity of the spiking was confirmed analytically for both the SRM 2585 and the dust. After spiking, samples were shaken for 2h on an orbital shaker and allowed to stand inside a fumehood for 6h before the gastro-intestinal extraction for the solvent to evaporate, thus facilitating compound interactions with the matrix (Ballesteros-Gómez et al., 2016).

3.2.2 Dialysis membrane

Approximately 16 cm of standard grade, flexible and transparent regenerated cellulose (RC) dialysis membrane with 3.5 kDa MWCO and 18 mm flat width (1.1mL/cm) (Spectra/Por™ 3, SpectrumLabs Inc., USA) was used to encapsulate the Tenax TA® beads. The membrane length and flat width were selected for the sample volume to be added in the membrane using an online tool provided by SpectrumLabs Inc. (http://www.spectrumlabs.com/dialysis/dtCalc.html), allowing for tube sealing with 19mm metallic clips. MWCO selection for the RC membrane is primarily governed by the molecular weight (MW) of the biological molecules of the GI compartments and the target analytes of our study. The MWCO was selected to be over three-fold higher than the highest MW of the target analytes (*i.e.* BDE183 MW: 722 Da). The diffusion of PBDEs across the membrane was aided by the addition of 10mL of GIT fluid inside the RC membrane/Tenax TA® system.

3.2.3 Gastro-intestinal Extraction

The gastro-intestinal extraction test involved three compartments, namely stomach (1h; pH=2.5), small intestine (4h; pH=7) and colon (16h; pH=6.5) tested in sequential mode (Fig. 1). Fed CE-PBET conditions were achieved by the addition of dietary components into stomach and colon incubations as described in (Tilston et al., 2011) and all media were prepared in deionised H_2O (dH_2O). All experiments were conducted in triplicate. Gut media aliquots (80 mL) were added into clean, amber 100 mL Duran® glass bottles, sealed with PTFE-lined screw caps and stored at -20ºC prior use if necessary. Tenax TA® beads were cleaned prior use to remove fine particles by ultrasonication with 40mL acetone (x2), 40mL acetone:hexane 1:1 (x2) and 40mL hexane (x2) for 10 min in each sonication step. Tenax® TA was then allowed to air-dry at 105ºC overnight and was stored in a hexane-washed, Duran® bottle inside a desiccator. A short video demonstration of the Tenax TA® inclusion in the RC dialysis membrane is available online https://figshare.com/s/e7312fa7d177b35bc7d0 .Before employment, the RC dialysis membrane was soaked in ultra-pure H_2O at room temperature for 45 min under continuous stirring to remove any preservatives such as glycerine and sodium azide. The RC membrane was then thoroughly rinsed with dH_2O and one side sealed with a 19mm hexane-washed, metallic clip. Using a small glass funnel, Tenax TA® (0.5 g) was added inside the RC membrane, followed by 10 mL of stomach medium. The tubing was then sealed using

another metallic clip. Then, 0.5 g of indoor dust were added in the remaining 70 mL of stomach fluid and the RC membrane/Tenax TA® system was introduced to the bottle (Fig 1A). A solid-to-liquid (S/L) ratio 1:140 was achieved, thus preventing any bioaccessibility underestimation due to poor dissolution (Abdallah et al., 2012; Dean and Ma, 2007). The bottles were placed at 45^{o} angle inside a temperature-controlled waterbath at 37 ^{o}C and rotated at 130 rpm for 1h, mimicking the GIT peristaltic movement. After 1 h, the samples were removed from the waterbath and, due to the continuous character of CE-PBET, stomach fluid was converted to small intestine media (SI) by addition of bile salts (0.5 g/L) and pancreatine (1.78 g/L) with pH adjusted to 7 using saturated $NaHCO_3$. The small intestine incubation continued as above for 4h (Fig 1B). The RC membrane/Tenax TA® system was then removed from the bottle and was allowed to sediment for 15min. Due to its hydrophobic character, unsaturated Tenax TA® floats on top of the small intestine fluid inside the membrane (Fig. SI 1). Tenax TA® was trapped on the one side of the membrane, while the other side was carefully unsealed. The small intestine fluid inside the membrane was carefully collected (≈8 mL), was subsequently combined with the remaining 70 mL from the incubation and stored at +4 ^{o}C prior to liquid-liquid extraction (LLE).

The transition between the small intestine and colon compartments was achieved by physical transfer: the dust was recovered from the 70 mL of small intestine media by centrifugation (3500rpm, 15min), then added to 70 mL of colon medium. Using the same RC membrane and Tenax TA® as in the small intestine compartment, approximately 8 mL of pre-warmed colon medium were added and sealed with the metallic clips as described for the stomach compartment, re-introduced into the bottle where the indoor dust was re-suspended using the colon medium and incubated for 16h (Fig 1C). At the end of the colon incubation, the dust pellet was recovered by centrifugation as before and stored at -20^{o}C for extraction. Finally, Tenax TA® was recovered using clean cotton wool filtration, the colon fluid was passed through cotton wool, combined with the remaining 70 mL of colon fluid and stored at +4 ^{o}C for LLE (Fig 7). The cotton wool pieces from filtration together with the Tenax TA®, the RC membrane and the metallic clippers were collected in one bottle for ultra-sonication assisted extraction. More details on Tenax TA® filtration and recovery are available at SI.

3.2.4 Extraction and clean up

Before extraction, all samples were spiked with 200 ng of internal standard (ISTD) mix (100 μL of 2 ng/μL) prepared in toluene (PBDEs: BDE77 for BDE28, 47 and 100, BDE128 for

BDE153, 154 and 183 quantifications; nBFRs: ^{13}C-EH-TBB-d17, ^{13}C-BTBPE, ^{13}C-BEH-TEBP-d17 for EH-TBB, BTBPE and BEH-TEBP, respectively) and shaken on an orbital shaker for 1h. Gut fluids were subjected to a LLE using 30 mL hexane/ethyl acetate 3:1 v/v twice (Fig.8 – step 1). Two mL of acetone were added to enhance separation, when necessary. A gel-like emulsion bilayer (mainly lipid and carbohydrates) was developed, especially in the colon compartment. Oven-baked Na_2SO_4 (400 ^{o}C; powder) was added in the combined LLE extracts to absorb all remaining water residues and dissolve the gel-like emulsion. All samples were then allowed to settle for 1h at room temperature and the extracts were collected by centrifugation (3500rpm, 15min). The residual dust and the recovered Tenax TA® beads (together with the glass wool and the metallic clips) were subjected to ultra-sonication assisted extraction for 15 min using 30mL acetone/hexane 1:3 v/v twice (Fig. SI-2 – step 2 & 3). After each step, the extracts were collected by centrifugation (3500 rpm, 15 min).

All extracts collected from each step were combined, evaporated to 1mL hexane using Syncore ® Analyst evaporator (Buchi, Switzerland) and then loaded onto Florisil® cartridges (2g, 6mL), using a slightly modified method published elsewhere (Van den Eede et al., 2012b) (Fig. SI2 – step 4). Briefly, Florisil® cartridges were pre-cleaned with 10 mL ethyl acetate and 6 mL of hexane; our target analytes were eluted using 20 mL hexane. This eluate was further concentrated to 1mL (in hexane) and then subjected to SPE clean-up on 5% acidified silica (5% AS) (2 g, 6 mL). The 5% AS cartridges were pre-cleaned with 6 mL hexane and 3 mL dichloromethane and then all extracts from the Florisil® step were loaded onto the SPE silica column. Our target analytes were eluted using 16 mL hexane and 8 mL dichloromethane and after collection, all eluates were concentrated near dryness under a gentle stream of N_2, reconstituted in 100 μL of toluene and then filtered (0.45 μm). Finally, the samples were transferred to injection vials, biphenyl (40 ng) was added as an injection recovery standard and analysed by GC-EI-MS. Further details about sample preparation and instrumental analysis are available at SI.

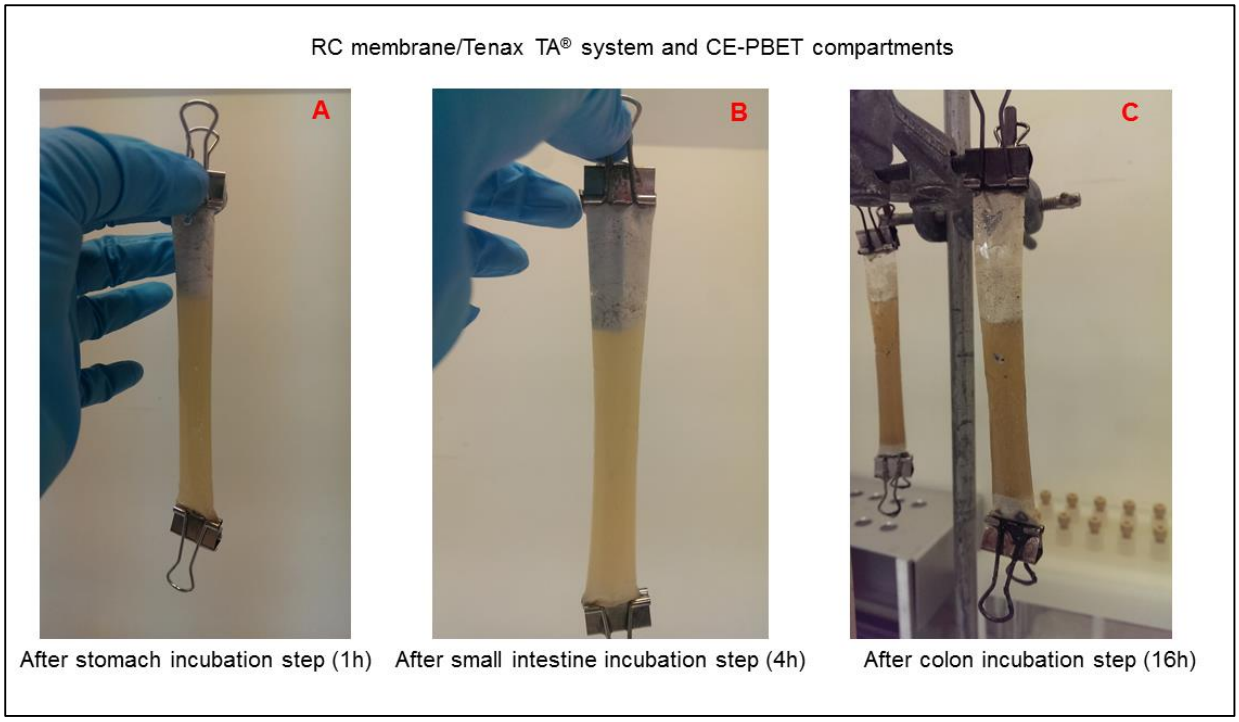


Figure 7– Stepwise representation of RC membrane /Tenax TA system after incubation of each CE-PBET compartments, namely (A) stomach, (B) small intestine and (C) colon. Please note the unsaturated Tenax TA floating on top of the water based gut medium (A &B), while the saturated part sediments after the end of colon incubation (C).

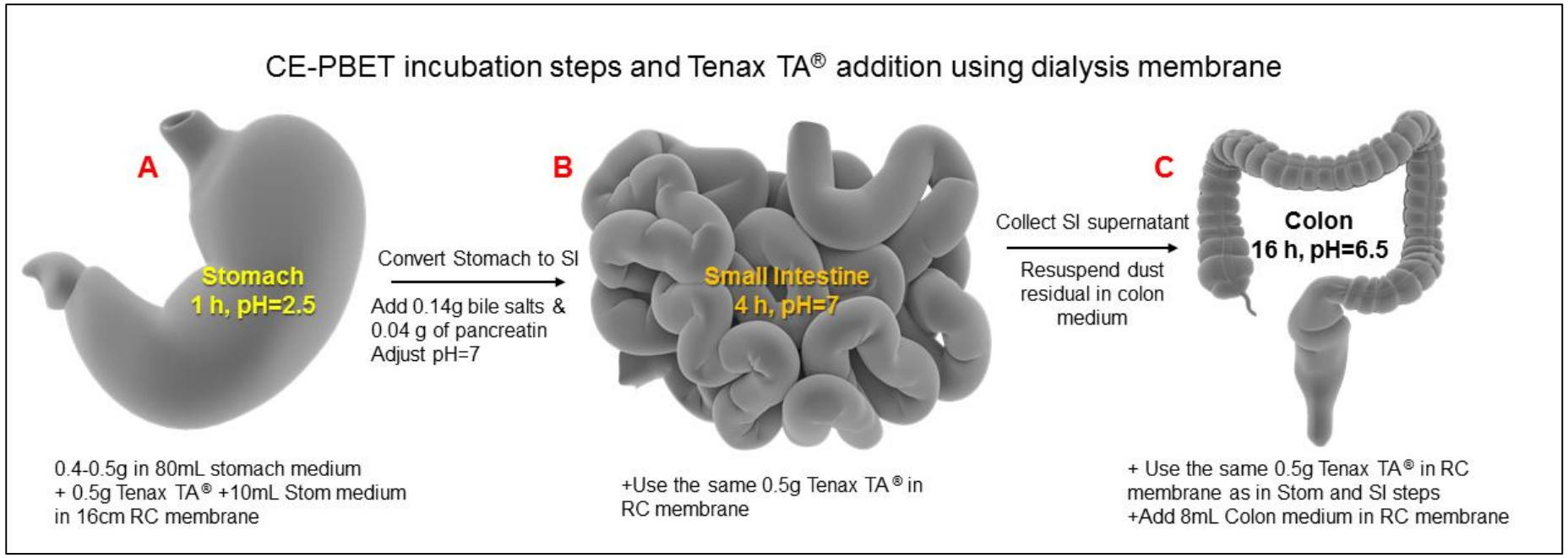


Figure 8 – Schematic representation of CE-PBET gut compartments and parameters (*i.e.* stomach (1h, pH=2.5), small intestine (SI) (4h, pH=7) and colon (16h, pH=6.5)) using 0.5g Tenax TA® added in 16cm of RC dialysis membrane

3.2.5 Data analysis

Bioaccessibility can be expressed as a mass (*e.g.* ng of a contaminant solubilised in the GI tract), a concentration (ng/g of a contaminant in dust) or as a fraction expressed in percentage (BAF%) (Guney and Zagury, 2016). In our study, bioaccessibility was determined according to (García-Alcega et al., 2016) using Eq. 2, where mass FR (SI+colon+Tenax TA®) is set as the sum of FR mass (ng) determined in small intestine (SI), colon and Tenax TA® compartments of CE-PBET system and mass (dust residual) is the mass (ng) determined in the dust residual collected after 16h-incubation of CE-PBET colon compartment which is considered as the non-bioaccessible fraction.

$$Bioaccessibility\ \%\ (BAF\%) = \frac{mass\ FR\ (SI + Colon + Tenax\ \text{TA®})}{mass\ FR\ (SI + Colon + Tenax) + mass\ (dust\ residual)}\ x\ 100$$

(Eq.2)

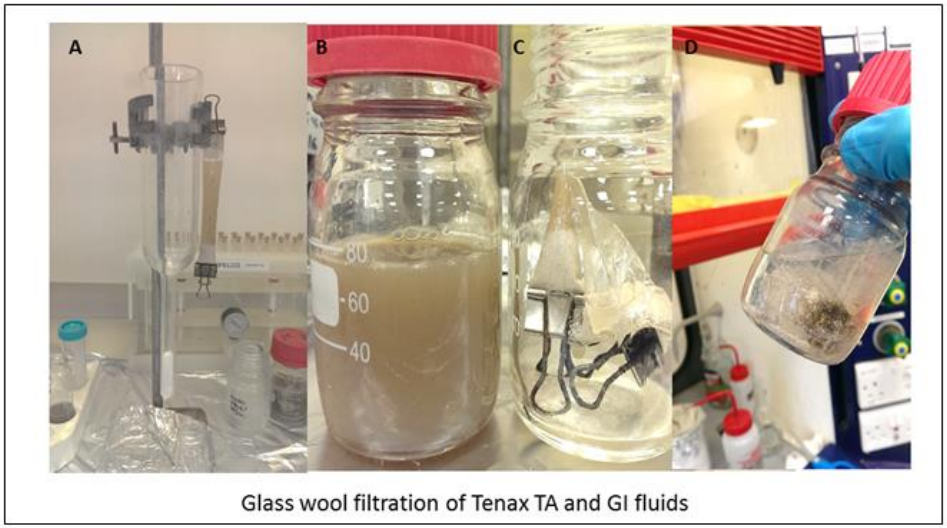
GraphPad Prism® version 7.04 for Windows (GraphPad Software, La Jolla CA, USA) was used for statistical analysis. Prior to statistical analysis, all BAF% were converted into fractions and arc-sine transformed. This mathematical transformation is necessary for statistical analysis of results set in percentages in order to equalise variances (R. R. Sokal and Rohlf, 1995). Multiple t-tests (unpaired; $p<0.05$) were performed to assess statistically significant differences among the different Tenax TA® amounts added (sections 3.1 and 3.2), whereas ordinary two-way ANOVA (Uncorrected Fisher's test, $p<0.05$) was performed to assess statistical differences for bioaccessibility with and without the addition of Tenax TA® in SRM 2585 method validation (section 3.3).

3.2.6 Quality assurance and quality control

All samples were analysed in triplicate together with oven-baked, laboratory-grade sand (procedural blank) and SRM 2585 (n=3, NIST, USA) was used for method validation and QC testing. Concentrations of our target analytes in method blanks were all below method limit of detection (mLOD) (0.05 ng/µL). RC membrane and Tenax TA® blanks were extracted for FR background contamination prior use and all values were found below mLOD. Extraction efficiency (%) was assessed for SI, colon, Tenax TA® and residual dust compartments by spiking experiments (see SI Table 2). Briefly, 100 ng of native PBDEs (100 µL of 1 ng/µL) in iso-octane and 200 ng of native EHFRs (100 µL of 2 ng/µL) in toluene were spiked to SI and

colon media, Tenax TA® (0.5 g) and dust (0.5 g). All samples were shaken on an orbital shaker for 1h. Finally, 30 mL of the corresponding extraction medium was added in each compartment, following the same sample preparation processes as before. Extraction efficiency values for all target analytes were >60% in all CE-PBET compartments, except BDE100 efficiency which was 52% and 54% in Tenax TA® and residual dust, respectively. Despite the moderately lower extraction efficiency for BDE100 in comparison to the other target analytes, the relative standard deviation (RSD%) of the method for BDE100 was 6%. Given the low deviation and variability, no correction was performed for BDE100.Glass test tubes were cleaned by soaking for at least 12 h in an alkali solution. After washing, the tubes were rinsed with water and dried at 100 ºC for at least 12 h and burnt at 400°C to remove all traces of contamination.

Sample preparation and Tenax TA® recovery



Glass wool filtration of Tenax TA and GI fluids

Step1: SI and colon fluids

- Collect SI and Colon fluids (spin 15 min at 3500rpm)
- Spike 200ng ISTDs
- Add 30mL Hex/EtOAc 1:3 (x2)
- R'n'R shake for 1h
- Subject for LLE (x2)
- Collect extracts + add Na_2SO_4
- Spin 15 min at 3500rpm
- Collect organic phase
- Subject to Florisil® fractionation & SPE clean-up (F1 only)

Step 2: Tenax TA® recovery

- After SI incubation, filter SI fluids using glass wool
- After colon incubation, filter Tenax TA® from RC membrane using glass wool filtration
- Collect glass wool filters from SI and colon fluid filtration in one bottle
- Chop RC membrane in 4 smaller pieces
- Add the 19mm metallic clippers for extraction as well

Step 3: Tenax TA® and residual dust

- Spike 200ng ISTDs
- Add 30mL Ace/Hex1:3 (x2)
- R'n'R shake for 1h
- Subject for ultrasonication (x2)
- Collect extracts + add Na_2SO_4
- Spin 15 min at 3500rpm
- Collect organic phase
- Subject to Florisil® fractionation & SPE clean-up (F1 only)

Step 4: Before Florisil ® fractionation

- Collect all extracts (approx 50-60mL each) from SI, colon, Tenax TA® and residual dust
- Concentrate to 1mL using a BUCHI Syncore® evaporator
- Extracts ready (1mL in hexane) for Florisil® fractionation & SPE clean-up (F1 only)

Figure 9 – Schematic representation of sample preparation of CE-PBET fluids and residual dust, as well as Tenax TA recovery using glass wool filtration

Table 15 – Extraction efficiency (%) for small intestine and colon compartment using LLE, Tenax and residual dust with ultrasonication assisted extraction. All samples were assessed in triplicates (n=3).

	Small Intestine (n=3)			Colon (n=3)			Tenax (n=3)			Residual dust (n=3)		
Target analyte	AVG%	STDEV	RSD%*	AVG%	STDEV	RSD%	AVG%	STDEV	RSD%	AVG%	STDEV	RSD%
BDE-28	74.8	6.0	8.0	76.8	9.2	12.0	66.7	0.1	9.0	71.9	6.5	9.0
BDE-47	87.7	2.9	3.3	82.9	1.9	2.3	77.1	0.1	8.5	68.0	5.7	8.5
BDE-100	69.2	9.4	13.6	77.7	10.5	13.5	54.2	0.1	6.0	52.0	3.1	6.0
BDE-153	58.6	0.03	4.4	77.7	0.1	16.6	89.0	0.1	10.0	92.9	6.0	6.5
BDE-154	96.7	0.0	2.6	79.3	13.2	0.2	103.7	0.1	10.0	86.0	5.8	6.7
BDE-183	92.2	0.1	13.8	66.2	0.1	17.9	90.3	0.00	0.1	65.5	0.0	0.1
EH-TBB	113.2	0.1	6.8	85.5	0.01	0.7	103.0	0.2	19.0	103.4	0.2	18.8
BTBPE	80.4	0.1	13.3	61.9	0.04	6.4	82.2	0.2	14.0	68.1	0.1	13.7
BEH-TEBP	64.1	0.01	1.6	98.0	0.1	6.9	76.0	0.2	16.0	83.7	0.1	16.4

*RSD%= (STDEV/AVG)*100

3.3 Results and discussion

3.3.1 Tenax TA® optimisation

The addition of Tenax TA® in CE-PBET considerably increased the bioaccessible fraction (%BAF) of all target analytes, illustrating the value of Tenax TA® as an adsorbent matrix for HOCs. Different masses of Tenax TA® were added to the CE-PBET system to optimise the adsorbent sink to ensure exhaustive FR desorption from indoor dust. PBDE-spiked indoor dust samples (n=3) were tested under four different conditions; (A) without any Tenax TA® addition (control) and with three different amounts of Tenax TA®, namely 0.25 g (B), 0.5 g (C) and 0.75 g (D). The same length of RC dialysis membrane (16cm) and mass of dust (0.5 g) was used in all treatments. Our results show that Tenax TA® enhanced gut bioaccessibility for PBDEs by approximately two-fold (Fig. 10) and the bioaccessible fraction was significantly different ($p<0.001$) between the controls (no Tenax) and with Tenax TA® addition, for all target analytes (Fig. 2). For example, with no Tenax TA® (control), the bioaccessible fraction of the low brominated PBDEs, BDE28 and BDE47, was 37.7% and 32.8%, respectively, whereas their BAF% increased with 0.25 g Tenax TA® inclusion to 55.1% and 54.9%, respectively. A trend to decreasing BAF% with increasing degrees of bromination for PBDEs can be seen for the control treatments and the different amounts of Tenax (Fig 2). Such findings are in agreement with Fang and Stapleton (2014), where a negative relationship between gut bioaccessibility and PBDE physicochemical properties such as degrees of bromination, MW and log Kow was described (Fang and Stapleton, 2014).

Few studies describe the influence of Tenax TA® inclusion on gut bioaccessibility of organic pollutants from solid matrices such as indoor dust or soil. CE-PBET and Tenax TA® were employed to assess FR gut bioaccessibility and for a wide range of low and high MW FRs present in indoor dust including BDE47, BDE100 and BDE183; in their experimental design, Fang and Stapleton (2014) used 0.5 g of Tenax as an absorptive sink but the effects of varying Tenax TA® content were not reported (Fang and Stapleton, 2014).

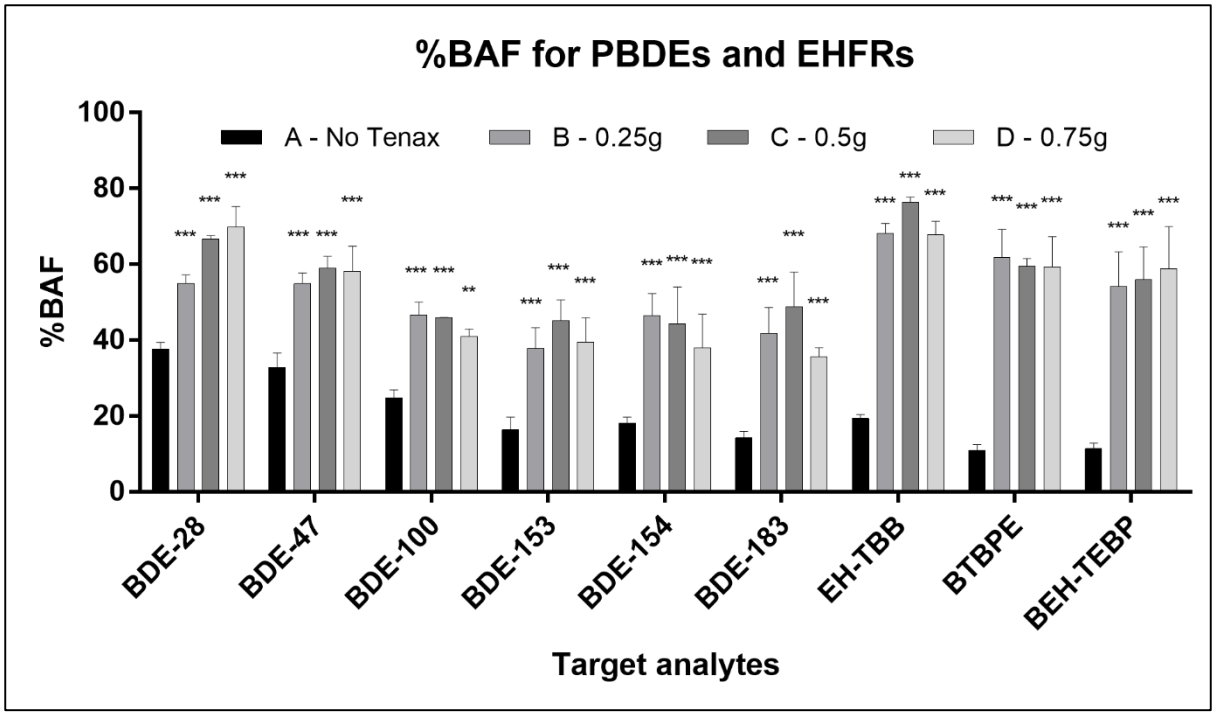


Figure 10 – CE-PBET bioaccessibility fraction (%BAF) of PBDEs without any Tenax TA® addition (control, A) and CE-PBET with Tenax TA® addition in three different amounts; i.e. 0.25g (B), 0.5g (C) and 0.75g (D). Statistically significant differences shown here (**; $p<0.01$ and ***; $p<0.001$) were established between the control (A) and all Tenax TA® treatments (B, C, D). Bar charts represent average values of triplicates. Error bars represent one standard deviation.

In a study assessing PAHs bioaccessibility in soils from China, 0.25 g of Tenax TA® were added into a PBET *in vitro* system (Li et al., 2015). According to Li et al (2015), this mass was five-fold higher than the small intestine organic matter (OC), thus allowing sufficient sorption capacity for the PAHs mobilized during their study (Li et al., 2015). Varying the content of Tenax TA® (0.25, 0.5 and 0.75 g) in the CE-PBET system studied here, showed few statistically significant differences for our analyte recoveries. Here, statistically significant differences among the three Tenax TA® amounts tested were found only for BDE28 bioaccessibility as an exception; some increase in BDE28 BAF% with Tenax TA® content, rising from 55.1% with 0.25 g Tenax TA® to 66.7% with 0.5 g (0.25 g vs 0.5 g; $p=0.017$) and 69.9% with 0.75 g Tenax TA® added (0.25 g vs 0.5 g; $p=0.006$) was observed. These results reflect the physicochemical properties of this FR as a low MW tri-BDE congener; Tenax TA® is a hydrophobic sink and the calculated log K_{ow} (EpiWeb) shows that BDE28 (log Kow 5.88) is less hydrophobic than BDE47 (log Kow 6.77) and hence greater amounts of the adsorbent may be needed to capture all of the released BDE28. For all other

analytes, there were no statistically significant differences in BAF% among the varying Tenax TA® amounts tested. Given the a) high sorption capacity of Tenax TA®, b) the broad range of physical properties (MW, water solubility and log K_{ow}) of our FRs mobilised from the ingested matrix and c) the relatively high Tenax TA® mass recovery (Fig 11), 0.5 g of Tenax TA® were selected and subsequently used below. Our results show that in order to maintain a constant sorptive gradient for the low MW PBDEs, a larger mass of Tenax TA® is required, since 0.25 g of Tenax TA® was not enough to sustain an exhaustive *in vitro* gut extraction for all target analytes.

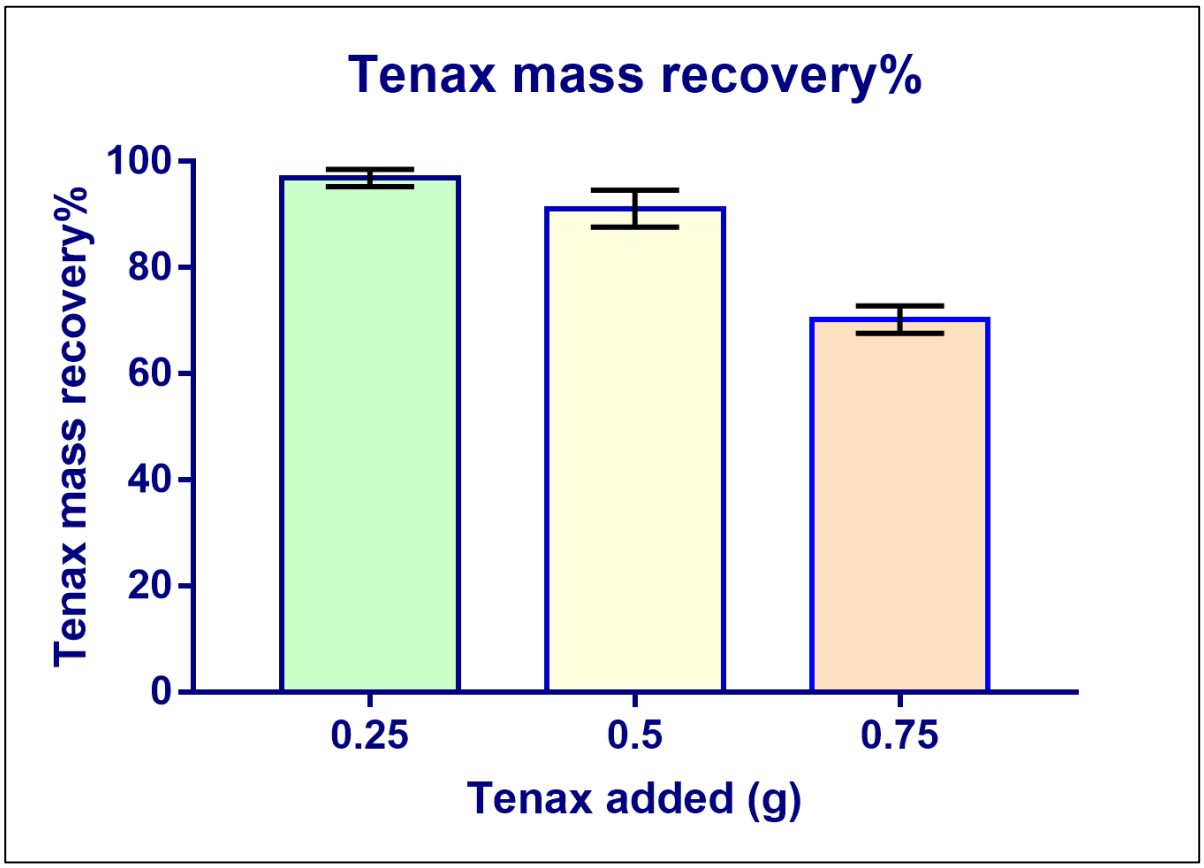


Figure 11 Bar chart presenting Tenax TA mass recovery% in different amounts of Tenax TA tested (n=3). Error bars represent one standard deviation

3.3.2 Tenax TA® sorption capacity to PBDEs and EHFRs

An assessment of PBDE release via the gut and Tenax TA® sorption capacity with respect to the three CE-PBET compartments was conducted per batch, not in sequential mode. Briefly, a fresh Tenax TA® sample (0.5 g) was incubated using a new RC dialysis membrane before the initiation of each CE-PBET compartment. All Tenax TA® samples were then collected and subjected to extraction and clean up, along with the gut fluids and the residual dust as previously described. The scope of this assessment was to evaluate Tenax TA® absorption

threshold relative to each CE-PBET compartment. Given the destructive character of the CE-PBET format per batch in section 3.2, Eq. 1 was not suitable for calculating PBDE sorption capacity on the different Tenax TA® batches. Hence, a modification of Eq.2 was used and PBDE sorption capacity (%) was determined using equation 2 (Eq. 3), where mass FR in Tenax TA® is the FR mass (ng) determined in each Tenax TA® sample incubated per batch during each CE-PBET compartment and mass FR in gut fluid is FR mass (ng) determined in CE-PBET gut fluids separately.

$$Sorption\ (\%) = \frac{\text{mass FR in Tenax TA®}}{mass\ FR\ \text{in Tenax TA®}\ + mass\ \text{FR in gut fluid}}\ x\ 100$$

(Eq. 2)

Shown in figure 12 and 13 are the results from PBDEs and EHFRs sorption to Tenax TA® in the three different CE-PBET compartments with respect to their incubation step. PBDE sorption to Tenax TA® results should not be considered as total PBDE bioaccessibility, but as the component attributable to Tenax TA® as an absorptive sink. Within the stomach compartment, BDE28 and BDE47 presented higher sorption on Tenax TA® (43.7 % and 25.6%, respectively) compared to PBDEs with higher bromine content such as BDE154 and BDE183 where Tenax TA® sorption ranged from 7.0 % to 8.8 %, respectively. Colon sorption to Tenax TA® was similar to small intestine for BDE28 (60.0 % and 66.2 %, respectively, whereas it was found repeatedly higher than small intestine for all the other target analytes (Fig.3) without any considerable differences, except BDE183 sorption on Tenax TA® which was nearly two-fold higher in the colon in comparison to small intestine (52.6 % and 36.1 %, respectively). Hence, both the "solvent" capacity of the medium and the "sink" capacity of the Tenax TA® are required to achieve optimum extraction of FRs from dust as a matrix. Besides Tenax TA®, our results further support the idea of dietary components addition in CE-PBET acting as additional mechanism liaising FR mobilisation, especially in the lipid-rich colon compartment as reported by (Tilston et al., 2011).

Desorption processes occurring in the GIT are usually dynamic, hence allowing organic contaminants mobilisation from the matrix to the gut fluids. As a result, FRs are readily absorbed via the gastro-intestinal membrane barrier towards blood and lymph circulation (Oomen et al., 2000). Tenax TA® inclusion in CE-PBET utilises the strong sorption properties and affinity of Tenax TA® to organic pollutants, mimicking the GIT absorption potential *in vivo* (Cui et al., 2016). Time-dependent kinetics and the duration of CE-PBET incubation steps have been established and discussed previously by (Tilston et al., 2011) with

respect to the GIT absorption processes *in vivo*. Therefore, in the present experimental design, kinetic tests assessing FR time-dependent release and sorption on Tenax TA were not practiced. Our overall goal was to assess FR sorption capacity with respect to the three CE-PBET compartments given the already established and validated gut absorption time settings. Moreover, bioaccessibility is governed by a) the physicochemical properties controlling diffusion, sorption and partitioning potential of the organic pollutant, b) the nature of the solid matrix where the pollutant sorption occurs (e.g. indoor dust and organic matter) and c) the *in vitro* test configuration and settings affecting a method's performance, hence pollutant bioaccessibility (Collins et al., 2015; Reichenberg and Mayer, 2006).

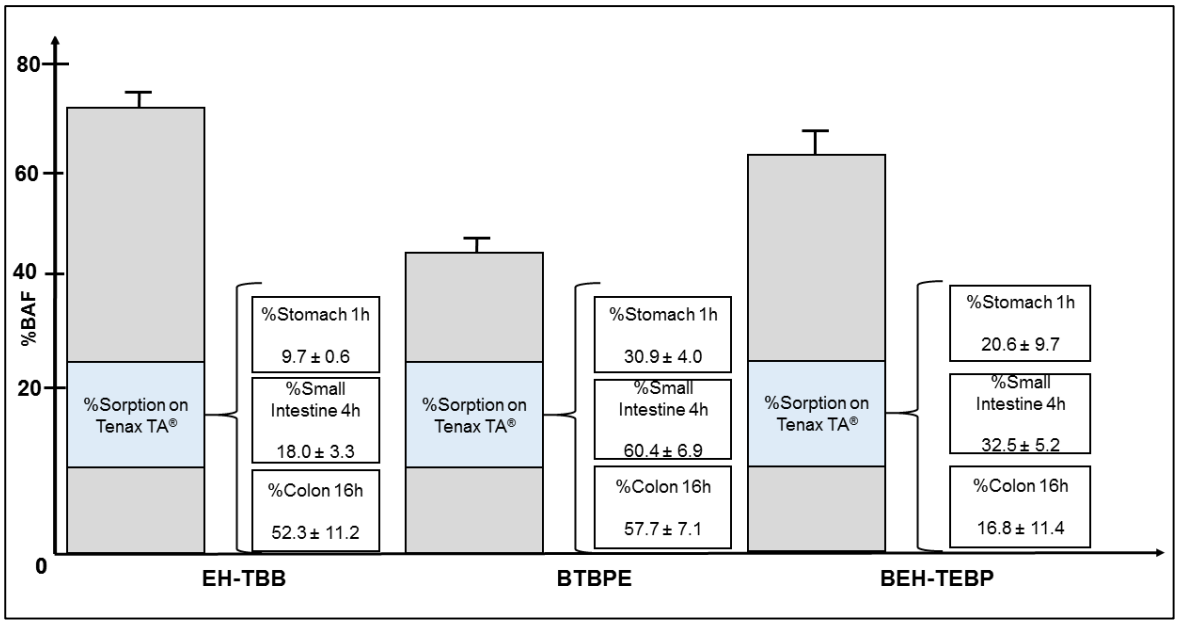


Figure 12 – Bar charts presenting selected EHFRs bioaccessibility (BAF%), EHFRs sorption on Tenax TA® and release separately in stomach (1h), small intestine (SI; 4h) and colon (16h) compartments. Bar charts represent average BAF% and %sorption values of triplicates. Error bars represent one standard deviation.

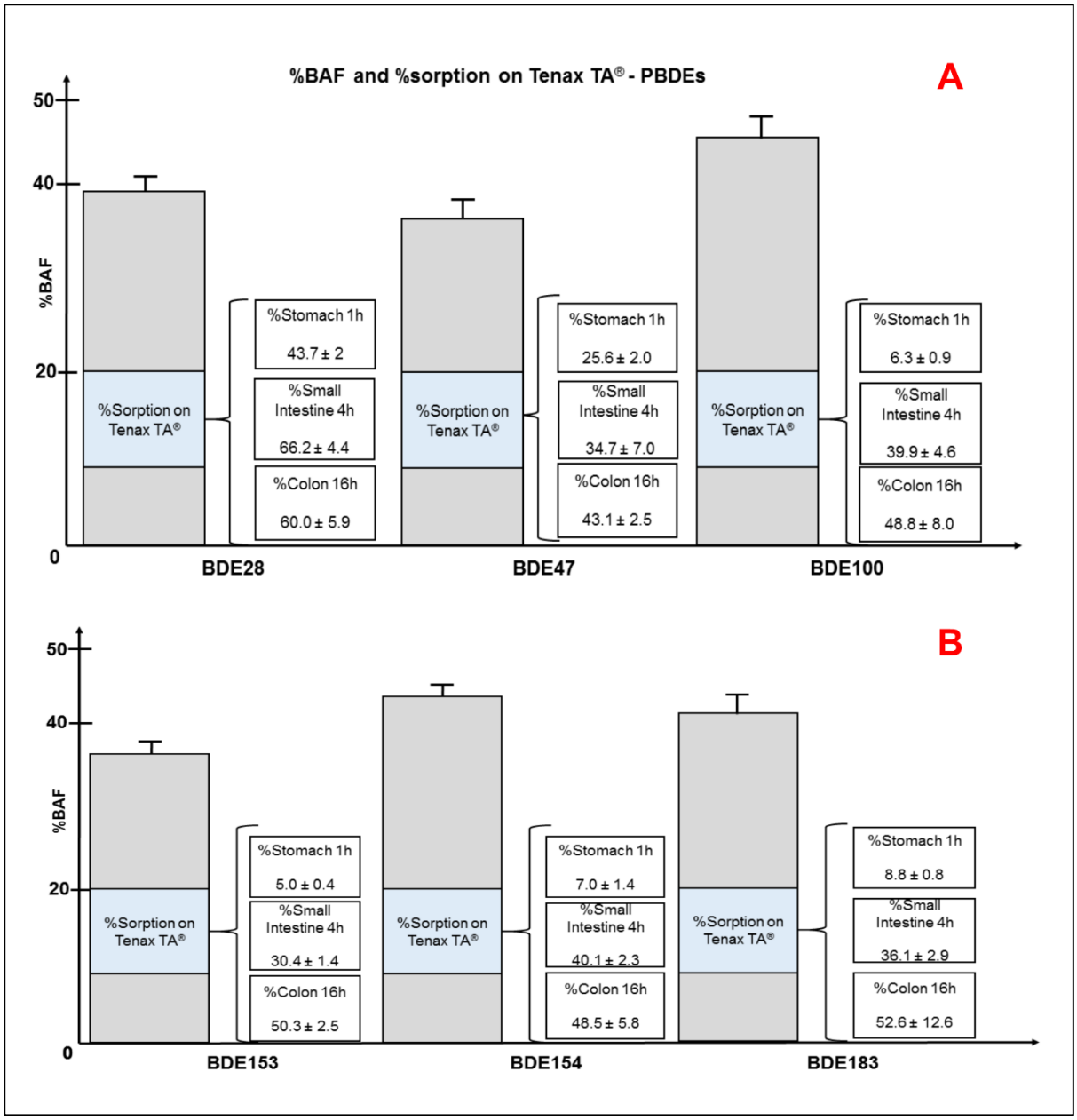


Figure 13 – Bar charts presenting PBDEs bioaccessibility (BAF%), PBDEs sorption on Tenax TA® and release separately in stomach (1h), small intestine (SI; 4h) and colon (16h) compartments. Bar charts represent average BAF% and %sorption values of triplicates. Error bars represent one standard deviation.

3.3.3 Method validation using SRM 2585

The above CE-PBET parameters were validated using SRM 2585 as a well-characterised and homogenous dust sample and the results are shown in Fig. 14. Bioaccessibility was studied using a) CE-PBET without the adsorption sink, b) CE-PBET with the addition of 0.5 g of Tenax TA® and c) FR-spiked SRM 2585 (100 ng) to evaluate greater FR contamination levels under environmentally realistic conditions using the same homogenous dust sample. As observed for dust samples from houses in Reading, statistically significant differences ($p=0.03$) were found for all target analytes when comparing CE-PBET with and without Tenax TA® addition (Fig 4). Again, BAF% using Tenax TA® rose between approximately two fold (BDE153 and BDE183) with greater increases seen for the less hydrophobic FRs such as BDE28 and BDE47 (nearly 3-fold bioaccessibility increases, respectively). No statistically significant spiking effect was found between the two SRM 2585 treatments (spiked and non-spiked) which both included 0.5 g of Tenax TA® and different FR contamination levels did not present any considerably different bioaccessibility values from the same dust matrix.

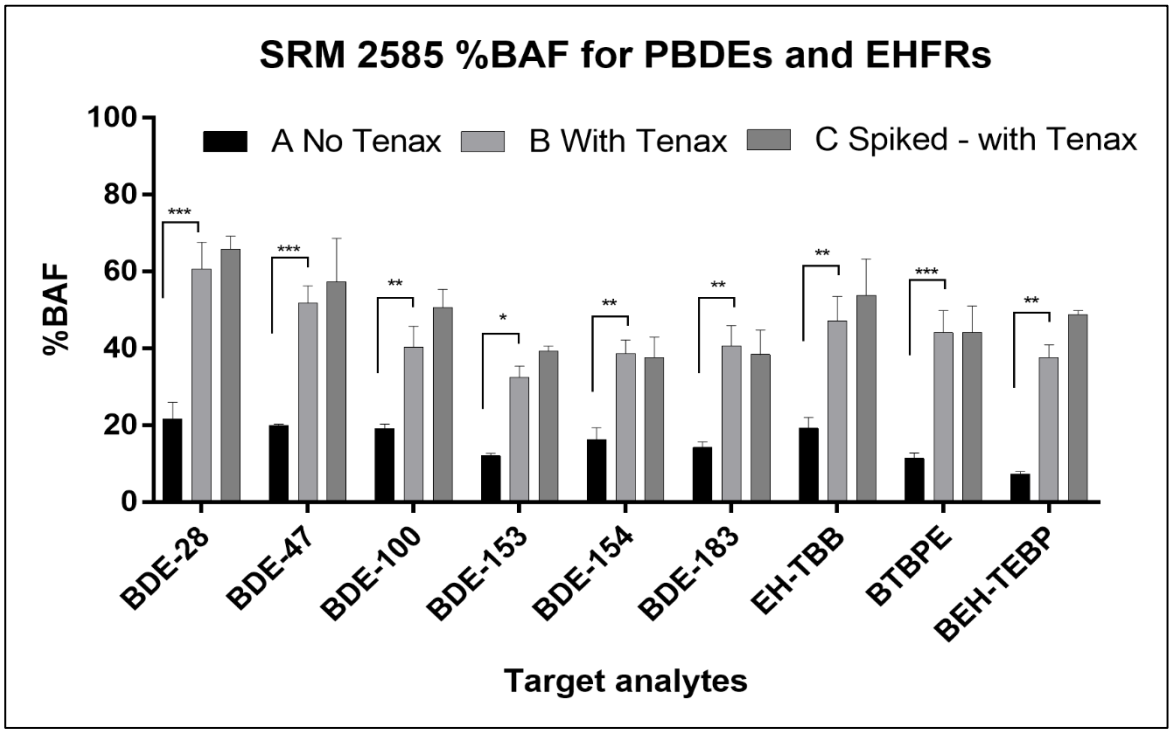


Figure 14 – CE-PBET bioaccessibility fraction (%BAF) using SRM 2585 without Tenax TA® inclusion (A), with Tenax TA® inclusion (B) and artificially spiked SRM 2585 and Tenax TA® (C). Statistically significant differences shown here (*; $p<0.05$, **; $p<0.01$ and ***; $p<0.001$) were established between SRM 2585 BAF% values without (A) and with

Tenax TA® (B) inclusion. Bar charts represent average values of triplicates. Error bars represent one standard deviation.

3.3.4 Proposing a unified test approach

This study describes an efficient method to physically separate Tenax TA® as an absorbent sink and indoor dust for *in vitro* bioaccessibility testing, and our model allows assessment of FRs (and potentially other HOCs) bioaccessibility from a solid matrix using artificial gastro-intestinal fluids. Previous methods used a self-designed stainless steel sieve to separate and recover Tenax TA® beads (Fang and Stapleton, 2014; ISO, 2015; Li et al., 2015; C. Li et al., 2016). Our approach, using RC dialysis tubing provides some important benefits. Dialysis tubing is readily available, reproducible (quality controlled) and can be sourced with a wide range of molecular weight cut offs. This allows investigators to select a membrane with a MW cut off sufficient to permit free diffusion of the analytes of interest, whilst restricting passage of larger macromolecules such as enzymes or proteins that may be added to simulated GI fluids. By restricting the passage of these unwanted materials, the sorption capacity of the Tenax TA® is predominantly used for the organic pollutants rather than media components and clean up and desorption is thus simplified. The tubing functions effectively to physically separate the Tenax TA® from the solid matrix (dust) and has high pH and temperature tolerance. Our study also shows the benefits of using an adsorption sink in the CE-PBET system. Compared to controls with no Tenax TA®, inclusion of the resin increased gut bioaccessibility for PBDEs with diverse physicochemical profiles. For the low brominated BDE28, 0.25 g of Tenax TA® were insufficient for exhaustive *in vitro* gut absorption, illustrating that the amount of Tenax TA® added to the modified CE-PBET system should be optimized with respect to the physicochemical properties (e.g. LogKow, water solubility) of the target analytes tested. Other than BDE28, for the (hydrophobic) FR's studied here, 0.5 g of Tenax TA® was shown to be an appropriate amount to add in order to ensure released pollutants were readily adsorbed.

3.4 Conclusion

Under the influence of the ISO 16751 method on the environmental availability of non-polar compounds being currently approved for registration, we propose a novel test format for assessing *in vitro* bioaccessibility of PBDEs with Tenax TA® addition as an adsorptive sink.

Our data also show that the existing default assessment of risk (*i.e.* all the ingested pollutant in a solid matrix being bioavailable) is an overestimate and that the BAF% varies between ~60% (BDE47) and ~50% (BDE153). Well designed *in vitro* bioaccessibility tests thus provide a simple approach for initial human risk assessments from ingested solid matrices giving a conservative, yet realistic indication of risk.

Supporting information

Chemicals and reagents

Native standard solutions of BDE 28, 47, 77, 100,128, 153, 154, and 183 were purchased from Cambridge Isotope laboratories Inc. (UK). Purity of all standards was >98% unless otherwise stated. Standard stock solutions were prepared in toluene for all compounds. Sodium sulphate (anhydrous, granular/powder, 99% pure), high purity grade Silica gel pore size 60 Å, 70-230 mesh, 63-200 μm (product code: #60741, Sigma-Aldrich), Florisil® 100-200 mesh (product code: #10104980, Acros Organics), concentrated Sulfuric acid (H_2SO_4) 96% analytical grade (Fisher Scientific, UK), Tenax® TA Porous Polymer Adsorbent, 60-80 mesh (product code: #11982, Sigma-Aldrich**),** Standard grade regenerated cellulose (RC) Spectra/Por™ 3 (18mm flat width, 1.1mL/cm dialysis membrane MWCO 3.5 kDa) (Spectrum Labs Inc., USA, product code: #11425859; FisherScientific, UK), micro centrifuge filters lined with 0.45μm pore size nylon filter 1.5mL volume capacity (product code #516-0236, VWR) and 19mm Small Silver Binder Clips (product code: #WW-376137, Staples Inc, UK.). Analytical grade inorganic salts were provided from Fisher Scientific (Loughborough, UK). All biological reagents used for media preparation and organic solvents used for extraction and clean-up steps were of HPLC grade and were obtained from Sigma-Aldrich (Gillingham, UK). Empty, pre-fritted polypropylene filtration tubes (6 mL) for silica SPE and Florisil cartridge preparation (2 g, 6 mL) were purchased from Sigma-Aldrich (UK). For 5% acidified silica gel preparation, concentrated sulphuric acid (H_2SO_4, >96%) was used and was purchased from Fisher Scientific. Briefly, 1.9 mL of pure sulphuric acid was added drop-wise to 50 g of hexane-washed, oven-dried silica gel under continuous and vigorous stirring. Glass test tubes were cleaned by soaking for at least 12 h in a phosphate-free, alkali solution. After washing, the tubes were rinsed with deionised water, dried at 100 ºC for at least 12 h and burned at 400°C to remove all traces of organic contamination.

Target analytes and analytical characteristics

Table SI 1 – Target analytes and physicochemical properties calculated from EPIWEB.

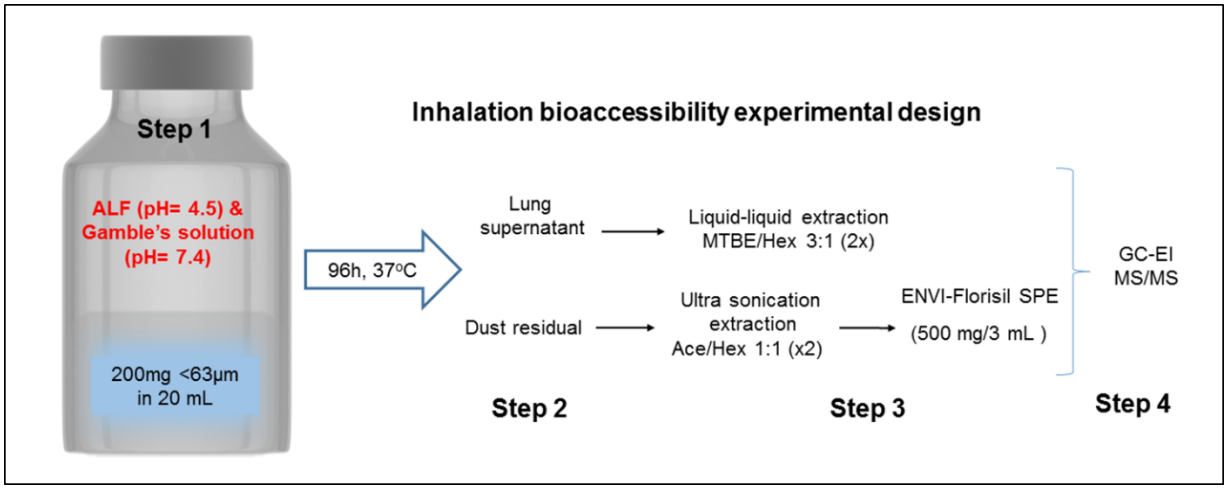
Abbreviation	Full name	Molecular folrmula	Category	MW (Da)	Log K_{ow}	Water solubility (mg/L) 25oC method 1	Vapour SUBCOOLED LIQUID pressure (mm Hg, 25oC)
BDE-28	2,4,4'-Tribromodiphenyl ether	C12 H7 Br3 O1	PBDEs	406.895	5.88	0.02642	9.16E-06
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	C12 H6 Br4 O1	PBDEs	485.791	6.77	0.001461	1.58E-06
BDE-100	2,2',4,4',6-Pentabromodiphenyl ether	C12 H5 Br5 O1	PBDEs	564.688	6.84	0.000394	3.10E-08
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether	C12 H4 Br6 O1	PBDEs	643.584	8.55	4.15E-06	1.86E-07
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether	C12 H4 Br6 O1	PBDEs	643.584	8.55	4.15E-06	1.41E-07
BDE-183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	C12 H3 Br7 O1	PBDEs	722.48	9.44	2.16E-07	2.45E-08
EH-TBB	2-ethylhexyl-2,3,4,5-tetrabromobenzoate	C15 H18 Br4 O2	EHFRs	549.918	7.73	1.14E-05	4.58E-06
BTBPE	1,2-bis(2,4,6-tribromophenoxy)ethane	C14 H8 Br6 O2	EHFRs	687.636	8.31	6.55E-07	3.17E-08
BEH-TEBP	Bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate	C24 H34 Br4 O4	EHFRs	706.14	9.34	1.98E-09	2.28E-09

Instrumental analysis

A Thermo Trace GC Ultra system equipped with a Thermo TG-SQC capillary column (15 m x 0.25mm x 0.25 μm) coupled to a Thermo ITQ 1100 mass spectrometer in electron ionisation mode ((EI-MS) was connected through a heated transfer line (300^{o}C). The injection temperature was set at 92 °C, hold 0.04 min, ramp 700 °C/min to 295 °C and 5μL of cleaned extracts in toluene were injected for GC analysis. Injection was performed under a pressure of 0.19 bar until 1.25 min in pulsed splitlless mode 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.50 min, ramp 10°C/min to 300°C, hold 3 min, ramp 40 °C/min to 310 °C, hold 5 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min.

Chapter 4

In vitro inhalation bioaccessibility of plasticisers present in indoor dust using artificial lung fluids



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In preparation

Abstract

Plasticisers are additives imparting durability, elasticity and flexibility in the manufacture of everyday consumer products. The lack of migration stability has resulted into their classification as major indoor contaminants. Despite their extensive use, the process of assessing human exposure and possible health effects arising from indoor dust contamination especially in children only began the past decade with limited results so far. This is the first study assessing the *in vitro* bioaccessibility (*i.e*. uptake) of traditional phthalate esters including dimethyl phthalate (DMP), diethyl phthalate (DEP) and di-(2-ethylhexyl) phthalate (DEHP) and alternative plasticisers used as phthalate substitutes in polymer materials such as bis(2-ethylhexyl) terephthalate (DEHT) and cyclohexane-1,2-dicarboxylic acid diisononyl ester (DINCH) present in indoor dust with respect to inhalation as an alternative route of exposure. Serving as surrogates to phthalate pulmonary release after dust inhalation *in vivo*, two separate artificial lung fluids, mimicking two different interstitial conditions were used, namely artificial lysosomal fluid (ALF, pH=4.5) representing the fluid that inhaled particles would contact after phagocytosis by alveolar and interstitial macrophages within the lung and Gamble's solution (GMB, pH=7.4) as a fluid for deep dust deposition within the interstitial fluid of the lung. Low molecular weight (MW) and short-chained phthalates such as DMP and DEP were found to be highly bioaccessible (>75%) in both artificial pulmonary media tested (*i.e.* Gamble's solution and ALF), regardless of the medium's pH and chemical composition, whereas high MW compounds such as DEHP, DINCH and DEHT were <5% bioaccessible. Such findings support the hypothesis of hydrophobicity and water solubility primarily influencing inhalation bioaccessibility of organic pollutants. Hence, compared to Gamble's solution, ALF as the artificial pulmonary fluid with the highest organic content formulation is more representative for future inhalation bioaccessibility studies of organics.

Keywords: bioaccessibility, inhalation, phthalate esters, alternative plasticisers, indoor dust

Highlights

- First study on *in vitro* inhalation bioaccessibility of organics from house dust
- Gamble's solution and artificial lung fluid were used as pulmonary surrogate media
- Low MW phthalates DMP and DEP were >75% bioaccessible in both lung fluids
- Alternative plasticisers DINCH and DEHT were <5% bioaccessible
- Inhalation bioaccessibility was highly influenced by hydrophobicity

4.1 Introduction

Oral bioaccessibility (*i.e.* uptake) of phthalate esters (PEs) has been studied using physiologically-based extraction tests (PBET). In two studies using indoor dust from China, the short-chain and low MW PEs such as DMP and DEP gave bioaccessibility values between 26 and 30%, compared to BBzP and DEHP whose oral bioaccessibilities were close to 10% (Kang et al., 2012; Wang et al., 2013). However, (He et al., 2016b) reported DMP was 83% bioaccessible through the GIT using indoor dust samples from different environments such as offices, hotels and classrooms, whereas DEHP was very poorly bioaccessible at around 1.9% from all the different microenvironments studied. Unlike dust ingestion and oral bioaccessibility of phthalate esters, limited studies exist on indoor dust particle inhalation, dissolution and the potential uptake of organic pollutants using artificial pulmonary fluids. Therefore, the need to establish and validate *in vitro* pulmonary tests is essential due to the prevalence of PEs in the indoor environment and their potential adverse health effects for humans, and especially children (Bornehag et al., 2004; Guo and Kannan, 2011; Hauser and Calafat, 2005),(Carlstedt et al., 2013). Elucidating the dissolution and absorption potential of plasticisers via the lung will improve our understanding of the importance of this alternative exposure route.

Human exposure to plasticisers in the indoor environment is a growing concern for human health with respect to their potential adverse effects on reproduction, endocrine and thyroid homeostasis (Hauser and Calafat, 2005) (Matsumoto et al., 2008; Rudel and Perovich, 2009; SCHER, 2007) . This is the first study reporting the *in vitro* uptake of phthalate esters and alternative plasticisers present in indoor house dust via two artificial lung fluids, mimicking two different interstitial conditions in the lung. Artificial lysosomal fluid (ALF, pH=4.5) represents the fluid that inhaled particles would contact after phagocytosis by alveolar and interstitial macrophages within the lung. Gamble's solution (GMB, pH=7.4) is a surrogate fluid for deep dust deposition within the interstitial fluid of the lung (Dean et al., 2017; Hedberg et al., 2010). Such fluids have been previously used in i*n vitro* inhalation bioaccessibility studies to investigate human exposure to water soluble metal particles and their fractions including Zn, Ni, Cu and Fe (Boisa et al., 2014; Hedberg et al., 2010; S.-W. Li et al., 2016; Wragg and Klinck, 2007).

To bridge this knowledge gap, the main objectives of the present study are: 1) to evaluate the *in vitro* pulmonary uptake (*i.e.* bioaccessibility) of PEs and alternative plasticisers present in indoor dust by employing two different artificial pulmonary fluids, *i.e.* Gamble's solution and artificial lysosomal fluid representing the healthy and inflammatory status of the tracheobronchial environment, respectively and 2) to assess the factors influencing inhalation bioaccessibility of plasticisers via indoor dust.

4.2 Material and methods

4.2.1 Sampling and dust particle properties

Ten indoor dust samples were collected from pre-existing vacuum cleaner bags (houses) in Norway (Oslo) as a part of the A-TEAM cohort sampling during November 2013 – April 2014 (Papadopoulou et al., 2016) (Table SI-3). All dust samples were passed through a methanol-washed sieve (<63 μm) with respect to inhalable and thoracic aerodynamic diameter particle cut off as suggested by the International Organization for Standardization (ISO) (International Organization for Standardization, 1995). Oven-baked Na_2SO_4 (granular) was sieved as a field blank, according to (Abdallah and Covaci, 2014). All dust samples were kept in hexane-washed amber glass bottles and stored at 4°C until analysis. Specific surface area and dust particle size were determined by laser diffraction spectroscopy (Mastersizer 3000, Malvern Ltd., UK) and the average of ten dust samples was 235.6 m^2/kg and 49.2 μm, respectively. Additional particle size properties such as total carbon (TC%) and nitrogen (TN%) content were determined (Thermo Flash 2000), while organic matter content (OMC%) was assessed by loss-on-ignition (LOI) as described in (Yu et al., 2012).

Table 16 - Dust particle properties of indoor dust samples from Norwegian houses (n=10)

Sample name	Organic matter content OMC%	STDEV	Nitrogen content %	STDEV	Total carbon content%	STDEV	*Median Particle size (μm)	*Specific surface area (m^2/kg)
N04	19.525	0.736	0.607	0.078	7.872	0.638	44.0	196.1
N08	57.832	4.010	3.706	0.119	22.625	0.709	39.8	284.1
N13	10.236	0.651	0.243	0.006	10.372	0.256	11.8	652.1
N14	64.288	0.903	5.541	0.256	28.718	1.093	68.3	147.5
N19	32.404	0.917	1.116	0.061	8.589	0.736	102.0	117.4

N20	39.423	0.724	1.554	0.218	20.782	2.137	44.9	186.4
N29	69.620	0.456	4.028	0.038	32.922	0.344	39.8	202.6
N30	57.605	2.440	1.340	0.113	22.380	1.055	65.5	132.6
N31	25.566	0.647	1.276	0.088	16.057	0.569	43.9	194.0
N40	62.909	0.531	1.854	1.620	19.798	1.779	32.2	243.4

*All experiments were conducted in triplicate, apart from particle size and specific surface area where the results presented here are from one replicate

4.2.2 Dust extraction and clean-up

The analytical method used for dust extraction was based on (Giovanoulis et.al, 2017). Briefly, 100mg of dust was extracted with 10 mL acetone: n-hexane (1:1 v/v) using microwave-assisted extraction which is considered as an effective and high productivity extraction technique for solid matrices such as indoor dust (see SI Fig 1). Methanol-washed, high quality Teflon vessels were used for the microwave extraction and all dust samples were spiked with 400ng internal standard (ISTD) mix prepared in n-hexane (DMP-d_4, DnBP-d_4 and DEHP-d_4). After the extraction medium addition, all samples were heated to controlled temperature with microwave power. When the microwave-assisted extraction cycle was completed, physical separation between the indoor dust (matrix) and the organic solvent phase was achieved by centrifuging at 1500rpm for 2 min. Then, all supernatants were transferred to oven-baked, transparent glass tubes using a disposable Pasteur pipette and the resulting extracts were then concentrated to 0.5mL under a gentle nitrogen (N_2) stream; the N_2 stream was passed through a glass Pasteur pipette tip (150mm) containing charcoal in order to eliminate any traces of external contamination. Finally, the solvent was exchanged to n-hexane (4ml) to avoid possible breakthrough of phthalates on ENVI-Florisil SPE (500 mg/3 mL) cartridges and then concentrated to 1mL. Prior to solid phase extraction (SPE), all ENVI-Florisil cartridges were pre-treated by two washing steps: a) with methyl tert-butyl ether (MTBE) (3ml) and b) with n-hexane (6ml). The 1mL extract was loaded onto the ENVI-Florisil cartridge and 9mL of n-hexane was added as a cleaning elution step. During the second elution, all target analytes were eluted using 9 mL acetone: n-hexane (1:1) and the resulting eluate was concentrated to 1ml with a gentle N_2 flow at room temperature, using a similar filtration technique as described above. Finally, all extracts were transferred to GC vials and biphenyl (300ng) was added as an injection recovery standard prior to GC-MS/MS analysis (Table SI-1).

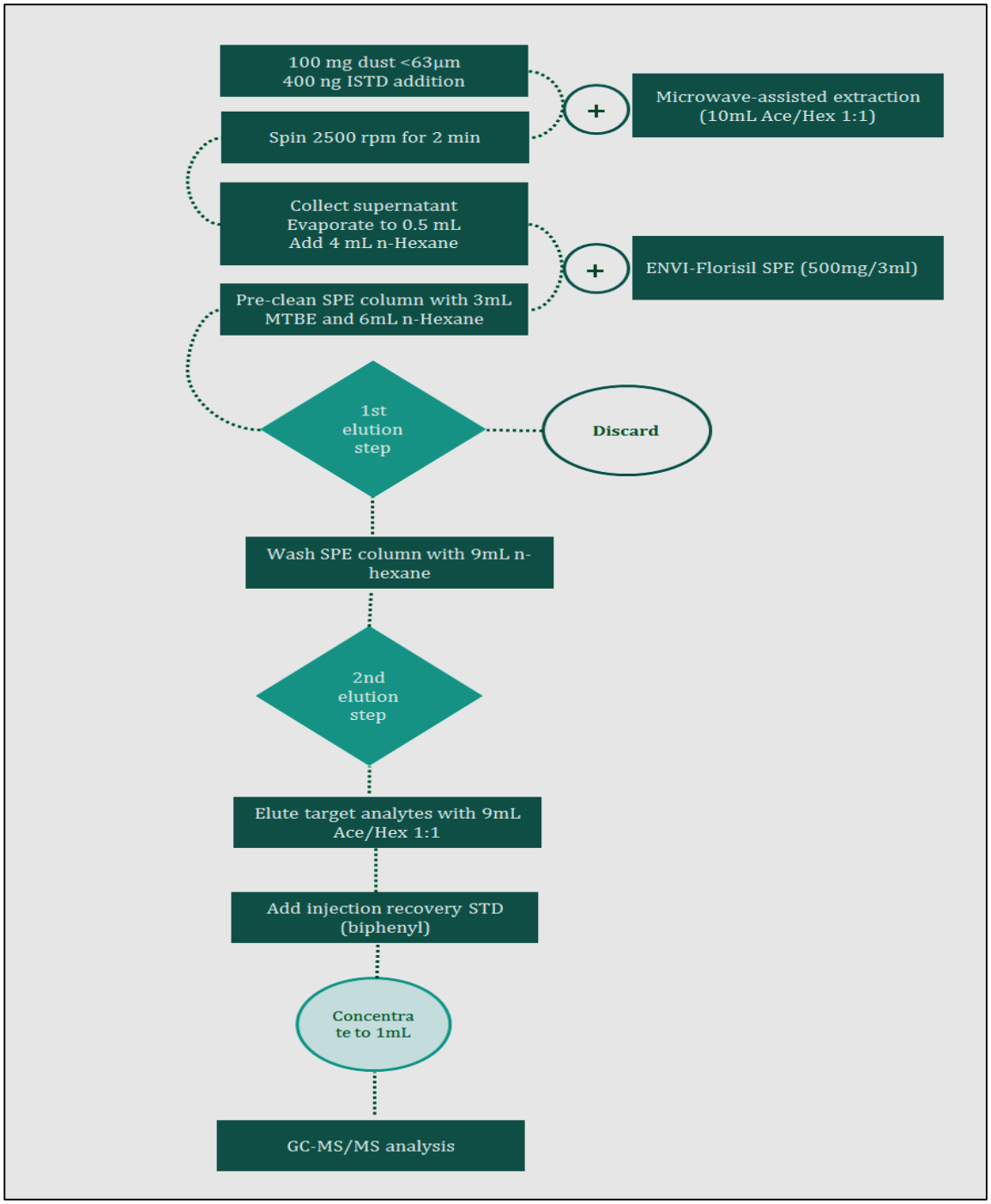


Figure 15 – Sample preparation flowchart for indoor dust extraction of all target analytes according to (Giovanoulis et.al., 2017)

4.2.3 Artificial lung fluid extraction

The lung fluid extractions involved two different artificial lung fluids, namely Gamble's (GMB) solution and artificial lysosomal fluid (ALF), (Fig. 16). All lung fluid extraction experiments were conducted in duplicate. Both media (1L) were prepared in ultra-pure H_2O

(18.2 Ω) as described in (Hedberg et al., 2010) (SI table 4) and pH adjustment was achieved using either 1M HCl or 1M NaOH.All media were freshly prepared 24h before the initiation of each test, were checked for background phthalate contamination and stored in oven-baked Duran® glass bottles at 4°C. According to Boisa et al (2014), the experimental volume for simulated lung fluid extraction tests should be 20mL, given the pulmonary fluid volume capacity of healthy non-smoking adults (0.3 mL/kg; 70kg body mass) (Boisa et al., 2014). In order to maintain a 1:100 solid-to-liquid (S/L) ratio between the incubated matrix and the lung fluid, 0.2 g of indoor dust (SI Table 3) were combined with 20 mL of each artificial lung fluid separately, as suggested by (Schaider et al., 2007) (Fig SI 2). Prior to securely capping them, the samples (including procedural blanks) were covered with oven-baked aluminium foil to avoid background phthalate contamination and were then continuously incubated for 96h to mimic human alveolar clearance capacity (Lindström et al., 2006; Wragg and Klinck, 2007), under continuous shaking (60rpm) at 37°C inside a thermostatic chamber. After 96h, the lung solutions and the incubated dust were collected by physical separation; the samples were centrifuged at 1500 rpm for 3 min. Using disposable glass Pasteur pipettes, the supernatants (lung fluid) were transferred into clean glass vials, while the residual dusts were stored at 4^{O}C. Prior to extraction, all samples were spiked with 400ng internal standard (ISTD) mix prepared in n-hexane (DMP-d_4, DnBP-d_4 and DEHP-d_4). All the supernatants were subjected to liquid-liquid extraction (LLE) using 7mL Hexane: MTBE 3:1, twice. To avoid any water residue and remove any gel-like emulsion formulated during LLE, sufficient amount of oven-baked Na_2SO_4 (powder) was added to all the extracts, followed by 1 min vortexing and organic phase collection after centrifugation (1500rpm for 2min). Solvent exchange to n-hexane followed and the samples were concentrated to 1ml under a gentle N_2 stream at room temperature as described above.

Ultrasonication-assisted extraction was employed for the residual dusts for 10min using 7mL of Acetone: Hexane 1:1, twice. All the extracts were collected by centrifugation at 1500 rpm for 3 min, combined, concentrated to 1ml under a gentle N_2 stream before clean-up through ENVI-Florisil SPE (500 mg/3 mL) cartridges, similarly to the dust extraction procedure described above. Briefly, all the Florisil® columns were pre-cleaned with MTBE and conditioned with n-hexane. The residual dust extracts in n-hexane were loaded onto the Florisil® columns, the first hexane eluate was discarded, while the second eluate was collected using 9 mL of MTBE. The resulting eluate was concentrated to 1ml under a gentle N_2 flow at room temperature, with a similar filtration technique as described above. Finally, all extracts (in 1mL) were transferred to oven-baked GC vials and biphenyl (300ng) was

added as an injection recovery standard prior to GC-MS/MS analysis. Further details of sample preparation and instrumental analysis are available in SI.

Table 17 - Chemical composition (g/L) of artificial lung fluid (ALF; pH=4.5) and Gamble's solution (GMB; pH=7.4) as suggested by (Hedberg et al., 2010)

Chemical ingredients	Artificial lung fluid (g/L)	Gamble's solution (g/L)
$MgCl_2$	0.050	0.096
NaCl	3.21	6.02
KCl	-	0.298
Na_2HPO_4	0.071	0.126
Na_2SO_4	0.039	0.063
$CaCl{\cdot}2H_2O$	0.128	0.368
$C_2H_3O_2Na{\cdot}H_2O$ (sodium acetate)	-	0.701
NaHCO3	-	2.60
$C_6H_5Na_3O_7{\cdot}2H_2O$ (sodium citrate)	0.077	0.097
NaOH	6.00	-
Citric acid	20.80	-
Glycine	0.059	-
$C_4H_4O_6Na_2{\cdot}2H_2O$ (Na_2Tartrate$\cdot 2H_2O$)	0.090	-
$C_3H_5NaO_3$ (NaLactate)	0.085	-
$C_3H_5O_3Na$ (NaPyruvate)	0.086	-
pH	4.50	7.40

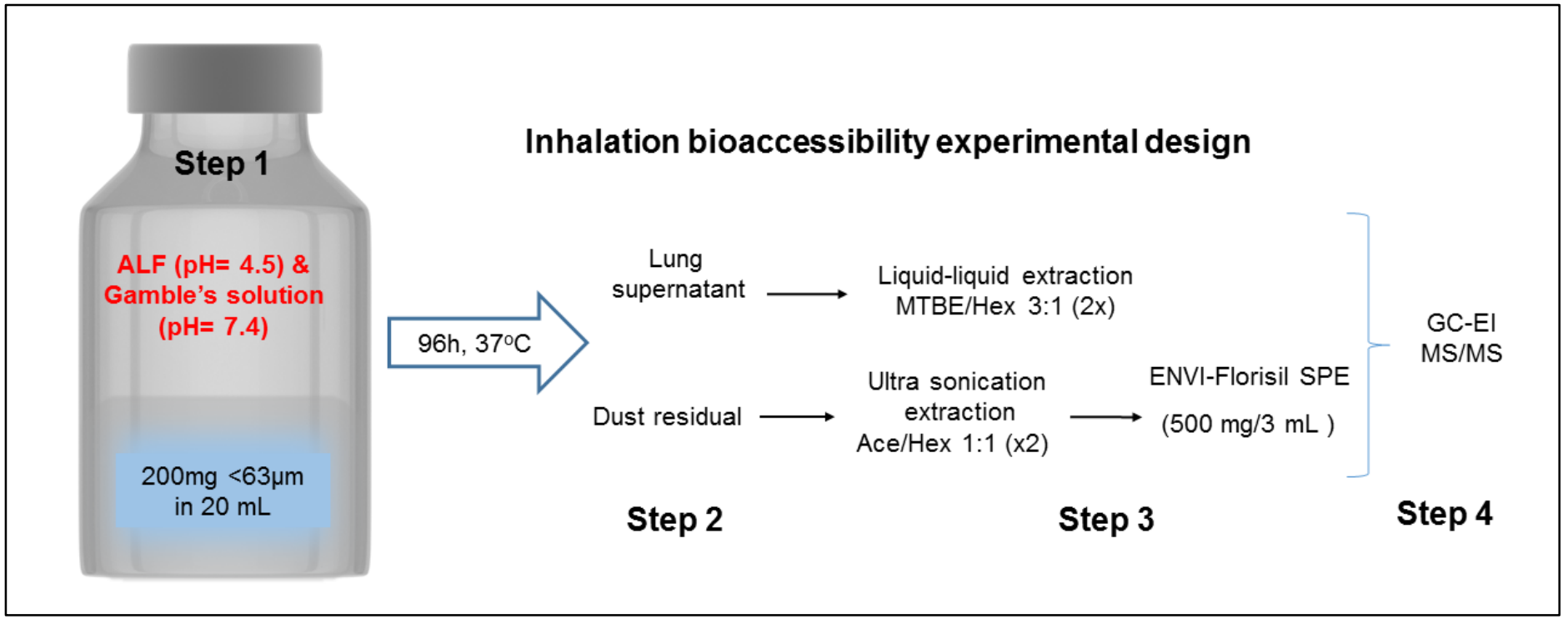


Figure 16 - Schematic representation of inhalation bioaccessibility test using two separate artificial lung fluids, namely a) Gamble's solution (pH=7.4) and b) artificial lysosomal fluid (pH=4.5). Shown in the graph are the different steps of the experimental procedure; lung fluid incubation for 96h at 37°C (step 1), sample collection using centrifugation for 3 min at 1500rpm (step 2), sample preparation and clean-up (step 3) and GC-EI MS/MS instrumental analysis (step 4).

4.2.4 Data analysis

Bioaccessibility can be expressed as a mass (*e.g.* ng of a contaminant solubilised in the respiratory tract), a concentration (ng/g of a contaminant in dust) or as a fraction - expressed as a percentage (BAF%) (Guney and Zagury, 2016). In our study, inhalation bioaccessibility (IBAF) was determined using Eq. 4, where mass phthalate ester (lung) is set as the organic compound mass (ng) determined in the lung supernatant of the *in vitro* pulmonary system and mass (dust residual) is the mass (ng) determined in the dust residual collected after the 96h-incubation of the *in vitro* pulmonary system which is considered as the non-bioaccessible fraction.

$$\text{IBAF\%} = \frac{mass\ PE\ \left(\begin{matrix} lung \\ supernatant \end{matrix}\right)}{mass\ PE\ (\text{lung supernatant}) + mass\ (dust\ residual)}\ x\ 100 \quad \text{(Eq. 4)}$$

GraphPad Prism® version 7.00 for Windows, (GraphPad Software, La Jolla CA, USA) was used for statistical analysis. Prior to statistical analysis, all data were checked for normality using the Shapiro–Wilk test and not all data passed the normality test. All data were arc-sine transformed, as this mathematical transformation is necessary for statistical analysis of results set in percentages in order to equalise variances as proposed by (Rr R. Sokal and Rohlf, 1995) . Ordinary two-way ANOVA (Uncorrected Fisher's test, $p<0.05$) was performed to assess statistically significant differences of phthalate esters between both artificial lung fluids. Spearman's correlation ($p<0.05$) was employed to assess statistical dependence and correlation between artificial lung fluids and the physicochemical properties of all target analytes. Minitab® version 17 for Windows (Minitab Inc., USA) was used for general linear model in order to assess the statistical relationship between inhalation bioaccessibility (IBAF%), plasticisers dust concentration, $\log K_{ow}$, organic matter content (OMC%) and dust particle size.

4.2.5 Quality assurance and quality control

All samples were analysed together with SRM 2585 (NIST, USA), used for method validation and QC testing during lung fluid testing (n=4) and dust extractions (n=5), respectively. Oven-baked, uncontaminated sand was used as a procedural blank during dust extractions and the results were blank-corrected for all target analytes by subtraction of the

mean blank values from the raw concentration values (expressed in ng/g) according to (Abdallah and Covaci, 2014). Four blank lung fluid samples (two from each lung fluid) with no added matrix were sequentially incubated and analysed as procedural blanks and all IBAF% results were blank corrected for all target analytes. Extraction efficiency for all target analytes ranged from 70 – 120% for both lung fluids, apart from BzBP which was equal to 268% and 242% for Gamble's and ALF, respectively (Table SI 6). Method limits of detection (mLOD) were calculated as three times the standard deviation of the lung fluid blanks (SI Table 7). Glass test tubes were cleaned by soaking for at least 12 h in an alkali solution. After washing, the tubes were rinsed with water and dried at 100 ºC for at least 12 h and burned at 400°C to remove all traces of contamination.

Table 18 – Method accuracy for indoor dust extraction based on SRM 2585 (n=5)

Concentration (ng/g)	SRM 2585 AVG (n=5)	STDEV	* RSD%	Matrix blank (ng/g)†
DMP	2596	243.2	9.4	4.7
DEP	7848	733.0	9.3	41.5
DiBP	5438	429.4	7.9	58.7
DnBP	32069	2589.2	8.1	78.2
BzBP	85456	5040.0	5.9	5.9
DEHP	575020	26960.3	4.7	297.3
DEHT	25101	8521.1	33.9	209.9
DiNP	199333	15279.1	7.7	127.7

*DINCH and DPHP not detected in SRM 2585; all values (ng/g) were blank-corrected

*RSD% =STDEV/AVG*100; † Uncontaminated sand was used as a matrix blank for dust extractions

Table 19 – Extraction efficiency for all target analytes for Gamble's solution (GMB) (n=2) and artificial lysosomal fluid (ALF) (n=2)

	GMB Recovery%				ALF Recovery%			
	R1	R2	AVG	STDEV	R1	R2	AVG	STDEV
DMP	86.7	90.1	88.4	2.4	84.0	90.8	87.4	4.8
DEP	109.3	114.7	112.0	3.8	106.7	108.4	107.5	1.2
DiBP	81.2	81.5	81.3	0.2	78.5	82.5	80.5	2.9
DnBP	79.5	81.2	80.4	1.2	71.2	77.7	74.5	4.6
BzBP	268.8	267.3	268.1	1.0	264.8	262.1	263.5	1.9
DEHP	118.5	135.6	127.0	12.1	93.0	102.0	97.5	6.3
DINCH	105.9	116.9	111.4	7.8	108.9	110.5	109.7	1.2
DEHT	101.6	107.1	104.4	3.9	94.8	95.7	95.2	0.7
DPHP	121.1	133.9	127.5	9.0	115.1	119.6	117.3	3.2
DiNP	116.0	112.9	114.4	2.2	111.3	107.2	109.2	2.9

4.2.6 Instrumental analysis

Analyses used a GC/MS-MS system with electron impact ionization mode (EI). The chromatographic separation of plasticizers was obtained on a cross-linked 5% - phenyl/95% - dimethylpolysiloxane capillary column 30 m × 0.25 mm (i.d.) × 0.25m film thickness using a gradient temperature; Agilent Technologies. The GC column oven temperature programme was: 45^{O}C for 1min followed by ramps of 15^{O}C/min to 300^{O}C, which was maintained for 6min. Ion source temperature was 250^{O}C and the split-splitless injection volume was 1µl in Multiple reaction monitoring (MRM) with two ion transitions (quantification and qualification) per selected analyte being used; compounds were quantified by calibration with the use of a deuterated internal standards. The data were analysed using MassHunter software version B.04.00 for quantitative analysis (Agilent Technologies, Inc. 2008).

4.3 Results and discussion

4.3.1 Concentration of plasticisers in indoor dust

All ten plasticisers examined in our study were detected in all the Norwegian dust samples (Fig.1; Table SI 5). DEHP was the most abundant phthalate ester in the samples, showing the highest median concentration of all the target analytes (225 µg/g), which is comparable to house dust concentrations reported from the US (Guo and Kannan, 2011; Rudel et al., 2003), Kuwait (Albar et al., 2017) and Denmark (Langer et al., 2010), but is three and four-fold lower than house dust concentrations reported from Germany (Abb et al., 2009; Fromme et al., 2004) and Bulgaria (Kolarik et al., 2008), respectively and nearly two-fold higher than another study from USA (California) (Dodson et al., 2015). Our results confirm the ongoing use of DEHP in consumer products in the house environment, despite bilateral legislative measures taken from the EU under the REACH regulation framework (European Commission, 2015) and the US EPA action plan under the Toxic Substances Control Act (TSCA) (US EPA, 2012). This may be an inadvertent consequence of recycling processes or

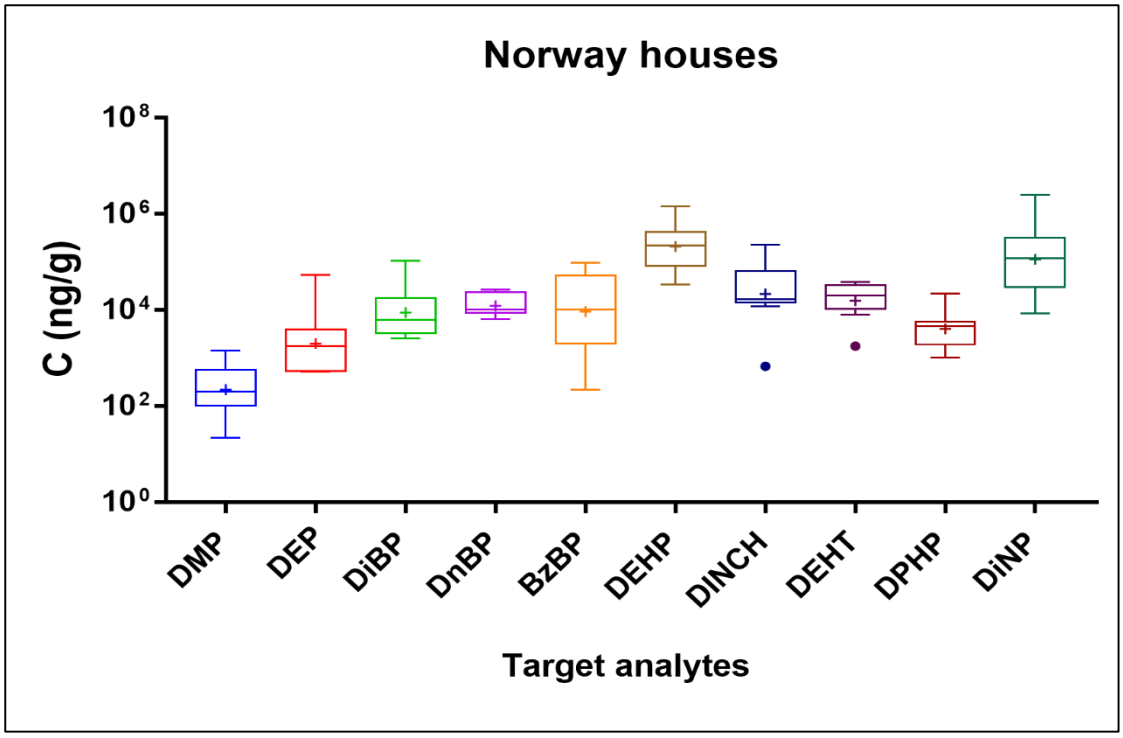


Figure 17 - Boxplots of indoor dust concentrations for phthalate esters and alternative plasticisers from Norwegian houses (N=10). Shown in the whiskers are 25th and 75th percentiles, median (central line), mean (+ symbol) and outlier (● symbol) values. All data shown are log transformed. Please note the linear scale for concertation (ng g^{-1}) on y axis.

the continued use of phthalates in consumer products such a children's toys (Ionas et al., 2014). DiNP which has been used as a DEHP-alternative since the early 2000s (Bui et al., 2016), was the second most prevalent phthalate ester with a median concentration of 120 µg/g, in agreement with levels from German (Abb et al., 2009) and Canadian houses (Kubwabo et al., 2013) and gave the highest maximum concentration among all target analytes (2,500 µg/g), nearly two-fold higher than the greatest DEHP level found in an individual dust sample (1,500 µg/g). Possibly due to the increasing demand for alternative plasticizers as a result of legislative restrictions on DEHP in consumer products (*e.g.* toys), alternative plasticisers including DEHT and DINCH were found in considerable and comparable levels (DEHT median: 20 µg/g and DINCH median: 17 µg/g, respectively), albeit both were an order of magnitude lower than DEHP and DiNP.

Other traditional phthalate esters such as BzBP, DnBP and DiBP were at three to five-fold lower concentrations than DEHT and DINCH in our Norwegian house dust samples. Our results reflect the Nordic indoor environment where hard-surfaced wooden flooring is prevalent (Roos and Hugosson, 2008), leading to potentially higher use of DEHP alternatives in order to meet the ongoing demands of the flooring industry. Compared with levels previously reported, BzBP, DnBP and DiBP median concentrations (10.6 µg/g, 10.3 µg/g and

6.4 μg/g, respectively) were comparable with a recent study from USA (Dodson et al., 2015), though the median concentration of BzBP was three and four-fold lower than house dust levels reported from Germany (Fromme et al., 2004) and Canada (Kubwabo et al., 2013) respectively. Such results may be attributed to the limited use of BzBP (used as a PVC plasticiser) and DnBP (used as plasticiser in cellulose plastics and latex adhesives) (Bornehag et al., 2005), and the growing worldwide trend on the use of non-phthalate alternatives (Bui et al., 2016). In our study, DEP and DMP presented the lowest median concentrations from all target analytes with DEP median concentration (1.8 μg/g) nine-fold higher than DMP (0.2 ug/g). DEP median concentration in our Norwegian indoor dust samples was in agreement with studies from the USA and China (Dodson et al., 2015; Guo and Kannan, 2011), Canada (Kubwabo et al., 2013), Denmark (Langer et al., 2010) and Saudi Arabia (Albar et al., 2017), while DMP levels were in agreement with data from Sweden (Bergh et al., 2011), but considerably lower than reported from the USA (Guo and Kannan, 2011). This may be related to the primary usage of DEP and DMP in personal care products, as well as due to their short-chain character, low MW and high vapour pressures favouring their partitioning primarily to the gas phase rather than on coarse dust particles (Weschler et al., 2008).

Phthalate ester concentrations have recently been reported in floor and surface dust, sampled with dust collection filters, from the same Norwegian population group (n=61) (Xu et al., 2016). The range of phthalate esters and alternative plasticisers levels in vacuum cleaner bags, floor and surface dust (collected from the living room) from Giovanoulis et al (2017) is of the same order as the vacuum cleaner dust in the present study (n=10, Norwegian house dust).

4.3.2 Inhalation bioaccessibility

This is the first study reporting *in vitro* pulmonary uptake of phthalate esters and alternative plasticisers from indoor dust, using two artificial pulmonary fluids, namely Gamble's solution and artificial lysosomal fluid (ALF) to evaluate phthalate bioaccessibility via inhalation; Gamble's solution is representative of the interstitial fluid of the deep lung and ALF is representative of the more acidic environment following phagocytosis by alveolar and interstitial macrophages within the lung (Boisa et al., 2014; Hedberg et al., 2010). Inhalation bioaccessibility values for the low MW phthalates DMP and DEP reached 80% in both pulmonary media (Fig. 2). Such findings support the hypothesis that inhalation is an

important alternative route of exposure for low MW and short-chained phthalate esters (Bui et al., 2017). Across all the target analytes, there were no statistically significant differences

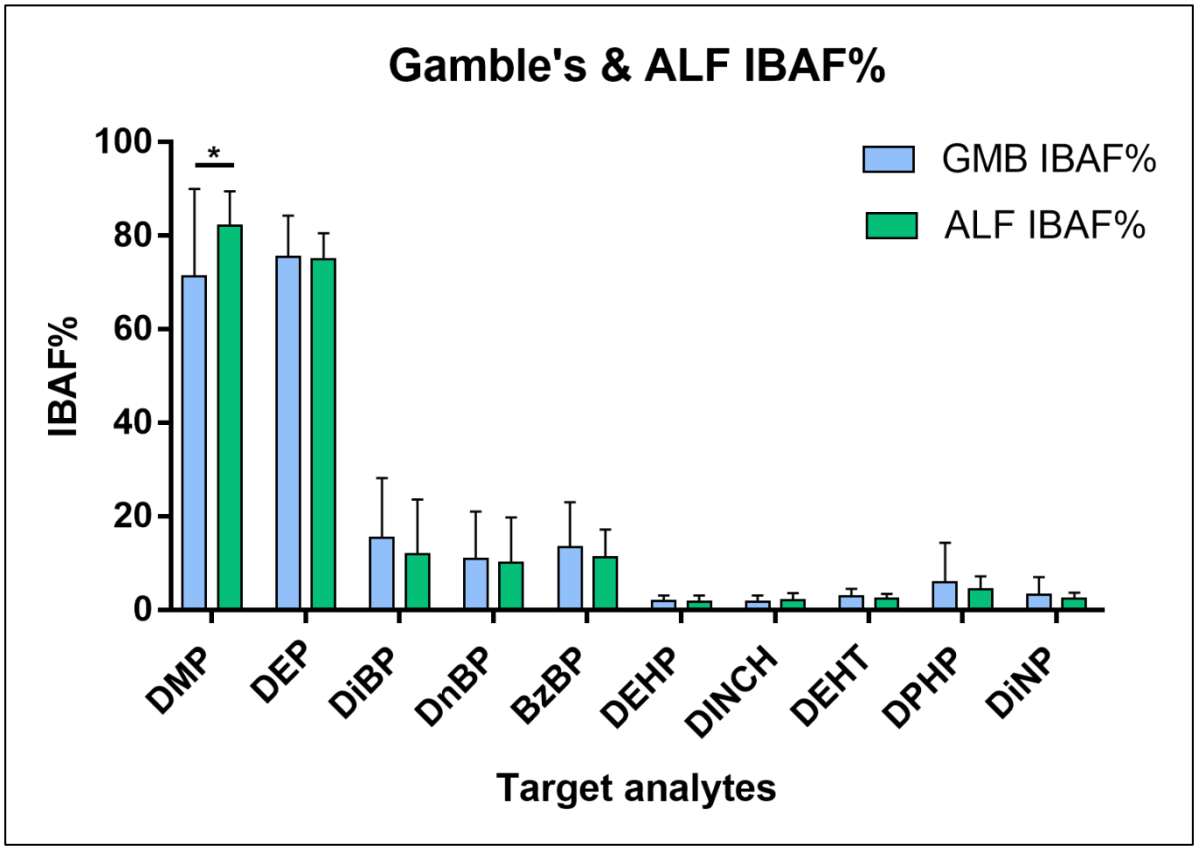


Figure 18 – *In vitro* inhalation bioaccessibility (IBAF%) of phthalate esters and alternative plasticisers present in indoor dust samples (N=10), using two different simulated lung fluids, namely Gamble's solution (GMB) and artificial lysosomal fluid (ALF). Statistically significant differences shown here (*; $p<0.05$). Bar charts represent average values in duplicates. Error bars represent 1STDEV.

for the uptake of plasticisers between the two media despite their differing pH's (Gamble's pH = 7.4; ALF pH = 4.5), apart for DMP where a statistically significant difference was found ($p=0.017$) with IBAF% of 71.3% and 82.1% for Gamble's solution and ALF, respectively. Our findings show that the phthalate esters are liberated from the inhaled dust particles into "normal" lung fluids (Gamble's solution) from where they can be absorbed and that the dust particles themselves need not undergo phagocytosis before the plasticisers are released from the dust matrix; the majority of DMP was also released into the deep lung fluid though greater amounts were liberated into the lysosomal media. Similar to gastro-intestinal bioaccessibility of organic pollutants which is partly governed by the pollutant's physico-chemical properties such as MW and log Kow (Collins et al., 2015), bioaccessibility of inhaled phthalates tended to decrease with increasing MW and log Kow (>4), ranging from 15% to 10% for DiBP, DiNP and BzBP in Gamble's solution and ALF, respectively (Fig 18 & 19). Less than 5% bioaccessibility was found for high MW phthalate esters such as DEHP

and its alternatives plasticisers DEHT (an isomeric alternative of DEHP) and DINCH, supporting research showing that dust ingestion and dermal uptake are the dominant exposure routes for very hydrophobic phthalate esters (Bui et al., 2016; Kang et al., 2012; Wormuth et al., 2006). Given the novelty of our study, there are no previous reports on the *in vitro* pulmonary uptake of plasticisers. In a study assessing human exposure to phthalates from indoor dust in China, Kang et al (2012) reported oral bioaccessibility of DMP and DEP via dust ingestion was 32% and 25%, respectively, whereas dermal absorption of DEP and DnBP directly from air has been also proposed by (Weschler et al., 2015). Importantly, our findings demonstrate that inhalation is a neglected additional and considerable route of exposure for low MW and short-chained phthalate esters.

Table 19 Blank values calculated in mass (ng) and method limits of detection (mLOD) for Gamble's and artificial lysosomal fluid (ALF)

Mass (ng)	Gamble's solution					Artificial lysosomal fluid (ALF)				
	R1	R2	AVG	STDEV	mLOD* (ng)	R1	R2	AVG	STDEV	mLOD (ng)
DMP	0.36	0.35	0.35	0.01	0.03	0.23	0.44	0.33	0.14	0.43
DEP	16.77	13.83	15.30	2.08	6.24	9.47	22.90	16.19	9.49	28.48
DiBP	2.42	1.47	1.95	0.67	2.01	1.22	2.26	1.74	0.74	2.21
DnBP	9.08	4.28	6.68	3.40	10.19	4.03	5.83	4.93	1.27	3.81
BzBP	1.60	0.46	1.03	0.81	2.43	0.05	0.90	0.47	0.60	1.80
DEHP	5.85	5.13	5.49	0.51	1.52	8.26	12.22	10.24	2.80	8.40
DINCH	1.20	3.85	2.53	1.87	5.62	0.21	0.06	0.14	0.11	0.33
DEHT	0.57	0.77	0.67	0.14	0.43	0.58	0.47	0.53	0.08	0.23
DPHP	1.65	0.23	0.94	1.00	3.00	0.16	0.17	0.17	0.01	0.03
DiNP	3.51	1.75	2.63	1.24	3.73	3.15	3.95	3.55	0.56	1.69

*method limit of detection (mLOD) = 3 x STDEV of blank

4.3.3 Method validation using SRM 2585

As we propose a novel method to assess bioaccessibility of inhaled matrices and considering that the pulmonary media were designed for nanoparticles and trace elements *in vitro* bioaccessibility studies (Hedberg et al., 2010; Klara Midander, 2010; Wragg and Klinck, 2007), we performed detailed method validation. Standard reference material SRM 2585 for organic contaminants in house dust (NIST, USA) was used for the validation and duplicate samples were sequentially incubated alongside the Norwegian house dust samples, following the same experimental and analytical conditions. The results of the SRM 2585 method validation step (Table 18) confirmed our findings with IBAF>75% for low MW phthalates, while DEHP and DiNP were the least bioaccessible compounds (IBAF% <5%) as the most

hydrophobic of our target analytes, following a comparable pattern with our Norwegian house dust bioaccessibility results. The SRM 2585 batch that was purchased in our study was prepared using a pool of dust samples collected during mid to late 1990s. Therefore, DINCH and DPHP were not detected, since their use in consumer products as traditional phthalate alternatives peaked after 2010 (Bui et al., 2016).

Table 20 - Lung fluid method validation using SRM 2585 (n=4)

Target analytes†	GMB IBAF% (n=2)	STDEV	ALF IBAF% (n=2)	STDEV
DMP	89.9	1.8	89.5	0.3
DEP	80.7	1.2	73.7	1.0
DiBP	17.6	2.7	8.0	0.6
DnBP	9.8	1.3	6.2	0.5
BzBP	18.5	3.6	13.2	0.6
DEHP	3.1	1.6	2.0	0.2
DEHT	4.9	1.6	4.6	0.6
DiNP	3.9	1.0	3.5	0.3

†DINCH and DPHP not present in SRM 2585

4.3.4 Factors affecting inhalation bioaccessibility

Further analysis of the bioaccessibility results for each individual Norwegian house dust sample (Fig.3) illustrates an apparent discrepancy with the dust collected for house 3 (H3) and the subsequent bioaccessibilities for DMP, BzBP, DnBP and DiBP in both Gamble's solution and ALF (20%<IBAF%<45%). This sample had little DMP (0.02 μg/g) and BzBP (0.22 μg/g), while concentrations for DiBP (3.4 μg/g) and DiBP (10.6 μg/g) were below the 25% percentile. The resulting differences in bioaccessibility suggest that a contaminant's concentration in the studied matrix (e.g. dust) may influence the *in vitro* release into media; such phenomena should be further explored but there may be a fraction of the phthalate esters that bind strongly to the dust matrix and which is thus less easily released during the incubation.

Table 21 –Spearman's correlation between inhalation bioaccessibility in two different artificial lung fluids and the physicochemical properties of plasticisers studied here

	GMB IBAF%		ALF IBAF%	
Physico-chemical properties†	Spearman's ρ	p value	Spearman's ρ	p value
MW	-0.561	0.096	-0.561	0.096
Log Kow	-0.705	0.027*	-0.705	0.027*
Log Koa	-0.588	0.081	-0.624	0.060
Vapour pressure	-0.535	0.115	-0.559	0.098

LogP	-0.818	0.006**	-0.782	0.011*
Water solubility	0.661	0.044*	0.636	0.054
Polarizability	-0.535	0.115	-0.559	0.098

*levels of statistical significance was set $p<0.05$

† Physicochemical properties of plasticisers studied here can be found at Table SI 2

Analysis of variance by general linear models (GLM) among factors which could potentially influence bioaccessibility - including phthalate dust concentration, log kow, organic matter content (OMC) and particle size - revealed a statistically significant interaction ($p<0.05$) only for log Kow (Fig. 19), explaining 70.6% and 69.5% of variance for Gamble's solution and ALF, respectively. Considering the diverse chemical composition and pH of the two artificial pulmonary media studied here and to further elucidate possible physico-chemical properties governing inhalation bioaccessibility, Spearman's correlation was performed between bioaccessibility and various physico-chemical properties of our target analytes, including molecular weight (MW), water solubility and log Kow. Water solubility and IBAF% were moderately correlated ($\rho<0.65$) with a statistically significant correlation established only for Gamble's solution ($p=0.044$) which is representative of the interstitial fluid of the deep

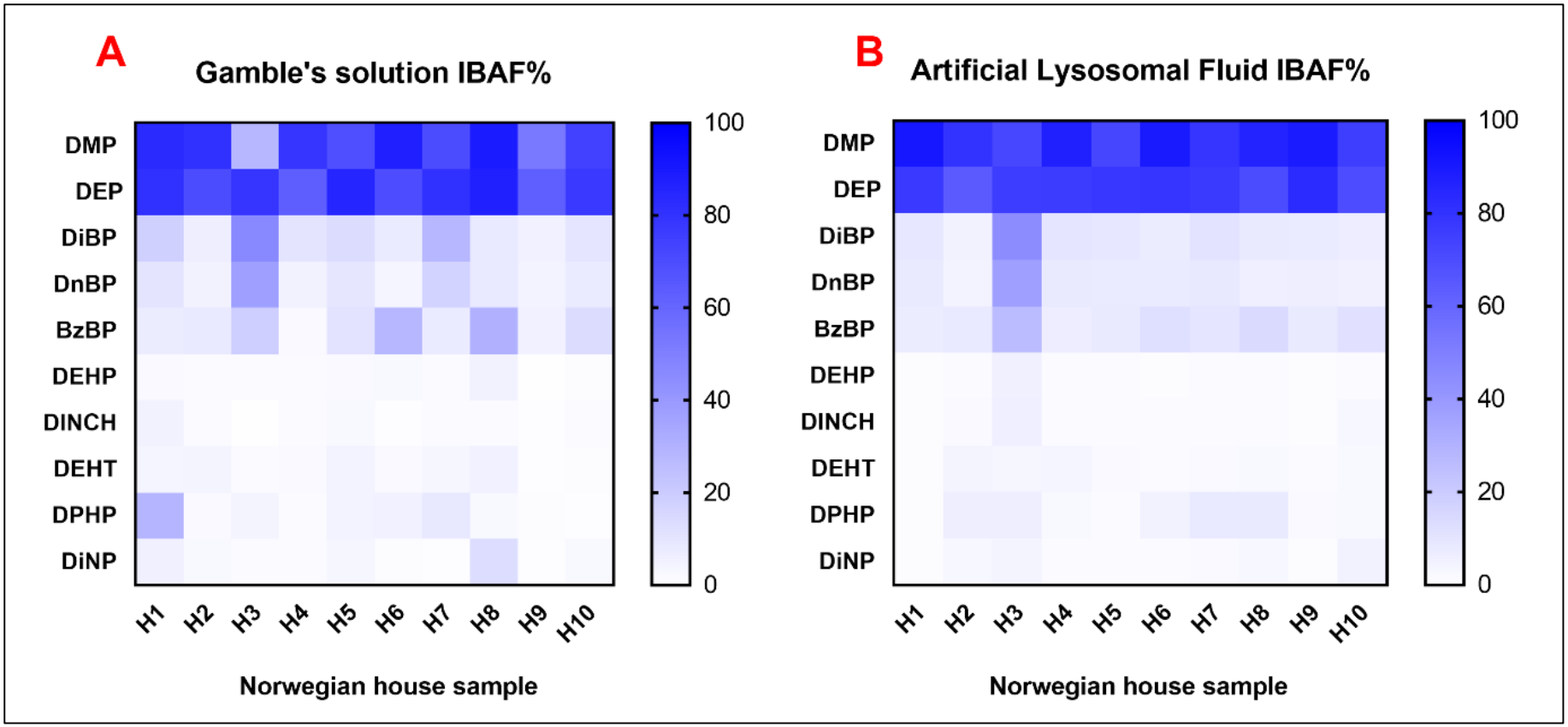


Figure 19 - Heat map presenting inhalation bioaccessibility for each separate Norwegian house dust sample (N=10) in two different simulated lung fluids, namely Gamble's solution (GMB) and artificial lysosomal fluid (ALF). Colour gradient represents average values in duplicates.

lung. Hydrophobicity and lipophilicity (represented by log Kow and log P, respectively) presented strong and statistically significant negative correlations ($\rho > -0.7$; $p < 0.05$) with IBAF for both artificial pulmonary media. For other properties such as MW and vapour pressure, a statistically significant correlation was not achieved (Table 2). However, compared to log Kow, higher statistically significant correlations were found between inhalation bioaccessibility and logP in both pulmonary media (Table 2). According to (Rutkowska et al., 2013), lipophilicity is a physicochemical property which encodes two major structural contributions, namely a bulk term reflecting hydrophobic and dispersive forces (*i.e.* hydrophobicity) and a polar term reflecting more directional electrostatic interactions and hydrogen bonds (*i.e.* polarity). Therefore, lipophilicity should be considered as the driving force behind inhalation uptake of phthalates during the design and data interpretation of dissolution studies employing artificial biological fluids (Marques et al., 2011).

4.4 Conclusion

This is the first study exploring the *in vitro* inhalation bioaccessibility for a wide range of traditional phthalate esters and their alternative plasticisers present in indoor dust from Norwegian houses. Low MW and short-chained phthalates such as DMP and DEP were found to be highly bioaccessible (>75%) in both artificial pulmonary media tested (*i.e.* Gamble's solution and ALF), regardless of the medium's pH and chemical composition. Therefore, inhalation can be potentially considered as a considerable route of exposure for such compounds, including compounds with comparable physico-chemical properties, e.g. chlorinated organophosphates (PFRs) (Schreder et al., 2016). A statistically significant relationship between Gamble's solution and ALF was found only for the bioaccessibility of DMP, whereas the inhalation bioaccessibility of heavier (in terms of MW) and more hydrophobic plasticisers did not exceed 5%. Also, dust particle properties such as organic matter content and particle size did not present any statistically significant interaction with the *in vitro* pulmonary uptake of plasticisers. Our results suggest that 1) inhalation bioaccessibility of organic pollutants is primarily governed from pollutant hydrophobicity and water solubility and 2) the artificial pulmonary fluid formulation of ALF, due to its higher organic content is more representative for inhalation studies of organics may play a

pivotal role in surrogate studies regarding *in vivo* lung tissue function and inflammation triggered by phthalate ester exposure. Finally, the unexplored experimental approach and employment of surrogate biological fluids (*e.g.* gastric, sweat etc.) should be explored, aiming towards a conservative, yet realistic risk assessment of plasticisers.

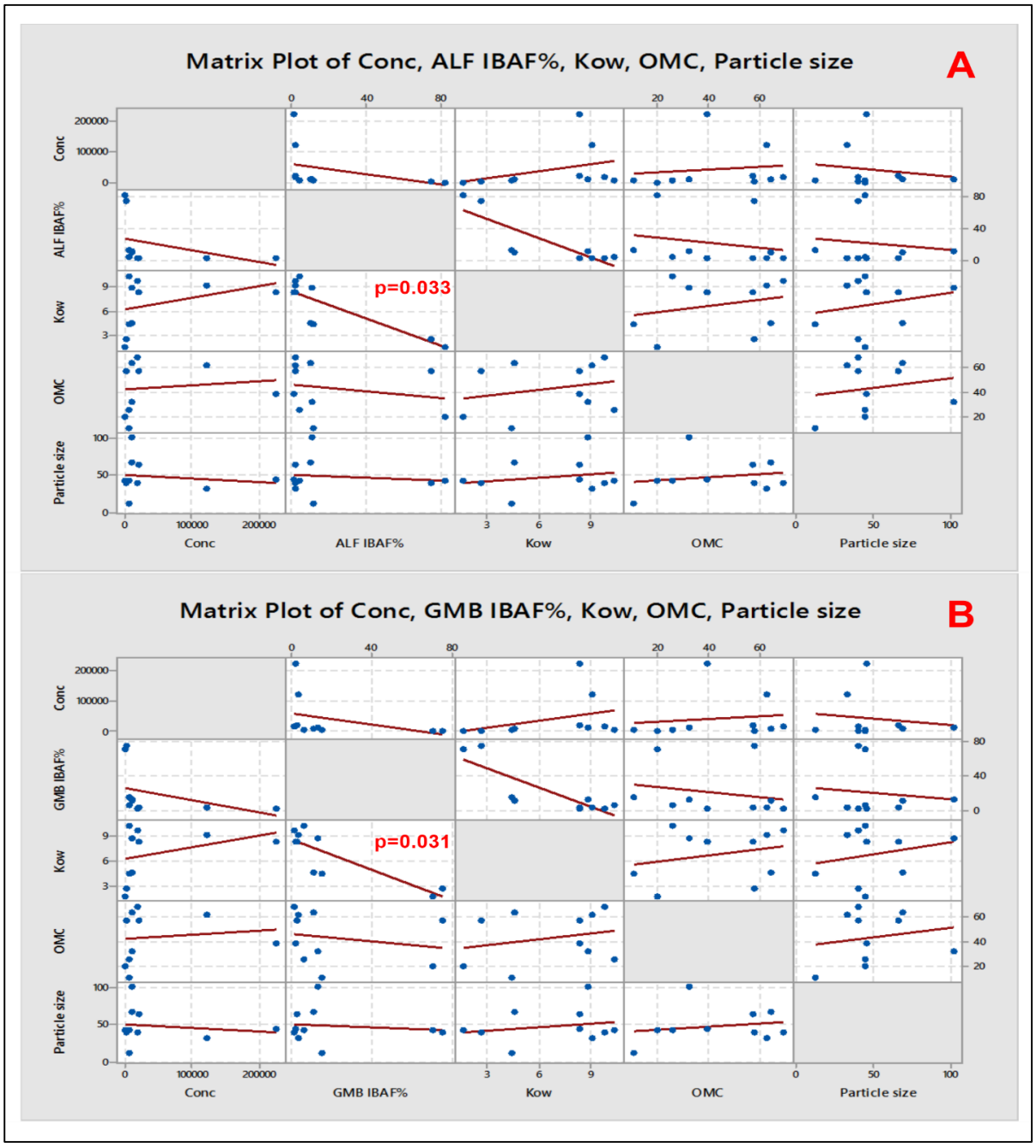


Figure 20 – Matrix plots showing the interactions between the bioaccessibility of the two artificial pulmonary fluids, namely artificial lysosomal fluid (ALF) (A) and Gamble's solution (GMB) (B) and independent variables including analyte concentration in the dust (Conc), log Kow, organic matter content (OMC) and particle size. The parallel lines show no statistically significant interaction ($p>0.05$), whereas the stepper lines show a statistically significant interaction ($p<0.05$)

Supporting information

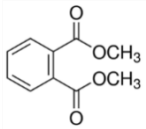
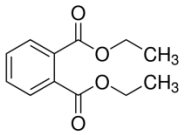
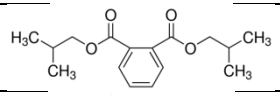
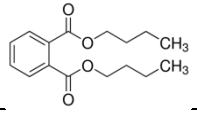
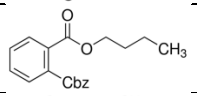
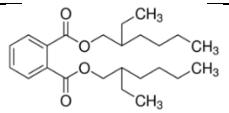
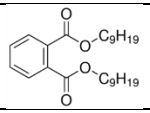
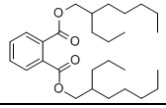
Materials and methods

Chemicals and reagents

Dimethyl phthalate (99 + %), diethyl phthalate (99%), diisobutyl phthalate (99%), di-n-butyl phthalate (99%), benzyl butyl phthalate (98%), bis(2-ethylhexyl) phthalate (99.5%), diisononyl phthalate (ester content ≥99 %, mixture of C9 isomers), bis(2-ethylhexyl) terephthalate (≥96%) were purchased from Sigma Aldrich (Schenelldorf, Germany). Cyclohexane-1,2-dicarboxylic acid diisononyl ester (99%) were supplied from Wuhan Yitongtai Science and Technology Co., Ltd (China) and bis(2-propylheptyl) phthalate from Toronto Research Chemicals (TRC, Canada). The quantification internal standards (IS), dimethyl phthalate-3,4,5,6-d4 (98 atom % D), dibutyl phthalate-3,4,5,6-d4 (98 atom % D) and bis(2-ethylhexyl)phthalate-3,4,5,6-d4 (98 atom % D), and the pre-injection recovery IS, biphenyl (99.5%) were also purchased from Sigma Aldrich (Schenelldorf, Germany). Methyl tert-butyl ether (MTBE), acetone, n-hexane and water were GC chromatographic grade bought from Merck. Standard Reference Material (SRM) 2585 - Organic Contaminants in House Dust was purchased from National Institute of Standards and Technology (NIST, USA).

Target analytes and analytical characteristics

Table SI-1. Retention time during chromatographic separation, names, abbreviations, CAS numbers and chemical structures of selected analytes used in Multiple Reaction Monitoring (quantifier - qualifier ion transitions & collision energies).

Name	Abbreviation	Chemical structure	CAS number	Used as	Retention time (R_t) (min)	MRM1 quantifier	Collision energy (eV)	MRM2 qualifier	Collision energy (eV)
Dimethyl phthalate	DMP		131-11-3	Target	11.7	162.9→77.1	40	162.9→92	40
Diethyl phthalate	DEP		84-66-2	Target	12.9	149.2→93.2	15	149.2→65.2	35
Diisobutyl phthalate	DiBP		84-69-5	Target	14.9	149→65	27	149→93	20
Di-n-butyl phthalate	DnBP		84-74-2	Target	15.6	149.1→65	25	149.1→93.1	25
Benzyl butyl phthalate	BBzP		85-68-7	Target	18.2	149.1→121	15	149.1→65	10
Bis(2-ethylhexyl) phthalate	DEHP		117-81-7	Target	19.3	279→149	2	279→167	2
Diisononyl phthalate	DiNP		28553-12-0	Target	20.2-22.2	293→149	10	293→85.1	5
Bis(2-propylheptyl) phthalate	DPHP		53306-54-0	Target	21.5	307→149.1	5	307→167	5

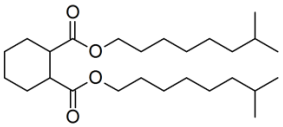
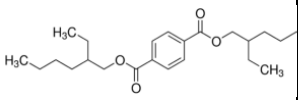
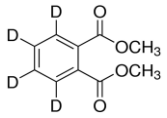
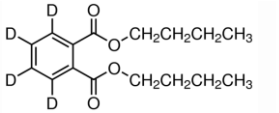
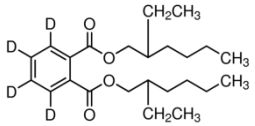
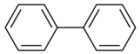
Cyclohexane-1,2-dicarboxylic acid diisononyl ester	DINCH		166412-78-8	Target	20.2-21.8	155.2→81.1	1	281.2→155.1	2
Bis(2-ethylhexyl) terephthalate	DEHT		6422-86-2	Target	20.8	261.1→149.1	1	167→79.1	5
Dimethyl phthalate-3,4,5,6-d_4	DMP-d_4		93951-89-4	ISTD	11.7	166.9→137.1	7.5	166.9→139	8
Dibutyl phthalate-3,4,5,6-d_4	DBP-d_4		93952-11-5	ISTD	15.6	153.1→97.1	15	153.1→69	15
Bis(2-ethylhexyl)phthalate-3,4,5,6-d_4	DEHP-d_4		93951-87-2	ISTD	19.3	152.9→69	30	152.9→97	15
Biphenyl			95-52-4	Injection recovery STD	11.2	154→153	15	154→152	40

Table SI 2- Physicochemical properties of phthalate esters and alternative plasticisers analysed in the present study

Name	Category	Molecular folrmula*	MW (Da)*	Log K_{ow}**	LogKoa estimated **	Water solubility (mg/L) 25oC **	Vapour pressure SUBCOOLED LIQUID (mm Hg, 25oC) **	Polarizability ($\pm 0.5\ 10^{-24}$ cm3) *	LogP*
DMP	Phthalate	C10 H10 O4	194.184	1.66	6.694	2014	3.08E-03	19.7	1.64
DEP	Phthalate	C12 H14 O4	222.237	2.65	7.023	287.2	2.10E-03	23.4	2.7
DiBP	Phthalate	C16 H22 O4	278.344	4.46	8.412	5.061	0.00241	30.7	4.46
DnBP	Phthalate	C16 H22 O4	278.344	4.61	8.631	2.351	2.01E-05	30.8	4.82
BzBP	Phthalate	C19 H20 O4	458.589	8.86	15.099	3.56E-05	1.10E-08	53.8	8.5
DEHP	Phthalate	C24 H38 O4	390.556	8.39	12.557	0.001132	1.42E-07	45.4	8.71
DINCH	Alternative	C26 H48 O4	424.657	9.82	12.36	8.83E-06	1.77E-06	49.0	9.69
DEHT	Alternative	C24 H38 O4	390.556	8.39	11.707	0.0002387	2.14E-05	45.4	9.55
DPHP	Phthalate	C28 H46 O4	446.662	10.36	13.184	2.24E-06	2.29E-07	52.8	10.83
DiNP	Phthalate	C26 H42 O4	418.609	9.15	12.22	3.59E-05	3.21E-06	49.1	9.04

*Data obtained from ACD/Labs Perceptra platoform; **: Data calculated from EPIWEB

Table SI-3 – Sample codes, country of origin, source, collection year and dust mass used per sample (g) for indoor dust extraction and artificial lung fluid incubation

Sample code	Country	Source	Collection year	Mass used (g) for dust extraction	Mass (g) used for artificial lung fluid incubations			
					ALF - R1	ALF - R2	GMB R1	GMB R2
H1	Norway	House	2013	0.100	0.202	0.203	0.201	0.201
H2	Norway	House	2013	0.101	0.203	0.201	0.201	0.202
H3	Norway	House	2013	0.099	0.201	0.201	0.201	0.201
H4	Norway	House	2013	0.100	0.201	0.202	0.203	0.202
H5	Norway	House	2013	0.101	0.201	0.202	0.202	0.203
H6	Norway	House	2013	0.101	0.201	0.201	0.202	0.202
H7	Norway	House	2013	0.100	0.201	0.202	0.202	0.201
H8	Norway	House	2013	0.101	0.203	0.202	0.203	0.201
H9	Norway	House	2013	0.100	0.202	0.202	0.203	0.201
H10	Norway	House	2013	0.100	0.202	0.201	0.201	0.201

Results

Table SI-4 – Descriptive statistics for all target analytes present in indoor dust samples from Norwegian houses (N=10). Concentrations in μg/g.

Target analyte	Minimum	25% Percentile	Median	75% Percentile	Maximum	Geometric mean	Mean	Std. Deviation	DF%
DMP	0.022	0.103	0.202	0.632	1.441	0.221	0.411	0.489	100
DEP	0.522	0.535	1.817	4.723	54.215	2.007	7.557	16.639	100
DiBP	2.585	3.279	6.366	19.243	106.045	8.871	19.349	31.905	100
DnBP	6.487	8.968	10.305	23.619	26.727	12.277	13.88	7.634	100
BzBP	0.217	2.033	10.618	53.379	96.565	9.345	27.23	33.325	100
DEHP	34.207	82.135	224.976	441.794	1463.954	210.403	360.981	431.19	100
DINCH	0.676	14.752	17.064	65.214	229.461	21.849	48.37	67.842	100
DEHT	1.767	10.748	20.052	32.879	38.707	15.841	20.755	12.429	100
DPHP	1.015	2.098	4.685	5.823	22.098	4.058	5.923	6.341	90
DiNP	8.583	31.293	121.611	366.619	2473.949	114.775	401.927	758.975	100

Chapter 5

General discussion

The aim of this thesis was to explore the human exposure and uptake *(i.e*. bioaccessibility) of legacy and emerging flame retardants via indoor dust using artificial human body fluids. The two main research pillars of this PhD thesis were: a) developing and establishing a unified approach with the inclusion of an absorptive sink for the assessment of oral bioaccessibility via dust ingestion as a predominant route of exposure, whilst b) inhalation was explored with respect to human exposure and *in vitro* uptake of phthalate esters and alternative plasticisers, especially the short-chain ones with low MW. EU regulation for the registration, evaluation, authorisation and restriction of chemicals (REACH) requires *in vitro* alternatives which can help reduce the amount of animal testing required. Hence, the research methods and the accompanying results discussed in chapters 2, 3 and 4 are meant to liaise collectively with formerly established testing strategies towards constructive enforcement of *in vitro* bioaccessibility methods and more effective implementation of the "precautionary principle" framework by orchestrating and adopting more efficient *in vitro* alternatives for chemical testing.

In chapter 1, the conceptual difference between bioavailability and bioaccessibility is introduced, followed by a meticulous review of existing literature regarding human exposure and risk assessment to FRs, PEs and alternative plasticisers. Orchestrated under the precautionary principle for organic pollutant assessment and in an attempt to efficiently combine the BARGE criteria for *in vitro* bioaccessibility testing with the ISO 1675 method (ISO, 2015), our systematic review puts forward the necessity for the development of a high-throughput, robust and unified *in vitro* method for oral bioaccessibility testing of organic pollutants. The inclusion of colon microbiota serving as a biologically active "sink" has been previously proposed by (Van de Wiele et al., 2005), studying the estrogenic potency of soil-bound PAHs to humans. Our comprehensive literature regarding "infinite sink" materials and bioaccessibility testing, screened among fundamentally diverse materials as candidates including Tenax TA®, silicone rods and cyclodextrins. Given its large surface area and high sorption capacity for HOCs, Tenax TA® was proposed as the most suitable resin to serve as a non-biological absorption sink and was employed in a novel bioaccessibility method described in chapter 3. Employing a biologically active (*i.e.* colon microbiota) and Tenax TA® as an absorption sink collectively are not recommended, since each one of them serves a profoundly different objective; microbiota inclusion is an ideal solution for the assessment of microbial transformation mechanisms and the underlying effects of organic compounds and their metabolites to the colon environment. However, the inclusion of Tenax TA® as a non-

biologically active absorption sink in the CE-PBET configuration is perceived as an exhaustive method can potentially improve *in vitro* bioaccessibility estimates, while it better mimics the sorption/desorption processes in the human GIT *in vivo*.

In chapter 2, we reported levels of legacy and alternative FRs in house dust samples from Norway and the UK, as well as from British stores and offices. Our findings reveal the predominant character of PFRs in the indoor (house and office) environment with considerable concentrations of monomeric PFRs in indoor dust collected from two geographically diverse areas, Norway and the UK. These findings are mainly governed by the growing use of PFRs in the global market as PBDEs substitutes (van der Veen and de Boer, 2012b). Our dust sample collection was conducted during summer 2013 – spring 2014, which can be considered as a transition period for the flame retardant industry between the banning and phase-out of PBDEs from North America and Europe in 2012 (Dodson et al., 2012; Newton et al., 2015), followed by the emerging prevalence of alternative FRs, including monomeric and oligomeric PFRs (Tao et al., 2016; Xu et al., 2016). Indoor dust is a suitable and well-established matrix not only for monitoring concertation trends and long-term human exposure to PBDEs (Allen et al., 2008), as well as for the identification of newer alternative FRs such as halogen-free oligomeric PFRs including iDPP, EHDPHP and TXP (monomeric PFR), liaised by the support of recent advances in analytical and instrumentational methods (Covaci et al., 2011). In our study, monomeric PFRs were found to be considerably higher in all samples compared to EHFRs, PBDEs and oligomeric PFRs, while the estimated human intakes for FRs for British toddlers were found to be higher than toddlers in Norway and below the suggested RfD values. This is the first study reporting human exposure via dust ingestion for most oligomeric PFRs, including EHDPH, iDPP and TXP; the work presented in chapter 2 acts an open reminder to the environmental and toxicological communities worldwide on the need for advanced and state-of-the-art analytical and instrumental methods as well as the importance of continuous FR screening via indoor dust as a long-established repository sink for organic compounds in the indoor environment.

In chapter 3, we presented a method development of the CE-PBET model with the inclusion of Tenax TA® as an "infinite" sink to the test configuration. With the Tenax TA® inclusion we succeed in 2- and 3-fold increase in FR absorption for the high and low brominated PBDEs, respectively compared to CE-PBET with no Tenax TA® present. While previously published gut bioaccessibility methods involved a self-designed stainless steel sieve for the

separation and the recovery of Tenax TA® beads (Fang and Stapleton, 2014; Li et al., 2015), in our experimental configuration the RC dialysis tubing provides successful physical separation of the Tenax TA® from the solid matrix (dust), has high pH and temperature tolerance and the selected 3.5 kDa MWCO of the RC membrane permits successful sorption on the Tenax TA® even of highly brominated PBDEs such as BDE 154 and BDE183, thus increasing their overall gut bioaccessibility by two-fold via passive diffusion in all CE-PBET compartments. Concerning the Tenax TA® mass loading, our results show that 0.25g were not enough to sustain an exhaustive gut extraction for the low brominated and more water soluble compounds such as BDE28. Hence, 0.5g of Tenax TA® were used in our method settings. Our study proposes that Tenax TA® mass loading should be thus optimised with respect to the physicochemical properties of the analytes of interest.

However, the case of selected EHFRs including EH-TBB, BTBPE and BEH-TEBP should be considered carefully and the available data cannot be fully interpreted as with the PBDEs. This is mostly attributed to instrumental limitations, rather than inefficiencies and inappropriateness of the study design. Two coeluting compounds were noted during GC-EI/MS analysis and their chromatographic separation was unattainable; a) EH-TBB co-elutes with BDE99, thus the latter analyte was excluded from the target compound list (Stapleton et al., 2008) and b) the 13C-labelled standard of BTBPE co-elutes with the native compound, making peak confirmation and peak integration rather challenging especially in full scan mode (NCI and SIM mode were not available in the GC-MS instrument configuration used during these analyses). Finally, even though a moderate gas flow (1.0 mL/min) and a GC column with high temperature tolerance and low-bleed were used during instrumental analysis, BEH-TEBP analytical performance did not meet the expected results presenting low reproducibility. Hence, the reader is advised to consider all the aforementioned limitations when assessing the presented data for the selected EHFRs in chapter 3.

In an attempt to explore alternative routes of exposure, we presented in chapter 4 the first *in vitro* study assessing the inhalation bioaccessibility for established phthalate esters and alternative plasticisers from in indoor dust from Norwegian houses. Low MW and short-chained phthalates such as DMP and DEP were found to be highly bioaccessible (>75%) in both artificial pulmonary media tested (*i.e.* Gamble's solution and ALF), regardless of the medium's pH and chemical composition. Among the studied physicochemical properties, only hydrophobicity was found to significantly influence the *in vitro* pulmonary uptake

($p<0.05$), setting $LogK_{ow}$ as the main driving force governing inhalation bioaccessility of organic pollutants. Dust particles properties such as dust particle size and OMC failed to present any statistically significant relationship with inhalation bioaccessibility. Our results reveal that pulmonary uptake may contribute substantially to human exposure for semi-volatile organic compounds with low MW, hydrophobicity and water solubility. The influence of particle properties

Chapter 6

Future perspectives

To our knowledge, no biomonitoring studies exist where *in vitro* bioaccessibility testing and different exposure routes (e.g. ingestion, inhalation) are collectively combined. Such study design tactics can provide a holistic approach to scientists regarding a more conservative human risk assessment of organics. Prior to embarking into any future biomonitoring studies proper quantification of exposure should be ensured, while development and validation of new analytical methods and commercially available reference standards for emerging compounds are essential. The work presented in this PhD thesis identified the weaknesses and limitations of previously established *in vitro* bioaccessibility tests for organic pollutants; our effort was towards establishing and validating novel bioaccessibility methods. Our main goal was to provide well-designed and state-of-the-art experimental settings with a leap towards an integrated and more comprehensive approach not only used by the scientific niche of environmental scientists, but to deliver *in vitro* methods directly applicable for regulators and policy decision makers to enforce and implement. Finally, the above listed points in addition to a) dust ingestion as a substantial route of exposure for FRs together with b) the requirement of Tenax TA® inclusion as an absorptive sink maintaining a desorption gradient similar to the *in vivo* GIT situation are the two central take-home messages supported by our results.

In vitro cell culture-based models of human adenocarcinoma monolayers such as HT-29 and Caco-2 have been employed in the past in absorption and bioavailability studies, mimicking the permeability potential of the human intestinal barrier (Cavret and Feidt, 2005; Dean and Ma, 2007; Tirelli et al., 2007). Such *in vitro* models combined with *ex vivo* and *in silico* approaches can be beneficial in understanding and elucidating the permeability potential of the intestinal barrier, by predicting GIT absorption, bioavailability and potential metabolism of organic pollutants (Pelkonen et al., 2001). However, such epithelium cell lines, given their current *in vitro* permeability configuration settings, present a finicky character during cell culture and maintenance with debatable viability success (Le Ferrec et al., 2001). Therefore, more effort and research should be encouraged in the future towards refining, re-designing and validating such approaches collectively against *in vivo* bioavailability studies.

One should bear in mind that the pulmonary media used in this study were initially designed for uptake studies of nanoparticles and elements present in soil. Such inorganic compounds tend to be more water soluble, thus their bioaccessibility patterns tend to differ compared those of organic pollutants; when designing this study, we were fully aware of the pulmonary

fluid formulations. Considering the novel character of *in vitro* pulmonary uptake studies for organic pollutants, there is a lot of room for further development towards a more representative and physiologically-relevant to the situation *in vivo* of a) the pulmonary fluid formulation with potential addition of biologically relevant surfactants such as dipalmitoylphosphatidylcholine (DPCC) (Boisa et al., 2014) and mucin type V (Adler et al., 2013; Heo et al., 2009), increasing thus the *in vitro* absorption capacity for less hydrophobic compounds and b) the *in vitro* test settings such as lung volume capacity, dust loading, incubation time etc.. Given all the above, future inhalation bioaccessibility studies should be primarily focused on in vivo validation younger children (<6 years old) who are the most vulnerable age group regarding exposure to phthalate esters through inhalation, rather than adults, whose phthalate exposure is primarily governed by dust ingestion and dietary intake (Giovanoulis, 2017).

Formerly unexplored routes of exposure such as inhalation were also investigated as part of a holistic future perspective for the testing of uptake of chemicals of emerging concern such as phthalate esters and alternative plasticisers. Hence, coming back to the main aims of the present PhD thesis and the necessity of *in vitro* bioaccessibility tests for fine tuning risk assessments discussed in chapter 1, we conclude that robust, high-throughput and rigorous *in vitro* uptake assays mimicking the human processes *in vivo* are essential. Further development and validation *in vivo* should be encouraged, testing for a broad and physico-chemically diverse range of established and emerging organic compounds. Overall, the aforementioned *in vitro* tests presented in this thesis will form the foundation upon which an integrated and robust testing strategy for chemicals of emerging concern can be built, whilst other contaminated matrices other than indoor dust such as soil may be tested in the future. Future research on suitable *in vitro* bioaccessibility tests for the screening of pollutants of emerging concern, as well as the development and validation of *in vitro* tests addressing alternative routes of exposure such as inhalation and dermal uptake, may lead to a comprehensive testing strategy within a realistic and conservative risk assessment framework, in conjunction high-throughput analyses such as non-targeted screening, metabolism and integrated *in silico* approaches for human exposure to consumer chemicals.

References

Abb, M., Heinrich, T., Sorkau, E., Lorenz, W., 2009. Phthalates in house dust. Environ. Int. 35, 965–970. doi:10.1016/j.envint.2009.04.007

Abb M., J.V. Breuer, C. Zeitz & W. Lorenz. 2010. Analysis of pesticides and PCBs in waste wood and house dust. Chemosphere, 81, 488-493.

Abdallah M.A.E., E. Tilston, S. Harrad & C. Collins. 2012. In vitro assessment of the bioaccessibility of brominated flame retardants in indoor dust using a colon extended model of the human gastrointestinal tract. Journal of Environmental Monitoring, 14, 3276-3283.

Abdallah, M.A.-E., Covaci, A., 2014. Organophosphate Flame Retardants in Indoor Dust from Egypt: Implications for Human Exposure. Environ. Sci. Technol. 48, 4782–4789. doi:10.1021/es501078s

Adler, K.B., Tuvim, M.J., Dickey, B.F., 2013. Regulated Mucin Secretion from Airway Epithelial Cells. Front. Endocrinol. 4. doi:10.3389/fendo.2013.00129

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29, 683–689. doi:10.1016/S0160-4120(03)00121-1

Albar, H.M.S.A., Ali, N., Shahzad, K., Ismail, I.M.I., Rashid, M.I., Wang, W., Ali, L.N., Eqani, S.A.M.A.S., 2017. Phthalate esters in settled dust of different indoor microenvironments; source of non-dietary human exposure. Microchem. J. 132, 227–232. doi:10.1016/j.microc.2017.02.008

Ali, N., Ali, L., Mehdi, T., Dirtu, A.C., Al-Shammari, F., Neels, H., Covaci, A., 2013. Levels and profiles of organochlorines and flame retardants in car and house dust from Kuwait and Pakistan: Implication for human exposure via dust ingestion. Environ. Int. 55, 62–70. doi:10.1016/j.envint.2013.02.001

Ali, N., Harrad, S., Muenhor, D., Neels, H., Covaci, A., 2011. Analytical characteristics and determination of major novel brominated flame retardants (NBFRs) in indoor dust. Anal. Bioanal. Chem. 400, 3073–3083. doi:10.1007/s00216-011-4966-7

Ali, N., Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2012. Assessment of human exposure to indoor organic contaminants via dust ingestion in Pakistan. Indoor Air 22, 200–211. doi:10.1111/j.1600-0668.2011.00757.x

Allen, J.G., McClean, M.D., Stapleton, H.M., Webster, T.F., 2008. Critical factors in assessing exposure to PBDEs via house dust. Environ. Int. 34, 1085–1091. doi:10.1016/j.envint.2008.03.006

Allen-King, R.M., Groenevelt, H., Mackay, D.M., 1995. Analytical Method for the Sorption of Hydrophobic Organic Pollutants in Clay-Rich Materials. Environ. Sci. Technol. 29, 148–153. doi:10.1021/es00001a019

Alves, A., Kucharska, A., Erratico, C., Xu, F., Hond, E.D., Koppen, G., Vanermen, G., Covaci, A., Voorspoels, S., 2014. Human biomonitoring of emerging pollutants through non-invasive matrices: state of the art and future potential. Anal. Bioanal. Chem. 406, 4063–4088. doi:10.1007/s00216-014-7748-1

Anh, H.Q., Nam, V.D., Tri, T.M., Ha, N.M., Ngoc, N.T., Mai, P.T.N., Anh, D.H., Minh, N.H., Tuan, N.A., Minh, T.B., 2016. Polybrominated diphenyl ethers in plastic products, indoor dust, sediment and fish from informal e-waste recycling sites in Vietnam: a comprehensive assessment of contamination, accumulation pattern, emissions, and human exposure. Environ. Geochem. Health 1–20. doi:10.1007/s10653-016-9865-6

Bakker M.I., R. de Winter-Sorkina, A. de Mul, P.E. Boon, G. van Donkersgoed, J.D. van Klaveren, B.A. Baumann, W.C. Hijman, S.P.J. van Leeuwen, J. de Boer & M.J. Zeilmaker. 2008. Dietary intake and risk evaluation of polybrominated diphenyl ethers in The Netherlands. Molecular Nutrition & Food Research, 52, 204-216.

Ballesteros-Gómez, A., Aragón, Á., Van den Eede, N., de Boer, J., Covaci, A., 2016. Impurities of Resorcinol Bis(diphenyl phosphate) in Plastics and Dust Collected on Electric/Electronic Material. Environ. Sci. Technol. doi:10.1021/acs.est.5b05351

Ballesteros-Gómez, A., Brandsma, S.H., de Boer, J., Leonards, P.E.G., 2014. Analysis of two alternative organophosphorus flame retardants in electronic and plastic consumer products: Resorcinol bis-(diphenylphosphate) (PBDPP) and bisphenol A bis (diphenylphosphate) (BPA-BDPP). Chemosphere, Flame Retardants in the Environment - Papers presented at 6th International Symposium on Flame Retardants (BFR2013), San Francisco from April 7-10 116, 10–14. doi:10.1016/j.chemosphere.2013.12.099

Batterman, S., Godwin, C., Chernyak, S., Jia, C., Charles, S., 2010. Brominated flame retardants in offices in Michigan, U.S.A. Environ. Int. 36, 548–556. doi:10.1016/j.envint.2010.04.008

Bearr, J.S., Mitchelmore, C.L., Roberts, S.C., Stapleton, H.M., 2012. Species specific differences in the in vitro metabolism of the flame retardant mixture, Firemaster® BZ-54. Aquat. Toxicol. 124–125, 41–47. doi:10.1016/j.aquatox.2012.06.006

Bekö, G., Weschler, C.J., Langer, S., Callesen, M., Toftum, J., Clausen, G., 2013. Children's Phthalate Intakes and Resultant Cumulative Exposures Estimated from Urine Compared with Estimates from Dust Ingestion, Inhalation and Dermal Absorption in Their Homes and Daycare Centers. PLoS ONE 8, e62442. doi:10.1371/journal.pone.0062442

Bellinger, D.C., 2013. Prenatal Exposures to Environmental Chemicals and Children's Neurodevelopment: An Update. Saf. Health Work 4, 1–11. doi:10.5491/SHAW.2013.4.1.1

Ben, Y.-J., Li, X.-H., Yang, Y.-L., Li, L., Di, J.-P., Wang, W.-Y., Zhou, R.-F., Xiao, K., Zheng, M.-Y., Tian, Y., Xu, X.-B., 2013. Dechlorane Plus and its dechlorinated analogs from an e-waste recycling center in maternal serum and breast milk of women in Wenling, China. Environ. Pollut. 173, 176–181. doi:10.1016/j.envpol.2012.09.028

Bergh C., R. Torgrip, G. Emenius & C. Östman. 2011. Organophosphate and phthalate esters in air and settled dust – a multi-location indoor study. Indoor Air, 21, 67-76.

Bergh, C., Torgrip, R., Emenius, G., Östman, C., 2011. Organophosphate and phthalate esters in air and settled dust – a multi-location indoor study. Indoor Air 21, 67–76. doi:10.1111/j.1600-0668.2010.00684.x

Berntssen M.H.G., P.A. Olsvik, B.E. Torstensen, K. Julshamn, T. Midtun, A. Goksoyr, J. Johansen, T. Sigholt, N. Joerum, J.V. Jakobsen, A.K. Lundebye & E.J. Lock. 2010. Reducing persistent organic pollutants while maintaining long chain omega-3 fatty acid in farmed Atlantic salmon using decontaminated fish oils for an entire production cycle. Chemosphere, 81, 242-252.

Betts, K.S., 2013. Exposure to TDCPP Appears Widespread. Environ. Health Perspect. 121, a150. doi:10.1289/ehp.121-a150

Boisa, N., Elom, N., Dean, J.R., Deary, M.E., Bird, G., Entwistle, J.A., 2014. Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10 size fraction of soil. Environ. Int. 70, 132–142. doi:10.1016/j.envint.2014.05.021

Bornehag, C.-G., Lundgren, B., Weschler, C.J., Sigsgaard, T., Hagerhed-Engman, L., Sundell, J., 2005. Phthalates in Indoor Dust and Their Association with Building Characteristics. Environ. Health Perspect. 113, 1399–1404. doi:10.1289/ehp.7809

Bornehag, C.G., Nanberg, E., 2010. Phthalate exposure and asthma in children. Int. J. Androl. 33, 333–345. doi:10.1111/j.1365-2605.2009.01023.x

Bornehag, C.-G., Sundell, J., Weschler, C.J., Sigsgaard, T., Lundgren, B., Hasselgren, M., Hägerhed-Engman, L., 2004. The Association between Asthma and Allergic Symptoms in Children and Phthalates in House Dust: A Nested Case-Control Study. Environ. Health Perspect. 112, 1393–1397.

Brandon, E.F.A., Oomen, A.G., Rompelberg, C.J.M., Versantvoort, C.H.M., van Engelen, J.G.M., Sips, A.J.A.M., 2006. Consumer product in vitro digestion model: Bioaccessibility of contaminants and its application in risk assessment. Regul. Toxicol. Pharmacol. 44, 161–171. doi:10.1016/j.yrtph.2005.10.002

Brandsma, S.H., Sellström, U., de Wit, C.A., de Boer, J., Leonards, P.E.G., 2013. Dust Measurement of Two Organophosphorus Flame Retardants, Resorcinol Bis(diphenylphosphate) (RBDPP) and Bisphenol A Bis(diphenylphosphate) (BPA-BDPP), Used as Alternatives for BDE-209. Environ. Sci. Technol. 47, 14434–14441. doi:10.1021/es404123q

Brasche S. & W. Bischof. 2005. Daily time spent indoors in German homes--baseline data for the assessment of indoor exposure of German occupants. International journal of hygiene and environmental health, 208, 247-253.

Brommer S., S. Harrad, N.V.d. Eede & A. Covaci. 2012. Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. Journal of Environmental Monitoring, 14, 2482-2487.

Brommer, S., Harrad, S., 2015. Sources and human exposure implications of concentrations of organophosphate flame retardants in dust from UK cars, classrooms, living rooms, and offices. Environ. Int. 83, 202–207. doi:10.1016/j.envint.2015.07.002

Brommer, S., Harrad, S., Eede, N.V. den, Covaci, A., 2012. Concentrations of organophosphate esters and brominated flame retardants in German indoor dust samples. J. Environ. Monit. 14, 2482–2487. doi:10.1039/C2EM30303E

Bruce S., B. Noller, V. Matanitobua & J. Ng. 2007. In vitro physiologically based extraction test (PBET) and bioaccessibility of arsenic and lead from various mine waste materials. Journal of Toxicology and Environmental Health-Part a-Current Issues, 70, 1700-1711.

Bui, T.T., Alves, A., Palm-Cousins, A., Voorspoels, S., Covaci, A., Cousins, I.T., 2017. Estimating uptake of phthalate ester metabolites into the human nail plate using pharmacokinetic modelling. Environ. Int. 100, 148–155. doi:10.1016/j.envint.2017.01.007

Bui, T.T., Giovanoulis, G., Cousins, A.P., Magnér, J., Cousins, I.T., de Wit, C.A., 2016. Human exposure, hazard and risk of alternative plasticizers to phthalate esters. Sci. Total Environ. 541, 451–467. doi:10.1016/j.scitotenv.2015.09.036

Butte W. & B. Heinzow. 2002. Pollutants in house dust as indicators of indoor contamination. Rev Environ Contam Toxicol, 175, 1-46.

Calabrese E.J., E.J. Stanek, R. Barnes, D.E. Burmaster, B.G. Callahan, J.S. Heath, D. Paustenbach, J. Abraham & L.A. Gephart. 1996. Methodology to estimate the amount

and particle size of soil ingested by children: implications for exposure assessment at waste sites. Regulatory toxicology and pharmacology : RTP, 24, 264-268.

Cao, Z., Xu, F., Covaci, A., Wu, M., Wang, H., Yu, G., Wang, B., Deng, S., Huang, J., Wang, X., 2014a. Distribution Patterns of Brominated, Chlorinated, and Phosphorus Flame Retardants with Particle Size in Indoor and Outdoor Dust and Implications for Human Exposure. Environ. Sci. Technol. doi:10.1021/es501224b

Cao, Z., Xu, F., Covaci, A., Wu, M., Yu, G., Wang, B., Deng, S., Huang, J., 2014b. Differences in the seasonal variation of brominated and phosphorus flame retardants in office dust. Environ. Int. 65, 100–106. doi:10.1016/j.envint.2013.12.011

Carlstedt, F., Jönsson, B. a. G., Bornehag, C.-G., 2013. PVC flooring is related to human uptake of phthalates in infants. Indoor Air 23, 32–39. doi:10.1111/j.1600-0668.2012.00788.x

Cave M.R., J. Wragg, I. Harrison, C.H. Vane, E. De Groeve, T. Van De Wiele, C.P. Nathanail, M. Ashmore, R. Thomas, J. Robinson & P. Daly. 2010a. Comparison of Batch Mode and Dynamic Physiologically Based Bioaccessibility Tests for PAHs in Soil Samples. 44, 2654-2660.

Cave, M.R., Wragg, J., Harrison, I., Vane, C.H., Wiele, T.V. de, Groeve, E.D., Nathanail, C.P., Ashmore, M., Thomas, R., Robinson, J., Daly, P., 2010. Comparison of Batch Mode and Dynamic Physiologically Based Bioaccessibility Tests for PAHs in Soil Samples. Environ. Sci. Technol. 44, 2654–2660. doi:10.1021/es903258v

Cavret, S., Feidt, C., 2005. Intestinal metabolism of PAH: in vitro demonstration and study of its impact on PAH transfer through the intestinal epithelium. Environ. Res. 98, 22–32. doi:10.1016/j.envres.2004.10.010

Cequier, E., Ionas, A.C., Covaci, A., Marcé, R.M., Becher, G., Thomsen, C., 2014. Occurrence of a Broad Range of Legacy and Emerging Flame Retardants in Indoor Environments in Norway. Environ. Sci. Technol. 48, 6827–6835. doi:10.1021/es500516u

Cequier, E., Sakhi, A.K., Marcé, R.M., Becher, G., Thomsen, C., 2015. Human exposure pathways to organophosphate triesters — A biomonitoring study of mother–child pairs. Environ. Int. 75, 159–165. doi:10.1016/j.envint.2014.11.009

Chemtura Corp, 2013. Great Lakes solutions Flame Retardants Product Guide.

Cheng Z., X.-P. Nie, H.-S. Wang & M.-H. Wong. 2013. Risk assessments of human exposure to bioaccessible phthalate esters through market fish consumption. Environment International, 57-58, 75-80.

Collins C.D., M. Mosquera-Vazquez, J.L. Gomez-Eyles, P. Mayer, V. Gouliarmou & E. Blum. 2013. Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils? Environmental Pollution, 181, 128-132.

Collins, C.D., Craggs, M., Garcia-Alcega, S., Kademoglou, K., Lowe, S., 2015. 'Towards a unified approach for the determination of the bioaccessibility of organic pollutants.' Environ. Int. 78, 24–31. doi:10.1016/j.envint.2015.02.005

Collins, C.D., Mosquera-Vazquez, M., Gomez-Eyles, J.L., Mayer, P., Gouliarmou, V., Blum, F., 2013. Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils? Environ. Pollut. 181, 128–132. doi:10.1016/j.envpol.2013.05.053

Cornelissen, G., van Noort, P.C.M., Govers, H.A.J., 1997. Desorption kinetics of chlorobenzenes, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: Sediment extraction with Tenax® and effects of contact time and solute hydrophobicity. Environ. Toxicol. Chem. 16, 1351–1357. doi:10.1002/etc.5620160703

Correia-Sá, L., Schütze, A., Norberto, S., Calhau, C., Domingues, V.F., Koch, H.M., 2017. Exposure of Portuguese children to the novel non-phthalate plasticizer di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH). Environ. Int. doi:10.1016/j.envint.2017.02.001

Costa, L.G., Giordano, G., 2007. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. NeuroToxicology 28, 1047–1067. doi:10.1016/j.neuro.2007.08.007

Covaci, A., Harrad, S., Abdallah, M.A.-E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: A review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532–556. doi:10.1016/j.envint.2010.11.007

Cui, X.-Y., Xiang, P., He, R.-W., Juhasz, A., Ma, L.Q., 2016. Advances in in vitro methods to evaluate oral bioaccessibility of PAHs and PBDEs in environmental matrices. Chemosphere 150, 378–389. doi:10.1016/j.chemosphere.2016.02.041

Cunha S.C., K. Kalachova, J. Pulkrabova, J.O. Fernandes, M.B.P.P. Oliveira, A. Alves & J. Hajslova. 2010. Polybrominated diphenyl ethers (PBDEs) contents in house and car dust of Portugal by pressurized liquid extraction (PLE) and gas chromatography–mass spectrometry (GC–MS). Chemosphere, 78, 1263-1271.

Darnerud P.O. 2003. Toxic effects of brominated flame retardants in man and in wildlife. Environment International, 29, 841-853.

de Wit, C.A., Herzke, D., Vorkamp, K., 2010. Brominated flame retardants in the Arctic environment — trends and new candidates. Sci. Total Environ. 408, 2885–2918. doi:10.1016/j.scitotenv.2009.08.037

Dean, J.R., Elom, N.I., Entwistle, J.A., 2017. Use of simulated epithelial lung fluid in assessing the human health risk of Pb in urban street dust. Sci. Total Environ. 579, 387–395. doi:10.1016/j.scitotenv.2016.11.085

Dean, J.R., Ma, R., 2007. Approaches to assess the oral bioaccessibility of persistent organic pollutants: A critical review. Chemosphere 68, 1399–1407. doi:10.1016/j.chemosphere.2007.03.054

Derouet, D., Morvan, F., Brosse, J.C., 1996. Chemical modification of epoxy resins by dialkyl(or aryl) phosphates: Evaluation of fire behavior and thermal stability. J. Appl. Polym. Sci. 62, 1855–1868. doi:10.1002/(SICI)1097-4628(19961212)62:11<1855::AID-APP10>3.0.CO;2-Y

Deutschle, T., Reiter, R., Butte, W., Heinzow, B., Keck, T., Riechelmann, H., 2008. A Controlled Challenge Study on Di(2-ethylhexyl) Phthalate (DEHP) in House Dust and the Immune Response in Human Nasal Mucosa of Allergic Subjects. Environ. Health Perspect. 116, 1487–93.

Dodson, R.E., Camann, D.E., Morello-Frosch, R., Brody, J.G., Rudel, R.A., 2015. Semivolatile Organic Compounds in Homes: Strategies for Efficient and Systematic Exposure Measurement Based on Empirical and Theoretical Factors. Environ. Sci. Technol. 49, 113–122. doi:10.1021/es502988r

Dodson, R.E., Perovich, L.J., Covaci, A., Van den Eede, N., Ionas, A.C., Dirtu, A.C., Brody, J.G., Rudel, R.A., 2012. After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ. Sci. Technol. 46, 13056–13066. doi:10.1021/es303879n

Doelman, C.J., Borm, P.J., Bast, A., 1990. Plasticisers and bronchial hyperreactivity. Lancet Lond. Engl. 335, 725.

de Wit C.A. 2002. An overview of brominated flame retardants in the environment. Chemosphere, 46, 583-624.

Dean J.R. & R.L. Ma. 2007. Approaches to assess the oral bioaccessibility of persistent organic pollutants: A critical review. Chemosphere, 68, 1399-1407.

Denys S., J. Caboche, K. Tack, G. Rychen, J. Wragg, M. Cave, C. Jondreville & C. Feidt. 2012. In Vivo Validation of the Unified BARGE Method to Assess the Bioaccessibility of Arsenic, Antimony, Cadmium, and Lead in Soils. Environmental Science & Technology, 46, 6252-6260.

Domingo J.L., A. Bocio, G. Falcó & J.M. Llobet. 2007. Benefits and risks of fish consumption: Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology, 230, 219-226.

Duan L., T. Palanisami, Y. Liu, Z. Dong, M. Mallavarapu, T. Kuchel, K.T. Semple & R. Naidu. 2014. Effects of ageing and soil properties on the oral bioavailability of benzo a pyrene using a swine model. Environment International, 70, 192-202.

EBFRIP, 2008. European Brominated Flame Retardant Industry Panel (EBFRIP) - Legislation on BFRs in Norway [WWW Document]. URL http://www.ebfrip.org/main-nav/european-regulatory-centre/national-regulations/norway (accessed 7.12.16).

ECB. 2002. European Chemicals Bureau - European Union Risk Assessment Report: bis (pentabromophenyl) ether. Office for Official Publications of the European Communities, Luxembourg.

ECHA, 2008a. EU RISK ASSESSMENT – tris(1,3-dichloro-2-propyl)phosphate TCPP CAS 13674-84-5 [WWW Document]. URL https://echa.europa.eu/documents/10162/13630/trd_rar_ireland_tccp_en.pdf (accessed 9.12.16).

ECHA, 2008b. EU RISK ASSESSMENT – tris(2-chloro-1-(chloromethyl)ethyl) phosphate TDCP CAS 13674-87-8 [WWW Document]. URL https://echa.europa.eu/documents/10162/13630/trd_rar_ireland_tccp_en.pdf (accessed 9.12.16).

ECHA, 2008c. EU RISK ASSESSMENT – tetraekis(2-chlorethyl)-dichloroisopentyldiphosphate (V6) CAS 38051-10-4 [WWW Document]. URL https://echa.europa.eu/documents/10162/c38476f5-ebfc-43b2-8800-83f04e623c74 (accessed 9.12.16).

ECHA, 2012. Bis(pentabromophenyl) ether (decabromodiphenyl... - Candidate List of substances of very high concern for Authorisation - ECHA [WWW Document]. URL https://echa.europa.eu/candidate-list-table/-/dislist/details/0b0236e1807dd2e6 (accessed 9.7.16).

ECHA, 2013. ANNEX XV – IDENTIFICATION OF TRIXYLYLPHOSPHATE (TXP) CAS 25155-23-1 AS SVHC [WWW Document]. URL https://echa.europa.eu/documents/10162/953524f2-7965-430c-be61-0bb02f08f83c (accessed 9.12.16).

ECHA, 2015. Trixylyl phosphate CAS 25155-23-1 Draft background document for trixylyl phosphate in the context of ECHA's seventh recommendation for the inclusion of substances in Annex XIV [WWW Document]. URL https://echa.europa.eu/documents/10162/953524f2-7965-430c-be61-0bb02f08f83c (accessed 9.12.16).

Ertl H. & W. Butte. 2012. Bioaccessibility of pesticides and polychlorinated biphenyls from house dust: in-vitro methods and human exposure assessment. Journal of exposure science & environmental epidemiology, 22, 574-583.

Ertl, H., Butte, W., 2012. Bioaccessibility of pesticides and polychlorinated biphenyls from house dust: in-vitro methods and human exposure assessment. J. Expo. Sci. Environ. Epidemiol. 22, 574–583. doi:10.1038/jes.2012.50

European Commission, 2015. EC amendment Annex II to Directive 2011/65/EU of the European Parliament and of the Council as regards the list of restricted substances [WWW Document]. URL http://ec.europa.eu/transparency/regdoc/rep/3/2015/EN/3-2015-2067-EN-F1-1.PDF (accessed 2.21.17).

European Commission, 2016. Regulation amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(pentabromophenyl)ether - Reference: G/TBT/N/EU/375 [WWW Document]. URL http://ec.europa.eu/transparency/regcomitology/index.cfm?do=search.documentdetail&cq8QnqGc5Vt5Bt5NkaBcEtHa7CE1pE/6NbmH3ijdpBXVqHZGdIwy2rS97ztb5t8b (accessed 9.26.16).

Fang, M., Stapleton, H.M., 2014. Evaluating the Bioaccessibility of Flame Retardants in House Dust Using an In Vitro Tenax Bead-Assisted Sorptive Physiologically Based Method. Environ. Sci. Technol. doi:10.1021/es503918m

Fang, M., Webster, T.F., Gooden, D., Cooper, E.M., McClean, M.D., Carignan, C., Makey, C., Stapleton, H.M., 2013. Investigating a Novel Flame Retardant Known as V6: Measurements in Baby Products, House Dust, and Car Dust. Environ. Sci. Technol. 47, 4449–4454. doi:10.1021/es400032v

Franken, C., Lambrechts, N., Govarts, E., Koppen, G., Den Hond, E., Ooms, D., Voorspoels, S., Bruckers, L., Loots, I., Nelen, V., Sioen, I., Nawrot, T.S., Baeyens, W., Van Larebeke, N., Schoeters, G., 2017. Phthalate-induced oxidative stress and association with asthma-related airway inflammation in adolescents. Int. J. Hyg. Environ. Health. doi:10.1016/j.ijheh.2017.01.006

Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Dietrich, S., Sievering, S., Burghardt, R., Schuster, R., Bolte, G., Völkel, W., 2013. Phthalates in German daycare centers: Occurrence in air and dust and the excretion of their metabolites by children (LUPE 3). Environ. Int. 61, 64–72. doi:10.1016/j.envint.2013.09.006

Fromme, H., Lahrz, T., Piloty, M., Gebhart, H., Oddoy, A., Rüden, H., 2004. Occurrence of phthalates and musk fragrances in indoor air and dust from apartments and kindergartens in Berlin (Germany). Indoor Air 14, 188–195. doi:10.1111/j.1600-0668.2004.00223.x

García-Alcega, S., Rauert, C., Harrad, S., Collins, C.D., 2016. Does the source migration pathway of HBCDs to household dust influence their bio-accessibility? Sci. Total Environ. 569–570, 244–251. doi:10.1016/j.scitotenv.2016.04.178

Gerberding J. 2004. Toxicological Profile for Polybrominated Biphenyls and Polybrominated Diphenyl Ethers (PBBs and PBDEs). U.S. Department of Health and Human Services, Public Health Service Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, Georgia.

Gevao, B., Al-Ghadban, A.N., Bahloul, M., Uddin, S., Zafar, J., 2013. Phthalates in indoor dust in Kuwait: implications for non-dietary human exposure. Indoor Air 23, 126–133. doi:10.1111/ina.12001

Giovanoulis, G., 2017. What contributes to human body burdens of phthalate esters? : An experimental approach.

Gouliarmou V., C.D. Collins, E. Christiansen & P. Mayer. 2013. Sorptive Physiologically Based Extraction of Contaminated Solid Matrices: Incorporating Silicone Rod As Absorption Sink for Hydrophobic Organic Contaminants. Environmental Science & Technology, 47, 941-948.

Gouliarmou, V., Collins, C.D., Christiansen, E., Mayer, P., 2013. Sorptive Physiologically Based Extraction of Contaminated Solid Matrices: Incorporating Silicone Rod As Absorption Sink for Hydrophobic Organic Contaminants. Environ. Sci. Technol. 47, 941–948. doi:10.1021/es303165u

Gouliarmou, V., Mayer, P., 2012. Sorptive Bioaccessibility Extraction (SBE) of Soils: Combining a Mobilization Medium with an Absorption Sink. Environ. Sci. Technol. 46, 10682–10689. doi:10.1021/es301515s

Gron C., A. Oomen, E. Weyand & J. Wittsiepe. 2007. Bioaccessibility of PAH from Danish soils. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 42, 1233-1239.

Guney, M., Zagury, G.J., 2016. Bioaccessibility and other key parameters in assessing oral exposure to PAH-contaminated soils and dust: A critical review. Hum. Ecol. Risk Assess. Int. J. 22, 1396–1417. doi:10.1080/10807039.2016.1185691

Guo L., Y. Qiu, G. Zhang, G.J. Zheng, P.K.S. Lam & X. Li. 2008. Levels and bioaccumulation of organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) in fishes from the Pearl River estuary and Daya Bay, South China. Environmental Pollution, 152, 604-611.

Guo, Y., Kannan, K., 2011. Comparative Assessment of Human Exposure to Phthalate Esters from House Dust in China and the United States. Environ. Sci. Technol. 45, 3788–3794. doi:10.1021/es2002106

Hack A. & F. Selenka. 1996. Mobilization of PAH and PCB from contaminated soil using a digestive tract model. Toxicology Letters, 88, 199-210.

Harper, C., 2003. Handbook of Building Materials for Fire Protection. McGraw Hill Professional.

Harrad S., C. Ibarra, M.A.-E. Abdallah, R. Boon, H. Neels & A. Covaci. 2008. Concentrations of brominated flame retardants in dust from United Kingdom cars, homes, and offices: Causes of variability and implications for human exposure. Environment International, 34, 1170-1175.

Hauser, R., Calafat, A.M., 2005. Phthalates and Human Health. Occup. Environ. Med. 62, 806–818. doi:10.1136/oem.2004.017590

Hauser, R., Duty, S., Godfrey-Bailey, L., Calafat, A.M., 2004. Medications as a source of human exposure to phthalates. Environ. Health Perspect. 112, 751–753.

Hazrati S. & S. Harrad. 2006. Causes of Variability in Concentrations of Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers in Indoor air. Environmental Science & Technology, 40, 7584-7589.

He, R., Li, Y., Xiang, P., Li, C., Zhou, C., Zhang, S., Cui, X., Ma, L.Q., 2016a. Organophosphorus flame retardants and phthalate esters in indoor dust from different microenvironments: Bioaccessibility and risk assessment. Chemosphere 150, 528–535. doi:10.1016/j.chemosphere.2015.10.087

Hedberg, Y., Gustafsson, J., Karlsson, H.L., Möller, L., Wallinder, I.O., 2010. Bioaccessibility, bioavailability and toxicity of commercially relevant iron- and chromium-based particles: in vitro studies with an inhalation perspective. Part. Fibre Toxicol. 7, 23. doi:10.1186/1743-8977-7-23

Henry T.R. & M.J. DeVito. 2003. NON-DIOXIN-LIKE PCBs: EFFECTS AND CONSIDERATION IN ECOLOGICAL RISK ASSESSMENT. pp. 49-49.

Heo, H.J., Lee, S.Y., Lee, M.N., Lee, H.J., Seok, J.H., Lee, C.J., 2009. Genistein and curcumin suppress epidermal growth factor-induced MUC5AC mucin production and gene expression from human airway epithelial cells. Phytother. Res. 23, 1458–1461. doi:10.1002/ptr.2801

Heudorf, U., Mersch-Sundermann, V., Angerer, J., 2007. Phthalates: Toxicology and exposure. Int. J. Hyg. Environ. Health, Children's Environment in Central Europe - Threats and ChancesResults of an International Workshop held on November 21-24, 2006 in Osnabrück 210, 623–634. doi:10.1016/j.ijheh.2007.07.011

Hsu, N.-Y., Lee, C.-C., Wang, J.-Y., Li, Y.-C., Chang, H.-W., Chen, C.-Y., Bornehag, C.-G., Wu, P.-C., Sundell, J., Su, H.-J., 2012. Predicted risk of childhood allergy, asthma,

and reported symptoms using measured phthalate exposure in dust and urine. Indoor Air 22, 186–199. doi:10.1111/j.1600-0668.2011.00753.x

Hu X., Y. Zhang, Z. Ding, T. Wang, H. Lian, Y. Sun & J. Wu. 2012. Bioaccessibility and health risk of arsenic and heavy metals (Cd, Co, Cr, Cu, Ni, Pb, Zn and Mn) in TSP and PM2.5 in Nanjing, China. Atmospheric Environment, 57, 146-152.

Jaakkola, J.J., Oie, L., Nafstad, P., Botten, G., Samuelsen, S.O., Magnus, P., 1999. Interior surface materials in the home and the development of bronchial obstruction in young children in Oslo, Norway. Am. J. Public Health 89, 188–192. doi:10.2105/AJPH.89.2.188

Jaakkola, J.J.K., Knight, T.L., 2008. The Role of Exposure to Phthalates from Polyvinyl Chloride Products in the Development of Asthma and Allergies: A Systematic Review and Meta-analysis. Environ. Health Perspect. 116, 845–53.

James K., R.E. Peters, B.D. Laird, W.K. Ma, M. Wickstrom, G.L. Stephenson & S.D. Siciliano. 2011. Human Exposure Assessment: A Case Study of 8 PAH Contaminated Soils Using in Vitro Digestors and the Juvenile Swine Model. Environmental Science & Technology, 45, 4586-4593.

James, K., Peters, R.E., Laird, B.D., Ma, W.K., Wickstrom, M., Stephenson, G.L., Siciliano, S.D., 2011. Human Exposure Assessment: A Case Study of 8 PAH Contaminated Soils Using in Vitro Digestors and the Juvenile Swine Model. Environ. Sci. Technol. 45, 4586–4593. doi:10.1021/es1039979

Jones-Otazo, H.A., Clarke, J.P., Diamond, M.L., Archbold, J.A., Ferguson, G., Harner, T., Richardson, G.M., Ryan, J.J., Wilford, B., 2005. Is House Dust the Missing Exposure Pathway for PBDEs? An Analysis of the Urban Fate and Human Exposure to PBDEs. Environ. Sci. Technol. 39, 5121–5130. doi:10.1021/es048267b

Jonsson, R., 2005. The end consumer's choice of floorcovering in the Netherlands and the United Kingdom: a comparative pilot study of substitute competition. J. Wood Sci. 51, 154–160. doi:10.1007/s10086-004-0632-4

Juhasz A.L., E. Smith, J. Weber, M. Rees, A. Rofe, T. Kuchel, L. Sansom & R. Naidu. 2007. Comparison of in vivo and in vitro methodologies for the assessment of arsenic bioavailability in contaminated soils. Chemosphere, 69, 961-966.

Juhasz A.L., J. Weber, G. Stevenson, D. Slee, D. Gancarz, A. Rofe & E. Smith. 2014. In vivo measurement, in vitro estimation and fugacity prediction of PAH bioavailability in post-remediated creosote-contaminated soil. Science of the Total Environment, 473, 147-154.

ICL IP Inc., 2008. Syn-O-Ad® 8475 MSDS ICL-Industrial Products [WWW Document]. URL http://icl-ip.com/wp-content/uploads/2012/03/7071SA_enSYN-O-AD.pdf (accessed 9.12.16).

Ionas, A.C., Dirtu, A.C., Anthonissen, T., Neels, H., Covaci, A., 2014. Downsides of the recycling process: Harmful organic chemicals in children's toys. Environ. Int. 65, 54–62. doi:10.1016/j.envint.2013.12.019

ISO, 2015. ISO/DIS 16751 - Soil quality -- Environmental availability of non-polar organic compounds -- Determination of the potential bioavailable fraction and the non-bioavailable fraction using a strong adsorbent or complexing agent [WWW Document]. URL https://www.iso.org/standard/64272.html (accessed 7.21.17).

Kademoglou, K., Xu, F., Padilla-Sanchez, J.A., Haug, L.S., Covaci, A., Collins, C.D., 2017. Legacy and alternative flame retardants in Norwegian and UK indoor environment: Implications of human exposure via dust ingestion. Environ. Int. 102, 48–56. doi:10.1016/j.envint.2016.12.012

Kalantzi, O.I., Martin, F.L., Thomas, G.O., Alcock, R.E., Tang, H.R., Drury, S.C., Carmichael, P.L., Nicholson, J.K., Jones, K.C., 2004. Different Levels of Polybrominated Diphenyl Ethers (PBDEs) and Chlorinated Compounds in Breast Milk from Two U.K. Regions. Environ. Health Perspect. 112, 1085–1091. doi:10.1289/ehp.6991

Kanazawa, A., Saito, I., Araki, A., Takeda, M., Ma, M., Saijo, Y., Kishi, R., 2010. Association between indoor exposure to semi-volatile organic compounds and building-related symptoms among the occupants of residential dwellings. Indoor Air 20, 72–84. doi:10.1111/j.1600-0668.2009.00629.x

Kang Y., Y. Yin, Y. Man, L. Li, Q. Zhang, L. Zeng, J. Luo & M.H. Wong. 2013. Bioaccessibility of polychlorinated biphenyls in workplace dust and its implication for risk assessment. Chemosphere, 93, 924-930.

Kang Y., Y.B. Man, K.C. Cheung & M.H. Wong. 2012. Risk Assessment of Human Exposure to Bioaccessible Phthalate Esters via Indoor Dust around the Pearl River Delta. Environmental Science & Technology, 46, 8422-8430.

Kang, Y., Man, Y.B., Cheung, K.C., Wong, M.H., 2012. Risk Assessment of Human Exposure to Bioaccessible Phthalate Esters via Indoor Dust around the Pearl River Delta. Environ. Sci. Technol. 46, 8422–8430. doi:10.1021/es300379v

Kim, J.-W., Isobe, T., Muto, M., Tue, N.M., Katsura, K., Malarvannan, G., Sudaryanto, A., Chang, K.-H., Prudente, M., Viet, P.H., Takahashi, S., Tanabe, S., 2014. Organophosphorus flame retardants (PFRs) in human breast milk from several Asian countries. Chemosphere, Flame Retardants in the Environment - Papers presented at

6th International Symposium on Flame Retardants (BFR2013), San Francisco from April 7-10 116, 91–97. doi:10.1016/j.chemosphere.2014.02.033

Klara Midander, A. de F., 2010. Bioaccessibility studies of ferro-chromium alloy particles for a simulated inhalation scenario: a comparative study with the pure metals and stainless steel. Integr. Environ. Assess. Manag. 6, 441–55. doi:10.1002/ieam.32

Kocbach Bølling, A., Holme, J.A., Bornehag, C.G., Nygaard, U.C., Bertelsen, R.J., Nånberg, E., Bodin, J., Sakhi, A.K., Thomsen, C., Becher, R., 2013. Pulmonary phthalate exposure and asthma - is PPAR a plausible mechanistic link? EXCLI J. 12, 733–759.

Koch I., K.J. Reimer, M.I. Bakker, N.T. Basta, M.R. Cave, S. Denys, M. Dodd, B.A. Hale, R. Irwin, Y.W. Lowney, M.M. Moore, V. Paquin, P.E. Rasmussen, T. Repaso-Subang, G.L. Stephenson, S.D. Siciliano, J. Wragg & G.J. Zagury. 2013. Variability of bioaccessibility results using seventeen different methods on a standard reference material, NIST 2710. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 48, 641-655.

Kolarik, B., Naydenov, K., Larsson, M., Bornehag, C.-G., Sundell, J., 2008. The Association between Phthalates in Dust and Allergic Diseases among Bulgarian Children. Environ. Health Perspect. 116, 98–103.

Kriebel, D., Tickner, J., Epstein, P., Lemons, J., Levins, R., Loechler, E.L., Quinn, M., Rudel, R., Schettler, T., Stoto, M., 2001. The precautionary principle in environmental science. Environ. Health Perspect. 109, 871–876.

Kubwabo, C., Rasmussen, P.E., Fan, X., Kosarac, I., Wu, F., Zidek, A., Kuchta, S.L., 2013. Analysis of selected phthalates in Canadian indoor dust collected using household vacuum and standardized sampling techniques. Indoor Air 23, 506–514. doi:10.1111/ina.12048

Laird, B.D., Van de Wiele, T.R., Corriveau, M.C., Jamieson, H.E., Parsons, M.B., Verstraete, W., Siciliano, S.D., 2007. Gastrointestinal Microbes Increase Arsenic Bioaccessibility of Ingested Mine Tailings Using the Simulator of the Human Intestinal Microbial Ecosystem. Environ. Sci. Technol. 41, 5542–5547. doi:10.1021/es062410e

Lamoureux G.L. & D.G. Rusness. 1986. Xenobiotic Conjugation in Higher Plants. In: Xenobiotic Conjugation Chemistry, pp. 62-105. American Chemical Society.

Langer, S., Weschler, C.J., Fischer, A., Bekö, G., Toftum, J., Clausen, G., 2010. Phthalate and PAH concentrations in dust collected from Danish homes and daycare centers. Atmos. Environ. 44, 2294–2301. doi:10.1016/j.atmosenv.2010.04.001

Latendresse, J.R., Brooks, C.L., Capen, C.C., 1994. Pathologic Effects of Butylated Triphenyl Phosphate-Based Hydraulic Fluid and Tricresyl Phosphate on the Adrenal

Gland, Ovary, and Testis in the Fischer-344 Rat. Toxicol. Pathol. 22, 341–352. doi:10.1177/019262339402200401

Le Ferrec, E., Chesne, C., Artusson, P., Brayden, D., Fabre, G., Gires, P., Guillou, F., Rousset, M., Rubas, W., Scarino, M.L., 2001. In vitro models of the intestinal barrier. The report and recommendations of ECVAM Workshop 46. European Centre for the Validation of Alternative methods. Altern. Lab. Anim. ATLA 29, 649–668.

Lee L.K. & J. He. 2010. Reductive debromination of polybrominated diphenyl ethers by anaerobic bacteria from soils and sediments. Applied and environmental microbiology, 76, 794-802.

Legler, J., Brouwer, A., 2003. Are brominated flame retardants endocrine disruptors? Environ. Int. 29, 879–885. doi:10.1016/S0160-4120(03)00104-1

Lepom P., M. Berndt, A. Duffek & E. Warmbrunn-Suckrow. 2010. Oral Bioaccessibility of PBDEs in Dust Using an In Vitro Gastrointestinal Model. In: Brominated Flame Retardants 2010.

Leufroy A., L. Noel, D. Beauchemin & T. Guerin. 2012. Use of a continuous leaching method to assess the oral bioaccessibility of trace elements in seafood. Food Chemistry, 135, 623-633.

Li, C., Cui, X., Fan, Y., Teng, Y., Nan, Z., Ma, L.Q., 2015. Tenax as sorption sink for in vitro bioaccessibility measurement of polycyclic aromatic hydrocarbons in soils. Environ. Pollut. 196, 47–52. doi:10.1016/j.envpol.2014.09.016

Li, C., Sun, H., Juhasz, A.L., Cui, X., Ma, L.Q., 2016. Predicting the Relative Bioavailability of DDT and Its Metabolites in Historically Contaminated Soils Using a Tenax-Improved Physiologically Based Extraction Test (TI-PBET). Environ. Sci. Technol. 50, 1118–1125. doi:10.1021/acs.est.5b03891

Li, S.-W., Li, H.-B., Luo, J., Li, H.-M., Qian, X., Liu, M.-M., Bi, J., Cui, X.-Y., Ma, L.Q., 2016. Influence of pollution control on lead inhalation bioaccessibility in PM2.5: A case study of 2014 Youth Olympic Games in Nanjing. Environ. Int. 94, 69–75. doi:10.1016/j.envint.2016.05.010

Lindström, M., Falk, R., Hjelte, L., Philipson, K., Svartengren, M., 2006. Long-term clearance from small airways in subjects with ciliary dysfunction. Respir. Res. 7, 79. doi:10.1186/1465-9921-7-79

Liu, L.-Y., He, K., Hites, R.A., Salamova, A., 2016. Hair and Nails as Noninvasive Biomarkers of Human Exposure to Brominated and Organophosphate Flame Retardants. Environ. Sci. Technol. 50, 3065–3073. doi:10.1021/acs.est.5b05073

Ljung K., O. Selinus, E. Otabbong & M. Berglund. 2006. Metal and arsenic distribution in soil particle sizes relevant to soil ingestion by children. Applied Geochemistry, 21, 1613-1624.

Lopez, P., Brandsma, S.A., Leonards, P.E.G., de Boer, J., 2011. Optimization and development of analytical methods for the determination of new brominated flame retardants and polybrominated diphenyl ethers in sediments and suspended particulate matter. Anal. Bioanal. Chem. 400, 871–883. doi:10.1007/s00216-011-4807-8

Luongo, G., Östman, C., 2016. Organophosphate and phthalate esters in settled dust from apartment buildings in Stockholm. Indoor Air 26, 414–425. doi:10.1111/ina.12217

Marklund, A., Andersson, B., Haglund, P., 2003. Screening of organophosphorus compounds and their distribution in various indoor environments. Chemosphere 53, 1137–1146. doi:10.1016/S0045-6535(03)00666-0

Marques, M.R., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolution Technol 18, 15–28. doi:dx.doi.org/10.14227/DT180311P15

Matsukami, H., Suzuki, G., Takigami, H., 2015. Compositional Analysis of Commercial Oligomeric Organophosphorus Flame Retardants Used as Alternatives for PBDEs: Concentrations and Potential Environmental Emissions of Oligomers and Impurities. Environ. Sci. Technol. 49, 12913–12921. doi:10.1021/acs.est.5b03447

Matsumoto, M., Hirata-Koizumi, M., Ema, M., 2008. Potential adverse effects of phthalic acid esters on human health: A review of recent studies on reproduction. Regul. Toxicol. Pharmacol. 50, 37–49. doi:10.1016/j.yrtph.2007.09.004

Mayer, P., Hilber, I., Gouliarmou, V., Hale, S.E., Cornelissen, G., Bucheli, T.D., 2016. How to Determine the Environmental Exposure of PAHs Originating from Biochar. Environ. Sci. Technol. doi:10.1021/acs.est.5b05603

Meeker, J.D., Stapleton, H.M., 2010. House Dust Concentrations of Organophosphate Flame Retardants in Relation to Hormone Levels and Semen Quality Parameters. Environ. Health Perspect. 118, 318–323. doi:10.1289/ehp.0901332

Mendell, M.J., 2007. Indoor residential chemical emissions as risk factors for respiratory and allergic effects in children: a review. Indoor Air 17, 259–277. doi:10.1111/j.1600-0668.2007.00478.x

Mihajlović, I., 2015. Recent Development of Phosphorus Flame Retardants in Thermoplastic Blends and Nanocomposites, in: Visakh, P.M., Arao, Y. (Eds.), Flame Retardants, Engineering Materials. Springer International Publishing, pp. 79–114.

Moya, J., Bearer, C.F., Etzel, R.A., 2004. Children's Behavior and Physiology and How It Affects Exposure to Environmental Contaminants. Pediatrics 113, 996–1006.

Newton, S., Sellström, U., de Wit, C.A., 2015. Emerging Flame Retardants, PBDEs, and HBCDDs in indoor and outdoor media in Stockholm, Sweden. Environ. Sci. Technol. doi:10.1021/es505946e

Nerc & Dtz. 2009. Bioaccessibility Testing of Contaminated Land for Threats to Human Health Background to Bioaccessibility. pp. 1-11.

Oberdorster, G., 1995. Lung Particle Overload: Implications for Occupational Exposures to Particles. Regul. Toxicol. Pharmacol. 21, 123–135. doi:10.1006/rtph.1995.1017

OECD. 2013. OECD Guidelines for the Testing of Chemicals - Section 5 - Other Test Guideline. Organisation for Economic Co-operation and Development, http://www.oecd-ilibrary.org/docserver/download/9713181e.pdf?expires=1389763121&id=id&accname=guest&checksum=42C6F8B851A27AC408397DFED7EC7899 [Accessed 03/01/2014].

Oie, L., Hersoug, L.G., Madsen, J.O., 1997. Residential exposure to plasticizers and its possible role in the pathogenesis of asthma. Environ. Health Perspect. 105, 972–978.

Oomen A.G., A. Hack, M. Minekus, E. Zeijdner, C. Cornelis, G. Schoeters, W. Verstraete, T. Van de Wiele, J. Wragg, C.J.M. Rompelberg, A. Sips & J.H. Van Wijnen. 2002. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. Environmental Science & Technology, 36, 3326-3334.

Oomen A.G., A.J.A.M. Sips, J.P. Groten, D.T.H.M. Sijm & J. Tolls. 2000. Mobilization of PCBs and Lindane from Soil during in Vitro Digestion and Their Distribution among Bile Salt Micelles and Proteins of Human Digestive Fluid and the Soil. Environmental Science & Technology, 34, 297-303.

Oomen, A.G., Rompelberg, C.J.M., Bruil, M.A., Dobbe, C.J.G., Pereboom, D.P.K.H., Sips, A.J. a. M., 2003. Development of an In Vitro Digestion Model for Estimating the Bioaccessibility of Soil Contaminants. Arch. Environ. Contam. Toxicol. 44, 0281–0287. doi:10.1007/s00244-002-1278-0

Oomen, A.G., Sips, A.J.A.M., Groten, J.P., Sijm, D.T.H.M., Tolls, J., 2000. Mobilization of PCBs and Lindane from Soil during in Vitro Digestion and Their Distribution among Bile Salt Micelles and Proteins of Human Digestive Fluid and the Soil. Environ. Sci. Technol. 34, 297–303. doi:10.1021/es990446j

Ortega-Calvo, J.-J., Harmsen, J., Parsons, J.R., Semple, K.T., Aitken, M.D., Ajao, C., Eadsforth, C., Galay-Burgos, M., Naidu, R., Oliver, R., Peijnenburg, W.J.G.M., Römbke, J., Streck, G., Versonnen, B., 2015. From Bioavailability Science to

Regulation of Organic Chemicals. Environ. Sci. Technol. 49, 10255–10264. doi:10.1021/acs.est.5b02412

Papadopoulou, E., Padilla-Sanchez, J.A., Collins, C.D., Cousins, I.T., Covaci, A., de Wit, C.A., Leonards, P.E.G., Voorspoels, S., Thomsen, C., Harrad, S., Haug, L.S., 2016. Sampling strategy for estimating human exposure pathways to consumer chemicals. Emerg. Contam. doi:10.1016/j.emcon.2015.12.002

Patisaul, H.B., Roberts, S.C., Mabrey, N., McCaffrey, K.A., Gear, R.B., Braun, J., Belcher, S.M., Stapleton, H.M., 2013. Accumulation and Endocrine Disrupting Effects of the Flame Retardant Mixture Firemaster® 550 in Rats: An Exploratory Assessment. J. Biochem. Mol. Toxicol. 27, 124–136. doi:10.1002/jbt.21439

Pelkonen, O., Boobis, A.R., Gundert-Remy, U., 2001. In vitro prediction of gastrointestinal absorption and bioavailability: an experts' meeting report. Eur. J. Clin. Pharmacol. 57, 621–629. doi:10.1007/s002280100369

Pillai, H.K., Fang, M., Beglov, D., Kozakov, D., Vajda, S., Stapleton, H.M., Webster, T.F., Schlezinger, J.J., 2014. Ligand binding and activation of PPARγ by Firemaster® 550: effects on adipogenesis and osteogenesis in vitro. Environ. Health Perspect. 122, 1225–1232. doi:10.1289/ehp.1408111

Pollard, P.C., 1987. Dialysis: a simple method of separating labelled bacterial DNA and tritiated thymidine from aquatic sediments. J. Microbiol. Methods 7, 91–101. doi:10.1016/0167-7012(87)90029-7

Quintana, J.B., Rosende, M., Montes, R., Rodríguez-Álvarez, T., Rodil, R., Cela, R., Miró, M., 2017. In-vitro estimation of bioaccessibility of chlorinated organophosphate flame retardants in indoor dust by fasting and fed physiologically relevant extraction tests. Sci. Total Environ. 580, 540–549. doi:10.1016/j.scitotenv.2016.11.210

Quiros-Alcala L., A. Bradman, K. Smith, G. Weerasekera, M. Odetokun, D.B. Barr, M. Nishioka, R. Castorina, A.E. Hubbard, M. Nicas, S.K. Hammond, T.E. McKone & B. Eskenazi. 2012. Organophosphorous pesticide breakdown products in house dust and children's urine. J Expos Sci Environ Epidemiol, 22, 559-568.

Raffensperger, C., Tickner, J., 1999. Protecting Public Health and the Environment; Implementing The Precautionary Principle.

Reichenberg, F., Mayer, P., 2006. Two complementary sides of bioavailability: Accessibility and chemical activity of organic contaminants in sediments and soils. Environ. Toxicol. Chem. 25, 1239–1245. doi:10.1897/05-458R.1

Robinson, E.C., Hammond, B.G., Johannsen, F.R., Levinskas, G.J., Rodwell, D.E., 1986. Teratogenicity studies of alkylaryl phosphate ester plasticizers in rats. Fundam. Appl. Toxicol. Off. J. Soc. Toxicol. 7, 138–143.

Rodríguez-Navas, C., Rosende, M., Miró, M., 2017. In-vitro physiologically based extraction of solid materials: Do we have reliable analytical methods for bioaccessibility studies of emerging organic contaminants? TrAC Trends Anal. Chem. 91, 42–52. doi:10.1016/j.trac.2017.03.005

Roos, A., Hugosson, M., 2008. Consumer preferences for wooden and laminate flooring. Wood Mater. Sci. Eng. 3, 29–37. doi:10.1080/17480270802573586

Roosens L., M.A.-E. Abdallah, S. Harrad, H. Neels & A. Covaci. 2009. Exposure to Hexabromocyclododecanes (HBCDs) via Dust Ingestion, but Not Diet, Correlates with Concentrations in Human Serum: Preliminary Results. Environmental Health Perspectives, 117, 1707-1712.

Rosner, D., Markowitz, G., 2002. Industry challenges to the principle of prevention in public health: the precautionary principle in historical perspective. Public Health Rep. 117, 501–512.

Rostami I. & A.L. Juhasz. 2011. Assessment of Persistent Organic Pollutant (POP) Bioavailability and Bioaccessibility for Human Health Exposure Assessment: A Critical Review. Critical Reviews in Environmental Science and Technology, 41, 623-656.

Ruby M.V. & Y.W. Lowney. 2012. Selective Soil Particle Adherence to Hands: Implications for Understanding Oral Exposure to Soil Contaminants. Environmental Science & Technology, 46, 12759-12771.

Ruby M.V., A. Davis, R. Schoof, S. Eberle & C.M. Sellstone. 1996. Estimation of lead and arsenic bioavailability using a physiologically based extraction test. Environmental Science & Technology, 30, 422-430.

Ruby, M.V., Davis, A., Schoof, R., Eberle, S., Sellstone, C.M., 1996. Estimation of Lead and Arsenic Bioavailability Using a Physiologically Based Extraction Test. Environ. Sci. Technol. 30, 422–430. doi:10.1021/es950057z

Ruby, M.V., Fehling, K.A., Paustenbach, D.J., Landenberger, B.D., Holsapple, M.P., 2002. Oral Bioaccessibility of Dioxins/Furans at Low Concentrations (50−350 ppt Toxicity Equivalent) in Soil. Environ. Sci. Technol. 36, 4905–4911. doi:10.1021/es020636l

Rudel R.A., D.E. Camann, J.D. Spengler, L.R. Korn & J.G. Brody. 2003. Phthalates, Alkylphenols, Pesticides, Polybrominated Diphenyl Ethers, and Other Endocrine-Disrupting Compounds in Indoor Air and Dust. Environmental Science & Technology, 37, 4543-4553.

Rudel, R.A., Camann, D.E., Spengler, J.D., Korn, L.R., Brody, J.G., 2003. Phthalates, Alkylphenols, Pesticides, Polybrominated Diphenyl Ethers, and Other Endocrine-Disrupting Compounds in Indoor Air and Dust. Environ. Sci. Technol. 37, 4543–4553. doi:10.1021/es0264596

Rudel, R.A., Perovich, L.J., 2009. Endocrine disrupting chemicals in indoor and outdoor air. Atmospheric Environ. Oxf. Engl. 1994 43, 170–181. doi:10.1016/j.atmosenv.2008.09.025

Rutkowska, E., Pajak, K., Jóźwiak, K., 2013. Lipophilicity--methods of determination and its role in medicinal chemistry. Acta Pol. Pharm. 70, 3–18.

Schaider, L.A., Senn, D.B., Brabander, D.J., McCarthy, K.D., Shine, J.P., 2007. Characterization of Zinc, Lead, and Cadmium in Mine Waste: Implications for Transport, Exposure, and Bioavailability. Environ. Sci. Technol. 41, 4164–4171. doi:10.1021/es0626943

SCHER, 2007. European Comission. Scientific Committee on Health and Environmental Risks Opinion on risk assessment on indoor air quality [WWW Document]. URL http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_055.pdf (accessed 9.14.16).

Schreder, E.D., Uding, N., La Guardia, M.J., 2016. Inhalation a significant exposure route for chlorinated organophosphate flame retardants. Chemosphere 150, 499–504. doi:10.1016/j.chemosphere.2015.11.084

Scott W.C. & J.R. Dean. 2005. An assessment of the bioavailability of persistent organic pollutants from contaminated soil. Journal of Environmental Monitoring, 7, 710-715.

Semple, K.T., Doick, K.J., Jones, K.C., Burauel, P., Craven, A., Harms, H., 2004. Defining Bioavailability and Bioaccessibility of Contaminated Soil and Sediment is Complicated. Environ. Sci. Technol. 38, 228A–231A. doi:10.1021/es040548w

Shalat S.L., K.C. Donnelly, N.C.G. Freeman, J.A. Calvin, S. Ramesh, M. Jimenez, K. Black, C. Coutinho, L.L. Needham, D.B. Barr & J. Ramirez. 2003. Nondietary ingestion of pesticides by children in an agricultural community on the US/Mexico border: Preliminary results. Journal of Exposure Science and Environmental Epidemiology, 13, 42-50.

Siciliano S.D., B.D. Laird & C.L. Lemieux. 2010. Polycyclic aromatic hydrocarbons are enriched but bioaccessibility reduced in brownfield soils adhered to human hands. Chemosphere, 80, 1101-1108.

Sjodin A, O. Papke, E. McGahee, J.F. Focant, R.S. Jones, T. Pless-Mulloli, L.M. Toms, T. Herrmann, J. Muller, L.L. Needham & D.G. Patterson, Jr. 2008. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. Chemosphere, 73, S131-136.

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., Bergman, A., 1999. Flame retardant exposure: polybrominated diphenyl ethers in blood from Swedish workers. Environ. Health Perspect. 107, 643–648.

Smith E., J. Weber, A. Rofe, D. Gancarz, R. Naidu & A.L. Juhasz. 2012. Assessment of DDT Relative Bioavailability and Bioaccessibility in Historically Contaminated Soils Using an in Vivo Mouse Model and Fed and Unfed Batch in Vitro Assays. Environmental Science & Technology, 46, 2928-2934.

Sokal, R.R., Rohlf, F.J., 1995. Analysis of frequencies. Biometry Princ. Pract. Stat. Biol. Res. 685–793.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: the principles and practices of statistics in biological research, 3rd edn.,(WH Freeman: New York).

Stanek E.J.C.E.J.. 2000. Daily Soil Ingestion Estimates for Children at a Superfund Site. Risk Analysis: An International Journal., 20.

Stapleton H.M., S. Eagle, A. Sjödin & T.F. Webster. 2012. Serum PBDEs in a North Carolina Toddler Cohort: Associations with Handwipes, House Dust, and Socioeconomic Variables. Environmental Health Perspectives, 120, 1049-1054.

Stapleton, H.M., Allen, J.G., Kelly, S.M., Konstantinov, A., Klosterhaus, S., Watkins, D., McClean, M.D., Webster, T.F., 2008. Alternate and New Brominated Flame Retardants Detected in U.S. House Dust. Environ. Sci. Technol. 42, 6910–6916. doi:10.1021/es801070p

Stapleton, H.M., Eagle, S., Sjödin, A., Webster, T.F., 2012. Serum PBDEs in a North Carolina Toddler Cohort: Associations with Handwipes, House Dust, and Socioeconomic Variables. Environ. Health Perspect. 120, 1049–1054. doi:10.1289/ehp.1104802

Stapleton, H.M., Klosterhaus, S., Eagle, S., Fuh, J., Meeker, J.D., Blum, A., Webster, T.F., 2009. Detection of Organophosphate Flame Retardants in Furniture Foam and U.S. House Dust. Environ. Sci. Technol. 43, 7490–7495. doi:10.1021/es9014019

Stapleton, H.M., Klosterhaus, S., Keller, A., Ferguson, P.L., van Bergen, S., Cooper, E., Webster, T.F., Blum, A., 2011. Identification of Flame Retardants in Polyurethane Foam Collected from Baby Products. Environ. Sci. Technol. 45, 5323–5331. doi:10.1021/es2007462

State of Washington, 2016. House Bill 2545 [WWW Document]. Wash. State Legis. URL http://app.leg.wa.gov/billinfo/summary.aspx?year=2015&bill=2545 (accessed 7.7.16).

Stockholm Convention, 2009a. UNEP/POPS/POPRC.4/14 Listing of hexabromodiphenyl ether and heptabromodiphenyl ether.

Stockholm Convention, 2009b. UNEP/POPS/POPRC.4/18 Listing of tetrabromodiphenyl ether and pentabromodiphenyl ether.

Suzuki, G., Tue, N.M., Malarvannan, G., Sudaryanto, A., Takahashi, S., Tanabe, S., Sakai, S., Brouwer, A., Uramaru, N., Kitamura, S., Takigami, H., 2013. Similarities in the Endocrine-Disrupting Potencies of Indoor Dust and Flame Retardants by Using Human Osteosarcoma (U2OS) Cell-Based Reporter Gene Assays. Environ. Sci. Technol. 47, 2898–2908. doi:10.1021/es304691a

Tajima, S., Araki, A., Kawai, T., Tsuboi, T., Ait Bamai, Y., Yoshioka, E., Kanazawa, A., Cong, S., Kishi, R., 2014. Detection and intake assessment of organophosphate flame retardants in house dust in Japanese dwellings. Sci. Total Environ. 478, 190–199. doi:10.1016/j.scitotenv.2013.12.121

Tao S., Y. Lu, D.Y. Zhang, Y.F. Yang, Y. Yang, X.X. Lu & D.J. Sai. 2009. Assessment of Oral Bioaccessibility of Organochlorine Pesticides in Soil Using an In Vitro Gastrointestinal Model. Environmental Science & Technology, 43, 4524-4529.

Tao, F., Abdallah, M.A.-E., Harrad, S., 2016. Emerging and Legacy Flame Retardants in UK Indoor Air and Dust: Evidence for Replacement of PBDEs by Emerging Flame Retardants? Environ. Sci. Technol. doi:10.1021/acs.est.6b02816

Tilston, E.L., Gibson, G.R., Collins, C.D., 2011. Colon Extended Physiologically Based Extraction Test (CE-PBET) Increases Bioaccessibility of Soil-Bound PAH. Environ. Sci. Technol. 45, 5301–5308. doi:10.1021/es2004705

Tirelli, V., Catone, T., Turco, L., Di Consiglio, E., Testai, E., De Angelis, I., 2007. Effects of the pesticide clorpyrifos on an in vitro model of intestinal barrier. Toxicol. In Vitro, In Vitro Cytotoxicity Mechanisms. Proceedings of the 46th ETCS International Meeting and the 3rd International Joint Meeting of AICC and CELLTOX 21, 308–313. doi:10.1016/j.tiv.2006.08.015

Uemura H., K. Arisawa, M. Hiyoshi, S. Dakeshita, A. Kitayama, H. Takami, F. Sawachika, M. Yamaguchi & S. Sasai. 2010. Congener-specific body burden levels and possible determinants of polybrominated diphenyl ethers in the general Japanese population. Chemosphere, 79, 706-712.

UK Department for Business, Energy & Industrial Strategy, 2016. Consultations on furniture and furnishings fire safety regulations: proposed changes [WWW Document]. URL https://www.gov.uk/government/consultations/furniture-and-furnishing-fire-safety-regulations-proposed-changes-2016 (accessed 9.14.16).

UK Environment Agency, 2009a. An overview of the environmental risk evaluation reports for aryl phosphate esters [WWW Document]. URL https://www.gov.uk/government/publications/an-overview-of-the-environmental-risk-evaluation-reports-for-aryl-phosphate-esters (accessed 7.10.16).

UK Environment Agency, 2009b. Environmental risk evaluation report: Isodecyl diphenyl phosphate (iDPP) CAS no. 29761-21-5 [WWW Document]. URL https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290853/scho0809bquf-e-e.pdf

UK Environment Agency, 2009c. Environmental risk evaluation report: 2-Ethylhexyl diphenyl phosphate (EHDPHP) (CAS no. 1241-94-7) [WWW Document]. URL https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/290842/scho0809bqty-e-e.pdf

UK Environmental Agency. 2005. International workshop on the potential use of bioaccessibility testing in risk assessment of land contamination. In: Science Report SC040054, Environment Agency, Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol, BS32 4UD.

US EPA, 2010. An Exposure Assessment of Polybrominated Diphenyl Ethers (PBDE) (Final) (Reports & Assessments No. EPA/600/R-08/086F).

US EPA, 2012. US EPA Phthalates Action Plan under Toxic Substances Control Act [WWW Document]. URL https://www.epa.gov/sites/production/files/2015-09/documents/phthalates_actionplan_revised_2012-03-14.pdf (accessed 2.21.17).

USDA. 2012. United States Departent of Agriculture National Agricultural Library - Nutrient Data Laboratory. http://ndb.nal.usda.gov/ndb/foods/list [Accessed 01/05/2012].

USEPA, 1997. EXPOSURE FACTORS HANDBOOK (1997 FINAL REPORT) [WWW Document]. URL http://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=12464&CFID=186151631&CFTOKEN=30802087&jsessionid=8630fc2d1ae6e420aa6331196837422577 31 (accessed 4.1.15).

USEPA. 2008. Standard Operating Procedure for an In Vitro Bioaccessibility Assay for Lead in Soil. EPA 9200.1-86.

Van de Wiele, T., Vanhaecke, L., Boeckaert, C., Peru, K., Headley, J., Verstraete, W., Siciliano, S., 2005. Human Colon Microbiota Transform Polycyclic Aromatic Hydrocarbons to Estrogenic Metabolites. Environ. Health Perspect. 113, 6–10. doi:10.1289/ehp.7259

Van de Wiele, T.R., Verstraete, W., Siciliano, S.D., 2004. Polycyclic aromatic hydrocarbon release from a soil matrix in the in vitro gastrointestinal tract. J. Environ. Qual. 33, 1343–1353.

Van den Eede, N., Dirtu, A.C., Ali, N., Neels, H., Covaci, A., 2012a. Multi-residue method for the determination of brominated and organophosphate flame retardants in indoor dust. Talanta 89, 292–300. doi:10.1016/j.talanta.2011.12.031

Van den Eede, N., Dirtu, A.C., Neels, H., Covaci, A., 2011. Analytical developments and preliminary assessment of human exposure to organophosphate flame retardants from indoor dust. Environ. Int. 37, 454–461. doi:10.1016/j.envint.2010.11.010

van der Veen, I., de Boer, J., 2012a. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. Chemosphere 88, 1119–1153. doi:10.1016/j.chemosphere.2012.03.067

Venier, M., Audy, O., Vojta, Š., Bečanová, J., Romanak, K., Melymuk, L., Krátká, M., Kukučka, P., Okeme, J., Saini, A., Diamond, M.L., Klánová, J., 2016. Brominated flame retardants in the indoor environment — Comparative study of indoor contamination from three countries. Environ. Int. 94, 150–160. doi:10.1016/j.envint.2016.04.029

Wang H.-S, Y.-G. Zhao, Y.-B. Man, C.K.C. Wong & M.-H. Wong. 2011a. Oral bioaccessibility and human risk assessment of organochlorine pesticides (OCPs) via fish consumption, using an in vitro gastrointestinal model. Food Chemistry, 127, 1673-1679.

Wang H.-S., Y.-B. Man, F.-Y. Wu, Y.-G. Zhao, C.K.C. Wong & M.-H. Wong. 2010. Oral Bioaccessibility of Polycyclic Aromatic Hydrocarbons (PAHs) through Fish Consumption, Based on an in Vitro Digestion Model. Journal of Agricultural and Food Chemistry, 58, 11517-11524.

Wang H.-S., Z.-J. Chen, W. Wei, Y.-B. Man, J.P. Giesy, J. Du, G. Zhang, C.K.-C. Wong & M.-H. Wong. 2013a. Concentrations of organochlorine pesticides (OCPs) in human blood plasma from Hong Kong: Markers of exposure and sources from fish. Environment International, 54, 18-25.

Wang P. & A.A. Keller. 2008. Particle-Size Dependent Sorption and Desorption of Pesticides within a Water−Soil−Nonionic Surfactant System. Environmental Science & Technology, 42, 3381-3387.

Wang W., F.-Y. Wu, M.-J. Huang, Y. Kang, K.C. Cheung & M.H. Wong. 2013d. Size fraction effect on phthalate esters accumulation, bioaccessibility and in vitro cytotoxicity of indoor/outdoor dust, and risk assessment of human exposure. Journal of Hazardous Materials, 261, 753-762.

Wang W., M.-J. Huang, F.-Y. Wu, Y. Kang, H.-S. Wang, K.C. Cheung & M.H. Wong. 2013b. Risk assessment of bioaccessible organochlorine pesticides exposure via indoor and outdoor dust. Atmospheric Environment, 77, 525-533.

Wang W., M.-J. Huang, J.-S. Zheng, K.C. Cheung & M.H. Wong. 2013c. Exposure assessment and distribution of polychlorinated biphenyls (PCBs) contained in indoor

and outdoor dusts and the impacts of particle size and bioaccessibility. Science of the Total Environment, 463–464, 1201-1209.

Wang Z., J. Zhao, L. Song, H. Mashayekhi, B. Chefetz & B. Xing. 2011b. Adsorption and Desorption of Phenanthrene on Carbon Nanotubes in Simulated Gastrointestinal Fluids. Environmental Science & Technology, 45, 6018-6024.

Wang, J., Tian, M., Chen, S.-J., Zheng, J., Luo, X.-J., An, T.-C., Mai, B.-X., 2011. Dechlorane Plus in house dust from E-waste recycling and urban areas in South China: Sources, degradation, and human exposure. Environ. Toxicol. Chem. 30, 1965–1972. doi:10.1002/etc.587

Wang, W., Wu, F.-Y., Huang, M.-J., Kang, Y., Cheung, K.C., Wong, M.H., 2013. Size fraction effect on phthalate esters accumulation, bioaccessibility and in vitro cytotoxicity of indoor/outdoor dust, and risk assessment of human exposure. J. Hazard. Mater. 261, 753–762. doi:10.1016/j.jhazmat.2013.04.039

Watanabe I. & S. Sakai. 2003. Environmental release and behavior of brominated flame retardants. Environ Int, 29, 665 - 682.

Webster L., M. Russell, P. Walsham, L.A. Phillips, I. Hussy, G. Packer, E.J. Dalgarno & C.F. Moffat. 2011. An assessment of persistent organic pollutants in Scottish coastal and offshore marine environments. Journal of Environmental Monitoring, 13, 1288-1307.

Weschler, C.J., Bekö, G., Koch, H.M., Salthammer, T., Schripp, T., Toftum, J., Clausen, G., 2015. Transdermal Uptake of Diethyl Phthalate and Di(n-butyl) Phthalate Directly from Air: Experimental Verification. Environ. Health Perspect. 123. doi:10.1289/ehp.1409151

Weschler, C.J., Salthammer, T., Fromme, H., 2008. Partitioning of phthalates among the gas phase, airborne particles and settled dust in indoor environments. Atmos. Environ. 42, 1449–1460. doi:10.1016/j.atmosenv.2007.11.014

Whitehead T.P., F.R. Brown, C. Metayer, J.-S. Park, M. Does, M.X. Petreas, P.A. Buffler & S.M. Rappaport. 2013. Polybrominated diphenyl ethers in residential dust: Sources of variability. Environment International, 57–58, 11-24.

Wilkes, C.E., Summers, J.W., Daniels, C.A., Berard, Mark, T., 2005. PVC Handbook. Hanser Gardner Pubns.

Wong M.H., A.O. Leung, J.K. Chan & M.P. Choi. 2005. A review on the usage of POP pesticides in China, with emphasis on DDT loadings in human milk. Chemosphere, 60, 740-752.

Wong, L.I.L., Reers, A.R., Currier, H.A., Williams, T.D., Cox, M.E., Elliott, J.E., Beischlag, T.V., 2015. The Effects of the Organic Flame-Retardant 1,2-Dibromo-4-(1,2-

dibromoethyl) Cyclohexane (TBECH) on Androgen Signaling in Human Prostate Cancer Cell Lines. J. Biochem. Mol. Toxicol. n/a-n/a. doi:10.1002/jbt.21784

Woolgar, P.J., Jones, K.C., 1999. Studies on the Dissolution of Polycyclic Aromatic Hydrocarbons from Contaminated Materials Using a Novel Dialysis Tubing Experimental Method. Environ. Sci. Technol. 33, 2118–2126. doi:10.1021/es980638z

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What Are the Sources of Exposure to Eight Frequently Used Phthalic Acid Esters in Europeans? Risk Anal. 26, 803–824. doi:10.1111/j.1539-6924.2006.00770.x

Wragg, J., Klinck, B., 2007. The bioaccessibility of lead from Welsh mine waste using a respiratory uptake test. J. Environ. Sci. Health Part A 42, 1223–1231. doi:10.1080/10934520701436054

Xing G.H., Y. Yang, J.K.Y. Chan, S. Tao & M.H. Wong. 2008. Bioaccessibility of polychlorinated biphenyls in different foods using an in vitro digestion method. Environmental Pollution, 156, 1218-1226.

Xu, F., Giovanoulis, G., Van Waes, S., Padilla Sanchez, J.A., Papadopoulou, E., Magnér, J., Haug, L.S., Neels, H., Covaci, A., 2016. A Comprehensive Study of Human External Exposure to Organophosphate Flame Retardants via Air, Dust and Hand wipes: the Importance of Sampling and Assessment Strategy. Environ. Sci. Technol. doi:10.1021/acs.est.6b00246

Yu Y., D. Yang, X. Wang, N. Huang, X. Zhang, D. Zhang & J. Fu. 2013. Factors influencing on the bioaccessibility of polybrominated diphenyl ethers in size-specific dust from air conditioner filters. Chemosphere.

Yu Y., S. Han, D. Zhang, T. Van de Wiele, M. Lu, D. Wang, Z. Yu, M. Wu, G. Sheng & J. Fu. 2009. Factors Affecting the Bioaccessibility of Polybrominated Diphenylethers in an in Vitro Digestion Model. Journal of Agricultural and Food Chemistry, 57, 133-139.

Yu, Y.-X., Pang, Y.-P., Li, C., Li, J.-L., Zhang, X.-Y., Yu, Z.-Q., Feng, J.-L., Wu, M.-H., Sheng, G.-Y., Fu, J.-M., 2012. Concentrations and seasonal variations of polybrominated diphenyl ethers (PBDEs) in in- and out-house dust and human daily intake via dust ingestion corrected with bioaccessibility of PBDEs. Environ. Int. 42, 124–131. doi:10.1016/j.envint.2011.05.012

Zander, J., 2010. The Application of the Precautionary Principle in Practice: Comparative Dimensions. Cambridge University Press.

Zhang, J. (Jim), Smith, K.R., 2003. Indoor air pollution: a global health concern. Br. Med. Bull. 68, 209–225. doi:10.1093/bmb/ldg029

Zhang, Y., Pignatello, J.J., Tao, S., 2016. Bioaccessibility of nitro- and oxy-PAHs in fuel soot assessed by an in vitro digestive model with absorptive sink. Environ. Pollut. 218, 901–908. doi:10.1016/j.envpol.2016.08.021

Zhang, Y., Pignatello, J.J., Tao, S., Xing, B., 2015. Bioacessibility of PAHs in Fuel Soot Assessed by an in Vitro Digestive Model: Effect of Including an Absorptive Sink. Environ. Sci. Technol. 49, 3905–3912. doi:10.1021/es505898v

Zheng, J., Luo, X.-J., Yuan, J.-G., Wang, J., Wang, Y.-T., Chen, S.-J., Mai, B.-X., Yang, Z.-Y., 2011. Levels and sources of brominated flame retardants in human hair from urban, e-waste, and rural areas in South China. Environ. Pollut. 159, 3706–3713. doi:10.1016/j.envpol.2011.07.009

Zheng, X., Xu, F., Chen, K., Zeng, Y., Luo, X., Chen, S., Mai, B., Covaci, A., 2015. Flame retardants and organochlorines in indoor dust from several e-waste recycling sites in South China: Composition variations and implications for human exposure. Environ. Int. 78, 1–7. doi:10.1016/j.envint.2015.02.006

Zhu, J., Feng, Y.-L., Shoeib, M., 2007. Detection of dechlorane plus in residential indoor dust in the city of Ottawa, Canada. Environ. Sci. Technol. 41, 7694–7698.

Appendix I

Curriculum vitae

My PhD research is a part of an EU-funded Marie Curie Innovative Training Network (ITN) called "Advanced Tools For Exposure Assessment and Biomonitoring" (http://www.ateam-research.com/). Our main objectives are to develop analytical methods and human biomonitoring methods for assessing human exposure to emerging organic compounds such as halogenated flame retardants and phthalate esters present in everyday consumer products.

Short Biography:

Katerina Kademoglou was born in Serres, Greece on March 12, 1986. In 2009, she received a five-year Bachelor of Science degree in Biological Applications and Technology from the University of Ioannina in Greece. During that time, she conducted her BSc thesis on the feeding habits of *Atherina boyeri* in the lagoons of Nestos River in NE Greece under the guidance of prof. Ioannis Leonardos from the University of Ioannina and Dr. Manos Koutrakis, senior researcher at the National Fisheries Institute at Nea Peramos (Kavala) in Greece. In 2010, Katerina enrolled at Wageningen University in the Netherlands, following a two-year Master of Science degree in Environmental Sciences with specialisation in Environmental Toxicology. She conducted her Master Thesis on the "Effects of xenobiotics on Thyroid-stimulating hormone (TSH) synthesis and activity" at the department of Toxicology of Wageningen University under the leadership of Prof Ivonne Rietjens and the supervision of PhD candidate Barae Jomaa. In 2012, Katerina completed her MSc curriculum with an internship at the National Water Research Institute at Monza (Italy), performing algal toxicity tests assessing the trophic status of Lake Occhito (Italy) according to OECD and USEPA standard procedures. In 2013, she was awarded a Marie-Curie doctoral research fellowship within the EU-funded Innovative Training Network (ITN) named "Advanced Tools for Exposure Assessment and Biomonitoring" and was enrolled as a PhD student at the University of Reading in the UK, conducting her PhD research on the "*In vitro* bioaccessibility of emerging flame retardants (FRs) via air, dust and diet" under the guidance of Prof Chris D. Collins and Prof Adrian C. Williams. From 2013 until the completion of her

PhD thesis, Katerina has attended several national and international conferences presenting her PhD research.

EDUCATION

May 2013 – March 2017 PhD in Environmental Science

Marie Curie Action - Innovative Training Network (ITN) "A-TEAM"

Department of Geography and Environmental Sciences, University of Reading (UoR), Reading - UK

PhD thesis: In vitro bioaccessibility of flame retardants (FRs) via air, dust and diet

Human exposure assessment and uptake (i.e. bioaccessibility) of emerging flame retardants (FRs) via indoor dust using in vitro simulated human gut and lung fluids techniques

PhD thesis Supervisor: Prof. Chris Collins (UoR)

2010 – 2012: MSc Environmental Sciences – Environmental toxicology

Wageningen University and Research Centre (WUR), Wageningen - The Netherlands

MSc thesis: Effects of xenobiotics on Thyroid-stimulating hormone (TSH) synthesis and activity

Method development and validation of in vitro bioassays towards an in vitro screening strategy of potential thyroid-disrupting chemicals on established mammalian cell lines, MSc Thesis Grade: 7.5/10

MSc thesis Supervisor: Prof. dr. ir. I.M.C.M. Rietjens (WUR)

2003 - 2009: 5-year BSc degree in Biology (Grade: 6.68/10, Very Good)

Department of Biological Applications and Technologies, University of Ioannina (UoI), Ioannina – Greece

BSc thesis title: Feeding behaviour and intraspecific competition of Atherina boyeri (Risso, 1810) in the Lagoons of Nestos River (Greece), BSc Thesis Grade: 9.5/10

BSc thesis Supervisor: Prof. Ioannis Leonardos (UoI)

HONOURS AND AWARDS

February 2017 2016 Best Postgraduate Research Output

Award for best PhD student publication in 2016 within the School of Archaeology, Geography and Environmental Science at the University of Reading for the paper "Legacy and alternative flame retardants in Norwegian and UK indoor environment: Implications of human exposure via dust ingestion", (2016), Environment International, doi: 10.1016/j.envint.2016.12.012

April 2016 "Athena Swan Network" Silver Award

Athena SWAN Charter is a scheme to recognise and share good practice on gender equality in science, technology, engineering, mathematics and medicine (STEMM) in UK higher education employment. During the preparation phase of Athena SWAN application, I was a postgraduate representative of the department of Geography and Environmental Science at the University of Reading to the departmental committee. I had a leading role in assessing and reporting qualitative and quantitative data on gender equality and well-being of PhD students at departmental level.

September 2013 Dr. Emmanuel Lahaniatis fellowship for young scientists

The 5,000 euro scholarship was awarded for young researcher scientific excellence by the scientific organising committee of the 17th International Symposium on Environmental Pollution and its Impact on Life in the Mediterranean Region (MESAEP) in Istanbul, Turkey

May 2013 Marie Curie Doctoral Fellowship

PhD research within the EU-sponsored (FP7) Marie Curie Innovative Training Network "Advanced Tools for Exposure Assessment and Biomonitoring (A-TEAM)" made up of eight partners across Europe including the University of Reading.

The aim of the ATEAM project was to assess prospective chemicals for exposure potential in humans with respect to biologically-relevant monitoring of external exposure.

RESEARCH INTERESTS

- Human exposure to emerging organic pollutants
- Occurrence, fate and effects of emerging contaminants in the environment
- Investigation of bioavailability and biological effects of environmental pollutants
- Endocrine and thyroid disruption in humans and the wildlife