Does the source migration pathway of HBCDs to household dust influence their bio-accessibility?

García-Alcega, Sonia; Rauert, Cassandra; Harrad, Stuart; Collins, Chris D.

DOI:
10.1016/j.scitotenv.2016.04.178

Document Version
Peer reviewed version

Citation for published version (Harvard):
García-Alcega, S, Rauert, C, Harrad, S & Collins, CD 2016, 'Does the source migration pathway of HBCDs to household dust influence their bio-accessibility?', *Science of the Total Environment*, vol. 569-570, pp. 244-251. https://doi.org/10.1016/j.scitotenv.2016.04.178

TITLE

Does the source migration pathway of HBCDs to household dust influence their bio-accessibility?

Authors

Sonia García-Alcega[a], Cassie Rauert[b], Stuart Harrad[b], Chris D. Collins[a]

Affiliation

[a]Soil Research Centre, University of Reading, Reading, RG6 6AH, UK

[b]School of Geography Earth and Environmental Sciences, University of Birmingham, Birmingham, B15 2TT, UK

Corresponding author

Chris D. Collins

Soil Research Centre University of Reading, Reading, RG6 6AH, UK

Tel +44(0)118 378 8910

c.d.collins@reading.ac.uk

Abstract

A study was conducted to assess the human bioaccessibility of dust contaminated with hexabromocyclododecane (HBCD) via two migration pathways a) volatilisation with subsequent partitioning to dust particles, and b) abrasion of treated textile fibres directly to the dust. This was achieved using previously developed experimental chamber designs to generate dust samples contaminated with HBCDs emitted from a HBCD treated textile curtain. The generated dust samples were exposed to an *in vitro* colon extended physiologically based extraction test (CE-PBET). The bioaccessibility of the HBCDs which were incorporated within dust as a result of volatilisation from the curtain material with subsequent partitioning to dust was higher than in dusts contaminated with HBCDs via abrasion of the curtain (35% and 15% respectively). We propose this occurs due to a stronger binding of HBCDs to treated fabric fibres than that experienced following volatilisation and sorption of HBCDs to dust particles.

Keywords

Flame retardants; Indoor environment; *In vitro* digestion model; Oral exposure; Volatilisation; Abrasion

1. Introduction

Since the ban of polybrominated diphenyl ethers (PBDEs), hexabromocyclodecanes (HBCDs) have been used as a substitute brominated flame retardant (BFR) primarily in polystyrene insulation, building materials and textiles as well as in electronics and upholstery (Covaci et al., 2006). HBCDs are not chemically bound to the polymer as they are incorporated into the product by an additive process (Bakker et al., 2008; Boutrup et al., 2011).Therefore they can migrate into the environment contaminating indoor dust (Roosens et al., 2009). In the same way as PBDEs, HBCDs can enter the environment by emission during their production or when the product is being added to a manufactured product, but also by leaching during the entire lifetime of the product (Covaci et al., 2006).

Exposure to HBCDs is of concern because humans spend about 90% of their time indoors (Jones-Otazo et al., 2005; Allen et al., 2007) and HBCDs are persistent, neu-

rotoxic, endocrine disruptors and can alter immunological and reproductive systems (Roze et al., 2009; Saegusa et al., 2009; Palace et al., 2010). Rather than residing in air, almost all the semi volatile organic compounds (SVOC) such as HBCDs are re-adsorbed on the surface of furniture, walls and settled dust (Weschler and Nazaroff, 2008; Zhang et al., 2011). Dust is an important pathway of exposure to HBCDs, especially for children whose HBCDs uptake can be 10 times higher via dust ingestion than through diet (Abdallah et al., 2008), they are also more susceptible to toxicants as they are undergoing development (Roze et al., 2009).

Limited information is available on the migration mechanisms of BFRs from treated products to dust (Rauert et al., 2014b). To date, the hypothesized migration pathways consist of: (1) volatilisation of BFRs from the treated product followed by partitioning to dust; (2) physical abrasion of the treated product and (3) transfer by direct contact between the treated product and dust (Webster et al., 2009; Rauert et al., 2014b). Test chamber experiments have previously been used to study the migration of phthalates simulating volatilization with partitioning to dust and transfer by direct contact between the treated product and dust migration pathways (Clausen et al., 2004; Schripp et al., 2010). More recently, Rauert et al. examined volatilization of HBCDs with subsequent partitioning to dust (Rauert et al., 2014a) and abrasion of fibres (Rauert et al., 2014b) from a textile treated with the HBCD technical formulation using an in-house developed test chamber. The same group have also used chamber techniques to investigate the mass transfer of PBDEs from a plastic TV casing to indoor dust via three migration pathways (volatilisation, abrasion and direct contact between the source and dust respectively) (Rauert and Harrad, 2015). These workers concluded that for high molecular weight PBDEs like BDE-209 the principal migration pathway to the dust is by source contact followed by abrasion, they did not detect substantial transfer by volatilisation.

Oral bioaccessibility measures the fraction of compounds which are desorbed from the ingested matrix into the gastrointestinal fluids *in vitro* (Collins et al., 2015), providing an estimation of bioavailability (EPA , 2007). Bioaccessibility studies avoid the use of animal experiments, which are ethically and economically problematic, difficult to conduct and still may not represent human conditions (Hamel et al., 1999; Oomen et al., 1999; Ruby et al., 1993; Ruby et al., 1999). A pollutant's bioaccessibil-

ity may vary depending on numerous factors such as the characteristics of the dust, physicochemical properties of the compounds, gastrointestinal conditions (fed or unfed state) (Yu et al., 2008;Yu et al., 2013; Camenisch et al., 1998; Testa et al., 2000) and also the process by which chemicals are incorporated into the dust (Yu et al., 2012).

Abdallah et al. (2012) used the colon extended physiologically based extraction test (CE-PBET) to assess the bioaccessibility of different FRs from dust. A similar CE-PBET model was used by Fang et al. (2014) who added tenax beads as a chemical sink. Both groups of researchers observed a decreasing trend in bioaccessibility with increasing log K_{OW} of FRs. The CE-PBET model has been chosen for the present work as it is deemed a realistic model of the human gastrointestinal tract.

The test chamber developed by Rauert et al. (2014a) was utilised to generate dusts containing elevated HBCD concentrations via: (1) volatilisation from a HBCD formulation treated textile with subsequent partitioning to dust and (2) abraded treated textile fibres entering the dust directly. It was hypothesised that dust contaminated via the volatilisation with partitioning pathway (1) would have a more homogeneous distribution of HBCD through the dust sample than dust contaminated via the abrasion pathway (2) with HBCD contamination in abraded dusts being more heterogeneous owing to the HBCDs primarily associated with the presence of the textile fibres. We also hypothesised that the HBCD-treated fabric fibres in the dust contaminated via pathway (2) would reduce the bioaccessibility as the HBCDs may be more strongly bound to the fibre than HBCDs that have partitioned to dust particle surfaces via volatilisation (pathway 1).

2. Material and methods

2.1. Materials and reagents

Solvents used during the extraction and analysis were all of analytical grade; *n*-hexane, acetone, methanol and dichloromethane were purchased from Merck (Darmstadt, Germany). Indoor dust SRM 2585 was purchased from NIST (Gaithersburg, MD, USA). Empty polypropylene filtration SPE cartridges (3 mL) were obtained from Sigma-Aldrich (Gillingham, UK). Silica gel (40 µm pore size) was purchased from J.T.Baker (London, UK). Anhydrous sodium sulfate (Na_2SO_4) and con-

centrated sulfuric acid (H_2SO_4, 98%) were purchased from Merck (Darmstadt, Germany). Standards of individual HBCDs (α-HBCD, β-HBCD, γ-HBCD), labelled ^{13}C HBCDs (α-, β-, γ-) and d_{18} γ-HBCD were purchased from Wellington Laboratories (Guelph, ON, Canada). Glass fibre filters (GFFs, 12.5cm diameter, 1 µm pore size) were purchased from Whatman (Maidstone, UK). Florisil (60-100 mesh) and silica gel (60Å, 60-100 mesh) were purchased from Sigma-Aldrich (Dorset, UK). Oxygen-free nitrogen gas was purchased from BOC Gases (Manchester, UK).

For the CE-PBET model, analytical grade inorganic salts were obtained from Fisher Scientific (Loughborough, U.K) and organic components were purchased from Sigma-Aldrich (Dorset, UK). All glassware was cleaned by soaking for at least 12 h in a phosphate-free alkali solution, rinsed with water followed by distilled water and dried at 100°C for at least 12 h.

2.2. Low level dust procurement

As described previously (Rauert et al., 2014a), a bulk house dust sample obtained from a private residence in Belgium containing low concentrations of HBCDs was utilised in these chamber experiments. Bulk dust samples were collected with the residents' own vacuum cleaner. The contents of the vacuum cleaner bag were emptied and sieved with a 500 µm mesh size hand held sieve. The bulk dust was further sieved to <250 µm before use, as larger particles are considered less relevant for assessing bioaccessibility from dust ingestion as they are unlikely to adhere to the skin on a hand hence be available for ingestion from hand-mouth contact (U.S.EPA, 1999; U.S.EPA, 2003; U.S.EPA, 2005; Duggan et al., 1985; Duggan and Inskip, 1984; Yamamoto et al., 2006). The dust sample was homogenised via vortex and 100-200 mg subsamples were extracted and analysed to determine indigenous HBCDs concentrations. Concentrations of HBCDs are listed in Table 1. NIST dust standard reference material (SRM 2585) was used for accuracy and precision measurement (see section 2.9). Indicative HBCD concentrations in SRM-2585 and our determinations for SRM-2585 and the Belgian dust are shown in Table 1.

Table 1. Mean HBCD concentrations (ng g^{-1}) in SRM-2585 and Belgian (control) dust samples.

		α-HBCD	β-HBCD	γ-HBCD
SRM-2585	Indicative values [a, b, c]	13.2 – 19.0	3.6 – 4.3	68.2 ± 120
	1	21	6.2	130
	2	24	7.7	130
	3	18	5.3	110
	average ± SD	21 ± 3	6 ± 1	123 ± 12
	Standard error	2	1	7
	CV (%)	14	17	10
Belgian (control) dust	1	75	10	36
	2	59	12	23
	3	25	3.0	12
	4	25	6.0	28
	5	40	8.0	32
	6	38	7.0	49
	7	42	8.0	55
	average ± SD	43 ± 18	8 ± 3	34 ± 15
	Standard error	7	1	6
	CV (%)	42	37	44

[a](Van den Eede et al. 2012); [b](Schreder & La Guardia 2014); [c](Keller et al. 2007)

2.3. HBCD treated curtains

Fabric curtains treated with the HBCD technical formulation were obtained from the National Institute for Environmental Studies (NIES), Tsukuba, Japan. Concentrations of HBCDs in these curtains were previously reported at 18,000 mg kg^{-1} for α-HBCD, 7,500 mg kg^{-1} for β-HBCD and 17,000 mg kg^{-1} for γ-HBCD (Kajiwara and Takigami, 2013).

2.4. Test chamber experimental design

2.4.1. Test chamber apparatus

A cylindrical in-house designed and built test chamber was utilised for these investigations. Details of the chamber setup have been described previously (Rauert et al. 2014a; Rauert et al., 2014b). Briefly, the chamber was constructed from stainless steel with dimensions of 10 cm diameter and 20 cm height. A removable, aluminium mesh shelf was placed halfway down the chamber or 3 cm above the chamber floor, depending on the experimental design, to allow separation of the HBCD 'source' and an aliquot of dust. The desired temperature of the chamber was obtained by placing it into a hot water bath with chamber internal temperature monitored by a LogTag TRIX-8 temperature data logger (LoggerShop Technology, Dorset, UK).

2.4.2. Volatilisation with partition to dust particles

Belgian (control) dust (1.2 g) pre-characterised for HBCDs was weighed onto a glass fibre filter (GFF) and placed on the chamber floor. A 4 x 4 cm piece of curtain treated with HBCDs (the HBCDs source) was placed on the mesh shelf located in the middle of the chamber. The experiment was undertaken for 1 week at 35 °C to simulate the worst case scenario of high volatilisation rates from curtains heated in warm countries during summer time. The chamber was sealed from the outside environment during the experiment to retain all volatilised analytes inside the chamber and encourage their partitioning to the dust. The configuration of these experiments is shown in Figure 1A (Rauert et al. 2015). After the experiment, the chamber was cooled and maintained at room temperature for five hours before opening to reduce the loss of volatiles with chamber lid removal. Post-experiment, the dust was homogenised by vortexing and 1-2 subsamples of 0.05 g each were extracted and analysed for HBCDs. Concentrations of HBCDs in these dusts are listed in Table 2. The remainder of the dust sample was subjected to the bioaccessibility test.

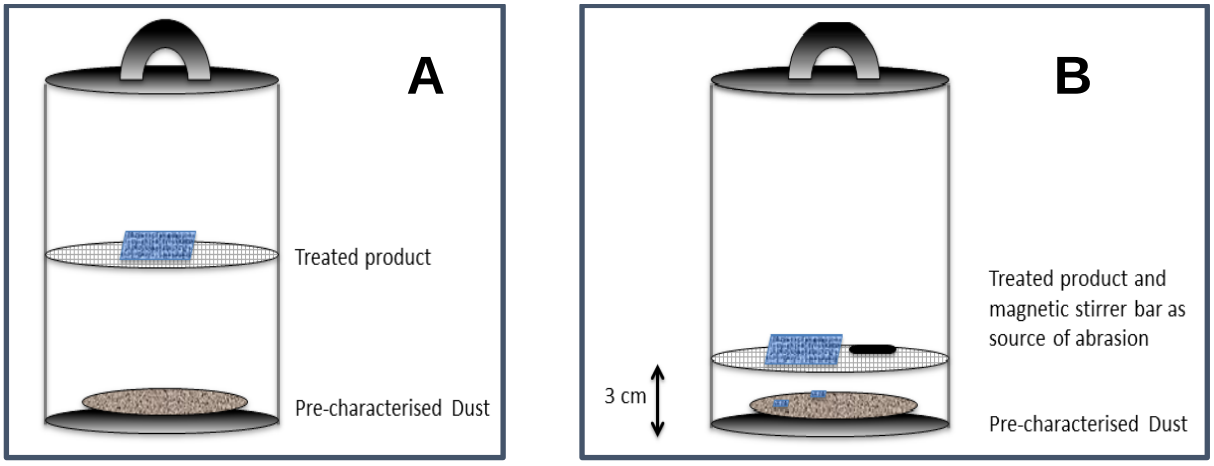

Figure 1. Schematic of test chamber experiment for generating dust samples contaminated with HBCDs via: a) volatilisation from a HBCD source with subsequent partitioning to dust and b) abrasion of a HBCD source, followed by transfer to dust of abraded particles/fibres. Figure modified from Rauert et al. (2015)**.**

Table 2. Concentrations (ng g^{-1}) of HBCDs determined in dust samples contaminated via volatilisation and subsequent partitioning to dust.

		α-HBCD	**β-HBCD**	**γ-HBCD**
Volatilisation 1	Analysis 1	190	74	280
Volatilisation 2	Analysis 1	4500	1600	5900
	Analysis 2	400	130	500
	Standard error	1674	600	2205
	CV (%)	118	120	119
Volatilisation 3	Analysis 1	1200	400	1500
	Analysis 2	940	300	1200
	Standard error	106	41	122
	CV (%)	17	20	16
Volatilisation 4	Analysis 1	5200	1800	6700

	Analysis 2	2966	940	3900
	Standard error	1117	430	1400
	CV (%)	39	44	37
Volatilisation 5	Analysis 1	820	280	1100
	Analysis 2	450	170	1200
	Standard error	185	55	50
	CV (%)	41	35	6

2.5.3. Abrasion of textile fibres to dust

Pre-characterised dust (0.5 g) was placed on a GFF on the chamber floor. The mesh shelf was lowered to 3 cm above the chamber floor and a piece of 2 x 2 cm curtain treated with HBCDs was placed on the shelf. The chamber was placed on a magnetic stirrer plate and the abrasion of the curtains was generated with a magnetic stirrer bar, 40 mm x 8 mm, (Fisher Scientific, Leicestershire, UK) which was also placed on the mesh shelf. The rotating stirrer bar (200 rpm) was in direct contact with the curtain encouraging the loosening of fibres and particles to migrate through the mesh into the dust. The abrasion experiments were conducted for 24 hours at room temperature to minimise losses of HBCDs via volatilisation. The chamber experimental design for these experiments is illustrated in Figure 1B.

Post experiment, any fibres observable with the naked eye were removed to reduce the presence of large (>250 µm) particle sizes and the dust was homogenised by vortexing. One aliquot of this abraded dust (0.05 g) was extracted and analysed for HBCDs. Elevated HBCD concentrations were observed (7000-20000 ng g^{-1}) and in order to reduce the HBCD concentration to within an order of magnitude of the volatilised dusts (to remove concentration differences as a factor influencing bioaccessibility), the abraded dusts were diluted with the addition of an aliquot of the original Belgian dust, containing minimal levels of HBCDs (Table 1). Five abrasion dusts of 1.2 g each were prepared mixing 0.03-0.4 g of the chamber generated dust with the original Belgian dust. The dusts were thoroughly mixed by vortexing and three subsamples of 0.05 g each were extracted and analysed for HBCDs. The remainder of the

dust sample was exposed in the bioaccessibility model. HBCD concentrations of the dusts are shown in Table 3.

Table 3. Concentrations (ng g^{-1}) of HBCDs determined in dust samples contaminated via abrasion.

		α-HBCD	β-HBCD	γ-HBCD
Abrasion 1	Analysis 1	470	170	840
	Analysis 2	290	96	410
	Analysis 3	570	350	4100
	Standard error	82	75	1165
	CV	32	64	133
Abrasion 2	Analysis 1	3600	1100	5000
	Analysis 2	610	220	840
	Analysis 3	320	99	400
	Standard error	1048	315	1466
	CV	120	116	122
Abrasion 3	Analysis 1	6800	2300	10000
	Analysis 2	430	140	570
	Standard error	3185	1080	4715
	CV	125	125	126
Abrasion 4	Analysis 1	700	200	850
	Analysis 2	1100	500	2600
	Analysis 3	250	90	310
	Standard error	246	123	691
	CV	62	81	96
Abrasion 5	Analysis 1	3100	830	3300
	Analysis 2	230	72	280
	Analysis 3	450	160	610
	Standard error	922	239	9

CV	127	117	119

Abraded and volatilised dusts display a large heterogeneity of HBCDs concentrations between replicates of the same dust. This difference in concentrations will not affect the bioaccessibility analysis because the bioaccessibility is calculated as a ratio of phases in the CE-PBET (see equation 1) assuming the contaminated matrix is the same in each case. A t-test comparing the HBCDs concentrations in volatilised and abraded dusts was not significant ($p>0.05$) (Table S2, Supporting Information).

2.5. Incubation with CE-PBET

The prepared dusts (original low HBCD level Belgian dust, 5 simulated abrasion dusts and 5 volatilisation with partitioning to dust samples), the SRM-2585 dust reference material and uncontaminated sand as a procedural blank were exposed in the CE-PBET model in triplicate. Bioaccessibility experiments were conducted using the model in a fed state to represent the maximum bioaccessibility scenario and a solid to liquid (S/L) ratio between the dust matrix and the gastrointestinal solutions of 1/167. Lower S/L ratios have shown saturation phenomena of the chemicals in the GI solutions (Van de Wiele et al., 2007) which may result in underestimated bioaccessibility values and S/L ratios between 1/150 and 1/250 have been suggested as optimum (Yu et al., 2011). 0.3 g of each dust was exposed in 50 mL of stomach solution at pH 2.5 which also contained food components and the samples were incubated for 1h at 37°C in water bath with constant shaking. After this time, the stomach solutions with the dusts were converted to small intestine solutions by adding pancreatin (1.78 g/L) and bile salts (0.5 g/L) and increasing the pH to 7 by addition of NaCl. Samples were returned to the water bath for a further 4h. After this incubation, the samples were centrifuged (20°, 3000 rpm, 10 min) keeping the liquid phase for extraction of HBCDs (i.e. bioaccessible fraction in the small intestine) and introducing the pellet into the colon medium. Colon solutions were incubated during 16h at 37°C water bath under constant shaking and then were centrifuged as above. HBCDs from the liquid phase were extracted and considered as bioaccessible fraction in the colon. HBCDs from the pellet were also extracted; these are considered as the non bioaccessible fraction or residue. The bioaccessibility protocol is depicted in Figure 2.

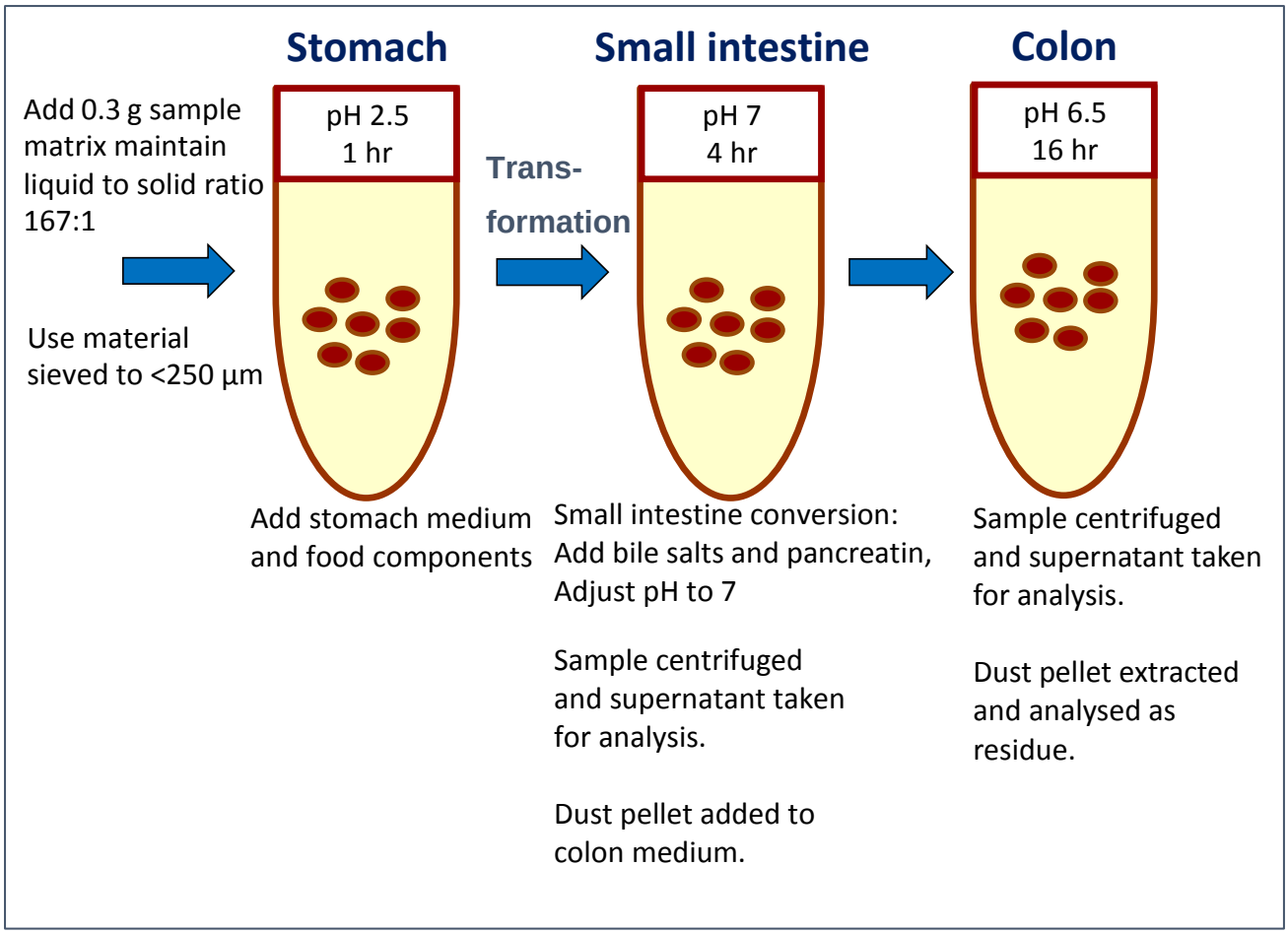

Figure 2. Schematic procedure of CE-PBET modified from Collins et al. (2015).

Bioaccessibility was determined using Equation 1, where *mHBCDs supernatants* is the sum of the mass (ng) of HBCD determined in the small intestine and colon supernatant phases of the CE-PBET and *mHBCDs pellet* is the mass (ng) determined in the pellet from the colon solution (residue from the centrifugation of the colon solution which is considered as the non bioaccessible fraction) of the CE-PBET.

$$Bioaccessibility = \frac{mHBCDs\ supernatants}{mHBCDs\ supernatants + mHBCDs\ pellet}\ x100 \qquad (1)$$

2.6. HBCDs extraction

Dust extraction and purification was performed using previously published methods (Rauert et al., 2014a, Rauert et al., 2014b). Dusts were loaded into 66 mL cells containing 1.5 g of Florisil and Hydromatrix and were extracted with pressurised liquid extraction (ASE 350, Dionex Europe, UK). Each cell was fortified with 4 ng of ^{13}C-labelled α, β and γ-HBCD as internal (surrogate) standards prior to extraction with hexane:dichloromethane (1:1 v/v) at 90^{o}C and 1500 psi. The cell was heated for 5 min, held static for 4 min and purged for 90 s, with a flush volume of 50%, for 3 cycles. The collected ASE extracts and chamber inner surface solvent rinses were concentrated to 0.5 mL using a ZymarkTurbovap II (Hopkinton, MA, USA) before purification. Clean-up was conducted by loading onto SPE cartridges filled with 4 g of pre-cleaned acidified silica (44% concentrated sulfuric acid w/w). The analytes were eluted with 30 mL of hexane:dichloromethane (1:1, v/v), with the eluate evaporated to dryness under a gentle stream of nitrogen. Samples were reconstituted to 100 *μ*L with 2 ng of d_{18}-γ-HBCD in HPLC grade methanol, used as recovery standard.

Extracts from the small intestine, colon and the residual pellet were fortified with 4 ng of ^{13}C-labelled γ-, α-, and β-HBCD. Samples were extracted via a single liquid-liquid extraction with the addition of 30 ml of acetone-hexane (1:1), followed by incubation in a water bath for 1 hour at 37°C and sonicated for 30 min. The hexane layer was removed and evaporated to 1 mL under a steady stream of nitrogen. Sample clean-up was performed with 0.5 mL of concentrated sulfuric acid, added to sample vials, and vortexed for 1 minute. The hexane (top) layer was removed for acid silica clean-up. Empty solid phase extraction (SPE) cartridges were prepared for use by addition of 1 g of acidified silica (44% w/w), then 0.5 g of anhydrous sodium sulfate. The acid silica was prepared following a previously reported method (Ali et al., 2011) where 50 g of silica gel was washed with 80 mL of *n*-hexane before sonication in an ultrasonic bath for 30 minutes. The silica was heated to 160°C overnight, then after cooling to room temperature, 22 mL of concentrated sulphuric acid (98 %) was slowly added under continuous stirring. HBCDs were eluted from the prepared SPE columns with 15 mL of *n*-hexane and 3 mL of DCM. These extracts were evaporated and reconstituted to 100 µL with 2 ng of d_{18}-γ-HBCD in HPLC grade methanol added as a recovery standard and introduced into LC-MS vials.

2.7. HBCDs analysis

Target HBCDs were separated with a dual pump Shimadzu LC-20AB Prominence liquid chromatography (Shimadzu, Kyoto, Japan) equipped with a SIL-20A autosampler, and a DGU-20A3 vacuum degasser. A Varian Pursuit XRS3 C18 reversed phase analytical column (150 mm x 4.6 mm i.d., 3 µm particle size) was used for separation of target HBCDs (α-, β- and γ-). A mobile phase program based upon (mobile phase A) 1:1 methanol/water and (mobile phase B) methanol at a flow rate of 0.18 mL min^{-1} was applied for elution of the target compounds.

Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an ESI ion source operated in negative ion mode. MS/MS detection, operated in multiple reaction monitoring (MRM) mode, was used for quantitative determination of the HBCD diastereomers, ^{13}C-, and d_{18}- labelled analogues. Recoveries of HBCDs were 87-94% (Table S1).

2.8. Accuracy and Precision

As a measure of accuracy and precision of the method, SRM-2585 was analysed on a regular basis. Due to the lack of an appropriate reference material for HBCDs, this SRM was analysed and concentrations compared to the reported values in the literature were measured (Table 1). The SRM was analysed with every 20 samples as an ongoing method performance check. Three 0.3 g of laboratory-grade sand were run through the CE-PBET model in triplicate as a method blank. Concentrations of HBCDs were close to or <LOQs (0.5 ng g^{-1}).

2.9. Statistical analysis

The differences between the averages within groups were analysed using Microsoft Excel 2010 with t-tests assuming equal variances. The correlation between two variables were analysed by simple linear regression. A p-value of less than 0.05 was considered to indicate statistical significance.

3. Results and discussion

3.1. Chamber generated dust samples

Table 2 lists concentrations of HBCDs in two replicate analyses of the dusts generated via the volatilisation with subsequent partitioning to dust (pathway 1), from 5 experimental runs. Table 3 presents the concentrations of two to three analyses of the 5 dusts generated via abrasion (pathway 2). The large variation in HBCD concentrations in the repeat analyses of all dust samples demonstrate the heterogeneity of the HBCDs throughout the dusts. Following volatilisation with subsequent partitioning of HBCDs to dusts the CV between analyses was relatively low; majority of the dusts (17-44%), with one dust above 100%. The CV of the abraded dusts was above or close to 100% in all dusts. This result is perhaps expected for the dusts generated via abrasion as HBCD concentrations are dependent on the location of the HBCD treated textile fibres within the dust. The migration of BFRs to dust via volatilisation has been suggested to result in a uniform or homogeneous distribution of BFRs in dust compared with transfer via abrasion of source material(s) (Webster et al., 2009; Suzuki et al., 2009).

HBCD concentrations in the SRM-2585 dust had lower variability than the original Belgian dust or the dusts generated by abrasion or volatilization with subsequent partitioning to dust (Table 1). The CVs of the HBCD diastereomers in the SRM were 14, 17 and 10% for α, β and γ HBCD respectively whereas the CVs of the Belgian dust were 39, 77 and 78% respectively. The CV of the three HBCD diastereomers in abrasion generated and volatilised with subsequent partitioning to dust samples varied between dust replicates (30-120%). One explanation of this greater heterogeneity in the Belgian dust compared to the SRM is due to the differences in particle size of the dusts. The Belgian dust was sieved at <250 µm whereas the dust reference material was sieved <90 µm (Poster et al., 2007). This difference in particle size may result in a more homogeneous distribution of HBCDs, as larger particles may have originated from abrasion of fibres/particles from a treated source (i.e. creating isolated areas of high HBCD concentrations).

There was no significant difference ($p > 0.05$) in the concentrations between volatilisation dusts and abrasion dusts showing that we achieved our objective of generating dusts that contained statistically similar concentrations of HBCDs from two migration pathways. This is critical in order to eliminate variation in the bioaccessibility resulting from concentration differences between the two dusts.

3.2. Bioaccessibility of HBCDs

3.2.1. SRM-2585

Three aliquots (0.3 g) of the SRM-2585 dust were subjected to the CE-PBET method as another QC check. The amount of HBCDs (ng) of each compartment were converted to ng g^{-1} dust (for the CE-PBET 0.3 g dust were used) and the recovered concentrations obtained in the stomach, colon and residue compartments were consistent with the indicative values of SRM-2585 for γ-HBCD (Table 4). The concentration of α- and β-HBCDs in the stomach and colon samples were <LOQ in the three replicates and γ-HBCD was only 2.0-8.6% bioaccessible. This low bioaccessibility can be attributed to the strong bonds between HBCDs and the dust as a consequence of the long residence time (long period of time in contact with the dust). The different parent material as source of HBCDs between the SRM-2585 (which is a pooled sample of indoor dusts) and the piece of curtain could also affect to decrease the bioaccessibility.

Table 4. Indicative mass (ng) of HBCDs in SRM-2585, in small intestine, colon and residue compartments and bioaccessibility of HBCDs from three replicate analyses of the SRM (LOD = limit of detection)**.**

		α-HBCD	β-HBCD	γ-HBCD
SRM-Indicative values (whole dust)		19 ± 3.7	4.3 ± 1.1	120 ± 22
SRM-1	Small intestine	<2.2	<1.9	2.2
	Colon	<2.2	<1.9	4.2
	Residue	17	5.5	68
	Bioaccessibility (%)	< LOD	< LOD	8.6
SRM-2	Small intestine	<2.2	<1.9	<2.2
	Colon	<2.2	<1.9	7.1
	Residue	<2.2	<1.9	170
	Bioaccessibility (%)	< LOD	< LOD	4.0
SRM-3	Small intestine	<2.2	<1.9	23

	Colon	<2.2	<1.9	4.3
	Residue	<2.2	<1.9	140
	Bioaccessibility (%)	< LOD	< LOD	2.0

3.2.2. Dusts generated by volatilisation with partitioning to particles and abrasion

The mean bioaccessibility of HBCD from both migration pathways compared to the Belgian dust (control) are shown in Figure 3 and in Table 5. In general, the samples contained higher concentrations in the residue compartment with concentrations ranging between 10-31%, 3-10% and 63-86% in the stomach, colon and residue respectively. These results suggest therefore that a substantial proportion of ingested HBCDs stay bound to dust and pass through the digestive system without being absorbed into the gastrointestinal tract.

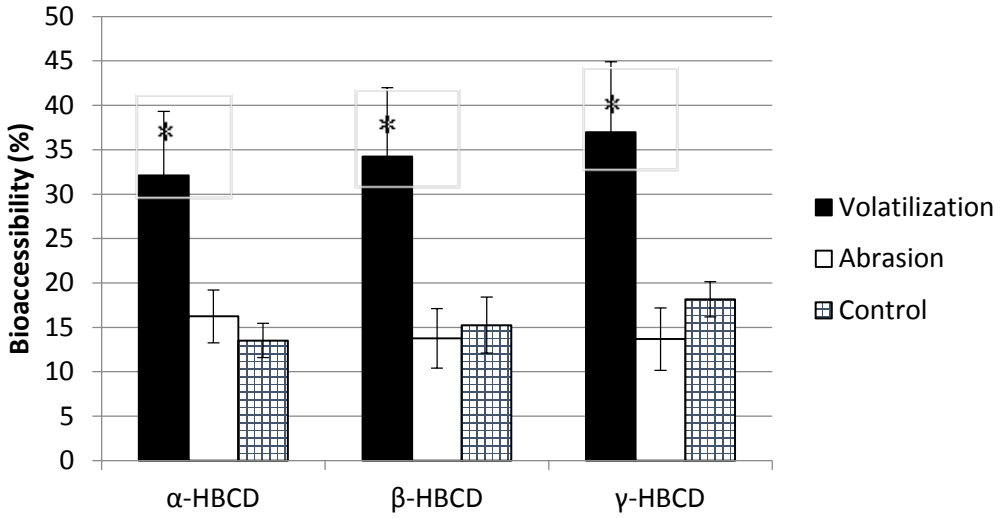

Figure 3. Average bioaccessibility of HBCDs from the control, and dusts contaminated via volatilisation with subsequent partitioning and abrasion. The y-error bars represent the standard error. (*) denotes a statistically significant difference ($p<0.05$) in bioaccessibilities (%) between volatilisation and abrasion generated dusts**.**

Table 5. HBCD distribution (%) in small intestine, colon and residue compartments and the bioaccessible fraction (%) of dusts when contaminated via volatilisation or abrasion, and Belgian

dust (control). Five dusts contaminated by abrasion and five dusts contaminated by volatilisation were exposed to the CE-PBET in triplicate (5x3 dusts per migration pathway).

		α-HBCD	β-HBCD	γ-HBCD
Volatilisation	Small Intestine	22	30	12
	Colon	3.4	2.3	11
	Residue	74	48	78
	Bioaccessibility (%)	32	34	37
	Standard error	7	8	8
Abrasion	Small Intestine	13	12	11
	Colon	4.6	8.5	3.3
	Residue	83	80	86
	Bioaccessibility (%)	16	14	14
	Standard error	3	3	4
Belgian dust	Small Intestine	3.8	1.7	10
	Colon	3.2	0.4	3.1
	Residue	42	72	59
	Bioaccessibility (%)	12	16	19
	Standard error	2	3	2

The bioaccessibility of all 3 HBCD diastereomers was significantly higher in dusts contaminated via the migration by volatilization pathway (1) compared to the abrasion pathway (2) ($p=0.02$, 0.01 and 0.007 for α, β and γ respectively). Dusts contaminated via volatilisation with subsequent partitioning had a significantly higher bioaccessibility than in the Belgian dust (control) in the case of α and β HBCDs ($p=0.02$ and 0.01 respectively), whereas the bioaccessibility in abrasion generated samples compared to the control dust was not significantly different for any of the diastereomers ($p>0.05$). A stronger bond between HBCDs and dust particles/fibres is expected when these hydrophobic compounds migrate to the dust in the guise of abraded source fibres/particles than via volatilisation and subsequent partitioning to dust. This stronger bond can be explained by the residence time of the compounds in the matrix. In abraded material this residence time is longer because HBCDs are already sorbed to the fibres. By comparison, when they migrate by volatilisation they are newly incorporated into the dust and are desorbed less strongly. This may contribute to the lower bioaccessibility in the control dust and in the SRM-2585 than in the volatilisation with partitioning dust samples. The strong binding between HBCDs

and curtain fibres may make HBCDs less bioaccessible to the biological fluids in a parallel manner to the influence of organic matter on the bioaccessibility of PBDEs (Yu et al., 2013).

The mean bioaccessibility of HBCD diastereomers in the present study from chamber generated dust was 14 to 37%, lower than that previously reported (72-90%) by Abdallah et al. (2012). Abdallah et al. simulated the digestion in an unfed state therefore we would have expected the bioaccessibility of lipophilic compounds such as HBCDs to be lower in unfed state than in the present study. In addition, Abdallah et al. (2012) used the same dust sample in 10 replicates which was homogeneous in HBCD concentration. In the present study for instance, we used 12 different samples and the volatilised and abraded dusts had a greater heterogeneity in concentrations of HBCDs. It is thus likely that the highly variable bioaccessibility values obtained for the chamber generated dusts stem from a non-homogeneous distribution of HBCD concentrations throughout these samples. The bioaccessibility of the SRM-2585 and the control Belgian dust were consistent for the triplicate assays. (Fang and Stapleton, 2014) reported up to 80% bioaccessibility of OPFRs and suggested that the increased bioaccessibility (when compared to PBDEs) was due to a difference in log K_{OW}. Following this, as HBCDs have a similar log K_{OW} to PBDEs, they should present a similar level of bioaccessibility. Our results are in line with the previously reported PBDE bioaccessibilities (20-58% for tri-hepta-BDEs) (Abdallah et al., 2012; Yu et al., 2013).

4. Conclusion

This study is the first to test the hypothesis that the migration pathway via which HBCDs enter dust influences the bioaccessibility of HBCDs from dust ingestion. HBCDs were more bioaccessible from dust samples contaminated via volatilisation with subsequent partitioning to dust (mean 35%) than in dust contaminated via abraded source fibres (mean 15%). We believe this difference in bioaccessibility results from fibres released from the curtains during the abrasion process. These fibres which are not bioaccessible contain high amount of HBCDs.

Volatilisation is considered as the main migration pathway for SVOCs compounds from treated products to indoor environment. These results have shown that compounds released to indoor air and deposited into the dust become more bioaccessi-

ble than when migrated by abrasion. Previous research suggested that BDE-209 in dusts is originated from polymers via abrasion whereas the lower brominated PBDEs (with higher vapour pressures) enter the dust through volatilising to the air. This difference in migration may be one of the reasons of why BDE-209 has been shown to be less bioaccessible than other PBDEs. Future research should consider including in the risk assessment of SVOCs the migration pathways in order to determine the worse exposure case scenario.

Although the mean bioaccessibilities of HBCDs from dusts contaminated with abraded source fibres were significantly lower ($p < 0.05$) than those in dusts contaminated as a result of volatilisation followed by deposition, more data are required to confirm this result. The results from this study were obtained under only one specific BFR usage scenario, HBCDs present in a treated textile. Future research should investigate the behaviour of different BFRs and the influence of different source materials. In conclusion, these results suggest that exposure estimates of HBCDs to humans from ingested dust that do not take into account bioaccessibility and factors such as the mode of HBCD incorporation into the dust are potentially incorrectly calculating exposure and should be updated.

Acknowledgements

The research leading to these results has received funding from the European Union Seventh Framework Program (*FP7/2007-2013*) under grant agreement No 264600 (INFLAME project). We also thank Natusuko Kajiwara and HidetakaTakigami from the National Institute for Environmental Studies, Japan, for supplying the HBCD treated curtain samples.

References

Abdallah, M., Harrad, S. & Covaci, A. (2008). Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, UK: implications for human exposure. *Environmental Science & Technology,* **42,** 6855-6861.

Abdallah, M., Tilston, E., Harrad, S. & Collins, C. (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.

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. *Analytical and Bioanalytical Chemistry,* **400,** 3073-3083.

Allen, J. G., McClean, M. D., Stapleton, H. M., Nelson, J. W. & Webster, T. F. (2007). Personal Exposure to Polybrominated Diphenyl Ethers (PBDEs) in Residential Indoor Air. *Environmental Science & Technology,* **41,** 4574-4579.

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

Boutrup, S., Dam, M., Mannio, J., Jörundsdóttir, H. Ó. & Hedlund, B. (2011). Brominated flame retardants (BFRs) in the nordic environment.

Camenisch, G., Alsenz, J., van de Waterbeemd, H. & Folkers, G. (1998). Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *European Journal of Pharmaceutical Sciences,* **6,** 313-319.

Clausen, P. A., Hansen, V., Gunnarsen, L., Afshari, A. & Wolkoff, P. (2004). Emission of Di-2-ethylhexyl Phthalate from PVC Flooring into Air and Uptake in Dust: Emission and Sorption Experiments in FLEC and CLIMPAQ. *Environmental Science & Technology,* **38,** 2531-2537.

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. *Environment international,* **78,** 24-31.

Covaci, A., Gerecke, A. C., Law, R. J., Voorspoels, S., Kohler, M., Heeb, N. V., Leslie, H., Allchin, C. R. & de Boer, J. (2006). Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. *Environmental Science & Technology,* **40,** 3679-3688.

Duggan, M., Inskip, M., Rundle, S. & Moorcroft, J. (1985). Lead in playground dust and on the hands of schoolchildren. *Science of The Total Environment,* **44,** 65-79.

Duggan, M. J. & Inskip, M. (1984). Childhood exposure to lead in surface dust and soil: a community health problem. *Public health reviews,* **13,** 1-54.

EPA), U. S. E. P. A. U. (2007). Guidelines for Neurotoxicity Risk Assessment.

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. *Environmental science & technology,* **48,** 13323-13330.

Hamel, S. C., Ellickson, K. M. & Lioy, P. J. (1999). The estimation of the bioaccessibility of heavy metals in soils using artificial biofluids by two novel methods: mass-balance and soil recapture. *Science of The Total Environment,* **243–244,** 273-283.

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. *Environmental Science & Technology,* **39,** 5121-5130.

Kajiwara, N. & Takigami, H. (2013). Emission behavior of hexabromocyclododecanes and polybrominated diphenyl ethers from flame-retardant-treated textiles. Environmental Science: Processes & Impacts,**15**, 1957-1963.

Keller, J.M. et al., 2007. Standard reference materials available from the National Institute of Standards and Technology for the analysis of brominated flame retardants. In *Proceedings of the 4th International workshop on brominated flame retardants*. pp. 24–27.

Oomen, A. G., Sips, A. J. A. M., Groten, J. P., Sijm, D. T. H. M. & Tolls, J. (1999). 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.

Palace, V., Park, B., Pleskach, K., Gemmill, B. & Tomy, G. (2010). Altered thyroxine metabolism in rainbow trout (Oncorhynchus mykiss) exposed to hexabromocyclododecane (HBCD). *Chemosphere,***80,** 165-169.

Poster, D. L., Kucklick, J. R., Schantz, M. M., Vander Pol, S. S., Leigh, S. D. & Wise, S. A. (2007). Development of a House Dust Standard Reference Material for the Determination of Organic Contaminants. *Environmental Science & Technology,***41,** 2861-2867.

Rauert, C. & Harrad, S. (2015). Mass transfer of PBDEs from plastic TV casing to indoor dust via three migration pathways—A test chamber investigation. *Science of The Total Environment,***536,** 568-574.

Rauert, C., Harrad, S., Stranger, M. & Lazarov, B. (2014a). Test chamber investigation of the volatilization from source materials of brominated flame retardants and their subsequent deposition to indoor dust. *Indoor air,***25,** 393-404.

Rauert, C., Harrad, S., Suzuki, G., Takigami, H., Uchida, N. & Takata, K. (2014b). Test chamber and forensic microscopy investigation of the transfer of brominated flame retardants into indoor dust via abrasion of source materials. *Science of The Total Environment,***493,** 639-648.

Rauert, C., Lazarov, B., Harrad, S., Covaci, A. & Stranger, M. (2014c). A review of chamber experiments for determining specific emission rates and investigating migration pathways of flame retardants. *Atmospheric Environment,***82,** 44-55.

Roosens, L., Abdallah, M. A., Harrad, S., Neels, H. & Covaci, A. (2009). Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: preliminary results. *Environ Health Perspect.,***117,** 1707-12. doi: 10.1289/ehp.0900869. Epub 2009 Jul 13.

Roze, E., Meijer, L., Bakker, A., Van Braeckel, K., Sauer, P. & Bos, A. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. *Environ Health Perspect,***117,** 1953 - 1958.

Ruby, M. V., Davis, A., Link, T. E., Schoof, R., Chaney, R. L., Freeman, G. B. & Bergstrom, P. (1993). Development of an in vitro screening test to evaluate the in vivo bioaccessibility of ingested mine-waste lead. *Environmental Science & Technology,***27,** 2870-2877.

Ruby, M. V., Schoof, R., Brattin, W., Goldade, M., Post, G., Harnois, M., Mosby, D. E., Casteel, S. W., Berti, W., Carpenter, M., Edwards, D., Cragin, D. & Chappell, W. (1999). Advances in Evaluating the Oral Bioavailability of

Inorganics in Soil for Use in Human Health Risk Assessment. *Environmental Science & Technology,***33,** 3697-3705.

Saegusa, Y., Fujimoto, H., Woo, G.-H., Inoue, K., Takahashi, M., Mitsumori, K., Hirose, M., Nishikawa, A. & Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1, 2, 5, 6, 9, 10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. *Reproductive Toxicology,***28,** 456-467.

Schreder, E.D. & La Guardia, M.J., 2014. Flame Retardant Transfers from U.S. Households (Dust and Laundry Wastewater) to the Aquatic Environment. *Environmental Science & Technology*, 48(19), pp.11575–11583.

Schripp, T., Fauck, C. & Salthammer, T. (2010). Chamber studies on mass-transfer of di (2-ethylhexyl) phthalate (DEHP) and di-< i> n</i>-butylphthalate (DnBP) from emission sources into house dust. *Atmospheric Environment,***44,** 2840-2845.

Suzuki, G., Kida, A., Sakai, S.-i. & Takigami, H. (2009). Existence State of Bromine as an Indicator of the Source of Brominated Flame Retardants in Indoor Dust. *Environmental Science & Technology,***43,** 1437-1442.

Testa, B., Crivori, P., Reist, M. & Carrupt, P.-A. (2000). The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and examples. *Perspectives in Drug Discovery and Design,***19,** 179-211.

Tilston, E. L., Gibson, G. R. & Collins, C. D. (2011). Colon Extended Physiologically Based Extraction Test (CE-PBET) Increases Bioaccessibility of Soil-Bound PAH. *Environmental Science & Technology,***45,** 5301-5308.

U.S.EPA (1999). Short sheet: IEUBK model soil/dust ingestion rates; EPA 540-F-00-007, OSWER 9285.7-33;U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response: Washington, DC. .

U.S.EPA (2003). Superfund Lead-Contaminated Residential Sites Handbook; Final ; OSWER 9285.7-33;U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Lead Sites Workgroup: Washington, DC. .

U.S.EPA (2005). Estimation of Relative Bioavailability of Arsenic in Soil and Soil-Like Materials by in Vivo and in Vitro Methods; USEPA Review Draft; U.S. Environmental Protection agency, Region 8: Denver, CO.

Van den Eede, N. et al., 2012. Multi-residue method for the determination of brominated and organophosphate flame retardants in indoor dust. *Talanta*, 89, pp.292–300.

Van de Wiele, T. R., Oomen, A. G., Wragg, J., Cave, M., Minekus, M., Hack, A., Cornelis, C., Rompelberg, C. J., De Zwart, L. L. & Klinck, B. (2007). Comparison of five in vitro digestion models to in vivo experimental results: lead bioaccessibility in the human gastrointestinal tract. *Journal of Environmental Science and Health Part A,***42,** 1203-1211.

Webster, T. F., Harrad, S., Millette, J. R., Holbrook, R. D., Davis, J. M., Stapleton, H. M., Allen, J. G., McClean, M. D., Ibarra, C., Abdallah, M. A.-E. & Covaci, A. (2009). Identifying Transfer Mechanisms and Sources of Decabromodiphenyl Ether (BDE 209) in Indoor Environments Using Environmental Forensic Microscopy. *Environmental Science & Technology,***43,** 3067-3072.

Weschler, C. J. & Nazaroff, W. W. (2008). Semivolatile organic compounds in indoor environments. *Atmospheric Environment,***42,** 9018-9040.

Yamamoto, N., Takahashi, Y., Yoshinaga, J., Tanaka, A. & Shibata, Y. (2006). Size distributions of soil particles adhered to children's hands. *Archives of Environmental Contamination and Toxicology,* **51,** 157-163.

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. *Environment International,* **42,** 124-131.

Yu, Y., Pang, Y., Zhang, X., Li, C., Yu, Z. & Fu, J. (2011). Optimization of an in vitro method to measure the bioaccessibility of polybrominated diphenyl ethers in dust using response surface methodology. *Journal of Environmental Sciences,* **23,** 1738-1746.

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

Zhang, X., Diamond, M. L., Robson, M. & Harrad, S. (2011). Sources, emissions, and fate of polybrominated diphenyl ethers and polychlorinated biphenyls indoors in Toronto, Canada. *Environmental science & technology,* **45,** 3268-3274.