UNIVERSITY OF BIRMINGHAM

INVESTIGATING THE SOURCES AND MAGNITUDE OF HUMAN EXPOSURE TO HALOGENATED ORGANIC POLLUTANTS USING ADVANCED METHODS FOR ENVIRONMENTAL ANALYSIS

by
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ABSTRACT

Analytical methods based on LC-ESI-MS/MS or LC-APPI-MS/MS were developed and/or validated for the separation and determination of BDE-209 and TBBP-A, in addition to HBCD diastereomers, enantiomers and degradation products in a wide range of samples including air, dust, diet, simulated GIT fluid, human serum and breast milk. The obtained concentrations were used to estimate the exposure of adults, toddlers and nursing infants to the target BFRs via inhalation, dust ingestion and diet using different exposure scenarios and the relative importance of each exposure pathway was assessed for the studied age groups. Causes of variability in concentrations of HBCDs in indoor dust were elucidated and forensic microscopy techniques were used to study the mechanisms of transfer of BDE-209 and HBCDs to indoor dust. A colon-enhanced physiologically based extraction model was developed and applied for the first time to study the bioaccessibility of target BFRs from human GIT. Exposure via dust ingestion, but not diet, correlated significantly ($p<0.01$) with ΣHBCDs in serum of 16 adults. The levels of target BFRs were reported for the first time in 28 human milk samples from the UK. The relationship between adult intake of BFRs and the observed body burdens was studied using a pharmacokinetic model. Although no enantioselective enrichment was detected in either dust or diet, enrichment of (-)-α-HBCD was observed in both human serum and milk which may be attributable to enantioselective absorption, metabolism and/or excretion.

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CONFERENCE PRESENTATIONS

OP = oral presentation; **PP** = Poster presentation and **CA** = contributing author.

1. **Abdallah, M. A. E.**; Harrad, S., Isomer-specific determination of hexabromocyclododecane in indoor air and dust in Birmingham, UK. *the 2nd Network Conference on Persistent Organic Pollutants*, **2007**; Birmingham, UK. **PP**
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3. Harrad, S.; **Abdallah, M. A. E.**, Vapour:particle phase distribution of Hexabromocyclododecane (HBCD): Are PUF disk passive samplers suitable for monitoring HBCD in indoor air? *The IEH Eleventh Annual UK Review Meeting on Outdoor and Indoor Air Pollution Research, Cranfield University*, **2008**; Cranfield, UK. **PP**
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6. **Abdallah, M. A. E.**; Harrad, S.; Covaci, A., The Effect of Light on Hexabromocyclododecanes (HBCDs) in Indoor Dust. *The 28th International*

Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2008), **2008**; Birmingham, UK. **OP-Otto Hutzinger presentation award**

7. **Abdallah, M. A. E.**; Harrad, S.; Covaci, A., Hexabromocyclododecanes and Tetrabromobisphenol-A in Indoor Air and Dust in Birmingham, UK: Implications for Human Exposure. *The 28th International Symposium on Halogenated Persistent Organic Pollutants (Dioxin 2008),* **2008**; Birmingham, UK. **OP**

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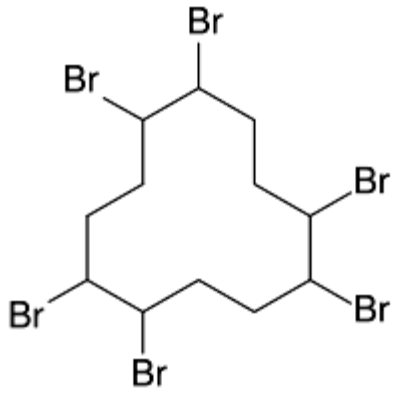
ABBREVIATIONS

ABS	Acrylonitrile-butadiene-styrene
APPI	Atmospheric pressure photoionisation.
BDE-209	Decabromodiphenyl ether.
BFR	Brominated flame retardant.
BSE	Back scattered electrons.
BSEF	Bromine science and environmental forum
Bw	Body weight.
COT	Committee on toxicity.
DAD	Diode array detector.
Dw	Dry weight.
ECNI	Electron capture negative ionisation.
EDS	Energy dispersive spectrometry.
EF	Enantomeric fraction.
EI	Electron impact.
EPA	Environment protection agency.
EPS	Expanded polystyrene.
ESEM	Environmental scanning electron microscopy.
ESI	Electrospray ionisation.
GC	Gas chromatography.
GIT	Gastrointestinal tract.
HBCD	Hexabromocyclododecane.
HIPS	High impact polystyrenes.
HPLC	High pressure liquid chromatography.
IS	Internal standard.
ITD	Ion trap detector.
K_{ow}	n-octanol/water partition coefficient.
LOD	Limit of detection.
LOQ	Limit of quantification.
Lw	Lipid weight.

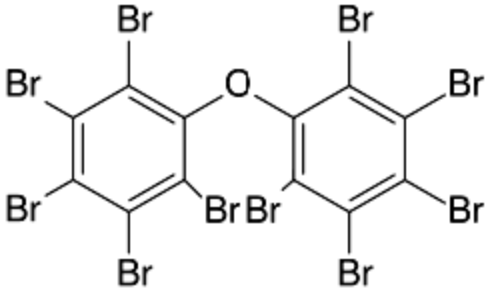
m/z	Mass to charge ratio.
MRM	Multiple reaction monitoring.
MS	Mass spectrometry.
MT	Metric tonnes.
PAH	Polycyclic aromatic hydrocarbon.
PBDE	Polybrominated diphenyl ether.
PBET	Physiologically based extraction test.
PBT	Persistent, bioaccumulative and toxic.
PCB	Polychlorinated biphenyls.
PCDD/F	Polychlorinated dibenzo-p-dioxin/furan.
PK	Pharmacokinetic.
PME	Public microenvironment.
POP	Persistent organic pollutant.
PUF	Polyurethane foam.
QA/QC	Quality assurance/quality control.
RDS	Recovery determination standard.
RRT	Relative retention time.
RSD	Relative standard deviation.
SD	Standard deviation.
SE	Secondary electrons.
SES	Sampling evaluation standard.
STP	Sewage treatment plant.
T_3	Triiodothyronine.
T_4	Thyroxine.
TBBP-A	Tetrabromobisphenol A.
TDI	Tolerable daily intake.
Ww	Wet weight.
XPS	Expanded polystyrene.

CHAPTER I
INTRODUCTION

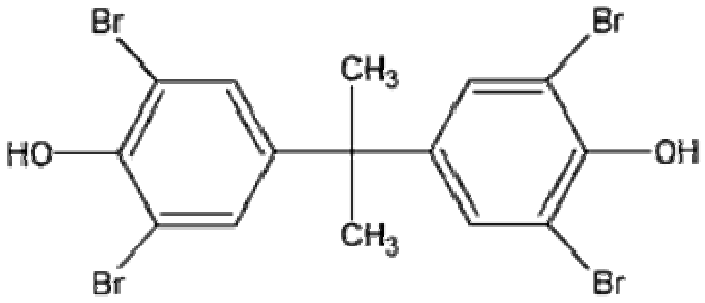
Every year, fires kill more than 4,000 people, injure more than 20,000, and result in property damage exceeding an estimated $11 billion in the USA while in Europe, over 4,000 Europeans lost their lives in fires and fire losses came to more than €15 billion in 2005 ([Anon], 2008). Fire incidence has decreased in the last quarter of a century partly because of the fire prevention regulations requiring the presence of flame retardant chemicals in many industrial products including plastics, polymers, textiles, building materials, electric and electronic equipments (Birnbaum and Staskal, 2004). Flame retardants were first applied in ancient Egypt where alum was used to flame retard wooden objects (Hindersinn, 1990). Currently, there are 4 major groups of flame retardants on the market: inorganic, halogenated organic, organophosphorous and nitrogen based compounds (van Esch, 1997). Brominated flame retardants (BFRs; a subgroup of the halogenated organic class of flame retardants) are currently the largest market group of flame retardants because of their low cost and high performance efficiency. The most widely used BFRs are tetrabromobisphenol A (TBBP-A) with a global demand of 170,000 metric tons (MT) in 2004, alongside decabromodiphenyl ether and hexabromocyclododecane (HBCD) for which the worldwide market demands in 2001 were 56,100 and 16,700 MT respectively. These chemicals are used to flame retard a wide variety of products including: construction materials, upholstery, plastic housings for electronic devices and printed circuit boards (BSEF, 2009a). While TBBP-A is mainly used as a "reactive" flame retardant chemically bound to the polymer matrix; both BDE 209 and HBCD are used as additives mixed with the polymer matrix during manufacture and thus are believed to be more easily released to the environment from flame retarded products (Alaee et al., 2003a).



Hexabromocyclododecane



Decabromodiphenyl ether



Tetrabromobisphenol-A

Figure 1.1: Chemical structures of the BFRs studied in this thesis.

1.1 Mechanism of action of BFRs.

In order for a solid substance to burn, it has to be preheated by means of an external source. This results in thermal decomposition (pyrolysis phase) of the material with release of flammable gases which then react with atmospheric O_2 to produce visible flames and generate more heat (burning phase). If the generated heat is sufficient, the material will continue decomposing and the burning process becomes self-propagating. BFRs act by slowing down or preventing the burning phase via reducing heat generation and production of further flammable gases. Upon exposure to high temperatures, BFRs release $Br^{\cdot}$ radicals which react with the hydrocarbon molecules of flammable gases to produce HBr. The produced HBr reacts with $OH^{\cdot}$ and/or $H^{\cdot}$ radicals to form H_2O, H_2 and $Br^{\cdot}$ radicals which can restart the cycle.

$$RBr \longrightarrow R^{\cdot} + Br^{\cdot}$$

$$Br^{\cdot} + RH \longrightarrow R^{\cdot} + HBr$$

$$HBr + OH^{\cdot} \longrightarrow H_2O + Br^{\cdot}$$

$$HBr + H^{\cdot} \longrightarrow H_2 + Br^{\cdot}$$

Flame retardant efficiency depends on its bromine content and its control of bromine release. Bromine is released in a narrow temperature range resulting in optimal concentrations in the flame zone. In contrast, chlorine is released over a wider temperature range, making chlorinated flame retardants less effective (van Esch, 1997).

1.2 Hexabromocyclododecane (HBCD).

1.2.1 Physicochemical properties.

HBCD is a white odourless solid prepared by bromination of cyclododecatri-1,5,9-ene (CDT). Theoretically, 16 stereoisomers (6 pairs of enantiomers and 4 meso forms) can be formed by bromination of all four CDT isomers. So far, only 8 of those have been identified in commercial HBCD formulations (Heeb et al., 2005). Technical grade HBCD consists mainly of the γ-HBCD diastereoisomer (ranging between 75 and 89%), while α- and β-HBCDs are present in considerably lower amounts (10-13% and 0.5-12%) respectively (Becher, 2005). The two other stereoisomers identified in a low melting technical HBCD product, termed δ- and ε -HBCD (0.5 and 0.3 % of ΣHBCDs respectively), are meso forms (i.e. achiral). The presence of these two meso forms in the technical product is probably caused by impurities in the starting material (Heeb et al., 2005). A summary of the physicochemical properties of HBCD technical product is given in table 1.1.

1.2.2 Applications and uses.

The primary application of HBCD is as an additive to extruded (XPS) and expanded (EPS) polystyrene foams used for thermal insulation in the building industry. Typical HBCD levels in EPS and in XPS are 0.67% and 2.5% w/w respectively making HBCD the most suitable flame retardant for these applications since any other flame retardant would likely need higher load levels in the polystyrene foam (BSEF, 2009a).

Table 1.1: Physicochemical properties of technical HBCD (KEMI (National Chemicals Inspectorate), 2007).

Property	**Value**
Chemical formula	$C_{12}H_{18}Br_6$
Molecular weight	641.7 g $mole^{-1}$
Boiling point	Decomposes at >190 °C
Melting point	179-181 °C α-HBCD 170-172 °C β-HBCD 207-209 °C γ-HBCD
Density	2.24 g cm^{-3}
Vapour pressure	6.27 x 10^{-5} Pa at 21°C
Water solubility (20 °C)	7.60 x 10^{-8} mole L^{-1} α-HBCD 2.29 x 10^{-8} mole L^{-1} β-HBCD 0.33 x 10^{-8} mole L^{-1} γ-HBCD 10.31 x 10^{-8} mole L^{-1} ΣHBCDs
n-octanol/water partition coefficient.	Log K_{ow} = 5.62 (technical product) 5.07 ± 0.09 α-HBCD 5.12 ± 0.09 β-HBCD 5.47 ± 0.10 γ-HBCD

HBCD is also used in textiles where it is applied as a back coating to the fabric, encapsulated in a polymer matrix. Typical HBCD levels in the polymer backcoat are 6-15% w/w. About 2 % of the total use of HBCD is in high impact polystyrene (HIPS) where it ranges from 1-7% w/w. HBCD flame-retarded HIPS are used in electric and electronic equipments e.g. audiovisual equipment cabinets and refrigerator lining. Other minor HBCD applications are in polypropylene, adhesives, latex binders, unsaturated polyester and PVC (wires, cables and textile coatings) (KEMI (National Chemicals Inspectorate), 2007). HBCD is used more extensively in Europe than in North America, where it has been substituted for some of the non-foam applications for which PBDEs were formerly used (Birnbaum and Staskal, 2004).

1.2.3 Toxicology and health effects.

The toxicological database for HBCD is limited. The direct acute and chronic toxicity of HBCD appears to be low (Law et al., 2005). However, HBCD has an antagonistic effect on detoxification enzymes that may increase the toxicity of other compounds (Ronisz et al., 2004). It has also been suggested that HBCD may induce cancer by a non-mutagenic mechanism (Helleday et al., 1999, Ronisz et al., 2004) and can disrupt the thyroid hormone system (Yamada-Okabe et al., 2005, Darnerud, 2003, Darnerud, 2008, van der Ven et al., 2006). A recent study has identified HBCD as an endocrine disruptor of concern for human health at relatively low exposure levels (van der Ven et al., 2009). Neonatal exposure to HBCD can induce developmental neurotoxic effects, such as aberrations in spontaneous behaviour, learning, and memory function (Law et al., 2005, Eriksson et al., 2002, Eriksson et al., 2004b). HBCD can also alter the normal uptake of the neurotransmitters in rat brains (Mariussen and Fonnum, 2003, Dingemans et al., 2009) and it has an inhibitory effect on the glutamine and dopamine uptake in rat brain (Lilienthal et al., 2009). This effect is additive to the effects of other BFRs and methyl mercury (Andersen et al., 2006). Further studies are needed on the toxicological effects of HBCD. To date, no information exists on the relative human toxicity of the different HBCD diastereomers and enantiomers. Also, until recently, toxicological testing has been conducted with technical HBCD products rich in γ-HBCD, whereas internal exposure may be dominated by another isomer (Covaci et al., 2006, Ryan et al., 2006, Thomsen et al., 2008).

1.2.4 Environmental levels and behaviour.

HBCD has been detected in almost every environmental medium and is now considered to be a ubiquitous contaminant (Covaci et al., 2006).

1.2.4.1 Air and dust.

The high affinity for organic carbon and low vapor pressure of HBCD causes the majority of the airborne fraction to be sorbed to particulate matter and only a minor fraction is found in the gas phase (Covaci et al., 2006). HBCD has been detected in both urban and rural air across Sweden (5- 610 pg m^{-3}) and at very remote sites in northern Finland (2-280 pg m^{-3}), Sweden (5-170 pg m^{-3}) and USA (0.1-11 pg m^{-3}) suggesting that

it can undergo long-range atmospheric transport (de Wit, 2002, de Wit et al., 2006, Remberger et al., 2004, Hoh and Hites, 2005). This suggestion has been supported by the detection of HBCD, after atmospheric transport from Western Europe and eastern North America, in various animals from remote regions such as Greenland and Svalbard in the Arctic Ocean (de Wit, 2002, de Wit et al., 2006, Law et al., 2006b, Verreault et al., 2005, Vorkamp et al., 2005).

Very little is known about the levels of HBCDs in indoor air. High concentrations of ΣHBCDs (0.2-150 μg m^{-3}) were found in air samples collected near the breathing zone of occupationally exposed workers at an industrial plant producing expandable polystyrene (Thomsen et al., 2007b). Recently, Takigami et al. (Takigami et al., 2009) found low concentrations (< 9 ng m^{-3}) of ΣHBCDs in air from 2 hotel rooms following their report of 6.7 and 280 pg ΣHBCDs m^{-3} in air from two homes (Takigami et al., 2007) in Japan.

HBCDs have also been determined in 19 house dust samples from USA (4.5-130,200 ng g^{-1} dw) (Stapleton et al., 2008), while its concentration in different private houses and offices in the UK was found to be between 940-6,900 ng g^{-1} dw (Santillo et al., 2003). Other studies have estimated the concentrations of HBCD in 18 offices from different EU countries (3-3,700 ng g^{-1} dw) (Leonards et al., 2001) and in 23 offices and private homes from Belgium (20-58,000 ng g^{-1} dw) (Greenpeace report, 2003). Takigami et al. (Takigami et al., 2009) reported HBCD concentrations of 72-1,300 ng g^{-1} dw in 8 dust samples taken from a Japanese hotel. All the previous measurements of HBCDs in indoor environment were performed using different GC/MS techniques which do not allow the separation of different HBCD isomers. Hence all the results are given as ΣHBCDs with no isomer-specific data.

1.2.4.2 Soil, sediment and sewage sludge.

The hydrophobic nature of HBCD allows it to bind strongly to solid particles such as soil, sediment and sewage sludge. Therefore, it was detected at various concentrations in several studies. High HBCD concentrations were determined in surface soil samples collected near an XPS producing plant in Sweden (140-1,300 ng g^{-1} dw) (Remberger et al., 2004) and other HBCD processing plants in Germany and Belgium (111-23,200 ng ΣHBCD g^{-1} dw) (Petersen et al., 2004). However, much lower concentrations (1.7-5.6 ng

ΣHBCD g^{-1} dw) are reported in soil samples collected away from point sources in China (Yu et al., 2008). Low concentrations (< 4 ng ΣHBCDs g^{-1} dw) were reported in sediment samples without known HBCD point sources (Remberger et al., 2004, Christensen et al., 2004, Evenset et al., 2007) which might be due to long-range atmospheric transport. However, much higher levels (up to 2430 ng g^{-1} dw) were found in sediments downstream of HBCD production sites and industrial areas (Guerra et al., 2009, Morris et al., 2004, Verslycke et al., 2005, Marvin et al., 2006, Schlabach et al., 2004).

The widespread detection of HBCD in sewage sludge (Remberger et al., 2004, Law et al., 2006b, Morris et al., 2004, Sellström et al., 1999) has been attributed to diffuse leaching from flame-retarded products into wastewater streams (Morris et al., 2004). Application of HBCD-contaminated sludge to agricultural or other land may redistribute the BFR to the soil–sediment compartment and further into aquatic or terrestrial food chains (Law et al., 2005).

1.2.4.3 Aquatic invertebrates, fish and marine mammals.

HBCD was determined in various aquatic invertebrates (Morris et al., 2004, Verslycke et al., 2005, Tomy et al., 2004, Bethune et al., 2005). Highest concentrations (769 and 727 ng g^{-1} lw) were found in starfish and mysid shrimps from the Rivers Tees (UK) (Morris et al., 2004) and Scheldt (Netherlands) (Verslycke et al., 2005) respectively, close to current or past production facilities for HBCDs. Concentrations of HBCDs in invertebrates were lower than in fish collected from corresponding locations suggesting biomagnification along the food web (Tomy et al., 2004, Covaci et al., 2006).

Several studies have reported on HBCD concentrations in both freshwater and marine fish. Levels of the BFR exhibited a wide variation (0.1-10,275 and 0.1-180 ng g^{-1} lw in freshwater and marine fish respectively) depending on the fish species, collection site and the analysed tissue(s) (Covaci et al., 2006). Concentrations of HBCD in fish downstream of HBCD point sources were higher than in those from upstream reference sites (Janak et al., 2005, Morris et al., 2004, Verslycke et al., 2005, Allchin and Morris, 2003). Strong regional differentiation was observed in HBCD levels in herring from the Baltic Sea where lower concentrations were measured in the northern Baltic compared to the Baltic Proper, suggesting ongoing inputs into the southern part of the Baltic Sea (Asplund et al., 2004). HBCD was determined in several species of marine mammals (Covaci et al.,

2006). Highest concentrations were found in the blubbers of porpoises from the northwest coast of Scotland (1,009-9,591 ng g^{-1} lw) and the Irish Sea (466-8,786 ng g^{-1} lw). Average levels in blubbers of porpoises from other sites were 1,379 ng g^{-1} lw on the south coast of Ireland, 1,143 ng g^{-1} lw in the southern part of North Sea, 959 ng g^{-1} lw in the northern part of North Sea and 121 ng g^{-1} lw near the coast of Spain (Zegers et al., 2005). The average HBCD concentrations in blubbers of common dolphins were 1,223 ng g^{-1} lw on the west coast of Ireland, 433 ng g^{-1} lw on the coast of France and 184 ng g^{-1} lw on the coast of Spain (Zegers et al., 2005). Lower average concentrations were reported in bottlenose dolphins (7.38 ng g^{-1} lw), bull sharks (78 ng g^{-1} lw), Atlantic sharpnose sharks (55 ng g^{-1} lw) from the coast of Florida (USA) (Johnson-Restrepo et al., 2008) and in white-sided dolphins (130 ng g^{-1} lw) from east US coasts (Peck et al., 2008). Similar levels (median = 90 ng g^{-1} lw) were measured in blubbers from striped dolphins caught in the Pacific Ocean near Japan (Marsh et al., 2005). Recently, a significant downturn in levels of HBCDs in the blubber of 138 harbour porpoises (Phocoena phocoena) stranded or bycaught in the UK during 2003-2006 was reported (Law et al., 2008a), where the maximum ΣHBCDs (12,800 ng g^{-1} lw) was almost half that measured by the same group (21,400 ng g^{-1} lw) in 85 porpoises collected from the same area from 1994-2003 (Law et al., 2006c). Possible contributory factors to the observed decrease include the closure in 2003 of a HBCD manufacturing plant in NE England which had considerable emissions, and two voluntary schemes intended to reduce emissions of HBCD to the environment from industry.

1.2.4.4 Birds.

Very few studies have investigated the occurrence of HBCD in birds (Covaci et al., 2006). A wide concentration range (34-300 ng ΣHBCDs g^{-1}) was measured in guillemot eggs from the Baltic Sea collected in the period 1969-2001. The temporal trend showed a peak in the mid 1970s followed by a decrease, then concentrations increased during the late 1980s. No significant change was observed at the last 10 year period of the study (Sellstrom et al., 2003). Higher concentrations were reported in the eggs of white-tailed seaeagle (320-3100 ng g^{-1} lw) collected from Sweden (Janak et al., 2008) and in peregrine falcon eggs (34-2400 ng g^{-1} lw) from Sweden (Lindberg et al., 2004) and the UK (71-1150 ng g^{-1} lw) (Morris et al., 2004). On the other hand, Knudsen et al. reported

much lower concentrations of ΣHBCDs (0.4-27 ng g^{-1} lw) in eggs of four seabird species from Northern Norway and Svalbard (Knudsen et al., 2005) while higher levels (0.2-230 ng g^{-1} lw) were measured in peregrine falcon eggs in south Greenland (Vorkamp et al., 2005).

HBCD was also measured in livers of cormorants (138-1320 ng g^{-1} lw) (Morris et al., 2004) and sparrow hawks (84-19,200 ng g^{-1} lw) (De Boer et al., 2004) from the UK. Much lower levels were reported in guillemot muscle tissue (43-92 ng g^{-1} lw) from Sweden (Lundstedt-Enkel et al., 2005) and glaucous gull plasma (5-83 ng g^{-1} lw) from Svalbard (Verreault et al., 2005).

1.2.4.5 Terrestrial mammals.

HBCD was found (ΣHBCDs = 41 ± 5.6 ng g^{-1} lw) in adipose tissue samples of 20 male and female polar bears from central East Greenland. However, it was not detected in any blood, liver or brain samples from the same group of animals (Gebbink et al., 2008). Kunisue et al. measured HBCDs in liver and adipose tissue samples from raccoon dogs (Nyctereutes procyonoides) collected from different parts of Japan where concentrations ranged from 0.2-10, <0.005-3.7 and <0.005-20 ng g^{-1} lw for α-, β- and γ-HBCDs respectively. Either α- or *γ*-HBCD were dominant in the analysed samples (Kunisue et al., 2008).

1.2.4.6 Human body Burdens.

Several studies have detected HBCDs in various human matrices. HBCD was measured in human milk samples from Sweden (ΣHBCDs = 0.2-2.4 ng g^{-1} lw), Norway (0.2-20 ng g^{-1} lw) (Covaci et al., 2006), North America (0.3-10 ng g^{-1} lw, mainly α-HBCD) (Ryan et al., 2006), Japan (1-4 ng g^{-1} lw, α-HBCD predominant in 13 samples while γ-HBCD predominated in 2 samples. β-HBCD<LOD in all samples.) (Kakimoto et al., 2008), Russia (0.2-1.67 ng g^{-1} lw) (Polder et al., 2008a), Belgium (1.5 ng g^{-1} lw, only α-HBCD detected) (Colles et al., 2008) and USA (0.2-0.9 ng g^{-1} lw) (Schecter et al., 2008a). A study of temporal trends of HBCD concentrations in breast milk of Swedish mothers showed that ΣHBCDs increased four to five times over the period 1980 to 2002 but seems to have stabilized in 2003/04 (Fangstrom et al., 2008). By comparison, the time

trend of ΣHBCDs appeared to be related to that of industrial HBCD consumption level in Japan over the period 1973-2006 (Kakimoto et al., 2008). Recently, a study of HBCDs in 33 breast milk samples from Spain reported higher concentrations than previously observed (up to 188 ng ΣHBCDs g^{-1} lw). The HBCD isomer profiles were dominated by the γ-isomer except for 6 samples, where a dominance of α-HBCD was observed. Interestingly, enantiomer-sepecific analysis revealed an enrichment of the α-(-)-enantiomer, while no clear preference was observed in the case of γ-HBCD (Eljarrat et al., 2009b).

HBCD was also measured in samples of maternal serum (up to 7.4 ng g^{-1} lw) and cord serum (0.2-4.3 ng g^{-1} lw) collected from the Netherlands (Meijer et al., 2008). Median ΣHBCDs levels of 4.1 and 2.6 were reported in male and female serum samples collected from Norway (Thomsen et al., 2008). Serum ΣHBCDs levels of 6-856 ng g^{-1} lw were reported in occupationally exposed workers in an industrial plant producing expandable polystyrene in Sweden while HBCD was not detected (LOD= 1 ng g^{-1} lw) in persons from a reference group with no occupational exposure. The contribution of γ-HBCD to ΣHBCDs in serum was 39% but no clear correlation was established between serum levels and average HBCD concentrations in airborne dust samples collected near the subjects' breathing zone (Thomsen et al., 2007b).

Low concentrations of HBCDs (up to 7.5 ng g^{-1} lw) were also reported in adipose tissue samples from the Czech Republic (Pulkrabova et al., 2009). In adipose tissue samples from New York, ΣHBCDs up to 2.41 ng g^{-1} lw were reported. γ-HBCD was the dominant isomer accounting for 83% of ΣHBCDs, followed by α-HBCD (17%). However, 35% of the human tissue samples contained α-HBCD concentrations higher than γ-HBCD suggesting that some individuals are exposed to elevated concentrations of α-HBCD (Johnson-Restrepo et al., 2008).

1.2.5 HBCD isomer profiles.

The stereoisomer profile of HBCDs in most abiotic samples (including air, soil, sewage sludge and sediment) appears broadly similar to that of the technical HBCD formulation, with γ-HBCD being the predominant isomer (figure 1.2). However, some studies (Morris et al., 2004, Hoh and Hites, 2005, Schlabach et al., 2004, Yu et al., 2008) have reported a

higher contribution of α-HBCD than expected from the technical mixture. The reason behind this difference was stated as thermal isomerisation during the processing of HBCDs and/or stereoisomer-specific processes in the environment (Covaci et al., 2006). On the other hand, α-HBCD was the predominant isomer (figure 1.2) in the majority of biotic samples (including aquatic invertebrates, fish, marine mammals and birds). The average percent contribution of α -HBCD was higher in fish (80%) than in invertebrates (70%) (Covaci et al., 2006). These findings together with the accumulation of α-HBCD in higher amounts in liver than muscles, suggest that α-HBCD is much more resistant to biotransformation by liver microsomal enzymes than β- and γ-HBCD (Janak et al., 2005, Zegers et al., 2005). However, a possible bioisomerisation of γ-HBCD to α-HBCD in liver cannot be excluded (Law et al., 2006a). Similar results were reported by Janak et al. using an in vitro biotransformation assay (Janak et al., 2006) where α-HBCD was the least biotransformed and most bioaccumulative diastereomer. Interestingly, they also reported faster biotransformation of (+)-α-HBCD than (-)-α-HBCD, the latter being the most bioaccumulative enantiomer in the studied samples.

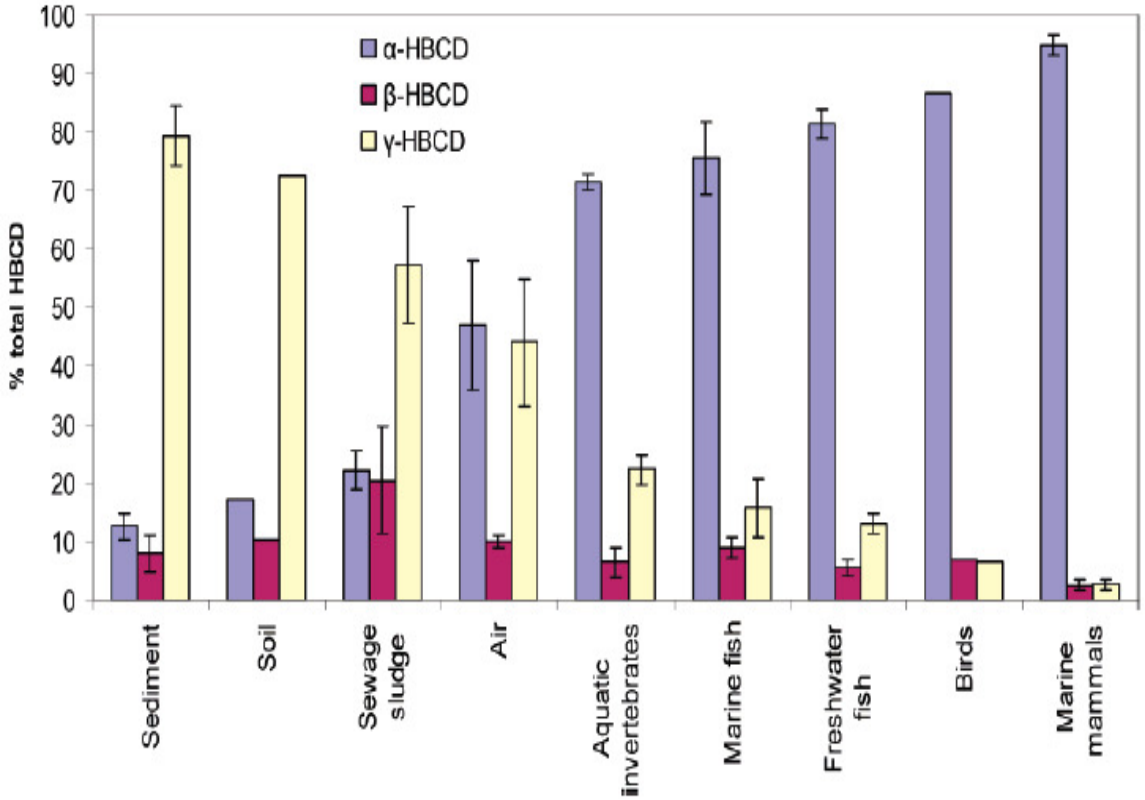


Figure 1.2: Average % contribution of HBCD diastereomers to ΣHBCDs in different biotic and abiotic media. Error bars represent standard deviations. (Covaci et al., 2006).

1.2.6 Regulatory aspects.

HBCD has undergone an EU Risk Assessment for Environment and Human Health under the existing substances regulation (ESR), led by the Swedish government. The Risk Assessment has assigned HBCD as having persistent, bioaccumulative and toxic (PBT) properties. This conclusion was based on an apparent increase in environmental concentration observed in recent years despite not meeting the criterion for persistence (KEMI (National Chemicals Inspectorate), 2007). Based on its PBT properties, the European Chemicals Agency decided to include HBCD in the candidate list for substances of very high concern (SVHC) list, within the REACH framework (ECHA, 2008). This was followed by inclusion of HBCD on the list of substances added to a proposal to revise the restriction of hazardous substances (RoHS) directive (RoHs directive, 2008). HBCD is also under consideration for inclusion as a persistent organic pollutant (POP) under the framework of the Stockholm Convention on Persistent Organic Pollutants (Stockholm convention on POPs, 2008). HBCD has also been identified by the U.K. Chemical Stakeholders Forum as persistent, bioaccumulative, and toxic chemicals and is included on the OSPAR list of chemicals for priority action (KEMI (National Chemicals Inspectorate), 2007).

1.3 Tetrabromobisphenol-A (TBBP-A).

1.3.1 Physicochemical properties.

TBBP-A is a white crystalline powder at 20 ºC. A summary of the physicochemical properties of TBBP-A is given in table 1.2.

Table 1.2: Physicochemical properties of TBBP-A (EU Risk Assessment Report, 2006).

Property	**Value**
Chemical formula	$C_{15}H_{12}Br_4O_2$
Molecular weight	543.9 g $mole^{-1}$
Boiling point	~316 ºC (decomposes at 200-300 ºC)
Melting point	181-182 ºC
Density	2.12 g cm^{-3}
Vapour pressure	6.24 x 10^{-6} Pa at 25 °C

Table 1.2 *(continued)*: Physicochemical properties of TBBP-A (EU Risk Assessment Report, 2006).

Property	**Value**
Water solubility	pH 5 - 2.7 x 10^{-7} mole L^{-1} at 25°C pH 7 - 23.2 x 10^{-7} mole L^{-1} at 25°C pH 9 - 43.0 x 10^{-7} mole L^{-1} at 25°C pure water 4.4 x 10^{-7} mole L^{-1} at 25°C
Log K_{ow}	5.90
Acid dissociation constants (pKa)	pKa_1= 7.5 pKa_2 = 8.5

1.3.2 Applications and uses.

BSEF (BSEF, 2009a) reported that 58 % of TBBP-A is used as a reactive FR in epoxy, polycarbonate and phenolic resins in printed circuit boards, 18 % is used for the production of TBBP-A derivatives and oligomers, while 18 % is used as additive FR in the manufacture of acrylonitrile-butadiene-styrene (ABS) resins or high impact polystyrene (HIPS). However, BFR industry spokespersons claim that, since it was not effective, TBBP-A was never used as an additive FR in HIPS (EU Risk Assessment Report, 2006), while the European Flame Retardants Association (Flame Retardant Fact Sheet - (TBBP-A), 2007) indicates that TBBP-A is "possibly" used in HIPS. TBBP-A is used primarily as an intermediate in the manufacture of epoxy and polycarbonate resins, where it becomes bound covalently in the polymer and is thus an integral part of the product. The only potential for exposure that remains originates from un-reacted TBBP-A, if an excess has been added during the production process. TBBP-A is also used as a reactive FR in polycarbonate and unsaturated polyester resins. Polycarbonates are used in communication and electronics equipment, electronic appliances, transportation devices, sports and recreation equipment, lighting fixtures and signs. Unsaturated polyesters are used for making simulated marble floor tiles, bowling balls, furniture parts, coupling compounds for sewer pipes, automotive patching compounds, buttons, and for encapsulating electrical devices. The resin used in the printed circuit boards cannot be recycled, although the copper content can be recovered in copper smelters (EU Risk Assessment Report, 2006).

Commercial FR epoxy resins contain up to approximately 20 % bromine. The main use of these resins is in the manufacturing of rigid epoxy laminated printed circuit boards. There are two main types of rigid or reinforced laminated printed circuit boards that are commonly used (Brominated flame retardants, 1999). These are usually either based on glass fibre reinforced epoxy resin (designated FR4) or cellulose paper reinforced phenolic resin (designated FR2), but a range of types are available. The FR4-type laminate is by far the most commonly used laminate and is typically made by reaction of around 15-17 % TBBP-A in the epoxy resin (Brominated flame retardants, 1999).

As an additive FR, TBBP-A is generally used with antimony oxide for optimum performance (H. Hakk, 2001). Antimony oxide is not used generally in conjunction with TBBP-A in reactive FR applications (EU Risk Assessment Report, 2006). TBBP-A is considered as an alternative additive FR to the octabromodiphenyl ether (Octa-BDE) mixture in ABS. The use of Octa-BDE in this application is no longer allowed in the EU (Directive 2002/95/EC, 2003). It is therefore possible that the amount of TBBP-A used in this application in particular could increase in the future. As an additive FR, it does not react chemically with the other components of the polymer, and, therefore may leach out of the polymer matrix after incorporation, with consequent implications for human exposure. Concentrations commonly found in these applications are between 10 and 20 %, depending on the polymer. ABS resins are used in automotive parts, pipes and fittings, refrigerators, business machines, and telephones. HIPS resins are used in packaging, consumer products, disposables, electrical and electronic equipment, furniture, building and construction materials (Environmental Health Criteria, 1995). The largest additive use of TBBP-A is found in television casings with approximately 450 tonnes of TBBP-A used per year. Other uses include: PC monitor casings, components in printers, fax machines and photocopiers, vacuum cleaners, coffee machines and plugs/sockets (EU Risk Assessment Report, 2006).

1.3.3 Toxicology and health effects.

Oral administration studies with rats and mice indicate that TBBP-A has a low acute toxicity, with $LD_{50} > 5$ g kg^{-1} and > 4 g kg^{-1} for rats and mice, respectively (Environmental Health Criteria, 1995). Due to its structural resemblance to the thyroid hormone thyroxin (T_4) and the known xenoestrogen Bisphenol A, the major concern

regarding TBBP-A is its potential as endocrine disruptor. The thyroid hormonal activity of TBBP-A was examined by Kitamura et al. using rat pituitary GH3 cell lines, in which release of growth hormone is thyroid hormone-dependent. In those experiments, TBBP-A stimulated the production of growth hormone and enhanced the proliferation of GH3 cells. TBBP-A similarly also enhanced proliferation of the rat pituitary MtT/E-2 cell lines, whose growth is oestrogen-dependent. These results suggest that TBBP-A acts both as thyroid hormone and oestrogen agonist (Kitamura et al., 2002). These findings are similar to those of Ghisari et al. who observed a growth of GH3 cells which could not be counteracted by the inhibiting growth effect of the anti-oestrogen ICI. These data also indicate that the effect of TBBP-A is thyroid hormone-like and estrogen receptor-mediated (Ghisari and Bonefeld-Jorgensen, 2005). In an *in vitro* study, TBBP-A proved to be a rather potent inhibitor of the sulphation of estradiol by oestrogen sulphotransferase, an important inactivation pathway of estradiol. Inhibition of this enzyme may lead to increased bioavailability of estradiol *in vivo* (Kester et al., 2002). The resulting weak oestrogen-like properties are confirmed by several other studies (Korner et al., 1998, Meerts et al., 2001, Samuelsen et al., 2001, Olsen et al., 2003).

While TBBP-A produced a thyromimetic effect on the GH3 pituitary cell line, an anti-thyroidal effect was observed on Chinese hamster ovary cells transiently transfected with T_3 receptors, as well as an inhibition of the binding of triiodothyronine (T_3) to thyroid hormone receptors (Kitamura et al., 2005). Moreover, TBBP-A was shown to be a potent *in vitro* inhibitor for the binding of T_4 to transthyretin, the thyroid hormone-binding transport protein in plasma. The binding of TBBP-A is 10 times stronger than that of the natural ligand T_4 (Meerts et al., 2000, Hamers et al., 2006).

TBBP-A is also immunotoxic, which was demonstrated by *in vitro* inhibition of the expression of CD25, a receptor essential for the proliferation of activated T-cells (Mariussen and Fonnum, 2003). Furthermore, TBBP-A neurotoxicity was determined by inhibition *in vitro* of neurotransmitter uptake into synaptosomes and dopamine uptake into synaptic vesicles (Pullen et al., 2003) and generation of free radicals (Reistad et al., 2005). Additionally, TBBP-A was reported to interfere with cellular signalling pathways (Strack et al., 2007).

1.3.4 Environmental levels and behaviour.

1.3.4.1 Air and dust.

Concentrations of TBBP-A in air from domestic and office indoor environments exceed those in outdoor air, which arises presumably from the use of TBBP-A in printed circuit boards and HIPS enclosures for electronic goods. Elevated atmospheric concentrations (several orders of magnitude above those found in outdoor air) are reported for specific occupational environments such as electronics dismantling plants.

In Sweden, TBBP-A was detected in air at a plant for recycling of electronics (dismantling of computers, TV sets etc.) and offices equipped with computers (Bergman et al., 1999). The mean concentration in 12 samples from the recycling plant was 29.7 ng m^{-3}. In 4 offices equipped with computers, the mean concentration was 0.035 ng m^{-3}. TBBP-A could not be detected in 2 outdoor samples. Sjödin et al. (Sjodin et al., 2001) further investigated the presence of TBBP-A in both indoor and outdoor air in Sweden. They reported mean concentrations of 0.036 ng m^{-3} in six office microenvironments containing computers, 0.093 ng m^{-3} from two teaching halls and 0.035 ng m^{-3} from two computer repair facilities. Concentrations were below detection limits (unreported) in outdoor air indicating indoor sources of TBBP-A. They also reported TBBP-A concentrations in air within an electronic products recycling plant to be several orders of magnitude higher than those found in the other indoor microenvironments investigated (mean of 30 ng m^{-3} in the dismantling hall and 140 ng m^{-3} in the shredder). The study found that TBBP-A was present primarily in the particle phase rather than in the vapour phase (Sjodin et al., 2001). This may suggest that passive air sampling devices that sample primarily the vapour phase only (e.g. PUF disk samplers) may not be appropriate for monitoring TBBP-A. The importance of electronic goods as an emission source is underlined by Tollbäck et al. (Tollback et al., 2006), who reported that the TBBP-A concentration in air from a dismantling hall within a Swedish electronics recycling plant was 13.8 ng m^{-3}. Inoue et al. (Inoue et al., 2006b) reported a mean concentration of 0.2 ng m^{-3} in indoor air from 26 microenvironments in Kanagawa and Tokyo, Japan, where TBBP-A was found above the limit of detection (0.1 ng m^{-3}) in 14 out of the 26 analysed samples. Takigami et al. (Takigami et al., 2007) studied TBBP-A in air sampled simultaneously inside and outside 2 houses in Hokkaido, Japan. Concentrations in the

matched outdoor and indoor samples were 7.1 and 9 pg m^{-3}, respectively for the first house, and 9.5 and 16 pg m^{-3} for the second. Xie et al. (Xie et al., 2007) investigated the presence of TBBP-A in outdoor air from a rural site in northern Germany, over the Wadden Sea and off the Northeast Atlantic. Comparable concentrations of TBBP-A were found in the northern German site (ranging from < 0.04 to 0.85 pg m^{-3}) and over the Wadden Sea (ranging from 0.31 to 0.69 pg m^{-3}). Concentrations of TBBP-A in the Arctic ranged from < 0.04 to 0.17 pg m^{-3}. The latter higher Arctic concentration was present in a sample collected off the West Norwegian coast, indicating an input source from land to ocean. Interestingly, Alaee et al. (Alaee et al., 2003b) reported TBBP-A to be present at 70 pg m^{-3} in the airborne particulates collected in 2000 in the Arctic (Dunai, Russia).

Information on the presence of TBBP-A in indoor dust is far less extensive than that available on other additive BFRs, such as PBDEs and HBCDs. What little data do exist, suggests that concentrations are at the low end of those found for PBDEs and HBCDs. This is consistent with the fact that TBBP-A is used primarily as a reactive FR and as such its release from treated goods is likely to be less facile than for compounds whose use pattern is largely or exclusively as additive FRs. Takigami et al. (Takigami et al., 2007) reported concentrations of 490 and 520 ng g^{-1} in two samples of domestic dust from Hokkaido, Japan. Similar concentrations were detected by Santillo et al. (Santillo et al., 2003), who reported TBBP-A to be present above the detection limit in 4 out of 10 pooled samples of UK domestic dust. Concentrations in these four samples ranged from 190 to 340 ng g^{-1}, exceeding substantially concentrations reported in an earlier study of dust in offices from the European Parliament building, where concentrations in those 9 out of 16 samples where TBBP-A was detectable fell between 5 and 47 ng g^{-1}. More recently, Chernyak et al. (Chernyak SM et al., 2007) monitored the change in TBBP-A concentrations in indoor dust from a newly constructed building in Michigan, USA, over the period following the building's construction, furnishing, and occupation. A continuous increase in TBBP-A concentration in dust from 0.4 to 2.0 ng g^{-1} was observed over the study period of 1 year, suggesting that the dust samples had yet to reach saturation with TBBP-A over this period. The increase in TBBP-A concentration in dust was less dramatic than for other BFRs, such as BDE 209. Yu and Hu reported concentrations of TBBP-A ranging between 18.9 and 39.6 μg g^{-1} in dust samples from

computers in Chinese offices (n = 4). However, these results should be interpreted with care since the analysis was performed using HPLC-DAD (Yu and Hu, 2007).

1.3.4.2 Soil, sediment and sewage sludge.

To date, there appears to be very few reports on concentrations of TBBP-A in soil. Jin et al. (Jin et al., 2006) determined TBBP-A at 0.12 ng g^{-1} in a soil sample taken outside a TBBP-A producing plant in China. Given its reported propensity for partitioning to the atmospheric particulate phase (Sjodin et al., 2001) and the magnitude of its octanol-water partition coefficient (log K_{ow} = 5.90), one would anticipate that soil would constitute a major sink. The extent to which this is true will be influenced strongly by the extent to which TBBP-A undergoes edaphic degradation, coupled with the rate of release from treated goods and manufacturing facilities and subsequent atmospheric transport and deposition, but this dearth of information on soil contamination requires rectification. The concentration of TBBP-A in Chinese soil measured by SPE followed by HPLC-ITD-MS was 25.2 ± 2.7 ng g^{-1} (n = 4) (Peng et al., 2007). In another Chinese study, Yu and Hu (Yu and Hu, 2007) have reported TBBP-A concentrations ranging between 1.4 and 1.8 µg g^{-1} in soil collected near a garbage discharge site.

As with soil, the physicochemical properties of TBBP-A suggest that sewage sludge and sediment are theoretically important sinks. The available data support this idea, and reflect also the comparatively facile release to such matrices from industrial plants that either manufacture or use TBBP-A. To illustrate, in 1983, Watanabe et al. (Watanabe et al., 1983) reported (for the first time) TBBP-A to be present at 20 ng g^{-1} dw in a sample of sediment from the Neya River in Japan. The concentrations of TBBP-A and its dimethylated derivative in sediment were higher downstream (270 ng g^{-1} and 1500 ng g^{-1}) than upstream (34 and 24 ng g^{-1}) of a plastic factory using TBBP-A in Sweden (Sellstrom and Jansson, 1995). TBBP-A was also found in sewage sludge of the waste water treatment plant of the factory. Morris et al. (Morris et al., 2004) determined TBBP-A in river and estuarine sediment samples from Belgium, the Netherlands and the UK. The highest concentration of TBBP-A (9.8 µg g^{-1} dw) was found in freshwater sediments from the River Skerne (UK) close to a BFR manufacturing site. The mean concentration in the River Tees, further downstream, was 25 ng g^{-1} dw. The same study also provided an indication of the likely range of concentrations to be found in locations not influenced

directly by industrial emissions; reporting concentrations of TBBP-A in sediments from the Scheldt basin (0.1-67 ng g^{-1} dw), from the western Scheldt (0.1–3.2 ng g^{-1} dw), from other Dutch rivers (0.1-6.9 ng g^{-1} dw) and from other UK rivers (2-5 ng g^{-1} dw). Even lower concentrations of TBBP-A were found in sediments from the Norwegian lakes Mjøsa and Losna (0.04-0.13 ng g^{-1} dw) .

TBBP-A was also quantified in influents, effluents and sewage sludge from the Netherlands (mean values of < 6.9, 42 and 79 ng g^{-1} dw respectively) and the UK (mean values of 7.5, < 3.9 and 57 ng g^{-1} dw respectively). A maximum concentration of 192 ng g^{-1} dw was quantified in a secondary treated and dewatered sludge sample from Cork, Ireland (Morris et al., 2004). These concentrations are consistent with those reported in a survey of 57 Swedish sewage sludge samples, where concentrations ranged from < 0.3 to 220 ng g^{-1} dw (Oberg et al., 2002). De Wit et al. (de Wit et al., 2007) investigated sludge samples from 50 Swedish sewage treatment plants (STPs) for TBBP-A. TBBP-A concentrations were below LOQ (not reported) in 12 samples, while the mean concentration was 32 ng g^{-1} dw. Higher concentrations were found in a few sludge samples from STPs with known or suspected point sources (textile industries, extruded polystyrene production) but in some cases the point source was unknown. However, TBBP-A concentrations were significantly lower in digester sludge samples than in the raw sludge samples. This indicates that anaerobic biodegradation of TBBP-A does occur in the digester process.

The first comprehensive study on BFRs in source-separated compost and digestate from Switzerland showed mean TBBP-A levels of 510 ng g^{-1} dw (Brandli et al., 2007). The concentrations observed were at or above the levels found in background soils, which are the main recipient of compost and digestate. Where actually applied, compost can contribute considerably to the total input of organic pollutants to the soil. Chu et al. (2005) reported concentrations of TBBP-A ranging from 2.1-28.3 ng g^{-1} dw in sludge samples collected from wastewater treatment and pollution control plants in Ontario, Canada (Chu et al., 2005). In the same study, TBBP-A was detected above the detection limit of 0.05 ng g^{-1} dw in only 3 of 55 surface sediment samples from Lake Erie and in only one sample could TBBP-A be determined quantitatively at a concentration of 0.51 ng g^{-1} dw. Importantly, the authors found that TBBP-A can undergo debromination,

although it is not clear whether such degradation is the consequence of debromination of TBBP-A during the wastewater/sludge treatment process or other abiotic (e.g. photocatalyzed) debromination processes (Chu et al., 2005). This finding is in agreement with the debromination of TBBP-A in estuarine sediments reported by Voordeckers et al. (Voordeckers et al., 2002). TBBP-A was also detected at a concentration level of 300 ng/g dw in sewage sludge produced from the wastewaters of the Montreal area (Quade et al., 2003). TBBP-A has been reported at modest concentrations in Canadian sludges. Lee and Peart (Lee and Peart, 2002) have reported a median concentration of 12.4 ng g^{-1} (range < 1-46.2 ng g^{-1} dw) in sewage sludge from 34 Canadian sewage treatment plants. Quade et al. (Quade et al., 2003) have reported very low concentrations of TBBP-A in sediment from the Detroit river (range 0.60-1.84 ng g^{-1} dw). Sewage sludge collected from Southern Ontario had the same range of concentrations (14.3-43.8 ng g^{-1} dw) (Lee and Peart, 2002).

In Asia, low concentrations of TBBP-A (< 0.2-1.6 ng g^{-1}) were determined in sediments sampled before treatment in water treatment plants in Japan. It was evident that TBBP-A had leached from all the landfill sites including the oldest site that had been closed 25 years ago, and in which plastic wastes were buried. This means that TBBP-A had not fully degraded during that time and suggests that it may be useful as a marker to locate illegally dumped plastic wastes (Suzuki and Hasegawa, 2006).

1.3.4.3 Biotic samples.

Despite the extensive production and use of TBBP-A (BSEF, 2009a), data for biotic matrices are scarce (table 1.3). Law et al. (2006c) determined TBBP-A in the blubber of 68 porpoises (*Phocoena phocoena*) stranded in UK waters between 1994 and 2003. TBBP-A was detected in only 18 samples, with concentrations between 6 and 35 ng g^{-1} ww (Law et al., 2006c). Morris et al. (Morris et al., 2004) determined TBBP-A in a variety of aquatic biota from the North Sea, including five cormorant (*Phalacrocorax carbo*) liver samples from England. Levels of the flame retardant ranged from 2.5-14 ng g^{-1} lw (Morris et al., 2004). TBBP-A was also detected in mysid shrimps (*Neomysis integer*) from two sites in the Scheldt estuary at concentrations of 0.8 and 0.9 ng g^{-1} lw (Verslycke et al., 2005).

Table 1.3: Mean and range of TBBP-A concentrations (ng g^{-1} lw) in biological matrices.

Species	Tissue	Location	Concentrations	Reference
Invertebrates				
Common whelk	Whole	North Sea	5.0 – 96	(Morris et al., 2004)
Sea star	Whole	Scheldt estuary	< 1 – 2	
Sea star	Whole	Tees estuary	205	
Hermit crab	Whole	North Sea	< 1 - 35	
Mysid shrimp	Whole	Scheldt estuary	0.8 – 0.9	(Verslycke et al., 2005)
Fish				
Whiting	Muscle	North Sea	< 97 to 245 (mean 136)	
Cod	Liver	North Sea	< 0.3 - 1.8	
Hake	Liver	Atlantic	< 0.2	(Morris et al., 2004)
Eel	Muscle	Scheldt estuary	< 0.1 - 13 (mean 1.6)	
Eel	Muscle	Dutch rivers	< 0.1 - 1.3 (mean 0.3)	
Yellow eel	Muscle	Scheldt basin	< 0.1 - 2.1	
Yellow eel	Muscle	Dutch rivers	< 0.1 - 1.0	
Perch, pike, smelt, vendace, trout	Muscle	Norway	1.0 - 13.7	(Schlabach et al., 2004)
Bull shark	Muscle	Florida, USA	9.5 ± 12.0	(Johnson-Restrepo et al., 2008)
Atlantic sharpnose shark	Muscle	Florida, USA	0.87 ± 0.50	
Birds				
Cormorant	Liver	Wales and England	2.5 - 14	(Morris et al., 2004)
Common tern	Eggs	Western Scheldt	< 2.9	
Peregrine falcon, White-tailed sea eagle, Osprey, Golden eagle,	Egg	Norway	< 0.003 – 0.013 (ng g^{-1} ww)	(Herzke et al., 2005)
Marine mammals				
Harbour seal	Blubber	Wadden Sea	< 14	(Morris et al., 2004)
Harbour porpoise	Blubber	North Sea	< 11	
Harbour porpoise	Blubber	North Sea	0.1 - 418	
Harbour porpoise	Blubber	Tyne/Tees	0.31	
Harbour porpoise	Blubber	UK	6 - 35 (ng g^{-1} ww)	(Law et al., 2006c)
Bottlenose dolphin	Blubber	Florida, USA	1.2 ± 3.0	(Johnson-Restrepo et al., 2008)

Recently, Johnson-Restrepo et al. have measured the concentrations of TBBP-A in three marine top-predators from coastal waters of Florida, USA. The overall mean concentrations (mean ± SD) of TBBP-A were 1.2 ± 3.0 ng g^{-1} lw, 9.5 ± 12.0 ng g^{-1} lw, and 0.87 ± 0.50 ng g^{-1} lw in bottlenose dolphin blubber (n = 15), bull shark muscle (n = 13) and Atlantic sharpnose shark muscle (n = 3), respectively. The highest concentration of TBBP-A (35.6 ng g^{-1} lw) was measured in bull shark muscle (Johnson-Restrepo et al., 2008).

Herzke et al. determined TBBP-A in two eggs from each of 4 different Norwegian birds of prey (osprey, golden eagle, white-tailed sea eagle and peregrine falcon), which have different feeding habits and habitats, sampled between 1992 and 2002. TBBP-A was detected in all 8 samples in a concentration range of < 3 - 13 pg g^{-1} ww, which indicates that TBBP-A is distributed widely in a broad range of feed items of birds of prey in Norway (Herzke et al., 2005).

1.3.4.4 Human body Burdens.

Detection of TBBP-A in humans can be hampered by the short biological half-life of the compound, estimated at 2 days (Hagmar et al., 2000a). This is in agreement with the phenolic structure of TBBP-A that can be rapidly conjugated and subsequently excreted (Hakk et al., 2000). Still, TBBP-A may accumulate in humans, but a continuous exposure to this BFR is required to maintain a detectable level in humans.

In general, reports of TBBP-A in human samples are scarce. The first report of TBBP-A being present in human tissues dates back to 1979. In Arkansas, USA, TBBP-A was found in human hair in the vicinity of TBBP-A manufacturing sites (Sellstrom and Jansson, 1995). However, the quality of analytical methodology available at that time remains questionable. More recent reports of TBBP-A in human samples are focused mainly on occupationally exposed adults (Jakobsson et al., 2002, Thomsen et al., 2001, Sjodin et al., 2003b), because of the high probability of exposure in occupational environments. Hagmar et al. reported TBBP-A concentrations of 1.1-4 ng g^{-1} lw in the serum of the workers engaged in the recycling process in Sweden (Hagmar et al., 2000a). TBBP-A was also detected in 80% of serum samples collected from computer technicians (<0.05-1.84 ng g^{-1} lw) in Norway, while the compound could not be measured in the serum of a control group comprising office clerks and hospital cleaners (Jakobsson et al.,

2002). Thomsen et al. analysed serum from Norwegians working at: an electronics dismantling facility, in the production of printed circuit boards and as laboratory personnel; the latter being a control group. Average TBBP-A levels were significantly higher ($p < 0.05$) in the dismantlers (1.3 ng g^{-1} lw) than in both other groups (0.54 and 0.34 ng g^{-1} lw, respectively). Time trends of TBBP-A levels in serum of non-occupationally exposed individuals from Norway were studied in the period 1977-1999. TBBP-A was not detected in pooled serum samples in the period 1977-1981, but a slight increase in serum concentration was observed from 1986 to 1999 (0.44 to 0.65 ng g^{-1} lw). Several age groups from 0 to >60 years were studied with the highest serum concentration of TBBP-A found in the serum pool from the group aged 0-4 years (Thomsen et al., 2002).In Japan, TBBP-A was detected in 14 out of 24 blood samples from adult with a mean concentration of 1.35 ng g^{-1} lw (J. Nagayama et al., 2000).

Johnson-Restrepo et al. measured TBBP-A levels in 20 adipose tissue samples from New York, USA. Average TBBP-A concentration was 0.048 (± 0.102) ng g^{-1} lw, with a maximum of 0.46 ng g^{-1} lw. TBBP-A correlated well with HBCDs levels in the same samples which might indicate common sources of the 2 BFRs (Johnson-Restrepo et al., 2008).

1.3.5 Regulatory aspects.

Currently, there are no restrictions on the production of TBBP-A or its derivatives. In 2003, a Directive on the handling of Waste Electrical and Electronic Equipment (WEEE) (Directive 2002/96/EC, 2003) was adopted and it contains a requirement for selective treatment of plastics containing BFRs, including TBBP-A.

In Europe, TPPB-A is on the 4th list of priority chemicals (Regulation 2364/2000/EC, 2000) foreseen under European Council Regulation (EEC) No 793/93 of 23 March 1993 regarding the evaluation and control of the risks of existing substances. In the context of the REACH legislation, TBBP-A will be one of the first substances to go through the registration procedure due to its high production volume (BSEF, 2009a). The EU environmental risk assessment for TBBP-A confirmed a risk in some scenarios for surface water, sediment and soil when TBBP-A is used as an additive to ABS plastics (EU Risk Assessment Report, 2006). BSEF indicates that risks from additive application are manageable through a Voluntary Emissions Control Action Programme (VECAP), to

which 89 % of TBBP-A additive customers in Europe have signed up and these users have already begun reducing their emissions (BSEF, 2009a).

1.4 Decabromodiphenyl ether (Deca BDE, BDE 209).

1.4.1 Physicochemical properties.

BDE 209 is a white to off-white crystalline powder at room temperature. A summary of the physicochemical properties of BDE 209 is given in table 1.4.

Table 1.4: Physicochemical properties of BDE 209 (EU Risk Assessment Report, 2002).

Property	**Value**
Chemical formula	$C_{12}Br_{10}O$
Molecular weight	959.2 g $mole^{-1}$
Boiling point	decomposes at >320 ºC
Melting point	300-310 ºC
Relative density	3.0 at 20 ºC
Vapour pressure	4.63 x 10^{-6} Pa at 21 °C
Water solubility (25 °C)	<1 x 10^{-10} mole L^{-1}
n-octanol/water partition coefficient.	Log K_{ow} = 6.27

1.4.2 Applications and uses.

Deca BDE is mainly used in plastics and textile applications. It is applied as an additive flame retardant which allows it to diffuse out of the treated material over time depending on conditions and use. The European Brominated Flame Retardant Industry Panel (EBFRIP) reported that for 2002, the total EU consumption of deca BDE was around 8,300 MT, with 5,800 MT (70%) used in plastic/polymer applications (mainly for electrical and electronic equipment) and 2,500 MT (30%) used in textile applications. Chemtura, a major bromine producer estimated DecaBDE sales into Europe in 2005 at ~7,800 MT (Pakalin et al., 2007). Deca BDE is used in a variety of polymer applications in different amounts depending on a number of factors such as the degree of flame retardancy required in the end product, the effectiveness of the flame retardant and synergist within a given polymer, the physical properties (e.g. colour, density, stability

etc.) and the use of the end product. Industry information indicates that deca BDE is used at loadings of 10-15% w/w in polymers and is always used in conjunction with antimony trioxide (EU Risk Assessment Report, 2002). The major application for deca BDE flame retarded polymers is in HIPS which is used in electrical and electronic equipment (EEE) e.g. TV back casings, printers, scanners, fax machines, and similar applications. It is also used in a large number of other polymers with end-uses in EEE such as: polypropylene (for electronics), acetate copolymers (EVA (ethylene-vinyl acetate) and other ethylene copolymers for wire and cable), EPDM (ethylene-propylene-diene terpolymer) and thermoplastic elastomers (for wire and cable) and polyester resins (for electronics). Other minor uses include styrenic rubbers, polycarbonates, polyamides and terphthalates, and small amounts are also reported to be used in hot melt adhesives (Pakalin et al., 2007).
Deca BDE is widely used in polypropylene drapery and upholstery fabric where the FR is back coated onto the textiles in a latex binder. It may also be used in some synthetic carpets, where the FR is encapsulated within the polymer fibers. Decabromodiphenyl ether makes up around 30-40% of the dry coating weight of the textile backcoat (EU Risk Assessment Report, 2002).

1.4.3 Toxicology and health effects.

Very little data is available on the adverse effects of BDE 209 on human health. In general, the lower brominated PBDEs are more toxic and bioaccumulative than the higher brominated congeners (Darnerud, 2003). The acute toxicity of Deca BDE products is low with oral and dermal LD-50 values varying between 2 and 5 g kg^{-1} bw (Norris et al., 1975). However, the results of a 2-year feeding study conducted by the US national toxicology program (NTP) revealed a dose-related increase in hepatic and pancreatic adenomas in rats while in mice, the combined incidence of hepatocellular adenomas and carcinomas was increased, although not dose-related. Also, the combined incidence of thyroid gland follicular-cell adenomas and carcinomas was slightly increased (NTP, 1986). BDE 209 was reported to accumulate in livers of rats (Morck et al., 2003), muscles and livers of rainbow trout (Stapleton et al., 2006) and in adipose tissues of grey seals (Thomas et al., 2005). The serum $t_{0.5}$ of BDE-209 ranged from 2 to 15 days in rats (Sandholm et al., 2003), grey seals (Thomas et al., 2005) and occupationally exposed

workers (Thuresson et al., 2006). BDE 209 was reported to be an endocrine disrupter in male mice following exposure during development where decreased levels of serum T3 and cell swelling of hepatocytes in male offspring were observed (Tseng et al., 2008). Teshima et al. reported decreased serum levels of both T3 and T4 in maternal Sprague-Dawley rats upon exposure to deca BDE during the period from late gestation to after lactation (Teshima et al., 2008). The neurodevelopmental effects of BDE 209 have been reviewed recently (Goodman, 2009). These effects suggested that deca BDE is a developmental neurotoxicant that can produce long-term behavioral changes following a discrete period of neonatal exposure in mice (Rice et al., 2007). Viberg et al. reported that neonatal exposure to PBDE 209 can induce persistent aberrations in spontaneous behaviour in mice that get worse with age. They also concluded that both lower and higher brominated diphenyl ethers can cause similar developmental neurotoxic effects in both mice and rats (Viberg et al., 2007). These findings were supported by Johansson et al. who reported neonatal exposure to BDE 209 to cause dose-response changes in spontaneous behaviour (hyperactivity and reduced or lack of habituation) and cholinergic susceptibility (reduced and/or hypoactive response to nicotine) in adult mice that worsen with age. They concluded that BDE 209 can be as potent as the lower brominated PBDEs in causing developmental neurotoxic defects (Johansson et al., 2008).

1.4.4 Environmental levels and behaviour.

1.4.4.1 Air and dust.

Due to its high molecular weight and very low vapour pressure, BDE 209 is expected to partition more to the particulate phase in air or sorb strongly to dust particles. The levels of BDE 209 in outdoor air in Europe, North America and Asia were reviewed previously (Law et al., 2006b, Law et al., 2008b, de Wit et al., 2006). The results of modelling suggest that deca BDE has much less potential for long range transport to reach remote areas than lower brominated BDEs (Wania and Dugani, 2003). However, BDE 209 was the predominant congener in air samples (median ΣPBDEs = 8.6 pg m^{-3}) collected from an island located in the central basin of the Baltic Sea (ter Schure et al., 2004). In outdoor air samples from 5 sites (urban, semiurban, agricultural, and remote) from Lake Michigan through the US, BDE 209 comprised 6-31% of ΣPBDEs (average = 100 pg m^{-3}) and was

mainly associated with the particle phase (Hoh and Hites, 2005). Another indication of the potential of BDE 209 for long range transport is its high contribution to ΣPBDEs (50% on average) in air samples collected at various locations around Lake Mjøsa in Norway where long range atmospheric transport from the UK was suggested as one of the possible reasons (Breivik et al., 2006). BDE 209 (11-54 pg m^{-3}) was the dominant PBDE congener in outdoor air samples collected from 4 sites in Izmir, Turkey. The authors also studied the phase partitioning of higher BDEs in air and concluded that when higher PBDEs (hexa to deca congeners) are emitted from their sources in the gas-phase, they may remain in that phase for several months before reaching equilibrium with atmospheric particles which indicate that "in addition to particle-bound transport, the gas-phase transport of highly brominated congeners (i.e. BDE-209) may also be important" (Cetin and Odabasi, 2008). In Asia, BDE-47, -99, -100, and -209 were the dominant congeners in particulate phase samples collected from the Bohai Sea to the high Arctic (ΣPBDEs = 2-199 pg m^{-3}), suggesting that the widely used commercial penta- and deca-BDE products were the original sources.

Few studies have reported BDE 209 concentrations in air from indoor microenvironments. BDE 209 was found in 1 out of 5 samples from houses in Sweden at a concentration of 260 pg m^{-3} comprising 62% of ΣPBDEs (Karlsson et al., 2007). Recently, a Greek study (Mandalakis et al., 2008) showed that BDE 209 was the main congener in air samples from automobile cabins (median ΣPBDEs = 201 pg m^{-3}; BDE 209 = 104 pg m^{-3}). Deca BDE was detected in 30% and 45% of indoor air samples collected from 20 bedrooms and living rooms from Boston, USA with average concentrations of 94.8 and 94.2 pg m^{-3} respectively (Allen et al., 2007). BDE 209 was determined in air samples near suspected sources in USA where concentrations up to 650,000 pg m^{-3} were recorded at an electronics recycling facility (Cahill et al., 2007). Similarly high concentrations were reported in particles collected from air in 2 electronic recycling facilities (Sjodin et al., 2001, Julander et al., 2005) in Sweden (average = 33,350 and 64,000 pg m^{-3}).

Several authors have reported levels of PBDEs in dust from different indoor microenvironments. The data available (table 1.5) generally show that BDE 209 is the most abundant BDE congener in indoor dust.

Table 1.5: Summary of BDE 209 concentrations (ng g^{-1} dw) in indoor dust.

Microenvironment	Country	Concentration	Reference
Houses (n=10)	UK	3,800-19,900	(Santillo et al., 2003)
House (n=1)	Denmark	260	
House (n=1)	Finland	100	
Houses (n=6)	Spain	58-1615	(Regueiro et al., 2007)
Houses (n=10)	Germany	6-410	(Sjodin et al., 2008)
Houses (n=10)	Australia	23-13000	
Houses (n=10)	UK	910-54000	
Houses (n=10)	USA	120-21,000	
Houses (n=68)	Canada	74-10,000	(Wilford et al., 2005)
Houses (n=16)	UK	120-520,000	(Harrad et al., 2008b)
Houses (n=7)	Canada	290-1,100	
Houses (n=17)	USA	530-3,300	
Houses (n=17)	USA	162-8,750	(Stapleton et al., 2005)
Houses (n=10)	Australia	95-1585	(Toms et al., 2009)
Houses (n=17)	Kuwait	0.8-338	(Gevao et al., 2006)
Houses (n=31)	Singapore	68-13,000	(Tan et al., 2007)
Houses (n=13)	Japan	14-3,200	(Kono et al., 2007)
Homes (n=19)	Japan	100-2600	(Suzuki et al., 2006)
Offices (n=14)	Japan	150-17,000	
Cars (n=60)	USA	4,380-3,570,000	(Lagalante et al., 2009)

1.4.4.2 Soil, sediment and sewage sludge.

The levels of PBDEs in agricultural soils after application of sewage sludge were studied by two groups. Both reported increased concentrations of ΣBDEs (dominated by BDE 209) in soil following sewage sludge amendment at the research stations. This increase ranged between 1.2-45 fold in 5 different sites in Spain (Eljarrat et al., 2008) with BDE 209 concentrations of 81-1,082 and 25-655 ng g^{-1} dw in sewage sludge and sludge-amended soils respectively. While in Sweden, sewage sludge amendment of soil in two farms increased ΣBDEs 2-13 fold with the highest increases for BDE 209. Deca BDE was the predominant BDE congener in 21 surface soil samples covering the whole territory of Taiyuan city, China (Li et al., 2008) where BDE 209 concentrations ranged from 0.01-209.54 ng g^{-1} dw. Zou et al. studied PBDEs levels in 42 soil samples from the Pearl River Delta (PRD), China (Zou et al., 2007) where BDE 209 was the most abundant congener in all samples with average concentrations of 2.4-66.6 and 25.7-102.0 ng g^{-1} dw in surface and point source (collected near e-waste dismantling sites) soils respectively.

Recently, Luo et al. reported high BDE 209 concentrations up to 48,600 ng g^{-1} dw in soils from an e-waste recycling region in Southern China (Luo et al., 2009).

Several authors have reported levels of BDE 209 in sewage sludge and sediments in the past few years. These data are summarised in table 1.6.

Table 1.6: Summary of BDE 209 levels (ng g^{-1} dw) in sewage sludge and sediment.

Sample	**Country**	**Concentration**	**Reference**
Sediment	Norway	0.9-145	(Zegers et al., 2003)
Sediment	Netherlands	6-380	
Sediment	Germany	2-15	
Sediment	Spain	2-132	(Eljarrat et al., 2004)
Sediment	Belgium	1-1,200	(Voorspoels et al., 2004)
Sediment	Netherlands	1.2-32	(Klamer et al., 2005)
Sediment	Belgium	315-8,410	(Covaci et al., 2005)
Sediment	Netherlands	240-1,650	(Verslycke et al., 2005)
Sediment	UK	0.6-3,190	(Allchin et al., 1999)
Sediment	Italy	2.1-15.3	(Guzzella et al., 2008)
Sediment	Norway	0.6-27	(Schlabach et al., 2004)
Sediment	Switzerland	1.1-7.4	(Kohler et al., 2008)
Sediment	USA	1-242	(Song et al., 2004, Song et al., 2005a, Song et al., 2005b)
Sediment	Australia	0.03-35.90	(Toms et al., 2008b)
Sediment	Japan	1.5-85	(Minh et al., 2007)
Sediment	Korea	0.22-493	(Moon et al., 2007)
Sediment	China	0.4-3580	(Mai et al., 2005)
Sediment	China	792-4,137 ng g^{-1} organic carbon	(Xiang et al., 2007)
Sediment	China	0.3-2,776	(Wang et al., 2009)
Sewage Sludge	Germany	97-2,220	(Knoth et al., 2007)
Sewage Sludge	Czech Republic	10-330	(Pulkrabová et al., 2007)
Sewage Sludge	Sweden	0.6-390	(Oberg et al., 2002)
Sewage Sludge	Netherlands	0.5-330	(de Boer et al., 2003)

Table 1.6 (continued): Summary of BDE 209 levels (ng g^{-1} dw) in sewage sludge and sediment.

Sample	Country	Concentration	Reference
Sewage Sludge	USA	85-4,890	(Hale et al., 2001)
Sewage Sludge	Spain	41-4,150	(Martinez et al., 2006)
Sewage Sludge	Spain	8-717	(Sanchez-Brunete et al., 2009)
Sewage Sludge	Australia	260-640	(Ricklund et al., 2008)
Sewage Sludge	Canada	55-1,800	
Sewage Sludge	UK	12,000	
Sewage Sludge	Germany	6.4-1,400	
Sewage Sludge	New Zealand	170-9,500	
Sewage Sludge	South Africa	310	
Sewage Sludge	USA	700-19,000	
Sewage Sludge	Sweden	790	
Sewage Sludge	Switzerland	270-390	
Sewage Sludge	Czech Republic	60-220	
Sewage Sludge	Kuwait	5-1596	(Gevao et al., 2008)

1.4.4.3 Abiotic debromination.

BDE-209 has been shown to undergo photolytic (Soderstrom et al., 2004, Eriksson et al., 2004a, Ahn et al., 2006) and microbial debromination (He et al., 2006) under selected controlled laboratory conditions to produce lower brominated derivatives. Gerecke et al. investigated the degradation of BDE 209 under anaerobic conditions in sewage sludge and reported debromination of deca BDE to produce nona BDEs 207 and 208 which in turn can undergo reductive debromination to produce 6 octa BDE congeners (Gerecke et al., 2005). BDE 209 was also reported to debrominate in anaerobic sediment with a corresponding increase in nona-, octa-, hepta-, and hexa-PBDEs (Tokarz et al., 2008). This was further confirmed by Kohler et al. who reported a shift in congener patterns of octa- and nona BDEs in sediments, compared to the respective congener patterns in technical PBDE products. The octaBDE 202 was detected in sediments, representing a transformation product that is not reported in any of the technical PBDE products indicating anaerobic debromination of BDE 209 in sediments (Kohler et al., 2008).

Stapleton et al. identified the octa BDE 202 as a product of photolytically mediated degradation of BDE 209 in house dust. This was confirmed by a change in nona BDEs congener profile and BDE 197/BDE 201 ratio from that in technical deca BDE products (Stapleton and Dodder, 2008).

1.4.4.4 Aquatic invertebrates, fish and marine mammals.

Few studies have reported concentrations of BDE 209 in aquatic invertebrates (Verslycke et al., 2005, Ramu et al., 2007, Liu et al., 2005). However, as the samples were not depurated, this may have been due to sediment particles in their gut rather than incorporated into the soft tissues as shown by Booij et al. who reported a 60-98% reduction of BDE 209 levels observed in mussels after 24 hours depuration (Booij et al., 2002).

BDE-209 generally accounted for less than 10% of the ΣPBDEs in fish and marine mammals in the few studies which reported it (Frederiksen et al., 2009). In the published literature related to PBDEs in aquatic biota, BDE-209 was either not detected or detected only in a few samples in concentrations around the limit of detection. This was attributed to poor bioavailability of BDE-209 to aquatic biota (Allchin et al., 1999) and relatively rapid biotransformation and/or excretion of the fully brominated BDE by aquatic biota (Eljarrat et al., 2007).

BDE-209 was detected in 14 out of 15 freshwater fish samples collected downstream of an industrial site in Spain at levels from 20-707 ng g^{-1} lw. However, it was not detected in any upstream samples (Eljarrat et al., 2007). Levels of BDE 209 up to 37 ng g^{-1} lw was reported in freshwater fish from Germany (Lepom et al., 2002). Deca BDE was also detected in three different fish species from the Baltic Sea with concentrations up to 116 ng g^{-1} lw (Burreau et al., 2004). Leonards et al. reported BDE 209 (1.9-17 ng g^{-1} lw) in 24% of fish samples collected from the western Scheldt estuary (Leonards et al., 2004). BDE-209 was also detected in 8 liver samples of bib, sole and whiting from the Scheldt estuary in concentrations ranging from 3 to 37 ng g^{-1} ww (Voorspoels et al., 2003).

1.4.4.5 Birds.

In general, detection frequency and levels of BDE-209 in bird tissues and eggs is higher than in aquatic biota. Higher PBDE congeners including BDE-183, BDE-197, BDE-196,

BDE-207, BDE-206 and BDE-209 as well as several unidentified octa- and nona-congeners were detected in almost all samples of peregrine falcon eggs breeding in Spain (8 unhatched eggs from 6 different females). The results seem to indicate the influence of habitat and feeding habits on the BDE congener patterns in birds of prey, perhaps suggesting that birds feeding in terrestrial habitats and on other birds may be more highly exposed to the higher brominated BDE congeners than marine species (Jiménez et al., 2005). This hypothesis was supported by Jaspers et al. who studied PBDEs in liver and muscle samples from 7 species of aquatic and terrestrial predatory birds from Flanders (Belgium). BDE 209 (52-85 ng g^{-1} lw) was only measured in the terrestrial birds indicating that terrestrial species may be more exposed to higher brominated BDE congeners than aquatic birds (Jaspers et al., 2006). Levels of deca BDE were studied in various tissues of birds of prey from Belgium where it was quantified only in 6 out of 44 liver samples (up to 190 ng g^{-1} lw) and in 19 out of 25 serum samples (up to 58 ng g^{-1} lw), but not in any other tissues (Voorspoels et al., 2006b). De Boer et al. studied the levels of BDE 209 in the tissues and eggs of various bird species from the UK (De Boer et al., 2004). BDE 209 was detected in terrestrial birds muscles (13-563 ng g^{-1} lw), livers (6-200 ng g^{-1} lw) and eggs (2-108 ng g^{-1} lw) (De Boer et al., 2004). Lindberg et al. measured BDE 209 (26-370 ng g^{-1} lw) in eggs from 3 different peregrine falcon populations from Sweden (Lindberg et al., 2004). Concentrations up to 9.7 ng g^{-1} lw of BDE 209 were determined in 19% of 43 eggs of 8 bird species from South Africa (Polder et al., 2008c). High concentrations of BDE 209 (up to 4.1 ppm lw) and other higher-brominated PBDEs were measured in 95 California peregrine falcon eggs (Holden et al., 2009). However, BDE-209 was detected in 2006 herring gull egg pools from the Laurentian Great Lakes (4.5-20 ng g^{-1} ww) and constituted 0.6-4.5% of Σ_{39}PBDEs. From 1982 to 2006, the BDE-209 doubling times ranged from 2.1 to 3.0 years (Gauthier et al., 2008). The uptake of BDE209 by top predators even in the aquatic environment was reported in south China where higher BDEs, including BDE 209 (up to 290 ng g^{-1} lw) were detected in egg samples from 2 waterbirds (little egret *Egretta garzetta* and black-crowned night heron *Nycticorax nycticorax*). Deca BDE was also found in plasma (up to 0.33 ng g^{-1} lw) of glaucous gulls (*Larus hyperboreus*) collected in 2004 from Svalbard (Verreault et al., 2005).

1.4.4.6 Terrestrial mammals.

The bioaccumulation of deca BDE in top terrestrial predators was confirmed by Voorspoels et al. who measured BDE 209 in adipose tissue (3.7-200 ng g^{-1} lw), liver (9.1-760 ng g^{-1} lw) and muscles (3.9-290 ng g^{-1} lw) of 33 red foxes from Belgium (Voorspoels et al., 2006a). BDE 209 was also detected in liver and adipose tissue samples of raccoon dogs from Japan at concentrations ranging from <0.1-160 and <0.1-11 ng g^{-1} lw respectively. Higher detection frequencies were reported in liver than in adipose tissue samples (Kunisue et al., 2008).

1.4.4.7 Bio-debromination.

BDE-209 is reported to debrominate in rainbow trout and carp to produce lower brominated congeners from nona- to penta-BDEs, which may have greater persistence and toxicity than the parent compound (Stapleton et al., 2006, Stapleton et al., 2004a). Deca BDE debromination to produce nona- and hepta-BDEs was reported in the European starlings (Van den Steen et al., 2007) and peregrine falcons (Holden et al., 2009). Reductive debromination of BDE 209 to lower BDE congeners was also observed in lactating cows (Kierkegaard et al., 2007). Several authors have studied the metabolism of BDE 209 in rats and reported hydroxy- metabolites with 5 to 9 bromine atoms (Morck et al., 2003, Sandholm et al., 2003, Norris et al., 1975, Riu et al., 2008). In humans, the potential for *in vivo* formation of lower BDEs from debromination of deca BDE was suggested in occupationally exposed rubber workers (Thuresson et al., 2005).

1.4.4.8 Human body Burdens.

Several studies have reported BDE 209 in different human matrices indicating the bioavailability of the fully-brominated compound to humans. However, more research is required to assess the extent of such bioavailability. A summary of BDE 209 levels reported in different human samples is given in table 1.7. Deca BDE was predominant in placenta and breast milk samples from Spain, while its presence in cord blood and placenta samples indicates prenatal exposure to BDE 209, which could continue after birth via breast milk (Gomara et al., 2007). Fangstrom et al. reported 7-year-old children to have higher blood concentrations of BDE-209 than their mothers (Fangstrom et al., 2005a). It is possible that the measured human levels of BDE 209 reflect current

exposure as a result of its low bioaccumulation potential caused by its relatively short half-life (~1 week) compared to lower brominated congeners (e.g. BDE 153; $t_{0.5}$=11.5 years) (Geyer et al., 2004, Sjodin et al., 2003b). However, the higher concentrations could also be related to the exposure of small children via ingestion of dust (Frederiksen et al., 2009).

Table 1.7: Median (Mean*) levels of BDE 209 (ng g^{-1} lw) in human tissue samples.

Sample	Country	Concentration	Reference.
Milk	Faroe Islands	0.6	(Fangstrom et al., 2005b)
Milk	Germany	0.1	(Vieth et al., 2004)
Milk	USA/Canada	0.43	(She et al., 2004)
Milk	Spain	2.9	(Gomara et al., 2007)
Milk	USA	0.92*	(Schecter et al., 2003)
Milk	Russia	0.19	(Polder et al., 2008a)
Milk	Norway	0.13	(Polder et al., 2008b)
Milk	Australia	0.31	(Toms et al., 2009)
Serum	Norway	5.0	(Thomsen et al., 2007a)
Serum	Belgium	11.1	(Covaci and Voorspoels, 2005)
Serum	Australia	3.2	(Toms et al., 2008a)
Serum	Sweden	2.5	(Thuresson et al., 2005)
Mother's serum	Faroe Islands	0.8	(Fangstrom et al., 2005a)
Children's serum	Faroe Islands	1.0	(Fangstrom et al., 2005a)
Maternal serum	Spain	1.1	(Gomara et al., 2007)
Paternal serum	Spain	1.1	
Cord serum	Spain	2.2	
Cord Serum	Belgium	28.1	(Covaci and Voorspoels, 2005)
Serum (occupational)	Sweden	4.8	(Sjodin et al., 1999)
Serum (occupational)	Sweden	35	(Thuresson et al., 2005)
Serum (occupational)	China	310	(Bi et al., 2007)
Plasma	Sweden	9.9	(Karlsson et al., 2007)
Placenta	Spain	1.0	(Gomara et al., 2007)

1.4.5 Regulatory aspects.

In 2008, the European court of justice ruled against the exemption of deca BDE from RoHS directive and decided that its use must be phased out with effect from July 1, 2008

(Judgment of the European Court of Justice on Joint Cases C-14/06 and C-295/06, 2008). BDE 209 will now go through REACH registration. From April 1, 2008, the Norwegian government has introduced a ban on deca BDE. The ban includes deca BDE as a substance, in preparations and in products such as cellular rubber, textiles and upholstery. Means of transport (e.g. cars) are exempted from the ban. A ban on deca BDE in electrical and electronic products has been in place in Norway since the 1st of July 2006 (Norwegian Pollution Control Authority (SFT), 2008).

1.5 Pathways of human exposure to HBCDs, TBBP-A and BDE 209.

Several pathways have been suggested for human exposure to BFRs including dermal contact, diet, inhalation, and dust ingestion. Very little is known about the extent of dermal absorption of BFRs via skin contact. However, it appears to be a very minor pathway of exposure (EU Risk Assessment Report, 2006, KEMI (National Chemicals Inspectorate), 2007, EU Risk Assessment Report, 2002). Air inhalation can be a significant exposure pathway to the studied BFRs for occupationally exposed individuals with concentrations up to 150 μg m^{-3} (Thomsen et al., 2007b), 140 ng m^{-3} (Sjodin et al., 2001) and 650 ng m^{-3} (Cahill et al., 2007) reported for ΣHBCDs, TBBP-A, and BDE 209 respectively in indoor air from different electronic dismantling and recycling facilities. However, non-occupational exposure to the target BFRs via inhalation of indoor air is unlikely to exert a major contribution to the overall exposure to these flame retardants due to the low levels of these compounds reported so far in both outdoor and indoor air (Covaci et al., 2006, Covaci et al., 2009, Frederiksen et al., 2009). Thus, for non-occupationally exposed individuals; the major intake of HBCDs, TBBP-A and BDE 209 is probably from food and indoor dust ingestion.

Diet was long believed to be the most important source of human exposure to BFRs as was the case for many other POPs (Sjodin et al., 1999). However, in the case of PBDEs; diet cannot account for the differences in internal human exposure observed between Europe and North America. The PBDE levels in comparable food items were similar across the Atlantic (except for meat products which contain slightly higher levels in North America). Therefore, it will be difficult to explain the concentration differences in human matrices solely on the basis of dietary exposure (even considering the difference

in dietary habits). In recent years, exposure from house dust was proposed as the 'missing' pathway to internal human exposure that can explain such differences. Large differences in PBDE levels in indoor dust have been observed across the Atlantic similar to the human internal exposure pattern (Frederiksen et al., 2009).

The low detection frequency and concentrations of target BFRs in food items makes it difficult to obtain an accurate estimate of the dietary exposure to these BFRs. The UK Food Standards Agency estimated the upper bound adult dietary intake from total diet using an average diet intake scenario to be 5.9, 1.6 and 4.5 ng kg^{-1} bw day^{-1} for ΣHBCDs, TBBP-A and BDE 209 respectively. While for toddlers, the upper bound dietary intake from total diet was 24, 7 and 10 ng kg^{-1} bw day^{-1} for ΣHBCDs, TBBP-A and BDE 209 respectively (UK Food Standards Agency, 2006). PBDEs were detected in all the studied 19 food groups. Meat products contained the highest ΣPBDEs, dominated by BDE 209 which was also the major BDE congener in all but two food groups (canned vegetables and fish). In general, the less brominated congeners such as BDE 47 were most abundant in fish, whilst the higher brominated congeners were predominant in meat. α-HBCD was detected in half of the food groups, being highest in fruit, vegetables and meat. β- and γ-HBCD were found less frequently, at lower levels and only in fruit and vegetables. TBBP-A was not found above the limit of detection in any samples (UK Food Standards Agency, 2006).

The significance of unintentional dust ingestion in indoor environments as an exposure pathway to BFRs has been confirmed by several studies in the past few years (Harrad et al., 2006b, Stapleton et al., 2005, Jones-Otazo et al., 2005, Wilford et al., 2005, Law et al., 2005, Covaci et al., 2009). Recently, Lorber et al. concluded that exposure to PBDEs (including BDE 209) in house dust accounted for 82% of the overall estimated adult PBDE intakes in USA (Lorber, 2008).

1.6 Aims.

From the above, a number of research gaps are identifiable with respect to aspects of the environmental fate and behaviour, pathways and magnitude of human exposure to HBCDs, TBBP-A, and BDE-209. In order to address these gaps, the aims of the current study are to:

1- Develop and validate sampling and analytical methodology for determination of HBCDs, TBBP-A and BDE 209 in several environmental matrices.
2- Report concentrations of the three main HBCD diastereomers, TBBP-A and BDE 209 in air and dust from different indoor microenvironments.
3- Study the causes of variability in concentrations and isomer profiles of HBCDs in indoor dust.
4- Estimate human exposure to the target compounds via inhalation and dust ingestion in Birmingham, UK and assessment of the relative importance of each exposure route to the overall exposure of adults and toddlers using different exposure scenarios.
5- Compare the levels of the target BFRs in indoor dust from different countries and their implications for human exposure.
6- Study the transfer mechanisms of BDE 209 and HBCDs to indoor dust.
7- Study the relationship between levels of HBCDs in indoor dust, diet and serum.
8- Assess the bioaccessibility of the studied BFRs from the human GIT following dust ingestion using a physiologically-based extraction test (PBET).
9- Report the levels of HBCDs, TBBP-A and BDE 209 in human milk samples from Birmingham, UK and study the relationship between external and internal exposure to the studied BFRs using a simple, one-compartment pharmacokinetic model.

CHAPTER II

SAMPLING AND ANALYTICAL METHODOLOGY

Analytical methodology for determination of BFRs in various biotic and abiotic samples studied in this thesis generally consists of four major steps: sampling, extraction, clean-up and analysis. In this chapter each of these steps will be discussed in detail. Validation of the developed and applied analytical procedures as well as the quality assurance/quality control measures employed in order to ensure the validity of the generated data will also be presented and explained.

2.1 Sampling.

2.1.1 Air sampling.

Air samples were collected in a total of 92 microenvironments within the West Midlands conurbation. Samples were taken under normal use conditions to reflect actual human exposure. The following microenvironment categories were sampled in this study: homes (living rooms, n=35), offices (n=30), Cars (n=21) and PMEs (3 Pubs, 1 restaurant and 2 supermarkets). Outdoor air sampling (n=5) was performed at the Elms Road Observatory Site (EROS) in Birmingham, UK. Different air sampling techniques were utilised depending on the physicochemical properties of the studied BFR and its relative distribution between the gaseous and particulate phase. Passive air sampling techniques are generally preferred for monitoring pollutants in indoor air, owing to their ease of use, inobtrusiveness during deployment and low cost (Nothstein et al., 2000) which allows simultaneous monitoring in several spatially distinct locations. Furthermore, they are noise-free, do not require electricity and provide time weighted average (TWA) concentrations which renders them more appropriate for assessment of chronic exposure to contaminants (Hazrati and Harrad, 2007). However, despite their proven track record for monitoring indoor air concentrations of contaminants such as PBDEs and PCBs (Harrad et al., 2006a), the fact that PUF disks effectively sample only the vapour phase, renders them inappropriate for contaminants that exist primarily in the particle phase. (Wilford et al., 2004). In this study, we found that HBCDs exist mainly in the gaseous

phase while TBBP-A and BDE-209 are associated primarily with the particulate phase of indoor air. Therefore, passive air samplers were used to sample HBCDs while active air samplers were applied to sample TBBP-A and BDE-209 in indoor air. All outdoor air samples were collected using high volume air sampling technique.

2.1.1.1 Active air sampling.

2.1.1.1.1 High volume active air sampling.

This was conducted using a Graseby–Andersen Hi–Vol sampler (Westech Instrument Services, Bedfordshire,UK) fitted with a total suspended particulate (TSP) inlet modified to hold a standard PTFE back coated glass-fibre filter (GFF, 25 cm x 20 cm, 1 μm pore size, Whatman, UK) and a pre-cleaned polyurethane foam (PUF) plug (8 cm diameter x 10 cm length, 503 cm^3 volume, 0.017 g cm^{-3} density). Prior to sampling, both the filter and PUF plug were treated with 10 ng of a sampling efficiency standard (SES) (d_{18}-α-HBCD) designed to provide a quantitative measure of the efficiency of sampling. Sampling was conducted for 2 hours at an accurately measured flow-rate of 0.75 m^3 min^{-1} yielding sample volumes of 90 m^3. The filter and PUF plug were analysed separately.

2.1.1.1.2 Low volume active air sampling.

A low volume, double inlet pump (Capex L20X) was operated for 24 h at a flow rate of 39 L min^{-1} to yield a single sample comprising approximately 56 m^3 air. The particulate phase was collected by employing a 47 mm membrane filter (1.0 μm pore size, Whatman, UK) housed in a standard open face 47 mm filter holder airside of the PUF plug sorbent. Two PUF plugs (4 cm diameter × 8 cm length) housed by a glass holder (3 cm diameter × 25 cm length), were used as a gas phase sorbent. Filters and PUF plugs were each treated with 10 ng of SES (d_{18}-α-HBCD) prior to each active sampling session. The designated flow rate of 39 L min^{-1} was achieved and maintained by using a flow meter (Platon 50 L min^{-1}) connected to an adjustable valve. The flow meter was calibrated using a Gilibrator air flow calibrator (Gilian) which is classified as a primary standard device. The PUF plug holders were covered with aluminium foil throughout sampling to minimise photodegradation. Following sample collection, the particulate and vapour phases were analysed separately.

2.1.1.2 Passive air sampling.

2.1.1.2.1 Passive air samplers for collecting gaseous phase.

Polyurethane foam disks (140 mm diameter, 12 mm thickness, 360.6 cm^2 surface area, 0.07 g cm^{-3} density, PACS, Leicester, UK) were sheltered by either one or two different size stainless steel housings (18 cm, one L bottom housing—not used in "part-sheltered'' configuration—and 23 cm, two L top housings respectively). PUF disks were washed in tap water, dried at room temperature and pre-extracted with 1:1 hexane:DCM for 8 hours in a Soxhlet apparatus prior to field deployment. PUF disks were treated with 10 ng of d_{18}-α-HBCD as a SES prior to sampling. Shelters were cleaned carefully and solvent rinsed to remove potential contamination.

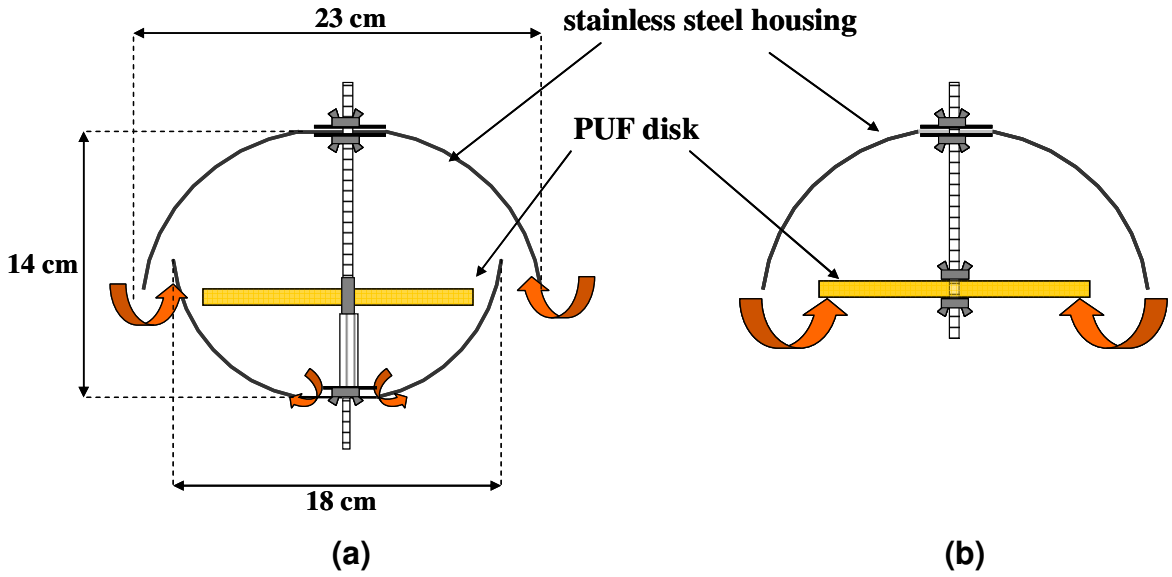


Figure 2.1: Diagram of PUF disk passive air samplers used to sample the gaseous phase (a) Fully-sheltered configuration. (b) Part-sheltered configuration.

2.1.1.2.2 Passive air samplers for collecting both gaseous and particulate phases.

As shown in Figure 2.2, samplers consist of two pieces of sampling media: a PUF disk (140 mm diameter, 12 mm thickness, 360.6 cm^2 surface area, 0.07 g cm^{-3} density, PACS, Leicester, UK) and a glass fiber filter (GFF, 12.5 cm diameter, 1 μm pore size, Whatman, UK) fully sheltered between two different size stainless steel housings (18 cm, one L bottom housing and 23 cm, two L top housing). To avoid any gravitational deposition of particles, the PUF disk was mounted to the top of the shelter with only the downward

face exposed. The GFF was suspended at the middle of the housing (supported by a stainless steel perforated disk mounted on the central screw) to trap the particulate matter. PUF disks were pre-extracted with 1:1 hexane:DCM for 8 hours in a Soxhlet apparatus while GFFs were preconditioned by heating at 450°C for 5 h prior to field deployment. Both sampling media were treated with 10 ng of d_{18}-α-HBCD as a SES prior to sampling. Shelters were cleaned carefully and solvent rinsed to remove potential contamination.

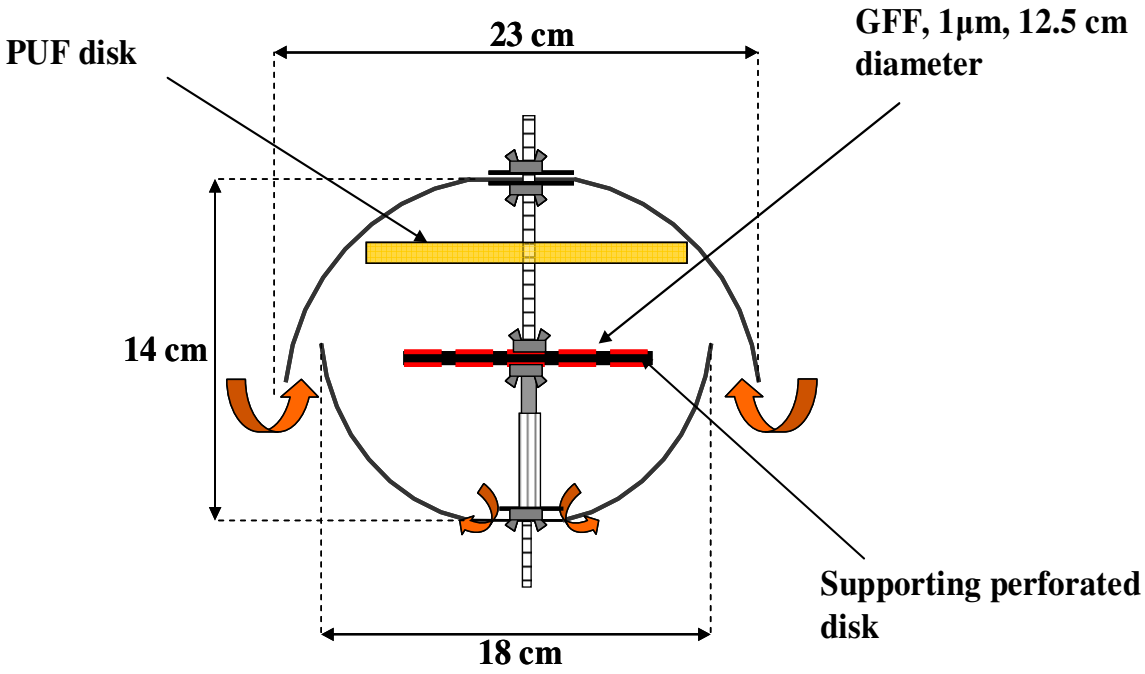


Figure 2.2: Diagram of passive air samplers used to sample both the gaseous and particulate phases.

2.1.1.3 Calibration of passive air samplers.

In order to obtain quantitative data on airborne contaminant concentrations from passive air samplers, one needs to know not only the mass of contaminant sequestered by the sampler over the course of its deployment, but the volume of air sampled over the same period. In order to achieve this, it is necessary to conduct a calibration exercise to determine the passive air sampling rates for each of the studied compounds.

2.1.1.3.1 Experimental conditions.

In summary, the calibration experiment involved simultaneous monitoring of α-, β-, γ-HBCDs, TBBP-A and BDE-209 in indoor air using passive air samplers over a variety of

exposure times, and calibration against time weighted average concentrations of the same compounds derived via low volume active air sampling. Fully-sheltered passive samplers (n = 8) were deployed over a 50 days period in a temporarily vacant office microenvironment at a height of 150 cm with a minimum distance between samplers of 50 cm. PUF disks were harvested at 10 d intervals over the 50 days of the experiment. To ensure that detectable concentrations were provided by the passive samplers at the 10 and 20 days sampling intervals, three and two samplers were harvested and combined for analysis at these times respectively. The analyte masses present in these combined samples were subsequently normalized to a single PUF disk equivalent mass for the purposes of the calibration. Concurrent with the deployment of the passive air samplers, a single active air sample was taken covering the full 50 d duration of the experiment. The same calibration experiment was conducted in identical fashion in the same room for the "Part-sheltered" and "Full-sheltered + filter" sampler configurations described in sections 2.1.1.2.1 and 2.1.1.2.2 respectively.

2.1.1.3.2 Passive samplers' uptake rates.

Time integrated active sampling-derived concentrations and masses collected by each Passive sampler during the calibration experiment were determined for each of the studied BFRs (Table 2.1).

Table 2.1: Concentrations (pg m^{-3}) of target BFRs in indoor air derived from low volume active air sampler.

	α- HBCD	**β- HBCD**	**γ- HBCD**	**Σ HBCDs**	**TBBPA**	**BDE-209**
Particulate phase	22	11	55	88	35	211
Gaseous phase	52	25	125	202	<LOQ	<LOQ

To determine the sampling rates of each compound, the equivalent air volumes sampled by each PUF disk over a given exposure period, V_{eq} (cm^3), were calculated using equation (1).

$$V_{eq} = \frac{M}{C_A} = k_A \; A_{PUF} \quad \Delta t \ldots\ldots\ldots\ldots\ldots (1)$$

where M is the mass of compound sequestered by the PUF disk (pg) within the deployment period (table 2.2), C_A is the concentration (pg cm^{-3}) of the target analyte in the air being sampled, k_A is the air side mass transfer velocity (cm sec^{-1}), A_{PUF} is the exposed macro surface area of the PUF disk (cm^2), and Δt is the sampling period (sec). The V_{eq} values were converted to m^3 units and plotted against the exposure time of the PUF disks in days (d). The slope of the linear regression plots obtained (Figure 2.3 shows a representative example of a plot for γ-HBCD and the part-sheltered configuration) is defined as the passive air sampling rate (R, m^3 d^{-1}) of the PUF disk samplers for the corresponding compound (i.e. $V_{eq} = R \; \Delta \; t$, where $R = k_A \; A_{PUF}$). Strong correlation (R values >0.987) between values of V_{eq} and PUF disk exposure time for each target BFR regardless of sampler housing configuration demonstrate linear uptake of studied compounds for the passive samplers over the 50 day calibration period.

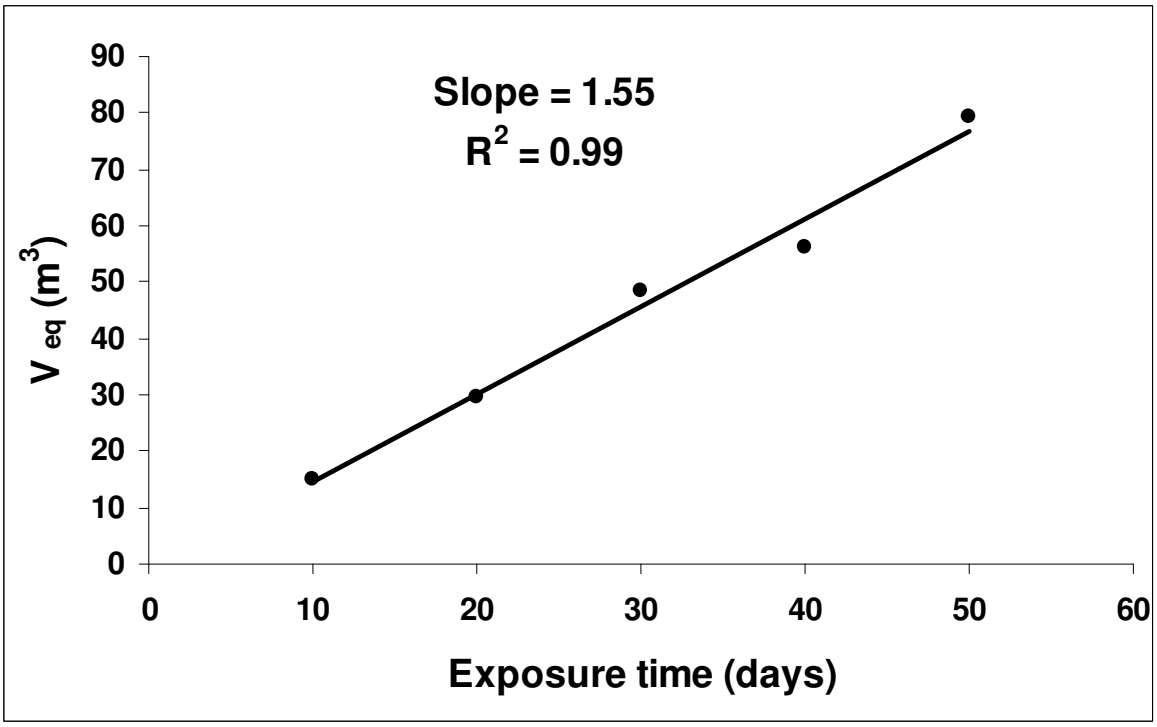


Figure 2.3: Plot of equivalent air volume (V_{eq}, m^3) versus exposure time (days) for γ-HBCD and the part-sheltered PUF disk passive sampler configuration. slope = passive air sampling rate (m^3 d^{-1}).

Table 2.2: Contaminant masses (pg) collected by different configurations of passive air samplers during the deployment period (days).

sampler configuration	Time (days)	α-HBCD	β-HBCD	γ-HBCD	TBBPA	BDE-209
Part-sheltered	10	1200	1050	3100	<LOQ	<LOQ
	20	2600	2200	6150	<LOQ	<LOQ
	30	3550	3300	10100	<LOQ	<LOQ
	40	5450	4300	11700	<LOQ	<LOQ
	50	5800	4900	16500	<LOQ	<LOQ
Full-sheltered	10	700	550	1750	<LOQ	<LOQ
	20	1400	1100	3500	<LOQ	<LOQ
	30	1950	1500	4900	<LOQ	<LOQ
	40	3100	2150	6600	<LOQ	<LOQ
	50	3650	2850	9700	<LOQ	<LOQ
Full-sheltered + filter	10 (PUF)	350	200	950	<LOQ	<LOQ
	20 (PUF)	800	400	2200	<LOQ	<LOQ
	30 (PUF)	1400	650	3450	<LOQ	<LOQ
	40 (PUF)	1650	800	4100	<LOQ	<LOQ
	50 (PUF)	2300	1100	5600	<LOQ	<LOQ
	10 (filter)	100	50	300	200	1100
	20 (filter)	250	150	700	450	2250
	30 (filter)	350	200	1050	750	3500
	40 (filter)	550	250	1350	900	4300
	50 (filter)	600	300	1600	1100	5400

Passive sampling rates for each sampler configuration are given in table 2.3. The greater sampling rates for the part-sheltered configuration are in line with previous observations for PBDEs (Hazrati and Harrad, 2007), and are attributable to the reduced air flow to the PUF disk caused by the bottom housing of the fully-sheltered configuration. While the fully-sheltered configuration is more appropriate for outdoor air where the bottom housing affords additional protection from rain to the PUF disk, it is also useful in indoor

microenvironments to provide some protection to the sampling media from curious children. The enhanced sampling rates of the part-sheltered device make it the configuration of choice for sampling office microenvironments. The sampling rates obtained using the fully-sheltered sampler (table 2.3) are within the range for tri- through hepta-PCBs and tri-through hexa-PBDEs reported for an identical fully-sheltered PUF disk configuration (Hazrati and Harrad, 2007).

Table 2.3: Passive sampling rates (m^3 day^{-1}) of the studied BFRs in indoor air for each sampler configuration.

Compound	**Part-sheltered configuration**	**Fully-sheltered configuration**	**Fully-sheltered + filter configuration**	
			PUF	Filter
α-HBCD	1.38	0.87	0.85	0.58
β-HBCD	1.54	0.89	0.86	0.58
γ-HBCD	1.55	0.91	0.87	0.6
TBBP-A	N/A	N/A	N/A	0.56
BDE-209	N/A	N/A	N/A	0.57

In addition to the passive air sampling rates, we also calculated air side mass transfer coefficients (k_A) for each HBCD diastereomer and sampler configuration, given that $k_A = R/A_{PUF}$. For the part-sheltered configuration these were 0.044, 0.049, and 0.050 cm s^{-1} for α-, β-, and γ-HBCD respectively, and for the fully-sheltered configuration – 0.028, 0.029, and 0.029 cm s^{-1} for α-, β-, and γ-HBCD respectively. These can be used to estimate passive air sampling rates for the same sampler configuration but fitted with PUF disks of different macro surface areas.

2.1.1.3.3 Comparison of concentrations derived using PUF disk samplers with those derived via active sampling.

Table 2.4 lists the concentrations of studied BFRs in 5 indoor microenvironments using different passive air sampler configurations. In all locations, the PUF disk passive sampler-derived concentrations (part and fully-sheltered configurations) of ΣHBCDs are approximately one third lower than those derived using active air samplers (sum of both vapour and particulate phases). While this may be due partly to the fact that the

monitoring periods for passive and active sampling were not identical (and thus the concentrations experienced may differ), it was evident that the PUF disk-derived concentrations approximate very closely to the concentrations recorded in the vapour phase only by the active air sampler. This indicates that the PUF disk samplers "capture" only those HBCDs associated with the vapour phase, and suggests that PUF disk samplers may not be appropriate for use at low temperatures where the majority of airborne HBCDs may be expected to reside in the particulate phase. Neither TBBP-A nor BDE-209 were above the LOQ in any of the PUF disks of the passive samplers indicating that these two BFRs are associated mainly with the particulate phase of air. However, both compounds were collected by the passive sampler filter and the results compared favourably (table 2.4) to those obtained from active air samplers.

Table 2.4: Concentrations of target BFRs in indoor air (pg m^{-3}) derived using passive and active air samplers.

	BFR	**Active sampler**	**Fully-sheltered**	**Part-sheltered**	**Fully-sheltered +filter**
Office 1	ΣHBCDs	239	171	182	219
	TBBP-A	14	n.m.	n.m.	11
	BDE-209	64	n.m.	n.m.	57
Office 2	ΣHBCDs	283	199	217	269
	TBBP-A	11	n.m.	n.m.	10
	BDE-209	97	n.m.	n.m.	88
Office 3	ΣHBCDs	377	299	286	391
	TBBP-A	21	n.m.	n.m.	17
	BDE-209	118	n.m.	n.m.	115
House 1	ΣHBCDs	290	206	214	279
	TBBP-A	18	n.m.	n.m.	13
	BDE-209	143	n.m.	n.m.	145
House 2	ΣHBCDs	176	138	145	163
	TBBP-A	8	n.m.	n.m.	5
	BDE-209	151	n.m.	n.m.	139

2.1.1.3.4 Environmental scanning electron microscopy (ESEM) as a tool for understanding the mechanism of particulate matter collection by passive air sampler filter.

A "Fully-sheltered + filter" passive air sampler was deployed in an office microenvironment for 30 days and the filter was collected. Small pieces of the filter were adhered to aluminum stubs using double-sided carbon sticky tabs (Agar Scientific) prior to coating with evaporated gold in an Emscope SC500 evaporation unit. Microscopic examination was conducted at high vacuum in an FEI XL-30 FEG environmental scanning electron microscope (ESEM). Results revealed that most of the particulate matter collected was on the upper side of the filter indicating that gravitational force plays the major role in particle entrapment by the filter. The observed particles exhibited wide variation in shapes and sizes with diameters reaching up to 20 μm (figure 2.4).

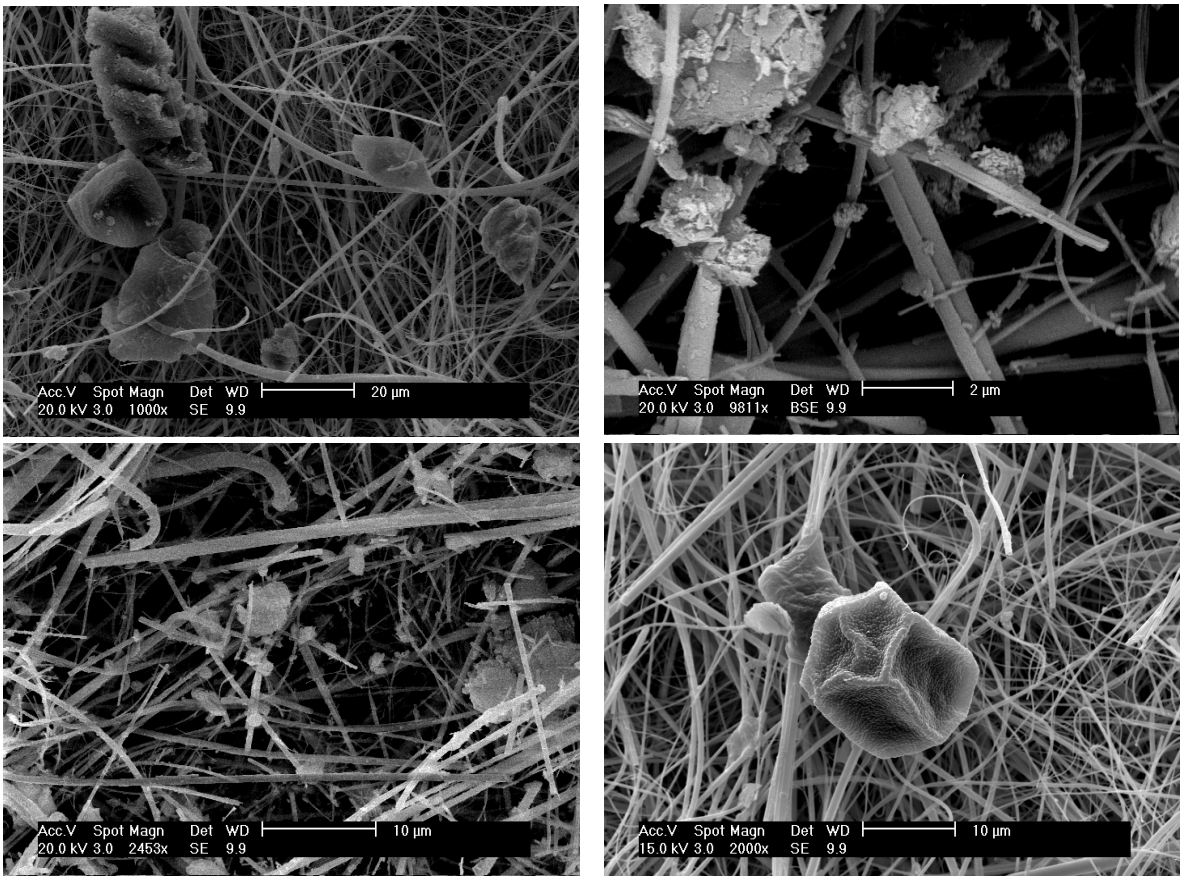


Figure 2.4: Environmental scanning electron microscopy (ESEM) images of a passive air sampler filter (upper side) showing different shapes and sizes of trapped particles.

2.1.2 Dust sampling.

Dust samples were collected using a Nilfisk Sprint Plus 1600 W vacuum cleaner or equivalent Nilfisk model available in the country sampled. All microenvironments comprised a convenience sample of acquaintances of the researchers. Samples were taken under normal room use conditions to reflect actual human exposure. Sampling was conducted according to a clearly defined standard protocol by one of the research team. One m^2 of carpet was vacuumed for 2 min in each location and in case of bare floors 4 m^2 were vacuumed for 4 min. Samples were collected using nylon sample socks (25 μm pore size) that were mounted in the furniture attachment tube of the vacuum cleaner. After sampling, socks were closed with a twist tie, sealed in a plastic bag and stored at -20 °C. Before and after sampling, the furniture attachment was cleaned thoroughly using an isopropanol-impregnated disposable wipe. A list of the microenvironments sampled in this study is given in table 2.5.

Table 2.5: Number of microenvironments sampled for dust in this study.

Country	Homes	Offices	Cars	Public micro-environments	Nurseries and primary schools
UK	45	35	35	6	43
USA	13	-	-	-	-
Canada	8	-	-	-	-
France	9	11	10	-	-
Belgium	16	-	-	-	-
Kazakhstan	10	10	11	-	-

2.1.3 Physiologically based extraction test (PBET) samples.

An in vitro physiologically based extraction test (PBET) using a laboratory digestion procedure designed to reproduce human gastro-intestinal tract (GIT) chemistry and function was applied to study the bioavailability of α-, β-, γ-HBCD diastereomers, TBBP-A and BDE-209 from the 3 main compartments of the GIT following ingestion of indoor dust. While the oral compartment was not studied because the residence time is so short, approximately 1 gram of a well-characterized, sieved and thoroughly homogenized

indoor dust sample was added to each of a simulated stomach, small intestine and colon compartments.

2.1.3.1 Stomach medium.

The stomach medium was prepared as described by Ruby et al. (Ruby et al., 1996). Briefly, the pH of 1 litre of deionised water was adjusted to the selected pH with 12 N HCl and adding 1.25 g of pepsin (activity of 800-2500 units mg^{-1}), 0.50 g of citrate (Fisher Chemical Co.), 0.50 g of malate (Aldrich Chemical Co.), 420 µL of lactic acid (synthetic syrup), and 500 µL of acetic acid (Fisher Chemical Co.). All chemicals were from Sigma Chemical Co. unless otherwise noted.

2.1.3.2 Small intestine medium.

The stomach medium was converted to the small intestine medium by the addition of saturated $NaHCO_3$ to increase the pH from 2.5 to 7.0 and 0.176 g bile salts and 0.05 g pancreatin (Ruby et al., 1996).

2.1.3.3 Colon medium.

The colon medium was prepared as described by Macfarlane et al. (Macfarlane et al., 1998b). In summary, The following components (in grams) were added to 1 liter of deionized water. starch (BDH), 5.0; porcine gastric mucin (Sigma type III), 4.0; xylan (oatspelt), 2.0; pectin (citrus), 2.0; guar gum, 1.0; arabinogalactan (larch wood), 2.0; inulin (chicory root), 1.0; yeast extract, 4.5; peptone water, 5.0; tryptone, 5.0; casein (BDH), 3.0; bile salts No.3, 0.4; $FeSO_4.7H_2O$, 0.005; NaCl, 4.5; $NaHCO_3$, 1.5; KCl, 4.5; KH_2PO_4, 0.5; $MgSO_4.7H_2O$, 1.25; $CaCl_2.6H_2O$, 0.15; cysteine, 0.8 and haemin, 0.05.

2.1.3.4 Procedure.

One gram of dust was added to 100 mL of GIT medium in a 250-mL funnel. The funnel was submerged half-way in a temperature-controlled water bath maintained at 37 °C (figure 2.5). The mixture was allowed to stand for 10 min, and then argon gas was purged through the reaction vessel. The flask contents were mixed gently using a magnetic stirrer to mimic the peristaltic movement of the human GIT. The pH was checked after 5 min, and every 10 min thereafter, and adjusted when necessary. After the specified incubation time, the flask contents were centrifuged and the supernatant was collected for analysis.

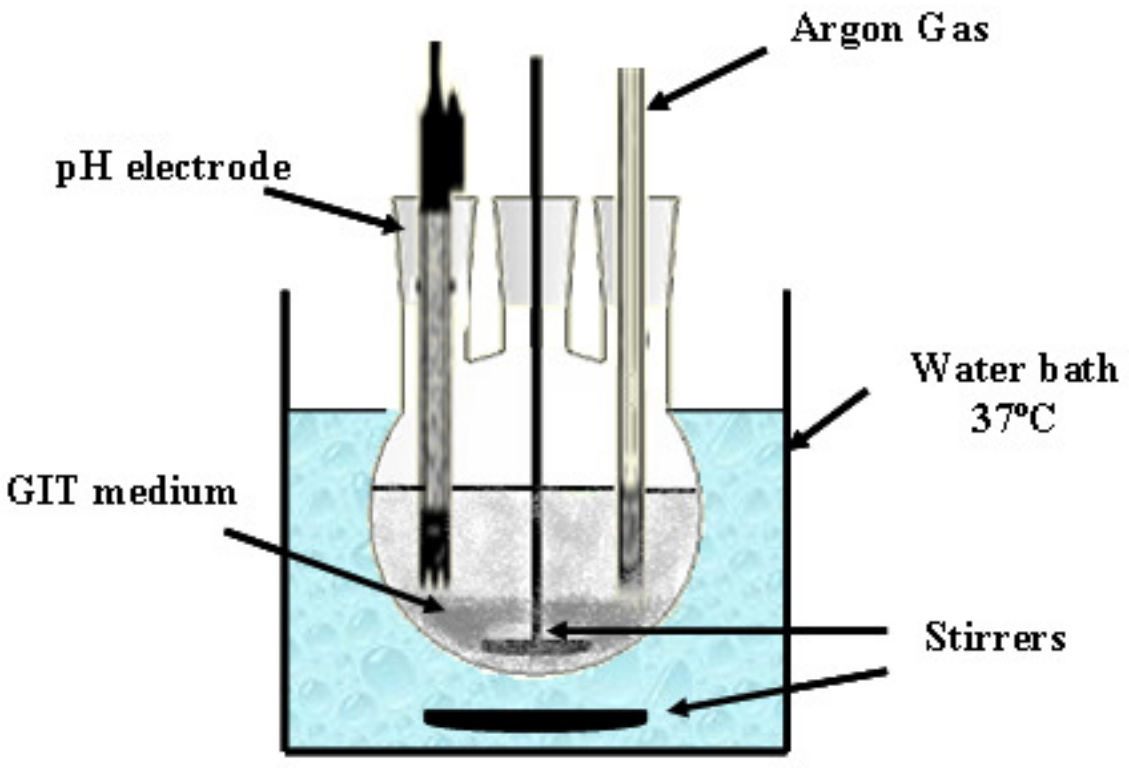


Figure 2.5: Simplified diagram of PBET experimental system

2.1.4 Human milk samples.

Human milk samples (n=28) were obtained from the milk bank of Birmingham Women's Hospital after the research proposal and experimental design were approved by a local research ethics committee (REC) according to the NHS guidelines. Informed consent was obtained from all milk donors who agreed to participate in this study. Milk samples (~50 mL) were obtained from the milk bank in 100 mL clean polypropylene containers and transferred to the laboratory in ice boxes prior to storage at -20 ºC until analysis.

2.1.5 Duplicate diet samples.

Duplicate diet samples (n = 165) were collected from 16 Belgian students (7 males and 9 females aged between 20 and 25 years) residing in Antwerp university housing. Participants were instructed to maintain their usual dietary habits and provided at the end of each day a duplicate of breakfast, lunch and dinner. For each participant, duplicate diet samples were collected for one week. Duplicate food samples were homogenised, freeze dried and kept at -20 °C. The water content of each sample was determined gravimetrically to permit calculation of concentrations on a wet weight (ww) basis.

HBCD concentrations (ng g^{-1} ww) in each sample were multiplied by the sample mass to provide an estimate of dietary intake.

2.1.6 Serum samples.

Following acquisition of the diet and dust samples, each participant donated 10 mL blood which was centrifuged to obtain serum. An aliquot (150 µL) of the samples was analyzed for triglycerides and total cholesterol in a clinical laboratory. The total lipid content was calculated using the formula of described by Phillips et al. (Phillips et al., 1989) and varied between 2.95 g L^{-1} and 10.10 g L^{-1}. The remaining serum (3 to 4.5 mL) was stored at -20 °C until analysis. The study was approved by the Ethics Committee of the University of Antwerp and informed consent was obtained from each participant.

2.2 Extraction.

2.2.1 Dust samples.

Dust samples were sieved through a 500 µm mesh size sieve and well-homogenised prior to extraction using pressurised liquid extraction (Dionex Europe, UK, ASE 300). Accurately weighted aliquots (typically between 100 and 300 mg) were loaded into pre-cleaned 66 mL cells containing 1.5 g florisil and hydromatrix (Varian Inc., UK) to fill the void volume of the cells, spiked with 25 ng of each of ^{13}C-labelled TBBP-A, α-, β-, γ-HBCD and 200 ng of ^{13}C-labelled BDE-209 as internal (surrogate) standards (i.e. standards used for determination of analyte concentrations). The ASE cells were extracted with hexane:dichloromethane (1:9, *v/v*) at 90 ˚C and 1500 psi. The heating time was 5 minutes, static time 4 min, purge time 90 s, flush volume 50%, with three static cycles.

2.2.2 Duplicate diet samples, human milk samples and PBET samples.

Samples were freeze dried to remove the water content without affecting the analytes concentrations by heating. Accurately weighted aliquots of the freeze-dried samples (typically 0.5 g, 2 g and 0.5 g for duplicate diet, human milk and PBET samples respectively.) were loaded into pre-cleaned 66 mL cells containing 1.5 g florisil, 3 g alumina, 5 g anhydrous Na_2SO_4 and hydromatrix (Varian Inc., UK) to fill the void volume of the cells, spiked with 25 ng of each of ^{13}C-labelled TBBP-A, α-, β-, γ-HBCD

and 100 ng of ^{13}C-labelled BDE-209 as internal standards. The ASE cells were extracted using the method as described in section 2.2.1. The lipid weight of human milk samples was determined gravimetrically on separate aliquots of the freeze-dried samples (typically 1g) using a standard procedure (The European Standard EN 1528-2, 1996).

2.2.3 Serum samples.

Extraction and clean-up of serum samples were carried out as described elsewhere (Covaci and Voorspoels, 2005). Samples were thawed and homogenised by shaking for 1 min. Internal standards (7.5 ng of each of ^{13}C-labelled α-, β- and γ-HBCDs) were added to each sample (typically 3.5 mL), together with 1 mL formic acid for protein denaturation and 3 ml Milli-Q water for dilution, mixed and sonicated for 20 min. SPE cartridges (OASIS HLB cartridges, 6 mL/500 mg) were washed with 5mL DCM and activated with 5mL methanol and 5mL Milli-Q water prior to sample application. After sample loading, the SPE cartridges were rinsed with 3 mL Milli-Q water. The sorbent bed was dried thoroughly under vacuum for 30 min. The SPE cartridges were eluted with 3 X 3 mL DCM in a separate tube.

2.2.4 Air samples.

PUF disks, PUF plugs and filters, were spiked with 25 ng of each of ^{13}C-labelled α-, β-, γ-HBCD, TBBP-A and BDE-209 as internal standards prior to soxhlet extraction with hexane: dichloromethane (1:1 v/v) for 8 hours.

2.2.5 Rationale for extraction techniques.

Pressurised liquid extraction (PLE) was preferred over traditional soxhlet for solid-liquid extraction and was used whenever possible due to shorter extraction time and less solvent consumption. However, PLE was not suitable for extraction of PUF disks and filters from air samples where the disks could not be shaped for reuse and the filters slightly degenerate. Therefore, traditional soxhlet was used for extraction of air samples.

2.3 Clean-up.

2.3.1 Dust and air samples.

The crude extracts were concentrated to 0.5 mL using a Zymark Turbovap® II (Hopkinton, MA, USA) then cleaned up by loading onto SPE cartridges filled with 8 g of

pre-cleaned acidified silica (44% concentrated sulfuric acid, *w/w*). The analytes were eluted with 25 mL of hexane:dichloromethane (1:1, *v/v*). The eluate was evaporated to dryness under a gentle stream of N_2, then reconstituted in 200 µL of d_{18}-γ-HBCD and ^{13}C-BDE 100 (25 pg µL^{-1} each in methanol) used as recovery determination (or syringe) standard to determine the recoveries of internal standards for quality assurance/quality control (QA/QC) purposes.

2.3.2 Duplicate diet samples, human milk samples and PBET samples.

The crude extracts were concentrated to 0.5 mL using a Zymark Turbovap® II (Hopkinton, MA, USA) then washed with 3 mL of 98% sulfuric acid. After phase separation, the hexane layer was transferred onto a florisil column topped with sodium sulfate and eluted with 25 mL of hexane:dichloromethane (1:1, v/v). The eluate was evaporated to dryness under a gentle stream of N_2 and the dried extract was reconstituted in 200 µL of d_{18}-γ-HBCD and ^{13}C-BDE 100 (25 pg µL^{-1} each in methanol) used as recovery determination (or syringe) standard to determine the recoveries of internal standards for QA/QC purposes.

2.3.3 Serum samples.

The extract was concentrated to ~ 1 mL under a gentle stream of N_2. The concentrated eluate was loaded onto a SPE cartridge (6 mL) filled (from bottom to top) with 2 g of acid silica (44% concentrated sulfuric acid, *w/w*) and 0.5 g anhydrous Na_2SO_4 (pre-washed with 5 mL DCM). The analytes were eluted with 8 mL DCM. The final eluate was concentrated under a gentle nitrogen stream until dryness, dissolved in 100 µL of d_{18}-γ-HBCD (25 pg µL^{-1} in methanol) used as recovery determination (or syringe) standard to determine the recoveries of internal standards for QA/QC purposes.

2.3.4 Rationale for clean-up techniques.

Sample clean-up with sulfuric acid is necessary to remove lipid interferences. Passing the concentrated extract through acid silica-filled SPE cartridges was preferred whenever possible due to short processing time. However, diet, human milk and PBET samples were found to contain high lipid concentrations, hence a 1:1 *v/v* sulfuric acid wash followed by passing over a florisil column was necessary for complete lipid removal.

2.4 Analysis.

2.4.1 LC-ESI-MS/MS analysis for determination of HBCDs and TBBP-A.

Separation of α-, β-, γ-HBCDs and TBBP-A was achieved using a dual pump Shimadzu LC-20AB Prominence high pressure liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with SIL-20A autosampler, a DGU-20A3 vacuum degasser and a Varian Pursuit XRS3 (Varian, Inc., Palo Alto, CA, USA) C_{18} reversed phase analytical column (150 mm × 2 mm i.d., 3 µm particle size). A mobile phase program based upon (a) 1:1 methanol/water and (b) methanol at a flow rate of 150 µL min^{-1} was applied for elution of the target compounds; starting at 50% (b) then increased linearly to 100% (b) over 7 min, held for 4 min followed by a linear decrease to 60% (b) over 4 min, held for 1 min and finishing with 100% (a) for 10 min. TBBP-A and the three HBCD diastereomers were baseline separated with retention times of 9.0, 10.6, 11.2 and 11.7 min for TBBP-A, α-, β- and γ-HBCD, respectively (figure 2.6).

Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization (ESI) ion source operated in negative ion mode. Direct infusion of the target compounds (TBBP-A, ^{13}C-TBBP-A, α-, β- and γ- HBCDs, native, d_{18}-labelled and ^{13}C- labelled isomers, 2 ng μL^{-1} each in methanol) into the MS/MS system was performed using a built-in Harvard syringe pump at a flow rate of 10 µL min^{-1}. The infusion experiments served for tuning the instrument and adjusting the source and the compound-specific parameters during the analytical method development (table 2.6). MS/MS detection operated in the multiple reaction monitoring (MRM) mode was used for quantitative determination of the target compounds based on *m/z* 640.6→79, *m/z* 652.4→79 and *m/z* 657.7→79 for the native, ^{13}C-labelled and d_{18}-labelled HBCD diastereomers, respectively and *m/z* 540.8→79, *m/z* 552.8→79 for the native and ^{13}C-labelled TBBP-A, respectively.

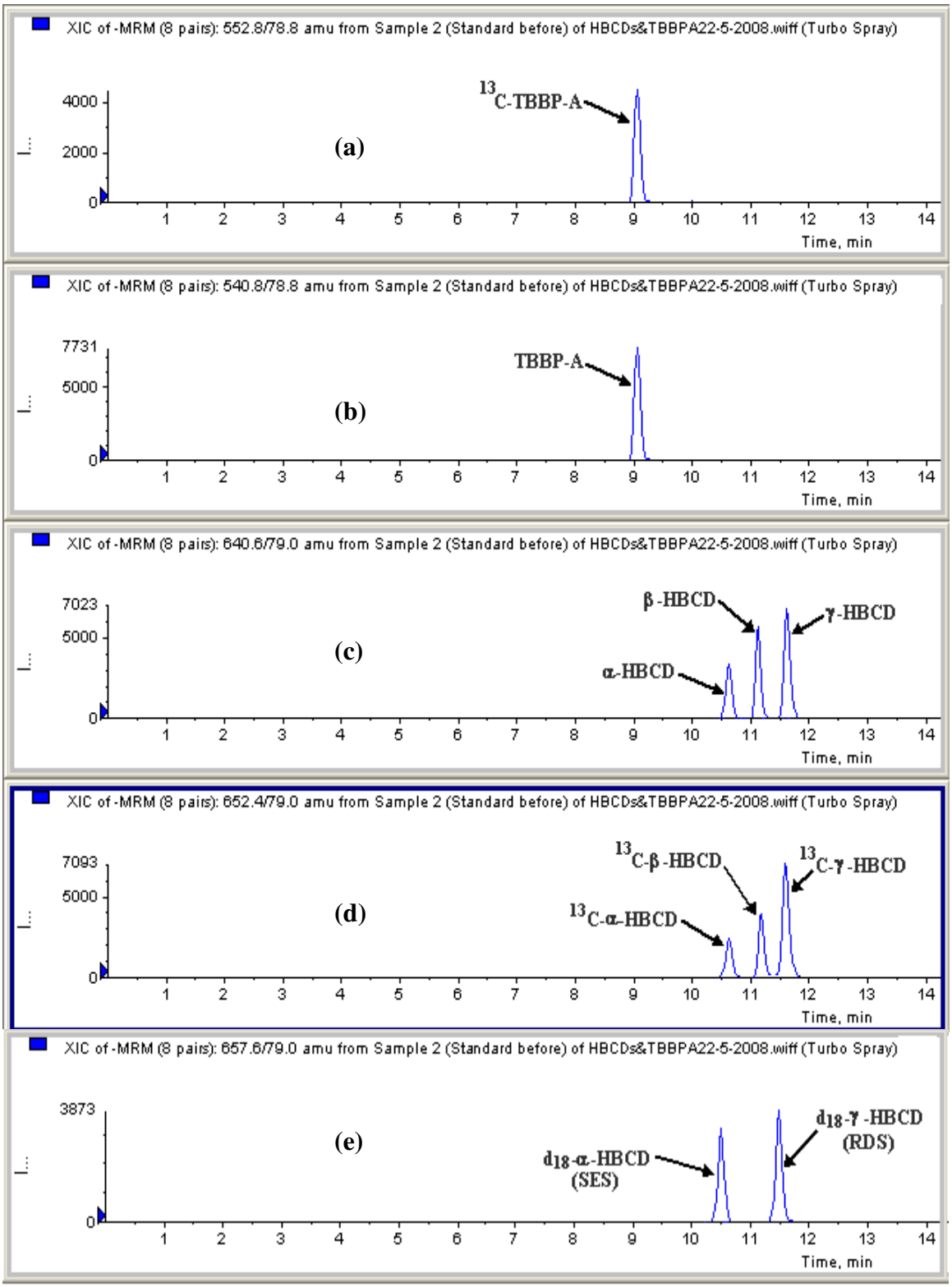


Figure 2.6: LC-ESI-MS/MS extracted ion chromatograms (20 pg on column) of (a) TBBP-A (b) ^{13}C-TBBP-A (c) HBCDs (d) ^{13}C-HBCDs and (e) d_{18}- HBCDs.

Table 2.6: Optimised MS/MS parameters for the analysis of HBCDs and TBBP-A.

Parameter	Value (units)
Curtain gas	35 (a.u.)*
Turbo gas temperature	500 (°C)
Ion spray voltage	- 4500 (V)
Declustering potential	-5 (V)
Focusing potential	-365 (V)
Collision gas	5 (a.u.)
Collision energy	40 (eV)
Cell entrance potential	-6 (V)
Collision cell exit potential	-10(V)

* a.u. – arbitrary units

2.4.2 Separation and identification of HBCD degradation products in indoor dust.

The thermal isomerisation and degradation of HBCDs at the high temperatures used during the GC/MS analysis have been reported previously (Koppen et al., 2008). These degradation products are observed in the GC–ECNI/MS chromatograms for both samples and standards (figure 2.7a,b) which makes it very difficult to determine if these degradation products are present originally in the samples or produced as a result of thermal degradation in the GC system.

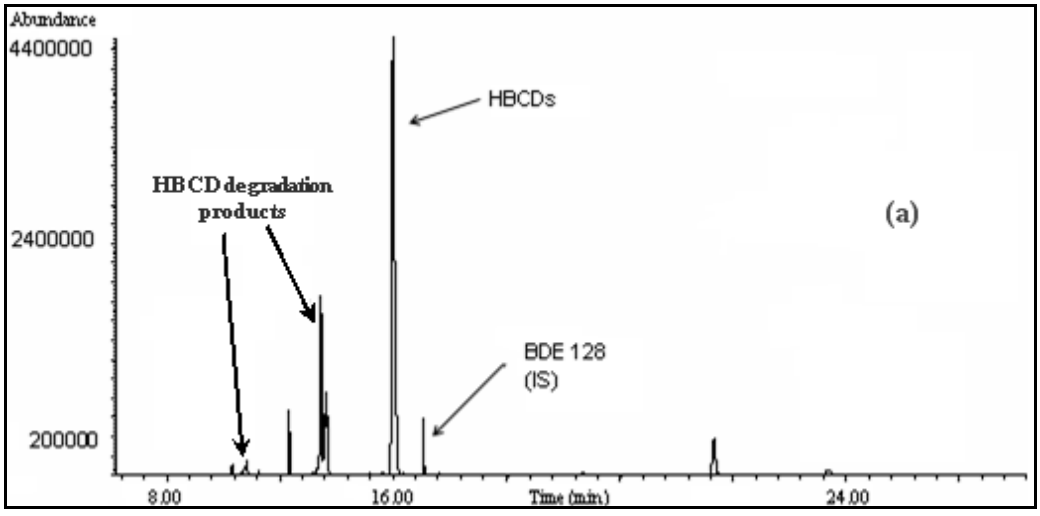


Figure 2.7a: GC/ECNI/MS chromatogram of *m/z* 79 of a dust sample showing HBCDs and degradation products.

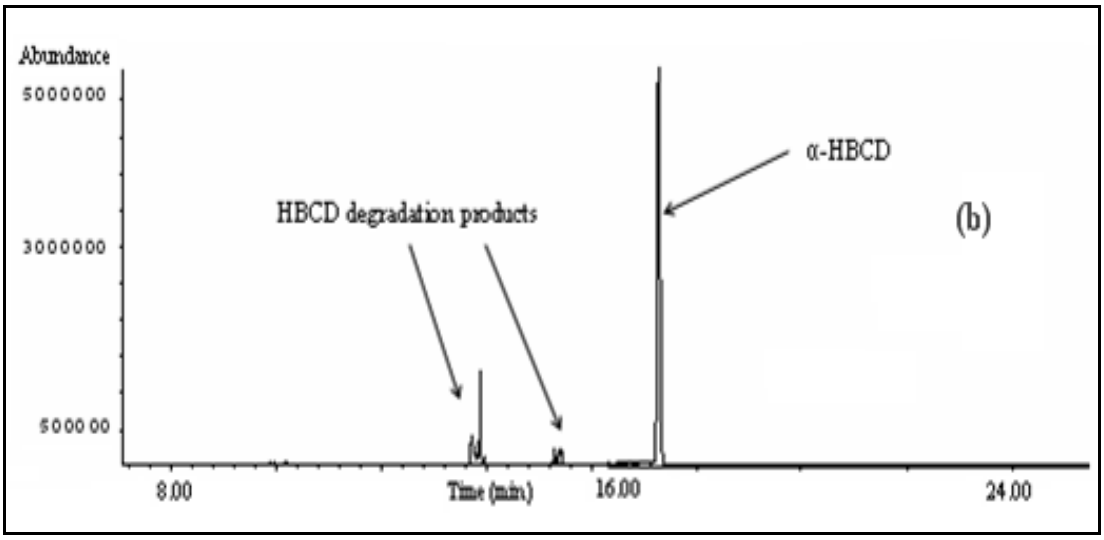


Figure 2.7b: GC/ECNI/MS chromatogram of *m/z* 79 for α-HBCD standard (1 ng μL^{-1} in iso-octane) showing degradation products.

The identity of the degradation products was investigated by GC–MS which separated the degradation products from HBCDs (figure 2.8) and provided their full scan mass spectra (figure 2.9). This was further confirmed by obtaining identical MS/MS chromatograms with appropriate isotope ratios for more than one MRM transition (figure 2.10).

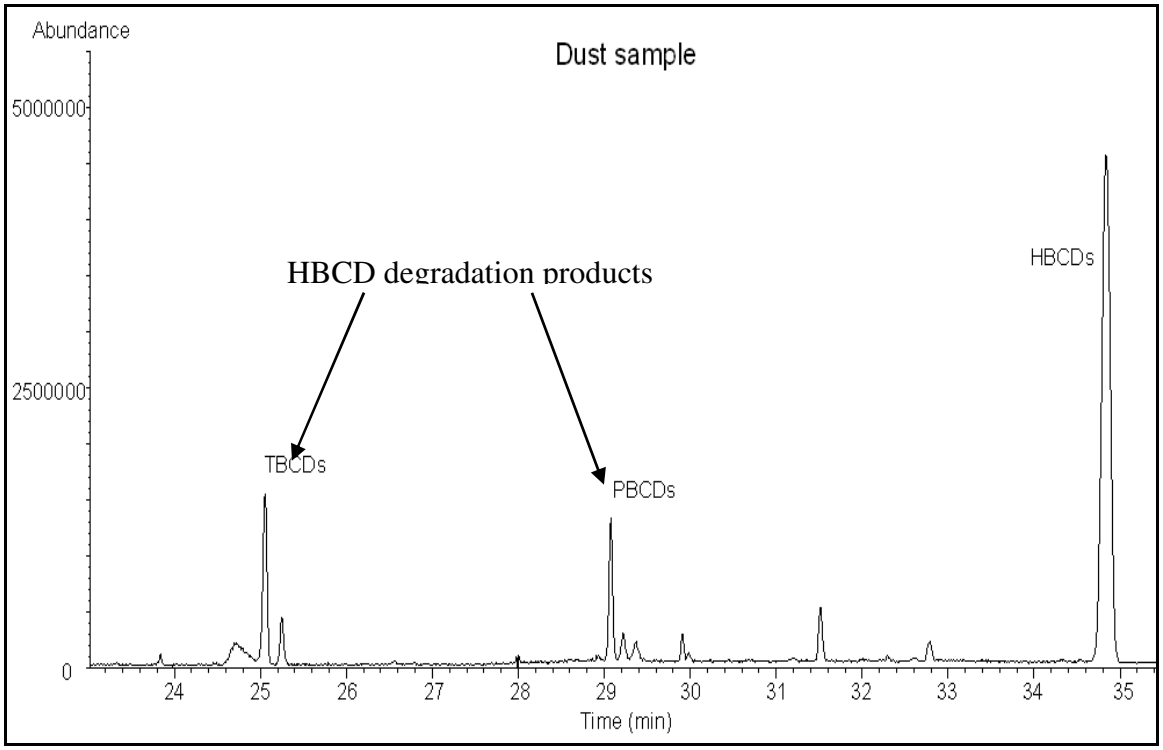


Figure 2.8: GC-EI/MS chromatogram of a dust sample showing HBCDs and the degradation products (PBCDs and TBCDs).

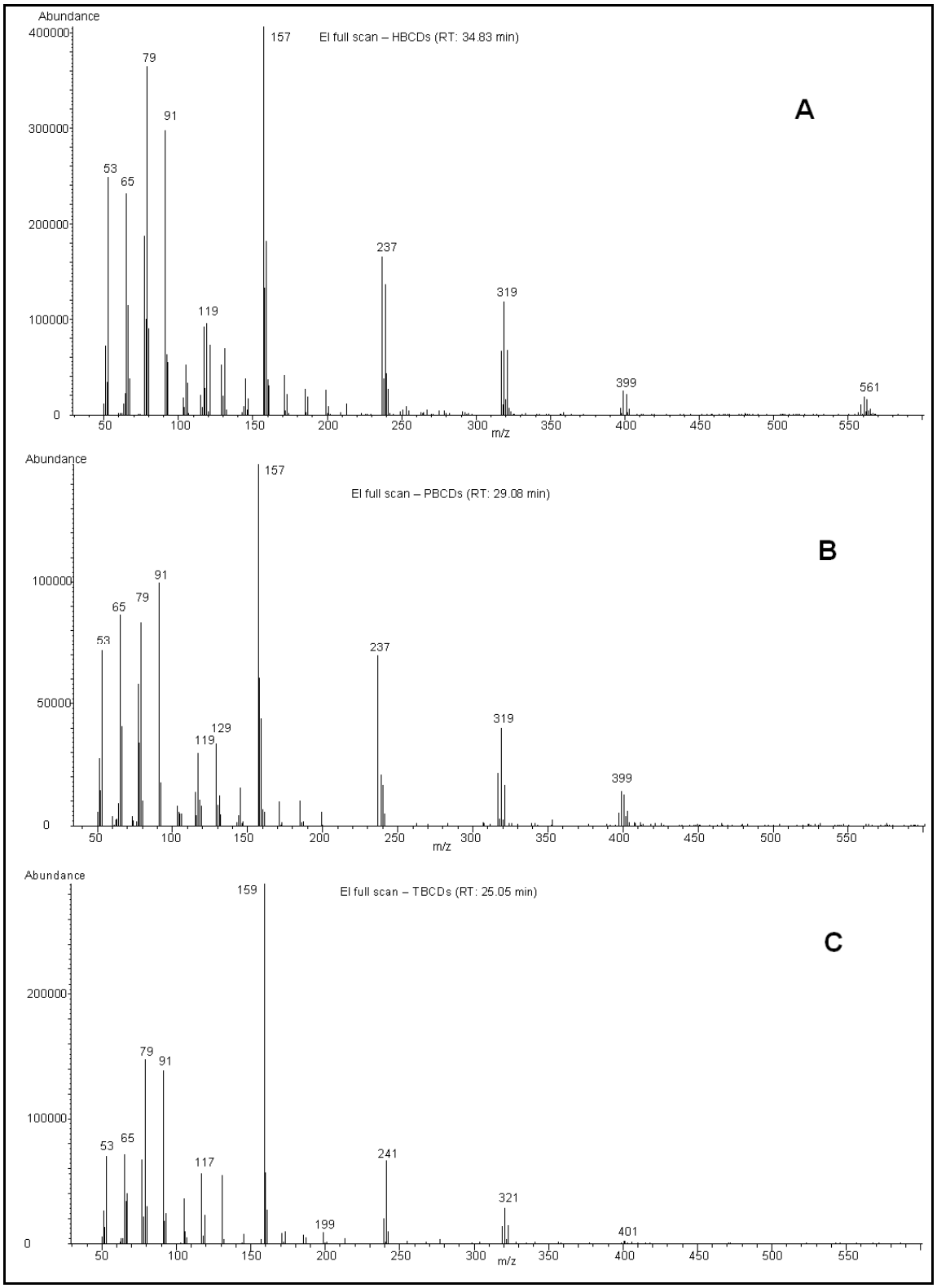


Figure 2.9: Full scan EI-MS spectra of (A) HBCDs, (B) PBCDs and (C) TBCDs in a dust sample.

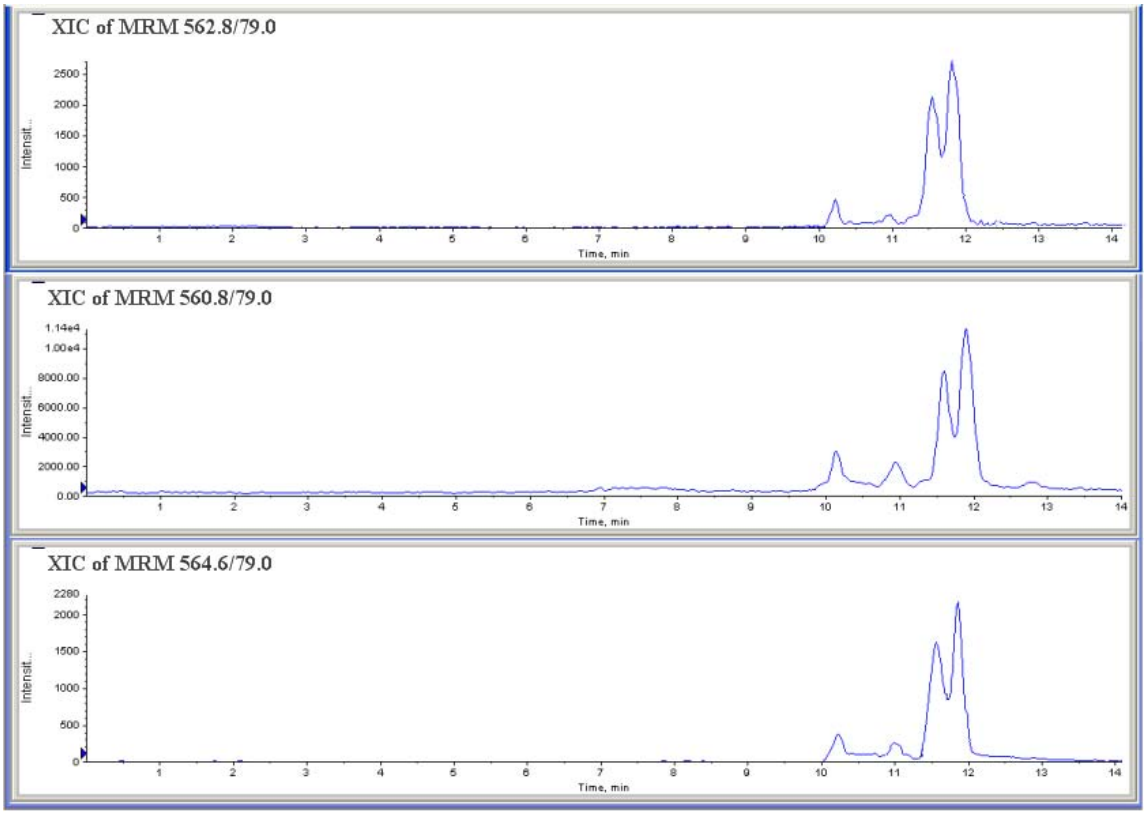


Figure 2.10: LC-MS/MS identical chromatograms of 3 MRM transitions monitoring HBCD degradation products (PBCDs).

The issue of whether these products are merely formed in the GC system may be resolved by the use of LC–MS/MS, during which no thermal degradation occurs and therefore, no degradation products are observed in the chromatograms of standards (figure 2.11). Two different degradation products were observed in almost all dust samples and in SRM 2585. The products thus identified are pentabromocyclododecenes (PBCDs) and tetrabromocyclododecadienes (TBCDs), which were monitored at transitions *m/z* 560.8→79 and *m/z* 480.4→79, respectively.

The mass spectra of HBCDs, PBCDs and TBCDs are very similar suggesting that these compounds have a similar structure (Figure 2.9). Furthermore, the low-mass fragment ions at *m/z* 53, 65/67, 79, 91, 105, and 117 strengthens further the assumption of an aliphatic backbone for the degradation products. At higher masses, the fragment ions at *m/z* 317 (2 Br) and *m/z* 399 (3 Br) are also in agreement with the mass spectrum of HBCDs. Unfortunately, GC-MS does not allow the correct identification of the number of PBCD or TBCD isomers, since, similar to HBCDs, these compounds also co-elute. No

peaks were detected for lower brominated (1-3 Br atoms) degradation products of HBCDs in the studied dust samples.

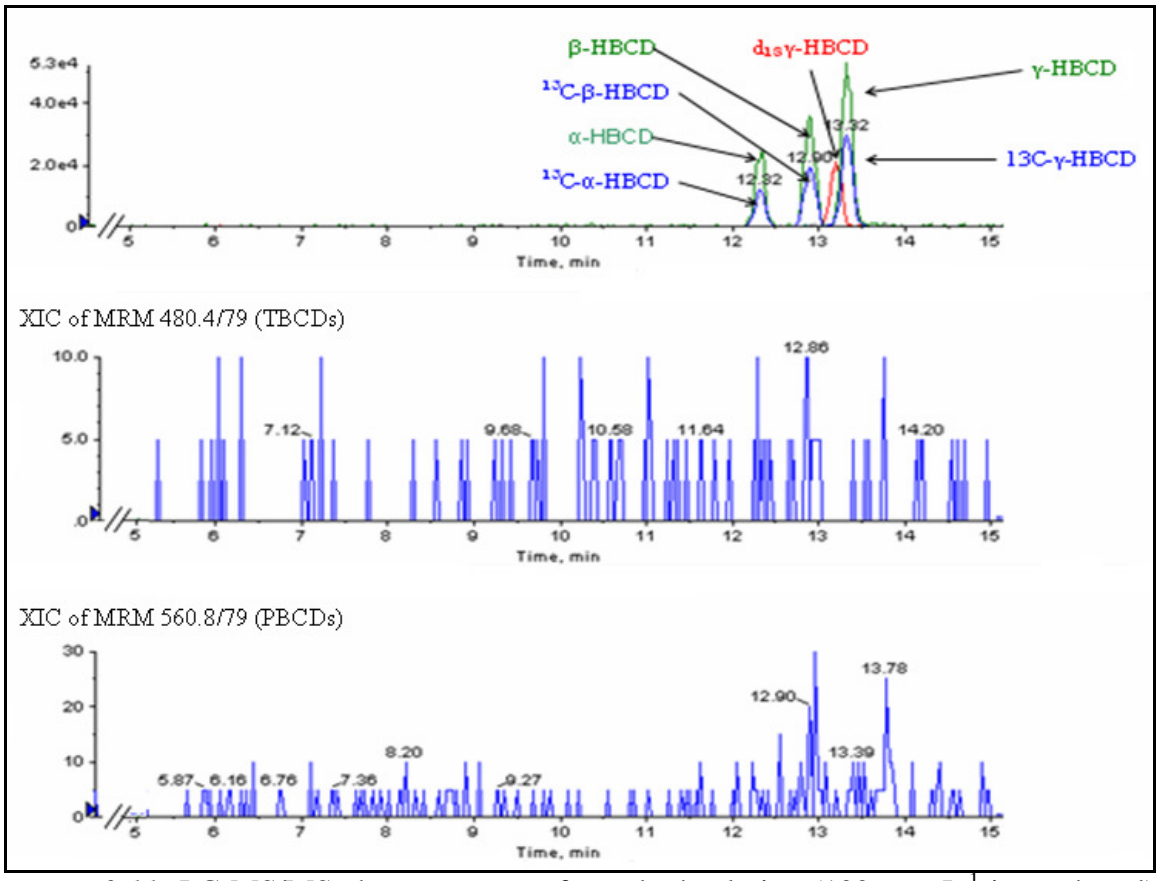


Figure 2.11: LC-MS/MS chromatogram of standard solution (100 pg μL^{-1} in methanol) of ^{12}C-, ^{13}C-HBCDs and d_{18}-γ-HBCD showing baseline separation of the 3 HBCD diastereomers and the absence of degradation products (MRM 480.4/79 for TBCDs and MRM 560.8/79 for PBCDs).

The LC-MS/MS chromatograms of PBCDs (figure 2.12) showed 4 well-resolved peaks indicating the presence of at least 4 major isomers. The PBCDs were well separated from the parent HBCDs on retention time basis (figures 2.11 and 2.12), with the PBCDs eluting between 10.13 and 11.90 min and the HBCDs between 12.32 and 13.32 min. The MS/MS technique enabled further structural confirmation through the monitoring of the PBCDs at *m/z* 560.8→79.0 (figure 2.10), which differs from that of the parent HBCDs monitored at *m/z* 640.9→79.0. These peaks were assigned the names of PBCD1 to PBCD4, with the first eluting isomer being PBCD1. PBCDs were reported previously by Barontini et al. (Barontini et al., 2001a, Barontini et al., 2001b) as thermal degradation

products of HBCDs having a molecular weight of 561 g/mol and a total number of 7 isomers detected and identified via GC-MS.

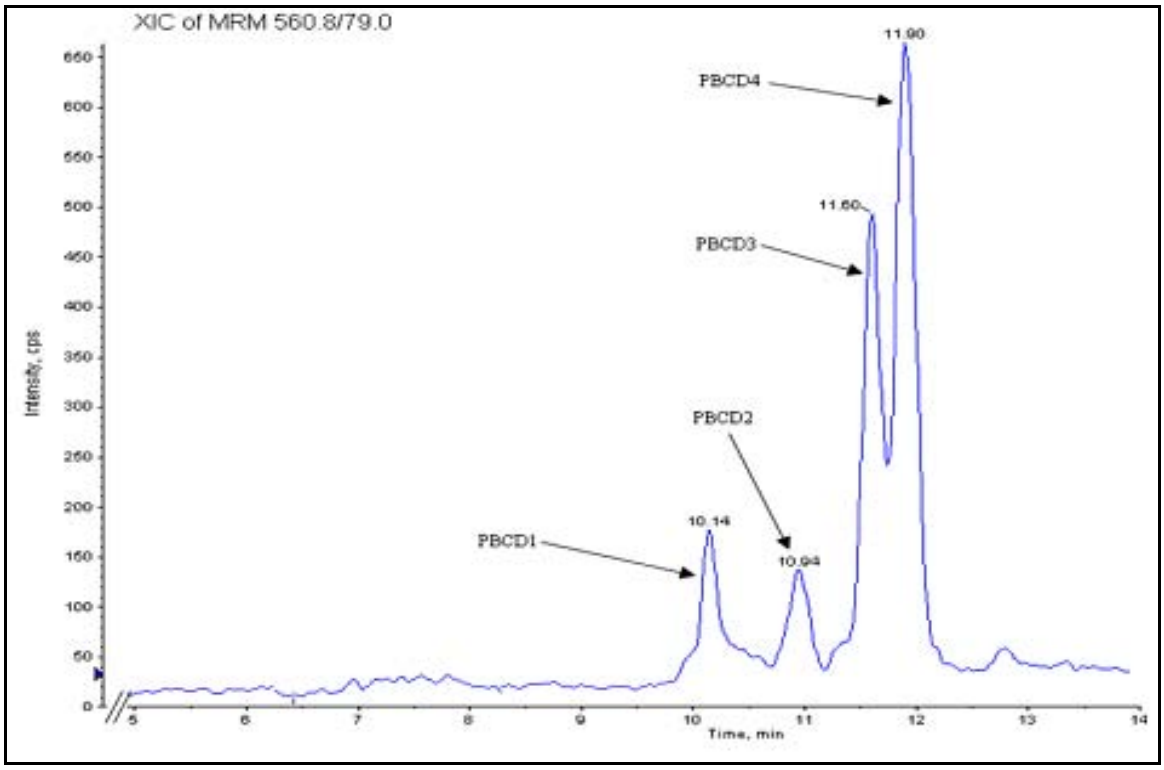


Figure 2.12: LC-MS/MS chromatogram of a dust sample showing PBCD isomers (MRM 560.8 to 79).

Hiebl and Vetter (Hiebl and Vetter, 2007) also detected PBCD (one isomer) as a metabolite/degradation product of HBCDs in chicken egg and in fish via GC-EI/MS. However, in the present study, 4 PBCD isomers were detected and identified via LC-MS/MS indicating that the degradation pathways of HBCDs under ambient environmental conditions are different from those induced under the drastic thermal conditions (300-400 °C) employed by Barontini *et al.* (Barontini et al., 2001a, Barontini et al., 2001b). This can also explain the difference in the profiles of degradation products obtained by GC-MS and LC-MS/MS analysis of dust samples. During the course of a GC run, the sample is exposed to high temperatures and the degradation products may be different from those originally present in the sample and detected as such by the LC-MS/MS.

Efforts were made to base-line resolve PBCD3 from PBCD4 on the LC column by slightly changing the solvent gradient and flow rate. However, since this was not

possible, no further trials were made to avoid alterations of the method parameters that might affect the separation and retention times of the main target compounds, in this case the α-, β- and γ- HBCD isomers.

Only two peaks were observed for TBCDs in chromatograms indicating the presence of 2 congeners (Figure 2.13). However, the first eluted peak (TBCD1) always was less intense than the second peak (TBCD2), which is also observed much more frequently in the dust samples. The detection of these 2 TBCD isomers in dust samples indicates that the degradation pathways of HBCDs in indoor dust are different from those in waste water sludge and freshwater sediments in which Davis et al. (Davis et al., 2006) reported only one TBCD isomer as a degradation product of HBCDs (via LC-APPI/MS). They concluded that HBCD degrades via a dibromoelimination reaction resulting in the loss of two bromines from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms.

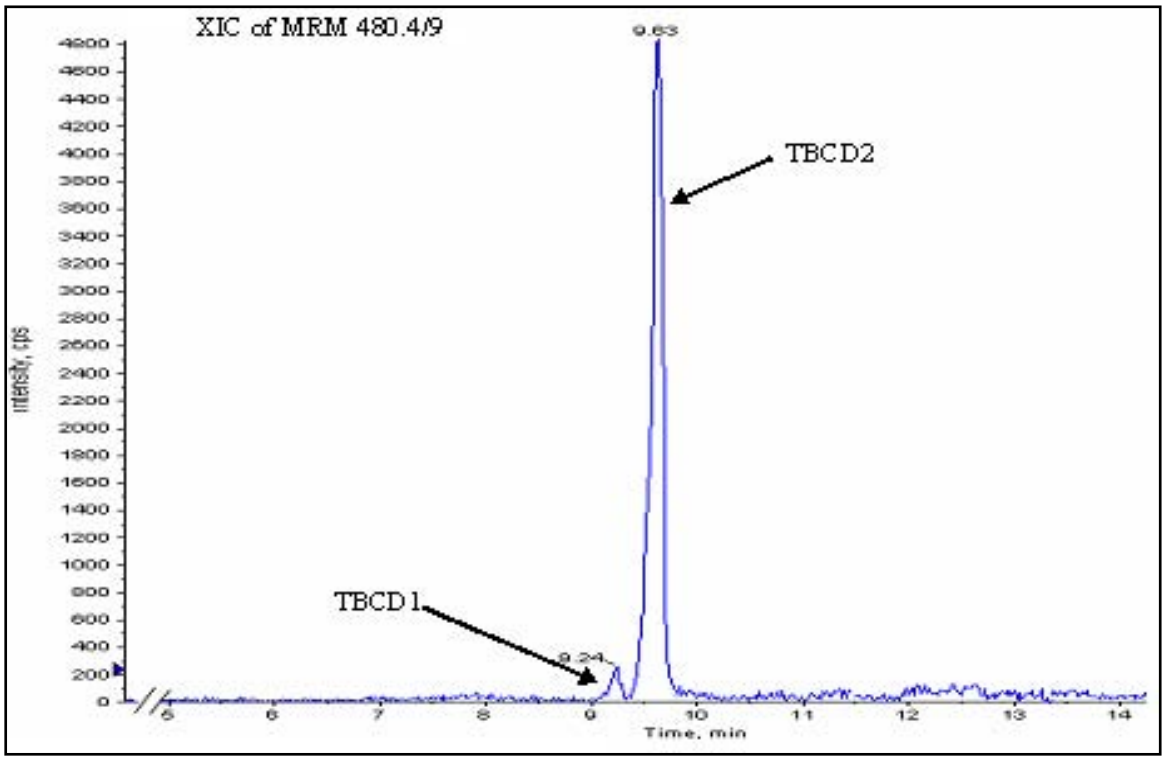


Figure 2.13: LC-MS/MS chromatogram of SRM 2585 (house dust) showing TBCD isomers (MRM 480.4 to 79).

However, the detection and identification of the 4 PBCDs together with the 2 TBCDs in the present study rules out vicinal debromination as the main degradation pathway of

HBCDs in indoor dust. The detection of degradation products, PBCDs and TBCDs, and their relationship to the parent HBCDs in the analyzed dust samples, favoUrs the elimination of HBr. This results in the formation of a double bond (adjacent to the initial carbon atoms containing Br) and is suggested to be a significant degradation pathway of HBCDs in indoor dust. This hypothesis is in agreement with the degradation pathway suggested by Hiebl and Vetter (Hiebl and Vetter, 2007) for HBCDs in chicken egg and white fish.

Accurate quantitation of the detected degradation products was not possible due to the lack of either native or labeled standards for these compounds which are (to the authors' knowledge) reported here in indoor dust for the first time. Furthermore, similar to HBCDs, the LC-MS/MS method allows the detection of different PBCD and TBCD diastereomers.

2.4.3 Determination of Chiral Signatures of HBCDs.

Separation of HBCD enantiomers was performed on a chiral permethylated *β*-cyclodextrin LC column (200 mm x 4 mm I.D., 5 µm particle size) (NUCLEODEX beta-PM, Macherey-Nagel; GmbH & Co, Düren, Germany). A mobile phase of (a) 1:1 methanol/water with 2 mM ammonium acetate and (b) 3:7 methanol/acetonitrile at a flow rate of 500 µL/min was applied for elution of the target compounds; starting at 50 % (b) then increased linearly to 100 % (b) over 4.5 min, held for 5.5 min, followed by a linear decrease to 65 % (b) over 4 min and held for 2 min. Mass spectrometric analysis was performed as described under 2.4.1. Good enantiomeric separation of the 3 main HBCD diastereomers was observed (figure 2.14). The order of elution of separated enantiomers was previously identified and confirmed in literature (Janak et al., 2005, Marvin et al., 2007, Heeb et al., 2005). ESI-based analysis of HBCDs is well-known to be affected by sample matrix that can cause enhancement or suppression of the target analytes signal and can adversely affect their quantification. However, these matrix-related effects can be accounted for by using isotopically-labelled isomers as internal standards due to their identical behavior to their native counterparts in the ESI (Tomy et al., 2005). Therefore, the enantiomer fractions (EFs) reported in this study are corrected using the responses of

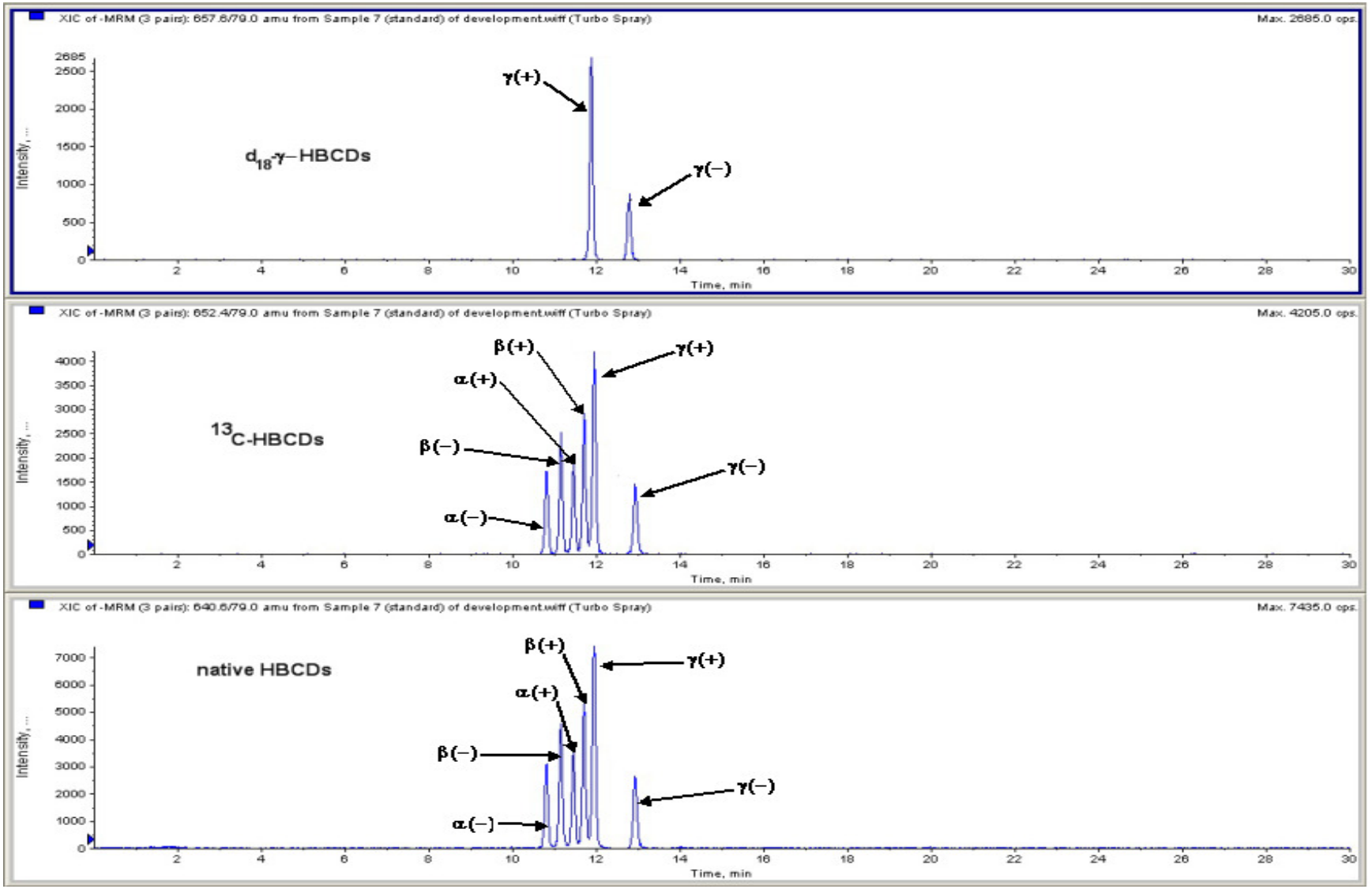


Figure 2.14: LC-ESI-MS/MS chromatogram showing enantiomeric separation of HBCDs standards (50 pg on-column each in methanol).

the corresponding ^{13}C-labelled standards as described by Marvin et al. (Marvin et al., 2007). The corrected EFs are calculated using equation 2.

$$EF_{corrected} = \frac{[(A^+ / A^+_{labelled}) x (pgA^+_{labelled})]}{[(A^+ / A^+_{labelled}) x (pgA^+_{labelled})] + [(A^- / A^-_{labelled}) x (pgA^-_{labelled})]} \quad ....(2)$$

Where A^+ is the peak area of the (+) enantiomer, $A^+_{labelled}$ is the peak area of the labelled (+) enantiomer, $pgA^+_{labelled}$ is the mass of labelled isomer added in picograms, A^- is the peak area of the (-) enantiomer, $A^-_{labelled}$ is the peak area of the labelled (-) enantiomer and $pgA^-_{labelled}$ is the mass of labelled isomer added in picograms.

2.4.4 LC-APPI-MS/MS analysis for determination of BDE 209.

BDE 209 was separated from other PBDEs using a dual pump Shimadzu LC-20AB Prominence liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with SIL-20A autosampler, a DGU-20A3 vacuum degasser and a Varian Pursuit XRS3 (Varian, Inc., Palo Alto, CA, USA) C_{18} reversed phase analytical column (250 mm × 4.6 mm i.d., 3 µm particle size). A mobile phase program based upon (a) 85:10:5 methanol/toluene/water and (b) 1:1 methanol/water at a flow rate of 500 µL min^{-1} was applied for elution of the target compound; starting at 85% (a) then increased linearly to 100% (a) over 30 min then held for 25 min. The column was equilibriated with 85% (a) for 5 min between runs. Mass spectrometric analysis was performed using a Sciex API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an atmospheric pressure photoionization (APPI) ion source operated in negative ion mode. Toluene was used as a doping agent introduced to a separate dopant port of the APPI source using a dedicated isocratic HPLC pump (Jasco PU-2800, Easton, MD, USA) at 12% of the flow rate of the mobile phase used. Direct infusion of the target compounds (BDE 209 and ^{13}C- labelled isomer, 2 ng µL^{-1} each in methanol) into the MS/MS system was performed using a built-in Harvard syringe pump at a flow rate of 10 µL min^{-1}. The infusion experiments served for the tuning and adjusting the source and the compound-specific parameters during method development (table 2.7).

Table 2.7: Optimized MS/MS parameters for the analysis of BDE 209.

Parameter	Value (units)
Curtain gas	25 (a.u.)*
Collision (CAD) gas	High
Temperature	400 (°C)
Ion spray voltage	- 1250 (V)
Probe nebuliser gas	60 psi
Auxiliary gas	30 psi
Declustering potential (DP)	-8 (V)
Collision energy	75 (eV)
Cell entrance potential	-12 (V)
Collision cell exit potential	-8(V)

- a.u. – arbitrary units

MS/MS detection operated in the MRM mode was used for quantitative determination based on *m/z* 486.6→78.8 and *m/z* 494.7→78.8 for the native and ^{13}C-labelled BDE 209 respectively (figure 2.15).

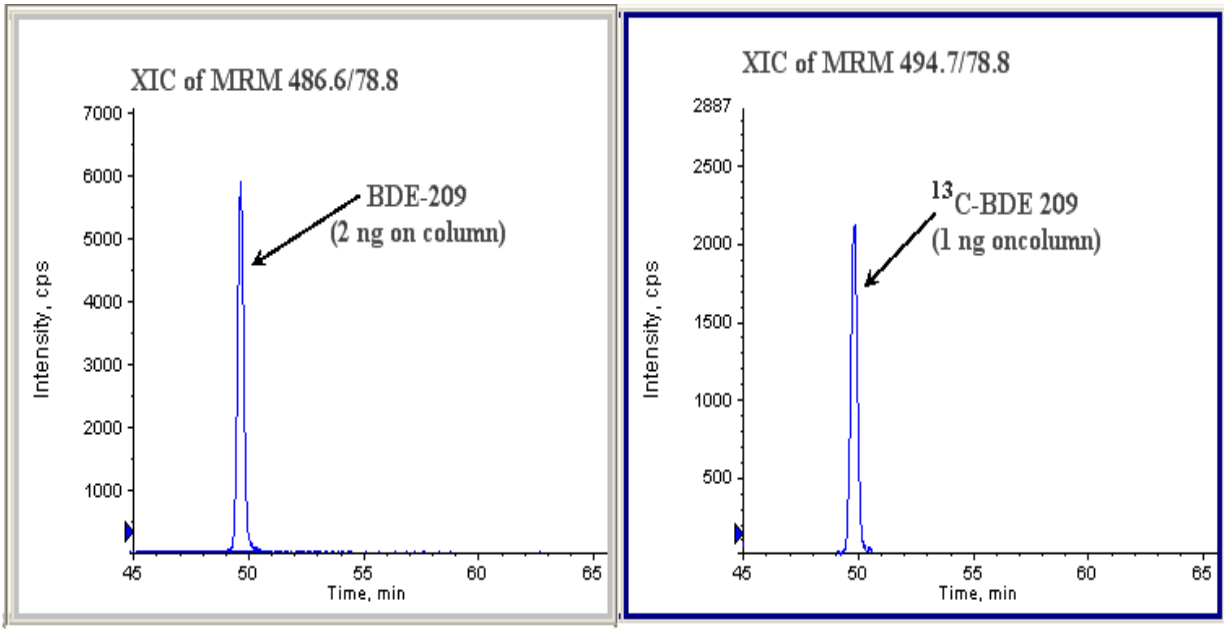


Figure 2.15: LC-APPI-MS/MS chromatograms of (a) BDE 209 and (b) ^{13}C-BDE 209.

2.4.5 GC-ECNI/MS analysis for determination of BDE 209 in dust samples.

This was conducted at the University of Antwerp, based on a method reported previously. (Covaci et al., 2005). Briefly, BDE 209 was determined using an Agilent 6890GC-5973MS equipped with a 15m DB-5 column (0.25 mm id, 0.10 μm film thickness) and operated in electron capture negative ionisation (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. Helium was used as carrier gas at constant flow (1.0 mL min^{-1}) with methane as moderating (or reagent) gas. The MS was operated in SIM mode and the electron multiplier voltage set at 2100 V. One μL of the extract was injected in solvent vent mode (injector temperature at 90 °C for 0.06 min, then increased at 700 °C min^{-1} to 305 °C, vent time 0.04 min, vent flow 75ml min^{-1}). The splitless time was 1.5 min. The GC temperature program was 90 °C for 1.5 min, then 15 °C min^{-1} to 295 °C for 15 min. Dwell times were 40 ms. Ions m/z 484.7/486.7 and 494.7/496.7 for BDE 209 and ^{13}C-BDE 209, respectively, were monitored for the entire run (figure 2.16).

2.4.6 Rationale for LC-MS/MS use for analysis.

Diastereomer-specific analysis of HBCDs is impossible using GC/MS techniques due to thermal rearrangement, isomeric interconversion and degradation of HBCDs at the high temperatures used during the course of GC analysis (Budakowski and Tomy, 2003, Morris et al., 2006). Therefore, LC-MS/MS is considered the method of choice (Covaci et al., 2007) for diastereomer- and enantiomer-specific determination of HBCDs where no high temperatures are required. This method also allows the use of isotopically labelled HBCDs as internal standards which perform a dual role by compensating for: (a) any variability in instrumental response of the mass spectrometer between injections and (b) matrix related ion suppression or enhancement effects that can occur in the ion source (Tomy et al., 2005).

For TBBP-A analysis, acidification and derivatisation steps are necessary before analysis by GC-based techniques. These two steps can produce errors and/or losses of the analyte, thus LC-MS/MS methods have the advantage that no derivatisation step is required (de Boer and Wells, 2006).

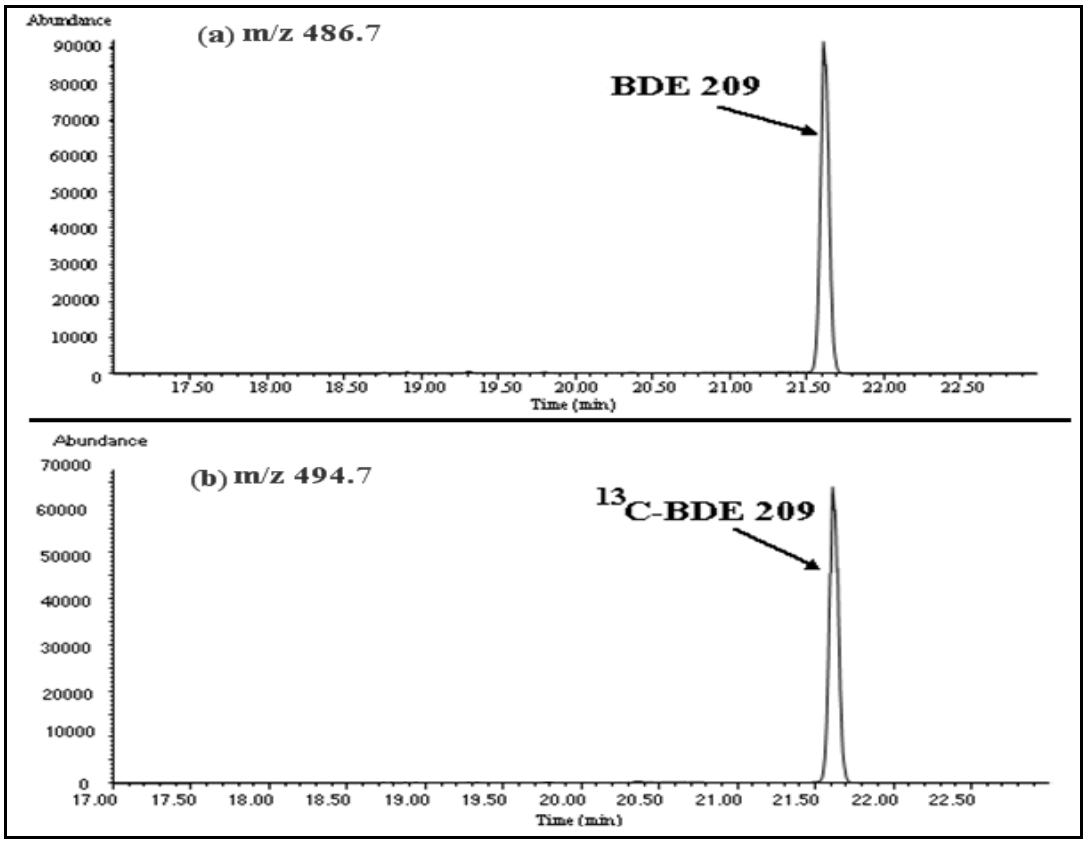


Figure 2.16: GC-ECNI/MS chromatograms of (a) BDE 209 and (b) ^{13}C-BDE 209 in a dust sample.

2.5 Validation and QA/QC Criteria.

2.5.1 Analyte identification and Quantification criteria.

Identification of each target compound and confirmation of the specific retention time (t_R) for each analyte peak to be used in quantification was carried out by separate injections of pure individual standards (2 ng on column) of each target compound.

A full 5-point calibration was conducted for each of the studied compounds in a concentration range of 20-5000 pg μL^{-1} to assess the linearity of the MS response. ^{13}C-labelled isomers were used as internal standards for each of the studied compounds because these labelled standards can compensate for both instrumental fluctuations of the MS system and matrix-related ion suppression or enhancement effects that can occur in the ion source (Covaci et al., 2007, Tomy et al., 2005). Excellent linearity was observed over the studied range as indicated by $R^2 > 0.99$ for all of the target compounds.

These 5 calibration points were used to calculate relative response factors (RRFs) for each of the target compounds. The RRF is defined as the instrument response for a unit amount of target pollutant relative to the instrument response obtained for the same amount of the internal standard (IS). RRF is calculated as in equation 3.

$$RRF = \frac{A_{NAT}}{A_{IS}} \times \frac{C_{IS}}{C_{NAT}} \quad \text{.....(3)}$$

Where A_{NAT} is the peak area for the native compound (i.e. the ^{12}C or ^{1}H isotope of the target compound. The term is used to distinguish it from the ^{13}C or deuterated isotope used as internal or recovery determination standards); A_{IS} is the peak area of the internal standard; C_{NAT} is the concentration of the native compound; and C_{IS} is the concentration of the internal standard. The relative standard deviation (RSD) of the RRFs calculated for each target compound at the 5 points of its calibration curve did not exceed 5%.

A calibration standard (50 pg μL^{-1} of native TBBP-A, BDE 209, α-, β-, γ- HBCDs, and their ^{13}C- labelled isomers in addition to d_{18}-γ-HBCD used as RDS and d_{18}-α-HBCD used as SES) was injected before and after each sample batch. The average RRFs of the target compounds from these two injections are calculated (These must be within ± 25% of the average RRFs obtained for that standards in the initial 5-point calibration) and used for calculating the concentrations of the target compounds in samples of this batch via equation 4.

$$Concentration = \frac{A_{NAT}}{A_{IS}} \times \frac{1}{RRF} \times \frac{M_{IS}}{SS} \quad \text{.....(4)}$$

Where A_{IS} = peak area of internal standard in sample; A_{NAT} = peak area of target pollutant in sample; RRF = relative response factor for the target pollutant; M_{IS} = mass of internal standard added to sample (pg or ng) and SS = sample size (m^3 or g).

For a given peak to be identified as a target pollutant in a sample, the following criteria needed to be met:

1. The signal to noise ratio (S/N) must exceed 3:1.
2. The bromine isotope ratios must be within ± 20% of the average for the 2 calibration standards ran before and after that sample batch.

3. The relative retention time (RRT) of the peak in the sample must be within ± 0.2% of the average value determined for the same congener in the 2 calibration standards ran before and after that sample batch.

2.5.2 Sampling evaluation standard (SES).

In order to assess any losses or breakthrough of analytes associated with both passive and active air sampling techniques used in this study, PUF disks and GFFs were treated with 20 ng of d_{18}-α-HBCD and BDE 128 prior to field deployment. d_{18}-α-HBCD was used as SES for α-, β-, γ- HBCDs and TBBP-A while BDE 128 was used as SES for BDE 209.

2.5.3 Recovery determination (syringe) standard (RDS).

The recoveries of both SES and IS during sampling or sample preparation were determined relative to the RDS added to the samples just before MS analysis. In this study, d_{18}-γ-HBCD was used as RDS for α-, β-, γ- HBCDs and TBBP-A while ^{13}C-BDE 100 was used as RDS for BDE 209. The recoveries of the IS in each sample were calculated using equation 5.

$$\%\ \text{IS Recovery} = \left[\left(\frac{A_{IS}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{IS}}\right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}}\right)_S\right] \text{x } 100 \text{.....(5)}$$

where $(A_{IS}/A_{RDS})_S$ = ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ = ratio of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration standards run before and after this batch of samples); $(C_{IS}/C_{RDS})_{STD}$ = ratio of concentration of internal standard to concentration of recovery determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ = ratio of concentration of recovery determination standard to concentration of internal standard in the sample.

For air samples, the recoveries of SES were calculated using equation 6.

$$\%\ \text{SES Recovery} = \left[\left(\frac{A_{SES}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{SES}}\right)_{STD} \times \left(\frac{C_{SES}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{SES}}\right)_S\right] \text{x } 100 \text{.....(6)}$$

where $(A_{SES}/A_{RDS})_S$ = ration of sampling evaluation standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{SES})_{STD}$ = ratio of recovery determination standard peak area to sampling evaluation standard peak area in the calibration standard (the average of values obtained for both calibration standards run before and after this batch of samples); $(C_{SES}/C_{RDS})_{STD}$ = ratio of concentration of sampling evaluation standard to concentration of recovery determination standard in the calibration standard and $(C_{RDS}/C_{SES})_S$ = ratio of concentration of recovery determination standard to concentration of sampling evaluation standard in the sample. A statistical summary of the IS and SES recoveries in all samples is given in table 2.8.

Table 2.8: Descriptive statistics of IS and SES recoveries (expressed as %) in the studied samples.

Standard	Average	SD*	Median	Minimum	Maximum
^{13}C-α-HBCD[a]	92	7	87	73	103
^{13}C-β-HBCD[a]	87	11	84	75	97
^{13}C-γ-HBCD[a]	88	12	87	68	106
^{13}C-TBBP-A[a]	94	6	85	70	105
^{13}C-BDE 209[a]	87	11	82	65	103
BDE 128[b]	89	10	86	74	97
d_{18}-α-HBCD [b]	93	12	81	75	105

* Standard deviation.
[a] IS and
[b] SES.

2.5.4 Accuracy and precision.

In the absence of an appropriate standard reference material for TBBP-A and HBCDs, the accuracy and precision of the analytical method for these contaminants (and BDE-209) was assessed as follows. HBCDs and BDE 209 were assessed via replicate analysis (n=10) of NIST standard reference material (SRM 2585, organic contaminants in house dust). The results obtained (table 2.9a) compared favourably with the certified value for BDE 209 and indicative values for HBCDs reported elsewhere (Keller et al., 2007). For TBBP-A, a standard addition or "matrix spike" method to SRM 2585 at 3 concentration levels (n=5 at each level) was used to assess the accuracy and precision of the method

and good results were obtained (table 2.9b). The low values of RSD obtained (<20%) indicate the good precision of the applied methods.

Table 2.9a: Concentrations (ng g^{-1}) of BDE 209 and HBCDS in NIST SRM2585 compared to the certified and indicative values (Keller et al., 2007)

	Average ± standard deviation (n=10)		**RSD (%) of measured values**
	Measured	**Indicative**	
α-HBCD	20 ± 3.1	19 ± 3.7	15.5
β-HBCD	4.3 ± 0.5	4.3 ± 1.1	11.6
γ-HBCD	121 ± 19	120 ± 22	15.7
	Measured	**Certified**	
BDE 209	2460 ± 95	2510 ± 190	3.8

Table 2.9b: Summary of standard addition method results for TBBP-A in SRM 2585*.

Mass added (ng)	**Mass recovered (ng)**	**Recovery (%)**	**RSD (%) (n=5)**
10	8.5	85	2.8
40	37.3	92.5	3.2
80	79.1	98.8	3.1

*Estimated TBBP-A concentration in SRM2585 = 245 ng g^{-1} ± 7.4 (this study).

In samples where particularly elevated concentrations of any of the target compounds were found (i.e. such that the internal standard "spiking" levels were inappropriately low), a second aliquot of the sample in question was analysed using a smaller quantity of sample and a higher amount of internal standards. The results of the second analysis are reported in this study.

2.5.5 Analysis of blanks, LODs and LOQs.

Instrumental limits of detection (LOD) were calculated for each of the studied compounds based on a 3:1 signal to noise ratio and were 2.1, 1.8, 1.6, 1.3 and 12.7 pg on column for TBBP-A, α-, β-, γ-HBCD and BDE 209 respectively.

None of the target compounds were detected in method blanks (n=10) for air samples consisting of a pre-cleaned PUF disk and a GFF (for passive samplers) and a GFF and PUF plug (for active samplers) treated as a sample. Method limits of quantification

(LOQ) for TBBP-A, individual HBCD diastereomers and BDE 209 were therefore governed by a 10:1 signal to noise ratio. Based on a sampled air volume of 30 m^3, this equates to 1.8 pg m^{-3} for TBBP-A, 3.3 pg m^{-3} for ΣHBCDs and 6.7 pg m^{-3} for BDE 209.

Similarly, all of the studied BFRs were below LOQ in method blanks (n=25, dust is replaced by 0.2 g of pre-extracted Na_2SO_4) for dust analysis. Detectable, but very low concentrations of HBCDs and BDE 209 (typically 0.1-0.8 ng g^{-1}) were observed in field blanks (n=12). These consisted of Na_2SO_4 (0.2 g) "sampled" using the vacuum cleaner according to the standard protocol and treated as a sample. Concentrations in samples in each batch of 10 were thus corrected for the contamination detected in the associated field blank. LOQs for individual HBCD diastereomers and BDE 209 were governed by the field blanks (Calculated as average + 3 *x* SD of the field blanks) and were typically 0.1 and 0.5 ng g^{-1} respectively, while for TBBP-A the LOQ was 0.05 ng g^{-1} based on signal to noise ratio of 10:1.

None of the target BFRs was detected in method blanks for human milk (n=5), diet (n=3) and serum (n=3) samples. Hence, the LOQs were estimated based on signal to noise ratio of 10:1 and were 1.2, 3.4 and 5.9 pg g^{-1} lw in human milk, 8.2, 14.3 and 27.6 9 pg g^{-1} ww in diet and 41.6, 50.4 and 78.8 pg g^{-1} lw in serum for TBBP-A, ΣHBCDs and BDE 209 respectively.

2.6 Statistical Analysis.

Statistical analysis of the data was conducted using Excel (Microsoft Office 2003) and SPSS version 13.0. In all instances, where concentrations were below the LOQ, concentrations were assumed to equal half the LOQ. The distribution of each data set was evaluated using both the Kolmogorov-Smirnov test and visual inspection of the quantile-by-quantile graphic plot in SPSS. The results revealed concentrations in all data sets to be log-normally distributed. Hence, further ANOVA and t-tests were performed on log-transformed concentrations. Levene test of homogeneity of variances and the F-test were performed on log-transformed data to evaluate statistical significance of the differences in variance between the tested data sets. Subsequently, the differences in means among the studied data sets were statistically evaluated using the suitable posthoc test (e.g. Bonferroni test if equal variances assumed or Games-Howell test if equal variances not

assumed). Confidence limits were preset to 95% while the significance levels in SPSS were set at 0.05.

CHAPTER III

BROMINATED FLAME RETARDANTS IN INDOOR AIR AND DUST FROM HOMES, OFFICES, CARS AND PUBLIC MICROENVIRONMENTS IN BIRMINGHAM, UK

3.1 Synopsis

In this chapter, concentrations of α-, β-, γ-HBCDs, TBBP-A and BDE-209 in indoor air and dust samples from different microenvironments including homes, offices, cars and public microenvironments (3 pubs, 1 restaurant and 2 supermarkets) from Birmingham, UK will be reported for the first time. The relationship between the diastereomer profiles of HBCDs in the analysed air and dust samples will be studied and compared to that in technical formulations. Levels of target BFRs will be compared to each others and to those reported in previous studies (whenever possible).

3.2 Sampling strategy

3.2.1 Indoor air sampling

Air samples were collected between February 2007 and January 2009 in a total of 92 microenvironments within the West Midlands conurbation. Samples were taken under normal room use conditions to reflect actual human exposure. We selected the following microenvironment categories for study: homes (living rooms, n=35), offices (n=30), Cars (n=21) and PMEs (n=6, 3 Pubs, 1 restaurant and 2 local supermarkets). Outdoor air sampling (n=5) was performed in December 2007 at the Elms Road Observatory Site (EROS) in Birmingham, UK.

PUF-disk passive air samplers (part and full-sheltered configurations) were calibrated, validated and used to sample HBCDs in indoor air (see section 2.1.1.2.1 for details). They were employed to provide a time-integrated sample over a 30 day sampling period. However, during method development, it became evident that, unlike HBCDs, airborne

TBBP-A and BDE-209 are present mainly in the particulate phase. This is in agreement with previous reports (Sjodin et al., 2001, Covaci et al., 2007). Hence, PUF disk samplers which sample mainly the vapour phase of air are unsuitable for monitoring these two BFRs. Furthermore, the PUF disk passive sampling rates derived for monitoring HBCDs in indoor air cannot be extrapolated to sampling outdoor air. For these reasons, low volume active air samplers (see section 2.1.1.1.2 for details) were used for the purposes of monitoring concentrations of HBCDs in outdoor air, TBBP-A and BDE-209 in both outdoor and indoor air in homes, offices and PMEs. However, active air samplers couldn't be used for collecting air samples from cars due to technical problems in electric supply to the pumps. Furthermore, the use of these samplers in a confined space like cars is unlikely to reflect the actual exposure because all the air inside the car is likely to be sampled within the first 2-3 hours while the rest of the sampling period will have a "diluting" effect on the final result. Therefore, a third configuration of passive air samplers (full sheltered + filter) was developed, calibrated, validated and used (see section 2.1.1.2.2 for details) to monitor the concentrations of all the target BFRs in air from cars.

3.2.2 Indoor dust sampling

Dust samples were collected between September 2006 and July 2008 from 111 microenvironments within the West Midlands conurbation and in Basingstoke, Hampshire. All microenvironments comprised a convenience sample of acquaintances of the authors. Samples were collected (see section 2.1.2 for detailed methodology) under normal room use conditions to reflect actual human exposure. The following microenvironment categories were selected for study: homes (living rooms, n=45), offices (n=30), cars (n=30) and PMEs (n=6).

3.3 Concentrations of target BFRs in air from different categories of indoor microenvironments.

Table 3.1 summarises the concentrations of α-, β-, γ-HBCDs, TBBP-A and BDE-209 in air samples from homes, offices, cars and PMEs. Indoor concentrations of HBCDs are an order of magnitude higher than those detected in outdoor air in this study (average (n=5) = 37 pg ΣHBCDs m^{-3}), and US outdoor air (maximum = 11 pg ΣHBCDs m^{-3}) (Hoh and

Hites, 2005). Although there were an insufficient number of samples from PMEs to permit meaningful statistical interpretation, concentrations in PMEs sampled are substantially above those detected in homes, offices and cars. While no statistically significant difference ($p<0.05$; see section 2.6 for details) was observed between ΣHBCDs in homes and offices, concentrations of ΣHBCDs in cars were significantly higher ($p<0.05$) than those measured in both homes and offices. It is interesting that median concentrations of ΣHBCDs reported here in air from each of the microenvironment categories studied are typically several times greater than the concentrations of Σtri-hexa-BDEs (i.e. those congeners predominant in the Penta-BDE formulation) reported in an earlier study of the same microenvironment categories in Birmingham, UK (Harrad et al., 2006b).The diastereomer profiles of HBCDs in the studied air samples (figure 3.1) showed predominance of the γ-isomer. However, unlike β-HBCD, the percent contribution of α-HBCD (23-29%) to ΣHBCDs was slightly higher than expected from the technical formulations.

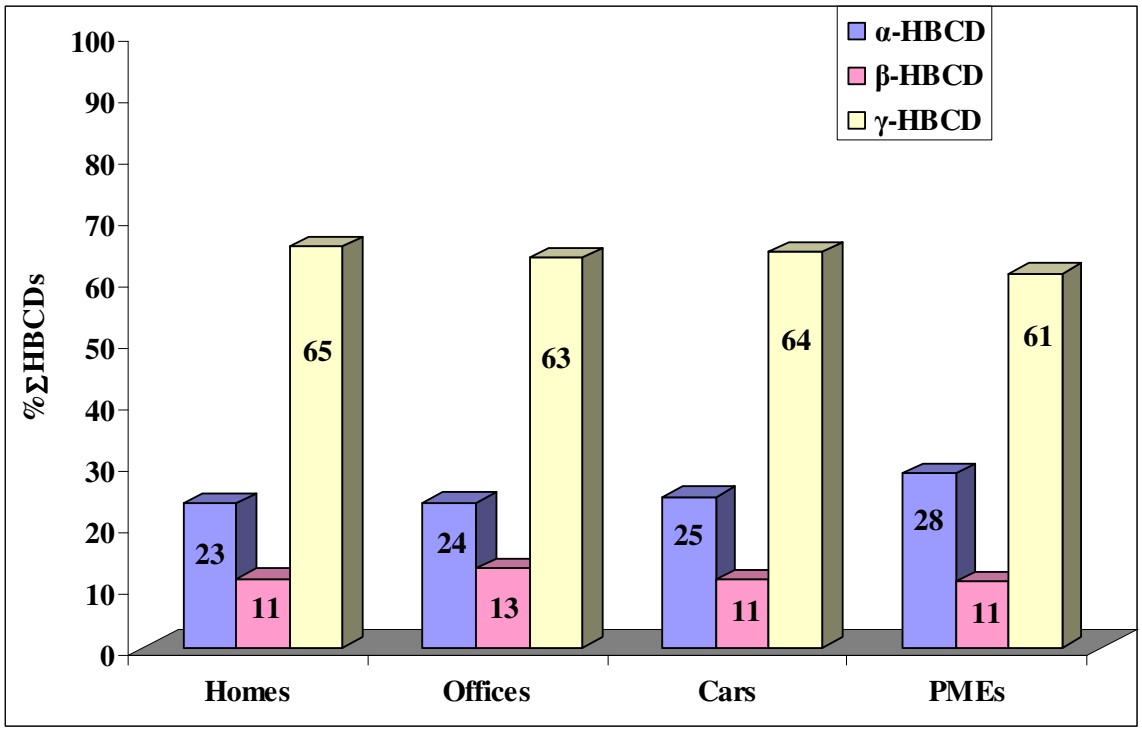


Figure 3.1: Average relative contribution (expressed as %) of HBCD diastereomers to ΣHBCDs in the studied air samples.

The concentrations of TBBP-A in indoor air (table 3.1) in this study exceeded by an order of magnitude those present in outdoor air – consistent with the presence of indoor

emission sources. While the relatively small sample numbers preclude statistical analysis of differences in contamination between microenvironment categories. Unlike HBCDs, there are no substantial differences apparent between concentrations in PMEs and those in homes and offices. The concentrations of TBBP-A in this study agree with previous reports of concentrations in offices and lecture halls from Sweden (Sjodin et al., 2001), but are lower than those reported from offices and houses from Japan (Inoue et al., 2006b).

Table 3.1: Summary of HBCDs, TBBP-A and BDE-209 concentrations (pg m^{-3}) in air samples from the studied microenvironments compared to Σtri-hexa-BDEs[a].

Location	Statistical parameter	α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209	Σtri-hexa-BDEs[a]
Homes, n=35, n=5 for TBBP-A and BDE-209	Average	59	22	170	250	16	397	52
	SD	77	8.7	140	240	5.2	171	61
	Median	37	22	120	180	15	366	24
	Minimum	14	5.0	39	67	8.9	189	4
	Maximum	430	54	710	1300	22	620	250
Offices, n=30, n=5 for TBBP-A and BDE-209	Average	43	24	120	180	16	650	170
	SD	20	5.9	68	90	12	202	280
	Median	36	23	110	170	11	682	71
	Minimum	18	14	43	70	4.1	341	10
	Maximum	87	34	370	460	33	853	1400
Cars, n=21	Average	90	40	237	367	3	1730	710
	SD	49	20	117	174	1	1250	1870
	Median	87	39	250	400	3	1310	41
	Minimum	14	7	49	70	n.d*	190	11
	Maximum	178	74	493	665	5	4020	4200
Public micro-environments, n=6	Average	250	28	550	900	26	n.m[$]	110
	SD	110	12	140	60	6.8	n.m	72
	Median	210	24	570	900	27	n.m	140
	Minimum	180	19	360	820	17	n.m	29
	Maximum	400	46	690	960	32	n.m	160
Outdoor air, n=5	Average	3.0	1.1	33	37	0.76	71	21[b]
	SD	0.51	0.09	1.9	2.4	0.06	10	
	Median	2.9	1.0	33	37	0.74	69	
	Minimum	2.3	0.94	31	34	0.69	59	
	Maximum	3.7	1.2	35	40	0.85	86	

a – from reference (Harrad et al., 2006b); b – from reference (Harrad and Hunter, 2006).

* not detected; [$] not measured.

Despite its very low vapour pressure, BDE-209 was detected in the studied air samples and –similar to TBBP-A- was associated mainly with the particulate phase which may be explained by its high molecular weight. While meaningful statistical analysis was not possible due to the small number of samples. BDE-209 concentrations in the studied cars were substantially higher than those in homes and offices (table 3.1). This was observed previously for Σtri-hexa-BDEs in an earlier study of the same microenvironment categories in Birmingham, UK (Harrad et al., 2006b). The high levels of BDE-209 in cars may be explained partially by the extensive application of deca BDE technical formulations (composed almost exclusively of BDE-209 (La Guardia et al., 2006)) in automobile interiors to flame retard various sectors including fabrics (where deca BDE is encapsulated in the backcoating), reinforced plastics, under the hood or dash and items of electric and electronic equipment (BSEF, 2009b). Levels of BDE-209 in air from homes (living rooms) in this study are broadly in line with those previously reported in Sweden (260 pg m^{-3}) (Karlsson et al., 2007) and USA (50-650 pg m^{-3}) (Allen et al., 2007). However, BDE-209 concentrations in air from cars in this study were significantly higher ($p<0.05$) than those reported in 33 cars from Greece (Mandalakis et al., 2008). This can be attributed to the extensive usage of deca BDE in upholstery fabrics and textiles in the UK (1,000-1,200 MT/year out of an estimated 1,500 MT/year in the EU for this application) to comply with the United Kingdom Furniture and Furnishing Fire Safety Regulations 1988 (EU Risk Assessment Report, 2002).

Based on their relative production volumes alone, one would anticipate concentrations to fall in the order TBBP-A>BDE-209>HBCDs>>Penta-BDE. While differences in vapour pressures will influence the relative atmospheric abundance of these BFRs, it is noteworthy that concentrations of TBBP-A in both indoor and outdoor air are much lower than those of BDE-209 and HBCDs and are similar to those of the tri-hexa-BDEs that constitute the bulk of the Penta-BDE formulation (Harrad et al., 2006b, Harrad and Hunter, 2006). This may be attributable to the widespread use of TBBP-A as a reactive flame retardant which makes its release from treated goods less facile than for an additive flame retardant like HBCD.

3.4 Concentrations of target BFRs in dust from different categories of indoor microenvironments.

Table 3.2 summarises the concentrations of HBCD diastereomers in dust samples from cars, homes, offices, and PMEs. Of particular interest is the very high concentration of 140,000 ng ΣHBCDs g^{-1} (29% α-, 18% β- and 53% γ-HBCD) detected in one UK house dust sample, which is the highest HBCD level reported to date.

Table 3.2: Summary of HBCDs, TBBP-A and BDE-209 concentrations (ng g^{-1}) in dust samples from the studied microenvironments compared to Σtri-hexa-BDEs[a].

Location	Statistical parameter	α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	Σtri-hexa-BDEs[a]	BDE-209
Homes, HBCDs (n=45); TBBP-A (n=35, n.d.*=1); BDE-209 (n=30)	Average	3200	1000	4200	8300	87	77	260000
	SD	11000	3900	13000	26000	71	68	580000
	Median	380	93	670	1300	62	46	8100
	Minimum	22	9.0	70	140	n.d.	7	n.d.
	Maximum	66000	26000	75000	140000	382	250	2200000
Offices, HBCDs (n=30); TBBP-A (n=30, n.d.=4); BDE-209 (n=20)	Average	610	210	760	1600	49	250	30000
	SD	780	300	910	1700	46	310	67000
	Median	220	84	470	760	36	100	6200
	Minimum	15	11	36	90	n.d.	16	620
	Maximum	2900	1300	3700	6600	140	1100	280000
Cars, HBCDs (n=30); TBBP-A (n=30, n.d. = 14); BDE-209 (n=20)	Average	3200	1400	14000	19000	6	2300	410000
	SD	2900	1600	16000	19000	8	5700	770000
	Median	2000	740	9600	13000	2	190	100000
	Minimum	54	16	27	190	n.d.	54	12000
	Maximum	8800	5200	56000	69000	25	22000	2600000
Public micro-environments, n=6	Average	1000	330	1400	2700	220	n.m.[$]	n.m
	SD	190	67	270	390	140	n.m.	n.m.
	Median	1000	310	1300	2700	230	n.m.	n.m.
	Minimum	810	270	1100	2300	52	n.m.	n.m.
	Maximum	1200	420	1700	3200	350	n.m.	n.m.

a – from reference (Harrad et al., 2008a); * not detected; [$] not measured.

We are unable currently to provide an explanation for this high concentration, based on a survey of potential BFR-treated items present in the room sampled. Statistical analysis reveals that concentrations of ΣHBCDs in car dust exceed significantly ($p<0.05$) those in both homes and offices. No significant differences were detected between concentrations of ΣHBCDs in dust from homes and offices. Such differences in contamination between microenvironment categories coincide with those observed by our group for Σtri-hexa-BDEs (Harrad et al., 2008a), but not BDE-209. The levels of the studied compounds in cars displayed no significant differences with respect to vehicle age or brand. Inspection of HBCD isomer profiles in the analysed dust samples (figure 3.2) revealed that the relative abundance of α-HBCD in indoor dust (7-69% of ΣHBCDs) was higher than that reported in HBCD commercial formulations (<20%). This was accompanied by a lower contribution of γ-HBCD (29-83%) while the contribution of β-HBCD (9-18%) was consistent with the proportion of this diastereomer in the HBCD technical formulations.

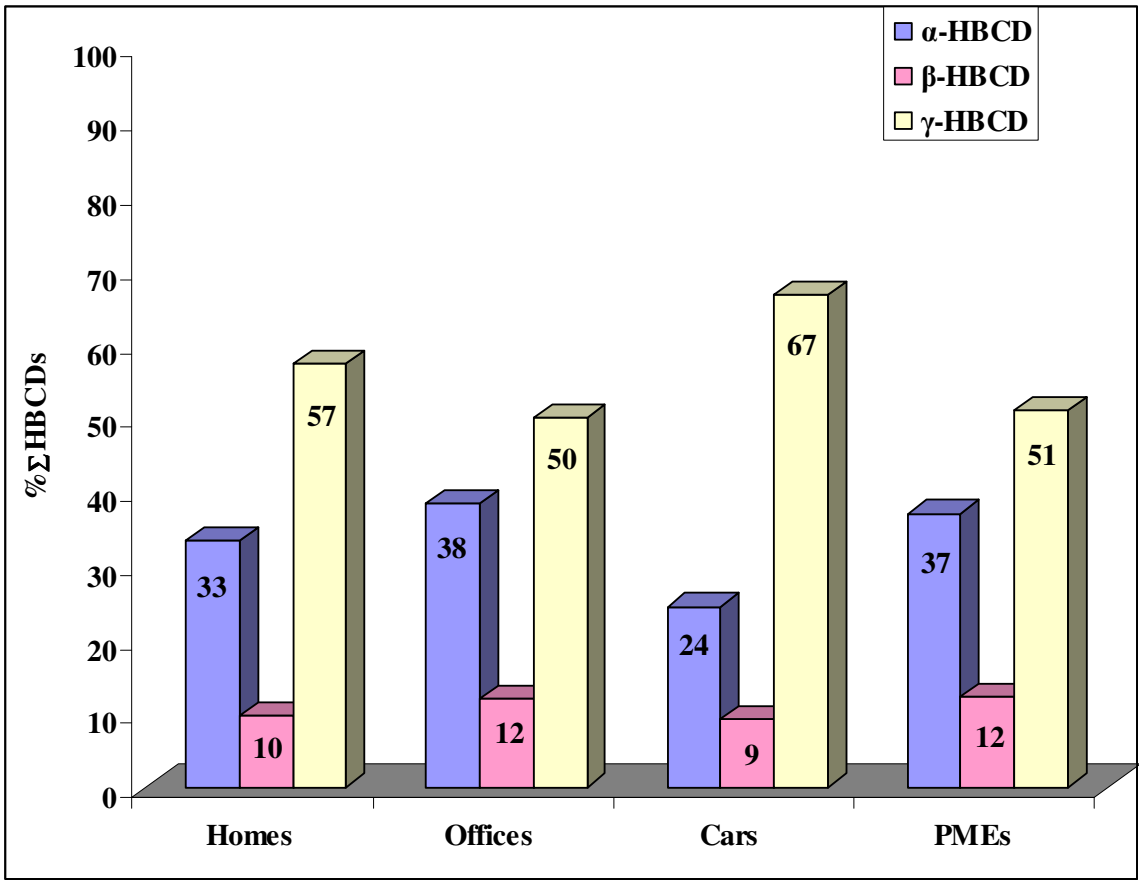


Figure 3.2: Average relative contribution (expressed as %) of HBCD diastereomers to ΣHBCDs in the studied dust samples.

This is consistent with release from HBCD-treated products where the high temperatures (>160 ºC) required to incorporate HBCD technical formulation into the product can cause a shift from γ- to α-HBCD (Koppen et al., 2008). This finding raises the possibility that the predominance of α-HBCD observed in humans (Covaci et al., 2006) may not solely be due to preferential *in vivo* biotransformation of β- and γ-HBCD (Zegers et al., 2005), but, at least partly attributable to the diastereomer pattern in dust.

Concentrations of TBBP-A in dust from the studied microenvironments are summarised in table 3.2. To our knowledge, this study is the first to report concentrations of TBBP-A in dust from cars and PMEs. The concentrations in office dust reported in this study are similar to those detected by Santillo *et al.* in office dust, where concentrations in 9 out of 16 samples where TBBP-A was detectable fell between 5 and 47 ng g^{-1}. Our TBBP-A concentrations in domestic dust also agree with those reported by Santillo *et al.* which ranged from 190 to 340 ng g^{-1} in 4 out of 10 pooled samples of UK domestic dust (Santillo et al., 2003). However, TBBP-A levels in domestic dust reported in this study are slightly lower than those reported by Takigami *et al*. in two domestic dust samples from Hokkaido, Japan (Takigami et al., 2007).

The levels of TBBP-A found in indoor dust samples are slightly lower than those of Σtri-hexa-BDEs (Harrad et al., 2008a), and substantially lower than those of both HBCDs and BDE-209. As noted earlier for air, this relative order of contamination is not a simple reflection of the respective production volumes of these BFRs. We believe this is consistent with the fact that TBBP-A is used predominantly as a reactive BFR and hence its release from treated goods is likely to be less facile than for additive BFRs, such as HBCDs and PBDEs.

In contrast to HBCDs (this study) and PBDEs (Harrad et al., 2008b), statistical analysis revealed that TBBP-A concentrations in car dust are significantly lower ($p<0.05$) than in homes and offices – there is no significant difference between concentrations in homes and offices. While there are too few samples from PMEs to draw firm conclusions, the concentrations of TBBP-A in dust from the sampled PMEs are markedly higher than in cars, homes, or offices. We believe this may be attributable to the comparatively large number of electronic items (e.g. TVs and video game machines) in these microenvironments.

BDE-209 concentrations (table 3.2) were the highest of the studied BFRs in the analysed dust samples. The levels of BDE-209 in domestic dust in this study (table 3.2) are higher than those reported in house dust from other countries, i.e. Germany (6-410 ng g^{-1}) (Sjodin et al., 2008), Australia (95-1585 ng g^{-1}) (Toms et al., 2009), Spain (58-1615 ng g^{-1}) (Regueiro et al., 2007), Canada (290-1100 ng g^{-1}) , USA (530-3300 ng g^{-1}) (Harrad et al., 2008b) and Japan (14-3200 ng g^{-1}) (Kono et al., 2007). This may be due to the particularly stringent fire safety regulations within the UK (the only countries within the EU that have regulations specifying a level of flame retardancy for domestic upholstery fabrics are the UK and Ireland) that appear to have resulted in the UK taking a disproportionately large share of the European market for deca BDE (e.g. 95% of all upholstery materials are flame retarded and over 50% of the total PBDEs used in the UK is used in the textile industry, whereas in most other countries the amounts used in this application would be much lower (EU Risk Assessment Report, 2002)).

Unlike HBCDs, TBBP-A and Σtri-hexa BDEs, there were no significant differences in BDE-209 concentrations between cars, offices and homes in this study. Our results of BDE-209 in car dust were consistent with the recently reported concentrations of deca BDE in previously owned automobiles from USA (Lagalante et al., 2009). Of particular note, are the highly elevated concentrations of BDE-209 found in dust from one car (2,600,000 ng g^{-1} = 0.26%), and two homes (0.22 and 0.14%). To our knowledge these are the highest concentrations of BDE-209 in indoor dust reported anywhere. In an effort to verify the highly elevated concentration of BDE-209 in the car sample and the home sample containing 1,400,000 ng g^{-1} BDE-209, we resampled both of these environments in January 2008, approximately 9 months after the original samples were taken. In both cases, there was only a slight decline in concentrations to 2,200,000 and 900,000 ng g^{-1} BDE-209, confirming the highly elevated contamination in these environments. While there were no apparent changes in the contents of the car, a number of changes in the living room contents occurred between the two samples. Specifically, the furniture, TV, VCR, and DVD player were replaced, suggesting that these were not the source of the elevated BDE-209 levels. Instead, the source appears to be either two items of electronic equipment present in the room during both sampling events, or the carpet or curtains which were not changed between samples.

3.5 Differences in HBCD diastereomer pattern between matched indoor air and dust samples.

There were differences in the diastereomer pattern of HBCDs observed in indoor air and dust samples (tables 3.1 and 3.22). To examine whether this was statistically significant, the percentage contributions of both α-HBCD and γ-HBCD to ΣHBCDs detected in matched pairs (n=25) of indoor air and dust samples (table 3.3) – i.e. collected from the same room at the same time (dust was collected at the end of the air sampling period) were compared. The results revealed that α-HBCD made a significantly greater contribution to ΣHBCD in dust than in air, with the opposite trend observed for γ-HBCD ($p<0.05$). On average, dust composition is 33% α-HBCD, 56% γ-HBCD, while for air; it is 22% α-HBCD, and 66% γ-HBCD. The relative abundance of β-HBCD was similar in both matrices. The pattern in air reflects what is likely to be present in HBCD-treated goods (bearing in mind the interconversion of HBCD stereoisomers at elevated temperatures (predominated by transformation of γ-HBCD to α-HBCD) encountered in technical processes required to incorporate HBCD into goods (Koppen et al., 2008)), and thus is likely a consequence of volatilisation from treated goods. In contrast, the even greater relative abundance of α-HBCD in dust from the same microenvironments is difficult to explain, but is consistent with the existence of processes that induce a post-depositional shift from γ-HBCD to α-HBCD in this matrix. It might also be a result of direct leaching from HBCD-treated products within the sampled microenvironments (where the abovementioned γ- to α-HBCD transformation has occurred during treatment). The faster degradation rate of γ-HBCD compared to α-HBCD reported previously (Davis et al., 2006) may also contribute to the observed HBCDs profiles in dust. The causes of this variability will be discussed in details in chapter 4 of this thesis.

Table 3.3: Concentrations of HBCDs in matched air and dust samples from the same microenvironment.

Sample no.	**Air samples concentration (pg m^{-3})**			**Dust samples concentration (ng g^{-1})**		
	α-HBCD	**β-HBCD**	**γ-HBCD**	**α-HBCD**	**β-HBCD**	**γ-HBCD**
1	38	15	155	876	314	2597
2	16	8	51	154	52	510
3	19	13	113	140	26	209
4	37	16	122	500	93	694
5	17	10	39	91	15	70
6	29	16	79	123	25	80
7	42	15	174	1397	491	1986
8	27	14	92	312	133	1012
9	45	18	128	1376	186	490
10	36	15	95	656	155	2083
11	72	22	222	2372	360	6100
12	20	13	80	171	73	936
13	22	19	102	112	31	188
14	19	9	49	76	17	91
15	42	18	101	318	74	342
16	32	23	116	168	94	376
17	29	17	74	190	73	121
18	38	24	106	587	143	872
19	29	20	110	217	74	502
20	52	25	116	823	176	1012
21	32	17	90	478	147	791
22	40	22	121	624	203	1128
23	22	11	65	97	43	209
24	39	22	138	629	380	2579
25	27	14	105	549	336	2180
Average	**34**	**17**	**101**	**571**	**148**	**1036**
SD	**13**	**4**	**40**	**536**	**131**	**1313**
Median	**32**	**16**	**105**	**318**	**94**	**694**
Minimum	**16**	**8**	**39**	**76**	**15**	**70**
Maximum	**72**	**25**	**222**	**2372**	**491**	**6100**

CHAPTER IV

CAUSES OF VARIABILITY IN CONCENTRATIONS AND ISOMER PROFILES OF HEXABROMOCYCLODODECANES IN INDOOR DUST

4.1 Introduction.

Our results of α-, β- and γ-HBCDs concentrations in dust from cars, homes, offices, and public microenvironments (see section 3.4 for details) showed considerable variability in the relative abundance of the α- and γ-diastereomers between dust samples. In some samples, the pattern resembled closely that in the commercial formulation (predominantly γ-HBCD), while others displayed a far higher contribution of α-HBCD. Furthermore, there was a significant difference in the relative abundance of the α-HBCD and γ-HBCD in matched indoor air and dust samples. On average, dust contained 33% α-HBCD and 56% γ-HBCD, while air had 22% α-HBCD, and 65% γ-HBCD. The relative abundance of the β-HBCD diastereomer was similar in both matrices (11-13%). We attributed the pattern in air to volatilization of HBCDs from treated goods. However, we could not explain the greater relative abundance of α-HBCD in dust from the same microenvironments, but suggested the existence of some post-depositional process effecting either conversion of γ- to α- in dust and/or preferential degradation of γ-HBCD. We hypothesized that such conversion and/or degradation might occur via either photolytic or biological pathways. As α-, β-, and γ-HBCD each exist as an enantiomeric pair (Heeb et al., 2005), the opportunity exists to examine the extent to which processes affecting their absolute concentrations and relative abundance are biologically-mediated. Specifically, while the relative abundance of each HBCD enantiomer should be unaffected by chemical or physical processes, it may be altered by enantioselective interaction with biological systems. We have also reported the presence of four pentabromocyclododecenes (PBCDs) and two tetrabromocyclododecadienes (TBCDs) in

indoor dust samples. We identified these as degradation products of HBCDs and concluded the elimination of HBr was an important degradation pathway for HBCDs in dust (see section 2.4.2 for details).

Our research group has reported that higher dust loadings (i.e. the mass of dust per m^2 floor) may "dilute" concentrations of PBDEs in dust (Harrad et al., 2008a). This assumes that: (a) PBDE emissions in the room remain constant, and (b) the sources of dust and of PBDEs are independent. When one room was monitored monthly for 10 months and after two samples that violated condition (a) were excluded, a significant ($p<0.05$) negative linear correlation was observed between dust loading and PBDE concentration in dust. If confirmed elsewhere, then such "dilution" of contamination may have implications for human exposure.

Another issue identified by our research group is that both within-room spatial and temporal variability in concentrations of PBDEs in dust exceeds that attributable to analytical variability alone. Such variation requires consideration when estimating human exposure via dust ingestion. In addition, temporal variability in PBDE concentrations could be linked to changes in room contents, thereby offering insights into potential emission sources (Harrad et al., 2008a). Furthermore, although such behaviour for PBDEs was not observed, it is possible that in rooms containing one or more potential point sources of a BFR, concentrations may be lower in dust sampled a greater distance from the source.

In light of the above, this chapter will discuss (a) the extent to which within-room spatial and temporal variability in contamination provides insights into the causes of contamination and influences estimates of exposure to HBCDs via dust ingestion; (b) the causes of the differences between diastereomer profiles in dust and indoor air; and (c) whether higher dust loadings can "dilute" concentrations of HBCDs in dust.

4.2 Sampling strategy

4.2.1 Within-room spatial and temporal variability in concentrations and diastereomer patterns of HBCDs in dust.

Within-room spatial variability in the concentrations of HBCDs in dust was studied in three homes and three offices. In each, five samples were taken on the same day at different locations within the room under study (see section 2.1.2 for details). Care was

taken to ensure no overlap between the areas covered by each sample. In addition, in one room where a TV identified as a likely HBCD-emitter was located, samples of dust were taken from inside the TV, and four separate 1 m^2 areas on a transect with increasing distance from the TV. Furthermore, in three homes, temporal variability in BFR concentrations in dust was studied by taking one sample per month over a period of either 9 or 10 months. In each room, care was taken to ensure the same area of the room was sampled on each occasion.

4.2.2 Temporal variation in concentrations, diastereomer pattern, and chiral signatures of HBCDs and concentrations of PBCDs in dust in presence and absence of light.

To examine the influence of exposure to light on the temporal evolution of the contamination of indoor dust with HBCDs and PBCDs, we took a homogenized aliquot of dust from a vacuum cleaner bag, sieved it through a 500 µm mesh sieve, homogenized it thoroughly, and stored it in a sealed dark glass container at -18 ˚C until use. Appropriate numbers of approximately 1.5 g aliquots of dust were placed on clean watch glasses with a transparent quartz lid and exposed to light under normal room usage conditions. Simultaneously in the same room, equal numbers of similar mass aliquots of dust were placed on watch glasses housed in the dark inside a sealed box to act as "controls" – figure 4.1 illustrates the experimental configuration.

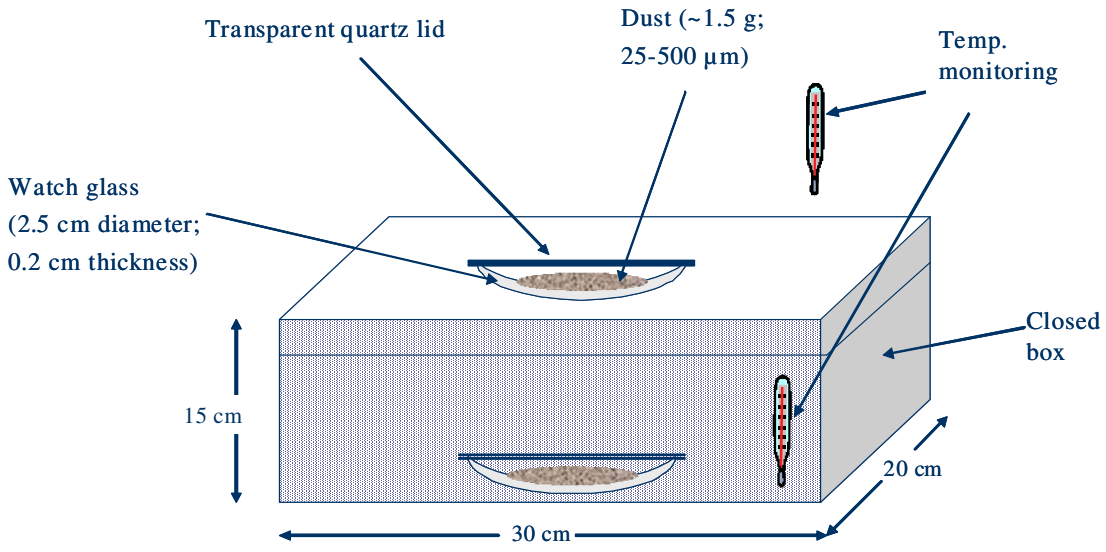


Figure 4.1: Experimental configuration for dust exposure experiments.

At the end of the specified exposure period, dust samples were weighed accurately prior to analysis. Two studies were conducted. The first analyzed one aliquot from both light-exposed and control groups every week for 5 weeks, while a later experiment took samples from a light-exposed group every day for 7 days – no controls were exposed in this latter study. While measurements were not made of the light intensity etc. in the room in which this experiment was conducted, conditions were consistent with many indoor environments at latitude: 52° 30' North, longitude: 1° 55' West, during December 2007 and January 2008. The characteristics of the room in which the experiments were conducted are given in table 4.1 as Home 2S, with artificial lighting in this 3.5 m x 2.5 m x 2.5 m high room provided by two 60 watt light bulbs. To verify that there were no significant differences in temperature experienced by the light-exposed and "control" samples, we recorded these temperatures every two hours for a 72 hour period. The mean temperatures were 19.6˚C (light-exposed) and 19.5˚C (controls). Hence, temperature is unlikely to be a factor influencing differences in diastereomer profiles between light-exposed and "control" samples.

Table4.1: Summary of room characteristics for temporal and spatial variability studies.

Sample Name	**Venti-lation**	**# Foam chairs**	**# Beds**	**# sofas**	**# TVs**	**# Stereos**	**# PCs**	**Other room contents**	**Floor type**
Home 1S	Natural	0	1	1	1	1	0	dehumidifier, alarm clock, phone/answering machine	carpet
Home 2S	natural	4	1	0	1	0	1	alarm clock	rug+carpet
Home 3S	natural	2	1	0	0	1	1	Printer	rug
Office 1S	natural	6	0	0	0	0	6	4 printers	rug
Office 2S	natural	40	0	0	0	0	2	2projector+dvd player	rug
Office 3S	natural	86	0	0	0	0	2	2projector+dvd player	rug
Home 1T	natural	0	1	1	0	1	0	dehumidifier, alarm clock, phone/answering machine	carpet
Home 2T	natural	3	1	0	1	0	2	microwave, fridge, toaster, kettle, scanner/printer, speaker set	bare floor

4.3 Within-room spatial variability in contamination of dust with HBCDs.

We studied within-room spatial variability of HBCDs in three homes and three offices. Table 4.2 shows concentrations and dust loadings recorded in each sample, along with relative standard deviation (RSD) of these parameters in each room. Table 4.2 also gives RSD values obtained for replicate analyses of sub-samples (n=5) of dust samples from home 1S1 and home 1S2. This provides a "benchmark" against which our measurements of within-room spatial variability may be measured, as it quantifies the variability of measurement due to our sample homogenization and sieving process, and our analytical procedures combined. The relatively low RSDs obtained (5-7% for ΣHBCDs) are reassuring that our choice (that concurs with that of other researchers – e.g. (Allen et al., 2008)) of a 500 µm mesh sieve yields homogenous dust samples. Comparison with these RSD values shows that the spatial variability in concentrations of ΣHBCDs in dust within home 3 and office 1, are similar to those attributable to sample preparation and analytical variability. For home 1 and office 2, the spatial variability (RSDs = 19 and 26% respectively) is not attributable wholly to methodological factors; while for home 2 and office 3, the spatial variability (RSDs = 47 and 100% respectively) exceeds substantially that attributable to sample preparation and analytical measurement. While it is unwise to draw firm conclusions on the basis of just six microenvironments, there appears less spatial variability in concentrations of ΣHBCDs than was observed for five of the same rooms for individual PBDE congeners (Harrad et al., 2008a). For rooms like home 2 and office 3, where substantial within-room spatial variability occurs, one could conclude that the entire surface of a room must be sampled to obtain a representative sample. However, the possibility of appreciable variation in exposure depending on the location within the room should be considered, and it is possible that a more accurate estimate of exposure may be obtained via sampling only in the most-frequented parts of the room. As highlighted for PBDEs (Harrad et al., 2008a), substantial within-room variations in concentrations are pertinent where a more contaminated area coincides with for example a toddler's playpen.

Table 4.2: Spatial variation in dust loadings (g m^{-2}), diastereomer Profiles, and Concentrations (ng g^{-1}) of HBCDs.

Sample/ Diastereomer	Concentration α-HBCD	% α-HBCD	Concentration β-HBCD	% β-HBCD	Concentration γ-HBCD	% γ-HBCD	Concentration ΣHBCDs	Dust Loading
Home 1S1	63	24	26	10	170	66	260	6.8
Home 1S2	56	24	22	9	150	66	230	6.1
Home 1S3	44	26	15	9	110	65	170	2.6
Home 1S4	74	26	30	10	180	64	290	4.6
Home 1S5	55	23	25	11	160	66	240	4.3
RSD[a]	19	5	24	7	18	1	19	33
Home 2S1	5500	29	2100	11	11000	60	19000	0.29
Home 2S2	5800	54	1600	14	3500	32	11000	0.34
Home 2S3	6100	51	2000	17	3900	32	12000	0.31
Home 2S4	3600	62	850	15	1300	23	5700	0.27
Home 2S5	4200	61	1100	15	1700	24	7000	0.26
RSD[a]	22	26	36	14	93	44	47	11
Home 3S1	540	40	170	13	630	47	1300	0.41
Home 3S2	620	41	180	12	730	48	1500	0.35
Home 3S3	580	41	160	11	690	48	1400	0.39
Home 3S4	470	37	130	10	680	53	1300	0.31
Home 3S5	520	40	140	11	630	49	1300	0.28
RSD[a]	11	4	12	7	6	5	8	16
Office 1S1	1700	69	290	12	470	19	2500	1.1
Office 1S2	1500	65	320	14	470	20	2300	0.73
Office 1S3	1600	69	290	13	400	18	2300	0.76
Office 1S4	1500	70	270	12	380	18	2200	0.76
Office 1S5	1700	67	310	12	520	20	2500	1.5
RSD[a]	7	3	8	7	13	7	7	34

Table 4.2 (continued): Spatial variation in dust loadings (g m^{-2}), diastereomer profiles, and concentrations (ng g^{-1}) of HBCDs.

Sample/ Diastereomer	Concentration α-HBCD	% α-HBCD	Concentration β-HBCD	% β-HBCD	Concentration γ-HBCD	% γ-HBCD	Concentration ΣHBCDs	Dust Loading
Office 2S1	880	36	290	12	1300	53	2500	0.91
Office 2S2	830	32	260	10	1600	59	2600	0.67
Office 2S3	790	53	170	12	530	35	1500	0.67
Office 2S4	830	36	240	10	1200	54	2300	0.42
Office 2S5	860	57	160	11	480	32	1500	0.37
RSD[a]	4	27	25	8	47	26	26	36
Office 3S1	3500	29	1300	11	7100	60	12000	0.49
Office 3S2	730	28	300	11	1600	61	2600	0.41
Office 3S3	630	28	250	11	1400	61	2300	0.39
Office 3S4	590	32	190	10	1100	58	1900	0.43
Office 3S5	650	29	280	12	1400	59	2300	0.47
RSD[a]	100	5	100	7	100	2	100	9
RSD for Home 1S1[b]	14	7.6	8.8	6.3	5.4	3.4	6.7	-
RSD for Home 1S2[b]	12	11	12	9.6	7.0	4.4	5.2	-

[a] Relative standard deviation

[b] Values given are based on five replicate analyses

It is interesting to examine the spatial variation in concentrations within home 2 and office 3, in the context of the proximity of each sample location to potential HBCD sources. While the thermal instability of HBCD has limited previously its use in high impact polystyrene deployed in electronic goods, recent development of a more thermally stable HBCD formulation means that the UL94-V2 flame retardancy standard can be met. This renders the use of HBCD in audiovisual equipment including TVs (for which only the lower UL94-HB standard is required in Europe) feasible (Weil and Levchik, 2007). This is consistent with the otherwise surprising finding that Home 2 contains a TV shown later (table 4.3) to be a substantial source of HBCDs. It is therefore unsurprising that sample Home 2S1 which was taken closest to the TV, contains both the highest concentration of ΣHBCDs, and the greatest proportion of γ-HBCD. Other samples were taken further away from the TV, with samples Home 2S4 and Home 2S5 the greatest distance away. Similarly, sample Office 3S1 was taken close to the part of the room containing a PC and related electronics. Other samples were taken further away from these items. We consider this explains why sample Office 3S1 contains around 4-5 times ΣHBCDs than other samples in that room. Conversely, it is clear that in rooms like home 3 and office 1, within-room spatial variability for ΣHBCDs is so low as to preclude the need to sample the entire room.

4.4 Influence of an HBCD source on spatial distribution of dust contamination within a room.

As discussed above, we identified a TV set as a source of HBCDs in Home 2 studied for both spatial and temporal variation (Home 2S and Home 2T – Tables 4.2 and 4.4, respectively). To investigate further, we sampled dust from inside the TV, and from four separate 1 m^2 areas on a transect with increasing distance from the TV. Great care was taken to minimize sample cross-contamination: the sample from inside the TV was taken 2 weeks before the other samples and the vacuum cleaner cleansed thoroughly before next use. Furthermore, to sample dust from the inside of the TV, it was moved carefully onto a cloth-covered table elsewhere in the room, with any fugitive dust retained carefully within the cloth. The other samples were taken in the order of furthest distance from the TV first. Table 4.3 shows: (a) the dramatic decline in ΣHBCD concentration with increasing distance from the TV (which cannot be attributable to variations in dust

loadings which are similar for all samples); and (b) the marked shift in diastereomer pattern from 57 % γ-HBCD, 24 % α-HBCD in the TV dust, to 25 % γ-HBCD, 59 % α-HBCD in the sample furthest away from the TV. It appears that fresh contamination of dust closest to the TV occurs at a rate that exceeds that at which the shift in diastereomer profile takes place.

Table 4.3: Variation in Concentrations (ng g^{-1}) and Diastereomer pattern of HBCDs in dust with distance from a TV

Distance from TV (m)	α-HBCD	% α-HBCD	β-HBCD	% β-HBCD	γ-HBCD	% γ-HBCD	ΣHBCDs
Inside TV	130000	24	100000	19	310000	57	540000
1	6800	29	3900	16	13000	55	24000
2	5200	30	3500	20	8600	50	17000
3	5100	52	1600	16	3200	32	9900
4	3400	59	900	16	1400	25	5700

4.5 Within-room temporal variability in contamination of dust with HBCDs.

We also studied within-room temporal variability in concentrations of HBCDs in three homes. Concentrations and dust loadings for these samples are given in table 4.4 along with the RSD values. Characteristics of the rooms studied are in table 4.1. In general, RSDs of concentrations of ΣHBCDs in these samples (27-190 %) exceed those in the spatial variability study. Alongside data on within-room spatial variability, these data provide the first empirically-derived indication of the uncertainty associated with an exposure assessment of HBCDs based on a single measurement of dust contamination from a given location within a given room at a given point in time. While variability in the entire dataset of 9-10 monthly samples within each room is not excessive, caution is advised given the range of concentrations within each room. In the rooms studied, the maximum ΣHBCDs concentration (ng g^{-1}) exceeded the minimum by a factor of 2.6, 224, and 4.0, respectively. Clearly, substantial variation in estimates of exposure is possible, depending when a given room is sampled.

Table 4.4: Temporal variability in concentrations (ng g^{-1}) of HBCDs and dust loadings (g m^{-2}) within same room.

Sample/Isomer	α-HBCD	β-HBCD	γ-HBCD	ΣHBCDs	Dust Loading
Home 1T1	43	18	110	180	13.5
Home 1T2	65	28	170	260	7.3
Home 1T3	44	15	110	170	2.6
Home 1T4	96	48	300	440	4.4
Home 1T5	84	43	250	370	3.4
Home 1T6	89	46	270	400	2.8
Home 1T7	74	38	210	320	2.2
Home 1T8	72	37	200	310	1.3
Home 1T9	74	38	210	320	2.9
Home 1T10	86	43	240	370	1.3
RSD[a]	23	30	29	27	91
Home 2T1	4200	1200	940	6400	0.38
Home 2T2	8400	2900	4400	16000	1.5
Home 2T3	10000	3600	2200	16000	0.50
Home 2T4	3100	1100	510	4700	0.47
Home 2T5	7300	2900	3900	14000	0.71
Home 2T6	9000	3000	2300	14000	0.47
Home 2T7	4200	1500	1100	6800	0.41
Home 2T8	4900	1800	700	7400	0.54
Home 2T9	9600	3700	5200	19000	0.18
RSD[a]	38	40	70	42	62
Home 3T1	210	31	68	310	0.35
Home 3T2	12	2.1	3.0	17	0.53
Home 3T3	130	19	39	190	0.53
Home 3T4	120	19	34	180	0.63
Home 3T5	100	14	31	150	0.60
Home 3T6	100	16	32	150	0.71

Table 4.4 (continued): Temporal Variability in Concentrations (ng g^{-1}) of HBCDs and Dust Loadings (g m^{-2}) within Same Room.

Sample/Isomer	α-HBCD	β-HBCD	γ-HBCD	ΣHBCDs	Dust Loading
Home 3T7	81	11	23	120	0.81
Home 3T8	86	12	24	120	0.75
Home 3T9	1300	400	2100	3800	0.63
RSD[a]	160	210	250	190	36

[a] Relative standard deviation

We examined temporal variations in concentrations of ΣHBCDs in dust samples from the same three rooms relative to changes in room contents during monitoring. As the intention of this study was to examine temporal variations under normal use conditions, these changes in room contents were instigated by the room occupants only. Characteristics of the rooms studied are in table 4.1. In Home 1T (a bedroom), the RSD of ΣHBCDs concentration was low – consistent with that observed for spatial variability in some rooms (table 4.2). Indeed there were no obvious changes in room contents likely to alter emissions of HBCDs.

The most obvious features of the temporal trend in Home 2T (a bedroom), relate to the fact that it contained a TV identified as an emitter of HBCDs (i.e. the same Home 2 in which spatial variation was studied – sample series Home 2S). The TV was introduced into the room at the beginning of the period when sample Home 2T2 was taken, and coincides with a period when ΣHBCDs concentration increases by a factor of 2.5. This higher concentration remains in sample Home 2T3, but falls in sample Home 2T4, before rising in samples Home 2T5 and 2T6. We believe this may be explained by the fact that for 19 days during period Home 2T4, the TV was removed temporarily when it was loaned elsewhere. A further fall in concentration occurs in samples home 2T7 and 2T8, we believe due to the fact that the position of the TV within the room altered. The last sample (home 2T9) sees another rise in ΣHBCDs concentration. This does not appear to be related to the TV on this occasion, but could be attributable to the introduction of a new mattress and curtains.

In sample series Home 3T (living room/kitchen), there are two notable events. In home 3T2, an order of magnitude fall in ΣHBCDs concentration occurred. This coincides with the introduction of a new rug. After this sample, concentrations appear to reach equilibrium, and there is very little temporal variation in ΣHBCDs concentrations until sample home 3T9, in which there is a marked rise in ΣHBCDs concentration that coincides with the introduction of a new DVD player.

4.6 Temporal evolution of concentrations, diastereomer pattern, and chiral signatures of HBCDs and concentrations of degradation products in dust in presence and absence of light.

We have reported previously a significantly greater abundance of α-HBCD in indoor dust samples compared to air samples collected simultaneously from the same microenvironment (see section 3.5). We hypothesized this difference in diastereomer pattern was due to either a photolytic isomerisation of γ-HBCD to α-HBCD or a selective degradation of γ-HBCD (see section 3.5). Table 4.5 reports concentrations and enantiomer fractions (EFs) of α-, β-, and γ-HBCD, as well as concentrations of ΣHBCDs and ΣPBCDs in dust samples exposed to and shielded (i.e. controls) from light for different time periods. There are three salient features:

(a) A significant shift from the γ-HBCD to the α-HBCD diastereomer in the presence of light that is complete within one week. No such change is observed in the absence of light.

(b) There is no significant change in enantiomer fractions.

(c) There is degradative loss of HBCDs, as evidenced by the decrease in concentrations of ΣHBCDs and the increase in concentrations of ΣPBCDs with time. While occurring also in the absence of light, this process is more substantial in its presence.

Combined, these data suggest a rapid photolytically-mediated shift in the diastereomer profile, a slower degradative loss of HBCDs via elimination of HBr, and that neither of these processes are enantioselective.

Assuming degradation of HBCDs in indoor dust follows first order kinetics, the concentrations of ΣHBCDs in the analyzed dust samples (table 4.5) were used to calculate the half-life ($t_{1/2}$) of the flame retardant in indoor dust.

Table 4.5: Temporal evolution of concentrations (ng g^{-1}), diastereomer pattern and enantiomer fractions (EF) of HBCDs and PBCDs in indoor dust in presence and absence of light.

Sample Description (exposure time)	**α-HBCD[a]**	**% α[b]**	**EF**	**β-HBCD[a]**	**% β[b]**	**EF**	**γ-HBCD[a]**	**% γ[b]**	**EF**	**ΣHBCDs[a]**	**ΣPBCDs[a]**
Dust before exposure	110±7	25	0.52	60±5	13	0.51	280±9	62	0.55	450±13	6±1
Light exposed (1 week)	170±9	40	0.51	64±8	15	0.52	190±8	45	0.60	420±13	11±3
Control (1 week)	120±5	28	0.53	54±3	12	0.52	260±6	60	0.59	430±9	8±1
Light exposed (2 weeks)	160±6	41	0.56	62±3	16	0.47	170±4	43	0.52	400±10	18±2
Control (2 weeks)	120±6	28	0.54	53±4	13	0.52	250±6	59	0.52	420±7	11±3
Light exposed (3 weeks)	170±4	43	0.53	53±2	14	0.54	170±6	43	0.51	390±8	28±5
Control (3 weeks)	120±8	28	0.51	50±3	12	0.47	260±12	60	0.56	420±21	16±3
Light exposed (4 weeks)	160±8	43	0.51	43±4	12	0.54	160±14	44	0.55	360±12	49±6
Control (4 weeks)	110±9	27	0.57	56±7	14	0.55	240±11	60	0.53	410±15	28±3
Light exposed (5 weeks)	140±9	43	0.54	46±6	14	0.53	140±14	43	0.57	330±7	68±12
Control (5 weeks)	110±8	28	0.57	48±10	12	0.52	230±14	60	0.53	380±17	37±8
Light exposed (1 day)	110	24	-	56	13	-	280	63	-	450	-
Light exposed (2 days)	120	28	-	59	13	-	260	59	-	440	-
Light exposed (3 days)	140	32	-	64	15	-	230	53	-	440	-
Light exposed (4 days)	150	36	-	59	14	-	220	51	-	430	-
Light exposed (5 days)	160	38	-	58	13	-	210	49	-	430	-
Light exposed (6 days)	160	39	-	61	15	-	200	47	-	420	-
Light exposed (7 days)	170	40	-	62	15	-	190	46	-	420	-
Light exposed sample room A[c]	290	35	-	120	15	-	410	50	-	830	120
Light shielded sample room A[c]	200	26	-	110	14	-	480	60	-	800	36
Light exposed sample room B[c]	570	37	-	240	16	-	710	47	-	1500	310
Light shielded sample room B[c]	360	23	-	200	13	-	710	64	-	1500	89

- denotes not measured in this sample; [a]value cited is average ± standard deviation (n=5); [b]value cited is average (n=5); [c]Refers to sample pairs taken from adjacent locations in same room, one shielded from light, the other not.

For light-exposed samples, a plot of ln (ΣHBCDs) against time (weeks) yielded a straight line (R^2 = 0.95) with a negative slope of 0.0569 $weeks^{-1}$ (figure 4.2). The $t_{1/2}$ of HBCDs in indoor dust is thus an estimated 12.2 weeks in the presence of light. In the absence of light (control samples), first order decay in ΣHBCD concentrations is also evident, but with a longer $t_{1/2}$ of 26 weeks. It is stressed that these calculated half-lives apply only to the specific conditions prevailing during our experiment, and will vary according to the specific conditions (e.g. light intensity) in other locations. Further evidence of the impact of exposure to light on concentrations and diastereomer patterns of HBCDs in dust is provided at the foot of table 4.5, where a higher relative abundance of γ-HBCD, together with lower concentrations of PBCDs is shown in two samples of dust taken from locations shielded from light (under furniture) compared to samples taken from adjacent light-exposed locations.

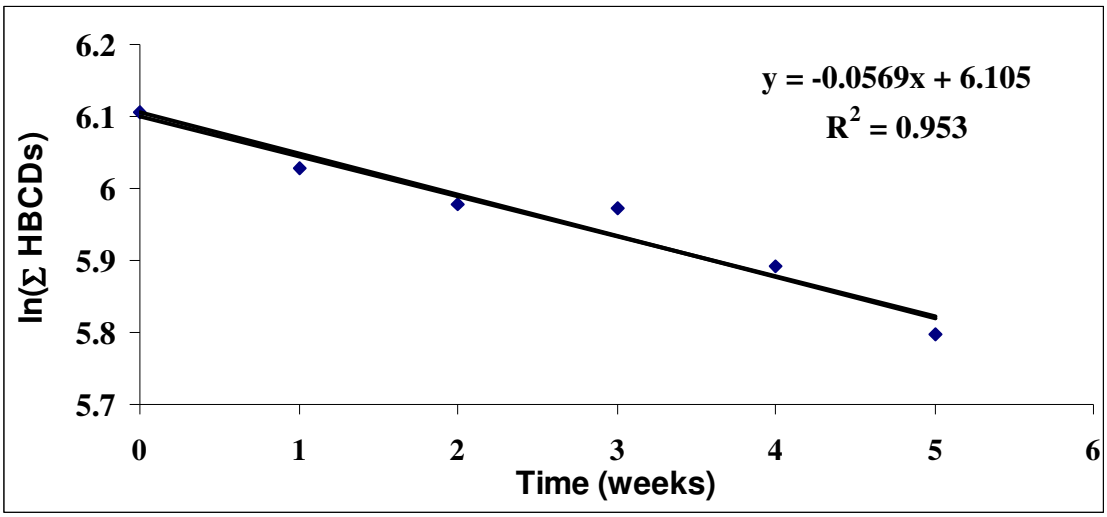


Figure 4.2: Estimation of the first order rate constant for the degradation of HBCDs exposed to light under experiment conditions.

The marked shift in HBCDs diastereomer pattern during the first week of exposure to light might be the result of direct isomerization of γ-HBCD to α-HBCD or there could be a series of isomeric interconversions that give rise to all stereoisomers from any given one similar to the thermally induced isomerization of HBCDs described elsewhere (Koppen et al., 2008). To further understanding of the photolytically induced isomerization of HBCDs in dust, standard solutions (2 ng μL^{-1} in methanol) of pure α-, β- and γ- HBCD were exposed to light in capped regular quartz cuvettes (i.e. those used

normally for spectrophotometric measurements) for one week with the % of each diastereomer monitored daily in each of the 3 standard solutions. This experiment was conducted in Home 2S – i.e. identical to where the dust exposure experiments were conducted. The results (table 4.6) reveal that each of the studied HBCD diastereomers can isomerize to produce different proportions of the other 2 diastereomers upon exposure to light. However, isomerization from γ-HBCD to α-HBCD is predominant. Table 4.6 also suggests net degradation occurs, with the loss over the duration of this experiment varying between 5.5% for the α-HBCD standard and 8.5% for the γ-HBCD standard.

Table 4.6: Effect of exposure to light on pure standards of HBCD diastereomers[a].

Time	α-HBCD			ΣHBCDs	β-HBCD			ΣHBCD	γ-HBCD			ΣHBCD
(days)	% α	% β	% γ	(ng μL^{-1})	% α	% β	% γ	(ng μL^{-1})	% α	% β	% γ	(ng μL^{-1})
0	100	0	0	2.00	0	100	0	2.00	0	0	100	2
1	98.4	0.7	0.9	2.00	1.2	98.2	0.6	1.98	3.5	0.7	95/8	1.97
2	97.8	0.8	1.4	1.96	3.6	95.5	0.9	1.95	7.5	0.8	91.7	1.94
3	97.0	1.0	2.0	1.94	6.2	92.5	1.3	1.94	10.6	1.4	88.0	1.91
4	96.5	1.2	2.3	1.91	11.7	85.8	2.5	1.91	15.6	2.9	81.5	1.88
5	94.9	1.9	3.1	1.91	16.0	80.4	3.7	1.89	22.2	3.8	74.0	1.86
6	92.9	3.1	4.0	1.90	20.1	75.5	4.5	1.88	26.9	4.7	68.4	1.85
7	90.5	4.0	5.5	1.89	23.9	71.3	4.8	1.88	32.4	5.5	62.2	1.83

[a]Percentages may not add to exactly 100 due to rounding.

4.7 Relationship between concentrations of HBCDs in dust and dust loadings.

Our data on the temporal variations in HBCD concentrations and dust loadings in homes 1T, 2T, and 3T (Table 4.4), where samples were taken from the same 1 m^2 area in the room every month for 9-10 months, offer an opportunity to evaluate the extent to which higher dust loadings dilute concentrations of these BFRs. Until now, estimates of human exposure to BFRs via the ingestion of indoor dust have been based on a "default" dust ingestion rate regardless of the dust loading of the room. However, this may not always be appropriate. To illustrate, consider two identically-dimensioned and ventilated rooms, containing identical BFRs emission strengths, but different dust loadings. In which will

exposure be greater? One may hypothesise there to be a lower mass-based concentration of BFRs due to dilution in a dustier room, but will the reduced exposure arising from the lower mass-based contaminant concentrations be mitigated to some degree by a greater dust ingestion rate in such a microenvironment?

As discussed elsewhere for PBDEs (Harrad et al., 2008a), a plot of dust loading versus ΣHBCD concentration should be linear with a negative slope, provided: (a) ΣHBCD emissions in the room remain constant throughout monitoring, and (b) the sources of the dust and of HBCDs are independent. For sample series Home 3T (Table 4.4), no significant relationship is observed ($p>0.05$). However, inclusion of sample home 3T9 violates condition (a) as it was taken when a substantial rise in ΣHBCD concentration occurred. If this sample is excluded, then there is a significant ($R=0.68$; $p<0.05$) negative correlation.

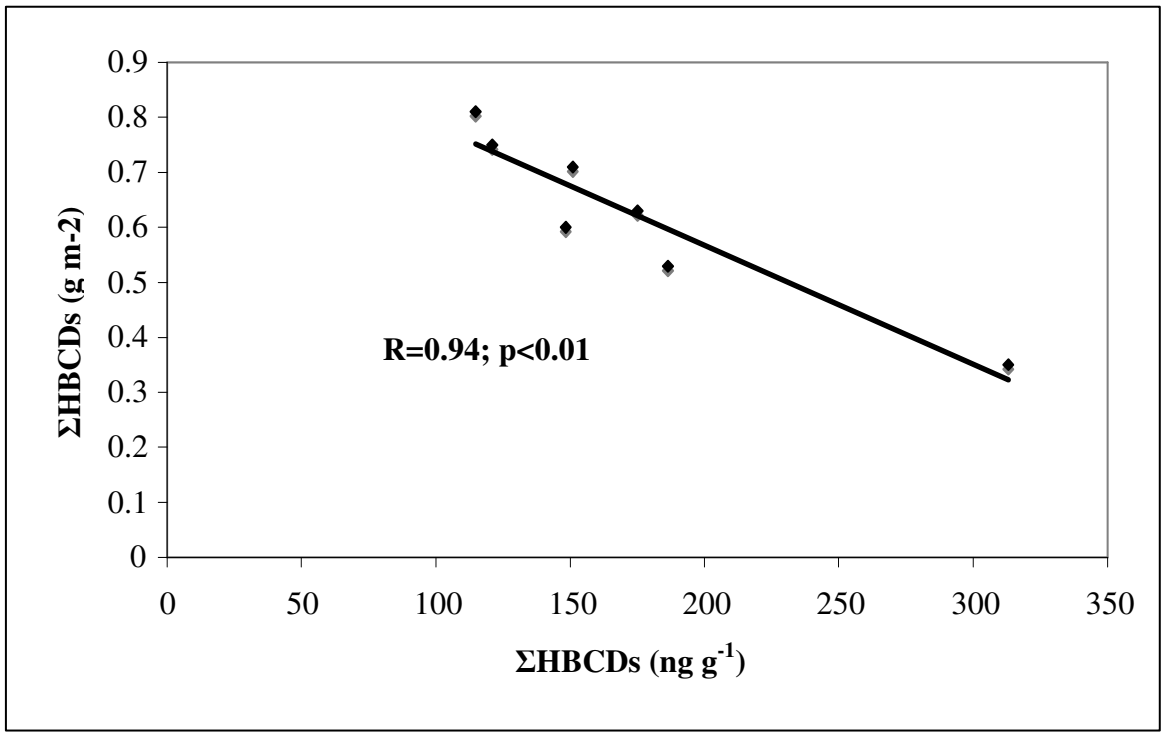


Figure 4.3: Linear correlation between ΣHBCDs (ng g^{-1}) in dust and dust loading (g m^{-2}) in sample group 3T (table 4.4).

Furthermore, if sample home 3T2 is also excluded (on the basis that during this period there is an order-of-magnitude fall in ΣHBCD concentration coinciding with the introduction of a new rug), then the significance of the negative correlation (figure 4.3)

rises ($R=0.94$; $p<0.01$). While the data in home 3T are consistent with the hypothesis that higher dust loadings dilute BFR concentrations, unless the source of BFRs and of dust are the same, no such relationships were evident in either home 1T or 2T.

CHAPTER V

ESTIMATION OF HUMAN INTAKE OF TARGET BROMINATED FLAME RETARDANTS VIA INDOOR AIR AND DUST IN BIRMINGHAM, UK; COMPARISON TO DIETARY INTAKE.

5.1 Synopsis.

In this chapter, the concentrations of α-, β-, γ-HBCD, TBBP-A and BDE-209 measured in indoor air (table 3.1) and dust (table 3.2) samples from homes, offices, cars and PMEs were combined with time-activity patterns to estimate the external exposure of UK adults and toddlers (6-24 months) to these BFRs via air inhalation and dust ingestion using different exposure scenarios based on different intakes and contamination levels. The estimated exposures were compared to dietary intakes of the target BFRs to determine the relative significance of each exposure pathway to the overall daily intakes of adults and toddlers.

5.2 Exposure estimation.

5.2.1 Inhalation exposure.

The algorithm given below (Currado and Harrad, 1998) was used to estimate both adult and toddler inhalation exposure to all target BFRs.

$$\Sigma_{Inhalation\ exposure} = [(C_H F_H) + (C_O F_O) + (C_C F_C) + (C_{PME} F_{PME}) + (C_{Oa} F_{Oa})]\ RR \quad \text{.....(5.1)}$$

Where $\Sigma_{Inhalation\ exposure}$ is the daily human exposure via inhalation (ng day^{-1}); $C_H/C_O/C_C/C_{PME}/C_{Oa}$ is the BFR concentration (ng m^{-3}) in homes/offices/ cars/public microenvironments/outdoor air, respectively. $F_H/F_O/C_C/F_{PME}/C_{Oa}$ is the respective average fraction of time spent in each environment, and RR is the daily respiration rate (m^3 day^{-1}).

To our knowledge, there exists no comprehensive dataset that describes time-activity patterns for the UK population. Hence, overall exposure estimates are based on the

assumption it occurs pro-rata to typical activity patterns - i.e. for adults 63.8% home, 22.3% office, 5.1% PMEs, 4.1% car, and 4.7% outdoors; for toddlers 86.1% home, 5.1% PMEs, 4.1% car, and 4.7% outdoors (Harrad et al., 2006b). In case of BDE-209, exposure via PMEs was excluded due to the absence of data on concentrations within this microenvironment category. Respiration rates for adults and toddlers were assumed to be on average 20 (Currado and Harrad, 1998) and 3.8 m^3 day^{-1} (Wilford et al., 2004), respectively.

5.2.2 Exposure via dust ingestion.

A similar algorithm to that given in equation 5.1 was used to estimate adult and toddler exposure to the studied BFRs via ingestion of indoor dust using the same time fractions cited in 5.2.1. Average adult and toddler dust ingestion figures of 20 and 50 mg day^{-1}, and high dust ingestion figures for adults and toddlers of 50 and 200 mg day^{-1} were used (Jones-Otazo et al., 2005). Various plausible dust ingestion exposure scenarios were estimated using 5^{th} percentile, median, average, and 95^{th} percentile concentrations of BFRs in the analysed dust samples (table 3.2). It is stressed that the range of exposure estimates via dust ingestion thus derived are only an indication of the likely range within the population. This is due to the highly uncertain nature of the ingestion rates used here (and in other studies) as they are based on only a small number of studies involving primary data collection (U.S. EPA, 2002., ECETOC, 2001, U.S. EPA, 1997).

5.3 External human exposure to HBCDs, TBBP-A and BDE-209.

Table 5.1 summarises the exposure estimates of UK adults and toddlers to the studied BFRs via inhalation and dust ingestion under different scenarios. It is evident that inhalation exposure to all target BFRs (0.2-14.6 ng day^{-1}) is substantially lower than that received via dust ingestion (0.4-78000 ng day^{-1}) which may be attributed to the low vapour pressure of these compounds. Despite its high molecular weight and very low vapour pressure, human inhalation exposure to BDE-209 is higher than that to ΣHBCDs, TBBP-A (table 5.1) and Σtri-to-hexa BDEs (Harrad et al., 2006b). This is probably caused by the extensive usage of deca BDE technical formulations in the UK (EU Risk Assessment Report, 2002).

Table 5.1: Summary of exposure estimates (ng day^{-1}) of UK adults and toddlers to target BFRs via air, dust and diet.

Air	Intake (ng day^{-1})	Adult						Toddler (6-24 months)					
		α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209	α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209
	5th %ile	0.5	0.2	1.3	2.3	0.2	4.6	0.1	0.0	0.3	0.5	0.0	0.8
	Average	1.2	0.6	3.2	5.0	0.3	9.4	0.2	0.1	0.6	1.0	0.1	1.7
	Median	0.8	0.4	2.6	3.9	0.3	8.9	0.2	0.1	0.5	0.8	0.1	1.5
	95th %ile	2.7	1.1	7.1	10.4	0.4	14.6	0.5	0.2	1.4	2.1	0.1	2.7
Dust	**Intake (ng day^{-1})**	**Adult**						**Toddler (6-24 months)**					
		Mean dust intake											
		α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209	α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209
	5th %ile	1.8	0.6	2.6	5.8	0.4	30	4.2	1.3	6.6	13.9	1.3	40
	Average	46.6	15.3	69.6	131.5	1.6	4300	144.7	47.2	212.0	403.8	4.4	14000
	Median	8.4	2.5	19.9	32.5	1.3	2300	22.8	6.3	51.8	86.9	3.3	6100
	95th %ile	109.2	48.5	331.1	468.7	3.2	24000	324.3	146.8	1042.6	1462.6	8.5	78000
		High dust intake											
	5th %ile	4.5	1.4	6.5	14.4	1.1	70	16.2	5.0	26.0	55.9	5.2	170
	Average	116.4	38.3	174.0	328.7	4.0	11000	557.5	178.9	736.7	1473.1	18.0	54000
	Median	21.1	6.2	49.7	81.3	3.1	5800	76.5	19.9	132.3	250.7	13.6	24000
	95th %ile	273.1	121.1	827.8	1171.7	8.1	61000	1250.7	559.3	3796.7	5428.6	34.9	310000
Diet[a]	**Average**	203	105	112	413	2.8[b]	315	120	57	67	240	0.4[b]	100
	High level	385	231	217	840	2.8[b]	910	240	110	140	500	0.4[b]	300

[a] Upper bound values (UK Food Standards Agency, 2006) assuming average adult and toddler weights of 70 and 10 kg respectively; toddler's uptake of BFRs via contaminated breast milk is not considered.

[b] Data from (de Winter-Sorkina et al., 2003).

In contrast to inhalation exposure, toddler exposure to the studied BFRs via dust ingestion is higher than adults. This is in agreement with toddlers ingesting more dust than adults due to their closer proximity to the ground and greater hand-to-mouth contact. Of particular interest is the comparison of our estimates of toddler exposure to ΣHBCDs via dust ingestion to the occupational exposure of Scandinavian workers in an industrial plant producing HBCD-treated expanded polystyrene (Thomsen et al., 2007b). Assuming that exposed workers in this study weigh 70 kg, inhale 20 m^3 day^{-1} over an 8 h working day, and that they are exposed continuously to air contaminated at the median value of 2.1 μg of ΣHBCDs m^{-3} (Thomsen et al., 2007b), then such workers are exposed to 200 ng ΣHBCDs kg bw^{-1} day^{-1}. By comparison, a UK toddler weighing 10 kg and ingesting 200 mg dust day^{-1} contaminated at the 95th percentile level reported in this study, will be exposed to 540 ng ΣHBCDs kg bw^{-1} day^{-1}. While it is stressed strongly that the dust exposure estimates cited here for comparison is a high-end exposure scenario, it is important to note that our findings suggest that some UK toddlers are being exposed at levels within the range of occupationally exposed workers. Although the toxicological implications of such exposures have yet to be elucidated fully, this finding provides substantial motivation for further studies investigating the magnitude and relative significance of pathways of human exposure to HBCDs.

Inspection of the HBCD isomer profile received via different exposure pathways (figure 5.1) reveals that dietary exposure is dominated by α-HBCD while γ-HBCD makes the major contribution to inhalation exposure. Predominance of the α-diastereomer in diet may be attributed to the higher levels and detection frequencies of HBCDs in fish, meat, eggs and dairy products where α-HBCD is the major diastereomer (UK Food Standards Agency, 2006, Knutsen et al., 2008, Remberger et al., 2004). Interestingly, the average diastereomer profile of HBCD exposure via dust ingestion falls somewhere between that for inhalation and diet with a 35% and 53% contribution of α- and γ-HBCDs respectively. This results in a higher contribution of α-HBCD (45%) than the other diastereomers to the overall external exposure of adults. However, the higher contribution of dust ingestion to the overall external exposure of toddlers to ΣHBCDs (table 5.1) results in a different diastereomer pattern of the overall HBCD intake in toddlers (figure 5.1) with a similar contribution of both α-HBCD (41%) and γ-HBCD (43%). This finding supports

our previous assumption of the possibility that the predominance of α-HBCD observed in humans (Covaci et al., 2006, Knutsen et al., 2008, Thomsen et al., 2007b) may not solely be due to *in vivo* biotransformation of β- and γ-HBCD (Zegers et al., 2005), but, at least partly attributable to the diastereomer pattern in dust.

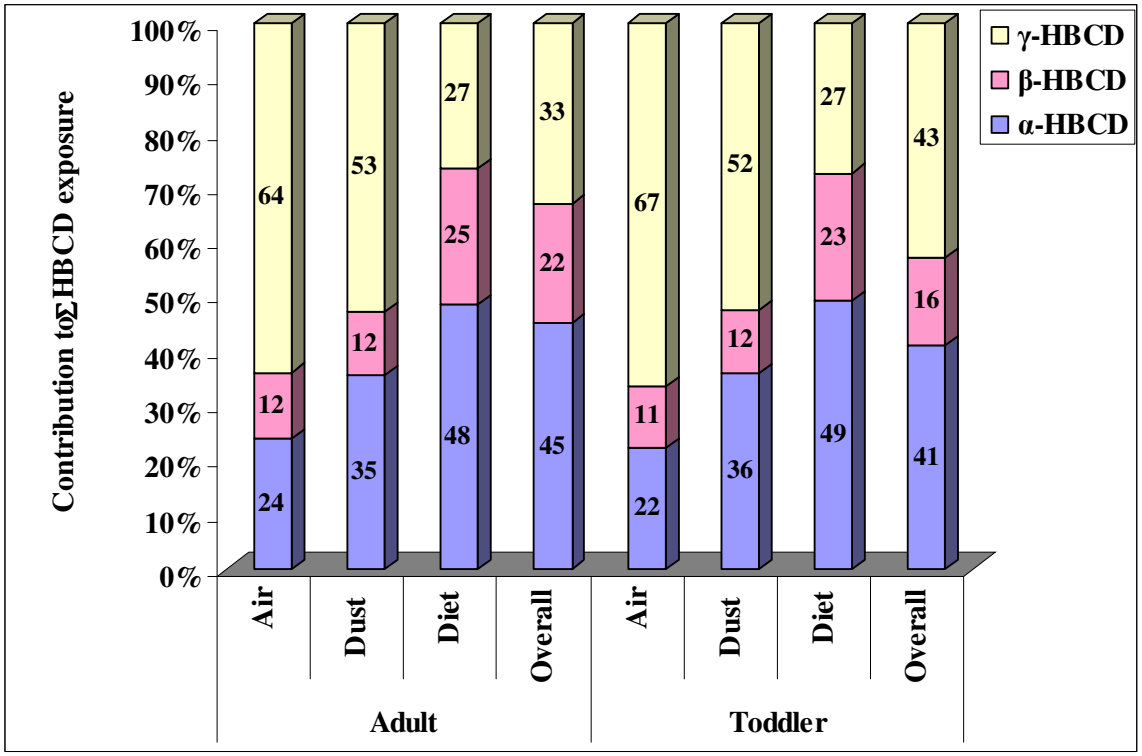


Figure 5.1: Average Contribution (%) of HBCD diastereomers to ΣHBCDs intake of adults and toddlers via different exposure pathways.

Despite increasing evidence of the potential risks of some BFRs to human health, no tolerable daily intakes (TDI) have been estimated for any of the studied compounds by the UK COT (Committee on Toxicity) so far (COT, 2004). However, the United States Environmental Protection Agency (U.S. EPA) has set a reference dose (RfD) of daily oral exposure to BDE-209 of 7 µg kg body weight^{-1} day^{-1}. This RfD is defined by the U.S. EPA as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (U.S. EPA, 2007). Our exposure estimates (table 5.1) indicate that UK toddlers (assuming an average body weight of 10 kg) ingesting 50 and 200 mg dust day^{-1} contaminated at the 95th percentile level will be exposed to 7.8 and 31 µg BDE-209 kg body weight^{-1} day^{-1}. While we are

aware that the number of children exposed at such high levels will likely be low; our high-end exposure scenario estimates for BDE-209 clearly exceed the U.S. EPA RfD which raises concern regarding the potential adverse health effects of such high exposure to BDE-209 via dust ingestion on some toddlers in the UK.

5.4 Contribution of different exposure pathways to the overall external exposure of adults and toddlers to HBCDs, TBBP-A and BDE-209.

The obtained estimates of exposure via air inhalation and dust ingestion are compared to upper bound dietary intakes of ΣHBCDs and BDE-209 from the whole diet by the UK population in 2004 (UK Food Standards Agency, 2006) (table 5.2). However, while dietary intakes of TBBP-A by the UK population (average upper bound exposure = 1.6 and 7 ng kg body weight^{-1} day^{-1} for adults and toddlers respectively) were reported in the same study (UK Food Standards Agency, 2006), TBBP-A was not found above the detection limit in any of the analyzed UK food samples. Hence, the exposure estimates derived from these data are based mainly on the quantification limit of the used method and appear substantially overestimated compared to the average dietary intake of TBBP-A by the Dutch population (0.04 ng kg body weight^{-1} day^{-1}) (de Winter-Sorkina et al., 2003). By comparison, the dietary exposure data from the Dutch survey appears more reliable since the flame retardant was detected and quantified in some of the analyzed food samples. Therefore, we have chosen to compare our estimates for human exposure to TBBP-A via air inhalation and dust ingestion to dietary intake of this compound by the Dutch population (de Winter-Sorkina et al., 2003) (table 5.2). Dust ingestion appears to be the major pathway of exposure to HBCDs, TBBP-A and BDE-209 for UK toddlers even at a mean dust intake scenario. Specifically, the exposure of a toddler weighing 10 kg and ingesting 200 mg dust day^{-1} contaminated at the 95th percentile level reported in this study will exceed ten-fold (for HBCDs), one hundred-fold (for TBBP-A) and one thousand-fold (for BDE-209) the exposure received via the diet (table 5.1). While this might represent only a small fraction of the population who are exposed at this high level via dust ingestion, nevertheless, it highlights the importance of dust ingestion as an exposure pathway to BFRs particularly for toddlers.

Table 5.2: Contribution (%) of different exposure pathways to the overall external exposure of adults and toddlers to ΣHBCDs, TBBP-A and BDE-209

		Average intake scenario[a]							
		Adult				**Toddler (6-24 months)**			
		5th %ile	**Average**	**Median**	**95th %ile**	**5th %ile**	**Average**	**Median**	**95th %ile**
ΣHBCDs	**Air**	0.6	0.9	0.9	1.2	0.2	0.2	0.2	0.1
	Dust	1.4	24	7.2	52.5	5.5	62.6	26.5	85.8
	Diet	98.1	75	91.9	46.3	94.3	37.2	73.2	14.1
TBBP-A	**Air**	5.9	6.4	6.8	6.3	0.0	2.0	2.6	1.1
	Dust	11.8	34	29.6	50.0	92.9	89.8	86.8	94.4
	Diet [c]	82.4	60	63.6	43.8	7.1	8.2	10.5	4.4
BDE-209	**Air**	1.3	1.7	0.2	0.1	0.5	0.2	0.0	0.0
	Dust	8.1	93.0	41.8	98.7	30.4	99.3	85.7	99.9
	Diet	90.6	6.8	56.5	1.3	69.1	0.7	14.1	0.1
		High intake scenario[b]							
ΣHBCDs	**Air**	0.3	0.4	0.4	0.5	0.1	0.1	0.1	0.0
	Dust	1.7	28.0	8.8	57.9	10.1	74.6	33.4	91.5
	Diet	98.1	71.6	90.8	41.5	89.9	25.3	66.5	8.4
TBBP-A	**Air**	4.9	4.2	4.8	3.5	0.0	0.5	0.7	0.3
	Dust	26.8	56.3	50.0	71.7	92.9	97.3	96.5	98.6
	Diet [c]	68.3	39.4	45.2	24.8	7.1	2.2	2.8	1.1
BDE-209	**Air**	0.5	0.6	0.1	0.0	0.2	0.1	0.0	0.0
	Dust	7.2	92.3	38.7	98.5	36.1	99.4	88.8	99.9
	Diet	92.3	7.6	60.7	1.5	63.7	0.6	11.1	0.1

[a] Calculated using average dust intakes of 20 and 50 mg day^{-1} for adults and toddlers and average level dietary intakes (UK Food Standards Agency, 2006); [b] Calculated using high dust intakes of 50 and 200 mg day^{-1} for adults and toddlers and high level dietary intakes (UK Food Standards Agency, 2006); [c] Calculated using data from (de Winter-Sorkina et al., 2003).

In contrast, inhalation is indicated as only a minor pathway of exposure. Specifically, under an exposure scenario assuming mean dust ingestion rates and average concentrations of both air and dust; inhalation contributes 0.9% and 0.2% of total daily exposure to HBCDs for adults and toddlers respectively, while for TBBP-A and BDE-209, inhalation exposure accounts for 6.4%, 2.0% and 1.7%, 0.2% of the total daily exposure of UK adults and toddlers respectively (table 5.2).

In summary, while dust ingestion constitutes the major exposure pathway for toddlers to all the studied BFRs, for adults, the relative significance of different exposure pathways for HBCDs appears somewhere between those for the Penta-BDE formulation (Harrad et al., 2006b, Harrad et al., 2008b) (principally diet, but with dust ingestion playing an important role for some individuals) and BDE-209 (for which dust and diet are broadly equally important). For TBBP-A, ingestion of indoor dust appears to make a greater contribution to overall exposure than is the case for HBCDs. We believe that the low exposure of the UK population to TBBP-A - despite its higher production volume and more extensive usage- compared to HBCDs and PBDEs likely reflects its less facile release from treated products due to its predominant use as a reactive flame retardant.

CHAPTER VI

THE SIGNIFICANCE OF CLASSROOM DUST INGESTION AS A PATHWAY OF EXPOSURE OF YOUNG CHILDREN TO BROMINATED FLAME RETARDANTS

6.1 Introduction.

While dietary exposure is an important pathway of human intake to BFRs (UK Food Standards Agency, 2006, de Winter-Sorkina et al., 2003, Harrad et al., 2004), Recent research has highlighted the potential significance of indoor dust ingestion as a pathway of human exposure to BFRs (Lorber, 2008, Frederiksen et al., 2009, Covaci et al., 2009, Covaci et al., 2006, Stapleton et al., 2008). Of particular concern is that normalised to body weight, young children are considered to ingest substantially more dust than adults due to their proximity to the ground and increased hand-to-mouth and object-to-mouth behaviour (Jones-Otazo et al., 2005, U.S. EPA, 2002.). The overall exposure of children aged 1-5 years to PBDEs in North America was found to be almost double that of adults. This was attributed to higher dust ingestion rates in children (Lorber, 2008). In the UK population, our results have shown that despite the higher inhalation and dietary intake of adults, the overall exposure of toddlers (6-24 months) to ΣHBCDs, TBBP-A and BDE-209 was higher than that of adults (table 5.1). This is caused mainly by the higher toddler intake of the studied BFRs via dust ingestion normalised to their low body weight. To date however, very little is known about the presence and levels of BFRs in dust from classrooms in child daycare centres and primary schools where young children spend a considerable fraction of their time. In addition, the increased number of electronic items and incorporation of information technology lessons at younger ages accompanied with the large number of foam containing items may increase the potential sources of some BFRs in classrooms. Therefore in this chapter, the concentrations of ΣHBCDs, TBBP-A and BDE-209 in indoor dust samples collected from 43 classrooms frequented by young children (age range <1-5 years) will be reported and compared to the levels of the studied

BFRs in house, office and car dust samples from Birmingham, UK (table 3.2). Children's exposure to BFRs via ingestion of classroom dust will then be estimated (see section 5.2.2) and compared to adult exposure via ingestion of office dust. Finally, the significance of dust ingestion compared to dietary intake as a pathway of children's exposure to BFRs will be evaluated.

6.2 Sampling.

Dust samples (n=43) were collected from classrooms in daycare centres and primary schools in the West Midlands of the UK, during winter 2007 /spring 2008 according to a standard protocol (see section 2.1.2 for details). Teachers of the sampled classrooms were asked to fill in a questionnaire detailing information about the classroom contents (e.g. foam chairs, electronics, carpets…etc), ventilation, vacuuming and time spent by children in the classroom.

6.3 Levels of BFRs in classroom dust.

A summary of the concentrations (ng g^{-1}) of the target BFRs in the studied classroom dust is given in table 6.1. The results show that ΣHBCDs and BDE-209 exhibit broadly similar levels in the analyzed dust samples while TBBP-A is detected at significantly ($p<0.05$) lower levels. This is in agreement with our previous finding in dust from homes, offices, cars and PMEs (table 3.2) and is consistent with the predominant use of TBBP-A as a reactive BFR which makes its release from treated goods less facile than for additive BFRs, such as HBCDs and PBDEs.

Table 6.1: Summary of HBCDs, TBBP-A and BDE-209 concentrations (ng g^{-1}) in classroom dust samples.

	α-HBCD	**β-HBCD**	**γ-HBCD**	**ΣHBCDs**	**TBBP-A**	**BDE-209**
Average	2148.7	981.1	5814.0	8943.8	202.5	8454.7
SD	2267.0	1302.7	12699.5	15546.6	280.8	21732.5
Median	1440.5	547.1	1728.6	4129.8	109.0	4997.4
Minimum	23.7	14.2	33.7	72.0	17.0	49.3
5^{th} %ile	92.4	51.8	191.8	364.9	20.3	137.8
95^{th} %ile	5927.5	3394.8	23469.8	36883.1	457.6	23988.1
Maximum	9946.7	6686.1	72221.6	88854.4	1406.3	88143.6

The average relative contribution of the main HBCD diastereomers to ΣHBCDs in the studied classroom dust samples was 37% α- (range 6-59%), 14% β- (range 4-24%) and 48% γ- HBCD (range 22-88%). This is consistent with our previous findings in house, office, car and public microenvironment dust samples and reflects the reported isomerisation of γ- to α-HBCD at the high temperatures used to incorporate HBCD technical mixtures (~78% γ-HBCD) into the flame retarded products (Koppen et al., 2008), as well as our previous finding of a photochemically induced isomerisation of γ- to α- HBCD in indoor dust upon exposure to UV light (see section 4.6 for details).

The concentrations of target BFRs in classroom dust were compared to those found in dust samples from homes, cars and offices (figure 6.1). Statistical analysis revealed that ΣHBCDs concentrations in classroom dust were significantly higher ($p<0.05$) than those in home and office dust.

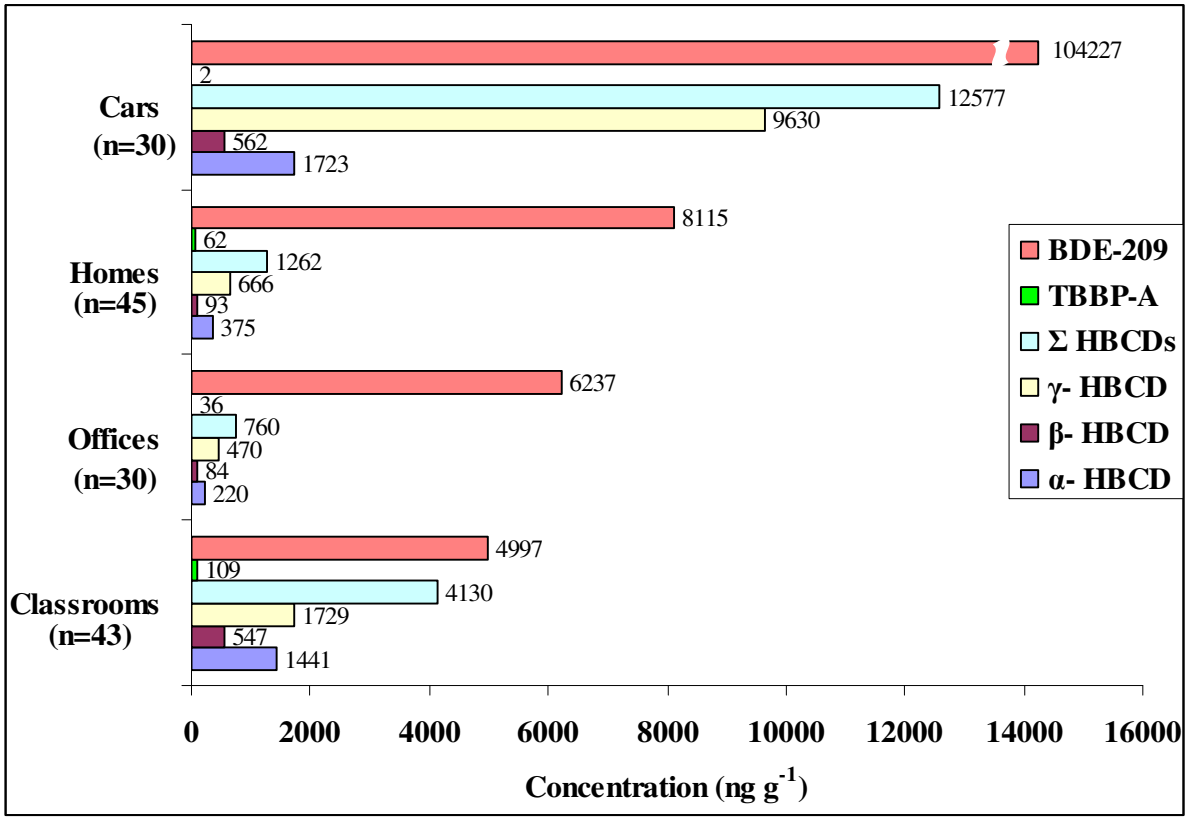


Figure 6.1: Median concentrations (ng g^{-1}) of target BFRs in dust from different microenvironments.

While TBBP-A levels in classrooms were significantly higher than those found in offices and cars, No statistically significant differences were observed among the concentrations

of BDE-209 in classroom, home and office dust which were all significantly lower than BDE-209 levels in car dust. No statistically significant correlations were established between the concentrations of any of the studied BFRs in classroom dust and the classroom contents, ventilation type or vacuuming rate.

6.4 Exposure of young children to the target BFRs via ingestion of classroom dust.

The concentrations of HBCDs, TBBP-A and BDE-209 in the analysed classroom dust samples were used to estimate the exposure of young children (<1-5 years) to the target BFRs via dust ingestion (see section 5.2.2 for details). Table 6.2 summarises the resulting exposure estimates using different intake scenarios.

Table 6.2: Summary of exposure estimates (ng day^{-1}) of young children to the target BFRs via ingestion of classroom dust*.

	Mean dust intake				High dust intake			
	5^{th} %ile	**Median**	**Average**	**95^{th} %ile**	**5^{th} %ile**	**Median**	**Average**	**95^{th} %ile**
α- HBCD	1.1	18.3	25.8	71.1	4.4	69.1	103.1	283.5
β- HBCD	0.6	6.6	11.8	40.7	1.5	26.3	47.1	163.0
γ- HBCD	2.3	20.7	69.8	281.6	9.2	83.0	279.1	1126.6
Σ HBCDs	4.4	49.6	107.3	443.6	17.5	198.2	429.3	1770.4
TBBP-A	0.2	1.3	2.4	4.7	1.0	5.2	9.7	22.1
BDE-209	1.6	62.0	102.5	485.2	6.3	239.9	405.8	1928.9

* Children spent 24% of their day in the classroom (average from our questionnaire information).

Statistical comparison revealed that children's exposure to all the studied BFRs via ingestion of classroom dust is significantly higher ($p<0.05$) than that of adults via ingestion of office dust.

6.5 Dust ingestion vs. dietary intake as pathways of children exposure to BFRs.

The concentrations of HBCDs, TBBP-A and BDE-209 in classroom dust were utilised alongside those from UK homes and cars (table 3.2) to calculate the overall daily exposure of young children (<1-5 years) to the target BFRs via dust ingestion (table 6.3). Assuming a typical average child weight of 20 kg, the obtained dust exposure estimates were compared (figure 6.2) to the estimated upper bound dietary exposure of this age

group to ΣHBCDs (16 ng kg body weight^{-1} day^{-1}) and BDE-209 (9.6 ng kg body weight^{-1} day^{-1}) from total diet in 2004 (UK Food Standards Agency, 2006).

Table 6.3: Summary of overall daily exposure estimates (ng day^{-1}) of young children to the target BFRs via ingestion of indoor dust.

Exposure scenario	BFR	5th %ile	Median	Average	95th %ile
Mean Dust Intake	α- HBCD	2.6	32.8	132.7	314.1
	β- HBCD	1.1	10.7	46.8	151.0
	γ- HBCD	5.2	61.7	231.7	1075.8
	Σ HBCDs	10.4	115.6	411.2	1549.2
	TBBP-A	1.0	4.1	4.4	11.1
	BDE-209	29.9	523.4	9236.0	54933.4
High dust intake	α- HBCD	10.6	131.1	530.7	1256.2
	β- HBCD	4.2	42.8	187.1	603.9
	γ- HBCD	20.7	247.0	926.9	4303.4
	Σ HBCDs	41.8	462.4	1644.7	6196.7
	TBBP-A	3.8	16.3	17.7	44.5
	BDE-209	119.8	2093.4	36943.8	219733.7

While dietary intakes of TBBP-A by different age groups of the UK population (average upper bound exposure = 1.6 and 7 ng kg body weight^{-1} day^{-1} for adults and toddlers respectively) were reported in the same study (UK Food Standards Agency, 2006), TBBP-A was not found above the detection limit in any of the analyzed UK food samples. Hence, the exposure estimates derived from these data are based mainly on the quantification limit of the used method and appear substantially overestimated compared to the average dietary intake of TBBP-A by the Dutch population (0.04 ng kg body weight^{-1} day^{-1}) (de Winter-Sorkina et al., 2003). Since TBBP-A was quantified in some of the analyzed food samples; the data from the Dutch survey appears more reliable. Therefore, we have chosen to compare our estimates for children's exposure to TBBP-A via dust ingestion (table 6.2) to the estimated dietary intake by the Dutch population (de Winter-Sorkina et al., 2003). Results revealed that dust ingestion is the predominant exposure pathway of children to all target BFRs (figure 6.2).

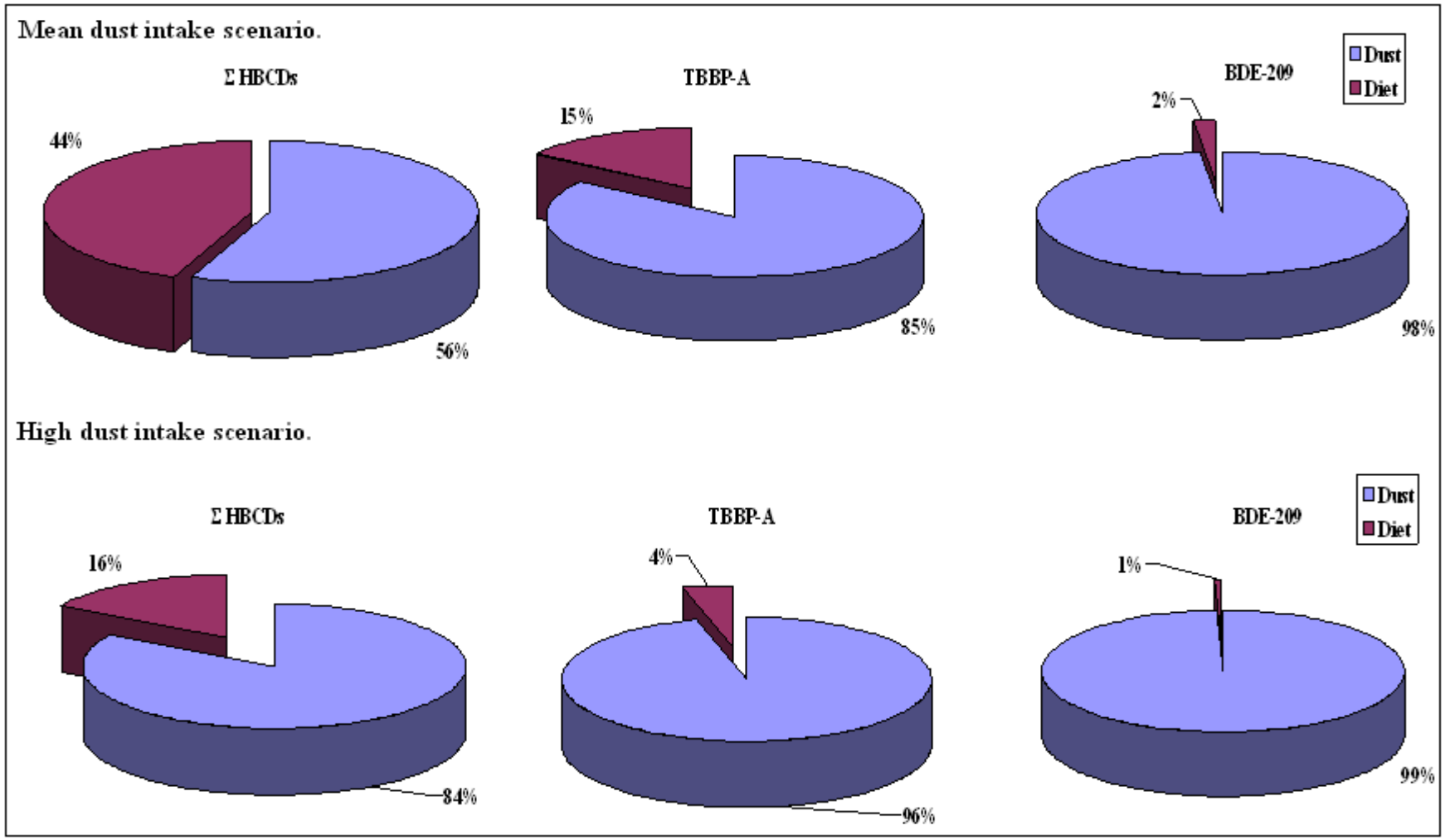


Figure 6.2: Relative significance (expressed as %) of dust (average estimates, table 6.3) and diet to the exposure (ng day^{-1}) of children to the studied BFRs.

While dust ingestion is the major pathway of exposure to BDE-209 (~98% compared to diet), dietary intake seems to play a more significant role in the external exposure of children to ΣHBCDs and TBBP-A (figure 6.2).

Interestingly, the exposure of a child weighing 20 Kg and ingesting 200 mg day^{-1} of dust contaminated at the 95^{th} percentile level to BDE-209 in this study (11 µg kg body $weight^{-1}$ day^{-1}) was found to exceed the U.S. EPA reference dose (U.S. EPA, 2007) of daily oral exposure to BDE-209 (7 µg kg body $weight^{-1}$ day^{-1}). Although the number of children exposed at such high contamination levels is likely to be very small, our results raise concerns regarding the potential adverse health effects of such high exposure to BDE-209 via dust ingestion on some children in the UK.

Finally, despite the current lack of conclusive toxicological evidence of the adverse health effects of combined exposure to different BFRs, our results indicate that on average, a UK child ingesting 50 mg dust is exposed to 10 µg day^{-1} of ΣBFRs in this study via both dust and diet.

CHAPTER VII

HEXABROMOCYCLODODECANES IN INDOOR DUST FROM CANADA, THE UNITED KINGDOM AND THE UNITED STATES: IMPLICATIONS FOR HUMAN EXPOSURE

7.1 Synopsis.

In this chapter, the concentrations of the three principal HBCD diastereomers in indoor dust from Canada, the United Kingdom and the United States will be reported. These concentrations will then be used to provide a preliminary assessment of human exposure to HBCDs via dust ingestion in the studied countries. Given the observed order-of-magnitude differences between North American and European exposure via this pathway to Σtri-hexa-BDEs (Harrad et al., 2008b), dust samples are examined from Toronto, Canada, Birmingham, UK, and Austin/Amarillo, USA. Furthermore, this study evaluates the extent to which the observed shift in diastereomer pattern thought to occur during incorporation of the commercial formulations into flame-retarded products is reflected in indoor dust.

7.2 Sampling strategy.

Dust samples were collected from homes in each city (n=31, 8, and 13 in Birmingham, Toronto, and Amarillo/Austin respectively) using a Nilfisk Sprint Plus 1600 W vacuum cleaner or equivalent Nilfisk model available in the country sampled. . Sampling was conducted in July-August 2006 (UK), September 2006 (Canada), and October 2006 (US); the homes selected in each city comprised a convenience sample of acquaintances of the authors. Sampling was conducted under normal room usage conditions according to a clearly-defined standard protocol by one of the research team (see section 2.1.2 for details). Samples from Canada and the United States were shipped to Birmingham, UK where they were stored at -20°C until analysis.

7.3 Levels of HBCDs in indoor dust.

All 3 HBCD diastereomers were detected and quantified in all dust samples. A statistical summary is given in table 7.1, Median concentrations were: in house dust from Birmingham, UK 730 ng ΣHBCDs g^{-1}; in house dust from Amarillo/Austin, USA 390 ng ΣHBCDs g^{-1} and in house dust from Toronto, Canada 640 ng ΣHBCDs g^{-1}.

Table 7.1: Summary of concentrations (ng g^{-1}) of HBCD diastereomers in indoor dust in this and other studies.

Location (reference)	**Statistical parameter/ Diastereomer**	**α-HBCD**	**β-HBCD**	**γ-HBCD**	**ΣHBCDs**
UK homes, n=31	Average	2800	470	2800	6000
(this study)	σ_{n-1}	12000	1500	7800	21000
	Median	170	66	440	730
	Geometric mean	290	81	560	1000
	Minimum	22	9	70	140
	Maximum	66000	7800	37000	110000
Canadian homes, n=8	Average	340	70	260	670
(this study)	σ_{n-1}	230	44	160	420
	Median	300	72	230	640
	Geometric mean	250	51	200	500
	Minimum	25	6	34	64
	Maximum	670	130	470	1300
US homes, n=13	Average	260	56	490	810
(this study)	σ_{n-1}	480	82	600	1100
	Median	80	28	300	390
	Geometric mean	110	29	290	450
	Minimum	17	6	79	110
	Maximum	1800	300	2000	4000
Belgian offices and homes, n=23	Average	-	-	-	4800
(Greenpeace report, 2003)	Minimum	-	-	-	<20
	Maximum	-	-	-	58000
Offices within EU, n=18	Minimum	-	-	-	<3
(Leonards et al., 2001)	Maximum	-	-	-	3700
UK offices and homes, n=10	Average	-	-	-	3160
(Santillo et al., 2003)	Minimum	-	-	-	940
	Maximum	-	-	-	6900

Table 7.1 (continued): Summary of concentrations (ng g-1) of HBCD diastereomers in indoor dust in this and other studies.

Location (reference)	Statistical parameter/ Diastereomer	α-HBCD	β-HBCD	γ-HBCD	ΣHBCDs
US homes, n=17	Average	-	-	-	140
(Stapleton et al., 2004b)	Minimum	-	-	-	<3
	Maximum	-	-	-	925
US homes, n=19	Average	-	-	-	354
(Stapleton et al., 2008)	Median	-	-	-	230
	Minimum	-	-	-	<4.5
	Maximum	-	-	-	13200

The range of concentrations was substantial, and of particular note is the very high concentration of ΣHBCDs (110,000 ng g^{-1}) detected in one UK house dust sample, which is the second highest ever reported (The highest being 140,000 ng g^{-1} reported in dust from a UK house). We are currently unable to discern an obvious reason for this elevated concentration, based on a survey of the number and type of potentially BFR-treated items (e.g. chairs, sofas, TVs, and other electronic items). There are only a few studies that report concentrations of ΣHBCDs (albeit non-diastereomer-specific) in indoor dust with which our data may be compared (see table 7.1). Our concentrations of ΣHBCDs are consistent broadly with previous studies in both Europe and the United States, but slightly higher. It is not possible to conclude without further analysis of a larger number of dust samples whether the higher concentrations in our study are a reflection of low sample numbers, or whether they are indicative of a temporal increase in concentrations related to possible increases in HBCD use.

7.4 International differences in HBCD contamination of house dust.

The ΣHBCDs data in house dust samples were evaluated statistically for differences in concentrations between the three countries studied. As a first step, the distribution of concentrations within each national dataset was evaluated using both Shapiro-Wilks and

Kolmogorov-Smirnov tests. The results – combined with visual inspection of frequency diagrams - revealed the data for both the UK and the US to be log-normally distributed, while that for Canada displayed a normal distribution. Hence, we performed two ANOVA tests: one on the log-transformed concentrations and another on the non-transformed concentrations. Neither analysis revealed any significant differences ($p>0.05$ as measured using Scheffe and Bonferroni post-hoc tests) in concentrations of ΣHBCDs among the three countries. This absence of a significant difference in HBCD concentrations between the UK and North America is slightly surprising given that 2001 European market demand for HBCDs was around 3.5 times greater than that in the Americas (BSEF, 2009a). However, firm conclusions cannot be drawn without updated information on market demand in the specific countries studied (as opposed to regional demand), while it cannot be excluded that a larger survey may reveal differences.

7.5 HBCD Diastereomer patterns.

In one low-melting-point technical-product, γ-HBCD was the most abundant diastereomer (81.6%), the α-diastereomer contributed 11.8%, with the majority of the remainder (5.8%) being β-HBCD (Law et al., 2005). An even greater predominance of the γ-diastereomer (98% of ΣHBCDs) in a commercial formulation has also been reported (Ryan et al., 2006). Hence, table 7.2 reveals that the relative abundance of α-HBCD in dust samples (range 4-67% ΣHBCDs) was higher generally than that reported to be present in commercial formulations (<20% α-HBCD) and that it constituted >50% of ΣHBCDs in 11 out of 58 samples.

As discussed earlier, although HBCD commercial formulations are dominated by the γ-diastereomer, the temperatures (160˚C-220˚C) required to incorporate the commercial formulation into treated materials can cause a marked shift towards predominance of the α-diastereomer in treated products (Koppen et al., 2008). The comparative abundance of the α-diastereomer in dust samples in this study is consistent with this finding. Clearly, this study does not provide unequivocal evidence of a causal link between ingestion of indoor dust and human body burdens of HBCD. However, it raises the possibility that the observed predominance of the α-diastereomer in humans (Covaci et al., 2006) may not be attributable solely to *in vivo* metabolism (Zegers et al., 2005), nor dietary exposure

(which the limited evidence to date suggests is predominantly α-HBCD) (UK Food Standards Agency, 2006).

Table 7.2: Summary of HBCD Diastereomer Patterns (% of ΣHBCD) in House and Office Dust in this study

Location (reference)	**Statistical parameter/ Diastereomer**	**α-HBCD**	**β-HBCD**	**γ-HBCD**
UK homes, n=31	Average	32	8	60
	σ_{n-1}	16	3	17
	Median	24	8	69
	Geometric mean	29	8	56
	Minimum	14	4	24
	Maximum	67	16	79
Canadian homes, n=8	Average	49	10	41
	σ_{n-1}	7	2	9
	Median	48	9	44
	Geometric mean	49	10	39
	Minimum	39	8	27
	Maximum	60	13	52
US homes, n=13	Average	28	7	65
	σ_{n-1}	15	3	17
	Median	23	7	69
	Geometric mean	23	6	63
	Minimum	4	3	34
	Maximum	57	12	94

7.6 Implications for human exposure.

The measured concentrations in house dust were used to estimate the exposure of adults and toddlers to ΣHBCD via dust ingestion in each of the three countries studied (table 7.3). To do so, we have assumed average adult and toddler dust ingestion figures of 20 and 50 mg day^{-1}, and high dust ingestion figures for adults and toddlers of 50 and 200 mg day^{-1} (Jones-Otazo et al., 2005). We have then estimated various plausible dust ingestion

exposure scenarios, using 5th percentile, median, average, and 95th percentile concentrations in the dust samples reported here. It is stressed that the range of exposure estimates via dust ingestion thus derived are only an indication of the likely range within the population. This is partly because of the relatively small number of dust samples analyzed (although as mentioned earlier, concentrations are in line with those reported elsewhere), and also due to the highly uncertain nature of the ingestion rates used here (and in other studies) as they are based on only a small number of studies involving primary data collection (ECETOC, 2001, U.S. EPA, 1997, U.S. EPA, 2002.). In addition, the exposure via ingestion of dust from other microenvironments like offices, cars or nurseries is not accounted for in these estimations.

Table 7.3: Summary of estimates of exposure (ng day-1) of adults and toddlers to ΣHBCDs via dust ingestion in Canada, the UK, and the US

Intake (ng day^{-1})[a]	**Adult**				**Toddler (6-24 months)**			
	5th %ile	**Median**	**Average**	**95th %ile**	**5th %ile**	**Median**	**Average**	**95th %ile**
Canada (mean)[b]	3.0	13	13	24	7.5	32	33	59
Canada (high)[c]	7.5	32	33	59	30	130	130	240
UK (mean)[b]	3.2	15	120	440	8.0	37	300	1100
UK (high)[c]	8.0	37	300	1100	32	150	1200	4400
US (mean)[b]	2.3	7.7	16	60	5.8	19	40	150
US (high)[c]	5.8	19	40	150	23	77	160	600

[a] Using dust ingestion rates from reference (Jones-Otazo et al., 2005).
[b] Mean dust ingestion rate for adults = 20 mg d^{-1}; for toddlers 50 mg d^{-1}.
[c] High dust ingestion rate for adults = 50 mg d^{-1}; for toddlers 200 mg d^{-1}.

Comparing the obtained estimates via dust ingestion to the UK estimated upper bound dietary intake from the whole diet in 2004 (413 ng ΣHBCDs day^{-1} for a 70 kg adult and 240 ng ΣHBCDs day^{-1} for a 10 kg toddler) (UK Food Standards Agency, 2006), it is clear

that ingestion of indoor dust represents an important pathway for human exposure to HBCD within the UK. Specifically, our estimates of UK exposure via dust ingestion range from 3.2 ng to 1100 ng ΣHBCDs person^{-1} day^{-1} for adults and from 8.0 to 4400 ng ΣHBCDs person^{-1} day^{-1} for toddlers.

The current absence of dietary exposure estimates for Canada and the United States prevents similar comparison for these countries. It is also instructive to compare our estimates of exposure via dust ingestion with data relating to the occupational exposure of Scandinavian workers in an industrial plant producing HBCD-treated expanded polystyrene (Thomsen et al., 2007b). Assuming that exposed workers in this study weigh 70 kg, inhale 20 m^3 day^{-1} over an 8 hour working day, and that they are continuously exposed to air contaminated at the median value of 2.1 μg ΣHBCDs m^{-3} (Thomsen et al., 2007b), then such workers are exposed to 200 ng ΣHBCDs kg bw^{-1} day^{-1}. By comparison, a UK toddler weighing 10 kg and ingesting 200 mg dust day^{-1} contaminated at the 95th percentile level reported in this study, will be exposed to 440 ng ΣHBCDs kg bw^{-1} day^{-1}. While it is stressed strongly that the dust exposure estimate cited here for comparison is a high-end exposure scenario, it is important to note that our findings suggest that some UK toddlers are being exposed at levels within the range of occupationally-exposed workers. Although the toxicological implications of such exposures have yet to be elucidated fully, this finding provides substantial motivation for further studies investigating the magnitude and relative significance of pathways of human exposure to HBCDs.

CHAPTER VIII

ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY (ESEM) AS A TOOL TO ELUCIDATE THE TRANSFER MECHANISM OF BDE-209 AND HBCDS TO INDOOR DUST

8.1 Introduction.

Despite the increasing evidence of the substantial implications of indoor dust for human exposure to BFRs, the mechanisms via which practically involatile chemicals like BDE-209 transfer into dust from treated goods are still unclear. Several mechanisms have been suggested for the transfer of BFRs from products to indoor air and dust including:

a) Volatilisation from treated goods and emission to air followed by deposition to dust particles (EU Risk Assessment Report, 2002, KEMI (National Chemicals Inspectorate), 2007). The volatilization aspect of this process will be enhanced at elevated temperatures. The results of chamber measurements (Kemmlein et al., 2003) for BFRs including penta-BDE, HBCD, TBBP-A and deca-BDE revealed that BFR emissions from a printed circuit board at 60 ºC are higher than at 23 ºC. This difference was 500-fold in the case of BDE-47. Emissions of HBCD from EPS and XPS insulation boards were also reported. For PBDEs, as the number of bromine atoms in the compound increased above six, emissions were increasingly affected by sink effects. Therefore, it was concluded that under actual indoor environmental conditions non-volatile compounds such as deca BDE would likely bind to house dust particles (Kemmlein et al., 2003).

b) Direct leaching of BFRs from treated products to dust by weathering or physical abrasion (EU Risk Assessment Report, 2002). The potential of both BDE-209 and HBCD to leach from plastics and mouldings is very small due to the high resistance to physical wear and tear. However, the release of these BFRs into indoor dust bound within particles or fibers released from disintegrating textiles and rubbers is more likely to occur (EU Risk Assessment Report, 2002, KEMI (National Chemicals Inspectorate), 2007).

In an attempt to understand the mechanism of transfer of both BDE-209 and HBCD to indoor dust and thereby explain the very high concentrations of these BFRs observed in some of the analysed samples (table 3.2), we hypothesized that volatilized and redeposited BFRs will be associated with dust particles containing organic matter (Weschler and Nazaroff, 2008) and will be distributed homogeneously in house dust. In contrast, BFRs originating from physical abrasion of treated textiles and other materials will remain bound within particles of the original polymer matrix and will be distributed heterogeneously throughout the debris. This is consistent with the fact that the deposition of the flame-retardant backcoatings on fabric is non-continuous and the distribution of flame-retardant compounds within fabrics is non-uniform (KEMI (National Chemicals Inspectorate), 2007).

To find an explanation for the high concentrations of some BFRs in indoor dust, we selected dust samples with high concentrations of BDE-209 (n=2) and HBCDs (n=2). In each of these samples the concentrations of other BFRs were at least an order of magnitude lower than the studied compound (table 8.1). We also examined a dust sample with low concentrations of all BFRs to act as a control.

Table 8.1: Concentrations (ng g^{-1}) of BFRs in dust samples examined by ESEM.

Sample #	**ΣHBCDs**	**BDE-209**	**Σtri-hexa-BDEs**	**Decaethane (LOQ=10ng/g)**	**TBE (LOQ= 5ng/g)**
Sample 1	40000	3400	720	110	< LOQ
Sample 2	18000	1700	74	1400	< LOQ
Sample 3	1400	2310000	196	93	< LOQ
Sample 4	2100	2600000	1100	150	29
Sample 5	16	27	38	< LOQ	< LOQ

8.2 Examination of dust samples using ESEM.

Small aliquots of dust samples were adhered to aluminum stubs using double-sided carbon sticky tabs (Agar Scientific) prior to coating with evaporated gold in an Emscope SC500 evaporation unit. Microscopic examination was conducted at high vacuum in an FEI XL-30 FEG environmental scanning electron microscope (ESEM). Samples were

first examined using secondary electron (SE) images produced by inelastic scattering of the electron beam which provides high resolution imaging and allows quick movement throughout the microscopic stage. This was followed by using backscattered electron (BSE) images produced by elastic collisions, which display a brighter image of atoms with higher atomic numbers (caused by stronger scattering of these electrons and thus appear brighter in backscatter images) (Goldstein et al., 2003). This was followed by using the X-ray energy dispersive spectrometry (EDS) system to examine specific areas of particles (which appeared brighter on the BSE images) and to create detailed elemental maps of the specified areas. The beam energy used for imaging varied between 1 and 20 kV and for EDS was 30 kV. Bromine was monitored at the K-α line =11.9 KeV for enhanced selectivity.

8.3 ESEM as a tool to elucidate causes of elevated concentrations of BDE-209 in indoor dust.

Examination of the most contaminated sample with BDE-209 in this study (sample 4 from a 9 year-old car, table 8.1) via backscattered electrons found a range of bromine-rich particles generally ranging in size from <1 to 20 μm. A representative example of these images is shown as Figures 8.1a and 8.1b. Inspection of Figure 8.1a shows the presence of a largish particle (on the order of 30 μm across). The unevenly distributed shiny white patches present on the surface of this particle are due to back-scattered electrons denoting the presence of Br. Figure 8.1b focuses on a small circled section of the particle shown in Figure 8.1a, showing at greater magnification some white patches on the particle surface that EDS analysis reveals as containing 27.9% Br (figure 8.2). We believe such particles arise as a result of abrasion of treated textile fabrics; as such material is surface-coated with the Deca-BDE formulation. We believe this to be a more plausible source of such highly contaminated particles than the abrasion of treated high impact polystyrene (HIPS) electronic housing, as: (a) HIPS is far more resistant to abrasion than fabrics, and (b) because Deca-BDE is incorporated within rather than on the surface of HIPS (EU Risk Assessment Report, 2002). Furthermore, the ESEM images are not consistent with contamination with BDE-209 arising via volatilisation from treated products and subsequent deposition on dust particles as this should result in a

more uniform distribution of bromine on all particles within a dust sample, rather than the presence of a small number of highly contaminated particles unevenly coated with Br.

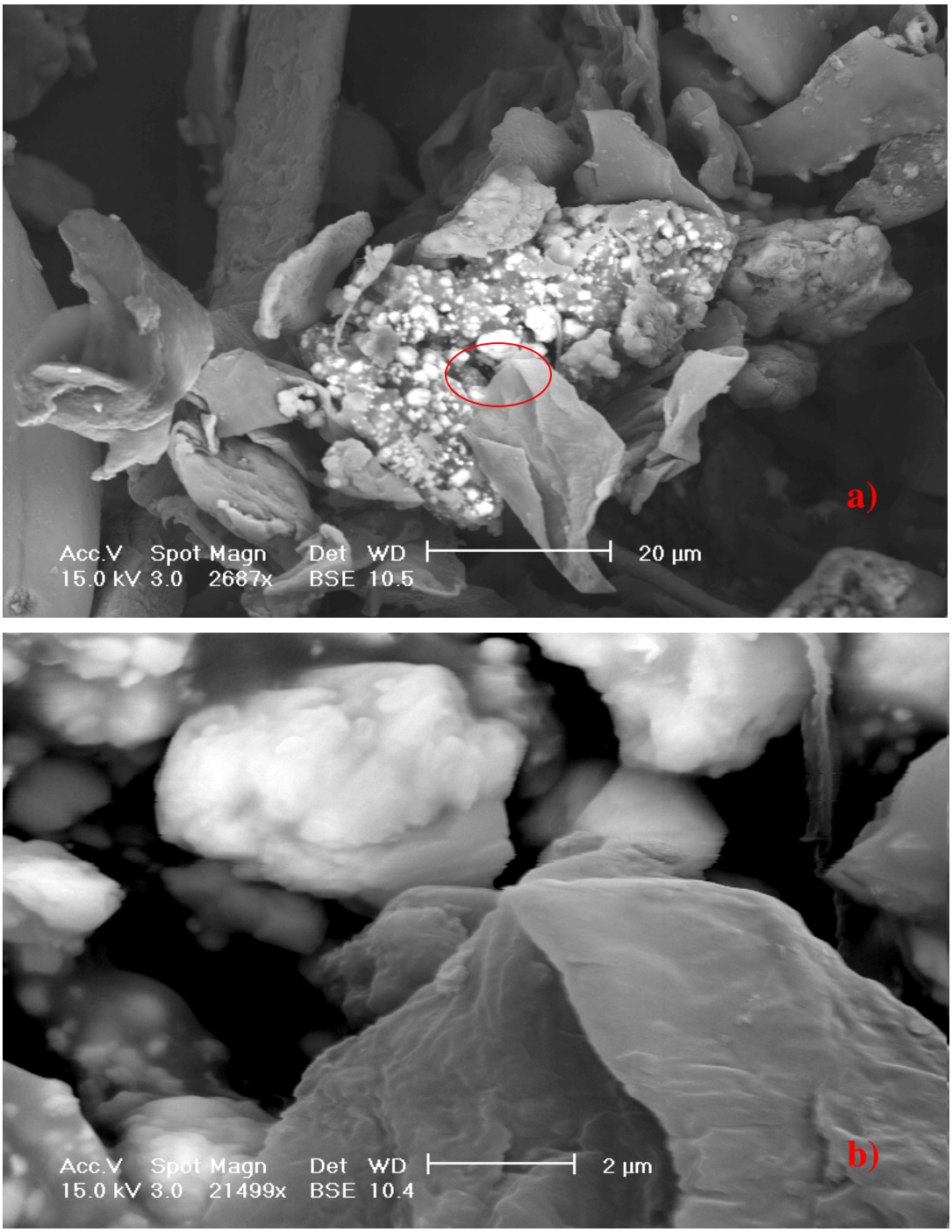


Figure 8.1: ESEM images of a car dust sample containing 0.26% BDE-209; (a) whole particle (20 µm scale), (b) enlargement of area circled in (a) (2 µm scale). Shiny white areas indicate presence of bromine.

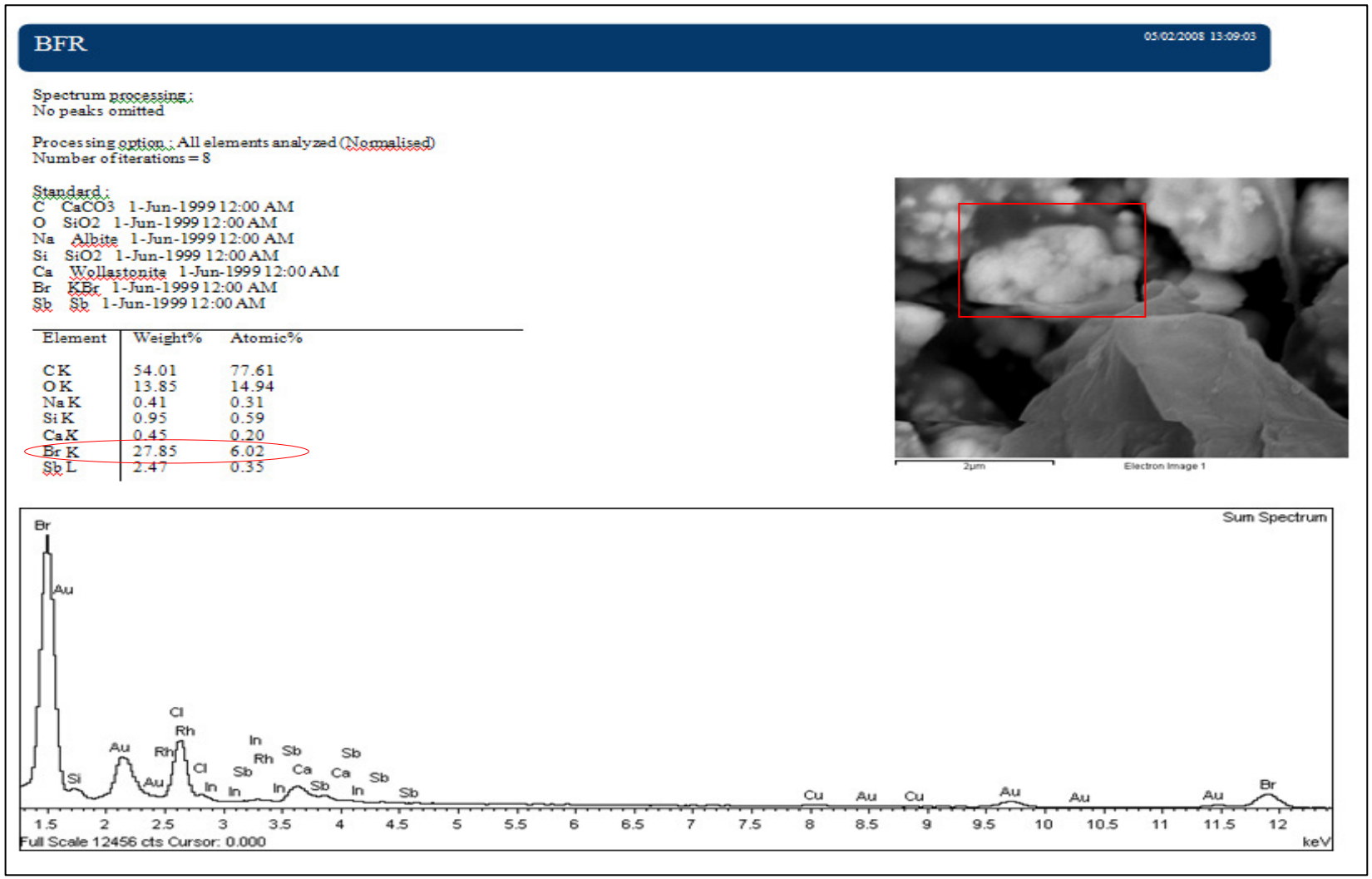


Figure 8.2: X-ray EDS report for sample in fig 8.1b showing a 27.85% w/w bromine content.

Given the highly elevated concentration of BDE-209 in this car sample and in the living room from which a sample containing 0.14% BDE-209 originated (sample 3, table 8.1), we resampled both of these environments in January 2008, approximately 9 months after the original samples were taken.

In both cases, there was only a slight decline in concentrations to 0.22% and 0.09% BDE-209, confirming the highly elevated contamination in these environments. While there were no apparent changes in the contents of the car, a number of changes in the living room contents occurred between the two samples. Specifically, the furniture, TV, VCR, and DVD player were replaced, suggesting that these were not the source of the elevated BDE-209 levels. Instead, the source appears to be either two items of electronic equipment present in the room during both sampling events, or more likely in view of the ESEM data, either the carpet or curtains which were not changed between samples.

8.4 ESEM as a tool to elucidate causes of elevated concentrations of HBCDs in indoor dust.

Examination of the most contaminated sample with ΣHBCDs in this study (sample 1 from an office, table 8.1) using BSE imaging showed no uneven distribution of bright patches. Similar results were obtained by careful examination of another house dust sample with high concentration of ΣHBCDs (sample 2, table 8.1). Using the x-ray EDS mapping tool to study the bromine distribution throughout the sample (by monitoring the K-α line =11.9 KeV) (figure 8.3). Both samples showed homogenous distribution of particles containing bromine (figure 8.3a). No localised bright patches with high bromine content were observed similar to those found in samples highly contaminated with BDE-209 (figure 8.3c). This indicates that the mechanism of HBCD transfer to dust in samples 1 and 2 is different from that observed for BDE-209 in samples 3 and 4 (table 8.1). We believe that HBCD was transferred to these dust samples by volatilisation and deposition to dust particles resulting in a homogenous distribution of bromine.

We only examined a small number of dust samples with high concentrations of BDE-209 and HBCDs which are both relatively non volatile at room temperature. Caution is therefore needed in extrapolating these results to other dust samples or to other BFRs.

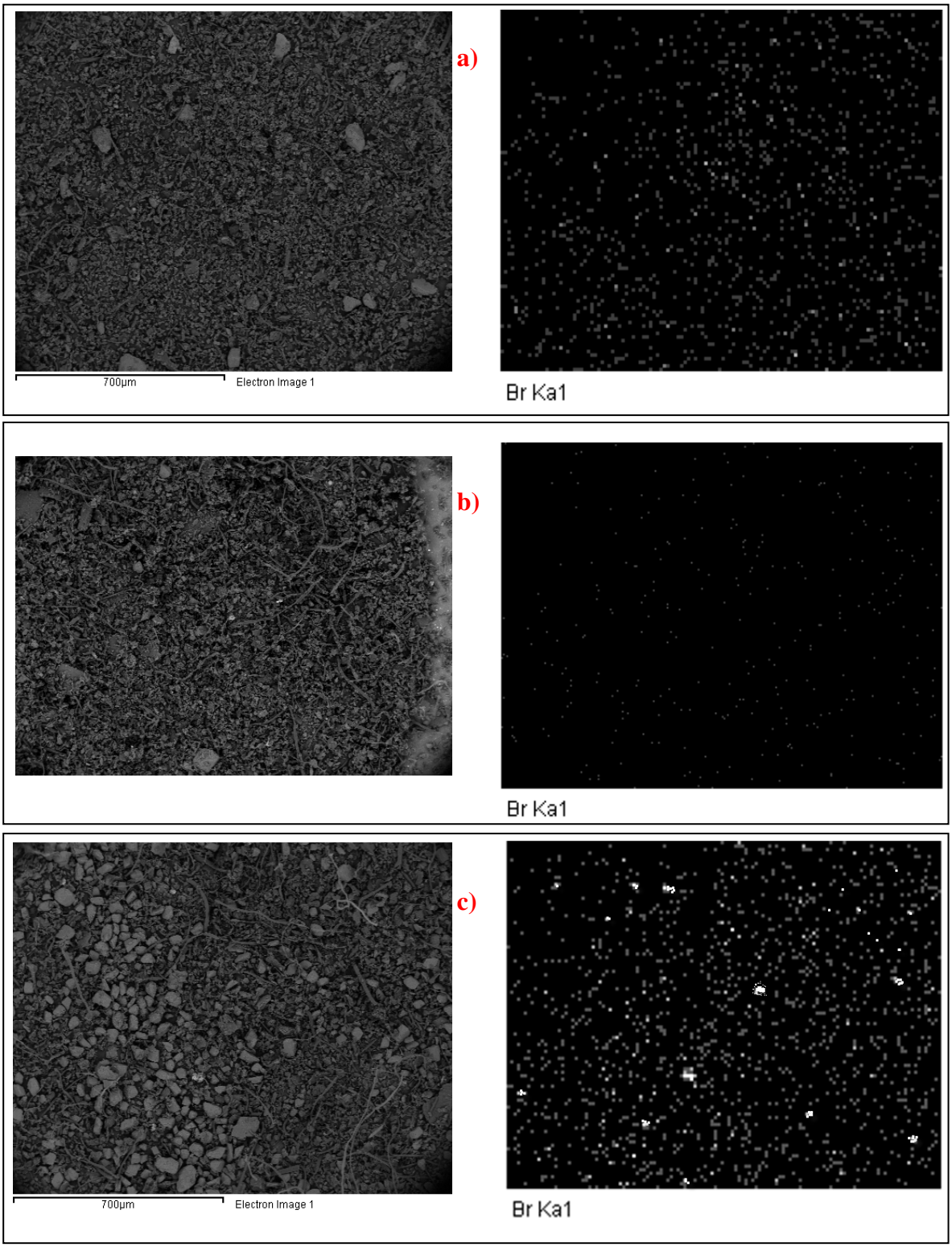


Figure 8.3: x-ray EDS mapping of (a) sample 1 showing homogenous distribution of bromine (b) sample 5 showing homogenous but very low bromine content (c) sample 4 showing localised bright patches rich in bromine.

It is possible, for example, that the more volatile Penta-BDE may transfer to dust via both volatilization and physical degradation of foam materials. Hence, more studies of other dust samples with varying degrees of contamination are needed to better understand the sources and distribution of BFRs in indoor dust.

CHAPTER IX

EXPOSURE TO HEXABROMOCYCLODODECANES VIA DUST INGESTION, BUT NOT DIET, CORRELATES WITH CONCENTRATIONS IN HUMAN SERUM

9.1 Introduction.

The production, use and environmental detection rates of HBCDs have increased over the early part of this decade in a variety of matrices, including guillemot eggs (Sellstrom et al., 2003), marine mammals (Law et al., 2006c), lake sediments (Kohler et al., 2008) and breast milk (Eljarrat et al., 2009a, Kakimoto et al., 2008). Most recently though, a decline in HBCD manufacturing emissions appears to have effected a stabilization in HBCD concentrations in porpoises from the UK (Law et al., 2008a) and in fish (Roosens et al., 2008). Despite these facts, only few studies have examined HBCD concentrations in matrices relevant to human exposure, such as food (Driffield et al., 2008, Fernandes et al., 2008, van Leeuwen and de Boer, 2008) or indoor dust (Stapleton et al., 2008). Likewise, we are aware of only one study in which the levels of HBCDs in serum of Norwegians were correlated to the consumption of highly contaminated fish (Thomsen et al., 2008). We have recently shown (see chapters 5 and 6 for details) that dust ingestion is a pertinent exposure pathway for HBCD, and a significant positive correlation was reported between concentrations of polybrominated diphenyl ethers (PBDEs) in house dust and diet with those in human milk (Wu et al., 2007). Yet, no study has examined the relationship between dust intake and serum concentrations for HBCDs. Moreover, no publication exists to date combining exposure to HBCDs via both diet and dust.

Against this background of limited data regarding human exposure assessments for HBCD, this study examines the relationship between individual body burden and contemporaneous exposure via two pathways (food and dust) for adults. To achieve this, concentrations of ΣHBCDs were measured in the blood serum of 16 Belgian adults and compared with contemporaneous duplicates of their dietary intake collected over a period

of 1 week, as well as dust samples from their bedrooms. The total intake of HBCDs for individual participants was calculated as the sum of dust ingestion and dietary intake and was correlated with the corresponding serum concentrations. Finally, diastereomeric and Enantiomeric patterns were determined to improve current knowledge concerning isomer- and enantiomer specific fate in the human body. Such knowledge may prove of particular value should evidence emerge of diastereomer- and/or enantiomer-specific toxicity.

9.2 Sampling strategy.

9.2.1 Participants.

Sixteen Belgian students (7 males and 9 females aged between 20 and 25 years) residing in university housing were recruited. Time spent outside the dormitory (and the corresponding exposure to HBCDs) was not accounted for, but it is highly plausible that the students used their room for domestic activities. The study was approved by the Ethics Committee of the University of Antwerp and all subjects gave informed consent before participating in the study. To minimize confounding due to previous exposures, participants were required to have resided in university housing for at least three years prior to the study and to have been resident in Belgium since childhood.

9.2.2 Sample collection.

Duplicate diet: Duplicate diet samples (n = 165) were collected between May - June 2007. Participants were instructed to maintain their usual dietary habits and provided at the end of each day an identical duplicate of what they had consumed for breakfast, dinner and additional snacks, such as deserts. Lunches were consumed at the university cafeteria; all daily menus were analyzed once and added up to each volunteer's dietary pattern according to their preference that day. Participants consuming each morning the same breakfast were only analyzed once. For each participant, duplicate diet samples were collected for one week.

Indoor dust: Dust samples were collected on the last day of duplicate diet collection, according to a standardized protocol by one of the research team (see section 2.1.2 for details).

Blood Serum: Following acquisition of the diet and dust samples, each participant donated 10 mL blood which was centrifuged to obtain serum. An aliquot (150 μL) of the samples was analyzed for triglycerides and total cholesterol in a clinical laboratory. The total lipid content varied between 2.95 g/L and 10.10 g/L. The remaining serum (3 to 4.5 mL) was stored at -20 °C until analysis.

9.3 Concentrations of HBCDs in food, dust and serum.

Food: Only 13 out of 165 duplicate diet samples contained concentrations of ΣHBCDs above LOQ at concentrations ranging between 0.01 – 0.35 ng/g ww (average 0.13) (table 9.1). HBCDs could only be detected in diet samples containing meat, milk, cheese and fish, with highest ΣHBCDs levels found in a duplicate diet sample that contained tuna. Following the protocol applied during the current study, complete meals were homogenized prior to analysis which might partially explain the high number of non-detcted due to dilution by low contaminated ingredients in a meal. The concentrations reported here are at the low end of those reported previously, but the range is in line with concentrations (0.02 – 0.3 ng/g ww) reported recently for the UK (Driffield et al., 2008)(table 9.1). In general, the highest concentrations of HBCDs (up to 5.0 ng/g ww) were reported in fish (Knutsen et al., 2008, Remberger et al., 2004) and European food samples are characterized by a lower detection frequency of HBCDs compared to PBDEs (D'Silva et al., 2006, Voorspoels et al., 2007). HBCD data in American foodstuffs are scarce (Schecter et al., 2008b).

Dust: HBCDs were detected in all dust samples with the three isomers being above LOQ (table 9.1). ΣHBCDs ranged between 33 – 758 ng/g dw (average 160, median 114). Concentrations are considerably lower compared to the limited European database, consisting mainly of UK studies (Abdallah et al., 2008a, Abdallah et al., 2008c, Abdallah et al., 2008b). Specifically, the UK values of HBCDs in house dust (table 3.2) were statistically higher (t-test on log transformed concentrations, $p<0.01$) than the values detected in this study. Although a Belgian Greenpeace study monitored several pooled and individual home dust samples with concentrations up to 57 600 ng/g dw, median values were below LOQ (20 ng/g dw), indicating that HBCDs were not present in the majority of samples (Greenpeace 2004).

Table 9.1: Descriptive statistics of individual HBCD isomers and ΣHBCD concentrations in food (ng g^{-1} ww), dust (ng g^{-1} dw) and serum (ng g^{-1} lw) from the present and related studies.

	Country		**Median**	**Average**	**SD***	**Range**	**Reference**
food	Belgium	ΣHBCDs	0.098	0.127	0.113	0.014 – 0.345[a]	Present study
(ng g^{-1} ww)	Sweden	ΣHBCDs	-	-	-	< 0.8 – 4.9	(Remberger et al., 2004)
	UK	ΣHBCDs	-	-	-	0.02 – 0.3	(Driffield et al., 2008)
	Norway	ΣHBCDs	-	-	-	0.12 – 5[b]	(Knutsen et al., 2008)
			-	-	-	0.03 – 0.15[c]	
			-	-	-	0.2 – 6[d]	
Dust	Belgium	ΣHBCDs	114	160	169	33 – 758	Present study
(ng g^{-1} dw)		α-HBCD	69	93	107	22 – 481	
		β-HBCD	14	19	19	4 – 87	
		γ-HBCD	31	48	50	7 – 190	
	UK	ΣHBCDs	1300	8300	26000	140 – 140000	(Abdallah et al., 2008a)
		α-HBCD	380	3200	11000	22 – 66000	
		β-HBCD	93	1000	3900	9 – 26000	
		γ-HBCD	670	4200	13000	70 – 75000	
	UK	ΣHBCDs	730	6000	-	140 – 110000	(Abdallah et al., 2008c)
		α-HBCD	170	2800	-	22 – 66000	
		β-HBCD	66	470	-	9 – 7800	
		γ-HBCD	440	2800	-	70 – 37000	

Table 9.1 (continued): Descriptive statistics of individual HBCD isomers and ΣHBCD concentrations in food (ng g^{-1} ww), dust (ng g^{-1} dw) and serum (ng g^{-1} lw) from the present and related studies.

	Country		**Median**	**Average**	**SD***	**Range**	**Reference**
Dust	Canada	ΣHBCDs	640	670	-	64 – 1300	(Abdallah et al., 2008c)
(ng g^{-1} dw)		α-HBCD	300	340	-	25 – 670	
		β-HBCD	72	70	-	6 – 130	
		γ-HBCD	230	260	-	34 – 470	
	US	ΣHBCDs	390	810	-	110 – 4000	(Abdallah et al., 2008c)
		α-HBCD	80	260	-	17 – 1800	
		β-HBCD	28	56	-	6 – 300	
		γ-HBCD	300	490	-	79 - 2000	
	Belgium	ΣHBCDs	< 20	4805	-	< 20 - 57554	(Greenpeace report, 2003)
	US	ΣHBCDs	230	354	8.6	< 4.5 - 130200	(Stapleton et al., 2008)
Serum	Belgium	ΣHBCDs	1.7	2.9	3.2	< 0.5 – 11.3	Present study
(ng g^{-1} lw)	Netherlands	ΣHBCDs	-	-	-	0.5 – 1.5	(Weiss et al., 2004)
	Norway	ΣHBCDs	4.1	9.6	-	< LOQ – 52[f]	(Thomsen et al., 2008)
		ΣHBCDs	2.6	3.7	-	< LOQ – 18[g]	
	Sweden	ΣHBCDs	0.46	-	-	< 0.24 – 3.4	(Weiss et al., 2006)

* Standard deviation.

[a] duplicate diets, [b] fish, [c] meat, [d] egg, [e] only α-HBCD detected, [f] men, [g] women.

Serum: Concentrations of ΣHBCDs measured in blood serum from each of the study participants fell in the range < 0.5 - 11 ng/g lw (average 2.9) (table 9.1). Seven out of 16 blood serum samples were below LOQ. Levels of ΣHBCDs in this study (table 9.1) are comparable with those reported for non-occupationally exposed populations (Thomsen et al., 2008, Weiss et al., 2004, Weiss et al., 2006), but lower than those detected in occupationally-exposed adults (Thomsen et al., 2007b).

9.4 HBCD isomeric patterns.

Food: The diastereomeric pattern in the majority of our food samples was dominated by γ-HBCD, with the exception of three duplicate diet samples containing fish, meat or cheese, where α-HBCD was dominant. Such predominance of the α-HBCD isomer has been documented previously in fish and meat (Fernandes et al., 2008, Knutsen et al., 2008, Roosens et al., 2008) and arises probably as a result of selective biotransformation of the different isomers (Heeb et al., 2008, Zegers et al., 2005). In contrast, a predominance of γ-HBCD in sugars and preserves was reported (Driffield et al., 2008). Our data agree thus with the hypothesis that α-HBCD dominates in comestibles of animal origin, while γ-HBCD is the most prevalent isomer in other foodstuffs, including ingredients used for food processing (Driffield et al., 2008).

Dust: The dominant isomer in dust was α-HBCD, followed by γ-HBCD (table 9.1). This underlines previous observations in indoor dust of an appreciable shift from the γ-HBCD dominated profile observed in the commercial HBCD formulation (Abdallah et al., 2008a, Abdallah et al., 2008c, Abdallah et al., 2008b). Recently, the isomerization of γ-HBCD to α-HBCD has been observed following exposure of dust to UV radiation from sunlight (Harrad et al., 2009). The higher proportion of α-HBCD in textiles, such as curtains, confirms the isomerization of γ-HBCD to α-HBCD during incorporation of HBCD in consumer products (Kajiwara et al., 2009) which may consequently lead to higher levels of α-HBCD in dust.

Serum. A diastereomeric shift towards α-HBCD is likely to occur in biotic samples due to preferential metabolism of β-HBCD and γ-HBCD by cytochrome P450 (Zegers et al., 2005). The presence of γ-HBCD in the dust and food samples of the present study and its complete absence in the corresponding serum samples (figure 9.1) is consistent with *in*

vivo transformation of γ-HBCD to α-HBCD, and with the observations of Weiss et al. (2006), where α–HBCD was the predominant isomer (97-99% of ΣHBCDs) in a pooled serum sample comprising blood from 53 individuals. Although α-HBCD was dominating, small amounts (1-3%) of γ-HBCD were also detected.

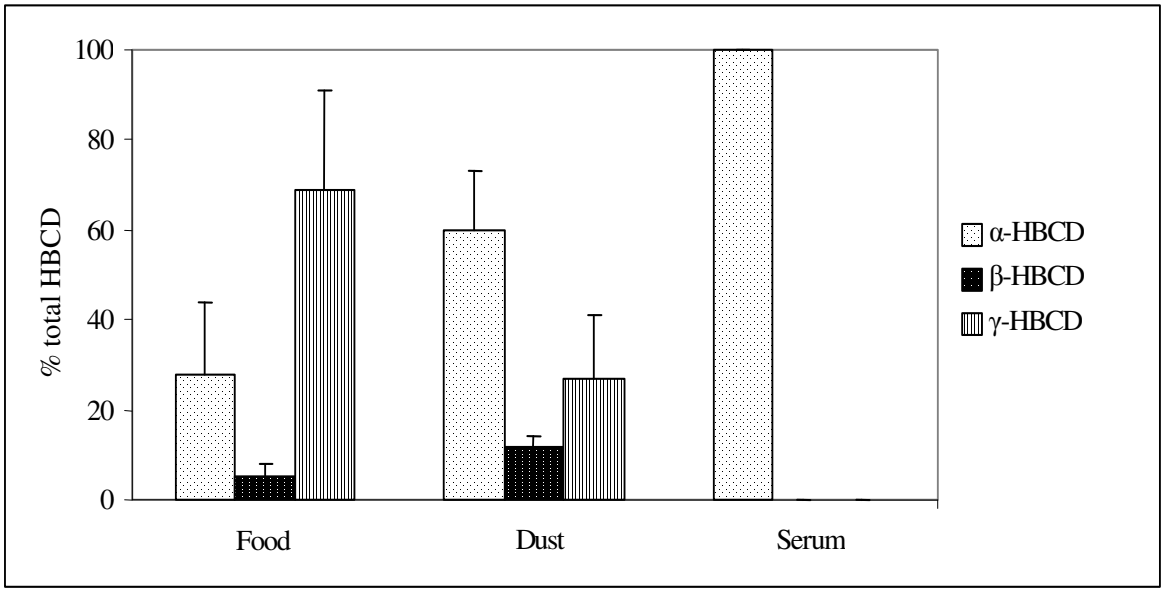


Figure 9.1: Average percentage distribution of individual HBCD isomers in food, dust and serum.

Some studies reported γ-HBCD to have a higher percentage of the total HBCDs in human tissues, such as adipose tissue (Johnson-Restrepo et al., 2008) and serum samples (Thomsen et al., 2007b). In contrast, a recent study reported the dominance of γ-HBCD in 24 out of 30 Spanish breast milk samples, while α-HBCD was predominant in the remainder (Eljarrat et al., 2009a). Interestingly, HBCD concentrations in this Spanish study are higher compared to similar studies (Antignac et al., 2008, Kakimoto et al., 2008, Polder et al., 2008a), indicating a higher exposed population. An increase in the percentage of γ-HBCD has also been seen in occupationally-exposed workers, with γ-HBCD making up to 40% of ΣHBCDs (Thomsen et al., 2007b). While the reasons for the different isomer profiles in human tissues from different studies are not yet clear, it is reasonable to hypothesize that they arise from a combination of differences in external exposures (e.g. α-HBCD predominated in both dust and diet of the present study), and

inter-individual variations in metabolism. More detailed studies are required to comprehend the cause(s) of the isomer profiles observed in humans.

9.5 Enantiomeric patterns.

The chiral signature (i.e. the relative abundance of the two enantiomers of a given isomer) of all detected isomers in food was racemic (EF = 0.5) or close to racemic in all samples above LOQ (table 9.2). Since this study is the first to suggest a racemic chiral signature of HBCDs in duplicate diets, comparison with other studies is not possible.

Table 9.2: Average ± SD enantiomeric fractions (EFs) of α-, β- and γ-HBCD in food, dust and serum. Racemic EF = 0.50, n.d. = not detected.

Diastereomer	Food (n=12)	Dust (n=9)	Serum (n=9)
α-HBCD	0.49 ± 0.04	0.52 ± 0.02	0.28 ± 0.02
β-HBCD	0.52 ± 0.02	0.48 ± 0.03	n.d.
γ-HBCD	0.51 ± 0.03	0.50 ± 0.02	n.d.

In dust samples, racemic or near-racemic chiral signatures were also observed for all isomers (table 9.2), consistent with recent observations (Harrad et al., 2009). Combined, these findings suggest that human exposure to HBCDs consists solely of racemic mixtures of HBCD isomers. The present study reports (-)-α-HBCD as the dominating enantiomer in human serum, with an average EF of 0.28 ± 0.02 (figure 9.2, table 9.2). Similar selective enantiomeric enrichment of (-) α-HBCD was reported in human serum (Weiss et al., 2006) and in human milk (Eljarrat et al., 2009a). The combination reported here of racemic signatures in dust and diet suggests that the directionally-consistent and non-racemic signatures for α-HBCD in serum are attributable to enantioselective absorption, metabolism and/or excretion as opposed to external exposure to non-racemic matrices.

9.6 Human intake of HBCDs.

Food: To date, relatively little is known about the magnitude of human exposure to HBCDs and the relative significance of different pathways. Table 9.3 compares the duplicate diet estimates of this study with previous estimates of dietary exposure obtained via either means (e.g. food basket surveys).

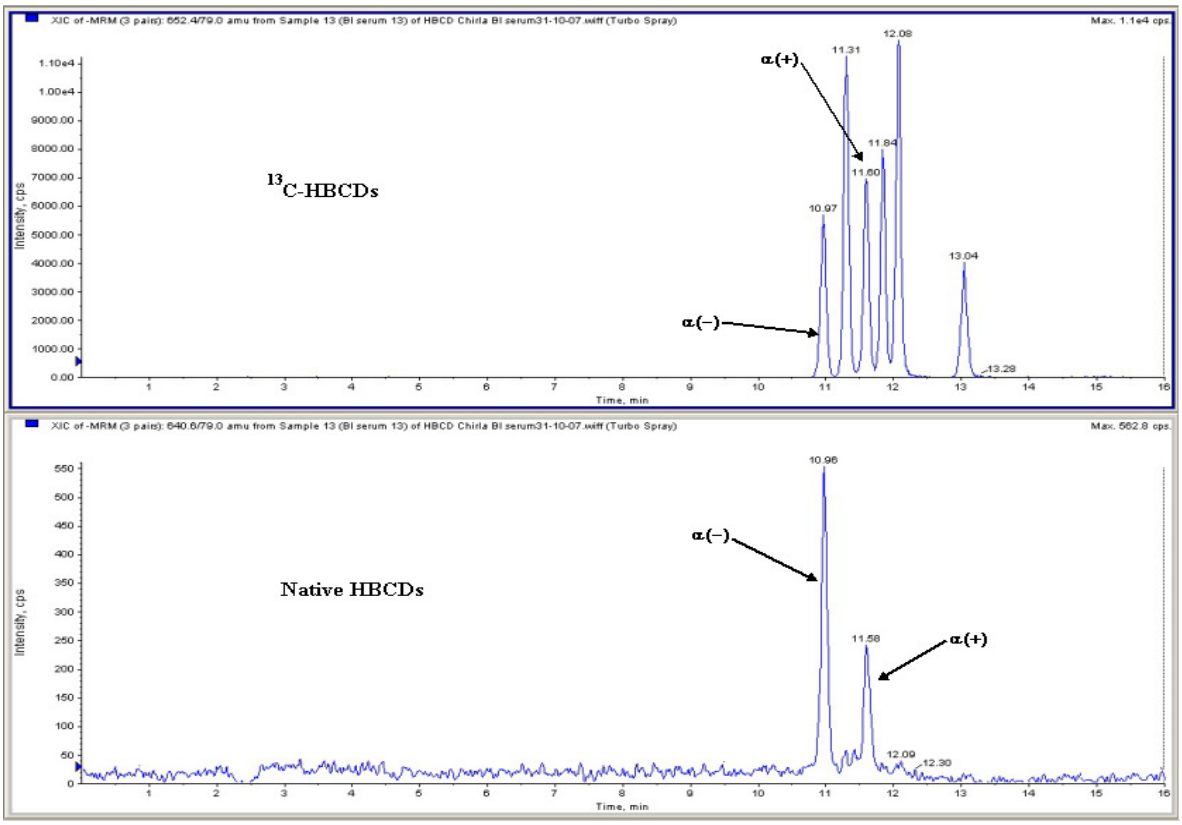


Figure 9.2: chiral chromatogram of native and ^{13}C-HBCDs in a serum sample.

Our dietary exposure estimates (1.2-20 ng ΣHBCDs/day; average 7.2 ng) are appreciably lower than those reported previously (200 – 500 ng ΣHBCDs/day) for the Netherlands (de Winter-Sorkina et al., 2003) and the UK (Driffield et al., 2008, UK Food Standards Agency, 2006), but are more consistent with those reported recently (4 to 81 ng ΣHBCDs/day) for Norway (Knutsen et al., 2008). This apparent discrepancy is likely due to: (a) the fact that our estimates are based on a short "snap-shot" in time of exposure for a small number of individuals; (b) the nature of the diets consumed in the present study, which consisted largely of lean meats and vegetables, with a low or no HBCD content; and (c) the fact that food basket studies provide conservative estimates of exposure when there is a low detection frequency of HBCDs and the exposure estimate for such samples was based on a concentration either equal to or half the method detection limit. Comparison between studies was challenging because the dietary exposure to HBCDs was assessed in the present study through duplicate diets.

Table 9.3: Intake (ng day^{-1}) of ΣHBCDs from food and dust ingestion in adults in this and related studies.

Intake	Country	Dust exposure	Median	Average	SD	Range	Reference
food (ng/day)	Belgium	-	5.5	7.2	5.2	1.2 - 20	present study
	Belgium	-	-	-	-	174	(de Winter-Sorkina et al., 2003)
	UK	-	-	-	-	354 - 474	(Driffield et al., 2008)
	Norway	-	16	18	-	4 -81	(Knutsen et al., 2008)
dust (ng/day)	Belgium	high	5.7	8.0	8.5	2.8 - 38	present study
		average	2.3	3.2	3.4	1.1 - 15	
	UK	high	81	329	-	14 - 1172	(Abdallah et al., 2008a)
		average	33	132	-	6 - 469	
	UK	high	37	-	-	8 - 1100	(Abdallah et al., 2008c)
		average	15	-	-	3 - 440	
	Canada	high	32	-	-	8 - 59	
		average	13	-	-	3 - 24	
	US	high	19	-	-	6 - 150	
		average	8	-	-	2 - 60	
food + dust (ng/day)	Belgium	high	13	15	8.9	5.2 - 42	present study
		average	8	10	5.5	3.6 - 20	

Dust: To estimate exposures via dust ingestion, we used an average adult dust ingestion rate of 20 mg day^{-1} and a high dust ingestion rate of 50 mg day^{-1} (Jones-Otazo et al., 2005). Multiplying these values by the concentrations of ΣHBCDs detected in dust from the rooms of individual participant yielded exposures between 1.1 - 15 ng ΣHBCDs/day (average 3.2 ng for an average dust ingestion rate) and 2.8 - 38 ng ΣHBCDs/day (average 8.0 ng for a high dust ingestion rate) (table 9.3). Such estimates are at the low end of those calculated for UK adults (6 - 469 ng ΣHBCDs/day) (Abdallah et al., 2008a). It is important to note that this study did not monitor dust ingestion in other microenvironments, such as cars and lecture/library halls, nor the potential exposure during weekends in the parental home, which can add to the present exposure through dust. The exact influence on exposure of such other microenvironments will vary from individual to individual, but overall would be likely to result in higher exposure given that concentration of HBCDs in dust from UK cars exceeded significantly those from homes and offices (Abdallah et al., 2008a).

Combined diet and dust: Combining HBCD intake via both dust ingestion and diet, individual exposures ranged from 4 – 20 ng day^{-1} (average dust ingestion scenario) and from 5 – 42 ng day^{-1} (high dust ingestion scenario) (table 9.3). Calculating the relative importance of dust and diet as pathways of exposure to ΣHBCDs, the major contributor to the total intake of HBCDs depended on the amount of dust ingested. Based on the average dust intake scenario, food intake is the most important contributor to total intake (mean 67%, range 23 – 93%) (figure 9.3). Conversely, if the high dust ingestion scenario exposure estimate is used, food and dust contribute equally to the overall exposure of adults to ΣHBCDs in this study (mean contribution of dust 51%, range 16 – 90%) (figure 9.3). This agrees with our previous reports on the UK population, for which the relative significance of different exposure pathways for HBCDs appears somewhere between those for the Penta- BDE (principally diet, but with dust ingestion playing an important role for individuals with high concentrations of dust in their indoor environment) and BDE-209 (for which dust and diet are broadly equally important) (Abdallah et al., 2008a, Harrad et al., 2008a).

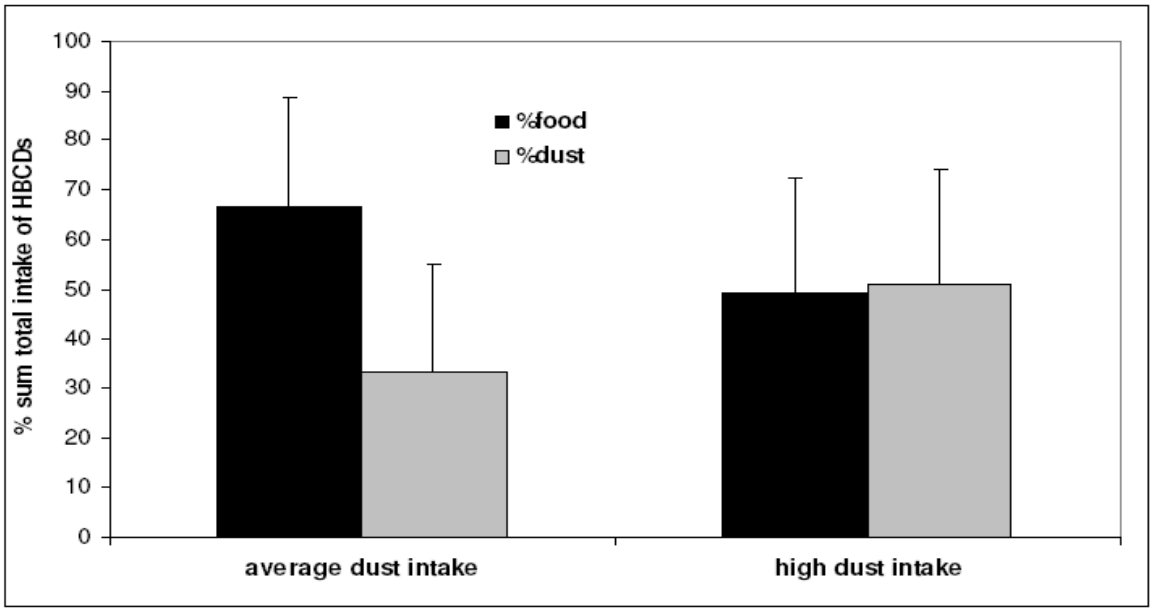


Figure 9.3: Contribution of dietary intake and dust ingestion to the the daily exposure of Belgian adults to ΣHBCDs.

9.7 Relationships between Exposure via Dust and Food Ingestion and Serum Concentrations.

To examine the relationship between the exposure of participants in this study with concentrations in their serum, we plotted serum concentrations of ΣHBCDs for a given individual against exposure: (a) via diet and dust (under an average dust ingestion scenario) combined; (b) via diet and dust (under a high dust ingestion scenario) combined; (c) via diet alone; and (d) via dust ingestion alone. No significant correlations were observed between serum concentrations and intake via diet alone (r_s = - 0.11, p=0.64) and combined food and dust exposure (under both average and high dust ingestion scenarios) (r_s = 0.34, p=0.20 and r_s = 0.47, p=0.07, respectively). Interestingly, the addition of dust ingestion to the combined food and dust exposure has increased the correlation between total HBCD intake and serum concentrations. Furthermore, HBCD concentrations in serum were correlated significantly with estimates of exposure via dust (r_s = 0.86, p<0.01) (figure 9.4). Yet, the relationship strongly depended on the high value (for both serum and dust). When this was removed, the correlation dropped to r_s = 0.81, but stayed significant p<0.01. The influence of dust ingestion on the serum concentrations is most probably attributed to the fact that it is a relatively constant exposure (as long as there are no changes in the BFR-treated products in the room), in

contrast to dietary intake, which is mostly influenced by irregular spikes in exposure through occasional ingestion of contaminated food. This is a highly relevant finding that adds to the growing weight of evidence that exposure to persistent organic chemicals in indoor dust exerts an important influence on human body burdens of such chemicals. In particular, it is in line with the correlation between concentrations of PBDEs in house dust and human milk reported previously for 12 individuals (Wu et al., 2007).

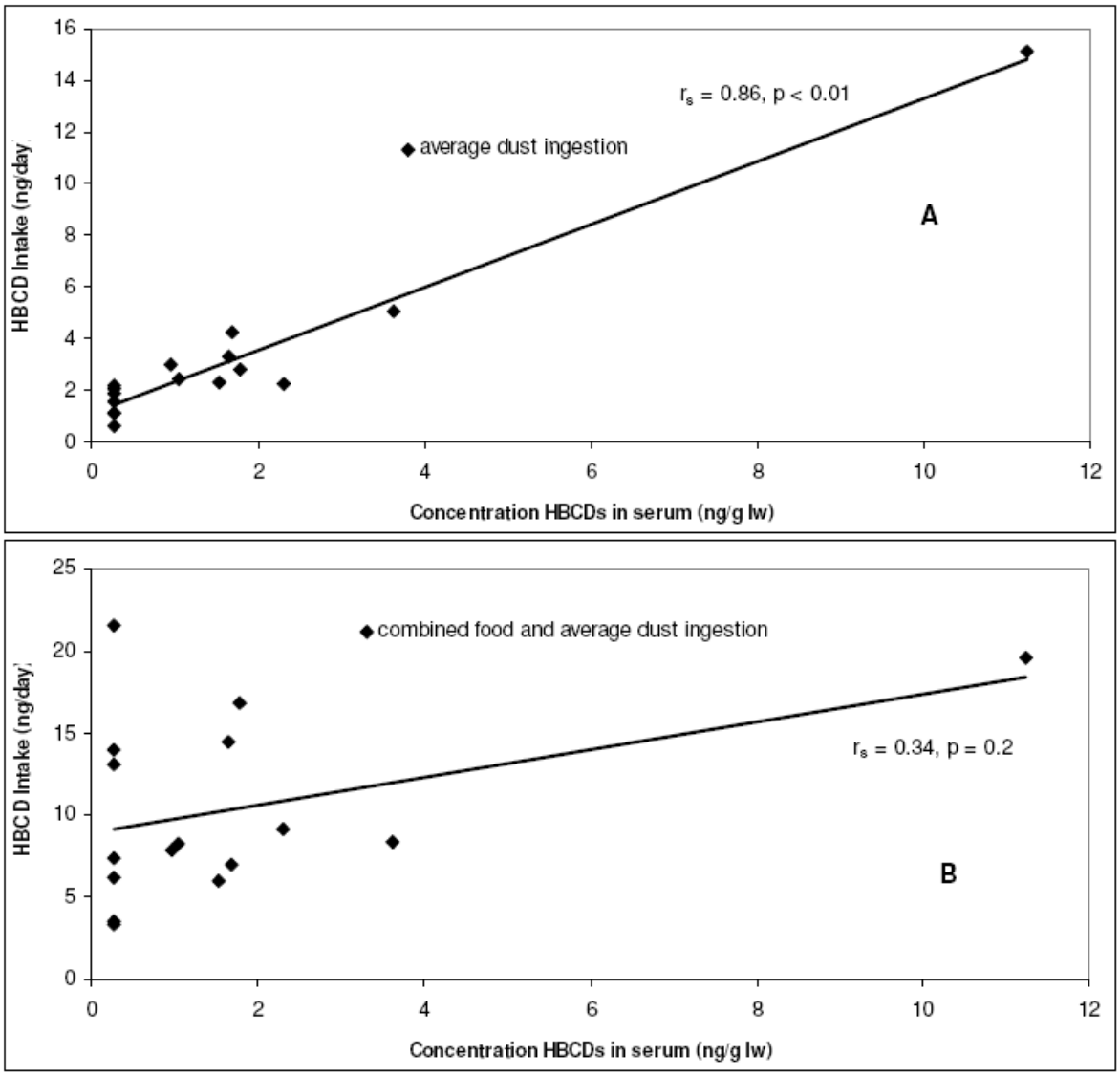


Figure 9.4: Relationship between serum concentrations of ΣHBCDs in serum and exposure to ΣHBCDs via dust ingestion (A) or combined food and average dust ingestion (B).

In contrast, while the study of Wu et al. (2007) reported a correlation between dietary exposure estimated from food frequency questionnaire and body burden for PBDEs, we found no such relationship for HBCDs. Significant correlations between dietary intake of HBCDs (especially from fish) and serum concentrations were already observed for populations exposed to high levels of HBCDs in the diet, e.g. fishermen (Thomsen et al., 2008). Although the current study reports daily exposure from food and dust to be approximately similar in magnitude, only dust exposure seems to correlate with serum concentrations. This suggests that episodic high dietary exposure of HBCDs, through irregular consumption of contaminated food items (e.g. eel), is a more important determinant of the body burden than continuous background dietary exposures at the low levels, as measured in this study. If this is true, one week of duplicate diets is too short to reflect true dietary intake of HBCDs and larger, more detailed studies are required to confirm this view. We believe that diet is an important contributor to human exposure to HBCDs, resulting from the consumption of highly contaminated food items (fatty fish, meat, etc). The sampling period in this study was too short to record consumption of such food items, leading to a background dietary exposure, which in turn has led to a lack of correlation between food exposure and serum.

To conclude, the exposure to HBCDs of the participants in this study via both dust ingestion and dietary intake is at the low end of that reported for previous studies. The relative contribution of the two exposure pathways is dependent on the dust ingestion rate assumed. Under an average dust ingestion scenario, diet is the major pathway, while under a high dust ingestion scenario, intake via dust and diet are roughly equal in importance. The importance of dust ingestion as an exposure pathway is emphasized by the significant correlation between exposure to HBCDs via dust ingestion and its concentration in serum. This suggests that people residing in houses with high concentrations of HBCDs in dust are potentially highly exposed. *In vivo* enantioselective absorption, metabolism and/or excretion of α-HBCD is demonstrated to be the cause of the substantial enrichment of (-)-α-HBCD in human tissues in this and other studies.

CHAPTER X

THE BIOACCESSIBILITY OF BROMINATED FLAME RETARDANTS FOLLOWING DUST INGESTION USING A COLON-ENHANCED PHYSIOLOGICALLY BASED EXTRACTION TEST

10.1 Introduction.

While *bioavailability* is defined as the fraction of an administered dose of a certain chemical compound that reaches the systemic circulation unchanged, *bioaccessibility* is the term used to define the fraction of the target compound introduced that dissolves in the gastrointestinal tract (GIT) medium and therefore, is available for absorption (Ruby et al., 1996). The former is measured *in vivo*, while the latter may be quantified *in vitro* via a physiologically based extraction test (PBET). PBET is an *in vitro* test system which incorporates human GIT parameters (including stomach, small intestine and colon pH and chemistry, solid-to-solution ratio, mixing and emptying rates) for predicting the bioaccessibility of chemicals from a solid matrix (Ruby et al., 1996). Assessment of bioaccessibility via PBET is an important tool when evaluating the risk to humans from persistent organic pollutants (POPs) and heavy metals (Dean and Ma, 2007). This approach seeks to mimic the major processes of human digestion to assess the released fraction of POPs and heavy metals from ingested substances consumed either accidentally or intentionally (Oomen et al., 2000). This tool has been applied successfully for estimation of the bioaccessibility of PCDDs/Fs, PAHs and PCBs from diet and surface soils (Dean and Ma, 2007).

Very little is known about the absorption of HBCDs from human GIT. In 1980, Yu and Atallah reported ~100% oral absorption of γ-HBCD in rats when administered as a solution dissolved in acetone:olive oil mixture. However, Arita et al. (1983) and Chengelis (2002) reported lower absorption (32-67%) by rats when HBCDs were administered orally (gavage) in the form of a suspension in olive oil (KEMI (National Chemicals Inspectorate), 2007). Therefore, the EU risk assessment draft concludes that

"When HBCD is properly dissolved in the vehicle; it is probably readily absorbed from the GIT. However, the exact extent of oral absorption is unknown; it is probably in the order of 50-100 %" (KEMI (National Chemicals Inspectorate), 2007). Accordingly, the bioaccessible fraction of HBCDs in the GIT following oral administration is likely to be all bioavailable. To our knowledge, there are no stereoisomer-specific studies of the absorption of HBCDs by humans or any other mammals from solid matrices following oral administration. While our results suggest that the observed shift from predominance of the γ-HBCD in abiotic samples to the α-isomer being predominant in most biotic and human samples (Covaci et al., 2006) may not solely be due to preferential *in vivo* biotransformation of β- and γ-HBCD (Zegers et al., 2005), but, at least partly attributable to other reasons like the diastereomer pattern in dust (table 3.2), other reasons may include preferential absorption and/or excretion of one or more of the 3 main HBCD diastereomers in humans.

Animal studies have shown that TBBP-A is fully (>95%) absorbed from rats GIT after oral administration as a solution in vegetable oil (Hakk et al., 2000, Meerts et al., 1999, EU Risk Assessment Report, 2006). However, no information is available on the bioavailability of TBBP-A following oral administration in a suspension or solid form.

A recent well-conducted study reported that the absorption of BDE-209 from the GIT of male rats following dust ingestion was similar to its absorption following administration as a solution in corn oil at two concentration levels (Huwe et al., 2008b). However, the extent of BDE-209 absorption from the GIT is very difficult to determine due to rapid excretion in faeces (~90% within 24 hours) and extensive metabolism (~65% of the excreted dose in the form of metabolites) (Morck et al., 2003, Huwe and Smith, 2007). Therefore, different assessments of BDE-209 bioavailability in rats following oral administration were reported in literature ranging from 4-26% (Huwe et al., 2008b, NTP, 1986, Morck et al., 2003, Sandholm et al., 2003, EU Risk Assessment Report, 2002).

Based on this scarce information, the aims of the current work are to:

- Determine the bioaccessibility of α-, β-, γ-HBCDs, TBBP-A and BDE-209 from the human stomach, small intestine and colon following ingestion of indoor dust.
- Investigate the effect of simulated GIT media on the enantiomeric fractions (EFs) of the 3 main HBCD diastereomers; and

- Assess the factors likely to affect the bioavailability of the studied BFRs from the human GIT following ingestion of indoor dust.

10.2 Selection of PBET parameters.

10.2.1 pH.

Gastric pH is quite variable among individuals, and depends strongly on nutritional status. Several gastric pH values ranging from 1-4 were previously utilized in PBET experiments to assess the bioaccessibility of POPs (Dean and Ma, 2007). In this study, we chose a gastric pH value of 2.5 to reflect a nutritional status intermediate between fasting and fed states (Ruby et al., 1996).

The pH of the small intestine compartment was adjusted to 7 which is consistent with average measured small intestinal pH values in humans (Murthy et al., 1980), while a colon pH of 6.5 was selected to represent an average pH of the ascending, transverse and descending colon segments measured in humans (Macfarlane et al., 1998a).

10.2.2 Stomach emptying rate.

The human stomach emptying rate is an exponential function of the stomach contents with the fastest emptying occurring after ingestion of a meal. In adults, ~80% of stomach emptying is achieved within the first hour after ingestion, with complete emptying occurring within 2 hours (Hunt and Spurrell, 1951, Podczeck et al., 2007). In children, 94% of emptying was reported to occur in 54-68 min after ingestion (Smith et al., 1993, Ruby et al., 1996). Therefore, a stomach incubation time of 1 hour was used in this study.

10.2.3 Small intestine transit time.

The passage of chyme (semi-fluid digested food material) from the top of the small intestine to the entrance to the large intestine in adults requires 3-5 hours (Ruby et al., 1996). In children, small intestine transit time following ingestion of a semisolid meal was reported to range 3.5-4.5 hours (Murphy et al., 1988). On the basis of these data, a small intestine transit time of 4 hours was selected in this study.

10.2.4 Colon residence time.

Although the colon residence time in humans normally ranges between 36-72 hours (Dean and Ma, 2007), a significant microbial growth was observed in the colon medium after 8 hours of incubation even after using pre-autoclaved medium components. This

microbial activity may exert significant effects on the levels and isomeric profiles of the target compounds in the studied medium despite the fact that these dust microbes would not flourish *in vivo* due to competition with a large, well-established community of gut microbes. For this reason we opted to use an 8 hour colon incubation time in our model. While we certainly recognize that as a possible source of inaccuracy in our model, we believe that this was the best method to avoid any undesired effects of dust microbes on the PBET results.

10.2.5 Solid to fluid ratio, mixing and temperature.

Solid to fluid ratios as low as 1:5-1:25 were reported to affect dissolution in previous studies of POPs bioaccessibility via GIT using similar PBET models (Dean and Ma, 2007). Therefore, a solid to fluid ratio of 1:100-1:120 was maintained in all our PBET experiments to avoid any effect of this parameter on the test results. All experiments were carried out at body temperature (37ºC) and the simulated GIT media were constantly shaken using a magnetic stirrer to simulate the peristaltic movement.

10.3 Experiment design.

A full description of the GIT media composition and experimental procedures is presented in section 2.1.3. A schematic diagram of the experiment design is given in figure 10.1. The supernatant representing the bioaccessible fraction of dust from each of the stomach, small intestine and colon media compartments (3 replicates each) were collected and analysed separately to determine the bioaccessibility from each single compartment. In addition, 1 gram dust was added to the stomach medium, incubated for 1 hour then the stomach medium was converted to small intestine medium by addition of saturated $NaHCO_3$ to increase the pH from 2.5 to 7.0 and 0.176 g bile salts and 0.05 g pancreatin (Ruby et al., 1996). After incubation for 4 hours, the small intestine medium was centrifuged and the supernatant collected and analysed to represent bioaccessibility from stomach and small intestine (3 replicates). Finally, the stomach+small intestine experiment was repeated and after centrifugation the residue was added to a fresh colon medium and incubated for 8 hours. This was followed by centrifugation and the supernatant was collected and analysed to assess the bioaccessible fraction from the whole GIT (3 replicates).

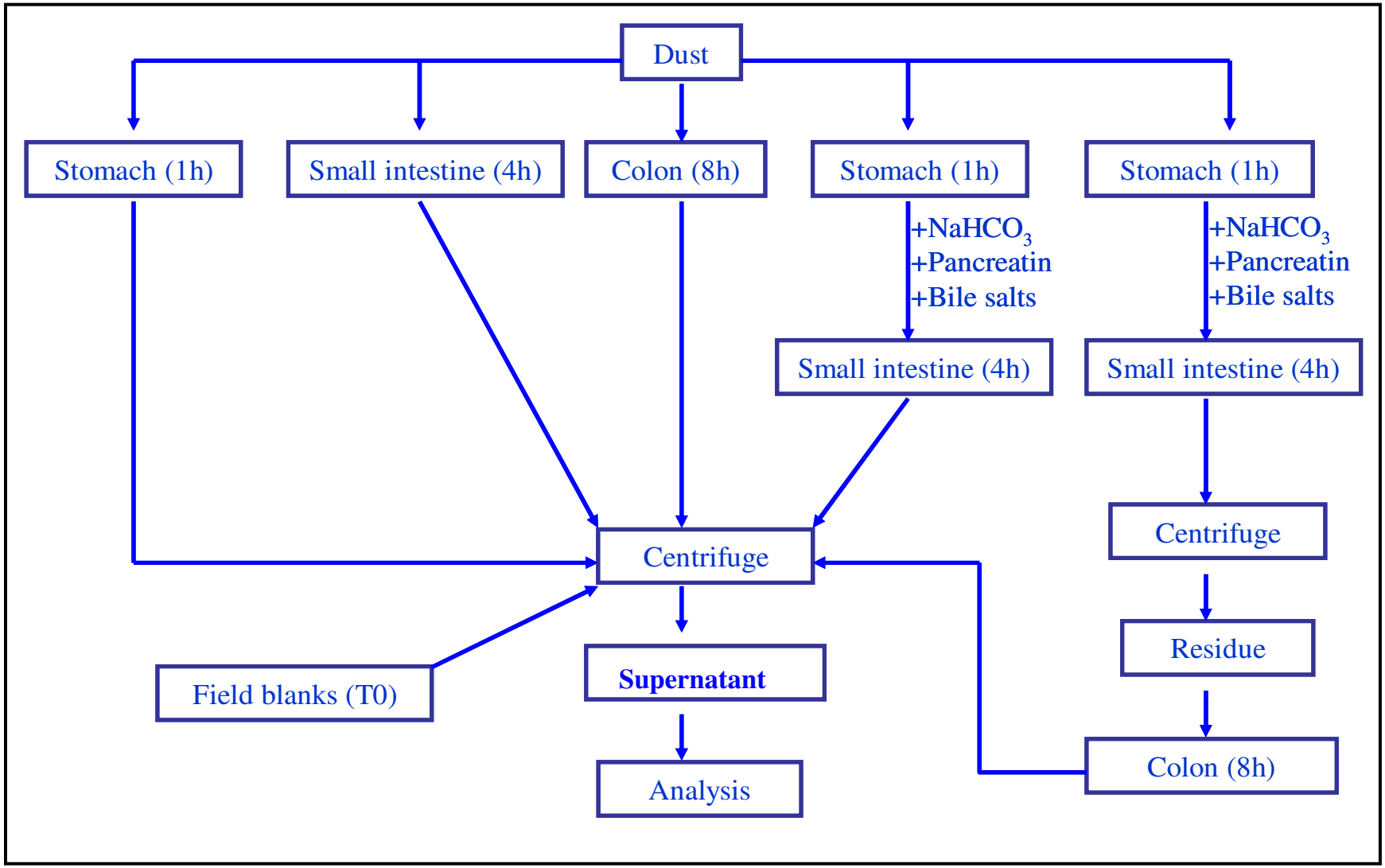


Figure 10.1: Schematic diagram of the PBET experiment design.

Field blanks were obtained by addition of dust to a freshly prepared GIT medium (3 replicates for each of stomach, small intestine and colon compartments), followed by immediate centrifugation (T0) and collection of the supernatant for analysis.

10.4 Bioaccessibility of the studied BFRs.

Table 10.1 shows the estimated bioaccessibility of each of the studied BFRs from different simulated GIT compartments. None of the target BFRs was above the quantification limit in any of the analysed field blanks. Therefore, results were not corrected for any background contamination.

Table 10.1: Bioaccessibility (expressed as %) of the studied BFRs from different GIT compartments.

Sample no.& ID	α-HBCD	β-HBCD	γ-HBCD	Σ HBCDs	TBBP-A	BDE-209
Stomach (1)	64	51	40	46	65	7
Stomach (2)	60	55	38	44	52	5
Stomach (3)	57	44	36	41	62	8
Stomach (average)	**60**	**50**	**38**	**43**	**60**	**7**
Sm.int. (1)	51	39	33	37	65	6
Sm.int. (2)	57	48	35	40	58	8
Sm.int. (3)	62	50	35	42	78	7
Sm.int. (average)	**57**	**46**	**34**	**39**	**67**	**7**
Colon (1)	57	52	34	40	68	5
Colon (2)	65	50	35	42	68	5
Colon (3)	54	40	33	37	77	6
Colon (average)	**59**	**47**	**34**	**40**	**71**	**5**
Stomach-Sm.int. (1)	77	61	51	56	77	10
Stomach-Sm.int. (2)	88	72	52	60	90	12
Stomach-Sm.int. (3)	82	74	52	59	76	12
Stomach-Sm.int. (average)	**82**	**69**	**51**	**59**	**81**	**11**
Stomach-Colon (1)	95	84	74	78	94	15
Stomach-Colon (2)	89	76	71	75	88	13
Stomach-Colon (3)	93	80	73	77	99	14
Stomach-Colon (average)	**92**	**80**	**72**	**77**	**94**	**14**

The bioaccessibility results of TBBP-A in the studied samples are in agreement with previous bioavailability studies in rats (EU Risk Assessment Report, 2006) showing that almost all (94%) the ingested dose was bioaccessible and available for absorption from human GIT. The bioaccessible fraction of BDE-209 from the whole GIT in our PBET model (14%) falls within the bioavailability range of BDE-209 (4-26%) reported in different animal studies (Huwe et al., 2008, NTP, 1986, Morck et al., 2003, Sandholm et al., 2003) and is in agreement with the very low water solubility and high lipophilicity of this compound.

The bioaccessibility of α-, β- and γ-HBCDs (table 10.1) shows that none of the studied HBCD diastereomers were 100% bioaccessible from dust as reported previously from solutions in rats (KEMI (National Chemicals Inspectorate), 2007). It is evident that the bioaccessibility of γ-HBCD is less than that of α- and β-isomers. This is likely to be associated with the lower water solubility of the γ-isomer (2 μg L^{-1}) compared to that of α- and β-HBCDs (49 and 15 μg L^{-1} respectively) which makes its dissolution from the solid phase more difficult than for the other diastereomers studied. We hypothesise that the effect of this limited bioaccessibility of γ-HBCD on the overall absorption of ΣHBCDs from the GIT will vary according to the % contribution of this isomer to ΣHBCDs in the ingested dust which has displayed previously a wide variability from 29-83% in 111 dust samples (see section 3.4). The volume of the GIT fluid at the time of dust ingestion is also likely to affect the bioaccessibility of ΣHBCDs from dust as a higher fluid volume will result in more dissolution of the poorly soluble γ-isomer. The distribution of HBCD diastereomers (expressed as % of ΣHBCDs) in the bioaccessible fraction differed from that in the original dust (figure 10.2) where the % contribution of γ-HBCD in the bioaccessible fraction is lower (accompanied by a higher contribution of α- and β-HBCDs) than that in dust. However, the fact that γ-HBCD is still predominant in the bioaccessible fraction (figure 10.2) indicates that the lower bioaccessibility of this isomer from ingested dust is not solely sufficient to explain the previously observed shift from predominance of γ-HBCD in abiotic samples to predominance of the α-isomer in human milk and plasma samples(Covaci et al., 2006).

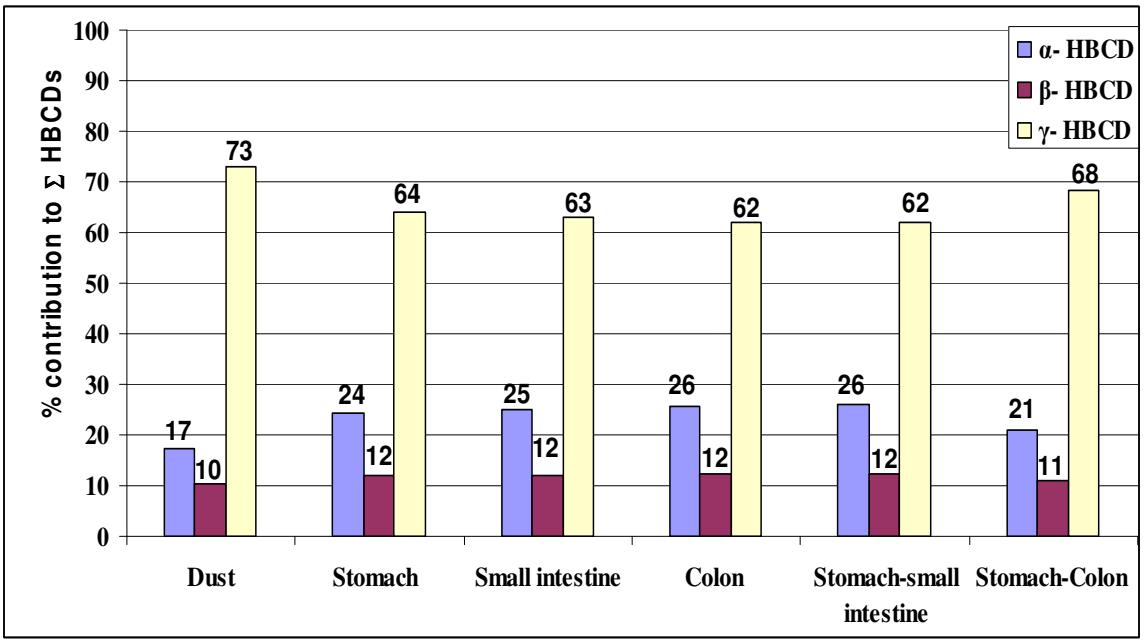


Figure 10.2: Average (n=3) contribution (expressed as %) of HBCD diastereomers to ∑HBCDs in the bioaccessible fraction of the studied GIT compartments compared to ingested dust.

Nevertheless, we hypothesise that this effect can vary with the concentration and isomer distribution of HBCDs in the ingested dust. The predominance of γ-HBCD reported recently in 24 out of 30 human milk samples from Spain while α-HBCD was dominant in the remaining 6 samples (Eljarrat et al., 2009a) may support our hypothesis that internal HBCD exposure is affected - to some extent - by the external exposure profile.

Careful inspection of the enantiomeric fractions (EFs) of the 3 main HBCD diastereomers in all the studied samples revealed no significant deviation from the racemic value (table 10.2) indicating the absence of enantioselective bioaccessibility processes. However, this does not rule out completely the occurrence of *in vivo* enantioselective absorption processes for HBCDs, as the GIT cell lining and bacterial flora are not included in our PBET model. The recently reported enrichment of the (-)-enantiomer of α-HBCD in human breast milk (Eljarrat et al., 2009a) and serum samples (see section 9.5 for details) indicates the presence of *in vivo* potentially enantioselective processes during HBCDs absorption, biotransformation and/or excretion.

Table 10.2: Enantiomeric fractions (EFs) of HBCDs in the the bioaccessible fraction of the studied GIT compartments compared to ingested dust.

	Enantiomeric fractions		
	α- HBCD	**β- HBCD**	**γ- HBCD**
Dust	**0.49**	**0.48**	**0.49**
Stomach	**0.51**	**0.50**	**0.48**
Small intestine	**0.50**	**0.51**	**0.52**
Colon	**0.51**	**0.48**	**0.49**
Stomach-small intestine	**0.49**	**0.47**	**0.52**
Stomach-Colon	**0.51**	**0.51**	**0.48**

While we are aware that the actual bioavailability of the studied BFRs may be affected by other factors *in vivo* like the bacterial flora, active and/or assisted transport via membranes. The preliminary results of our PBET model give a valuable insight into the different processes involved in the absorption of the studied BFRs from human GIT following dust ingestion which can be different from those encountered in animal studies when the tested compound is administered as a solution in vegetable oil.

In conclusion, the factors likely to affect the absorption of the studied BFRs from human GIT following dust ingestion include:

- Concentration (and isomer profile in case of HBCDs) in the ingested dust.
- Particle size of the ingested dust.
- Volume of GIT fluid and stomach emptying rate at the time of ingestion.

CHAPTER XI

BFRS IN HUMAN MILK: RELATIONSHIP BETWEEN INTAKE OF BFRS AND HUMAN BODY BURDENS USING A PHARMACOKINETIC MODEL

11.1 Introduction.

Several studies have reported different levels of BFRs in various human tissues including serum, placenta, liver, adipose tissue and human milk from different European and North American countries in the last few years (see chapter 1 for details). These human biomonitoring data provide a direct measurement of the internal dose of BFRs and represent a reliable estimate of human internal exposure resulting from various external exposure pathways (e.g. inhalation, ingestion of dust, diet and water) which is essential in the risk assessment of such compounds. However, the only available information on levels of BFRs in human samples from the UK is for the tri-to hexa-BDEs (major components of the pentabromodiphenyl ether commercial product) where the median concentrations for Σtri-to hexa-BDEs in human milk and serum samples collected in 2003 were 6.3 and 4.18 ng/g lw respectively (Kalantzi et al., 2004, Thomas et al., 2005). In addition, BDE-209 was detected in 11 out of 153 serum samples (LOD = 0.01 ng g^{-1} lw) at concentrations from 0.015-0.24 ng g^{-1} lw) (Thomas et al., 2006).

Currently, very little is known about the extent to which the contamination of indoor environments with BFRs influences human body burdens. Few studies have managed to establish significant positive correlations between the levels of BFRs in food or indoor dust and their concentrations in human milk or serum (Wu et al., 2007, Thomsen et al., 2008). However, such correlations could not be established in other studies (Roosens et al., 2009a, Toms et al., 2009). Recently, Lorber (2008) applied a pharmacokinetic model to predict the body burdens of PBDEs in American adults using intake data from different exposure pathways. The predicted body burdens were then compared to the reported

levels of PBDEs in human milk and serum and the relation between external and internal exposure was discussed (Lorber, 2008).

To address the paucity of information related to the levels of HBCDs, TBBP-A and BDE-209 in human matrices from the UK, we have chosen to study the concentrations of target BFRs in human milk samples due to its high lipid content (compared to blood or urine) which makes it an ideal matrix for measurement of POPs. Moreover, breast milk is a non-invasive medium for biomonitoring the exposure of breast-fed infants to BFRs. Therefore, this study reports –for the first time- on the concentrations of the three main HBCD diastereomers and their degradation products (previously identified as PBCDs and TBCDs; see section 2.4.2 for details) in addition to TBBP-A and BDE-209 in 28 human breast milk samples from Birmingham, UK. In addition, the diastereomeric and enantiomeric patterns of HBCDs in the studied milk samples will be discussed. The concentrations of target BFRs in breast milk are then used to estimate the dietary exposure of nursing infants to target BFRs using different scenarios. Finally, a simple, one-compartment pharmacokinetic model is applied to predict the body burdens of the studied BFRs in UK adults (using indoor air and dust levels measured in this study-see chapter 3 for details) and the obtained results are compared to the concentrations of target compounds measured in the analysed human milk samples.

11.2 Sampling strategy.

Breast milk samples (each comprising 50-100 ml) were obtained from 28 adult healthy volunteers via Birmingham Women's hospital Milk Bank after the study protocol was approved by Warwickshire Research Ethics Committee and the Research and Development Department in Birmingham Women's NHS foundation trust. Informed consent was obtained from all the participants before sample collection. Samples were kept in clean polypropylene plastic containers and transferred from the Milk Bank to the laboratory in special ice boxes then stored at -20ºC until the time of analysis. Due to ethical regulations, the samples were collected in a completely anonymous form with all participant information kept strictly confidential.

Table 11.1: Concentrations (pg g^{-1} lw) of target BFRs and enantiomeric fractions (EF) of HBCDs in the analysed human milk samples.

Sample	α-HBCD	EF-α	β-HBCD	EF-β	γ-HBCD	EF-γ	Σ HBCDs	Σ PBCDs[b]	Σ TBCDs[c]	TBBP-A	BDE-209
Milk 1	2473	0.28	91	nm[a]	131	0.52	2695	47	36	654	591
Milk 2	1683	0.33	346	0.48	555	0.48	2584	<LOQ[d]	<LOQ	286	<LOQ
Milk 3	1973	0.19	355	0.49	414	0.45	2742	<LOQ	69	144	420
Milk 4	751	0.24	97	nm	188	0.56	1036	<LOQ	<LOQ	<LOQ	<LOQ
Milk 5	2368	0.46	609	0.53	459	0.47	3435	<LOQ	<LOQ	<LOQ	<LOQ
Milk 6	1187	0.27	146	0.47	331	0.55	1664	<LOQ	147	66	124
Milk 7	3621	0.15	244	0.48	581	0.46	4445	122	114	95	86
Milk 8	6735	0.32	370	0.51	537	0.49	7642	<LOQ	310	<LOQ	584
Milk 9	1515	0.36	127	0.49	183	0.54	1825	<LOQ	154	129	815
Milk 10	11189	0.47	192	0.52	342	0.46	11723	<LOQ	102	<LOQ	<LOQ
Milk 11	1705	0.18	84	nm	128	0.58	1917	67	128	<LOQ	151
Milk 12	1479	0.24	292	0.51	211	0.47	1982	<LOQ	154	<LOQ	317
Milk 13	3236	0.22	285	0.54	364	0.59	3885	<LOQ	111	87	547
Milk 14	5887	0.33	391	0.47	632	0.52	6911	<LOQ	190	<LOQ	<LOQ
Milk 15	19713	0.49	695	0.51	1956	0.51	22365	202	333	<LOQ	485
Milk 16	5606	0.21	310	0.52	926	0.46	6842	<LOQ	<LOQ	<LOQ	<LOQ
Milk 17	17464	0.47	592	0.51	2199	0.48	20255	<LOQ	123	70	838
Milk 18	11165	0.36	750	0.47	1734	0.55	13648	83	363	108	89
Milk 19	4017	0.29	316	0.53	882	0.46	5215	<LOQ	172	<LOQ	154
Milk 20	1059	0.20	79	nm	559	0.47	1697	<LOQ	<LOQ	<LOQ	913
Milk 21	3270	0.30	395	0.48	915	0.61	4580	<LOQ	353	<LOQ	651
Milk 22	4255	0.19	497	0.51	618	0.55	5370	<LOQ	222	<LOQ	443
Milk 23	2279	0.29	184	0.47	550	0.52	3013	74	293	67	<LOQ

Table 11.1(continue): Concentrations (pg g^{-1} lw) of target BFRs and enantiomeric fractions (EF) of HBCDs in the analysed human milk samples.

Sample	α-HBCD	EF-α	β-HBCD	EF-β	γ-HBCD	EF-γ	Σ HBCDs	Σ PBCDs	Σ TBCDs[c]	TBBP-A	BDE-209
Milk 24	2275	0.25	321	0.47	581	0.59	3177	123	158	<LOQ	732
Milk 25	3107	0.48	161	0.52	503	0.48	3771	<LOQ	<LOQ	<LOQ	198
Milk 26	11025	0.18	509	0.46	2285	0.47	13819	<LOQ	261	<LOQ	405
Milk 27	4584	0.28	272	0.48	960	0.53	5816	<LOQ	113	<LOQ	252
Milk 28	1826	0.21	134	0.53	652	0.55	2612	<LOQ	171	<LOQ	<LOQ
Average	4909	0.29	316	0.49	728	0.51	5952	26	146	61	314
SD	4882	0.10	188	0.08	599	0.05	5521	51	114	134	300
Median	3171	0.28	301	0.50	557	0.52	3828	<LOQ	138	<LOQ	225
Minimum	751	0.15	79	0.46	128	0.45	1036	<LOQ	<LOQ	<LOQ	<LOQ
5^{th} %ile	1104	0.18	86	0.47	149	0.46	1675	<LOQ	<LOQ	<LOQ	<LOQ
95^{th} %ile	15267	0.48	665	0.53	2114	0.59	18003	122	346	236	830
Maximum	19713	0.49	750	0.54	2285	0.61	22365	202	363	654	913

[a] not measured.

[b] Pentabromocyclododecenes. (See section 2.4.2 for details).

[c] Tetrabromocyclododecadienes. (See section 2.4.2 for details).

[d] Limit of quantification which is 31, 26, 38 and 56 pg g^{-1} lw for PBCDs, TBCDs, TBBP-A and BDE-209, respectively.

11.3 Concentrations of BFRs in human milk.

11.3.1 HBCDs.

The 3 main HBCD diastereomers were detected in all of the analysed milk samples (table 11.1). Concentrations of ΣHBCDs ranged from 1 to 22 ng g^{-1} lw with an average and median values of 6 and 4 ng g^{-1} lw, respectively. ΣHBCDs concentrations in this study are in agreement with those reported in 85 human milk samples from Norway (0.4-20 ng g^{-1} lw) (Thomsen et al., 2005) and in 8 samples from Canada (0.4-19 ng g^{-1} lw) (Ryan et al., 2006). Lower levels of ΣHBCDs were measured in human milk samples from Sweden (0.2-2.4 ng g^{-1} lw) collected in 2001 (Aune et al., 2002). Similar low levels were reported in 37 breast milk samples (0.2-1.67 ng g^{-1} lw) from Russia (Polder et al., 2008a) and 26 samples (2.5-5 ng g^{-1} lw) from France (Antignac et al., 2008). HBCDs were also detected at low concentrations in human milk samples from Japan (1-4 ng g^{-1} lw) (Kakimoto et al., 2008) and USA (0.2-0.9 ng g^{-1} lw) (Ryan et al., 2006). However, a recent study reported much higher levels of ΣHBCDs (3-188, median 27 ng g^{-1} lw) in 33 human milk samples from Spain (Eljarrat et al., 2009a). This difference in ΣHBCDs levels reported in studies from various countries may be attributed to the difference in HBCD application and usage patterns in different countries at the various times of sample collection in the published reports. In addition, differences in personal exposure of the participants in those studies caused by different homes, working environment and diet in different countries and even within the same country can also result in different body burdens of HBCDs (Abdallah and Harrad, 2009).

11.3.1.1 HBCD diastereomer profiles.

α-HBCD was predominant in all the studied milk samples comprising 62-95% of ΣHBCDs while β- and γ-HBCDs ranged from 2-18% and 3-33% of ΣHBCDs respectively (figure 11.1). Few studies have reported on diastereomer profiles of HBCD in human milk. The predominance of α-HBCD was previously reported in human milk samples from USA, Canada (Ryan et al., 2006), Belgium (Colles et al., 2008), France (Antignac et al., 2008) and Japan (Kakimoto et al., 2008). α-HBCD was also reported as the major (or the only) HBCD diastereomer in human serum samples (Weiss et al., 2006, Thomsen et al., 2007b, Roosens et al., 2009b). The predominance of the α-diastereomer

in human tissues was suggested to be a result of preferential metabolism of β- and γ-HBCDs as reported in seal liver microsomes (Zegers et al., 2005). However, our results have shown that other factors may contribute to the prevalence of α-HBCD in humans including the intake via dust (see chapter 9) in addition to the physicochemical properties that may render α-HBCD more bioavailable than the other isomers (see chapter 10). A recent study reported higher adipose tissue deposition and lower faecal elimination rate of α- than γ-HBCD in female mice which can also result in higher body burdens of the α-isomer (Szabo et al., 2009).

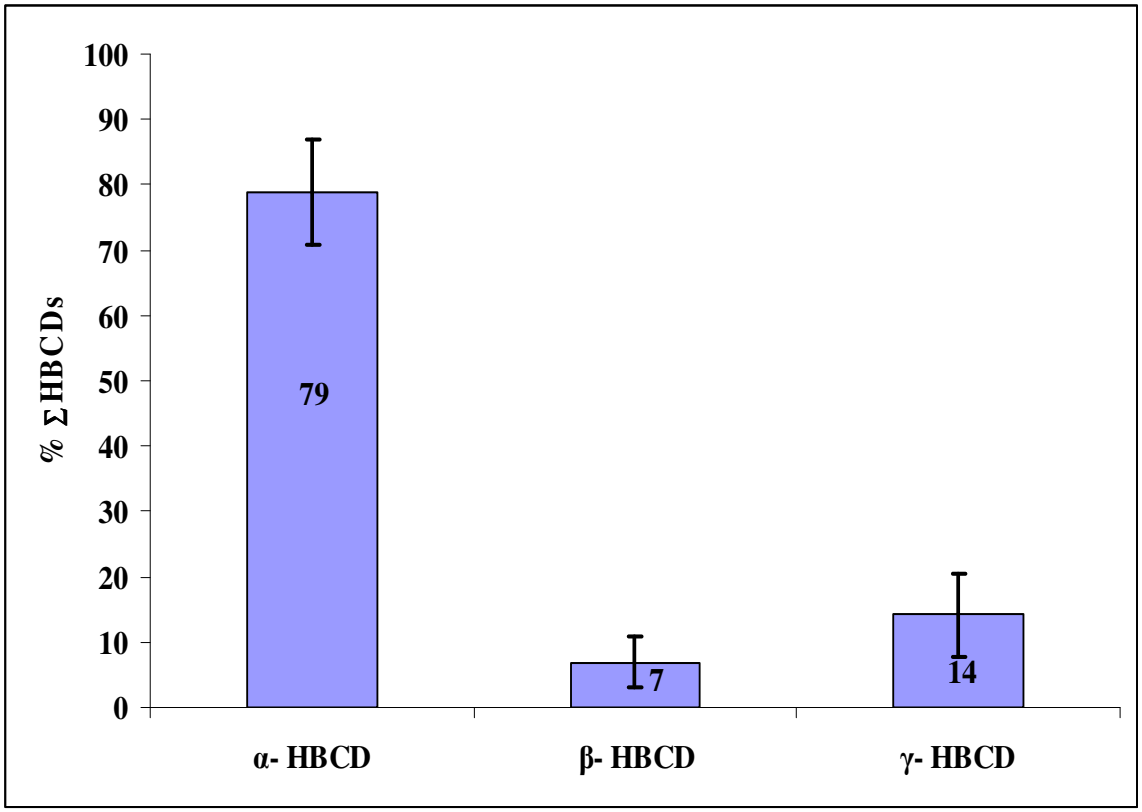


Figure 11.1: Average percentage contribution of HBCD diastereomers to ΣHBCDs in the analysed human milk samples (n=28). Error bars represent 1 standard deviation.

Interestingly, γ-HBCD was found to have the major contribution (54-100%) to ΣHBCDs in 24 out of 30 human milk samples from Spain, while α-HBCD predominated in the other samples. This inconsistency in the diastereomeric pattern was attributed to individual variability in metabolizing capacity or other unknown factors, such as the frequency of HBCD exposure (Eljarrat et al., 2009a).

11.3.1.2 HBCD enantiomer profiles.

Table 11.1 lists the enantiomeric fractions (EFs) of the 3 major HBCD diastereomers in the analysed milk samples. While the EFs of β-HBCD (average 0.49) and γ-HBCD (average 0.51) showed no significant deviations from the racemic value, significant enrichment of the (-)-α-HBCD enantiomer was evident from the EFs of this diastereomer (average 0.29; figure 11.2). The only study that examined the EFs of HBCDs in human milk samples from Spain has reported similar results (Eljarrat et al., 2009a) indicating the presence of potential enantioselective processes involved with the absorption, metabolism and/or excretion of HBCDs. This hypothesis is supported by the racemic chiral signatures of HBCDs in indoor dust (see chapters 4 and 9).

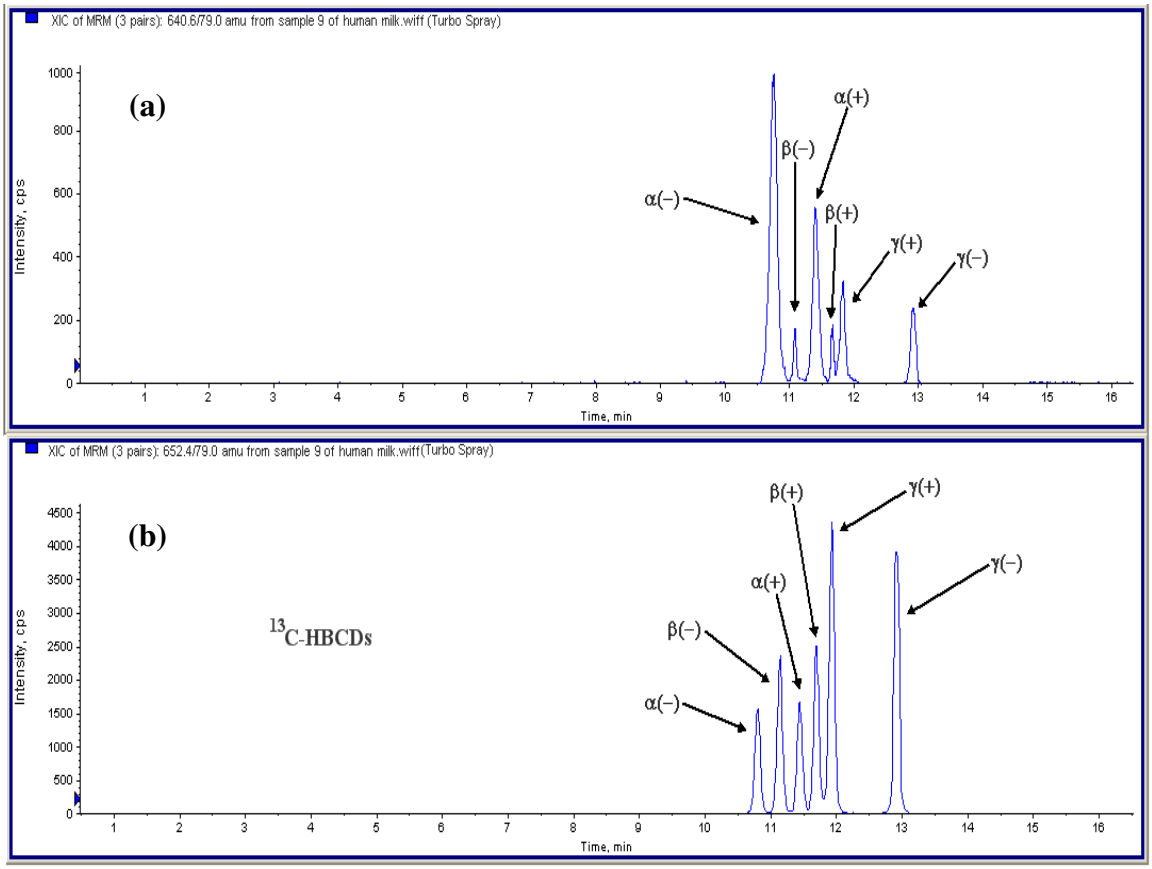


Figure 11.2: Chiral LC-ESI-MS/MS chromatograms of (a) native and (b) ^{13}C-HBCDs in a human milk sample.

Non-racemic EFs have also been reported for α-HBCD in marine biota. Enrichment of (-)-α-HBCD was observed in sole liver (EF = 0.43) and muscles (EF = 0.42) while higher accumulation of (+)-α-HBCD was observed in the livers of Bib (EF = 0.58) and whiting

(EF = 0.70) (Janak et al., 2005). A study of the enantiomer-specific accumulation of HBCDs in eggs of predatory birds revealed non-racemic signatures of α-HBCD (main HBCD diastereomer in the studied samples). While the eggs of peregrine falcon and common tern showed enrichment of (-)-α-HBCD, those of white-tailed sea eagle contained higher levels of the (+)-α-enantiomer. Interestingly, the EFs of α-HBCD in guillemot (EF = 0.53) differs considerably from that of its main prey, the herring (EF = 0.24), indicating that guillemots tend to accumulate relatively more (+)-α- compared to (-)-α-HBCD (Janak et al., 2008). Recently, enrichment of (-)-α-HBCD was also reported in the blubbers of white sided dolphins (Peck et al., 2008). It is obvious from those few studies that enantioselective processes may be involved in the uptake, metabolism and/or excretion of α-HBCD in fish, predatory birds and mammals. However, further studies are required for further understanding of the nature and extent of these processes and the factors affecting the preferential retention of a certain enantiomer within a given species.

11.3.1.3 HBCD degradation products.

We have previously separated and identified tetrabromocyclododecadienes (2 isomers) and pentabromocyclododecenes (4 isomers) as degradation products of HBCD in indoor dust (see section 2.4.2 for details). Recently, debromination of HBCD to produce tetra- and penta-brominated derivatives (in addition to other hydroxylated derivatives) was identified as a metabolic pathway of HBCD in Wistar rats (Brandsma et al., 2009). However, there exists -to date- no data on HBCD debromination products in human samples. Therefore, the presence of PBCDs and TBCDs was investigated in the analysed breast milk samples. PBCDs (3 isomers; figure 11.3a) were detected in 7 samples while TBCDs (2 isomer; figure 11.3b) showed higher detection frequency and were identified in 22 samples.

Due to the lack of any native or ^{13}C-labelled standards for TBCDs and PBCDs, semi-quantitative estimation of their concentrations in the analysed milk samples was performed using an average response factor of α-, β- and γ-HBCDs (table 11.1). While our results confirm the presence of lower brominated HBCD derivatives in humans, it is not yet clear whether the detected TBCDs and PBCDs originate from *in vivo* biotransformation or exist as a result of intake via ingestion of indoor dust. Very little is

known about the toxicological effects of TBCDs and PBCDs in humans probably due to the recent identification of these compounds and the lack of authentic standards. However, a recent study has compared the endocrine disrupting potency of HBCDs, PBCDs and PBDEs using a combination of two methods: a surface matching with the natural binder thyroxine (T_4) followed by approximation of free binding energies for various binding modes within human transthyretin receptor (hTTR). Results revealed higher binding affinities of PBCDs than the parent HBCDs. Interestingly, two trans-configured PBCD isomers showed higher binding affinities to hTTR than T_4 itself (Weber et al., 2009).

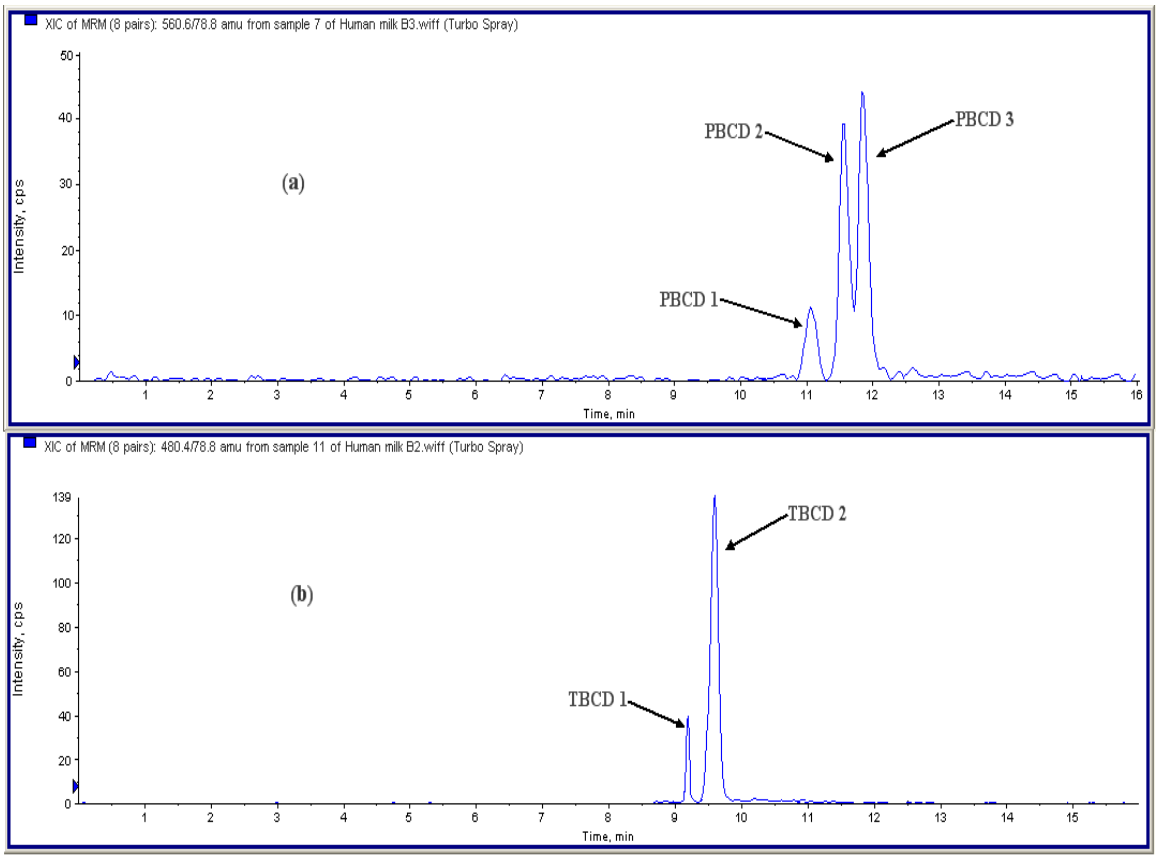


Figure 11.3: LC-ESI-MS/MS chromatograms of (a) PBCDs and (b) TBCDs in human milk (sample 15, table 11.1).

11.3.2 TBBP-A.

TBBP-A was detected in 36% of the studied samples with concentrations ranging from <LOQ to 0.65 ng g^{-1} lw (table 11.1). Consistent with its phenolic structure that can be rapidly conjugated and subsequently excreted, TBBP-A has a short human half-life of

approximately two days (Hakk et al., 2000, Hagmar et al., 2000a). Therefore, the detection of this flame retardant in some of the studied human milk samples is likely to reflect either recent or continuous exposure (Sjodin et al., 2003a, Covaci et al., 2009). Little information is available on the levels of TBBP-A in non-occupationally exposed humans probably due to the low detection frequency of this compound resulting from its short human half-life. Low levels of TBBP-A (0.44 to 0.65 ng g^{-1} lw) were reported in pooled serum samples of non-occupationally exposed adults collected from 1986 to 1999 in Norway (Thomsen et al., 2002). Relatively higher concentrations (average 1.35 ng g^{-1} lw) were measured in blood samples from Japan (J. Nagayama et al., 2000). Recently, low concentrations of TBBP-A (average 0.048; maximum 0.46 ng g^{-1} lw) were reported in human adipose tissue samples from USA (Johnson-Restrepo et al., 2008). To our knowledge, this study is the first to report on TBBP-A levels in human milk from non-occupationally exposed adults.

11.3.3 BDE-209.

BDE-209 was detected in 71% of the analysed human milk samples with an average and median concentration of 0.314 and 0.225 ng g^{-1} lw respectively (table 11.1). This is the first report of BDE-209 in human milk samples from the UK, which confirms the bioavailability of the fully-brominated compound to humans. Interestingly, the levels of BDE-209 in this study are generally in line with those reported in human milk samples from other European countries including Germany, Russia and Norway (see table 1.7 for details) but are significantly lower than BDE-209 concentrations measured in human milk samples from Spain (median = 2.9 ng g^{-1} lw) (Gomara et al., 2007) despite the substantially higher levels of BDE 209 reported in indoor dust samples from UK than the rest of Europe (Harrad et al., 2008a) and the reported higher usage of BDE 209 in the UK than other EU countries (EU Risk Assessment Report, 2002). Our results are also in agreement with those reported in human milk samples from Canada, USA, Australia and Faroe Islands (see table 1.7 for details).

The levels of BDE 209 (table 11.1) in the analysed human milk samples are significantly ($p<0.05$) lower than those of ΣHBCDs. Given the higher production volume of BDE 209 and its significantly higher concentrations in UK dust than ΣHBCDs (see table 3.2 for details), the observed lower body burdens of BDE 209 may be attributed to several

factors. These include its poor absorption from the GIT owing to its high molecular weight and poor solubility (see chapter 10 for details) combined with rapid elimination as evident from its short human half-life of 7-15 days (Sjodin et al., 2003a, Thuresson et al., 2006, Hagmar et al., 2000b). Furthermore, BDE 209 was reported to exist predominantly in plasma and liver and does not preferentially accumulate in fatty tissues such as milk and adipose tissue (Huwe et al., 2008a). In addition, the estimated dietary intake of BDE 209 by UK adults from total diet was lower than that of ΣHBCDs (UK Food Standards Agency, 2006).

Similar to TBBP-A, the detection of BDE 209 in milk is likely to reflect recent or continuous exposure due to the low bioaccumulation potential of the fully brominated compound caused by its relatively short half-life of 2 weeks (Sjodin et al., 2003a).

11.4 Nursing infants' dietary intake of BFRs via breast milk.

Breast milk has been recognized as a medium for direct transfer of POPs to nursing infants. One to three months old infants were reported to absorb more than 90% of most dioxin congeners present in their mothers' milk (McLachlan, 1993). To estimate the nursing infants' dietary intake of the studied BFRs via breast milk, equation 11.1 was used.

$$Di = \frac{C_{BFR}\ x\ F_{lipid}}{Bw} \quad ....(11.1)$$

Where ***Di*** is the estimated dietary intake (ng kg^{-1} bw day^{-1}); $\boldsymbol{C_{BFR}}$ is the concentration of target BFR in milk (ng g^{-1} lw); $\boldsymbol{F_{lipid}}$ is the daily lipid intake via breast milk (g lipid day^{-1}) and ***Bw*** is the infant's body weight (kg).

The infant's daily lipid intake via breast milk ($\boldsymbol{F_{lipid}}$) was calculated based on U.S. EPA guidelines (U.S. EPA, 2002.) which suggest an average intake of 702 ml milk per day for a 1 month old infant weighing 4.14 Kg. The median lipid content of the analysed milk samples was 3.47 g lipid per 100 ml of breast milk resulting in a daily lipid intake of 24.4 g lipid day^{-1}. Table 11.2 shows the estimated dietary intake of target BFRs via breast milk using different exposure scenarios.

Table 11.2: Estimated exposure (ng kg^{-1} bw day^{-1}) of a 1 month old infant to the target BFRs via breast milk.

	5th %ile	Average	Median	95th %ile
α- HBCD	6.44	28.62	18.49	89.02
β- HBCD	0.50	1.84	1.75	3.88
γ- HBCD	0.87	4.24	3.25	12.33
Σ HBCDs	9.77	34.71	22.32	104.97
Σ PBCDs	<LOQ	0.60	0.48	1.04
Σ TBCDs	<LOQ	1.08	0.91	2.05
TBBP-A	<LOQ	0.98	<LOQ	2.74
BDE-209	<LOQ	2.56	2.52	4.91

Figure 11.4 shows a comparison of the average dietary exposures of UK adults, toddlers (see table 5.1 for details) and infants (table 11.2) to target BFRs. Of particular interest is the average dietary exposure of an infant to ΣHBCDs via breast milk estimated at 35 ng kg^{-1} bw day^{-1} which exceeds the estimated upper-bound dietary intakes of both UK adults (6 ng Σ HBCDs kg^{-1} bw day^{-1}) and toddlers (24 Σ HBCDs kg^{-1} bw day^{-1}) (UK Food Standards Agency, 2006). A similar case is observed for TBBP-A although all the exposure estimates for the 3 age groups are less than 1 ng kg^{-1} bw day^{-1}.

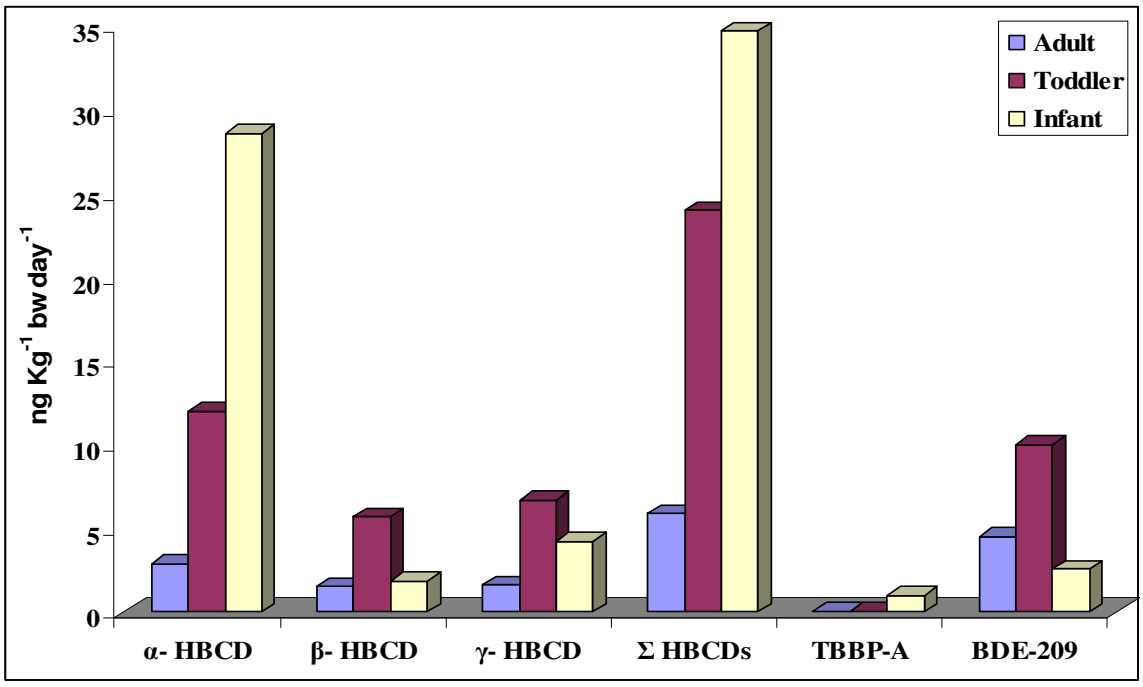


Figure 11.4: Average estimates of dietary exposure (ng kg^{-1} bw day^{-1}) of UK adults, toddlers and breast-fed infants to the target BFRs.

11.5 Comparison of BFR intake to human body burdens.

We have previously estimated UK adult intake of target BFRs via inhalation, dust ingestion and diet (see table 5.1 for details). To compare the estimated intakes to the body burdens measured in human milk samples, a simple one-compartment, first order pharmacokinetic (PK) model was used (Lorber, 2008). BFRs were hypothesized to accumulate in lipids (the single compartment in the model). Therefore, the change in BFR lipid concentration over time can be expressed by equation 11.2.

$$\frac{\delta C_{BFR}}{\delta t} = \frac{I_{BFR}(t) x\, AF_{BFR}}{BL(t) - K_{BFR}} \quad \text{.....(11.2)}$$

Where C_{BFR} is the compound specific concentration in lipids (ng g^{-1} lw); I_{BFR} is the daily intake of the target BFR (ng day^{-1}); AF_{BFR} is the absorption fraction; BL is body lipid mass (g) and K_{BFR} is the compound specific first order dissipation rate (day $^{-1}$). If K_{BFR} is assumed to be constant over time then equation 11.2 can be solved into:

$$C_{BFR}(t) = C_{BFR}(0)\; x\; e^{(-K_{BFR} \cdot t)} + [\frac{(I_{BFR}(t)\, x\, AF_{BFR})}{BL(t)}]\, x\, [\frac{(1 - e^{(-K_{BFR} \cdot t)})}{K_{BFR}}] \quad \text{.....(11.3)}$$

Where $C_{BFR}(0)$ is the studied BFR body lipid concentration at time 0. Assuming a constant dose over time at constant body lipid mass, the steady state BFR lipid concentration can be calculated from equation 11.4.

$$C_{BFR} = \frac{(I_{BFR}\, x\; AF_{BFR})}{BL\, x\, K_{BFR}} \quad \text{.....(11.4)}$$

To use equation 11.4 to convert daily adult intakes of BFRs via different exposure pathways to expected body burdens, the bioaccessible fractions of each target compound derived from our PBET model (see table 10.1) were used to substitute for AF_{BFR} in case of exposure via dust ingestion or diet, while the inhalable fraction was assumed to be 100% bioavailable. The body lipid mass was estimated based on a 25% body fat for an average adult weighing 70 Kg (U.S. EPA, 1997). Finally, K_{BFR} was calculated as $0.693/t_{0.5}$; where $t_{0.5}$ is the half-life of the studied BFR in the body lipid compartment.

Very little is known about the human half-lives of BFRs. While Hagmar et al. (2000) used serum samples from 4 occupationally exposed adults to determine half-lives of 2 days and 1 week for TBBP-A and BDE-209 respectively (Hagmar et al., 2000b), A more

detailed report estimated different $t_{0.5}$ values for various BFRs in different body compartments of non-occupationally exposed Swedish adults (Geyer et al., 2004). While the $t_{0.5}$ of TBBP-A in blood was estimated at 3.5 days which is in agreement with the results of Hagmar et al. (2000), a longer half-life ranging between 21-24 days was determined for the same compound in adipose tissue. Therefore, a $t_{0.5}$ of 22 days will be used for TBBP-A in our PK model. The $t_{0.5}$ of ΣHBCDs (α-HBCD + β-HBCD + γ-HBCD) in adipose tissue was reported in the same study (Geyer et al., 2004) and showed a wide variability from 23-219 days. This wide variability was explained on the basis of total body fat content of humans and individual host differences that affect bioaccumulation, uptake and elimination of BFRs (Geyer et al., 2004). Several studies have reported on the higher bioaccumulation potential and longer half-life of α-HBCD in marine biota and mammals (Janak et al., 2005, Janak et al., 2006, Szabo et al., 2009, Tomy et al., 2008, Covaci et al., 2006) in addition to the reported preferential biotransformation of the β- and γ- isomers in marine mammals (Zegers et al., 2005). Therefore, in the absence of any information about the diastereomer specific half-lives of HBCDs in human or animals, we opted to use different $t_{0.5}$ values for the different HBCD diastereomers. A half-life of 165 days (representing 75% of the maximum $t_{0.5}$ = 219 days reported in humans (Geyer et al., 2004)) was used for α-HBCD, while a $t_{0.5}$ = 55 days was used for the β- and γ- isomers (25% of 219 days). For ΣHBCDs, the half-life was calculated from equation 11.5.

$$t_{0.5}\sum HBCDs = f \cdot t_{0.5}\,\alpha HBCD + f \cdot t_{0.5}\,\beta HBCD + f \cdot t_{0.5}\,\gamma HBCD \quad \text{.....(11.5)}$$

Where *f* is the percent contribution of each isomer to ΣHBCDs in the studied matrix.

The estimated body burdens of the studied BFRs via inhalation, dust ingestion and diet are given in table 11.3. In general, good agreement was observed between the predicted and the observed body burdens given the simplicity of the model used (e.g. only one body compartment was studied) and the dearth of information regarding the half-lives of different BFRs in various compartments of the human body and the bioavailability of the studied compounds from human GIT following ingestion. In addition, the PK model used here does not estimate human exposure via routes such as dermal contact and water intake. This is due to the high uncertainty and complete absence of experimental data on the extent of BFR absorption via dermal contact and the expected minimal contribution

of water intake to the overall daily exposure to BFRs based on the very low solubility of the target compounds in water. However, the good agreement between the predicted and observed results especially for HBCDs and BDE-209 indicate that the studied exposure routes are the main pathways affecting internal human exposure and adult body burdens of BFRs. This is in agreement with the findings of Lorber (2008) who studied the exposure of Americans to PBDEs via different routes (Lorber, 2008). Our results are also in agreement with the American study (Lorber, 2008) regarding the predicted high contribution (~90%) of dust ingestion to the measured body burdens of BDE 209.

Table 11.3: Estimated adult body burdens from average daily exposure to target BFRs compared to observed levels in human milk samples.

	α- HBCD	**β- HBCD**	**γ- HBCD**	**Σ HBCDs**	**TBBP-A**	**BDE-209**
Average intake (ng day^{-1})						
Dust	46.64	15.33	69.63	131.59	1.64	4268.22
Diet	203.44	105.43	112.24	413.32	2.85	311.13
Air	1.24	0.62	3.29	5.47	0.39	9.41
Median intake (ng day^{-1})						
Dust	8.42	2.53	19.92	32.54	1.32	2267.74
Diet	203.44	105.43	112.24	413.32	2.85	311.13
Air	0.84	0.43	2.64	3.91	0.33	8.90
Average predicted body burdens (pg g^{-1} lw)						
Dust	583.82	55.63	227.37	1203.13	2.67	344.91
Diet	2546.46	382.51	366.50	3778.88	4.64	25.14
Air	16.91	2.82	14.91	65.00	0.67	5.43
Sum	3147.20	440.96	608.78	5047.01	8.37	375.48
Median predicted body burdens (pg g^{-1} lw)						
Dust	105.14	9.07	64.98	297.14	2.12	185.86
Diet	2546.46	382.51	366.50	3778.88	4.64	25.14
Air	10.88	1.81	11.79	46.31	0.52	5.14
Sum	2662.49	393.40	443.28	4122.33	7.63	216.14
Observed body burdens (pg g^{-1} lw)						
Average	4908.76	315.83	727.78	5952.36	60.93	314.15
Median	3171.32	301.26	557.41	3827.89	<LOQ	225.43

The good agreement between the observed and predicted body burdens for HBCD diastereomers using our PK model supports the previous reports of higher bioaccumulation potential and longer half-life of α-HBCD (165 days in this PK model) than the β- and γ-isomers (55 days in this PK model).

Interestingly, the average estimated body burdens of TBBP-A in the lipid compartment was much lower than the observed value (table 11.3) despite the use of a $t_{0.5}$ of 22 days as reported for human adipose tissue (Geyer et al., 2004). Given the high bioaccessibility of 94% estimated for this BFR (see table 10.2 for details), this difference may be attributed to a longer half-life of TBBP-A in human adipose tissue than reported, difference in the adult dietary intake of TBBP-A between UK and Netherlands (dietary intake of TBBP-A used in this PK model is estimated for Netherlands adults (de Winter-Sorkina et al., 2003) in the absence of reliable data from UK) or the individual variability in uptake, metabolism and excretion of BFRs. The latter explanation is more plausible since the average observed concentration in our study is 61 pg g^{-1} lw while the median value is below LOQ (table 11.3).

CHAPTER XII
SUMMARY AND CONCLUSIONS

Brominated flame retardants are a group of halogenated organic pollutants used to flame-proof a wide range of consumer products including construction materials, textiles, fabrics and polyurethane foam used in furniture, beddings, car interiors and carpets. BFRs are also applied to flame-retard printed circuit boards, high impact polystyrenes and acrylonitrile-butadiene-styrene used in the manufacture of external casing of electric and electronic equipment (BSEF, 2009a). The extensive application and use of BFRs has resulted in a growing scientific interest in studying the potential effects of these chemicals on the environment and human. Several studies have shown that BFRs do not remain bound to products but can migrate into air, dust, soil and water during various stages of manufacture, use and disposal. Such contamination (coupled with the persistence and hydrophobicity of BFRs) has resulted in their accumulation in fish, birds, marine mammals and other wildlife in addition to humans (Covaci et al., 2009, Hites, 2004, Covaci et al., 2006, Law et al., 2008b). This has raised concerns because of the potential adverse health impacts of BFRs including liver microsomal enzyme induction, endocrine disruption, developmental neurotoxic effects, reproductive toxicity, immunotoxicity and carcinogenicity (Darnerud, 2003). Such concerns have led to EU bans on the manufacture and new use of all three of the commercial PBDE formulations (Directive 2003/11/EC, 2003, Judgment of the European Court of Justice on Joint Cases C-14/06 and C-295/06, 2008) in addition to the recent inclusion of tri- to octa- BDEs as persistent organic pollutants (POPs) under the framework of the Stockholm Convention on POPs (Stockholm convention on POPs, 2008).

Given the above, the main aim of this work was to shed more light on the pathways and magnitude of human exposure to 3 widely used BFRs (HBCDs, TBBP-A and BDE-209) via indoor environments and investigating the relationship between the external exposure to these chemicals and human body burdens. Secondary objectives were to further understanding of the transfer of BFRs from treated products to the indoor environment

and the factors affecting the concentrations of the studied compounds in indoor air and dust.

The main achievements and outcomes of this thesis are summarised below:

- 3 passive air samplers were developed and validated for sampling the target compounds in both vapour and particulate phases of indoor air. Different extraction and clean-up methods were applied successfully to a wide range of samples including air, dust, diet, simulated GIT fluid, human serum and breast milk. Analytical methods based on LC-ESI-MS/MS or LC-APPI-MS/MS were developed and/or validated for the separation and determination of BDE-209 and TBBP-A, in addition to HBCD diastereomers, enantiomers and degradation products.

- Diastereomer-specific concentrations and EFs of HBCDs were measured in air and dust from different indoor microenvironments including homes, offices, cars, public microenvironments, nurseries and primary schools in Birmingham, UK. While no statistically significant difference ($p<0.05$) was observed between ΣHBCDs in air from homes (n=35; average = 250 pg m^{-3}) and offices (n=30; 180 pg m^{-3}), concentrations of ΣHBCDs in cars (n=21; 367 pg m^{-3}) were significantly higher ($p<0.05$) than those measured in both homes and offices. Statistical analysis also revealed that concentrations of ΣHBCDs in car dust (n=30; 19000 ng g^{-1}) exceed significantly ($p<0.05$) those in both homes (n=45; 8300 ng g^{-1}) and offices (n=30; 1600 ng g^{-1}). α-HBCD made a significantly greater contribution to ΣHBCD in dust than in air, with the opposite trend observed for γ-HBCD ($p<0.05$). On average, dust composition is 33% α-HBCD, 56% γ-HBCD, while for air; it is 22% α-HBCD, and 66% γ-HBCD. The relative abundance of β-HBCD was similar in both matrices.

- The causes of variability in concentrations and isomer profiles of HBCDs in indoor dust were studied and discussed. Within-room spatial and temporal variability of ΣHBCDs in dust exceeded that attributable to sampling and analytical variability alone and was linked to certain events involving potential HBCD-emitting sources. A TV set identified as a source of HBCDs was found to

influence both the concentrations and isomer profiles of the flame retardant within the room. A photolytically-mediated isomerisation of the three major HBCD diastereomers in indoor dust dominated by a γ- to α-HBCD shift was reported for the first time. This was accompanied by a slower degradative loss of HBCDs via elimination of HBr. Neither of these processes were enantioselective. The $t_{1/2}$ of HBCDs in indoor dust was estimated to be 12.2 and 26 weeks in light and dark respectively.

- TBBP-A and BDE-209 were present mainly in the particulate phase of air. Levels of both BFRs in air from homes (n=5; average=16 and 397 pg m^{-3} respectively) and offices (n=5; 16 and 650 pg m^{-3} respectively) reported in this study are generally in agreement with previous results from Sweden (Sjodin et al., 2001, Karlsson et al., 2007), USA (Allen et al., 2007) and Japan (Inoue et al., 2006a). However, BDE-209 concentrations in air from cars in this study (n=21; average= 1730 pg m^{-3}) were significantly higher ($p<0.05$) than those reported in Greek cars (Mandalakis et al., 2008). Concentrations of BDE-209 in UK domestic dust (n=30; average=260000 ng g^{-1}) were higher than those reported in house dust from other countries which may be attributed to the UK's disproportionately large share of the European market for Deca BDE due to its particularly stringent fire safety regulations (EU Risk Assessment Report, 2002). The concentrations of TBBP-A in indoor air and dust samples were substantially lower than those of both HBCDs and BDE-209 which is consistent with the predominant use of TBBP-A as a reactive flame retardant.

- Human intake of target BFRs via indoor air and dust was estimated using different exposure scenarios and compared to dietary exposure. Inhalation exposure to all target BFRs (0.2-14.6 ng day^{-1}) was substantially lower than that received via dust ingestion (0.4-78000 ng day^{-1}). In contrast to inhalation exposure, toddler (6-24 months) exposure to the studied BFRs via dust ingestion was higher than adults. This is consistent with toddlers ingesting more dust than adults due to their closer proximity to the ground and greater hand-to-mouth contact. The estimated exposure to ΣHBCDs of a UK toddler weighing 10 kg

under a high-end exposure scenario involving ingestion of 200 mg dust day^{-1} contaminated at the 95th percentile level reported in this study (540 ng kg bw^{-1} day^{-1}), was higher than that of occupationally exposed Scandinavian workers (median = 200 ng kg bw^{-1} day^{-1}) (Thomsen et al., 2007b). Using the same exposure scenario, the exposure of a UK toddler to BDE-209 via dust ingestion (31 µg kg bw^{-1} day^{-1}) exceeded the reference dose of daily oral exposure to BDE-209 (7 µg kg bw^{-1} day^{-1}) (U.S. EPA, 2007).

- Dust ingestion constituted the major exposure pathway for toddlers to all the studied BFRs. For adults, the relative significance of different exposure pathways for HBCDs appears somewhere between those for the Penta-BDE formulation (Harrad et al., 2006b, Harrad et al., 2008b) (principally diet, but with dust ingestion playing an important role for some individuals) and BDE-209 (for which dust and diet are broadly equally important). For TBBP-A, ingestion of indoor dust appeared to make a greater contribution to overall exposure than for HBCDs. The average diastereomer profile of HBCDs exposure via dust ingestion was somewhere between that for inhalation and diet with a 35% and 53% contribution of α- and γ-HBCD respectively resulting in a higher contribution of α-HBCD (45%) than the other diastereomers to the overall adult intake of HBCDs. This finding supports our hypothesis of the possibility that the predominance of α-HBCD observed in humans (Covaci et al., 2006, Knutsen et al., 2008, Thomsen et al., 2007b) may not solely be due to *in vivo* biotransformation of β- and γ-HBCD (Zegers et al., 2005), but, at least partly attributable to the diastereomer pattern in dust.

- The exposure of young children (<1-5 years) to all the studied BFRs via ingestion of classroom dust was significantly higher ($p<0.05$) than that of adults via ingestion of office dust. While dust ingestion was the major pathway of exposure to BDE-209 (~98% compared to diet), dietary intake seems to play a more significant role in the external exposure of children to ΣHBCDs (44%) and TBBP-A (15%). On average, a UK child ingesting 50 mg dust is exposed to 10 µg day^{-1} of ΣBFRs in this study via both dust and diet.

- The estimated average adult and toddler exposures to ΣHBCDs via ingestion of house dust in the UK (120 and 300 ng day^{-1}) were higher than those in Canada (13 and 33 ng day^{-1}) and USA (16 and 40 ng day^{-1}). However, no firm conclusions could be drawn due to the relatively small number of samples analysed from Canada and USA.

- Environmental scanning electron microscopy (ESEM) techniques were applied to elucidate the transfer mechanisms of BDE-209 and HBCD to indoor dust. Results revealed that high levels of BDE-209 in the examined dust are likely caused by direct leaching of the flame retardant from treated products to dust by weathering or physical abrasion. On the other hand, HBCD was transferred to the studied dust samples by volatilisation and re-deposition to dust particles.

- The relationship between combined exposure to the three main HBCD diastereomers via ingestion of food (duplicate diets) and indoor dust and HBCD concentrations in serum of 16 adults (20-25 years) was examined for the first time. The chiral signatures of HBCDs were also determined to help further understanding of source-to-human chain enantioselective degradation and/or metabolism. While γ- HBCD dominated in food, α-HBCD dominated in dust and was the sole isomer in serum. While exposure via dust ingestion correlated significantly ($p<0.01$) with ΣHBCDs concentrations in serum, no such correlation was evident with dietary exposure. Although no enantioselective enrichment was detected in either dust or diet, substantial enrichment of (-)-α-HBCD was observed in serum which may be attributable to enantioselective absorption, metabolism and/or excretion as opposed to external exposure to non-racemic matrices.

- The bioaccessibility of the target BFRs from the human GIT following dust ingestion was assessed using a colon-enhanced physiologically based extraction test (PBET). The overall bioaccessibility of TBBP-A, ΣHBCDs and BDE-209 were 94%, 77% and 14% respectively. The bioaccessibility of γ-HBCD was less than that of the α- and β-isomers. This is likely to be associated with the lower water solubility of the γ-isomer that makes its dissolution from the solid phase

more difficult than the other isomers. No change in the EFs of HBCDs was observed in the studied samples. However, this does not rule out completely the occurrence of *in vivo* enantioselective absorption processes for HBCDs, as the GIT cell lining and bacterial flora were not included in our PBET model.

- The concentrations of TBBP-A (average=61 pg g^{-1} lw), BDE-209 (average=314 pg g^{-1} lw), HBCDs (average ΣHBCDs=5952 pg g^{-1} lw), PBCDs (average ΣPBCDs=26 pg g^{-1} lw) and TBCDs (average ΣTBCDs=146 pg g^{-1} lw) were determined –for the first time- in 28 human milk samples from Birmingham, UK. α-HBCD was predominant in all the studied milk samples comprising 62-95% of ΣHBCDs. While the EFs of β-HBCD (average 0.49) and γ-HBCD (average 0.51) showed no significant deviations from the racemic value, significant enrichment of the (-)-α-HBCD enantiomer was evident from the EFs of this diastereomer (average 0.29).

- The levels of target BFRs in human milk were used to estimate nursing infants' intake of BFRs via breast milk. Of particular interest is the average dietary exposure of an infant to ΣHBCDs via breast milk estimated at 35 ng kg^{-1} bw day^{-1} which exceeds the estimated upper-bound dietary intakes of both UK adults (6 ng Σ HBCDs kg^{-1} bw day^{-1}) and toddlers (24 Σ HBCDs kg^{-1} bw day^{-1}) (UK Food Standards Agency, 2006).

- A single-compartment, first order pharmacokinetic (PK) model was applied to compare the estimated intakes of target BFRs via inhalation, dust ingestion and diet to the body burdens measured in human milk samples. Good agreement between the predicted and measured body burdens especially for HBCDs and BDE-209 was observed indicating that the studied exposure routes are the main pathways affecting internal human exposure and adult body burdens of BFRs. Interestingly, the estimated body burden of TBBP-A in the lipid compartment was much lower than the measured value which may be attributed to a longer TBBP-A half-life in human adipose tissue, differences between the adult dietary intake of TBBP-A in the UK and the Netherlands (the dietary intake of TBBP-A used in this PK model was for Netherlands adults (de Winter-Sorkina et al., 2003) in the

absence of reliable data from UK), or to individual variability in uptake, metabolism and excretion.

12.2 Research gaps and future perspectives.

The growing scientific interest in BFRs has driven progress in understanding many aspects related to the environmental fate and behaviour in addition to human exposure to this class of chemicals. However, significant research gaps still exist and further research is required to:

a) Derive accurate dust ingestion rates for various age groups to allow for accurate estimation of human intake of BFRs and other POPs via this important exposure pathway.

b) Study the possible differences between exposure estimates via dust ingestion derived from analysis of dust collected from dust bags, vacuuming of a specific standardized area of the room, vacuuming of the whole room, brushing and surface wiping. If significant, such differences would render more difficult comparison between exposure estimates derived from studies using different dust collection methods.

c) Provide more data on concentrations of various BFRs in indoor air and dust from different microenvironment categories (e.g. nurseries, day-care centres, Hospitals, sports gyms and public transport) in different countries using validated and reliable analytical techniques. More data are also required on dietary exposure to BFRs in particular for TBBP-A, HBCDs and BDE-209.

d) Further understanding of the mechanisms of transfer of BFRs from treated products to the indoor environment and the causes of variability in BFR levels in indoor air and dust. This may be achieved using advanced analytical techniques (e.g. ESEM), mathematical modeling, and controlled chamber experiments.

e) Investigating the extent of and the factors influencing the human bioavailability and/or bioaccessibility of BFRs via different exposure routes including ingestion, dermal contact and inhalation.

f) Consolidate and improve the database on human half-lives and toxicokinetic properties of BFRs.

g) Improve knowledge of the potential adverse effects of BFRs on human health to enable the determination of a tolerable daily intake (TDI) for these compounds.

h) Elucidate the relationship between the intake of BFRs via different exposure routes and human body burdens to evaluate the relative influence of each exposure pathway. This also highlights the need for validated, non-invasive indicators of body burden, especially for toddlers and young children, and evaluation of alternatives like hair, saliva, and faeces is required.

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