IN VITRO ASSESSMENT OF THE BIOACCESSIBILITY OF BFRs IN INDOOR DUST USING A COLON ENHANCED HUMAN GIT MODEL

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Introduction

The significance of indoor dust ingestion as a pathway of human exposure to BFRs has been recently highlighted especially for toddlers and school children[1-2]; with an increasing number of studies reporting on levels of different BFRs in indoor dust from various microenvironments in different countries in the past few years[3-4]. However, very little is known about the extent to which this contamination of indoor dust contributes to BFR human body burdens. This can be partly attributed to the dearth of information available about the bioavailabilty of BFRs to humans via different exposure pathways. A few studies have reported on the bioavailability of PBDEs in laboratory animals. The absorbed percentages of 5 major PBDEs in male rats following oral exposure[5] were different from those obtained from studies in aquatic species[6]. This was attributed mainly to inherent differences between species in addition to other factors such as the length of exposure and the exposure matrix. A more recent study reported bioavailability for 15 PBDEs administered in corn oil to male rats, and concluded that the involvement of different enzyme systems and gut microbial flora is likely to have a significant effect on the uptake and metabolism of different PBDEs[7]. These conclusions indicate that extrapolation of results obtained from animal studies to humans requires careful consideration due to species-specific parameters. However, no studies exist of the bioavailability of BFRs in human subjects due to inherent difficulties associated with such studies on humans. Therefore, *in vitro bioaccessibility* tests have recently gained increasing attention for determination of human uptake of various contaminants (e.g. heavy metals, PCDDs/Fs, PAHs and PCBs) via different exposure pathways[8]. While *bioavailability* refers to the fraction of total administered dose that reaches systemic circulation, *bioaccessibility* refers to the fraction of total target compound introduced that dissolves in the gastrointestinal tract (GIT) and therefore, is available for absorption[9]. Determination of bioaccessibility via physiologically based extraction tests (PBET) is a potentially valuable option for assessing the risk to humans from persistent organic pollutants (POPs) and metals. This approach seeks to mimic the processes of human digestion to assess the bioaccessibility of POPs and metals from ingested substances consumed either accidentally or intentionally[8]. In this work, we use a colon enhanced-physiologically based extraction test (CE-PBET) as an *in vitro* test system which incorporates human GIT parameters (including stomach, small intestine and colon pH and chemistry, enzymes, carbohydrates, solid-to-solution ratio, mixing and emptying rates) for assessing the bioaccessibility of PBDEs, HBCDs, and TBBP-A in indoor dust and further understand the factors likely to affect the bioavailability of the studied BFRs from human GIT.

Materials and Methods

Target BFRs: BDEs 47, 99, 100, 153, 154, 183, 209, TBBP-A, α-, β- and γ-HBCD.

Dust: A sieved and homogenised indoor dust sample with particle size range 25-500 μm was used throughout the study. Average concentrations of target BFRs were determined via analysis of 10 replicates, with results revealing very low variability (RSD < 5%). Average concentrations of ΣHBCDs, Σtri-hepta BDEs, BDE-209 and TBBP-A in the studied dust were 816, 63, 7580 and 11 ng g^{-1} respectively.

PBET:

(a) Stomach medium: Stomach medium was prepared as described by Ruby et al.[9]. 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), 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 noted otherwise.

(b) Small intestine medium: Stomach medium is converted to 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 [9].

(c) Colon medium: Colon medium was prepared as described by Macfarlane et al. [10]. In summary, the following components (in grams) were added to 1 litre of deionised 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.

(d) Procedure: One gram of dust was added to 100 mL of GIT medium in a 250 mL flask. The flask was submerged half-way in a temperature-controlled water bath maintained at 37 °C. The mixture was allowed to stand for 10 min, before argon gas was purged through the reaction vessel. The flask contents were mixed gently using a magnetic stirrer to match the peristaltic movement of the human GIT. The pH was checked after 5 min, and every 10 min thereafter, and the pH was adjusted when necessary. A flow chart of the CE-PBET model is given in figure 1.

Analysis: Samples were freeze-dried, accurately weighed, and spiked with 25 ng each of ^{13}C-labelled BDEs 47, 99, 153, 128 and 209, TBBP-A, α-, β- and γ-HBCDs prior to pressurised liquid extraction (Dionex ASE300, Dionex, UK). The crude extracts were concentrated then washed with 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. The dried extract was reconstituted in 200 µL of methanol containing 5 ng each of d_{18}-γ-HBCD and ^{13}C-BDE 100 used as recovery determination standards prior to LC-ESI-MS/MS analysis for HBCDs and TBBP-A and LC-APPI-MS/MS analysis for PBDEs. Further details can be found elsewhere[11-12].

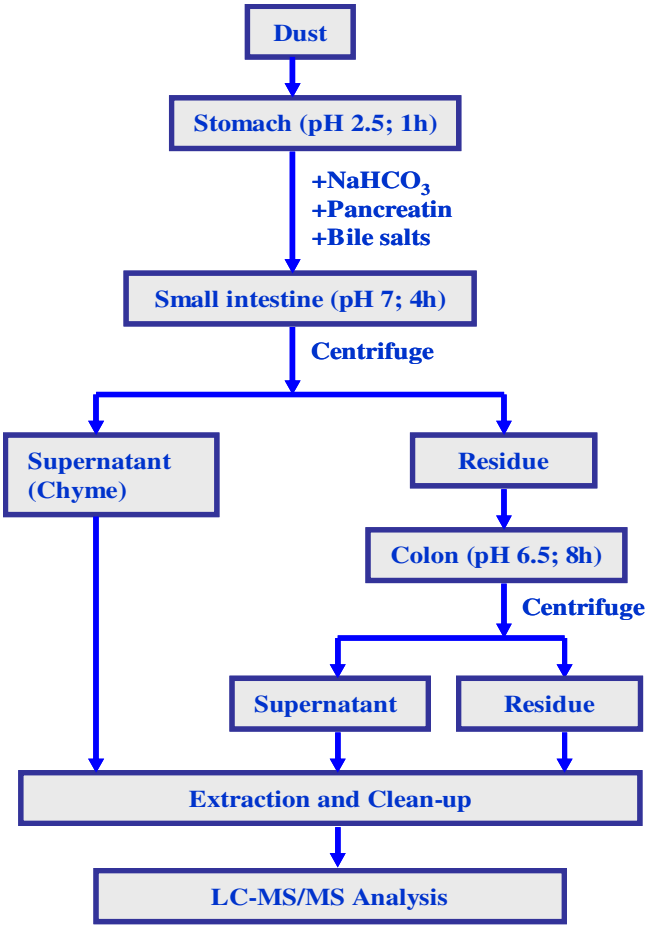


Figure 1: flowchart of the CE-PBET model operation.

Results and Discussion

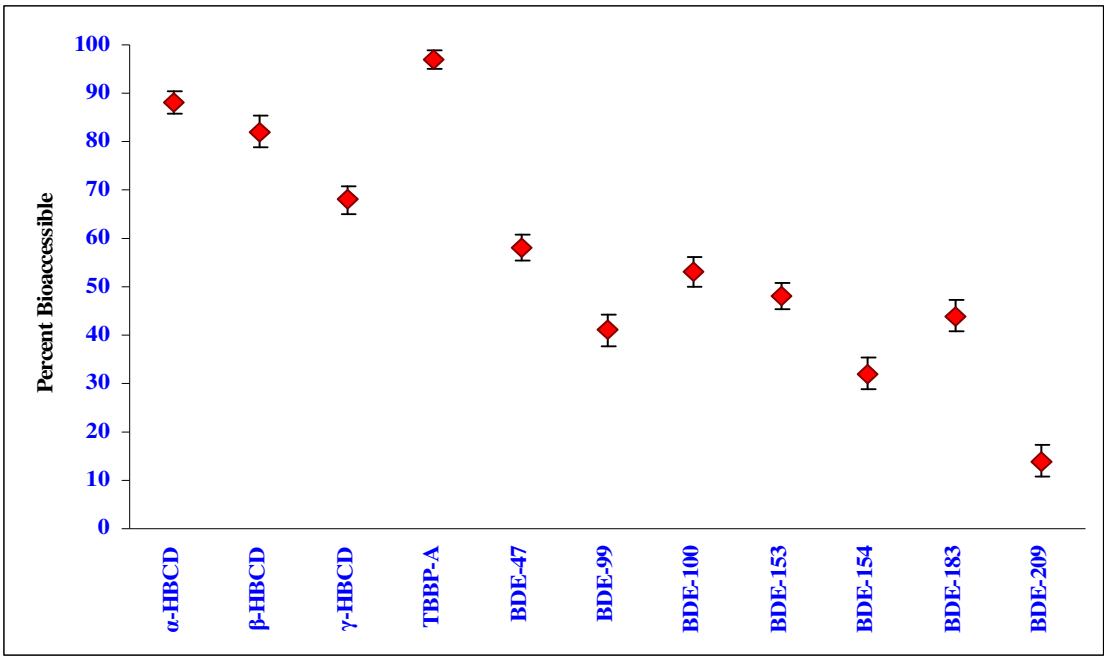
Figure 2 shows the average % bioaccessibility of target BFRs in indoor dust from human GIT. None of the studied compounds were 100% bioaccessible from dust as commonly assumed in exposure assessment models. None of the target BFRs was above the quantification limit in any of the analysed method or field blanks. Therefore, results were not corrected for any background contamination.

Bioaccessibility of the studied BFRs ranged from 14-92%. TBBP-A was the most bioaccessible (94%) which may be expected due to its phenolic structure and relative polarity compared to other BFRs. This is in agreement with animal studies which report TBBP-A to be almost completely (95%) bioavailable[13].

It is evident that the bioaccessibility of γ-HBCD is less than that of either its α- or β-isomers (figure 2). We hypothesise that this is because of the lower water solubility of the γ-isomer that makes its dissolution from dust more difficult than the other diastereomers studied. We believe 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 23-94% in 58 dust samples[14]. No significant changes in enantiomeric fractions (EFs) of the 3 main HBCD diastereomers were observed in any of the studied samples indicating the absence of enantioselective bioaccessibility. 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 reported enrichment of the (-) enantiomer of α-HBCD in human breast milk[15] and

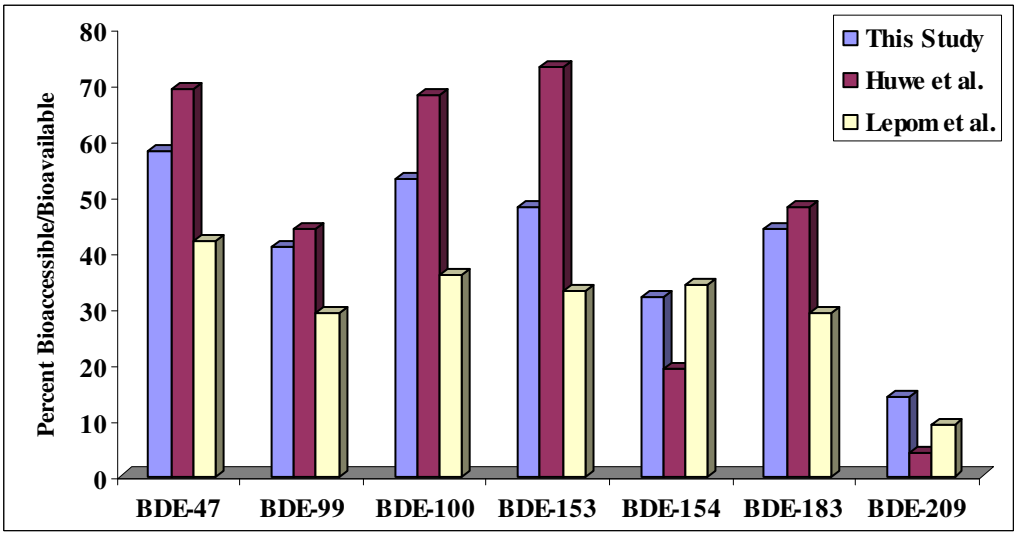
serum[16] indicates the presence of potential *in vivo* enantioselective processes associated with the absorption, biotransformation and/or excretion of HBCDs.

Figure 2: Average (n=3) percent bioaccessibility of target BFRs in indoor dust using CE-PBET



BDE-209 showed the lowest bioaccessibility of the studied compounds. The bioaccessible fraction of BDE-209 from the whole GIT in our CE-PBET model (14%) falls within the bioavailability range of BDE-209 (4-26%) reported in different animal studies [17-18] and is in agreement with the very low water solubility of this compound. Bioaccessibility of the studied tri-hepta BDEs ranged from 32-58%. Unlike bioavailability studies in pike[6], no decrease in the bioaccessibility with increasing level of bromination was observed. This is in agreement with the results of bioavailability studies in rats[5]. Comparison of the results obtained in our CE-PBET study to those obtained from a well-designed *in vivo* bioavailability study of PBDEs in indoor dust in male rats[17] shows a general agreement between the results for BDEs 47, 99, 100 and 183 (figure 3). The differences may be attributed to different enzyme systems, administered dose, particle size of dust and GIT fluid volume at the time of administration.

Figure 3: Comparison of the bioaccessibility/bioavailability of PBDEs in indoor dust from different studies



Interestingly, our bioaccessibility results from a single indoor dust sample are generally higher than those obtained by Lepom et al. using a PBET model without the colon compartment[19] (figure 3). This may be attributable to the characteristics of the specific dust samples examined in the two studies or by the enhanced bioaccessibility (and consequently bioavailability) of lipophilic compounds like POPs in the colon segment rich in carbohydrates which facilitates uptake of these compounds[20].

In conclusion, this preliminary study illustrates the potential of this approach. More detailed studies are now required to examine how the bioaccessibility of BFRs present in dust is influenced by factors such as the:

- Concentration and congener profile of BFRs in the ingested dust.
- Status of the GIT (fasting or fed) at the time of ingestion.
- Particle size of the ingested dust.
- Volume and components (carbohydrates, lipids or other food components) of GIT fluid and stomach emptying rate at the time of ingestion.

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