Preliminary Assessment of Bioaccessibility of HBCDs from Human GIT following Indoor Dust Ingestion Using a Physiologically Based Extraction Test (PBET)

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Abstract
An *in vitro* PBET which incorporates human gastrointestinal tract (GIT) parameters (including pH and chemistry, solid-to-solution ratio, mixing and emptying rates) was applied to study the bioaccessibility of α-, β- and γ-HBCDs from the 3 main GIT compartments following ingestion of indoor dust. While the oral compartment was not studied due to the short residence time in this compartment; 1 gram of a well-characterised, sieved and homogenised indoor dust sample was incubated at each of a simulated stomach (pH=2), small intestine (pH=7) and colon (pH=6.5) compartments for 1, 4 and 8 hours respectively. A "control" group was obtained from a similar experimental setup but no dust added. Samples were freeze dried, extracted using pressurised liquid extraction and analysed using LC-NI-ESI-MS/MS. No target compounds were above the detection limit (0.5 ng g^{-1}) in the control group. Results revealed that the average bioaccessibility of γ-HBCDs from the 3 studied GIT compartments (51%) was less than that for α- and β-HBCDs (82% and 69% respectively) which is probably due to the lower aqueous solubility of the γ-isomer (2 µg L^{-1}) compared to the α- and β-isomers (45 and 15 µg L^{-1} respectively). The average overall bioaccessibility of ΣHBCDs from the 3 GIT compartments studied was 77%. No significant change in the enantiomeric fractions was observed in any of the studied samples.

Introduction
Bioaccessibility is a term used to define the fraction of total target compound introduced that dissolves in the GIT medium and therefore, is available for absorption[1]. 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[1]. Assessment of bioaccessibility via PBET is an important tool when assessing the risk to humans from persistent organic pollutants (POPs) and metals. The approach seeks to mimic the processes of human digestion to assess the bioavailability of POPs and metals from ingested substances consumed either accidentally or intentionally. This tool has been applied successfully for estimation of the bioaccessibility of PCDDs/Fs, PAHs and PCBs from diet and surface soils[2].
HBCD is a brominated flame retardant widely used as an additive to expanded and extruded polystyrene foams for thermal insulation of buildings, back-coating of fabrics and to a lesser extent in HIPS. The commercial formulations consist mainly of α-, β-, and γ-diastereomers with γ- predominant. HBCD has low water solubility (49, 15, 2 µg L^{-1} for α-, β-, and γ-HBCD respectively), a fairly low vapor pressure (6.27 x 10^{-5} Pa) and is persistent. It can therefore bioaccumulate and undergo long-range transport[3]. The levels of HBCDs in different biotic and abiotic matrices have been reviewed [4, 5]. Of particular interest is the observed shift from predominance of the γ-HBCD in abiotic samples to the α-isomer being predominant in most biotic and human samples[4]. Our recent report on HBCDs in indoor dust highlighted the possibility that the predominance of α-HBCD observed in humans[4] may not solely be due to preferential *in vivo* biotransformation of β- and γ-HBCD[6], but, at least partly attributable to other reasons like the diastereomer pattern in dust[7]. Other reasons may include preferential absorption and/or excretion of one or more of the 3 main HBCD diastereomers. 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 less absorption % (32-67%) in rats when HBCDs were administered orally (gavage) in the form of a suspension in olive oil[3]. 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 %"[3].
Based on this scarce information, the aims of the current study are to:

- Determine the bioaccessibility of α-, β- and γ-HBCDs 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 isomers; and
- Assess the factors likely to affect the bioavailability of HBCDs from the human GIT following ingestion of indoor dust.

Materials and Methods

Dust: A well-characterised, sieved and homogenised indoor dust sample with particle size range 25-500 μm was used throughout the study. Average concentrations (n=10) of α-, β- and γ- HBCDs in the studied dust were 124, 74 and 518 ng g^{-1} respectively.

PBET:

(a)Stomach medium: Stomach medium was prepared as described by Ruby et al. [1]. 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 [1].

(c) Colon medium: Colon medium was prepared as described by Macfarlane et al. [8]. 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 (figure 1). 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. After the specified incubation time, the flask contents were centrifuged and the supernatant was collected for analysis.

Analysis: Samples were freeze-dried, accurately weighted then spiked with 25 ng of each of ^{13}C-labelled 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 and the dried extract was reconstituted in 200 μL of d_{18}-γ-HBCD used as recovery determination standard prior to LC-NI-ESI-MS/MS analysis. Details of both diastereomer- and enantiomer-specific LC-NI-ESI-MS/MS methods for HBCDs analysis can be found elsewhere[9].

Results and Discussion

Table 1 shows the average % bioaccessibility of HBCD diastereomers from the different GIT compartments studied. None of the studied HBCD diastereomers were 100% bioaccessible from dust as reported from solutions in rats previously [3]. It's evident that the bioaccessibility of the 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 that makes its dissolution from solid phase more difficult than 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 23-94% in 58 dust samples[10]. 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 rendering it more bioavailable. The isomer distribution of HBCD diastereomers (expressed as % of ΣHBCDs) exhibited a difference in the bioaccessible fraction from that in the original dust (figure 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 2) indicates that the lower bioaccessibility of this isomer from ingested dust is not alone 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[4]. 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 26 out of 30 human milk samples from Spain while α-HBCD was dominant in the remaining 6 samples[11] may support our hypothesis that internal HBCD exposure is affected - to some extent - by the external exposure profile.

No significant changes in enantiomeric fractions (EFs) of the 3 main HBCD diastereomers were observed in any of the studied samples (table 2) 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[11] and serum[12] indicates the presence of *in vivo* potentially enantioselective processes during HBCDs absorption, biotransformation and/or excretion.

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

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

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Tables:

Table 1: Average (n=3) % bioaccessibility of HBCD diastereomers from dust.

Sample ID	α- HBCD	β- HBCD	γ- HBCD	Σ HBCDs
Stomach	60	50	38	43
Small intestine	57	46	34	39
Colon	59	47	34	40
Stomach-Small intestine	82	69	51	59
Stomach-Small intestine-Colon	92	81	72	77

Table 2: Enantiomeric fractions (EFs) of HBCDs in the studied samples

	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

Figures:

Figure 1: Simplified diagram of PBET experimental system**.**

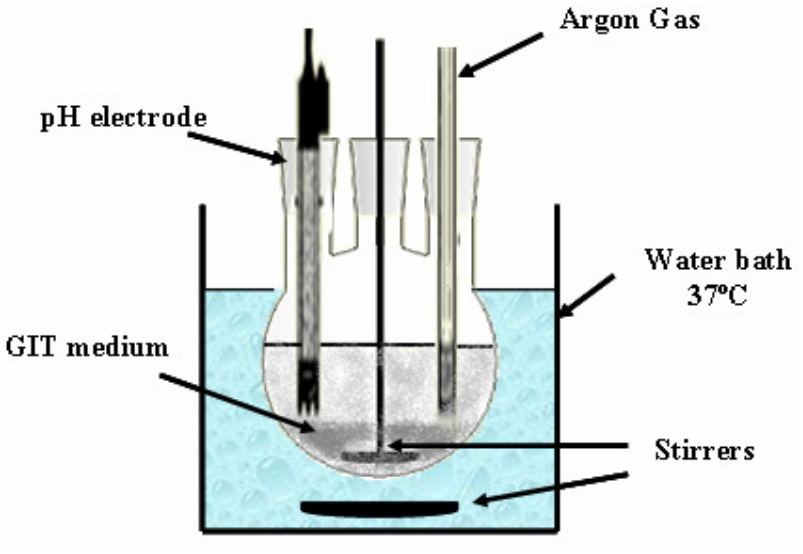

Figure 2: Average HBCDs isomer profiles in the studied samples