MODIFICATION AND APPLICATION OF AN *IN VITRO* HUMAN GASTROINTESTINAL TRACT FOR THE DETERMINATION OF ORAL BIOACCESSIBILITY OF POLYBROMINATED DIPHENYL ETHERS

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Introduction

Total contaminant concentrations are frequently used in risk assessment of contaminated sites to human health.[1] Such assessment, though advantageous for precautionary measures, may lead to overestimation of the amount of contaminant absorbed by humans.[1] These overestimations have significant implications for cost and sustainability of brownfield remediation; hence, the use of bioaccessible and bioavailable fractions of contaminants for site specific risk assessment is a very important parameter. The use of vertebrates and invertebrates for *in vivo* bioavailability studies of contaminants gives realistic measurements of the bioavailable fractions, however, ethical considerations of using mammals, the high cost, low sample through-put, and differing physiologies and ecologies, compromises the extrapolation of contaminant bioavailability in animals to humans; thus making *in vivo* methods unsuitable for routine laboratory testing purposes.[2] The use of *in vitro* bioaccessibility tests has recently gained much attention in studying human uptake of various contaminants such as heavy metals, PCDD/Fs, PAHs, PCBs, and PBDEs in various matrices following various approaches utilizing both 'fed' and 'fasted' state conditions.[2-7] It has recently been shown that the presence of food components in the gastrointestinal tract (GIT) during intestinal transit modulates the release of hydrophobic contaminants such as PBDEs.[8] Such release has been ascribed to the more hydrophobic character that food components give to the aqueous solution; similarly, soluble soil organic matter forms microscale hydrophobic environments in the aqueous phase, thus acting as a mobile sorbent for hydrophobic compounds such as PBDEs, PCBs and PAHs.[8] The types and concentrations of bile in GIT fluids, GIT extraction time, gastric pH and transit time in each GIT medium plays a significant role in in the release of PBDEs and other persistent organic pollutants.[8] To better understand the implication of the ingestion of dust from e-waste recycling sites for human exposure, the objectives of the present study include: (1) to determine the optimum GIT conditions for determining the bioaccessibility of PBDEs in the dust, (2) to apply the optimum conditions to determine the bioaccessibilities of eight environmentally relevant PBDE congeners, (3) to compare the effectiveness of two GIT procedures: the so called unified bioaccessibility method (fasted-state) of Europe and the FOREhST (fed-state) conditions reported by Lorenzi *et al.*[3] after parameter modifications, and (4) to determine the effectiveness of two extraction formats: batch and sequential, for studying the oral bioaccessibility of PBDEs.

Materials and methods

Sampling and analysis of total PBDEs in samples

Dust samples were collected from two e-waste dismantling/recycling facilities at various locations in South Africa. Sampling was carried out with a LG 1600W vacuum cleaner. The vacuum cleaner was fitted with dust collection bags at each sampling point and was copiously cleaned with wash solutions and isopropanol. Samples were stored in amber glass bottles at -10 °C. Dust samples were sieved through a 212 µm stainless steel sieve and extracted with 15 mL n-hexane:methanol mixture (1:3, v/v) in an ultrasonic water bath at 40 °C for 60 mins. Extracts were subjected to silica gel column chromatography prior to GC-EI-MS analysis. A Restek Rtx®-1614 capillary column (15 m × 250 µm × 0.1 µm) was used to effect separation and the MS was used in the selected ion monitoring (SIM) mode. The GC oven temperature programme was 90 ºC held for 2 mins, then increased at 20 ºC min^{-1} to 270 ºC, then ramped at 10 ºC min^{-1} to 325 ºC and held for 5 mins. Helium was employed as the carrier gas at a flow rate of 1.2 mL min^{-1} and a constant linear velocity of 58 cm s^{-1}. The ion source and transfer line temperatures were 230 ºC and 350 ºC, respectively. The ionization energy was 70 eV. An aliquot of 1 µL of each sample was injected in the pulsed splitless mode at an injector temperature of 285 ºC.

Preparation of gastrointestinal fluids

The physiologically based extraction tests in the present study were based on the Unified BARGE method (UBM) of Europe.[3] All GIT fluids were prepared in advance and stored at <4 ºC. The major modifications of

our methods compared to those of Lorenzi *et al*.[3] are basically prolonged extraction time, increased bile fluid for extraction (i.e. 9 mL) and optimum gastric pH for FORhEST. The analytical protocols involved GIT extractions followed by methanolic potassium hydroxide saponification (for the FOREhST protocol). Liquid-liquid extractions were employed for the recovery of PBDEs from the supernatants of the GIT fluid extracts; whereas ultrasonic-assisted extraction was employed for the determination of residual PBDEs in the matrix and for the total PBDE determination in dust samples. Silica gel column chromatography was applied for the purification of sample extracts prior to gas chromatographic mass-spectrometric determination of PBDEs. Factors, such as pH, incubation time, presence of food components, and dust-to-solution chemistry, responsible for the oral bioaccessiblities of PBDEs were optimized in this study. A mass-balance exercise was carried out to determine the percentage recovery of PBDEs in a standard reference material (SRM 2585) and in contaminated dust samples following the two *in vitro* GIT methods.[2] Equation 1 depicts the equation used to determine the recovery of each of the PBDE congeners following *in vitro GIT* extractions.

$$\%\, recovery = \frac{Average\ mass\ of\ PBDE\ congener\ in\ supernatants\ of\ modified\ FOREhST\ or\ UBM + average\ mass\ of\ PBDEs\ in\ solute\ after\ GIT\ \ extraction}{Average\ mass\ of\ PBDE\ congener\ in\ the\ extracted\ dust}$$

The % bioaccessibility was calculated as:

$$\text{Bioaccessibility (\%)} = \frac{Average\ amount\ of\ each\ PBDE\ in\ the\ supernatant\ of\ GIT\ medium}{Average\ amount\ of\ each\ PBDE\ \ originally\ present\ in\ extracted\ dust} \times 100$$

Results and discussion

Optimum gastric pH was obtained at pH 2.2 for all the BDE congeners (Fig. 1). However, there were reductions in the % bioaccessible fractions (%BAF) of BDE-183 and BDE-209 at pH > 2.33. The optimum pH for the BDE congeners represents the typical gastric pH after the ingestion of a meal. Equilibrium was attained within 8 hours of incubation for tri-hexaBDE, while BDE-183 and 209 attained equilibrium after 16 hours of incubation.

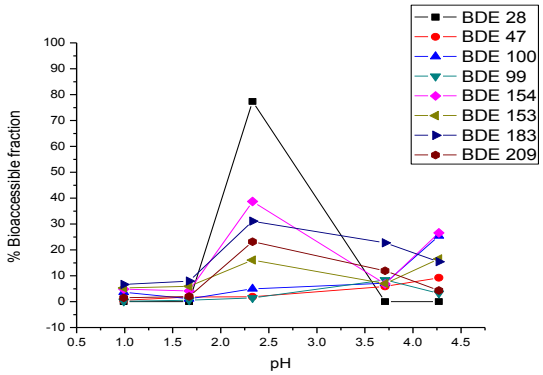

Fig. 1: Influence of gastric pH on the oral bioaccessibility of PBDEs in dust.

Following the mass balance exercise, most of the BDE congeners showed good recoveries ranging from 34.3-91.5% and 42.4-94.0% for the modified FOREhST (fed-state) and modified UBM (fasted-state) respectively in SRM 2585 dust samples.

In contaminated e-waste samples, the %BAF of PBDEs ranged between 6.5% (BDE-154) to 27.9% (BDE-183) for the "fasted-state" and 16.9% (BDE-153) to 92% (BDE-28) in the "fed-state" condition (Fig. 2).

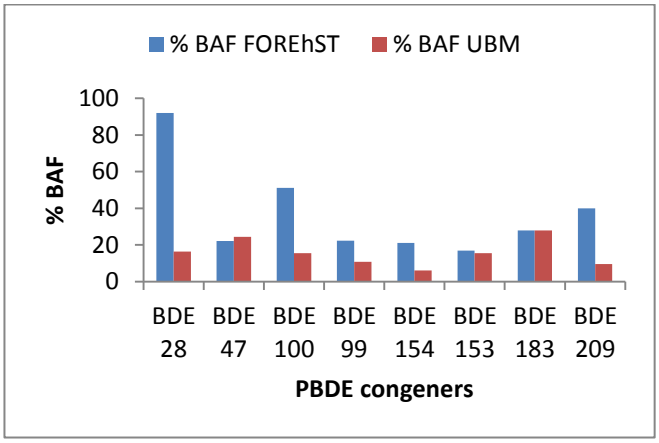

Fig. 2: %BAF of PBDE congeners following *in vitro* human GIT extraction using both the modified FOREhST and UBM extraction protocols for e-waste contaminated dust samples.

There were significant differences ($p < 0.05$) in the %BAF of PBDEs for both "fasted" and "fed" states in SRM 2585 samples. A positive correlation ($r = 0.62$) was obtained between the %BAF of the eight BDE congeners and their respective log K_{ow} in the "fasted-state" but not in the "fed-state" conditions. There were no statistical differences ($p < 0.05$) in the %BAF of PBDEs following batch and sequential extraction formats (Fig. 3).

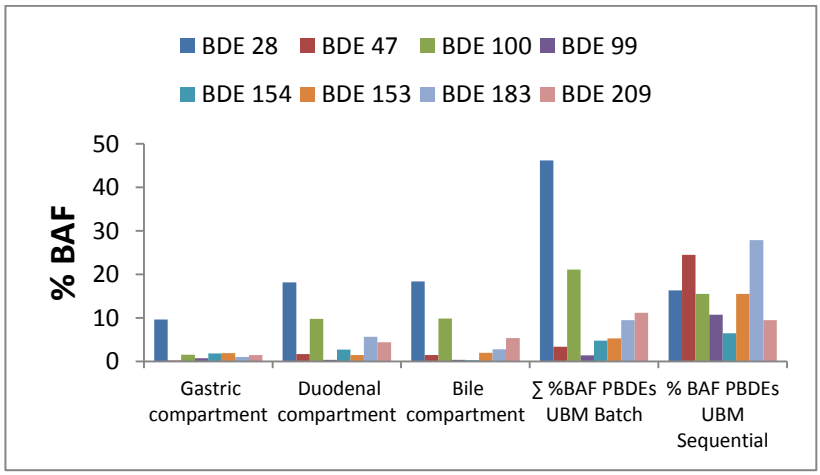

Fig. 3: %BAF of PBDEs in individual GIT media following batch extraction and comparison with sequential UBM extraction format.

We conclude that the oral bioaccessibility of PBDEs in dust are strongly influenced by the concentrations of PBDEs in the samples; and also characteristics such as dust pH and particle size; as well as volume and ratio of GIT fluid employed for incubation.

Acknowledgements
We thank the management and staff of all the e-waste recycling facilities for allowing us into their facilities for sampling. We are also extremely grateful to Restek Corporation for the gift of a Rtx®-1614 capillary GC column. OAA is grateful to the University of KwaZulu-Natal for funding this research through a doctoral scholarship.

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