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IN VITRO ORAL BIOACCESSIBILITY OF FRS IN INDOOR DUST USING TENAX-TA® ASSISTED COLON-EXTENDED PHYSIOLOGICALLY BASED EXTRACTION TEST (CE-PBET) COUPLED WITH A DIALYSIS MEMBRANE METHOD

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Introduction

Flame retardants (FRs) are man-made chemical compounds widely used in industry during the manufacturing of various commercial products such as computers, plastics, fabrics, textiles and polyurethane foam products in order to minimise or prevent fire (Covaci et al., 2003). Worldwide phase-out campaigns and legislative restrictions on the use of polybrominated diphenylethers (PBDEs) have resulted in the production of new PBDE-replacement products, also known as emerging FRs (e.g. EH-TBB, BEH-TEBP and BTBPE) (Ali et al., 2011).

Evaluating oral bioaccessibility (i.e. uptake) to xenobiotics via ingestion of abiotic matrices, such as indoor dust stands as a pivotal point in human exposure assessment to FRs where intestinal absorption is commonly considered as 100%, leading to potential risk overestimation. In vivo studies in rats can potentially bridge the uptake/bioavailability research gap, but are oftenly hindered due to ethical reasons (Oomen et al., 2003; Ruby et al., 2002).

As a result, more cost-efficient and less time-comsuming in vitro bioaccessibility alternatives are employed such as the colon-extended physiologically based extraction test (CE-PBET) in order to mimic the human uptake potential of organic compounds, including flame retardants (FRs), using physiologically-based simulated gastro-intestinal fluids (Abdallah et al., 2012; Tilston et al., 2011). Therefore, a need for the establishment of an in vitro colon-extended physiologically-based extraction test (CE-PBET) with the addition of a sorptive "sink" for the evaluation of in vitro bioaccessibility of FRs via the GIT is necessary in order to ensure high desoprtion gradient of xenobiotic release from the matrix and sustain a conservative, yet robust in vitro test (Collins et al., 2015).

Material and methods

In the present study, we demonstrate the feasibility of CE-PBET as developed by (Tilston et al., 2011) with the inclusion of Tenax TA® beads (60-80 mesh size) as a strong adsorbent resin to trap freelly-available FRs in the simulated GI juices, hence minimising FR re-absorption onto the dust particles. Compared to previously reported studies (Fang and Stapleton, 2014), 16cm (1.1mL/cm) RC Dialysis membrane with MWCO 3.5 kDa & 18mm flat width of a cellulose-based dialysis membrane (SpectrumLabs Inc., USA) was employed to encapsulate the Tenax TA®, allowing efficient separation between the Tenax TA® and the indoor dust (<250 μm) throughout the CE-PBET incubations, along with successful sorption of freelly-available FRs onto the Tenax TA® beads by passive diffusion (Fig.1). Parameters of the proposed method such as soption potential with respect to different amounts of Tenax TA® beads (i.e. 0.25, 0.5 or 0.75g) and matrix-dependent soption kinetics between the three-compartment CE-PBET (i.e. stomach, small intesting and colon) where assessed during one, four and 16 hours of incubation respectively, in order to further elucidated the factors controling GIT uptake of FRs on a mechanistic level.

After the GIT incubations were finished, all samples were separated by centrifugation at 3000rpm for 10min, followed by extraction and clean-up according to (Van den Eede et al., 2012). PBDEs (Tri- to Octa-BDEs) and selected nBFRs were selected as the target analytes of the present study. The supernatants of small intestine and colon media were subjected for a liquid-liquid extraction (LLE) using Hexane/Ethyl Acetate 3:1, while ultra-sonication assisted extraction using Acetone/Hexane 1:3 was used for the residual dust and recovered Tenax TA® beads. A selective and sensitive method to determine the analytes of interest was employed using a ThermoScientific® ITQ 1100 (GC-EI/MS).

Results and discussion

Preliminary results of the newly developed CE-PBET method are shown in Fig.2, where and indoor dust from a UK store was used, spiked with PBDEs and selected nBFRs in environmentally relevant levels. The PBDEs studied were from Tri- to Octa-BDEs and their nBFR alternatives, including EH-

TBB and BEH-TEBP for Penta-BDEs and BTBPE for Octa-BDEs. The encapsulation of Tenax into the RC dialysis membrane prevented the mixing of the incubated dust with the Tenax which can potentially lead to Tenax TA® loss and FR re-sorption onto the dust, while allowing the dissolved and freely-available FRs to be successfully absorbed by passive diffusion. Therefore, the dialysis membrane design is considered an efficient method which manages to successfully separate the Tenax TA® beads from dust matrix.

When compared to the "no sink" conditions, the inclusion of Tenax TA® beads during the GIT incubations has managed to increase the bioaccessible fraction (%BAF) between two- and three-fold for the low hydrophobic PBDEs (e.g. BDE-28) and the highly hydrophobic alternatives (e.g. EH-TBB and BEH-TEBP). Statistical analysis between the "no sink" conditions and the different Tenax masses (0.25g, 0.5g and 0.75g), has revealed a statistically significant relationship ($p<0.05$) for all target analytes (Fig.2). A statistically significant relationship ($p<0.05$) for 0.25g and 0.5/0.75g Tenax TA® inclusion was established for the case of BDE-28, unlike to the highly hydrophobic compounds. As a result, the use of 0.5g of Tenax TA® beads was considered to the sufficient for a successful sorption of a wide range of FRs with diverse physicochemical profiles.

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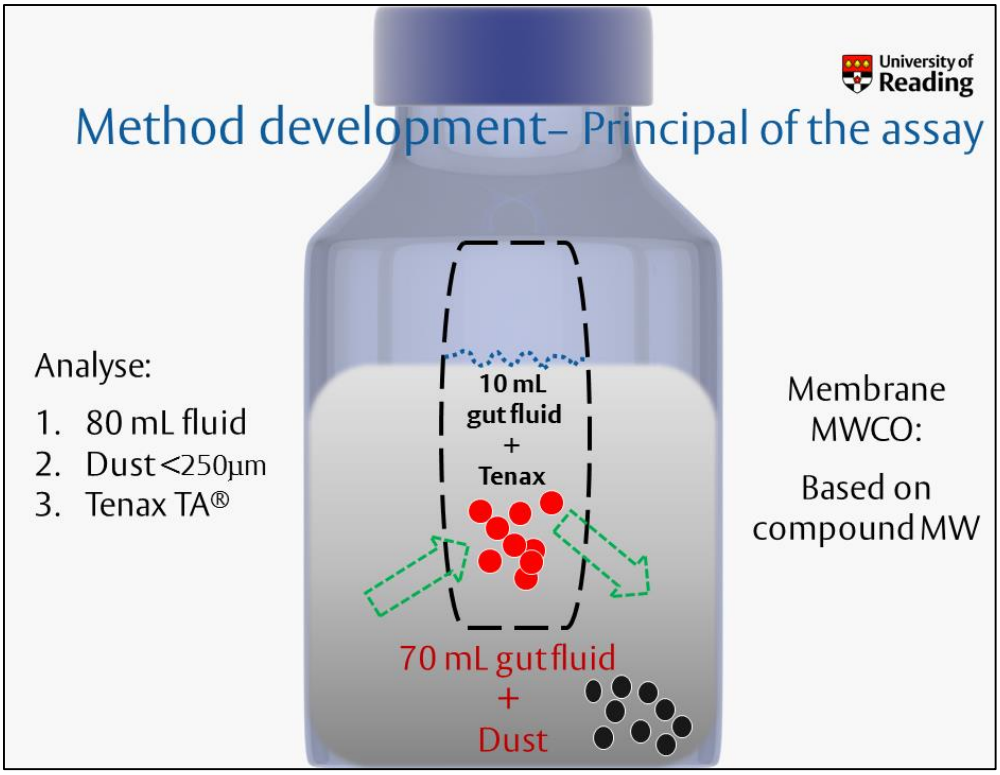

Fig. 1 Principal of the assay

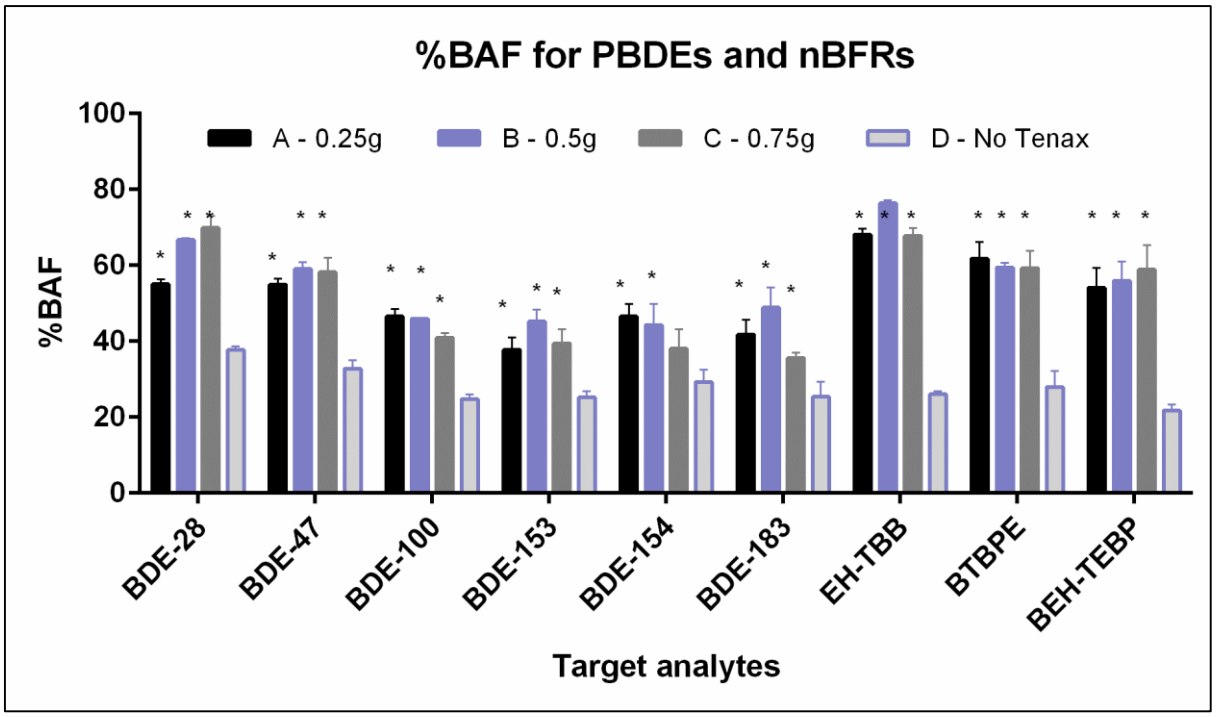

Fig. 2 Preliminary results of Tenax-assisted CE-PBET using different Tenax mass in spiked dust