

Bioaccessibility of PBDEs present in indoor dust: a novel dialysis membrane method with a Tenax TA® absorption sink

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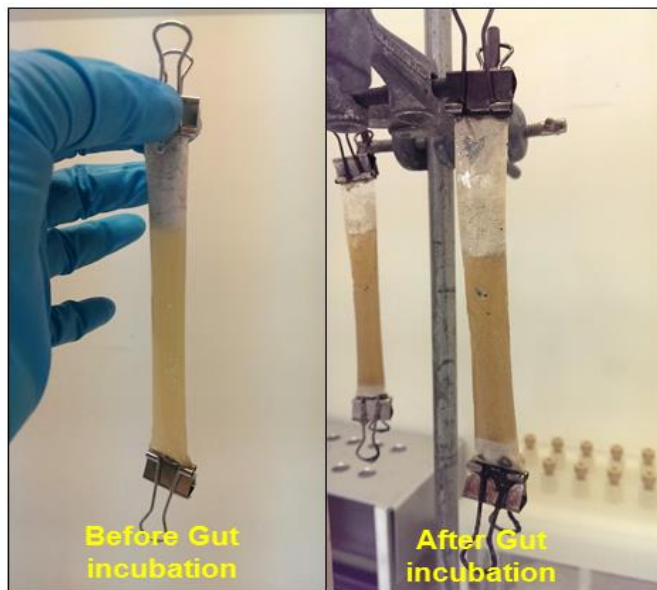
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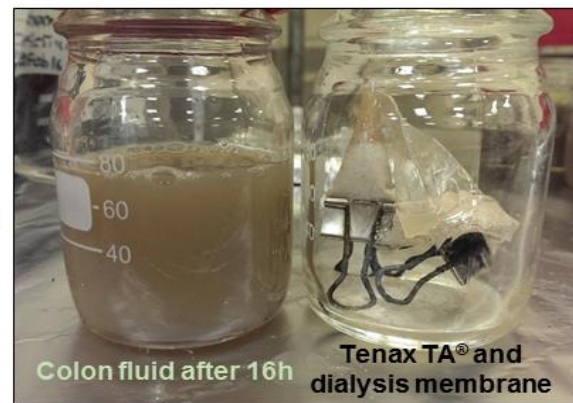
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Graphical abstract

Tenax TA[®]-assisted CE-PBET using Dialysis Membrane



Tenax TA[®] sedimented due to saturation



Successful physical separation between gut fluid and Tenax TA[®] after CE-PBET incubations

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Highlights

- First method employing dialysis membrane for physical separation between Tenax TA[®] and dust
- Tenax TA[®] used as an absorption sink trapped in dialysis membrane mimics the situation *in vivo*
- CE-PBET performance was tested under different Tenax TA[®] loadings (0.25, 0.5 & 0.75 g)
- Two to three-fold bioaccessibility increase with Tenax TA[®] inclusion for all PBDEs
- Colon sorption to Tenax TA[®] was similar to small intestine for BDE28, but was higher than small intestine sorption for other PBDEs

16 Bioaccessibility of PBDEs present in indoor dust: A novel dialysis membrane method with a
17 Tenax TA[®] absorption sink

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27

28

Abstract

29 Human uptake of flame retardants (FRs) such as polybrominated diphenyl ethers (PBDEs)
30 via indoor dust ingestion is commonly considered as 100% bioaccessible, leading to potential
31 risk overestimation. Here, we present a novel *in vitro* colon-extended physiologically-based
32 extraction test (CE-PBET) with Tenax TA[®] as an absorptive “sink” capable to enhance
33 PBDE gut bioaccessibility. A cellulose-based dialysis membrane (MW cut-off 3.5kDa) with
34 high pH and temperature tolerance was used to encapsulate Tenax TA[®], facilitating efficient
35 physical separation between the absorbent and the dust, while minimizing re-absorption of
36 the ingested PBDEs to the dust particles. As a proof of concept, PBDE-spiked indoor dust
37 samples (n=3) were tested under four different conditions; without any Tenax TA[®] addition
38 (control) and with three different Tenax TA[®] loadings (*i.e.* 0.25, 0.5 or 0.75 g). Our results
39 show that in order to maintain a constant sorptive gradient for the low MW PBDEs 0.5 g of
40 Tenax TA[®] are required in CE-PBET.. Tenax TA[®] inclusion (0.5 g) resulted in 40% gut
41 bioaccessibility c.f. for BDE153 and BDE183, whereas greater bioaccessibility values were
42 seen for less hydrophobic PBDEs such as BDE28 and BDE47 (~60%). When tested using
43 SRM 2585, our new Tenax TA[®] method did not present any statistically significant effect
44 ($p>0.05$) between non- spiked and PBDE- spiked SRM 2585 treatments. Our study describes
45 an efficient method where due to the sophisticated design, Tenax TA[®] recovery and
46 subsequent bioaccessibility determination can be simply and reliably achieved.

47

48 Keywords: bioaccessibility, Tenax TA[®], dialysis membrane, PBDEs, indoor dust

49

1. Introduction

51 Despite the strict legislative measures on the use of Penta-BDE and Octa-BDE formulations
52 in both the EU and USA in consumer products (*e.g.* carpets, electronic appliances and
53 furniture polyurethane foam) (Dodson et al., 2012; European Commission, 2003) and their
54 listing as persistent organic pollutants (POPs) under the Stockholm Convention (Stockholm
55 Convention, 2009a, 2009b), polybrominated diphenyl ethers (PBDEs) are legacy flame
56 retardants (FRs) being detected in considerable levels in indoor dust from China (Cao et al.,
57 2014; Sun et al., 2016), France (Raffy et al., 2017), the UK (Kademoglou et al., 2017; Tao et
58 al., 2016), the Czech Republic, USA and Canada (Venier et al., 2016). Under this regime,
59 human health concerns remain a critical issue, given the well-known PBDE potential to
60 induce endocrine and thyroid disruption (Legler, 2008) and neurodevelopmental disorders in
61 children (Bellinger, 2013; Costa and Giordano, 2007).

62 Total pollutant concentration of a contaminated solid matrix is perceived as the bioavailable
63 fraction after ingestion and it is frequently used in human risk and exposure assessment
64 (Semple et al., 2004). However, the assumption that 100% of the ingested toxicant within a
65 matrix being available is unrealistic (Collins et al., 2015). Animal bioavailability studies (*e.g.*
66 rodents or swine) are representative of the *in vivo* situation, but are often hindered due to
67 financial and ethical restrictions (Oomen et al., 2003; Ruby et al., 2002). To avoid risk
68 overestimation, bioaccessibility, *i.e.* the maximal fraction of an organic pollutant released
69 from an ingested matrix (*e.g.* dust) into the gastro-intestinal tract (GIT) fluids of the organism
70 has been proposed as a more realistic but conservative approach in human exposure
71 assessment of persistent organic pollutants (POPs), serving as a surrogate to bioavailability
72 (Brandon et al., 2006; Dean and Ma, 2007; Oomen et al., 2000). Several physiologically-
73 based extraction tests (PBET) have been proposed to assess organic pollutant release and
74 uptake from an ingested matrix via the GIT fluids *in vitro* (Brandon et al., 2006; Cave et al.,
75 2010; Gouliarmou and Mayer, 2012; Tilston et al., 2011; Van de Wiele et al., 2004), as a
76 substitute to *in vivo* studies (James et al., 2011) or for high-throughput estimates of
77 bioaccessibility when animal studies are not feasible (Rodríguez-Navas et al., 2017; Ruby et
78 al., 1996). Due to the non-polar and hydrophobic nature of hydrophobic organic compounds
79 (HOCs) such as PBDEs, sorption to indoor dust is likely to occur via volatilisation, abrasion
80 or fragmentation (Cao et al., 2014; García-Alcega et al., 2016), marking dust ingestion as a
81 potential major route of exposure to FRs for humans (Alves et al., 2014; Jones-Otazo et al.,
82 2005). Hence, *in vitro* bioaccessibility studies have been deployed, assessing human exposure

83 to contaminated indoor dust on a wide spectrum of HOCs including brominated flame
84 retardants (BFRs) (Abdallah et al., 2012), organophosphate FR (OPFRs) (He et al., 2016;
85 Quintana et al., 2017), pesticides and polychlorinated biphenyls (PCBs) (Ertl and Butte,
86 2012) and polybrominated diphenyl ethers (PBDEs) (Yu et al., 2012). However, the lack of
87 an adsorption sink in the various test formats may lead to risk underestimation due to the
88 absence of constant concentration gradient (Collins et al., 2015). Sink conditions better
89 mimic the sorption/desorption processes in the human GIT *in vivo* and, coupled with the
90 lipid-rich environment of the GI lumen and a long matrix:fluid contact time, may improve the
91 bioaccessibility estimates of HOCs, such as PBDEs (Collins et al., 2015; Zhang et al., 2015,
92 2016).

93 A colon-extended PBET system (CE-PBET) with a carbohydrate-rich colon compartment as
94 a “sink”, favouring polycyclic aromatic hydrocarbons (PAHs) desorption from soil has been
95 described (Tilston et al., 2011). Strong adsorbents such as silicone-activated contaminant
96 traps, cyclodextrins and silicone rods have also been proposed as “absorption sink” materials
97 in PBET systems, to improve bioaccessibility estimates for PAH-contaminated soil and
98 biochar (Gouliarmou et al., 2013; Mayer et al., 2016; Zhang et al., 2015). As part of the
99 International Organization for Standardization (ISO) guideline on bioavailability, an extended
100 (20h) Tenax-based extraction method achieved increased mobilisation (*i.e.* bioaccessibility)
101 of HOCs from soils and sediments onto this infinite sink and has been proposed for
102 standardisation (ISO, 2015; Ortega-Calvo et al., 2015). Tenax TA[®] is a versatile absorption
103 sink with large surface area and high sorption capacity for HOCs and was thus used as an
104 “infinite” sink in PBET systems, studying the uptake of FRs and PAHs via indoor dust (Fang
105 and Stapleton, 2014) and soil (Li et al., 2015), respectively. Cornelissen et al (1997)
106 employed Tenax TA[®] studying sorption/desorption kinetics of PAHs, alkylbenzenes and
107 PCBs from dredged sediments; the sink captured the organic pollutants from the solid matrix
108 but the Tenax TA[®] beads adhered to the glassware with consequent problems for physical
109 separation and recovery of Tenax TA[®] from the matrix (Cornelissen et al., 1997). The
110 variability in Tenax TA[®] mass recovery, its separation from the matrix and the design of an
111 appropriate vessel for Tenax TA[®] inclusion (*e.g.* stainless steel net) during PBET incubation
112 has discouraged further applications of Tenax TA[®] in environmental exposure studies (Li et
113 al., 2016; Mayer et al., 2016). In the work presented here, we describe a novel *in vitro*
114 method capable to overcome the aforementioned challenges concerning physical separation
115 and recovery of Tenax TA[®] from the matrix, while facilitating its successful inclusion and

116 performance as an adsorption sink in a previously established bioaccessibility test, namely
117 CE-PBET, for the assessment of oral bioaccessibility of PBDEs from indoor dust.

118 To separate aqueous and solid matrices, a regenerated cellulose (RC) dialysis tubing method
119 was employed, studying the sorption and dissolution of perchloroethane and PAHs from clay-
120 rich materials and sewage sludges, respectively (Allen-King et al., 1995; Woolgar and Jones,
121 1999). RC membranes present high pH and temperature tolerances, carry no fixed charge and
122 are highly resistant to halogenated hydrocarbons, such as PBDEs (Pollard, 1987). Tubing
123 characteristics including length, width, membrane sealing method and molecular weight cut
124 off (MWCO) have been evaluated. For example, 2.5 g of contaminated sewage sludge were
125 introduced into 10 cm of dialysis tubing with a 3.5 kDa MWCO (Woolgar and Jones, 1999).
126 Alternatively, 20 cm of dialysis tubing (29 mm width; 12-14 kDa MWCO) was used to
127 ensure that at least 30% of the analyte mass would remain in the solid phase after
128 equilibration (Allen-King et al., 1995). The solid material in the tubing was then introduced
129 inside glass bottles with synthetic groundwater spiked with the HOCs of interest. During
130 equilibration, all non-settling particles were retained inside the dialysis membrane, while
131 dissolved organic pollutants could permeate through the membrane and equilibrate across the
132 dialysis tubing by passive diffusion (Allen-King et al., 1995).

133 Our study aims are to systematically (a) develop an efficient method to separate Tenax TA[®]
134 and indoor dust as a matrix whilst enabling desorption of PBDEs to the Tenax TA[®] and (b)
135 optimise Tenax TA[®] as an absorption sink for PBDEs in a colon-extended gastro-intestinal
136 bioaccessibility *in vitro* system (CE-PBET).

137 **2. Materials and methods**

138 **2.1 Target analytes and indoor dust**

139 An indoor dust sample was collected in 2013 from a pre-existing vacuum cleaner bag in an
140 office at Reading (UK) and was used during method development tests and the results are
141 presented in sections 3.1 and 3.2. The dust sample was sieved to <250 µm, a particle cut off
142 likely to be ingested by humans (Yu et al., 2012), using a hexane-washed, metallic sieve and
143 stored in hexane-washed, amber glass bottles at +4 °C. Concentrations of all target analytes in
144 all dust samples were determined using methods described elsewhere (Kademoglou et al.,
145 2017). Briefly, 30 mg of dust was extracted with 2.5 mL hexane:acetone (3:1) using ultra-
146 sonication extraction for 10 min and vortexing for 1 min three times. The combined extract

147 was concentrated to 1 mL and loaded on aminopropyl (NH₂) silica cartridges (500 mg, 3 mL,
148 Agilent, USA) and further eluted with 10 mL hexane. The eluate was then further
149 concentrated, following a clean-up on an acidified silica cartridge (5%, 1 g, 6 mL) and elution
150 with 12 mL dichloromethane. The dust extracts were then evaporated, reconstituted with 100
151 µL of iso-octane and filtered using a micro centrifuge filter lined with 0.45 µm pore size
152 nylon filter (1.5 mL volume capacity) . Finally, the extracts were transferred to injection vials
153 and analyzed on GC-ECNI-MS. Standard reference material for indoor dust SRM 2585
154 (organic contaminants in house dust), purchased from the US National Institute of Standards
155 and Technology (NIST, USA), was used to assess method performance and the results are
156 presented in section 3.3. . Both SRM 2585 (used for method performance assessment; 0.5 g)
157 and dust samples (0.5 g) (used for method development) were spiked at environmentally
158 relevant concentrations (200 ng; 200µL of PBDEs native standard mix 1 ng/µL prepared in
159 iso-octane) and the validity of the spiking was confirmed analytically for both the SRM 2585
160 and the dust (Table SI 2). After spiking, samples were shaken for 2h on an orbital shaker and
161 allowed to stand inside a fumehood for 6h before the gastro-intestinal extraction for the
162 solvent to evaporate, thus facilitating compound interactions with the matrix (Ballesteros-
163 Gómez et al., 2016).

164 **2.2 Dialysis membrane**

165 Approximately 16 cm of standard grade, flexible and transparent regenerated cellulose (RC)
166 dialysis membrane with 3.5 kDa MWCO and 18 mm flat width (1.1mL/cm) (Spectra/Por™ 3,
167 SpectrumLabs Inc., USA) was used to encapsulate the Tenax TA® beads. The membrane
168 length and flat width were selected for the sample volume to be added in the membrane using
169 an online tool provided by SpectrumLabs Inc.
170 (<http://www.spectrumlabs.com/dialysis/dtCalc.html>), allowing for tube sealing with 19 mm
171 metallic clips. MWCO selection for the RC membrane is primarily governed by the
172 molecular weight (MW) of the biological molecules of the GI compartments and the target
173 analytes of our study. To maximize the rate of dialysis, the membrane with the largest
174 MWCO which will not cause excess loss of the desired analytes was used. Hence, the
175 MWCO was selected to be over three-fold higher of the MW of the heaviest target analyte
176 studied here (*i.e.* BDE183; MW= 722) (SpectrumLabs Inc., personal communication). The
177 diffusion of PBDEs across the membrane was aided by the addition of 10 mL of GIT fluid
178 (*i.e.* stomach, small intestine, colon) inside the RC membrane/Tenax TA® system.

179 2.3 Gastro-intestinal Extraction

180 The gastro-intestinal extraction test involved three compartments, namely stomach (1h;
181 pH=2.5), small intestine (SI) (4h; pH=7) and colon (16h; pH=6.5) tested in sequential mode
182 (Fig. 1). Fed CE-PBET conditions were achieved by the addition of dietary components such
183 as mucin, lipid-rich carbohydrates and bile salts into stomach, SI and colon incubations as
184 described in Table SI-3 according to (Tilston et al., 2011) and all media were prepared in
185 deionised H₂O (dH₂O). All experiments were conducted in triplicate. Gut media aliquots (80
186 mL) were added into clean, amber 100 mL Duran[®] glass bottles, sealed with PTFE-lined
187 screw caps and stored at -20°C prior use if necessary. Tenax TA[®] beads were cleaned prior
188 use to remove fine particles by ultrasonication with 40 mL acetone (x2), 40 mL
189 acetone:hexane 1:1 (x2) and 40 mL hexane (x 2) for 10 min in each sonication step. Tenax[®]
190 TA was then allowed to air-dry at 105 °C overnight and was stored in a hexane-washed,
191 Duran[®] bottle inside a desiccator. A short video demonstration of the Tenax TA[®] inclusion
192 in the RC dialysis membrane is available online
193 <https://figshare.com/s/e7312fa7d177b35bc7d0> (video used for demonstration purposes only;
194 the RC membrane is sealed using 19 mm metallic clips; see below). Before employment, the
195 RC dialysis membrane was soaked in ultra-pure H₂O at room temperature for 45 min under
196 continuous stirring to remove any preservatives such as glycerine and sodium azide. The RC
197 membrane was then thoroughly rinsed with dH₂O and one side sealed with a 19 mm hexane-
198 washed, metallic clip. Using a small glass funnel, Tenax TA[®] (0.5 g) was added inside the
199 RC membrane, followed by 10 mL of stomach medium. The tubing was then sealed using
200 another metallic clip. Then, 0.5 g of indoor dust were added in the remaining 70 mL of
201 stomach fluid and the RC membrane/Tenax TA[®] system was introduced to the bottle (Fig
202 1A). A solid-to-liquid (S/L) ratio 1:140 was achieved, thus preventing any bioaccessibility
203 underestimation due to poor dissolution of contaminants from dust (Abdallah et al., 2012;
204 Dean and Ma, 2007). The bottles were placed at 45° angle inside a temperature-controlled
205 waterbath at 37 °C and rotated at 130 rpm for 1h, mimicking the GIT peristaltic movement.
206 After 1 h, the samples were removed from the waterbath and, due to the continuous character
207 of CE-PBET, stomach fluid was converted to small intestine media (SI) by addition of bile
208 salts (0.5 g/L) and pancreatine (1.78 g/L) with pH adjusted to 7 using saturated NaHCO₃. The
209 small intestine incubation continued as above for 4h (Fig 1 B). The stomach medium was
210 converted to small intestine only outside the membrane, given the assumption that bile salts
211 and pancreatine would permeate to the inner barrier of the RC membrane during the 4-h
212 small intestine incubation step in order to reach a pH equilibrium between inside and outside

213 of the RC membrane/Tenax TA[®] system to sustain sorption/desorption by passive diffusion.
214 According to Spectrum Labs Inc. (USA) instructions to users, a first-order permeability rate
215 is observed provided that the RC tubing system is well stirred and the solvent (in our case gut
216 fluid) is changed several times during the dialysis procedure (Spectrum Labs Inc., personal
217 communication). The RC membrane/Tenax TA[®] system was then removed from the bottle
218 and was allowed to sediment for 15 min. Due to its hydrophobic character, Tenax TA[®] floats
219 on top of the small intestine fluid inside the membrane (Fig. SI 1). Tenax TA[®] was trapped
220 on the one side of the membrane, while the other side was carefully unsealed. The small
221 intestine fluid inside the membrane was carefully collected (\approx 8 mL), was subsequently
222 combined with the remaining 70 mL from the incubation and stored at +4 °C prior to liquid-
223 liquid extraction (LLE).

224 The transition between the small intestine and colon compartments was achieved by physical
225 transfer: the dust was recovered from the 70 mL of small intestine media by centrifugation
226 (3500 rpm, 15 min), then added to 70 mL of colon medium. Using the same RC membrane
227 and Tenax TA[®] as in the small intestine compartment, approximately 8 mL of pre-warmed
228 colon medium were added and sealed with the metallic clips as described for the stomach
229 compartment, re-introduced into the bottle where the indoor dust was re-suspended using the
230 colon medium and incubated for 16 h (Fig 1C). At the end of the colon incubation, the dust
231 pellet was recovered by centrifugation as before and stored at -20 °C for extraction. Finally,
232 Tenax TA[®] was recovered using clean cotton wool filtration, the colon fluid was passed
233 through cotton wool, combined with the remaining 70 mL of colon fluid and stored at +4 °C
234 for LLE (Fig SI-2). The cotton wool pieces from filtration together with the Tenax TA[®], the
235 RC membrane and the metallic clippers were collected in one bottle for ultra-sonication
236 assisted extraction. More details on the RC membrane/ Tenax TA[®] system, Tenax TA[®]
237 filtration and recovery are available at Fig. SI 1 and Fig.SI 2, respectively.

238 2.4 Tenax TA[®] sorption capacity

239 **An assessment of PBDE release via the gut and Tenax TA[®] sorption capacity with respect**
240 **to the three CE-PBET compartments was conducted per batch (*i.e.* a single Tenax TA[®]**
241 **sorption experiment was conducted separately relative to the CE-PBET compartment**
242 **and its incubation duration), not in sequential mode (*i.e.* continuous Tenax TA[®] sorption;**
243 **total incubation duration 21 h). Briefly, a fresh Tenax TA[®] sample (0.5 g) was incubated**
244 **using a new RC dialysis membrane before the initiation of each CE-PBET compartment.**
245 **Each Tenax TA[®] sample was finally harvested and subjected to extraction and clean up,**
246 **along with the gut fluids and the residual dust as described in section 2.5.2. 5 Extraction**
247 **and clean up**

248 Before extraction, all samples were spiked with 200 ng of internal standard (ISTD) mix (100
249 μL of 2 ng/ μL) prepared in toluene (BDE77 for BDE28, 47 and 100 and BDE128 for
250 BDE153, 154 and 184 quantifications, respectively) and shaken on an orbital shaker for 1h.
251 Gut fluids were subjected to a LLE using 30 mL hexane/ethyl acetate 3:1 v/v twice (Fig. SI2
252 – step 1). Two mL of acetone were added to enhance separation, when necessary. A gel-like
253 emulsion bilayer (mainly lipid and carbohydrates) was developed, especially in the colon
254 compartment. Oven-baked Na_2SO_4 (400 °C; powder) was added in the combined LLE
255 extracts to absorb all remaining water residues and dissolve the gel-like emulsion. All
256 samples were then allowed to settle for 1h at room temperature and the extracts were
257 collected by centrifugation (3500 rpm, 15 min). The residual dust and the recovered Tenax
258 TA[®] beads (together with the glass wool and the metallic clips) were subjected to ultra-
259 sonication assisted extraction for 15 min using 30 mL acetone/hexane 1:3 v/v twice (Fig. SI-2
260 – step 2 & 3). After each step, the extracts were collected by centrifugation (3500 rpm, 15
261 min). All extracts collected from each step were combined, evaporated to 1mL hexane using
262 Syncore[®] Analyst evaporator (Buchi, Switzerland) and then loaded onto Florisil[®] cartridges
263 (2 g, 6 mL), using a slightly modified method published elsewhere (Van den Eede et al.,
264 2012) (Fig. SI 2 – step 4). Briefly, Florisil[®] cartridges were pre-cleaned with 10 mL ethyl
265 acetate and 6 mL of hexane; our target analytes were eluted using 20 mL hexane. This eluate
266 was further concentrated to 1mL (in hexane) and then subjected to SPE clean-up on 5%
267 acidified silica (5% AS) (2 g, 6 mL). The 5% AS cartridges were pre-cleaned with 6 mL
268 hexane and 3 mL dichloromethane and then all extracts from the Florisil[®] step were loaded
269 onto the SPE silica column. Our target analytes were eluted using 16 mL hexane and 8 mL
270 dichloromethane and after collection, all eluates were concentrated near dryness under a
271 gentle stream of N_2 , reconstituted in 100 μL of toluene and then filtered using a micro

272 centrifuge filter lined with 0.45 µm pore size nylon filter (1.5 mL volume capacity) . Finally,
273 the samples were transferred to injection vials, biphenyl (40 ng) was added as an injection
274 recovery standard and analysed by GC-EI/MS. Further details about instrumental analysis are
275 available at SI.

276 **2.6 Data analysis**

277 Bioaccessibility can be expressed as a mass (*e.g.* ng of a contaminant solubilised in the GI
278 tract), a concentration (ng/g of a contaminant in dust) or as a fraction expressed in percentage
279 (BAF%) (Guney and Zagury, 2016). In our study, bioaccessibility was determined according
280 to (García-Alcega et al., 2016) using Eq. 1, where mass FR (SI+colon+Tenax TA[®]) is set as
281 the sum of FR mass (ng) determined in small intestine (SI), colon and Tenax TA[®]
282 compartments of CE-PBET system and mass FR (dust residual) is the FR mass (ng)
283 determined in the dust residual collected after 16h-incubation of CE-PBET colon
284 compartment which is considered as the non-bioaccessible fraction.

286 *Bioaccessibility* % (BAF%)

$$287 = \frac{\text{mass FR (SI + Colon + Tenax TA}^{\text{®}})}{\text{mass FR (SI + Colon + Tenax}^{\text{®}}) + \text{mass FR (dust residual)}} \times 100$$

285 (Eq.1)

288 GraphPad Prism[®] version 7.04 for Windows (GraphPad Software, La Jolla CA, USA) was
289 used for statistical analysis. Prior to statistical analysis, all BAF% were converted into
290 fractions and arc-sine transformed. This mathematical transformation is necessary for
291 statistical analysis of results set in percentages in order to equalise variances among
292 treatments (Sokal and Rohlf, 1995). Multiple t-tests (unpaired; p<0.05) were performed to
293 assess statistically significant differences among the different Tenax TA[®] amounts added
294 (sections 3.1 and 3.2), whereas ordinary two-way ANOVA (Uncorrected Fisher's test,
295 p<0.05) was performed to assess statistical differences for bioaccessibility with and without
296 the addition of Tenax TA[®] in SRM 2585 method validation (section 3.3).

297 **2.7 Quality assurance and quality control**

298 All samples were analysed in triplicate together with oven-baked, laboratory-grade sand
299 (procedural blank) and SRM 2585 (n=3, NIST, USA) was used for method validation and QC
300 testing. Concentrations of our target analytes in method blanks were all below method limit
301 of detection (mLOD) (0.05 ng/µL). RC membrane and Tenax TA[®] blanks were extracted for

FR background contamination prior use and all values were found below mLOD. No weight correction on bioaccessibility values with respect to potential Tenax TA[®] mass losses was employed in our study. According to the ISO 16751 method on organic pollutant bioavailability (2015), correction for such losses is recommended by air drying and weighing the dry amount of Tenax TA[®] after extraction (ISO, 2015). In our study separating Tenax TA[®] beads from the glass wool and the RC membrane post extraction was not feasible due to the character of Tenax TA[®] to adhere in any surface it comes in contact with during filtration (e.g. glass wool). Extraction efficiency (%) was assessed for SI, colon, Tenax TA[®] and residual dust compartments by spiking experiments (see SI Table 2). Briefly, 100 ng of native PBDEs (100 µL of 1 ng/µL) in iso-octane were spiked to SI and colon media, Tenax TA[®] (0.5 g) and dust (0.5 g). All samples were shaken on an orbital shaker for 1h. Finally, 30 mL of the corresponding extraction medium was added in each compartment, following the same sample preparation processes as before. Finally, biphenyl (40ng) was added as an injection recovery standard and the samples were analysed by GC-EI/MS. Extraction efficiency values for all target analytes were >60% in all CE-PBET compartments, except BDE100 efficiency which was 52% and 54% in Tenax TA[®] and residual dust, respectively. Such phenomena may be attributed to potential mass losses of Tenax TA[®] during glass wool filtration steps. Despite the moderately lower extraction efficiency for BDE100 in comparison to the other target analytes, the relative standard deviation (RSD%) of the method for BDE100 was 6%. Given the low deviation and variability, no correction was performed for BDE100 (Table SI 3). Glass test tubes were cleaned by soaking for at least 12 h in an alkali solution. After washing, the tubes were rinsed with water and dried at 100 °C for at least 12 h and burnt at 400°C to remove all traces of contamination.

3. Results and discussion

3.1 Tenax TA[®] optimisation

The addition of Tenax TA[®] in CE-PBET considerably increased the bioaccessible fraction (%BAF) of all target analytes, illustrating the value of Tenax TA[®] as an adsorbent matrix for HOCs. Different masses of Tenax TA[®] were added to the CE-PBET system to optimise the adsorbent sink to ensure exhaustive FR desorption from indoor dust. PBDE-spiked indoor dust samples (n=3) were tested under four different conditions; (A) without any Tenax TA[®] addition (control) and with three different amounts of Tenax TA[®], namely 0.25 g (B), 0.5 g (C) and 0.75 g (D). The same length of RC dialysis membrane (16cm) and mass of dust (0.5

334 g) was used in all treatments. Our results show that Tenax TA[®] enhanced gut bioaccessibility
335 for PBDEs by approximately two-fold (Fig. 2) and the bioaccessible fraction was
336 significantly different ($p < 0.001$) between the controls (no Tenax) and with Tenax TA[®]
337 addition, for all target analytes (Fig. 2). For example, with no Tenax TA[®] (control), the
338 bioaccessible fraction of the low brominated PBDEs, BDE28 and BDE47, was 37.7% and
339 32.8%, respectively, whereas their BAF% increased with 0.25 g Tenax TA[®] inclusion to
340 55.1% and 54.9%, respectively. A trend to decreasing BAF% with increasing degrees of
341 bromination for PBDEs can be seen for the control treatments and the different amounts of
342 Tenax (Fig 2). Such findings are in agreement with Fang and Stapleton (2014), where a
343 negative relationship between gut bioaccessibility and PBDE physicochemical properties
344 such as degrees of bromination, MW and log K_{ow} was described (Fang and Stapleton, 2014).
345 Few studies describe the influence of Tenax TA[®] inclusion on gut bioaccessibility of organic
346 pollutants from solid matrices such as indoor dust or soil. CE-PBET and Tenax TA[®] were
347 employed to assess FR gut bioaccessibility and for a wide range of low and high MW FRs
348 present in indoor dust including BDE47, BDE100 and BDE183; in their experimental design,
349 Fang and Stapleton (2014) used 0.5 g of Tenax as an absorptive sink but the effects of
350 varying Tenax TA[®] content were not reported (Fang and Stapleton, 2014). In a study
351 assessing PAHs bioaccessibility in soils from China, 0.25 g of Tenax TA[®] were added into a
352 PBET *in vitro* system (Li et al., 2015). According to Li et al (2015), this mass was five-fold
353 higher than the small intestine organic matter (OC), thus allowing sufficient sorption capacity
354 for the PAHs mobilized during their study (Li et al., 2015). Zhang et al (2017) reported fast
355 and efficient sorption only for high MW PAHs (*i.e.* 3 -5 benzene ring) using 0.1 g of Tenax
356 TA[®] studying PAH soil bioaccessibility; poor extraction efficiencies were noted for volatile
357 PAHs such as naphthalene, acenaphthylene and acenaphthene, possibly as a result of an air
358 drying step during Tenax TA[®] collection and separation from the gut fluid (Zhang et al.,
359 2017). Varying the content of Tenax TA[®] (0.25, 0.5 and 0.75 g) in the CE-PBET system
360 studied here, showed few statistically significant differences for our analyte recoveries. Here,
361 statistically significant differences among the three Tenax TA[®] amounts tested were found
362 only for BDE28 bioaccessibility as an exception; some increase in BDE28 BAF% with Tenax
363 TA[®] content, rising from 55.1% with 0.25 g Tenax TA[®] to 66.7% with 0.5 g (0.25 g vs 0.5 g;
364 $p = 0.017$) and 69.9% with 0.75 g Tenax TA[®] added (0.25 g vs 0.5 g; $p = 0.006$) was observed.
365 These results reflect the physicochemical properties of this FR as a low MW tri-BDE
366 congener; Tenax TA[®] is a hydrophobic sink and the calculated log K_{ow} (EpiWeb) shows that
367 BDE28 (log K_{ow} 5.88) is less hydrophobic than BDE47 (log K_{ow} 6.77) and hence greater

368 amounts of the adsorbent may be needed to capture all of the released BDE28. For all other
369 analytes, there were no statistically significant differences in BAF% among the varying
370 Tenax TA[®] amounts tested. In other words, for BDE28 being the least hydrophobic of our
371 target analyte list, we propose that Tenax TA mass loading greater than 0.25 g is required in
372 order to maximise the sorption potential of low MW PBDEs such as BDE28 to Tenax TA[®].
373 Given the a) high sorption capacity of Tenax TA[®], b) the broad range of physical properties
374 (MW, water solubility and log K_{ow}) of our FRs mobilised from the ingested matrix and c) the
375 relatively high Tenax TA[®] mass recovery (SI Fig 3), 0.5 g of Tenax TA[®] were selected and
376 subsequently used below. Our results show that in order to maintain a constant sorptive
377 gradient for the low MW PBDEs, a larger mass of Tenax TA[®] is required, since 0.25 g of
378 Tenax TA[®] was not enough to sustain an exhaustive *in vitro* gut extraction for all target
379 analytes.

380 **3.2 Tenax TA[®] sorption capacity to PBDEs in CEPBET components**

381

382 Here the influence of the gut media on Tenax sorption was being tested. Each compartment
383 (i.e. stomach, small intestine colon) was tested independently so Eq. 1 was not suitable for
384 calculating PBDE sorption capacity on the different Tenax TA[®] batches. Hence, Eq. 1 was to
385 determine the Tenax[®] loadings as fractions of the total concentration in each CE-PBET
386 compartment PBDE sorption capacity (%) was determined using equation 2 (Eq. 2), where
387 mass FR in Tenax TA[®] is the FR mass (ng) determined in each Tenax TA[®] sample for each
388 CE-PBET compartment and mass FR in compartment is FR mass (ng) determined in CE-
389 PBET gut fluids separately.

$$390 \text{ Tenax Sorption (\%)} = \frac{\text{mass FR in Tenax TA}^{\text{®}}}{\text{mass FR in Tenax TA}^{\text{®}} + \text{mass FR in compartment}} \times 100 \text{ (Eq. 2)}$$

391 Shown in figure 3 are the results from PBDEs sorption to Tenax TA[®] in the three different
392 CE-PBET compartments with respect to their incubation step. PBDE sorption to Tenax TA[®]
393 results should not be considered as total PBDE bioaccessibility, but as the component
394 attributable to Tenax TA[®] as an absorptive sink. Within the stomach compartment, BDE28
395 and BDE47 presented higher sorption on Tenax TA[®] (43.7 % and 25.6%, respectively)
396 compared to PBDEs with higher bromine content such as BDE154 and BDE183 where Tenax
397 TA[®] sorption ranged from 7.0 % to 8.8 %, respectively. Comparing stomach and colon
398 absorption, statistically significant relationships (p<0.01) were noted for all target analytes,
399 apart from BDE28 and BDE47 (p>0.05). Fundamental differences between stomach and

400 colon media formulae, ingredient concentrations (Table SI 3) and incubation times can be
401 considered as the driving factors for the interpretation of such results. Small intestine
402 absorption to Tenax TA[®] was similar to the colon for BDE28 (66.2 % and 60.0 %, respectively,
403 whereas it was found repeatedly lower than the colon for all the other target
404 analytes (Fig. 3) without any considerable differences. Tenax TA[®] sorption in the colon was
405 higher than SI overall, but was not statistically significant for individual compounds
406 except BDE183 sorption on Tenax TA[®] which was nearly two-fold higher in the colon in
407 comparison to small intestine (52.6 % and 36.1 %, respectively, $p=0.045$). We believe that
408 such findings are influenced by the addition of food components and bile salts as surfactants
409 to the small intestine and colon compartments (Table SI 3). Such biological phenomena are
410 able to enhance FR solubility and desorption potential from the dust to the gut (*i.e.* more
411 released and freely available FR in the gut fluids), promoting thus higher FR mobilisation and
412 sorption onto the Tenax TA sink ([Oomen et al., 2004; Zhang et al., 2015](#)). Compared to the
413 small intestine, incubation times and the concentration of compounds enhancing desorption
414 (e.g. mucin) are higher in the colon. All these factors combine to increase the release from the
415 dust that a higher concentration of PBDE is in solution and hence available for subsequent
416 sorption onto Tenax TA[®]. Hence, both the “solvent” capacity of the medium and the “sink”
417 capacity of the Tenax TA[®] are required to achieve optimum extraction of FRs from dust as a
418 matrix. Besides Tenax TA[®], our results further support the idea of dietary components
419 addition in CE-PBET acting as additional mechanism enhancing FR mobilisation, especially
420 in the lipid-rich colon compartment as reported by (Tilston et al., 2011).

421 3.3 Method performance using SRM 2585

422 The selected CE-PBET parameters as well as the overall performance of our new method
423 were assessed using SRM 2585 serving as a well-characterised and homogenous dust sample.
424 PBDE bioaccessibility was studied using a) CE-PBET without the Tenax TA[®] adsorption
425 sink, b) CE-PBET with the addition of 0.5 g of Tenax TA[®] and c) PBDE-spiked SRM 2585
426 (100 ng spike) to evaluate greater FR contamination levels under environmentally realistic
427 conditions using SRM 2585 as the same homogenous dust sample. As observed for BAF%
428 using a dust sample from Reading (section 3.1), statistically significant differences ($p=0.03$)
429 were found in %BAF% for all target analytes when comparing CE-PBET without Tenax TA[®]
430 addition (Fig. 4 A) and with Tenax TA[®] addition (Fig 4 B & C). The BAF% when Tenax
431 TA[®] was used as an adsorption sink rose between approximately two-fold (BDE153 and
432 BDE183) with greater increases seen for the low-brominated and less hydrophobic FRs such

433 as BDE28 and BDE47 (nearly 3-fold bioaccessibility increases, respectively) (Fig. 4 B & C).
434 No statistically significant effect ($p>0.05$) was found between the two SRM 2585 treatments
435 (spiked and non-spiked) which both included 0.5 g of Tenax TA[®] and different FR
436 contamination levels did not present any considerably different bioaccessibility values from
437 the same dust matrix (Fig. 4 B & C). Finally, compared to the control treatments (*i.e.* no
438 Tenax TA inclusion), the performance of the novel CE-PBET method described here using
439 SRM 2585 offers two to three-fold gut bioaccessibility increase for a wide range of PBDEs
440 with diverse physicochemical profiles, following a similar pattern to the indoor dust tested in
441 section 3.1.

442 **3.4 Proposing a unified test approach**

443 This study describes an efficient method to physically separate Tenax TA[®] as an absorbent
444 sink and indoor dust for *in vitro* bioaccessibility testing, and our model allows assessment of
445 FRs (and potentially other HOCs) bioaccessibility from a solid matrix using artificial gastro-
446 intestinal fluids. Previous methods used a self-designed stainless steel sieve to separate and
447 recover Tenax TA[®] beads (Fang and Stapleton, 2014; ISO, 2015; Li et al., 2015, 2016). Our
448 approach, using RC dialysis tubing provides some important benefits. Dialysis tubing is
449 readily available, reproducible (quality controlled) and can be sourced with a wide range of
450 molecular weight cut offs. This allows investigators to select a membrane with a MW cut off
451 sufficient to permit free diffusion of the analytes of interest, whilst restricting passage of
452 larger macromolecules such as enzymes or proteins that may be added to simulated GI fluids.
453 By restricting the passage of these unwanted materials, the sorption capacity of the Tenax
454 TA[®] is predominantly used for the organic pollutants rather than media components and clean
455 up and desorption is thus simplified. The tubing functions effectively to physically separate
456 the Tenax TA[®] from the solid matrix (dust) and has high pH and temperature tolerance. Our
457 study also shows the benefits of using an adsorption sink in the CE-PBET system. Compared
458 to controls with no Tenax TA[®], inclusion of the resin increased gut bioaccessibility for
459 PBDEs with diverse physicochemical profiles. For the low brominated BDE28, 0.25 g of
460 Tenax TA[®] were insufficient for exhaustive *in vitro* gut absorption, illustrating that the
461 amount of Tenax TA[®] added to the modified CE-PBET system should be optimized with
462 respect to the physicochemical properties (e.g. LogK_{ow} , water solubility) of the target
463 analytes tested. Other than BDE28, for the (hydrophobic) FR's studied here, 0.5 g of Tenax
464 TA[®] was shown to be an appropriate amount to add in order to ensure released pollutants
465 were readily adsorbed. A proposed rule can be a Tenax TA[®] mass loading of 0.5 g for

466 organic compounds with $\text{LogK}_{\text{ow}} < 6$ (e.g. BDE28, low MW PAHs etc.), while 0.25 g of
467 Tenax TA[®] mass loading can be employed for very lipophilic compounds such as penta- and
468 octa- BDEs ($\text{LogK}_{\text{ow}} > 6$).

469 **3.5. Future work**

470 Given the assumption that first-order permeability rates can be obtained provided that the RC
471 tubing system is well stirred and the gut fluid is changed several times during the dialysis
472 procedure, no kinetic characterisation was conducted in the present study. However, we
473 believe that further kinetic characterisation of the diffusion rates of the RC membrane system
474 should be encouraged and explored in the future. Additionally, the proposed unified gut
475 bioaccessibility method should be further examined against different matrices (e.g. soil) and
476 groups of emerging organic pollutants with diverse physicochemical properties. Fang and
477 Stapleton (2014) proposed their method to be employed for flame retardants with $\text{log Kow} > 5$
478 using 0.5 g of Tenax TA[®] mass loading in the test settings. Additionally, the ISO method on
479 bioavailability was designed for non-polar organic compounds with $\text{log Kow} > 3$ (ISO, 2015).
480 Potentially, our unified method on *in vitro* gut bioaccessibility could be proposed to a wide
481 range of organic pollutants and a rule of Tenax TA[®] mass loading could be established with
482 respect to a pollutant's log Kow and water solubility values, e.g. 0.25 g of Tenax TA[®] mass
483 loading should be used when testing for compounds with log Kow greater than 6 (*i.e.* more
484 hydrophobic), whereas 0.5 g of Tenax TA[®] mass should be employed for organic compounds
485 with log Kow lower than 6. Given the infinite sink inclusion, bioaccessibility parameters
486 including a reduction of the S/L ratio could be potentially explored on the basis of mass
487 transfer from the outside to inside of the membrane being quicker since the concentration
488 outside would reach earlier the solubility limit.

489 **3.6 Conclusion**

490 Under the influence of the ISO 16751 method on the environmental availability of non-polar
491 compounds being currently approved for registration, we propose a novel test format for
492 assessing *in vitro* bioaccessibility of PBDEs with Tenax TA[®] addition as an adsorptive sink.
493 Our data also show that the existing default assessment of risk (*i.e.* all the ingested pollutant
494 in a solid matrix being bioavailable) is an overestimate and that the BAF% varies between
495 ~60% (BDE47) and ~50% (BDE153). This study reveals that colon sorption to Tenax TA[®]
496 for low MW BDEs was similar compared to small intestine sorption for BDE28, unlike other
497 more hydrophobic PBDEs where colon sorption was higher than small intestine sorption.

498 Well designed *in vitro* bioaccessibility tests thus provide a simple approach for initial human
499 risk assessments from ingested solid matrices giving a conservative, yet realistic indication of
500 risk.

501 **Supporting Information**

502 Further details on chemicals and reagents, sample preparation and instrumental analysis are
503 provided at supporting information.

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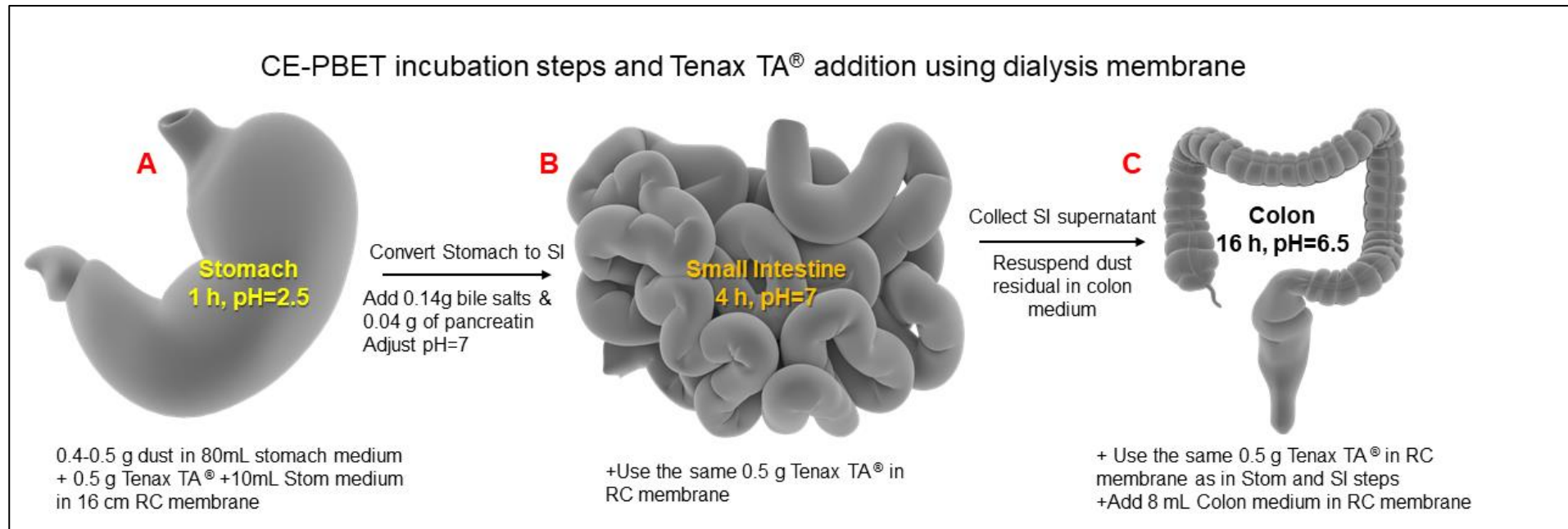
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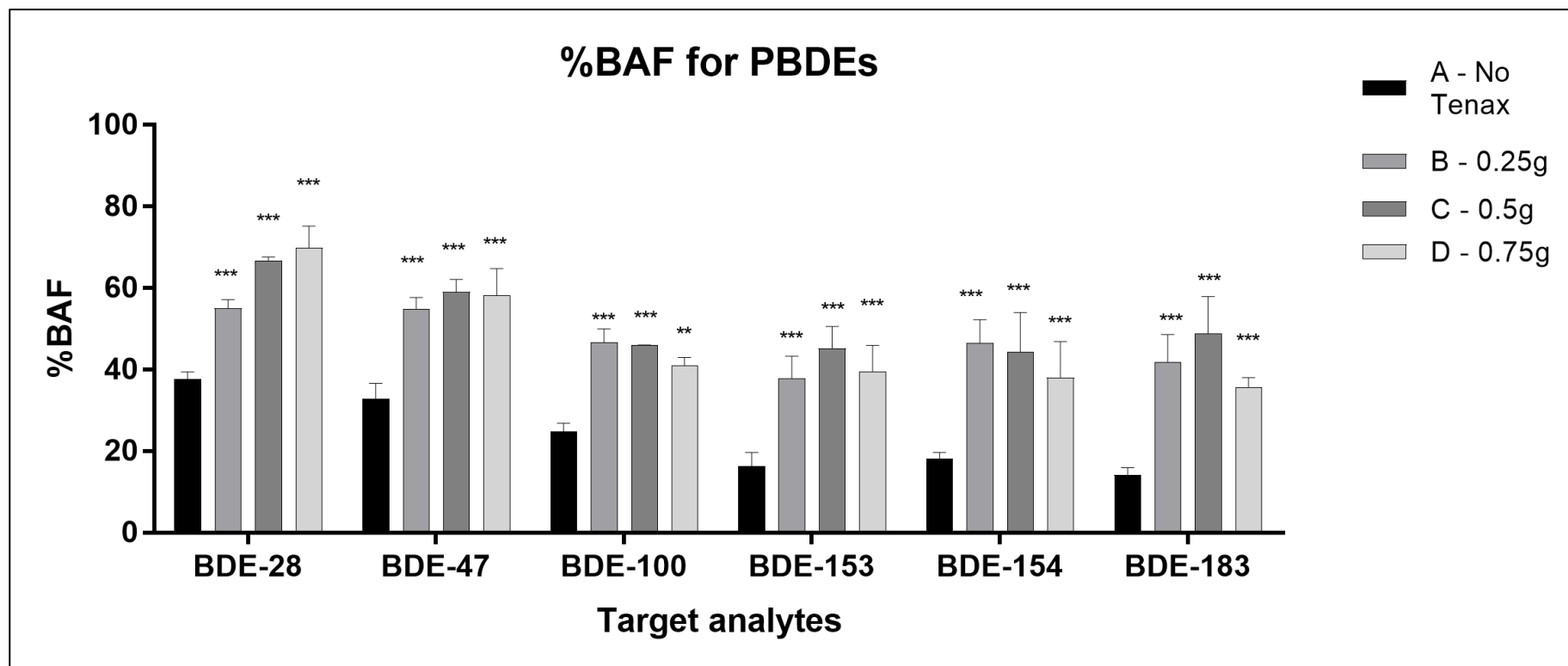
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Artwork and Tables with Captions



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699 **Figure 1** – Schematic representation of CE-PBET gut compartments and parameters (*i.e.* stomach (1 h, pH = 2.5), small intestine (SI) (4 h, pH = 7)
700 and colon (16 h, pH = 6.5)) using 0.5 g Tenax TA[®] added in 16 cm of RC dialysis membrane.



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702 **Figure 2** – CE-PBET bioaccessibility fraction (%BAF) of PBDEs without any Tenax TA[®] addition (control, A) and CE-PBET with Tenax TA[®]
 703 addition in three different amounts; i.e. 0.25 g (B), 0.5 g (C) and 0.75 g (D). Statistically significant differences shown here (**; p<0.01 and ***,
 704 p<0.001) were established between the control (A) and all Tenax TA[®] treatments (B, C, D). Bar charts represent average values of triplicates. Error
 705 bars represent one standard deviation.

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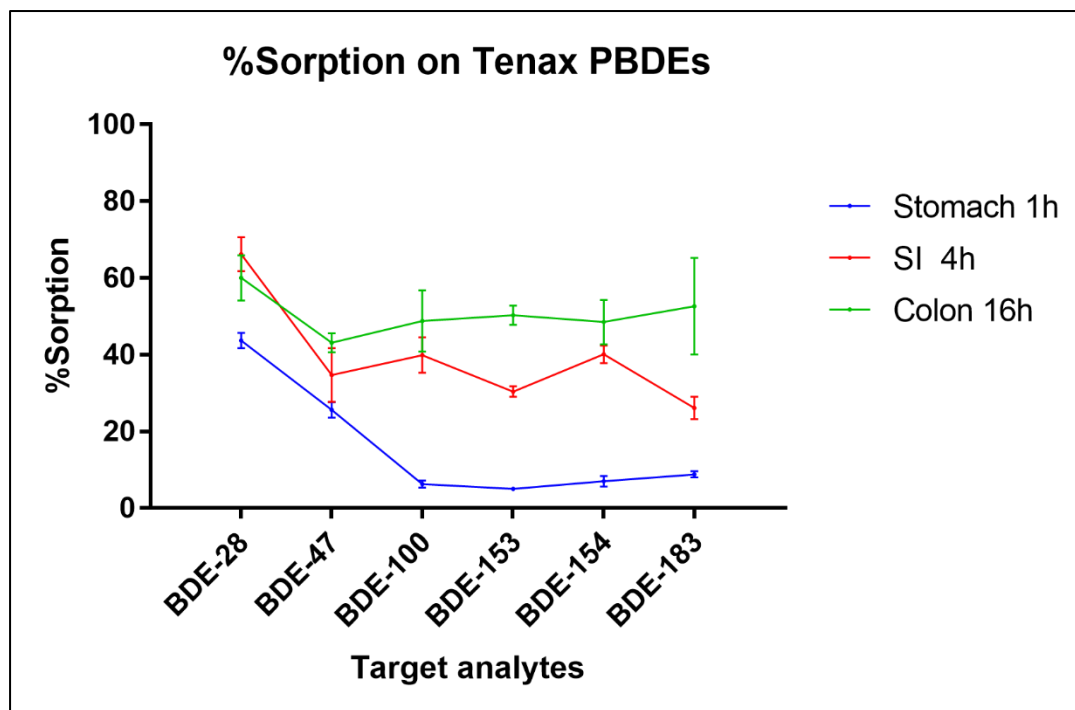
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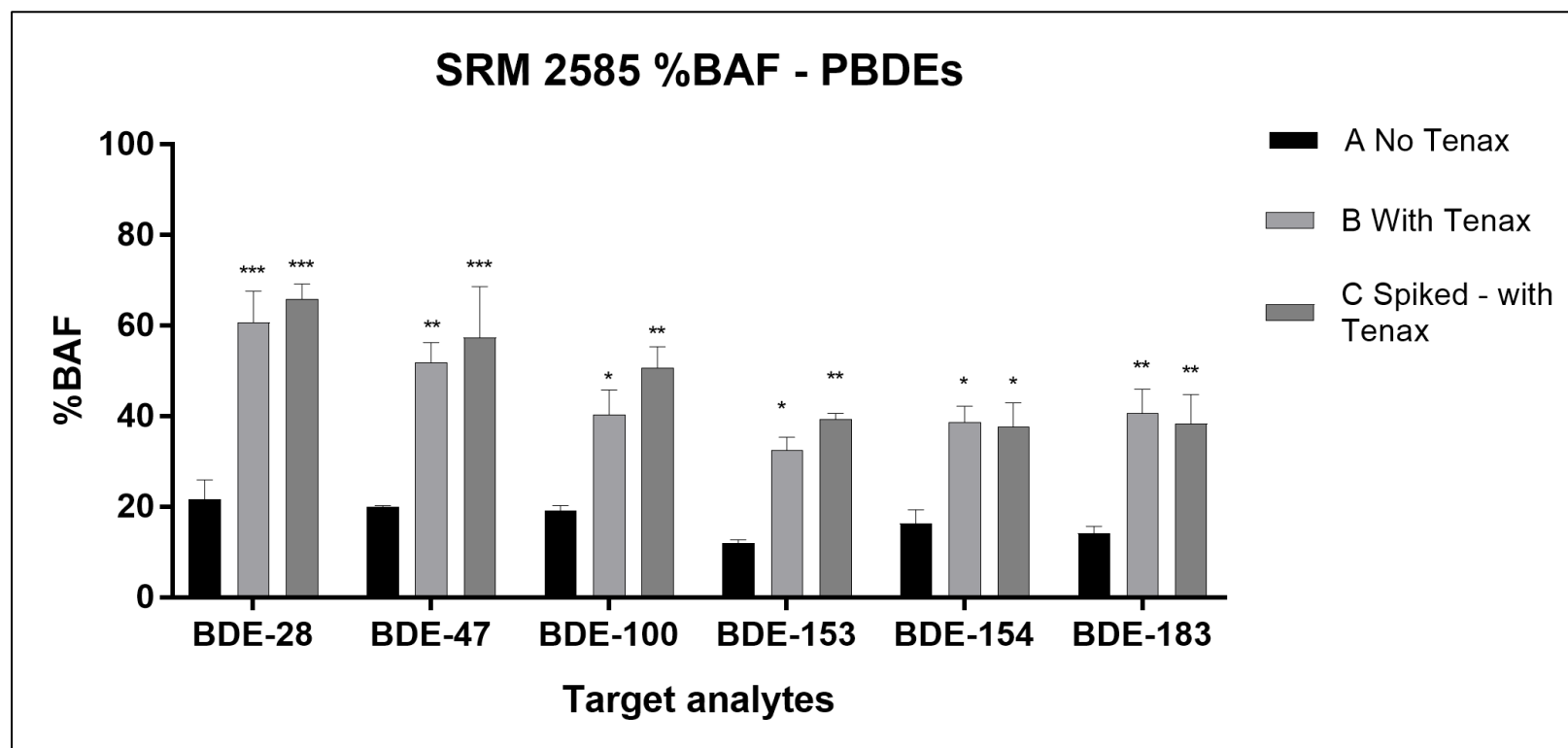
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713 **Figure 3** – Line plots presenting FR sorption on Tenax TA[®] separately in stomach (1h), small intestine (SI; 4h) and colon (16h) compartments. Line plots
 714 represent average values of triplicates. Error bars represent one standard deviation.

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718 **Figure 4 – Method performance of CE-PBET and bioaccessibility fraction (%BAF) using SRM 2585 without Tenax TA[®] inclusion (control; A), with Tenax**
 719 **TA[®] inclusion (B) and artificially spiked SRM 2585 and Tenax TA[®] inclusion (C). Statistically significant differences shown here (*; p<0.05, **; p<0.01 and**
 720 *****; p<0.001) were established between control treatments of SRM 2585 without Tenax TA[®] inclusion (A) and treatments B and C with Tenax TA[®] inclusion .**
 721 **Bar charts represent average values of triplicates. Error bars represent one standard deviation.**

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