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

Supporting information

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

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Materials and methods

Chemicals and reagents

Native standard solutions of BDE 28, 47, 77, 100,128, 153, 154, and 183 were purchased from Cambridge Isotope laboratories Inc. (UK). Purity of all standards was >98% unless otherwise stated. Standard stock solutions were prepared in toluene for all compounds. Sodium sulphate (anhydrous, granular/powder, 99% pure), high purity grade Silica gel pore size 60 Å, 70-230 mesh, 63-200 µm (product code: #60741, Sigma-Aldrich), Florisil® 100-200 mesh (product code: #10104980, Acros Organics), concentrated Sulfuric acid (H_2SO_4) 96% analytical grade (Fisher Scientific, UK), Tenax® TA Porous Polymer Adsorbent, 60-80 mesh (product code: #11982, Sigma-Aldrich**),** Standard grade regenerated cellulose (RC) Spectra/Por™ 3 (18mm flat width, 1.1mL/cm dialysis membrane MWCO 3.5 kDa) (Spectrum Labs Inc., USA, product code: #11425859; FisherScientific, UK), micro centrifuge filters lined with 0.45µm pore size nylon filter 1.5mL volume capacity (product code #516-0236, VWR) and 19mm Small Silver Binder Clips (product code: #WW-376137, Staples Inc, UK.). Analytical grade inorganic salts were provided from Fisher Scientific (Loughborough, UK). All biological reagents used for media preparation and organic solvents used for extraction and clean-up steps were of HPLC grade and were obtained from Sigma-Aldrich (Gillingham, UK). Empty, pre-fritted polypropylene filtration tubes (6 mL) for silica SPE and Florisil cartridge preparation (2 g, 6 mL) were purchased from Sigma-Aldrich (UK). For 5% acidified silica gel preparation, concentrated sulphuric acid (H_2SO_4, >96%) was used and was purchased from Fisher Scientific. Briefly, 1.9 mL of pure sulphuric acid was added drop-wise to 50 g of hexane-washed, oven-dried silica gel under continuous and vigorous stirring. Glass test tubes were cleaned by soaking for at least 12 h in a phosphate-free, alkali solution. After washing, the tubes were rinsed with deionised water, dried at 100 ºC for at least 12 h and burned at 400°C to remove all traces of organic contamination

Target analytes and analytical characteristics

Table SI 1 – Target analytes and physicochemical properties calculated from EPISuite 4.1™.

Abbreviation	Full name	Molecular formula	MW (Da)	Log K_{ow}	Water solubility (mg/L) 25 ºC	Vapour pressure (mm Hg, 25 ºC)
BDE-28	2,4,4'-Tribromodiphenyl ether	C12 H7 Br3 O1	406.895	5.88	0.02642	9.16E-06
BDE-47	2,2',4,4'-Tetrabromodiphenyl ether	C12 H6 Br4 O1	485.791	6.77	0.001461	1.58E-06

BDE-100	2,2',4,4',6-Pentabromodiphenyl ether	C12 H5 Br5 O1	564.688	6.84	0.000394	3.10E-08
BDE-153	2,2',4,4',5,5'-Hexabromodiphenyl ether	C12 H4 Br6 O1	643.584	8.55	4.15E-06	1.86E-07
BDE-154	2,2',4,4',5,6'-Hexabromodiphenyl ether	C12 H4 Br6 O1	643.584	8.55	4.15E-06	1.41E-07
BDE-183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	C12 H3 Br7 O1	722.48	9.44	2.16E-07	2.45E-08

Table SI 2 Concentrations in dust samples and analytical confirmation of dust and SRM 2585 concentrations after spiking with PBDEs (200 µL of PBDEs native standard mix 1 ng/µL). All concentration values are in ng/g.

Target analyte	*Concentration in UK dust before spiking	Concentration in UK dust after spiking	Expected concentration after spiking	§Recovery %	
BDE-28	11.4	186.1	211.4	88.0	
BDE-47	9.0	190.8	209.0	91.3	
BDE-100	2.5	188.1	202.5	92.9	
BDE-153	5.2	225.6	205.2	109.9	
BDE-154	2.2	178.4	202.2	88.2	
BDE-183	29.3	179.4	229.3	78.2	
Target analyte	*Concentration in SRM 2585	Concentration in SRM 2585 after spiking	Expected concentration after spiking	§Recovery %	*Ref value
BDE-28	15.3	190.3	215.3	88.4	46.9
BDE-47	446.5	589.2	646.5	91.1	497
BDE-100	35.8	188.1	235.8	79.8	43.8
BDE-153	137.5	363.8	337.5	107.8	119
BDE-154	99	264.7	299.0	88.5	83.5
BDE-183	52.5	204.6	252.5	81.0	43

*Taken from (Kademoglou et al., 2017)

§ Recovery %= (Concentration in dust after PBDE spiking / Expected concentration) x 100

Table SI 3 - Composition of 1 litre of media used in the fed CEPBET test. Fed state conditions achieved by addition of dietary components in stomach and colon media. From (Tilston et al., 2011)

Stomach, 1h, pH=2.5	
Reagent	Amount added in 1 L
Sodium malate (maleic acid)	0.5 g
Tri-sodium citrate	0.5 g
Lactic acid 85% w/w	420 µL
Acetic acid (glacial)*	500 µL
Pepsin (porcine)	1.25 g
Small Intestine, 4h, pH=7	
Bile salts	1.78 g
Pancreatine (porcine)	0.5 g
Colon, 16h, pH=6.5	
Mucin type II (porcine stomach)	4.0 g
Sodium chloride	4.5 g
Potassium chloride	4.5 g
Sodium bicarbonate	1.5 g
Magnesium sulphate hexahydrate	1.25 g
L-Cysteine Hydrochloride	800 mg
Potassium phosphate	500 mg
di-potassium phosphate	500 mg
Bile salts	400 mg
Calcium Chloride	189.0 mg
Haemin (>80%, bovine)	500 mg

Iron (II) sulphate heptahydrate	5.0 mg
Dietary components	
Starch (potato)	5.0 g
Peptone (casein)	34 g
Tryptone (vegetable)	6.1 g
Yeast extract	4.5 g
Casein	3.0 g
Pectin (citrus)	2.0 g
Xylan (beechwood)	2.0 g
Arabinogalactan (larch)	2.0 g
Guar gum	1.0 g
Inulin (chicory)	1.0 g

Instrumental analysis

A Thermo Trace GC Ultra system equipped with a Thermo TG-SQC capillary column (15 m x 0.25mm x 0.25 μm) coupled to a Thermo ITQ 1100 mass spectrometer in electron ionisation mode ((EI-MS) was connected through a heated transfer line (300ºC). The injection temperature was set at 92 °C, hold 0.04 min, ramp 700 °C/min to 295 °C and 5μL of cleaned extracts in toluene were injected for GC analysis. Injection was performed under a pressure of 0.19 bar until 1.25 min in pulsed splitlless mode 50 mL/min after 1.25 min. The GC temperature program was 90 °C, hold 1.50 min, ramp 10°C/min to 300°C, hold 3 min, ramp 40 °C/min to 310 °C, hold 5 min. Helium was used as a carrier gas with a flow rate of 1.0 mL/min.

RC membrane /Tenax TA® system

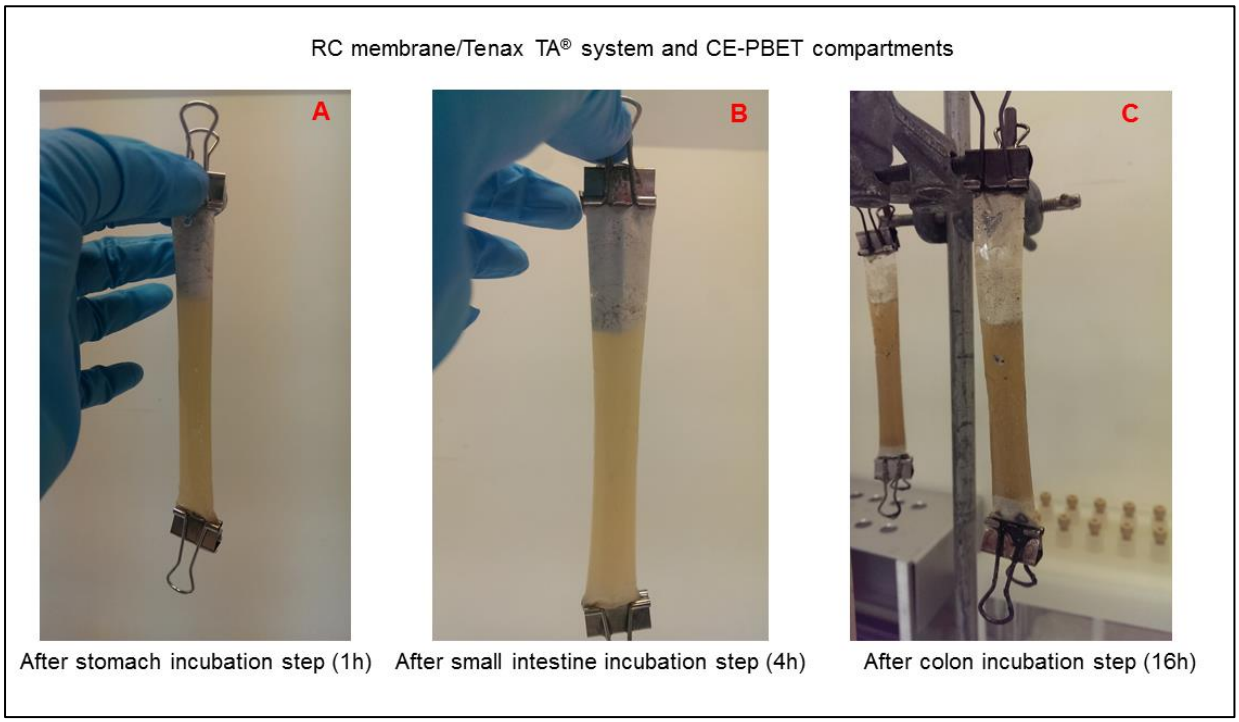
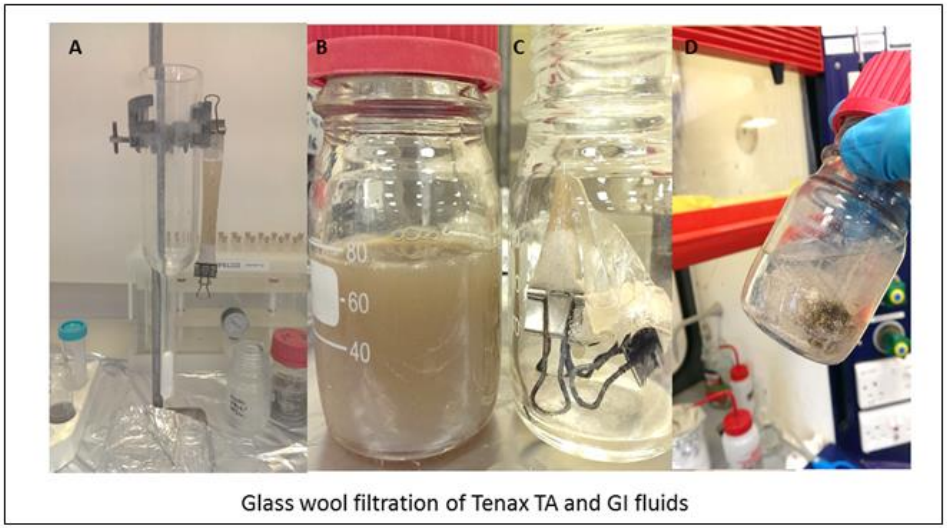


Figure SI 1– Stepwise representation of RC membrane /Tenax TA system after incubation of each CE-PBET compartments, namely (A) stomach, (B) small intestine and (C) colon. Please note the unsaturated Tenax TA floating on top of the water based gut medium (A &B), while the saturated part sediments after the end of colon incubation (C).

Sample preparation and Tenax TA® recovery



Glass wool filtration of Tenax TA and GI fluids

Step1: SI and colon fluids

- Collect SI and Colon fluids (spin 15 min at 3500rpm)
- Spike 200ng ISTDs
- Add 30mL Hex/EtOAc 1:3 (x2)
- R'n'R shake for 1h
- Subject for LLE (x2)
- Collect extracts + add Na_2SO_4
- Spin 15 min at 3500rpm
- Collect organic phase
- Subject to Florisil® fractionation & SPE clean-up (F1 only)

Step 2: Tenax TA® recovery

- After SI incubation, filter SI fluids using glass wool
- After colon incubation, filter Tenax TA® from RC membrane using glass wool filtration
- Collect glass wool filters from SI and colon fluid filtration in one bottle
- Chop RC membrane in 4 smaller pieces
- Add the 19mm metallic clippers for extraction as well

Step 3: Tenax TA® and residual dust

- Spike 200ng ISTDs
- Add 30mL Ace/Hex1:3 (x2)
- R'n'R shake for 1h
- Subject for ultrasonication (x2)
- Collect extracts + add Na_2SO_4
- Spin 15 min at 3500rpm
- Collect organic phase
- Subject to Florisil® fractionation & SPE clean-up (F1 only)

Step 4: Before Florisil ® fractionation

- Collect all extracts (approx 50-60mL each) from SI, colon, Tenax TA® and residual dust
- Concentrate to 1mL using a BUCHI Syncore® evaporator
- Extracts ready (1mL in hexane) for Florisil® fractionation & SPE clean-up (F1 only)

Figure SI 2 – Schematic representation of sample preparation of CE-PBET fluids and residual dust, as well as Tenax TA recovery using glass wool filtration

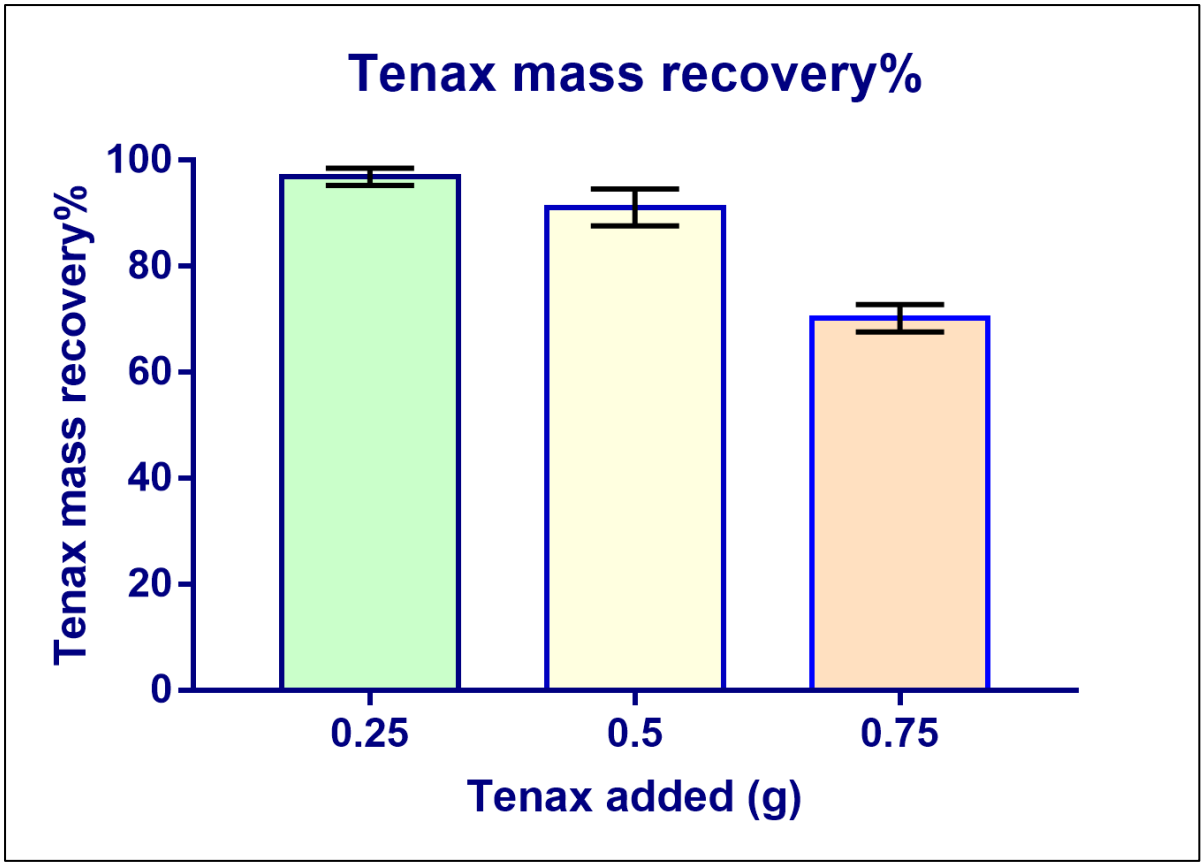


Figure SI 3 Bar chart presenting Tenax TA mass recovery% in different amounts of Tenax TA tested

Table SI 4 – Extraction efficiency (%) for small intestine and colon compartment using LLE, Tenax TA® and residual dust with ultrasonication assisted extraction. All samples were assessed in triplicates (n=3).

	Small Intestine (n=3)			Colon (n=3)			Tenax TA® (n=3)			Residual dust (n=3)		
Target analyte	AVG%	STDEV	RSD%*	AVG%	STDEV	RSD%	AVG%	STDEV	RSD%	AVG%	STDEV	RSD%
BDE-28	74.8	6.0	8.0	76.8	9.2	12.0	66.7	0.1	9.0	71.9	6.5	9.0
BDE-47	87.7	2.9	3.3	82.9	1.9	2.3	77.1	0.1	8.5	68.0	5.7	8.5
BDE-100	69.2	9.4	13.6	77.7	10.5	13.5	54.2	0.1	6.0	52.0	3.1	6.0
BDE-153	58.6	0.03	4.4	77.7	0.1	16.6	89.0	0.1	10.0	92.9	6.0	6.5
BDE-154	96.7	0.0	2.6	79.3	13.2	0.2	103.7	0.1	10.0	86.0	5.8	6.7
BDE-183	92.2	0.1	13.8	66.2	0.1	17.9	90.3	0.00	0.1	65.5	0.0	0.1

*RSD%= (STDEV/AVG)*100

References for SI

Kademoglou, K., Xu, F., Padilla-Sanchez, J.A., Haug, L.S., Covaci, A., Collins, C.D., 2017. Legacy and alternative flame retardants in Norwegian and UK indoor environment: Implications of human exposure via dust ingestion. Environ. Int. 102, 48–56. doi:10.1016/j.envint.2016.12.012

Tilston, E.L., Gibson, G.R., Collins, C.D., 2011. Colon Extended Physiologically Based Extraction Test (CE-PBET) Increases Bioaccessibility of Soil-Bound PAH. Environ. Sci. Technol. 45, 5301–5308. doi:10.1021/es2004705