Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting Information for:

Exploring variations of Hexabromocyclododecane concentrations in riverine sediments along the River Medway, UK

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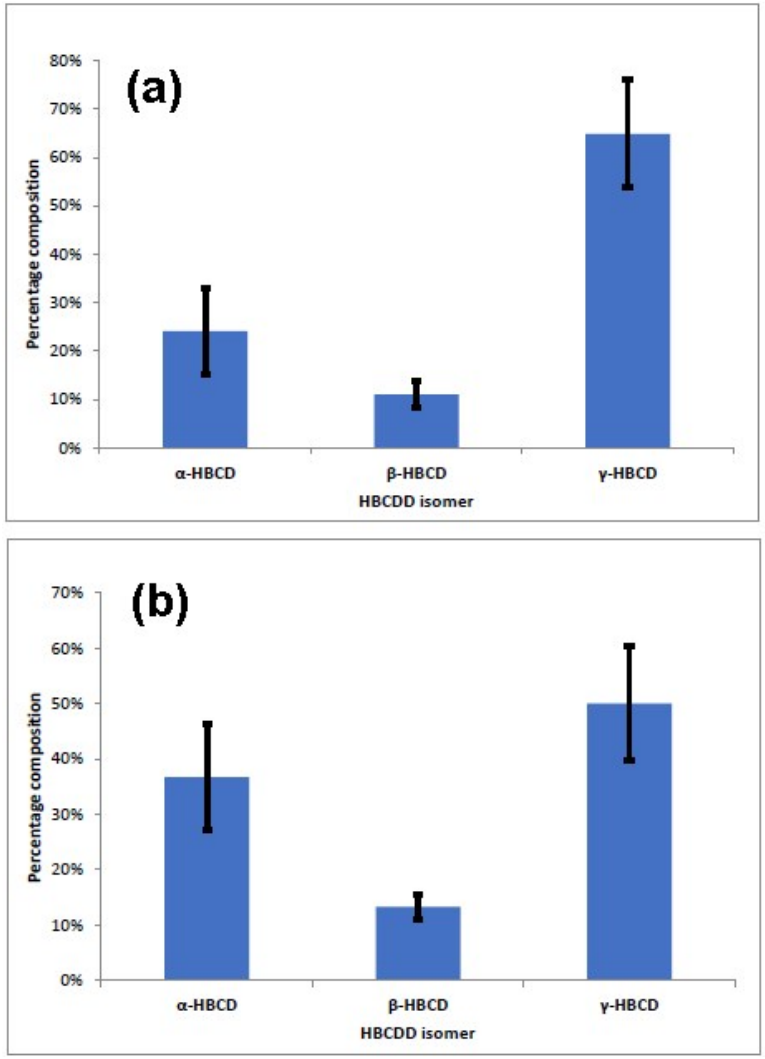
Table S1: Concentrations of HBCDD in the studied sediment samples from the river Medway.

Site	ng/g dw				Organic Carbon	ng/g OC			
	α-HBCDD	β-HBCDD	γ-HBCDD	ΣHBCDD		α-HBCDD	β-HBCDD	γ-HBCDD	ΣHBCDD
S1	0.87	0.27	2.22	3.36	0.0121	72	22	184	278
S2	0.88	0.28	1.67	2.83	0.0349	25	8	48	81
S3	0.74	0.21	0.99	1.94	0.0544	14	4	18	36
S4	1.58	0.92	10.68	13.19	0.0207	76	45	515	636
S5	1.27	0.57	4.49	6.33	0.0603	21	9	75	105
S6	1.38	0.54	5.74	7.65	0.0348	40	15	165	220
S7	1.72	0.91	6.44	9.07	0.0226	76	40	285	401
S8	1.79	1.07	15.02	17.88	0.0486	37	22	309	368
S9	2.29	1.72	15.10	19.12	0.0506	45	34	299	378
S10	1.25	0.81	5.31	7.37	0.0232	54	35	228	317
S11	3.00	2.18	22.12	27.31	0.0596	50	37	371	458
S12	3.21	2.41	14.44	20.05	0.0386	83	62	374	519
S13	1.53	0.74	3.00	5.27	0.0299	51	25	100	176
S14	1.12	0.46	1.93	3.51	0.0295	38	15	65	119
S15	0.72	0.31	1.16	2.19	0.0131	55	23	88	167
S16	0.37	0.14	0.44	0.96	0.0118	32	12	37	81
S17	0.36	0.11	0.35	0.82	0.0122	30	9	29	67
S18	0.54	0.29	1.24	2.06	0.0050	106	57	245	408

S19	1.01	0.40	1.95	3.36	0.0199	51	20	98	169
S20	2.93	1.95	11.40	16.29	0.0326	90	60	349	499
S21	1.89	0.94	6.61	9.44	0.0230	82	41	288	411
S22	6.26	3.52	29.36	39.14	0.0389	161	91	755	1006
S23	5.91	2.41	13.57	21.88	0.0431	137	56	315	508
S24	2.05	1.19	5.29	8.53	0.0304	67	39	174	281
S25	1.55	0.59	5.25	7.39	0.0326	48	18	161	227
S26	3.42	2.16	12.43	18.02	0.0394	87	55	315	457
S27	1.50	0.54	2.12	4.16	0.0235	64	23	90	177
S28	0.62	0.20	0.59	1.4	0.0363	17	5	16	39
S29	0.31	0.08	0.29	0.68	0.0235	13	3	12	29
S30	0.38	0.15	0.40	0.93	0.0303	13	5	13	31
S31	0.27	0.12	0.30	0.68	0.0261	10	4	11	26
S32	0.57	0.13	0.51	1.22	0.0319	18	4	16	38
S33	1.05	0.33	1.18	2.57	0.0309	34	11	38	83
S34	4.86	2.21	7.65	14.72	0.0398	122	55	192	369
S35	2.03	0.92	6.28	9.24	0.0378	54	24	166	245
S36	1.44	0.72	2.98	5.14	0.0416	35	17	72	124
S37	0.94	0.47	1.36	2.77	0.0363	26	13	37	76
S38	5.21	2.32	21.42	28.95	0.0415	126	56	517	698
S39	1.26	0.59	2.35	4.19	0.0295	43	20	79	142
S40	2.68	1.18	6.86	10.72	0.0304	88	39	225	352

Figure SI-1 Average HBCDD isomer profile in (a) tidal proportion and (b) non-tidal proportion of the river Medway (bar whiskers represent 1 standard deviation)



SI.2 Extended Method

As mentioned in the main text the samples were analysed according to the method reported by (Ganci et al, 2019). Details are provided below.

Chemical standards

Chemicals used in the analysis of the samples collected were all procured from Fisher Scientific, Loughborough, UK. These were of at least HPLC standard grade. High purity native, ^{13}C- and d_{18}- isotope labelled α-,β-, and γ-HBCDDs standards were purchased from Wellington Laboratories Inc. (Guelph, Canada). Florisil HyperSep™ SPE cartridges (1 g, 60 cm^3), concentrated sulphuric acid, copper powder and anhydrous sodium sulphate were also obtained from Fisher Scientific, Loughborough, UK.

Measurement of total organic carbon (TOC):

TOC was measured using the Loss on Ignition (LOI) method (Santisteban, 2004). Briefly, clean crucibles were dried in the oven at 105°C overnight, cooled in dessicator for 30min and their weight was recorded. Freeze-dried samples were added to crucibles and dried in the oven at 60°C overnight, cooled in dessicator and weight of crucibles with samples was recorded. Subsequently, crucibles with samples were placed in the furnace at 550°C and left for 6 hours. Finally, weight of cooled crucibles with ash was recorded. %TOC was calculated as:

$$\%TOC = \frac{pre\ ignition\ weight\ (g) - post\ ignition\ weight\ (g)}{pre\ ignition\ weight\ (g)}\ x\ 100$$

Sample extraction and clean up

2g of each sample were weighed into a clean glass extraction tube and mixed with 20µL of international standard mixture, as well as 2g of copper powder to remove sulphur. The samples were extracted using 4 mL of hexane:acetone (3:1 v/v) which were then vortexed for 5 minutes before ultrasonication for 20 minutes and were then put in a centrifuge for 5 minutes at 4,000 rotations per minute. The solvent layer was transferred to a clean dry test tube and the process was repeated twice for each sample. Under a gentle N2 stream, the combined solvent extract was evaporated to incipient dryness and reconstituted using 2ml of hexane.

Sulphuric acid (3 ml) was used to wash the extract. The organic phase was allowed to separate and transferred to another clean dry test tube. The

acid layer was washed with 2mL of hexane twice and the combined washings were again subject to a gentle stream of N2, until reduced to ~1mL. The sample was then loaded onto a conditioned HyperSep™1 g Florisil SPE cartridge onto which 1g of sodium sulphate was added. 20ml of Hexane:dichloromethane (1:1 v/v) was then used for elution of target compounds. The clean extract was concentrated under N2 and reconstituted using methanol:toluene (1:1 v/v) containing 250 pg/µl d_{18}- α-,β-, and γ-HBCDDs used as recovery determination (syringe) standrads for QA/QC purposes.

Instrumental Analysis

2 µL of each sample were analysed using a UPLC-Orbitrap-HRMS instrument. This comprised an UltiMate® 3000 ultra performance liquid chromatography system with a HPG-3400RS dual pump, TCC-3000 column oven, WPS-3000 auto sampler and a Q-Exactive™Plus Orbitrap mass spectrometer. Chromatographic separation was carried out on an Accucore™RP-MS column (100 × 2.1 mm, 2.6 µm) with mobile phases consisting of water (A) and methanol (B) at 400 µl min^{-1} using a dedicated gradient elution programme.

Time [min.]	% A (Water)	% B (Methanol)	Flow rate [µL min^{-1}]
0	35	65	400
3	35	65	400
4	15	85	400
6	15	85	400
7	0	100	500
10	0	100	500
11	15	85	400
13	15	85	400
14	35	65	400
17	35	65	400

HBCDD was then determined in the negative atmospheric pressure chemical ionisation mode [(–) APCI]. The Orbitrap was set to the following

settings : 70,000 FWHM (full width at half maximum at 200 m/z and scan rate of 3 Hz at 200m/z), AGC target $1e^6$, maximum injection time 100 ms, scan range 250 to 1000m/z, profile spectrum data type, sheath gas (nitrogen)flow rate 25 AU (arbitrary units), aux gas (nitrogen) flow rate 5 AU, discharge current 30µA, capillary temperature 250 °C, S-lens RF level 50 AU and aux gas heater temperature 320 °C.
Identification was carried out using the analyte accurate mass and quantification was conducted using Xcalibur™ software.

QA/QC

Method linearity was established using 5-point calibration curve for each target analyte over a concentration range of 20-1000 pg/µl (Table SI-2). The standard reference material SRM1944 (NIST) for sediment was used to evaluate the accuracy of the method for HBCDDs. One SRM sample was analysed for every 15 sediment samples. Results obtained for the SRM 1944 (19.6 ± 1.5 ng/g dw) were generally in good accordance with the certified levels (21.2 ± 0.9 ng/g dw). In addition, triplicate analysis of 5 samples revealed low RSD (6-9%) indicating good precision. Recoveries for internal standards were in the range of 90 to 120% for all samples, Limits of detection (LOD) and limits of quantification (LOQ) were estimated based on method described by Taylor (1984). In brief, standards were analysed in replicates (n = 10). The standard deviation of each measured concentration was plotted versus the specified concentration, giving a y-axis intercept of the resulting regression line corresponds to s0. The LOD is then defined as 3 × s0, while the LOQ is defined as 10 × s0.
To minimise blank contamination, all glassware was cleaned by soaking in a detergent solution overnight, before rinsing with deionised water. This was followed by washing with acetone, hexane, toluene and dichloromethane and subsequent baking at 120 °C overnight. One method blank (sodium sulfate instead of sediment) was analysed with every set of 6 samples. In addition, 6 filed blanks were collected alongside sediment samples from sites 1, 6, 14, 18, 29, 34. Each field blank constitutes ~2 gm of anhydrous sodium sulfate that were placed in a polyethylene sealable bag at the sampling site and then underwent all steps of sample transport, storage and processed as a sample. None of the target HBCDDs were detected in the blanks.

Table SI-2: QA/QC parameters

Compound	LOD pg/g	LOQ pg/g	Linearity	Range (pg/µl)	IS used	Recovery (%) (SD)
α-HBCDD	0.1	0.4	0.9913	20-1000	^{13}C- α-HBCDD	102 (13)
β-HBCDD	0.15	0.5	0.9964	20-1000	^{13}C-β-HBCDD	109 (8)
γ-HBCDD	0.1	0.4	0.9987	20-1000	^{13}C-γ-HBCDD	105 (12)

Determination of Internal (surrogate) Standard Recoveries

It is important to calculate the recoveries of internal standards (IS) as a QA/QC measure. The recoveries of IS (added before extraction) are calculated based on the recovery determination (syringe) standard (RDS), which is added in the final sample solvent for LC injection.

Internal standard (IS) recoveries are calculated as follows:

$$\% \text{ IS Recovery} = \left[\left(\frac{A_{IS}}{A_{RDS}}\right)_S \times \left(\frac{A_{RDS}}{A_{IS}}\right)_{STD} \times \left(\frac{C_{IS}}{C_{RDS}}\right)_{STD} \times \left(\frac{C_{RDS}}{C_{IS}}\right)_S\right] \text{x } 100$$

where $(A_{IS}/A_{RDS})_S$ = ratio of internal standard peak area to recovery determination standard peak area in the sample; $(A_{RDS}/A_{IS})_{STD}$ = ration of recovery determination standard peak area to internal standard peak area in the calibration standard (the average of values obtained for both calibration standards run for a batch of samples is used); $(C_{IS}/C_{RDS})_{STD}$ = ratio of concentration of internal standard to concentration of recovery

determination standard in the calibration standard; and $(C_{RDS}/C_{IS})_S$ = ratio of concentration of recovery determination standard to concentration of internal standard in the sample.

References

Ganci, A. P., Vane, C. H., Abdallah, M. A.-E., Moehring, M., Harrad, S. (2019). Legacy PBDEs and NBFRs in sediments of the tidal River Thames usingliquid chromatography coupled to a high resolution accurate massOrbitrap mass spectrometer. *Science of the Total Environment*. 658, 1355-1366.

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