SUPPORTING INFORMATION FOR
CURRENT-USE BROMINATED FLAME RETARDANTS IN
WATER, SEDIMENT, AND FISH FROM ENGLISH LAKES

Stuart Harrad, Mohamed Abou-Elwafa Abdallah, Neil L. Rose, Simon D. Turner,

Thomas A. Davidson

8 Tables, 2 Figures, 11 Pages

Table S1: Information about Sampling Locations

Site Name	Latitude	longitude	Altitude (m)	Lake Area (ha)	Mean Depth (m)	Max (Profundal) Depth (m)	Population density (people per sq km) of local/unitary authority of lake site	Population density (people per sq km) of local/unitary authorities within 25km of site
Fleet Pond	51.2890	-0.8257	68	26.4	0.5	0.8	250-499	>2500
Slapton Ley	50.2743	-3.6534	3	65.9	-	2.5	≤99	1000-2499
Wake Valley Pond	51.6695	0.0530	96	1	1.7	3.7	250-499	>10000
Holt Hall Lake	52.9154	1.0873	47	0.7	0.6	1	100-249	100-249
Thoresby Lake	53.2272	-1.0564	37	30.4	1.4	3.1	100-249	1000-2499
Edgbaston Pond	52.4546	-1.9209	127	7.2	0.9	2.1	>2500	>2500
Marton Mere	53.8093	-3.0004	7	10.8	1.35	4.15	>2500	>2500
Crag Lough	55.0058	-2.3672	244	10.1	1	2	≤99	≤99
Chapman's Pond	53.9344	-1.1201	16	0.6	2.13	4.5	500-999	1000-2499

Altitudes and areas from www.UKLakes.net

Population Density of England by local or unitary authority, 2007

Data from: <http://www.statistics.gov.uk/default.asp>

Regional Snapshots of Population data: <http://www.statistics.gov.uk/regionalsnapshot/>

Table S2: Concentrations (pg L⁻¹) of HBCD Diastereomers and TBBP-A in Operationally-Defined “Particulate” and “Freely-dissolved” Phases of Water Samples

	“Freely-Dissolved” phase					“Particulate” phase				
Location	α-HBCD	β-HBCD	γ-HBCD	ΣHBCDs	TBBP-A	α-HBCD	β-HBCD	γ-HBCD	ΣHBCDs	TBBP-A
Crag Lough	8.1	6.3	32	47	105	7.0	3.0	38	48	69
Fleet Pond	6.8	4.0	23	33	194	5.0	3.0	31	39	126
Wake Valley Pond	12	6.6	28	47	76	9.0	7.0	25	41	61
Holt Hall Lake	12	8.1	22	42	101	18	12	31	61	63
Slapton Ley	17	8.7	87	112	2083	18	9.0	107	134	1273
Thoresby Lake	9.2	4.4	15	28	763	14	7.0	23	44	472
Edgbaston Pool	25	13	75	113	1154	29	15	83	127	796
Marton Mere	20	8.7	54	82	286	20	8.6	57	86	146
Chapman’s Pond	25	10	27	62	742	23	9.0	25	57	438

Table S3: Bioaccumulation Factors (BAFs) for Fish Samples

Location	Species	α -HBCD	β -HBCD	γ -HBCD	Σ HBCDs	TBBP-A
Crag Lough	<i>Oncorhynchus mykiss</i>	14,000	3,500	1,300	3,900	
Crag Lough	<i>Perca fluviatilis</i>	3,500	1,300	950	1,500	
Crag Lough	<i>Perca fluviatilis</i>	8,000	1,000	360	2,000	12
Crag Lough	<i>Perca fluviatilis</i>	6,200	1,600	630	1,700	
Holt Hall Lake	<i>Carassius carassius</i>	7,200	1,300	1,100	3,000	
Holt Hall Lake	<i>Rutilus rutilus</i>	10,000	2,400	3,200	5,000	
Holt Hall Lake	<i>Carassius carassius</i>	6,100	1,300	1,500	2,800	
Holt Hall Lake	<i>Carassius carassius</i>	4,300	390	820	1,800	
Holt Hall Lake	<i>Carassius carassius</i>	10,000	680	1,200	3,700	
Chapman's Pond	<i>Cyprinus carpio</i>	3,500	2,100	1,400	2,300	
Chapman's Pond	<i>Carassius carassius</i>	9,400	1,800	1,500	4,700	
Chapman's Pond	<i>Carassius carassius</i>	2,700	820	1,000	1,700	0.7
Chapman's Pond	<i>Carassius carassius</i>	2,100	300	390	1,100	
Chapman's Pond	<i>Carassius carassius</i>	1,700	430	1,100	1,300	2.3
Slapton Ley	<i>Rutilus rutilus</i>	7,200	3,400	580	1,900	
Slapton Ley	<i>Scardinius erythrophthalmus</i>	2,300	2,000	420	850	
Slapton Ley	<i>Rudd/Roach hybrid</i>	4,500	2,000	430	1,200	
Slapton Ley	<i>Rutilus rutilus</i>	2,300	250	230	530	
Slapton Ley	<i>Rutilus rutilus</i>	1,300	460	110	310	
Slapton Ley	<i>Rutilus rutilus</i>	4,300	1,600	1,100	1,600	
Fleet Pond	<i>Perca fluviatilis</i>	1,600	960	210	620	
Fleet Pond	<i>Rutilus rutilus</i>	6,900	3,400	650	2,500	
Fleet Pond	<i>Tinca tinca</i>	5,100	590	180	1,200	
Fleet Pond	<i>Perca fluviatilis</i>	1,200	700	160	430	1.7

Fleet Pond	<i>Rutilus rutilus</i>	23,000	1,100	980	6,000	
Fleet Pond	<i>Perca fluviatilis</i>	4,200	450	620	1,300	
Thoresby Lake	<i>Esox lucius</i>	1,700	880	430	920	
Thoresby Lake	<i>Esox lucius</i>	6,200	660	910	2,600	
Marton Mere	<i>Esox lucius</i>	2,900	740	360	1,100	
Marton Mere	<i>Esox lucius</i>	13,000	700	470	3,500	
	Average	5,900	1,300	810	2,100	4.2
	σ_{n-1}	4,700	930	620	1,400	5.3

Details of Method Validation, QA/QC Procedures, and LC-MS/MS parameters

Water sample integrity

The HDPE containers used to collect water samples are washed sequentially before sample collection with dichloromethane, detergent and deionised water. Time from collection to delivery to the laboratory is between 2 hours and 7 days. The possibility of the hydrophobic target compounds undergoing sorptive loss to the HDPE container walls was examined. During the method development stage, each container was rinsed 3 times with 10 mL of dichloromethane after sample removal. The combined washings from each sample were collected and analysed for all target compounds. Both γ -HBCD and TBBP-A were detected in the washings (3:1 S/N ratio). However, none was above the LOQ (10:1 S/N ratio). With respect to possible sorptive and degradative losses of target compounds between collection and analysis; recoveries of the target compounds added to 20 L of Milli-Q water and stored in clean identical HDPE containers for 15 days (i.e. exceeding the combined time elapsed between sampling and commencement of analysis) ranged between (69-92%) without solvent washing and (72-88%) with solvent washings. Therefore, solvent rinsing of the HDPE container was found to have no significant effect on improving the recovery of target compounds and was not performed subsequently.

Method detection limits

Method detection limits were 1.6, 1.8, 1.2, 2.3 and 3.4 pg/injection for α -, δ -, β -, γ -HBCDs and TBBP-A respectively.

Matrix spikes

Matrix spikes to assess the recoveries of target compounds from water were carried out during method development at 3 concentration levels where the target compounds were added to 20 L of Milli-Q water and stored in clean identical HDPE containers for 15 days then treated as a sample. A summary of the results is given in table S4.

Table S4: Percent Recovery of target compounds from Milli-Q water.

Mass added (pg)	Recovery % (n=3)			
	α -HBCD	β -HBCD	γ -HBCD	TBBP-A
50	71- 89	76-84	69-91	72-85
500	74-87	73-88	74-86	76-83
5000	76-82	79-85	77-89	79-90

Analyte breakthrough

Analyte breakthrough was also assessed during method development using the matrix spike samples via spiking the 1st PUF plug with 50 ng d₁₈- α -HBCD and analyzing the 2 PUF plugs separately after sampling. Results revealed a 6-11% breakthrough from the first PUF plug. When a 3rd PUF plug was added, no breakthrough was observed from the 2nd to the 3rd plug. Therefore, only 2 PUF plugs were used for sampling.

Blanks

5 blanks were analysed for each matrix studied, for water this consisted of precleaned GFF and PUF plugs treated as a sample while for fish and sediment 1 g of precleaned sodium sulphate was used instead of the sample. None of the target compounds were detected in these blanks. We did not run field blanks in the strict sense, but as part of initial method validation 45 L of Milli-Q water were passed through GFF and 2 PUF plugs and treated exactly as a sample. None of the target compounds were above LOQ in these blanks (n=3).

Method reproducibility

During method development, 5 replicates were analysed for sediment samples and 7 replicates for fish samples. Replicate samples of water were not taken for operational reasons. Results are in table S5.

Table S5: Results of replicate analysis of sediment (pg/g dw) and fish (ng/g lw) samples.

Sample	Replicate 1		Replicate 2	
	ΣHBCDs	TBBP-A	ΣHBCDs	TBBP-A
Sediment 1	3951	3778	3936	3808
Sediment 2	2297	553	2308	544
Sediment 3	882	385	875	393
Sediment 4	1076	460	1094	462
Sediment 5	1216	331	1197	325
Fish 1	146	<LOQ	151	<LOQ
Fish 2	209	<LOQ	212	<LOQ
Fish 3	70	<LOQ	66	<LOQ
Fish 4	212	<LOQ	206	<LOQ
Fish 5	95	<LOQ	98	<LOQ
Fish 6	14	6	12	4
Fish 7	293	<LOQ	296	<LOQ

Method accuracy

For sediment and fish, due to the lack of appropriate standard reference material for TBBP-A and HBCDs, the accuracy and precision of the analytical method for HBCDs was assessed via replicate analysis (n=10) of NIST SRM 2585. The results obtained compared favourably with the indicative values reported elsewhere (1) (table S6). For TBBP-A, a standard addition or “matrix spike” method to SRM 2585 at 3 concentration levels (n=5 at each level) was used to assess the accuracy and precision of the method and good results were obtained (table S7).

Table S6: Concentrations of HBCD isomers in SRM2585 compared to the indicative values (*I*).

	Average concentration (ng g ⁻¹) ± standard deviation (n=7)		IS recovery %
	Measured	Indicative	
α-HBCD	21 ± 2.9	19 ± 3.7	74-102
β-HBCD	4.1 ± 0.7	4.3 ± 1.1	78-96
γ-HBCD	127 ± 16	120 ± 22	72-99

Table S7: Summary of standard addition method results for TBBP-A in SRM 2585*.

Mass added (ng)	Mass recovered (ng)	Recovery (%)	RSD (%) (n=5)
10	8.5	85	2.8
40	37.3	92.5	3.2
80	79.1	98.8	3.1

*Estimated TBBP-A concentration in SRM2585 = 245 ng g⁻¹ ± 7.4 (this study).

LC-MS/MS Parameters

Table S8: List of compounds studied and their MRMs.

Compound name	Abbreviation	Precursor ion	Fragment ion
Hexabromocyclododecane (α, β, γ, δ isomers)	HBCD (α, β, γ, δ isomers)	640.6	78.8
¹³ C- Hexabromocyclododecane (α, β, γ isomers)	¹³ C-HBCD (α, β, γ isomers)	652.6	78.8
d ₁₈ - Hexabromocyclododecane (α, γ isomers)	d ₁₈ -HBCD (α, γ isomers)	657.8	78.8
Pentabromocyclododecenes (4 isomers)	PBCDs	560.6	78.8
Tetrabromocyclododecadiene (2 isomers)	TBCDs	480.4	78.8

Tetrabromobisphenol-A	TBBP-A	540.6	78.8
¹³ C- Tetrabromobisphenol-A	¹³ C- TBBP-A	542.4	78.8

Reference

1. Keller, J. M.; Stapleton, H. M.; Heltsley, R.; Peck, A.; Kucklick, J. R.; Schantz, M.; Wise, S. A., SRMs available from NIST for the analysis of brominated flame retardants. *Poster presented at BFR2007, Amsterdam, The Netherlands. 2007.*

Figure S1: Sample Chromatogram of PBCDs in Sediment Extract

Figure S2: Sample Chromatogram of TBCDs in Sediment Extract