



Predatory Bird
Monitoring Scheme

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Liver concentrations of flame retardants in Eurasian otters (*Lutra lutra*) collected from Britain in 2010 & 2011: a Predatory Bird Monitoring Scheme (PBMS) Report

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Contents

1.	Executive Summary.....	4
2.	The Predatory Bird Monitoring Scheme.....	5
2.1.	Background.....	5
3.	Methods	7
3.1.	Collection of carcasses	7
3.2.	Analytical methods	7
3.3.	Data expression, format and analysis	8
4.	Results and Discussion	9
4.1.	Congener PBDE profile in otter the livers of otters from England & Wales that died in 2010 or 2011	9
4.2.	Other flame retardants	11
5.	Conclusions	13
6.	Acknowledgements	13
7.	References	14
8.	Appendix	16

1. Executive Summary

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's National Capability contaminant monitoring and surveillance work on avian predators. By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife.

The current study presents the results of a collaborative monitoring programme between the Predatory Bird Monitoring Scheme (PBMS) and the Cardiff University Otter Project (CUOP) in which the concentrations of Polybrominated Diphenyl Ethers (PBDEs) and some of the newer replacement flame retardants were determined in the livers of Eurasian otters (*Lutra lutra*). The principle aim of this work was to determine the current concentrations of PBDEs and other flame retardants that are accumulated by otters and whether there was any evidence of differences in accumulation between otters of different age, sex or provenance.

The otters that were analysed were from England and Wales and included adult and sub-adult males and females. Liver tissue was analysed using either Gas Chromatograph – Mass Spectrometry (GC-MS) or Liquid Chromatograph – Mass Spectrometry (LC-MS) techniques.

PBDEs were present in all otters analysed, while other newer flame retardants (replacements for some of the PBDEs) were detectable in 8 of the 64 livers tested. Individual PBDE congener profiles were dominated by BDE 47 (76% of sum PBDE concentrations wet weight) with BDE 153 and BDE 100 accounting for a further 21% of the PBDE tissue load. The concentrations of Σ PBDEs measured in the present study ranged between 3 and 718 ng/g wet weight and were within the range previously reported for Eurasian otters in England & Wales that had died between 1995 and 2005. There was no significant variation in liver Σ PBDE concentrations with age, sex, geographical provenance or year of death.

2. The Predatory Bird Monitoring Scheme

2.1. Background

The Predatory Bird Monitoring Scheme (PBMS; <http://pbms.ceh.ac.uk/>) is the umbrella project that encompasses the Centre for Ecology & Hydrology's long-term contaminant monitoring and surveillance work on avian predators. The PBMS is a component of CEH's National Capability activities.



By monitoring sentinel vertebrate species, the PBMS aims to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. The PBMS provides the scientific evidence needed to determine how chemical risk varies over time and space. This may occur due to market-led or regulatory changes in chemical use and may also be associated with larger-scale phenomena, such as global environmental change. Our monitoring also allows us to assess whether detected contaminants are likely to be associated with adverse effects on individuals and their populations.

Overall, the PBMS provides a scientific evidence base to inform regulatory decisions about sustainable use of chemicals (for example, the [EU Directive on the Sustainable Use of Pesticides](#)). In addition, the outcomes from the monitoring work are used to assess whether mitigation of exposure is needed and what measures might be effective. Monitoring also provides information by which the success of mitigation measures can be evaluated.

Currently, the PBMS has two key objectives:

- (i) to detect temporal and spatial variation in exposure, assimilation and risk for selected pesticides and pollutants of current concern in sentinel UK predatory bird species and in species of high conservation value
- (ii) in conjunction with allied studies, to elucidate the fundamental processes and factors that govern food-chain transfer and assimilation of contaminants by top predators.

Further details about the PBMS, copies of previous reports, and copies of (or links to) published scientific papers based on the work of the PBMS can be found on the [PBMS website](#).

Previously the PBMS has used the grey heron, *Ardea cinerea*, as a sentinel to assess how levels of contamination in the freshwater environment may be changing and to determine whether contamination may pose a risk to wildlife. However, the number of herons received each year by the PBMS is now relatively low and precludes detection of temporal and spatial variation. Consequently, the PBMS has developed a collaboration with the Cardiff University Otter Project (CUOP), one of the PBMS partners in the Wildlife Disease and Contaminant Monitoring and Surveillance (WILDCOMS) network

(<http://www.wildcoms.org.uk/>), to utilise Eurasian otters, *Lutra lutra*, in place of grey herons as a freshwater monitor. Fish comprise a high proportion of the diet of both otters and grey herons (Clavero *et al.*, 2003, Cook, 1978, Jedrzejewska *et al.*, 2001, Marquiss and Leitch, 1990) and so residues in both species are likely reflect contamination accumulated by freshwater and near shore fish.

The CUOP analyses the livers of the otters it collects for a selection of polychlorinated biphenyls (PCBs) and organochlorine insecticides but not for other persistent organic pollutants or inorganic contaminants. Linkage of the PBMS and CUOP provides cost-effective monitoring on the extent and variation in contamination of the freshwater environment for both POPs and inorganic contaminants. Previously, the PBMS has reported contamination of otters by inorganic elements such as lead and mercury (https://wiki.ceh.ac.uk/download/attachments/134414860/PBMS_Metals_Otters_2009.pdf?version=1&modificationDate=1327415435000). The current report describes the concentrations in otter livers of polybrominated diphenyl ether (PBDE) flame retardants and some of the newer replacement compounds.

There are 209 theoretically possible PBDE congeners, often classified by commercial mixtures that reflect the predominant congeners in the mixture, namely Penta- (PeBDE), Octa-(OBDE) and DEca-(DeBDE) formulations (Crosse *et al.*, 2012). PBDEs have been widely used as flame retardants in furniture foams and different plastics (Rahman *et al.*, 2001). PBDEs can enter the environment through direct emissions to air as gas or dust, by release to land and surface water, and via sewage and landfill. They are resistant towards acids and bases as well as heat and light and also to reducing or oxidising compounds; as a result they persist in the environment. PBDEs are of environmental concern because of their high lipophilicity, persistence and potential to bioaccumulate (Rahman *et al.*, 2001).

PBDEs have been detected in mustelid species including southern sea otters, *Enhydra lutris nereis* (Kannan *et al.*, 2008), and American river otters, *Lontra canadensis* (Basu *et al.*, 2007, Stansley *et al.*, 2010), and were quantified in a sample of Eurasian otters from Britain that died between 1995 and 2005 (Pountney, 2008). Some PBDE congener mixtures are known to be immunotoxic to mustelids (Martin *et al.*, 2007).

The aim of the current study was to quantify liver concentrations of PBDEs and other flame retardants (that are now replacing PBDEs) in the livers of a representative sample of Eurasian otters from across England and Wales and to determine whether contamination varied with sex, age or geographical regional.

The policy relevance of this work is that, because of rising environmental concentrations and concerns over toxicity, penta and octa BDEs have been phased out or banned in America and Europe since 2004 (Hale *et al.*, 2006, Vernier *et al.*, 2010, Pountney, 2008).

Pentabromodiphenyl ether, hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether) have been included in amendments to Annexes A/B/C of the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP, 2013). Determination of BDE concentrations in otters provides scientific evidence of whether such mitigation has been successful in terms of determining whether current exposure in otters is low. Furthermore, analysis of concentrations of replacement flame retardants in otters identifies whether these compounds are being emitted to the environment and accumulated by aquatic organisms.

3. Methods

3.1. Collection of carcasses

As part of the Cardiff University Otter Project (CUOP), otters found dead in England and Wales are examined to determine sex, weight and length. Age-class (adult, sub-adult or juvenile) is estimated from a combination of morphometric data and indicators of reproductive activity (Chadwick, 2006). Nutritional and reproductive status, lesions, growths and concretions are also noted.

Tissue samples are taken as part of the post-mortem examination, including the liver. A sub-sample of the liver is analysed for PCBs and organochlorine insecticides by the Environment Agency's National Laboratory Service, and the results of that analysis are published in reports produced for the Environment Agency².

During 2011, 34 otter livers were collected for PBDE analysis. They were from a stratified subset of animals found dead and collected by the CUOP; stratification was, where possible, by sex, age-class and provenance (Northern England, Eastern England, Wales and south-western England).

3.2. Analytical methods

The liver samples were analysed at the centralised analytical laboratories at the Centre for Ecology and Hydrology, Lancaster. Concentrations of 26 BDEs (6 tri-BDEs, 6 tetra-BDEs, 6 penta-BDEs, 4 hexa-BDEs, 2 hepta-BDEs, 5 octa-BDEs, 3 nona-BDEs and decabromodiphenylether) were quantified, together with concentrations of eight new flame retardants that are currently used as replacements to the phased out lower brominated PBDEs. The list of compounds that were determined is given in Table A1 in the appendix to this report, along with the limits of detection (LoD).

A sub-sample of each liver (~1 g) was thawed, weighed accurately, ground with sand and dried with anhydrous sodium sulphate. Each sample was spiked with labeled recovery standards (¹³C PBDEs and ¹³C BFRs) and Soxhlet extracted in DCM for 16 h. A small portion of the extract was evaporated to zero volume and the lipid content was determined gravimetrically. The remaining extract was cleaned using automated size exclusion chromatography followed by deactivated alumina column.

Tri- to hepta-BDEs, bromobenzenes, dechlorane plus and hexabromocyclododecane (HBCD) were analysed by GCMS, although HBCD was not quantified in otters that died in 2011 as quality control results and relatively high limits of detection indicated that the quantification of the compound was not adequate in that particular set of analysis. Each extract was spiked with labelled internal standards and 100µl of sample was injected into a

² the latest CUOP report can be downloaded at <https://publications.environment-agency.gov.uk/skeleton/publications/ViewPublication.aspx?id=23ef9515-459f-44f9-b3a7-d2239083b010>

GC-MS with programmable temperature vaporization (PTV) inlet. The PTV injector was kept at 55°C for 0.45 min, and heated to 325°C at a rate of 700°C min⁻¹ and kept at 325°C for 5 min. Then the temperature was reduced to 315°C min⁻¹ at a rate of 10°C min⁻¹. The GC-MS had a 25 m HT8 column (0.22 mm internal diameter and 0.25 µm film thickness, SGE Milton Keynes, UK) and the carrier gas was helium (2.0 ml min⁻¹). The temperature programme was: isothermal at 80°C for 2.4 min, 25°C min⁻¹ to 200°C, 5°C min⁻¹ to 315°C and was held at 315°C for 9.8 min.

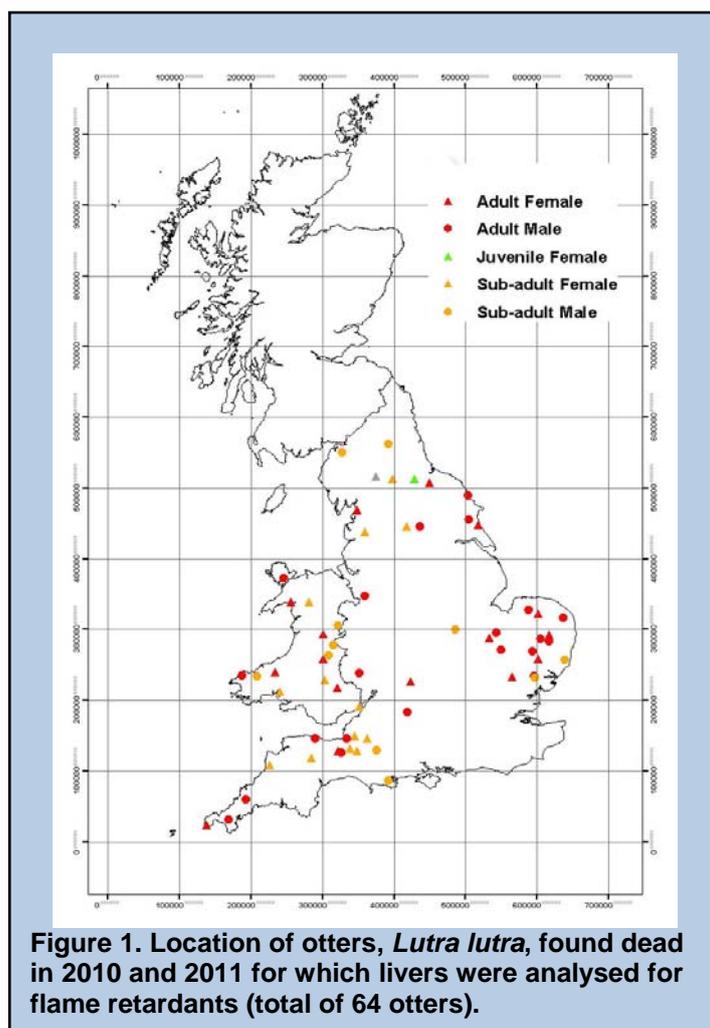
Octa-, to deca-BDEs, DBDPE, TBECH and OBIND (Table A1) were analysed by LCMS-MS. A fraction of the extract was solvent exchanged to methanol to allow for analysis on an LC-MS/MS system (ThermoFisherScientific) consisting of an Accela liquid chromatograph coupled to a Quantum Ultra TSQ triple quadrupole that was equipped with an atmospheric pressure chemical ionisation inlet (APCI) probe. Chromatographic separation was achieved on a Hypersil Gold PFP column (100 x 2.1 mm, 1.9 µm diameter particle size). Calibration standards were prepared in methanol. The Accela LC column oven was set to 30 °C. Solvent A was water/acetone/methanol (80:10:10, v/v/v) and solvent B was methanol/acetone (90:10, v/v). The injection volume was 5 µL and with method LC-BFR-1 the analytes except for Deca-BDE and DBDPE were eluted from the column using the following gradient and flow rate programmes: mobile phase B - 0 min, 30%; 1 min, 30%; 20 min, 70%; 30 min, 100%; 34.9 min, 100%; 35 min, 30%, held for 5 min; 0 min, 250 µL/min; 1 min, 250 µL/min; 20 min, 300 µL/min; 34.9 min, 300 µL/min; 35 min, 250 µL/min, held for 5 min. Deca-BDE and DBDPE were analysed in a separate short run with method LC-BFR-2 using a constant flow rate of 250 µL/min and the following gradient program: mobile phase B - 0 min, 80%; 1 min, 80%; 6 min, 100%; 6.1 min, 80%, held for 5 min. In both methods the MS/MS ion source parameters were as follows: discharge current 12 µA, vapouriser temperature 175 °C, sheath gas pressure 30, ion sweep gas pressure 0, auxiliary gas pressure 5, ion transfer capillary temperature 220 °C, collision gas pressure 2 mTorr, skimmer offset voltage 10 V.

Residues were quantified as recovery corrected concentrations using an internal standard correction method and calibration curves based on PBDE and FR standards (Greyhound Ltd, Birkenhead, UK and LGC Ltd, Teddington, UK).. Average recoveries for ¹³C-PBDE recovery standards for BDE 28, BDE 47, BDE 126, BDE 153, BDE 197 and BDE 209 ranged between 76% and 96%. The average recovery for ¹³C-HBB and ¹³C-HBCD recovery standards was 72% and 20%, respectively.

3.3. Data expression, format and analysis

Data for flame retardants analysed by GCMS that were previously determined in 30 otters that died in 2010 (Walker *et al.*, 2012) have, where appropriate, been incorporated with the new data generated for this report on otters that died in 2011, thereby increasing the total sample size to 64. The provenance of all the otters combined is shown in Figure 1. However, LCMS analysis of octa-, to deca-BDEs, DBDPE, TBECH and OBIND has only recently become available in the laboratory and was only conducted on the 34 otters that died in 2011.

Throughout this report, liver concentrations of flame retardants are reported as ng/g wet weight (wet wt). When all summed PBDE concentrations were calculated, individual congener concentrations below the limit of detection (non-detected) were assigned a zero value. All statistical tests were performed using Minitab (Version 16.1.0; Minitab Ltd., Coventry, UK). Residue data were skewed in their distribution towards lower concentrations and only a few otters had relatively high liver concentrations of contaminants. Therefore, concentrations were \log_{10} transformed prior to statistical analysis in order to satisfy the assumptions of the tests. Summary statistics of the residue data are therefore presented as geometric means.



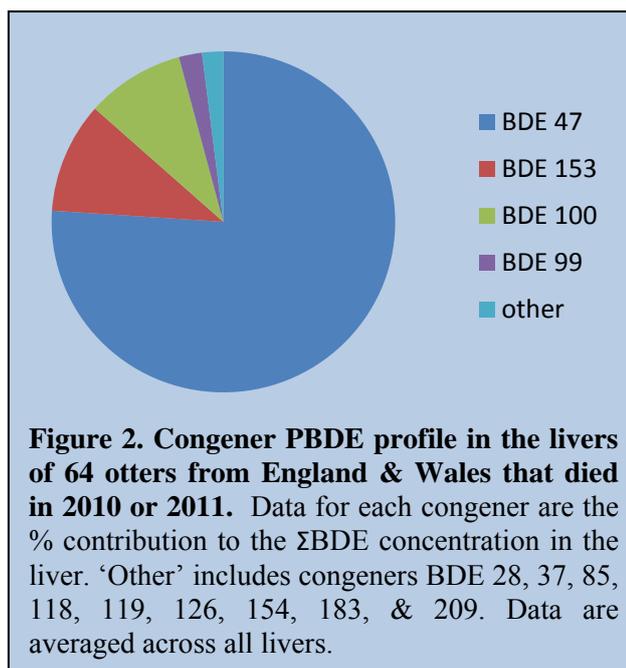
4. Results and Discussion

4.1. Congener PBDE profile in otter the livers of otters from England & Wales that died in 2010 or 2011

Although the heavier PBDE congeners were analysed for in otters that died in 2011 but not in 2010, concentrations of the extra congeners were almost always below the limit of detection. Therefore, data for otters from the two years have been combined when examining the overall PBDE congener profile.

PBDE residues were detected in the livers of all otters. PBDE residues were dominated by BDE congener 47 which accounted for, on average, 76% of the sum PBDE (Σ PBDE) concentration (Figure 2). BDEs 153 and 100 accounted for a further 21% of the Σ PBDE concentration (Figure 2). This concurs with an unpublished previous study of otters from England & Wales in which BDEs 47, 153 and 100 were the dominant congeners of mono- to hepta-BDEs (Pountney, 2008). However, the relatively high limit of detection for the higher brominated congeners in the present study means they may be under-represented in the PBDE profile.

The congeners of PBDEs can be grouped according to their level of bromination. The group with the highest concentration was the tetra-BDEs, although this was solely due to a single congener (BDE47 – Table 1). Sum Tri-BDE concentrations were low compared with other bromination groups while mean concentrations of Σ Penta-BDE, Σ Hexa-BDE, and Σ Hepta-BDE concentrations were similar to each other ranging between 4 and 11 ng/g wet wt. Nona-BDEs were not detected in any otters, while two had detectable residues of Deca-BDE and one contained an octa-BDE in their liver.



Sum PBDE concentrations in livers ranged from 3 to 718 ng/g wet wt. (Table 1). This was equivalent to 92 to 19,890 ng/g lipid weight and is similar to that (12.2 - 69882 ng/g lipid weight) found in otters from England and Wales that died between 1995 and 2005 (Pountney, 2008). Residues measured in the present study, and in the study by Pountney (2008) indicated a skewed distribution of residues with most being towards lower concentrations. Sum PBDE liver concentrations of a limited congener suite (BDE 28, 47, 66, 71, 99, 100, 153, & 154) measured in this study are also similar to those reported in a marine predatory mammal, harbour porpoises (*Phocoena phocoena*)(Covaci *et al.*, 2002).

A stepwise GLM analysis that included region, age-class, sex, and year as potential factors indicated, as with the study by Pountney (2008), that Σ PBDE concentrations did not significantly vary with these factors ($F_{\leq 2,56} \leq 1.73$, $P \geq 0.187$). Pountney (2008) found that the concentrations of higher brominated BDEs varied significantly between geographical regions but these higher brominated congeners were only detected in two otters in the current study.

Physiological and histopathological effects of PBDEs in wildlife have been demonstrated in a variety species, although the consequence on the individual and population is not clear (Hall *et al.*, 2003, Murvoll *et al.*, 2006, Raldua *et al.*, 2008, Sonne *et al.*, 2006). Martin *et al* (2007) observed reduced antibody production in ranch mink (*Mustela vison*) exposed to PBDE commercial mixture DE71 in their diet at 5ppm, with associated Σ BDE liver concentrations of 18505 ng/g lipid wt. Sensitivity to chemical contaminants can vary markedly between species and may vary with differences in congener profile, but only one of the 64 otters in the present study had a Σ BDE liver concentration of similar magnitude (19,890 ng/g lipid wt.) to that in adversely affected mink, and the remainder had concentrations below 10,000 ng/g lipid wt

4.2. Other flame retardants

The use of some technical mixtures of PBDEs has been, or is being replaced, by other flame retardants (Crosse *et al.*, 2012). It was possible to analyse for residues of nine of these non-PBDE flame retardants (see Table A1 in the appendix for full list of analytes). These flame retardants were at detectable concentrations in only a few of the samples. Hexabromocyclododecane (HBCD) was detectable in 4 out of 30 otters (2010 samples analysed only) although detection limits were relatively high and , 2-Dechlorane (DC2) was detected in 3 otters (two in 2010, one in 2011) while hexabromobenzene (6BrBz) was detected in 2 otters (both 2010). Concentrations, where detected, ranged from 0.317-104.5 ng/g wet wt., and in all cases concentrations were within one order of magnitude of the limit of detection.

Table 1. Geometric mean (Geomean), 95% confidence interval (95%CI), and range concentrations (ng/g wet wt.) of polybrominated diphenyl ethers (PBDEs) in the livers of otters that died in 2010 or 2011¹ and which had detectable residues

Compound ²	N ³	Geomean	95% C.I.		Min	Max
			Lower	Upper		
BDE 28	16	0.271	0.220	0.333	0.143	0.512
BDE 37	1	0.199	N/A ⁴	N/A	0.199	0.199
ΣTri-BDE	17	0.266	0.219	0.324	0.143	0.512
BDE 47	64	32.4	24.1	43.5	2.17	465
ΣTetra-BDE	64	32.4	24.1	43.5	2.172	465
BDE 100	62	4.00	2.94	5.44	0.432	146
BDE 119	1	0.550	N/A	N/A	N/A	N/A
BDE 99	47	1.31	0.949	1.80	0.265	37.5
BDE 118	2	0.817	N/A	N/A	0.563	1.19
BDE 85	3	1.10	0.131	9.30	0.413	2.02
BDE 126	3	1.63	0.313	8.44	0.887	3.30
ΣPenta-BDE	62	4.99	3.65	6.81	0.489	188
BDE 154	21	0.680	0.504	0.916	0.401	8.676
BDE 153	64	3.526	2.658	4.678	0.416	158.5
ΣHexa-BDE	64	3.66	2.75	4.87	0.416	159
BDE 183	3	12.0	1.49	96.1	5.73	29.8
ΣHepta-BDE	3	12.0	1.49	96.1	5.73	29.8
BDE 196	1	6.95	N/A	N/A	N/A	N/A
ΣOcta-BDE	1	6.95	N/A	N/A	N/A	N/A
Deca-BDE	2	17.6	N/A	N/A	8.70	35.5
ΣPBDEs	64	43.6	32.6	58.3	3.00	717

¹ Sample size for each compound listed in table was 64, except for Deca-BDE which was only quantified in the 34 samples received in 2011.

² BDE 17, 30, 32, 35, 49, 51, 66, 71, 77, 128, 138, 190, 197, 201, 202, 203, 208, 207, 206, 207, & 208 were not detected in any of the samples analysed.

³ N indicates number of samples with concentrations above the limit of detection.

⁴ Parameter not calculated as sample size too small.

5. Conclusions

PBDEs were detected in all otter livers analysed, while other flame retardants were detected in only a small number of samples. Congeners BDE47, BDE153 and BDE100 were dominant in the congener profile. The toxicological consequences of exposure to PBDEs in otters are uncertain given the lack of established links between liver PBDE concentrations and health effects in this species but concentrations were generally lower than those associated with adverse effects in mink. The general low level of detection of replacement flame retardants, suggests that there is little evidence to date of widespread contamination of otters with these compounds, although relatively high detection limits for some compounds may mean that low concentrations have not been detected.

6. Acknowledgements

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Polybrominated diphenyl ethers (PBDEs) in Eurasian otter (Lutra lutra) liver in Britain: 2010 & 2011

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8. Appendix

Table A1. List of analytes measured in otter livers, with IUPAC name, CAS number, and limit of detection (LoD) for this analysis.

Abbreviated name	IUPAC Name	CAS No.	LoD (ng/g wet wt.)
BDE 30	2,4,6-Tribromodiphenyl ether	155999-95-4	0.127
BDE 32	2,4',6-Tribromodiphenyl ether	189084-60-4	0.127
BDE 17	2,2',4-Tribromodiphenyl ether	147217-75-2	0.127
BDE 28	2,4,4'-Tribromodiphenyl ether	41318-75-6	0.127
BDE 35	3,3',4-Tribromodiphenyl ether	147217-80-9	0.127
BDE 37	3,4,4'-Tribromodiphenyl ether	147217-81-0	0.127
BDE 51	2,2',4,6'-Tetrabromodiphenyl ether	189084-57-9	0.508
BDE 49	2,2',4,5'-Tetrabromodiphenyl ether	243982-82-3	0.127
BDE 71	2,3',4',6-Tetrabromodiphenyl ether	189084-62-6	0.127
BDE 47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	0.127
BDE 66	2,3',4,4'-Tetrabromodiphenyl ether	189084-61-5	0.254
BDE 77	3,3',4,4'-Tetrabromodiphenyl ether	93703-48-1	0.127
BDE 100	2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	0.254
BDE 119	2,3',4,4',6-Pentabromodiphenyl ether	189084-66-0	0.254
BDE 99	2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	0.254
BDE 118	2,3',4,4',5-Pentabromodiphenyl ether	446254-80-4	0.254
BDE 85	2,2',3,4,4'-Pentabromodiphenyl ether	182346-21-0	0.254
BDE 126	3,3',4,4',5-Pentabromodiphenyl ether	366791-32-4	0.254
BDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	0.254
BDE 153	2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	0.254
BDE 138	2,2',3,4,4',5'-Hexabromodiphenyl ether	182677-30-1	0.508
BDE 183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	207122-16-5	0.508
BDE 128	2,2',3,3',4,4'-Hexabromodiphenyl ether		1.907
BDE 190	2,3',3,4,4',5,6-Heptabromodiphenyl ether	189084-68-2	3.813
BDE 202 ¹	2,2',3,3',5,5',6,6'-octabromodiphenyl ether		5.632
BDE 201 ¹	2,2',3,3',4,5',6,6'-octabromodiphenyl ether		12.03
BDE 197	2,2',3,3',4,4',6,6'-octabromodiphenyl ether		12.03
BDE 196	2,2',3,3',4,4',5,6'-octabromodiphenyl ether		3.718
BDE 203 ¹	2,2',3,4,4',5,5',6-octabromodiphenyl ether		2.155
BDE 208 ¹	2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether		10.22
BDE 207 ¹	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether		7.109
BDE 206 ¹	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether		5.140
BDE 209 ¹	decabromodiphenylether	1163-19-5	7.062
5BrMeBz	Pentabromomethylbenzene	87-83-2	0.127
5BrEtBz	Pentabromoethylbenzene	85-22-3	0.127
6BrBz	Hexabromobenzene	36355-01-8	0.127
DC1	Dechlorane plus 1	13560-89-9	0.508
DC2	Dechlorane plus 2		0.428
TBECH	tetrabromoethylcyclohexane		8.008
OBIND	octabromotrimethylphenylindane	155613-93-7	18.01
DBDPE	decabromodiphenylethane	1163-19-5	9.235
HBCD ²	Hexabromocyclododecane	3194-55-6	10.56

¹ BDEs 201, 202, 203, 206, 207, 208 & 209, TBECH, OBIND & DBDPE only analysed in samples from otters that had died in 2011 ² HBCD only analysed in samples from otters that had died in 2010