Predatory Bird Monitoring Scheme
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Liver concentrations of flame retardants in Eurasian otters (*Lutra lutra*) collected from Scotland between 2013 and 2015: a Predatory Bird Monitoring Scheme (PBMS) Report

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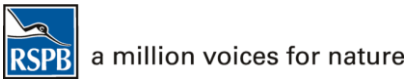

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Contents

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.

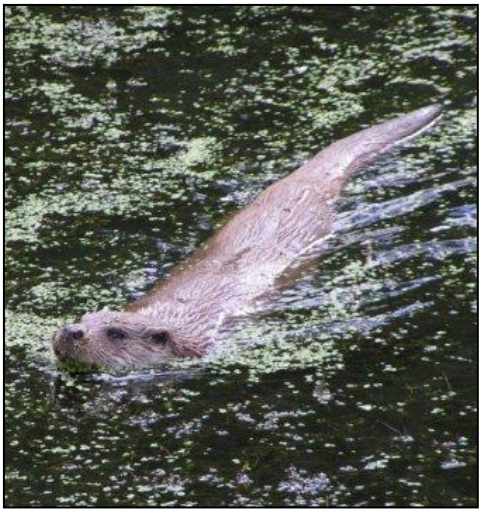
The current study presents the results of a study in which the concentrations of polybrominated diphenyl ethers (PBDEs) were determined in the livers of a small sample of Eurasian otters (*Lutra lutra*) that died in Scotland between 2013 and 2015. The principle aim of this work was to determine the current concentrations of PBDEs that are accumulated by otters in Scotland and whether these concentrations are likely to cause adverse effects in those individuals analysed.

The otters that were analysed included adult and sub-adult, males and females although there were insufficient sample numbers to test for differences among these demographic groups. Liver tissue was analysed using Gas Chromatograph – Mass Spectrometry (GC-MS).

PBDEs were detected in all otter livers analysed, with congeners BDE47, BDE153 and BDE100 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 lower than those associated with adverse effects in mink. The general low levels of PBDEs suggests that there is little evidence to date of toxicologically significant contamination of Scottish otters with these compounds. There is clear evidence that Scottish otters have significantly lower residues of the less-brominated PBDEs than those previously measured in otters from England and Wales. However, these results may not be representative of otters from throughout Scotland as the present sample came predominantly from the Inner Hebrides.

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 predatory 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), 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.

Previously, the PBMS has reported contamination of otters from England and Wales by inorganic elements such as lead and mercury (Walker *et al.*, 2011) and brominated flame retardants (Walker *et al.*, 2013). The current report describes the concentrations of polybrominated diphenyl ether (PBDE) flame retardants in otters from Scotland.

2.2. Polybrominated diphenyl ethers (PBDEs)

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 *et al.*, 2015). Some PBDE congener mixtures are known to cause immunotoxicity in mustelids (Martin *et al.*, 2007).

The aim of the current study was to quantify liver concentrations of PBDEs in the livers of a sample of Eurasian otters from Scotland and assess whether exposure, as determined from residue magnitude, was likely to have caused adverse effects in those individuals.

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, Pountney *et al.*, 2015, Vernier *et al.*, 2010). Penta-bromodiphnenyl ether, hexa-bromodiphenyl ether and hepta-bromodiphenyl 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.

3. Methods

3.1. Collection of carcasses

As part of the Cardiff University Otter Project (CUOP), otters found dead are collected for post mortem examination. Each otter is examined to determine sex and age. Age-class (adult, sub-adult or juvenile) is estimated from a combination of morphometric data and indicators of reproductive activity (Chadwick, 2007). Nutritional and reproductive status, lesions, growths and concretions are also noted. However, there were insufficient sample numbers to test for differences among demographic groups according to age and sex.

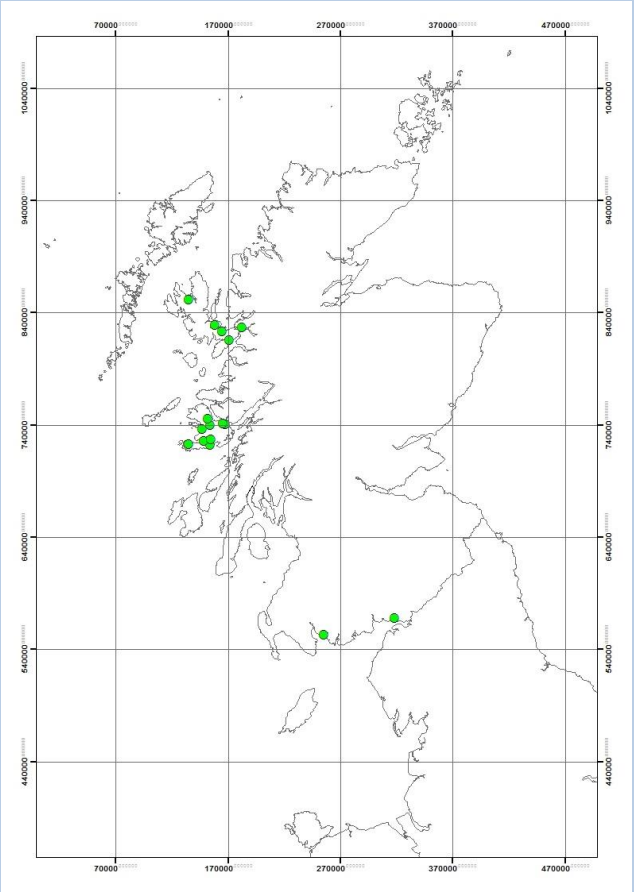


Figure 1. Location of otters, Lutra lutra, found dead in 2013 to 2015 for which livers were analysed for flame retardants (total of 16 otters).

Tissue samples, including the liver, are taken as part of the post-mortem examination. Between 2013 and 2015, livers were collected from 16 otters found dead in Scotland (Table 1 & Fig. 1) for analysis of PBDE, tri-BDE through nona-BDEs, residues (see analytical methods section).

Table 1 Summary of livers analysed from each year in which they were found for otters.

	Sub-Adult/Juvenile		Adult		
Year collected	Female	Male	Female	Male	Total
2013	1/0	0/0	1	1	3
2014	3/1	3/0	3	1	11
2015	0/0	0/0	1	1	2

3.2. Analytical methods

The liver samples were analysed at the centralized analytical laboratories at the Centre for Ecology and Hydrology, Lancaster. Concentrations of 32 BDEs (6 tri -BDEs, 6 tetra – BDEs, 6 penta-BDEs, 4 hexa-BDEs, 2 hepta-BDEs, 5 octa-BDEs and 3 nona-BDEs) were quantified. 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 (^{13}C PBDEs and ^{13}C 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.

All BDEs were analysed by GCMS. Each extract was spiked with labelled internal standards and 100µl of sample was injected into a 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.

Residues were quantified as recovery corrected concentrations using an internal standard correction method and calibration curves based on PBDE standards (Greyhound Ltd, Birkenhead, UK and LGC Ltd, Teddington, UK). Average recoveries for ^{13}C-PBDE recovery standards for BDE 28, BDE 47, BDE 126, BDE 153 and BDE 197 ranged between 94% and 98%.

3.3. Data expression, format and analysis

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 GraphPad Prism (Version 5.04 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). As often found for contaminant residues in wildlife, BDE residues in otters were left-skewed in distribution (preponderance of lower concentrations) and only a few otters had relatively high liver concentrations of contaminants. We therefore present summary statistics of BDE concentrations as geometric means.

4. Results and Discussion

4.1. Congener PBDE profile in the livers of otters from Scotland that died between 2013 and 2015

PBDE residues were detected in the livers of all otters. The residue profile was dominated by BDE congener 47 which accounted for, on average, 44% of the sum PBDE (ΣPBDE) concentration (Figure 2). BDEs 153 and 100 accounted for a further 32% of the ΣPBDE concentration (Figure 2). This concurs with previous studies of otters from England & Wales in which BDEs 47, 153 and 100 were the dominant congeners of mono- to hepta-BDEs (Pountney *et al.*, 2015, Walker *et al.*, 2013).

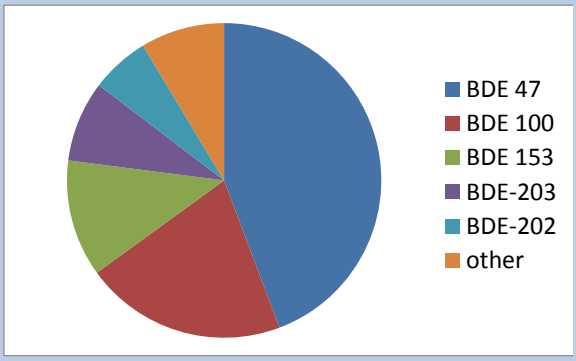


Figure 2. Congener PBDE profile in the livers of 16 otters from Scotland that died between 2013 and 2015. Data for each congener are the % contribution to the ΣBDE concentration in the liver. 'Other' includes congeners BDE 99, 118, 190, 196, 197, 201, 207, & 208. Data are averaged across all livers.

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 2). Tri-BDE concentrations were all below the limit of detection while geometric mean concentrations of ΣPenta-BDE, ΣHexa-BDE, and ΣOcta-BDE concentrations were similar to each other ranging between 0.8 and 1.6 ng/g wet wt. ΣHepta-BDE and Nona-BDEs were detected in only four otters with geometric mean concentrations of 1.7 and 2.0 ng/g wet wt., respectively.

PBDE residues measured in the present study, and in the studies by Pountney *et al.* (2015) and Walker *et al.* (2013) indicated a skewed distribution of residues with most being towards lower concentrations. Sum PBDE concentrations in livers ranged from 0.601 to 74.7 ng/g wet wt. (Table 2). This was equivalent to 15.8 to 1928 ng/g lipid weight[3]. Sum PBDEs in Scottish otters were significantly lower (student t-test on log-transformed data: t_{95}=3.72, P=0.0003) than those we measured in otters from England and Wales in our previous study (Walker et al. 2013); this comparison involved using summed congener concentrations calculated on the basis of exactly the same BDE congeners. Similarly, ΣTetra-, ΣPenta-, and ΣHexa-BDE concentrations were 16-, 3- and 3-fold lower, respectively, in Scottish otters compared to those we measured previously (Walker et al. 2013) in otters from England and Wales (T-test $t_{78} \geq 3.87$, $P \leq 0.0002$). More Scottish otters had detectable levels of the more brominated BDEs in their livers compared to those from England and Wales but this is likely

[3] Mean lipid content and the mean conversion factor for transforming wet weight to lipid weight concentrations in samples were 3.9% and 25.6, respectively.

due to improved analytical sensitivity in the current study; for example the limit of detection for BDE 197 was 0.139 and 12.03 ng/g wet weight in the current and previous study, respectively.

Physiological and histpathological effects of PBDEs in wildlife have been demonstrated in a variety of 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 5ppm PBDE commercial mixture DE71 in their diet which resulted in associated ΣBDE liver concentrations of 18,505 ng/g lipid wt. Sensitivity to chemical contaminants can very markedly between species and may vary with differences in congener profile, but the highest liver summed PBDE concentration in the 16 otters in the present study were approximately an order of magnitude lower than concentrations associated with adverse effects in mink.

Table 2. Geometric mean (Geomean), 95% geometric confidence interval (95%CI), and range of liver PBDE concentrations (ng/g wet wt.) in otters that died in Scotland between 2013 and 2015 [1] and which had detectable residues

Compound[2]	N[3]	Geomean	Lower 95% CI	Upper 95% CI	Min	Max
BDE 47	16	2.046	1.147	3.469	0.354	10.97
ΣTetra-BDE	16	2.046	1.147	3.469	0.354	10.97
BDE 100	13	1.447	0701	2.988	0.577	52.94
BDE 99	3	0.209	0.090	0.484	0.159	0.305
BDE 118	2	N/A[4]	N/A	N/A	0.145	1.081
BDE 118	2	N/A	N/A	N/A	0.145	1.081
ΣPenta-BDE	14	1.612	0.814	3.193	0.577	52.94
BDE 153	14	0.872	0.296	2.384	0.144	44.50
BDE 138	1	N/A	N/A	N/A	0.427	0.427
ΣHexa-BDE	15	0.932	0.339	2.561	0.144	53.65
BDE 183	1	N/A	N/A	N/A	0.369	0.369
BDE 190	4	1.642	0.069	39.15	0.278	9.746
ΣHepta-BDE	4	1.660	0.068	40.28	0.278	9.746
BDE 197	6	0.344	0.177	0.668	0.148	0.688
BDE 196	4	0.169	0.106	0.269	0.139	0.261
BDE 202	13	0.289	0.120	0.699	0.062	5.115
BDE 201	5	0.139	0.077	0.249	0.077	0.268
BDE 203	11	0.800	0.360	1.777	0.233	5.410
ΣOcta-BDE	16	0.808	0.354	1.845	0.062	11.24
ΣPBDEs	16	6.078	2.875	12.85	0.601	74.73

[1] 16 otters were analysed for all BDEs.
[2] BDE 17, 28, 30, 32, 35, 49, 51, 66, 71, 77, 85, 119, 126, 128, & 154 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, with congeners BDE47, BDE153 and BDE100 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 lower than those associated with adverse effects in mink. The general low level of detection of PBDEs suggests that there is little evidence to date of toxicologically significant contamination of Scottish otters with these compounds. This study did provide evidence that the (albeit relatively small number of) Scottish otters we sampled have significantly lower liver PBDE residues than those measured recently in otters from England and Wales and this may reflect lower human population density. However, these results may not be representative of otters from throughout Scotland as the present sample came predominantly from the Inner Hebrides.

6. Acknowledgements

We thank all those who submitted otters to the Cardiff University Otter Project (CUOP). This work would not have been possible without their efforts or those of a number of volunteers who assisted with post mortem examinations on the otters. We also thank Jacky Chaplow for preparing the map for this report.

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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	0.121
BDE 32	2,4',6-Tribromodiphenyl ether	189084-60-4	0.121
BDE 17	2,2',4-Tribromodiphenyl ether	147217-75-2	0.121
BDE 28	2,4,4'-Tribromodiphenyl ether	41318-75-6	0.121
BDE 35	3,3',4-Tribromodiphenyl ether	147217-80-9	0.121
BDE 37	3,4,4'-Tribromodiphenyl ether	147217-81-0	0.121
BDE 51	2,2',4,6'-Tetrabromodiphenyl ether	189084-57-9	0.121
BDE 49	2,2',4,5'-Tetrabromodiphenyl ether	243982-82-3	0.121
BDE 71	2,3',4',6-Tetrabromodiphenyl ether	189084-62-6	0.121
BDE 47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	0.121
BDE 66	2,3',4,4'-Tetrabromodiphenyl ether	189084-61-5	0.121
BDE 77	3,3',4,4'-Tetrabromodiphenyl ether	93703-48-1	0.121
BDE 100	2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	0.121
BDE 119	2,3',4,4',6-Pentabromodiphenyl ether	189084-66-0	0.121
BDE 99	2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	0.121
BDE 118	2,3',4,4',5-Pentabromodiphenyl ether	446254-80-4	0.121
BDE 85	2,2',3,4,4'-Pentabromodiphenyl ether	182346-21-0	0.121
BDE 126	3,3',4,4',5-Pentabromodiphenyl ether	366791-32-4	0.121
BDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	0.121
BDE 153	2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	0.121
BDE 138	2,2',3,4,4',5'-Hexabromodiphenyl ether	182677-30-1	0.121
BDE 183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	207122-16-5	0.121
BDE 128	2,2',3,3',4,4'-Hexabromodiphenyl ether		0.121
BDE 190	2,3',3,4,4',5,6-Heptabromodiphenyl ether	189084-68-2	0.121
BDE 202	2,2',3,3',5,5',6,6'-octabromodiphenyl ether		0.402
BDE 201	2,2',3.3',4,5',6,6'-octabromodiphenyl ether		0.402
BDE 197	2,2',3.3',4,4',6,6'-octabromodiphenyl ether		0.121
BDE 196	2,2',3.3',4,4',5,6'-octabromodiphenyl ether		0.402
BDE 203	2,2',3,4,4',5,5',6-octabromodiphenyl ether		0.121
BDE 208	2,2',3,3',4,5,5',6,6'-nonabromodiphenyl ether		2.008
BDE 207	2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether		2.008
BDE 206	2,2',3,3',4,4',5,5',6-nonabromodiphenyl ether		2.008