Predatory Bird Monitoring Scheme
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Polybrominated Diphenyl Ethers (PBDEs) in Eurasian otters (*Luta lutra*) collected from Britain in 2010: a Predatory Bird Monitoring Scheme (PBMS) Report

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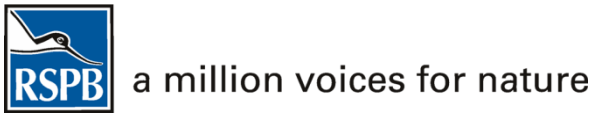
Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL



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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.

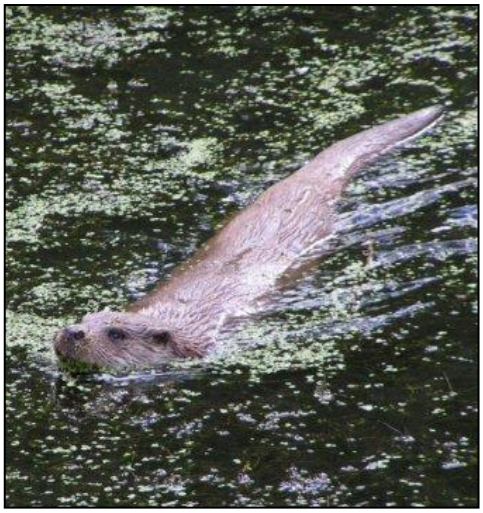
This is the first report on the findings of a collaborative study between the Predatory Bird Monitoring Scheme (PBMS) and the Cardiff University Otter Project (CUOP) in which the concentrations of Polybrominated Diphenyl Ethers (PBDEs) were determined in the livers of 30 Eurasian otters (*Lutra lutra*) found dead in 2010. The principle aim of this work was to determine the current concentrations of PBDEs accumulated by otters and whether there was any evidence of regional differences in sum PBDE concentrations.

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

PBDEs were present in all otters analysed, while other newer flame retardants (replacements for some of the PBDEs) were detectable in 7 of the 30 livers tested. Individual PBDE congener profiles were dominated by BDE 47 (78% of sum PBDE concentrations wet weight) with BDE 153 and BDE 100 accounting for a further 19% of the PBDE tissue load. The concentrations of ΣPBDEs 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.

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 (approximately 5/year) which limits ability to detect 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 utilize 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. The latest PBMS report on inorganic elements in otters can be downloaded from the PBMS website (http://pbms.ceh.ac.uk/), while this is the first PBMS report to summarize polybrominated diphenyl ether (PBDE) concentrations in otter livers.

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, added to 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 (Crosse *et al.*, 2012). They are resistant towards acids and bases as well as heat and light and also to reducing or oxidising compounds, so are, therefore, persistent in the environment. PBDEs are of environmental concern because of their high lipophilicity and high resistance to degradation processes, and because they are expected to readily bioaccumulate (Rahman *et al.*, 2001). Some PBDE congener mixtures are immunotoxic to mustelids (Martin *et al.*, 2007).

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).

The aim of this pilot study was to quantify liver concentrations of PBDEs in the livers of a representative sample of Eurasian otters collected from different regions of England and Wales and to determine whether there was evidence of regional variation in accumulation.

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). A motion to incorporate penta BDEs in the Stockholm Convention on Persistent Organic Pollutants (POPs) has been tabled and hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromodiphenyl ether) have been included in amendments to Annexes A/B/C (UNEP, 2010). Determination of BDE concentrations in otters provides scientific evidence of whether such mitigation has been successful in terms of ensuring exposure in otters is low.

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 analyzed 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 Agency[2].

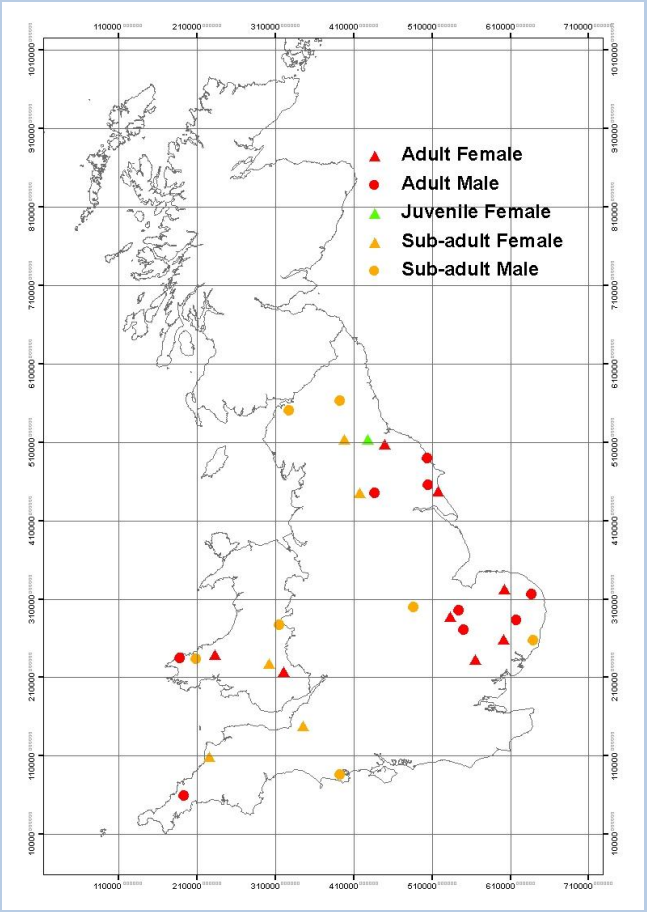


Figure 1. Location of otters, *Lutra lutra*, found dead in 2010 for which livers were analysed for flame retardants (total of 30 otters).

A sample of 30 otter livers were collected for PBDE analysis. They were from a stratified subset of animals found dead and collected by the CUOP in 2010; stratification was, where possible, by sex, age-class and provenance (Northern England, Eastern England, Wales and south-western England; Figure 1).

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 and 2 octa-BDEs) were quantified, together with concentrations of six new flame retardants that are currently used as replacements to the phased out lower brominated PBDEs. The list of compounds that were determined is

[2] the latest CUOP report can be downloaded at http://publications.environment-agency.gov.uk/pdf/SCHO0307BMKP-e-e.pdf.

given in Table 1 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 labelled 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.

The extract was spiked with labelled internal standards and 100µl of sample was injected into a GC-MS with programmable temperature vaporization (PTV) inlet. For PBDEs 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 at a rate of 10°C min^{-1}. For BFRs the PTV injector was kept at 60°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 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 (flow rate of 2.0 ml min^{-1}). For PBDEs 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. For other FRs the temperature programme was: isothermal at 80°C for 2.4 min, 30°C min-1 to 250°C and was held at 250°C for 33 min, 30°C min-1 to 315°C and isothermal at 315°C for 7 min.

Residues were quantified using an internal standard correction method and also calibration curves of the standard PBDEs and FRs (Greyhound Ltd, Birkenhead, UK and LGC Ltd, Teddington, UK), and were recovery corrected. Average recoveries for 13C-PBDE recovery standards range between 92% 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 that were below the limit of detection (non-detected) were assigned a zero value.

As with most chemical contaminants, the residue data were skewed towards lower concentrations with only a few otters having relatively high concentration. Summary statistics of the residue data are therefore presented as geometric means. Concentration values were log-transformed prior to statistical analysis in order to satisfy the assumptions of the tests. All statistical tests were performed using Minitab (Version 16.1.0; Minitab Ltd., Coventry, UK).

4. Results and Discussion

4.1. PBDEs

PBDE residues were detected in the livers of all 30 otters. PBDE residues were dominated by BDE congener 47 which accounted for, on average, 78% of the sum PBDE (ΣPBDE) concentration (Figure 2). BDEs 153 and 100 accounted for a further 19% of the ΣPBDE concentration (Figure 2). This concurs with an unpublished previous study of otters from England & Wales in which BDEs 47, 153, & 100 were the dominant congeners of mono- to hepta-BDEs (Pountney, 2008). However, the relatively high limit of detection for the higher brominated congeners (BDE 128, 190, 196 & 197) in the present study means they may be under-represented in the PBDE profile.

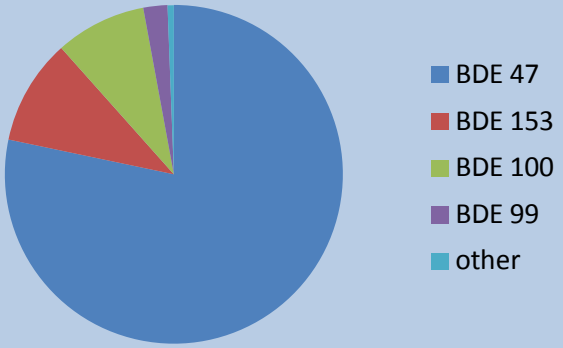


Figure 2. Congener PBDE profile in otter livers from England & Wales. Data for each congener are the % contribution to the ΣBDE concentration in the liver. 'Other' includes congeners BDE 28, 37, 85, 118, 119, 126, 154 & 183. Data are averaged across all livers.

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 1). Sum Tri-BDE concentrations were low compared with other bromination groups while mean concentrations of ΣPenta-BDE, ΣHexa-BDE, and ΣHepta-BDE concentrations were similar to each other ranging between 4 and 7 ng/g wet wt.

Sum PBDE concentrations in livers ranged from 3 to 718 ng/g wet wt. (Table 1). This is equivalent to 92 to 19,890 ng/g lipd 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 and sex as potential factors indicated, as with the study by Pountney (2008), that ΣPBDE concentrations did not significantly vary with these factors ($F \leq 1.12$, $P \leq 0.368$). Pountney (2008) did demonstrate that the concentrations of higher brominated BDEs did vary significantly according to geographical region, but these congeners were not quantified in our report.

Physiological and histpathological 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 ΣBDE liver concentrations of 18505 ng/g lipid wt. While it is important to note that sensitivity to chemical contaminants can very markedly between species, the maximum ΣBDE liver concentrations measured in the current study exceeds the residue levels associated with adverse effects noted in the study by Martin et al (2007). However, there were also significant differences in the congener profile in the study on ranch mink compared to those observed in otters in the current study, and this may affect toxicity.

Table 1. Geometric mean (Geomean), 95% confidence interval (95%CI), and range concentrations (ng/g wet wt.) of poly-brominated diphenyl ethers (PBDEs) in the livers of Eurasian otters received in 2010.

			95% C.I.			
Compound[1]	N[2]	Geomean	Lower	Upper	Min	Max
BDE 28	8	0.335	0.252	0.443	0.190	0.512
BDE 37	1	0.199	N/A[3]	N/A	0.199	0.199
ΣTri-BDE	9	0.316	0.240	0.416	0.190	0.512
BDE 47	30	39.24	25.47	60.45	2.585	465.1
ΣTetra-BDE	30	39.24	25.47	60.45	2.585	465.1
BDE 100	28	4.952	3.004	8.165	0.432	146.0
BDE 119	1	0.550	N/A	N/A	0.550	0.550
BDE 99	25	1.401	0.906	2.166	0.342	37.53
BDE 118	2	0.817	0.007	92.25	0.563	1.185
BDE 85	1	1.610	N/A	N/A	1.610	1.610
BDE 126	1	3.301	N/A	N/A	3.301	3.301
ΣPenta-BDE	28	6.435	3.981	10.40	0.489	188.0
BDE 154	14	0.712	0.453	1.118	0.418	8.676
BDE 153	30	3.873	2.439	6.149	0.416	158.5
ΣHexa-BDE	30	4.123	2.591	6.563	0.416	159.0
BDE 183	1	5.730	N/A	N/A	5.730	5.730
ΣHepta-BDE	1	5.730	N/A	N/A	5.730	5.730
ΣBDEs	30	50.56	32.26	79.25	3.001	717.8

[1] BDE 17, 30, 32, 35, 49, 51, 66, 71, 77, 128, 138, 190, 196 & 197 were not detected in any of the 30 samples analysed.

[2] N indicates number of samples with concentrations above the limit of detection.

[3] Parameter not calculated as sample size too small.

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 quantify the concentrations of six of these non-PBDE flame retardants during the same analysis run as the PBDEs (see appendix for full list of analytes). These flame retardants were at detectable concentrations in only a few (7/30) of the samples. Hexabromocyclododecane (HBCD) was detectable in 4 of the samples, while Hexabromobenzene (6BrBz) and 2-Dechlorane (DC2) were each detected in 2 otters. Concentrations, where detected, ranged from 0.317-104.5 ng/g wet wt., and in all cases concentrations were within 1 order of magnitude of the limit of detection.

5. Conclusions

PBDEs were detected in all otter livers analysed, while other flame retardants were detected in a smaller proportion 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.

6. Acknowledgements

We thank all the members of the public who have submitted otters to the Cardiff University Otter Project (CUOP). Their efforts are key to the success of the scheme. Zoe Deakin, Sarah Paul and a number of volunteers assisted with post mortem examinations on the otters. We also thank Jacky Chaplow for the map for this report.

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8. Appendix

Table 1. 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	0.158
BDE 32	2,4',6-Tribromodiphenyl ether	189084-60-4	0.158
BDE 17	2,2',4-Tribromodiphenyl ether	147217-75-2	0.158
BDE 28	2,4,4'-Tribromodiphenyl ether	41318-75-6	0.158
BDE 35	3,3',4-Tribromodiphenyl ether	147217-80-9	0.158
BDE 37	3,4,4'-Tribromodiphenyl ether	147217-81-0	0.158
BDE 51	2,2',4,6'-Tetrabromodiphenyl ether	189084-57-9	0.158
BDE 49	2,2',4,5'-Tetrabromodiphenyl ether	243982-82-3	0.158
BDE 71	2,3',4',6-Tetrabromodiphenyl ether	189084-62-6	0.158
BDE 47	2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	0.158
BDE 66	2,3',4,4'-Tetrabromodiphenyl ether	189084-61-5	0.317
BDE 77	3,3',4,4'-Tetrabromodiphenyl ether	93703-48-1	0.158
BDE 100	2,2',4,4',6-Pentabromodiphenyl ether	189084-64-8	0.317
BDE 119	2,3',4,4',6-Pentabromodiphenyl ether	189084-66-0	0.317
BDE 99	2,2',4,4',5-Pentabromodiphenyl ether	60348-60-9	0.317
BDE 118	2,3',4,4',5-Pentabromodiphenyl ether	446254-80-4	0.317
BDE 85	2,2',3,4,4'-Pentabromodiphenyl ether	182346-21-0	0.633
BDE 126	3,3',4,4',5-Pentabromodiphenyl ether	366791-32-4	0.633
BDE 154	2,2',4,4',5,6'-Hexabromodiphenyl ether	207122-15-4	0.317
BDE 153	2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	0.317
BDE 138	2,2',3,4,4',5'-Hexabromodiphenyl ether	182677-30-1	0.633
BDE 183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	207122-16-5	2.375
BDE 128	2,2',3,3',4,4'-Hexabromodiphenyl ether		9.896
BDE 190	2,3',3,4,4',5,6-Heptabromodiphenyl ether	189084-68-2	39.59
BDE 197	2,2',3,3',4,4',6,6'-Octabromodiphenyl ether		98.96
BDE 196	2,2',3,3',4,4',5,6'-Octabromodiphenyl ether		98.96
5BrMeBz	Pentabromomethylbenzene		0.169
5BrEtBz	Pentabromoethylbenzene		0.169
6BrBz	Hexabromobenzene		0.338
HBCD	Hexabromocyclododecane		10.56
DC1	Dechlorane plus 1		0.338
DC2	Dechlorane plus 2		0.338