Concentrations of PBDEs in a Top UK Predator, the European Otter (*Lutra lutra*), With Particular Attention to BDE209

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in a wide range of man-made products, and interest in levels of PBDEs in the environment has increased substantially in recent years because of their potential to affect hormone levels and alter behaviours[1, 2]. Of particular interest is their bioavailability and biomagnification capabilities and toxicity in wildlife. BDE209 is especially interesting because it is still in use, and in relatively immobile in the environment, yet has been found in animals in fairly remote locations. Declines in otter populations during the 1950s and 60s have been linked with levels of PCBs, dieldrin and other OCs[3], although these findings are not conclusive. As a top UK predator and a semi-aquatic species, the European otter (*Lutra lutra*) is an ideal candidate for studies of the biomagnification of organic pollutants, including 'new' compounds, such as PBDEs. In this study we investigate the concentrations of PBDEs in otter carcasses collected as part of the Environment Agency's monitoring programme in England and Wales.

Materials and Methods

Material for this work was derived from otters collected as part of an Environment Agency (EA) monitoring programme. Otter carcasses, reported by members of the public, were collected by the EA and sent for *post mortem* examination. *Post mortems* were carried out by either the Wildlife Veterinary Centre in Cornwall (Vic Simpson) or by the Cardiff University Otter Project (Elizabeth Chadwick). If the animal was in a suitable condition, i.e. not crushed or decomposed, several tissue samples, including liver, were collected. Collected liver samples were securely wrapped in foil before being placed in a plastic bag and deep frozen for storage and transportation. The Environment Agency's otter collection programme was set up in 1988, with the primary directive to measure and monitor levels of PCBs and OCs within the otter population.

During sample preparation for measurement of PBDEs, approximately 5mm of liver tissue was removed from the outer surface of the liver tissue sample and discarded (to reduce the potential influence of any possible contamination during *post mortem* examination and to avoid effects from the loss of liquids from the outer tissue during defrosting), and then approximately a 5g section was taken and homogenised with anhydrous sodium sulphate. These samples were then spiked with BDEs-51, 128 and 190 recovery standards and ^{13}C labelled BDE-209 isotope dilution standard before Soxhlet extraction with dichloromethane.

After extraction, an aliquot of the extract was removed and evaporated to dryness to calculate the extracted lipid content. The remaining extract was evaporated and transferred to hexane before being cleaned with several washes of 15% fuming sulphuric acid in concentrated sulphuric acid. A further clean up using gel permeation chromatography (GPC) was carried out before samples were evaporated

to 25µl of dodecane containing internal standards. Throughout the analytical procedure care was taken to keep exposure of samples to sources of light to a minimum.

Samples were analysed for BDEs 206, 207, 208 and 209 using a VG Autospec Ultima, running in EI+ mode. The GC used cool on-column injection, helium carrier gas, and was fitted with a 15 m DB5-MS column with a 2 m retention gap. Quantification was performed using ^{13}C BDE209 as an isotope dilution internal standard.

Samples were analysed for BDEs-17, 28, 32, 35, 37, 47, 49, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 166, 181, 183, 196 and 197 using an MD800 GC-MS running in NICI mode, with ammonia reagent gas. The GC used splitless injection, helium carrier gas, and was fitted with a 30 m DB5-MS column, with a 2 m retention gap.

For every nine liver samples, two blanks and one internal in-house reference material were also extracted and analysed. Results from the blanks were averaged and subtracted from liver sample values to account for background levels within the laboratory. Results from the blanks were also used to calculate limits of detection for individual samples, taking into account differences in the wet weight and lipid content of the samples.

Results and Discussion

Thirty-eight otter liver sample have been analysed, covering areas across England and Wales. Results indicate that BDE47 is by far the most dominant congener, with concentrations approximately ten-fold higher than those for any other BDE congener. The next highest concentrations were of BDE100, BDE99 and BDE153. Some of the most interesting findings relate to the relatively high concentrations of BDE209 within the liver samples, the results of which are shown in table 1.

Concentrations of BDE209 in the otters appear to be comparable across all the English and Welsh sites, with levels varying from below the limit of detection (typically $1.1 ng.g^{-1}$ wet weight) to around $30 ng.g^{-1}$ (wet weight). One exception to this was a particularly high concentration of almost $250 ng.g^{-1}$ (wet weight) found in an otter collected near Bridlington Bay in Humberside, North East England.

The pattern of congeners present, with high concentrations of BDE47, followed by slightly lower, but still significant concentrations of BDE99, 100 and 153 is similar to patterns seen in many studies of PBDEs within mammals and birds. Ratios of BDE99 and BDE100 need further study, but with far lower concentrations of BDE99 (typically at least half the concentration of BDE100) in many individuals it is possible that metabolism of this congener is common in the UK otter population. BDE209 has previously been observed in relatively few species[4, 5], with levels rarely much higher than the detection limit. Concentration of BDE209 in otter livers, although low compared with levels of other congeners, are much higher than would be expected based on results from other studies of PBDEs in biota. There are no known toxic effects of BDE209, although there is concern that it may debrominate to form more toxic PBDEs. High levels of BDE209, may, therefore, be of concern for otters, which appear to be susceptible to chemical influences on their populations.

Table 1. Concentrations of BDE209 in otter liver tissue, showing the year of collection and location.

Year	County	Area	BDE209 $ng.g^{-1}$ wet weight	BDE209 $ng.g^{-1}$ lipids
2005	Cornwall	SW England	9.4	45
2005	Cornwall	SW England	*	260
2005	Cornwall	SW England	8.0	173
2005	Cornwall	SW England	37	1241
2006	Cornwall	SW England	7.0	274
2006	Cornwall	SW England	2.5	*
2004	Devon	SW England	1.9	*
2005	Devon	SW England	*	66
2005	Devon	SW England	2.7	89
2006	Devon	SW England	*	122
2006	Devon	SW England	5.3	126
2004	Somerset	SW England	2.6	36
2005	Somerset	SW England	6.3	463
2005	Dorset	SW England	*	178
2005	Dorset	SW England	*	*
2006	Dorset	SW England	4.1	114
2006	Wiltshire	SW England	7.0	263
2004	Hampshire	SW England	1.8	38
1997	Dyfed	Wales	17	51
1999	Dyfed	Wales	*	71
2000	Dyfed	Wales	12	*
2001	Dyfed	Wales	1.7	91
2004	Dyfed	Wales	2.8	183
2004	Dyfed	Wales	6.2	95
2004	Powys	Wales	9.0	160
2004	Powys	Wales	*	151
2005	Powys	Wales	2.0	*
2004	Hereford and Worchester	Wales/Midlands	5.4	107
2006	Shropshire	Wales/Midlands	7.8	76
2004	Cheshire	Midlands	2.5	203
2003	Leicestershire	Midlands	7.8	123
2003	Bedfordshire	Midlands	6.6	152
2004	Northamptonshire	Midlands	5.0	*
2004	Northamptonshire	Midlands	7.9	*
2006	Norfolk	E England	1.3	38
2006	Essex	E England	4.3	82
2005	Humberside	NE England	248	6485
2005	Northumberland	NE England	3.6	*

* Below limit of detection

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