PBDEs in the European Environment Using Great Tit Eggs as a Biomonitoring Tool

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Introduction.
Polybrominated diphenyl ethers (PBDEs) are a group of chemicals that are widely used in different materials because of their flame retarding properties. Large production and use have led to their ubiquitous presence in the environment and in biota (Hites 2004). Although information is still limited, various toxic effects of PBDEs, including neurotoxicity and teratogenicity, have been reported in man and wildlife (Darnerud 2003; McDonald 2002). The bioaccumulative, persistent and toxic character of some congeners is of increasing concern and shows the need for more data on environmental levels of PBDEs (Birnbaum and Staskal 2004).

Until now, only few studies have been performed to monitor the concentrations of POPs on a large geographical scale using the same model system, for example passive air samplers (Gioia et al. 2006; Jaward et al. 2004) and pine needles (Jensen et al. 1992). The objective of the present study was to investigate the levels of PBDEs in the European environment using the eggs of a terrestrial bird species, the great tit (*Parus major*), as a biomonitoring tool. Great tits have several characteristics which make them very useful as a biomonitor for pollutants (Van den Steen et al. 2006). The chief asset of great tits as biomonitors is their ubiquity, which permits sampling of almost any wooded area across Europe (Eens et al. 1999). Because great tits are cavity-nesting birds, nest sites are often a limiting resource and they will readily nest in man-made nest boxes. Thus, breeding populations can be rapidly established and monitored, and eggs can be easily collected. Recently, we have shown that one randomly collected egg of great tits represents the contamination levels of PCBs, PBDEs and DDTs of the whole clutch (Van den Steen et al. 2006).

Materials and Methods.
In the breeding season of 2006, researchers from 14 European countries collected great tit eggs from different clutches (one random egg per clutch) from 23 sampling locations (Table 1). Sampling locations were situated in urban, agricultural and more remote wooded areas. The eggs were stored frozen until further treatment.

We analysed 5 to 8 eggs per sampling location. A homogenised sample of approximately 0.5 g was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (BDE 77 and 128). Further sample treatment and analysis were performed according to previously described methods (Dauwe et al. 2003). Briefly, after Soxhlet extraction, the lipid content was determined gravimetrically on an aliquot of the extract. The rest of the extract was cleaned up on a column filled with ~8 g acidified silica and eluted with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100 µl under a gentle nitrogen stream and transferred to an injection vial. PBDEs were analysed using a gas chromatograph coupled with a mass spectrometer (GC/MS) in electron capture negative ionisation (ECNI) mode, equipped with a HT-8 capillary column (25 m x 0.22 mm x 0.25 µm). In all samples, 7 PBDE congeners (BDE 47, BDE 49, BDE 99, BDE 100, BDE 153, BDE 154 + BB153, BDE 183) were analysed. Limits of quantification (LOQ) for the analysed compounds ranged between 0.1 and 1.4 ng/g lipid weight (lw). Besides PBDEs, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were also analysed (not reported here).

The quality assurance was done as described by Dauwe et al. (2003) and Van Den Steen et al. (2006) and consisted in daily check of calibration curves and regular analyses of procedural blanks, solvent blanks and SRM 1945, which has indicative values for PBDEs. Recoveries for individual PBDE congeners were between 87 and 104% (RSD < 12%). Procedural blank values were found to be consistent (RSD < 10%) and therefore the mean procedural blank value was used for subtraction. The method was validated through a good performance during the participation of the Toxicological Centre in interlaboratory studies for PBDEs.

Table 1: Location and characteristics of the different sampling locations

	Location	Country	Location characteristics
B1	Meeuwen-Gruitrode	Belgium (E)	Agricultural
B2	Mortsel	Belgium (N)	Urban
CZ	Kunin	Czech Republic (NE)	Agricultural
D1	Max Planck Institute for Ornithology in Seewiesen and Landscape conservation area Seebuchet, district of Starnberg	Germany (S)	Forest
D2	Bahrdorf	Germany (N)	Forest
D3	Lingen/Emsland	Germany (NW)	Forest
E1	Sagunto	Spain (E)	Orange plantation
E2	Hoya de Guadix	Spain (S)	Remote/agricultural
E3	Sierra Collserola close to Barcelona	Spain (NE)	Forest/urban
E4	Ciudad Real	Spain (Central)	Forest
EST	near Kilingi-Nõmme	Estonia (SW)	Forest
F	Toulouse	France (S)	Urban
FIN1	Harjavalta	Finland (S)	Forest
FIN2	Oulu	Finland (W)	Forest
H	Near Budapest	Hungary (N)	Urban
I1	Rome	Italy (Central)	Urban/agricultural
I2	Sicily	Italy (S)	Forest
N	Maridalen, Oslo	Norway (S)	Forest/urban
NL	Buunderkamp	The Netherlands (Central)	Forest
P	Choupal, Coimbra	Portugal (W)	Forest/urban/agricultural
PL1	Sobieszewo Island	Poland (N)	Agricultural/urban
PL2	40 km east from Krakow	Poland (S)	Forest
S	Lund	Sweden (S)	Forest/urban

Statistical calculations were performed using Statistica for Windows (Statsoft 1997) and GraphPad Instat 3.06 for Windows (GraphPad Software). The level of significance was set at $\alpha = 0.05$ throughout this study. A multiple regression was performed to investigate if there was a correlation between the sum PBDE concentrations and the latitude, the longitude and the number of inhabitants per km^2 of the closest city/town. The data were log transformed to meet the assumptions of normality. After standardization, we conducted a principal component analysis (PCA) to compare the congener profiles among the sampling locations. A Spearman rank correlation was performed to investigate if there was a correlation between the sum PBDEs and the sum PCBs.

Results and Discussion.

Levels of PBDEs

Sum PBDE concentrations ranged from 3.97 ± 0.74 ng/g lw to 136 ± 19 ng/g lw (Figure 1). As expected, PBDE concentrations in the great tit eggs were much lower compared to the levels in predatory bird eggs from Europe (Herzke et al. 2005; Jaspers et al. 2006; Lindberg et al. 2004). PBDEs levels were also lower than the concentrations reported by Van den Steen et al. (2006) in the eggs of great tits from several locations near Antwerp (Belgium). A multiple regression revealed no correlations between the sum PBDEs and the latitude, the longitude and the number of inhabitants per

km^2 ($F_{3,19}$ = 1.51, R^2 = 0.19, p = 0.25). However, figure 1 shows that PBDE concentrations in eggs from sampling locations near densely populated cities (E3, F, I1, S) are in the higher range of concentrations. A monitoring study across Europe using passive air samplers also found high PBDE values in urbanized source areas (Jaward et al. 2004). In addition to urbanization, local contamination sources could also play an important role.

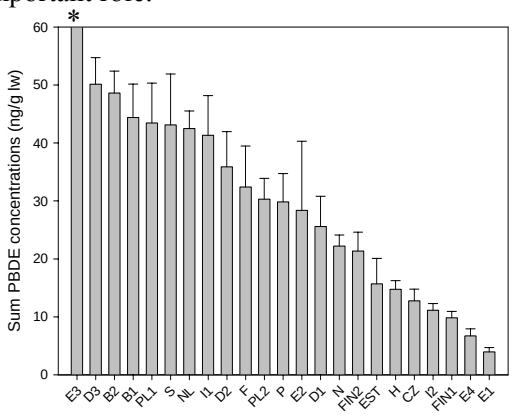

Figure 1: Sum PBDE concentrations (with standard errors) of the 7 analysed PBDE congeners in the eggs of great tits from different sampling locations in Europe, 5 ≤ n ≤ 8 per sampling location, locations are arranged from high to low concentrations, *136 ± 19 ng/g lw

Sum PBDE concentrations were significantly correlated with the sum PCB concentrations (Spearman rank correlation: n = 23, R = 0.71, p = 0.0002; Figure 2). This suggests similar exposure pathways of PCBs and PBDEs in great tit eggs. It can be observed that two sampling locations (CZ and E3; Figure 2) were highly divergent, suggesting different contamination sources of PCBs and PBDEs.

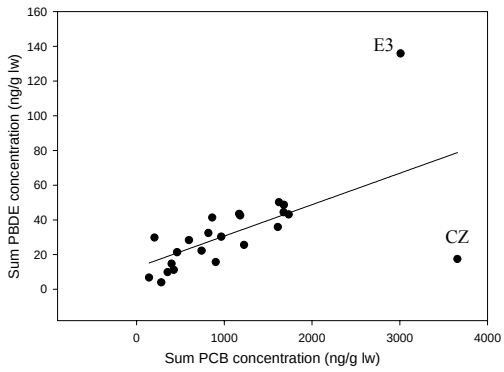

Figure 2: Relation between the mean sum PBDE and sum PCB concentrations in the eggs of great tits from different sampling locations in Europe (n = 23)

Profile differences among locations

A PCA revealed 2 factors (factor 1: BDE 47, BDE 99 and BDE 154 + BB 153; factor 2: BDE 100 and BDE 153) which together accounted for 75 % of the total variance. When plotting the corresponding factor scores, 2 sampling locations differed strongly from the other sampling locations. These 2 sampling locations were identified as E1 and F. The different congener profile of E1 can be explained by the low contamination levels of this location. Further investigation of the profile of F revealed that there was one egg sample with high levels of BDE 183 which was responsible for this divergent profile. When excluding this sample, profiles of the different sampling locations were quite similar (Figure 3). In most cases BDE 99 was the most abundant congener followed by BDE 47. Both congeners accounted for 66% of the total sum of PBDEs. A similar pattern has been observed in most

terrestrial bird species (Jaspers et al. 2006; Herzke et al. 2005). BDE 47 and BDE 99 are the major congeners in the Penta-BDE commercial mixture (WHO 1994). Although the Penta-BDE mixture has been withdrawn from the market in Europe in 2004 (Directive EEC 2003), the congeners present in this mixture are still ubiquitous in the environment (Hites 2004).

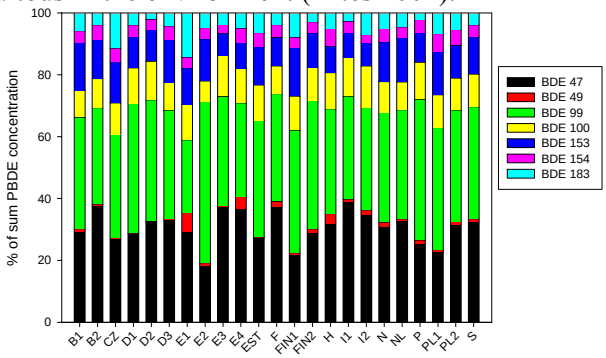

Figure 3: Contribution of PBDE congeners to the sum PBDE concentrations in the eggs of great tits from different sampling locations in Europe

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