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Brominated flame retardants and organochlorines in the European environment using great tit eggs as a biomonitoring tool

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ABSTRACT

Large-scale studies are essential to assess the emission patterns and spatial distribution of organohalogenated pollutants (OHPs) in the environment. Bird eggs have several advantages compared to other environmental media which have previously been used to map the distribution of OHPs. In this study, large-scale geographical variation in the occurrence of OHPs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), was investigated throughout Europe using eggs of a terrestrial residential passerine species, the great tit (*Parus major*). Great tit eggs from 22 sampling sites, involving urban, rural and remote areas, in 14 European countries were collected and analysed (5–8 eggs per sampling site). The environmentally most important congeners/compounds of the analysed pollutants were detectable in all sampling locations. For PCBs, PBDEs and OCPs, no clear geographical contamination pattern was found. Sum PCB levels ranged from 143 ng/g lipid weight (lw) to 3660 ng/g lw. As expected, PCB concentrations were significantly higher in the sampled urban compared to the remote locations. However, the urban locations did not show significantly higher concentrations compared to the rural locations. Sum PBDEs ranged from 4.0 ng/g lw to 136 ng/g lw. PBDEs were significantly higher in the urbanized sampling locations compared to the other locations. The significant, positive correlation between PCB and PBDE concentrations suggests similar spatial exposure and/or mechanisms of accumulation. Significantly higher levels of OCPs (sum OCPs ranging from 191 ng/g lw to 7830 ng/g lw) were

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detected in rural sampling locations. Contamination profiles of PCBs, PBDEs and OCPs differed also among the sampling locations, which may be due to local usage and contamination sources. The higher variance among sampling locations for the PCBs and OCPs, suggests that local contamination sources are more important for the PCBs and OCPs compared to the PBDEs. To our knowledge, this is the first study in which bird eggs were used as a monitoring tool for OHPs on such a large geographical scale.

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1. Introduction

The presence of organohalogenated pollutants (OHPs) in the environment has been a great cause of concern, because of their persistent character, bioaccumulative potential and adverse effects on both humans and wildlife (Vos et al., 2000). Different OHPs have, for example, been shown to cause effects on reproduction in birds through different mechanisms, such as eggshell thinning, embryotoxicity and effects on reproductive behaviour (Elliott and Martin, 1994; Fernie et al., 2008; Gilbertson et al., 1991; McCarty and Secord, 1999). However, sensitivity to the effects of OHPs varies among bird species, as for example passerine species have been shown to be less sensitive to the effects of DDE on eggshell quality than other groups of birds (Gill et al., 2003). There is also evidence of long-range transport of these substances to regions where they have never been used or produced. As a consequence these pollutants have been distributed worldwide and even remote locations, such as the polar regions, have been reached by these pollutants (Braune, 2007). Due to regulatory controls on the use of these compounds, there seems to be a decreasing temporal trend of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in biota (Jones and de Voogt, 1999). Nevertheless, concentrations in the environment are still prominent and may exert a potential health risk. Recently, the presence of polybrominated diphenyl ethers (PBDEs) in the environment has received much attention (Law et al., 2006). PBDEs are a group of chemicals that are widely used in different materials, such as plastics, textiles and foams, because of their flame retarding properties. Large-scale production and use have led to their ubiquity in the environment and in biota, in which PBDE levels have increased rapidly (Elliott et al., 2005; Hites, 2004; Norstrom et al., 2002). The production and use of the Penta- and Octa-BDE mixtures have recently been banned in Europe (Directive EEC, 2003). Currently, the Deca-BDE product is the only PBDE commercial mixture which can still be used in the EU. There is increasing evidence that BDE 209 in the Deca-BDE mixture may debrominate in the environment and in biota to form less-brominated BDE congeners which are more bioaccumulative and toxic than BDE 209 itself (Söderström et al., 2004; Stapleton et al., 2004; Van den Steen et al., 2007a).

Monitoring studies are essential to assess the current levels and risks of different OHPs in the environment. Birds' eggs have been used successfully to monitor OHPs in numerous studies (Donaldson et al., 1999; Elliott et al., 2005; Jaspers et al., 2005; Norstrom et al., 2002; Van den Steen et al., 2006; 2008). Eggs of most bird species can easily be collected and the removal of a single egg from a clutch is expected to have only a minor effect on the population level (Furness, 1993; Henny and Kaiser, 1996, Henny et al., 2004). Because eggs can readily be sampled from the same location each year, long-term monitoring studies using eggs are also feasible. Moreover, the study of widespread bird species enables monitoring on a larger spatial scale. Large-scale geographical studies are very valuable to obtain more information about the local usage, emission patterns and spatial distribution of OHPs. However, to date, few studies have monitored concentrations of OHPs in birds' eggs on a broad geographic scale. For example, double crested cormorant (*Phalacrocorax auritus*) and herring gull (*Larus argentatus*) eggs have been used to assess the spatial distribution of OHPs in the Great Lakes (Hebert et al., 1994; Ryckman et al., 1998). Other media that have been used to map the spatial distribution of OHPs are passive air samplers (Gioia et al., 2006; Jaward et al., 2004), pine needles (Jensen et al., 1992), tree bark (Simonich and Hites, 1995) and butter (Kalantzi et al., 2001). In contrast with these environmental media, birds are generally exposed to OHPs through the same routes of exposure (i.e. food) as humans. Another advantage of the use of birds is that they can be used as indicators of exposure and effects at the same time. Potential effects on reproduction and survival can be monitored and linked to the observed pollutants. Residential passerine bird species are particularly useful for monitoring local contamination with OHPs. In contrast with birds of prey and other species, residues in eggs of residential passerine species are expected to reflect local contamination much better because of their small home ranges, territories and foraging areas (Moore, 1966; Dauwe et al., 2006).

The great tit (*Parus major*) is a small insectivorous songbird species with a small foraging area close to the nest site (Cramp and Perrins, 1993). During the breeding season, they feed primarily on caterpillars, spiders and beetles (Cramp and Perrins, 1993). 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 in almost all the wooded areas of 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 rapidly be established and monitored, and eggs can easily be collected. Therefore, great tits are widely used as a study species in both behavioural and ecotoxicological research. They have, for example, been proven useful as long-term monitors of climate changes in Europe (Sanz, 2002; Visser et al., 2003). Great tits have large clutch sizes (up to 12 eggs; Cramp and Perrins, 1993) and one randomly collected egg has been shown to represent the contamination levels of PCBs, PBDEs and DDTs of the whole clutch (Van den Steen et al., 2006). In a previous study with great tit eggs, we investigated the small-scale geographical variation of OHPs in Flanders (Belgium; Van den Steen et al., 2008). In that study, levels of PCBs and PBDEs were highest in the industrialized sampling locations, whereas OCPs were highest in the rural locations. Previous studies with other monitoring tools, such as air samplers, have also shown that the levels of PCBs and PBDEs are linked to the degree of urbanization and industrial development and that OCP levels are highest in rural areas (Harner et al., 2006; Jaward et al., 2004). The objective of the present study was to investigate the geographical variation in the occurrence of different OHPs (PCBs, PBDEs and OCPs) in the European environment using the eggs of great tits. This was done to gather more information about background levels in Europe, to provide a baseline for long-term biomonitoring and to identify potential contamination sources. In addition, we investigated the influence of the type of sampling location on the presence of OHPs. To achieve this, we analysed great tit eggs from 22 sampling locations, involving urban, rural and remote locations, from 14 countries in Europe. Based on previous studies, urban sampling locations were expected to have the highest levels of PCBs and PBDEs, while OCPs were expected to be highest in the rural sampling locations.

2. Materials and methods

In the breeding season of 2006 (April–May), researchers from 14 European countries (Fig. 1) collected great tit eggs in 22 existing nest box populations. In agreement, one random egg per clutch (5–8 eggs per sampling site, see Table 1) was gathered. The eggs were collected before incubation, labeled individually and stored in a freezer (−20 °C) until transported to the laboratory. Eggs were transported on dry ice and stored frozen until further analysis. In total, 145 great tit eggs were analysed for PCBs, PBDEs and OCPs. A questionnaire was sent to the collectors in order

Fig. 1. Map of Europe with the sampling locations. Different types of sampling locations have different symbols: (sub-) urban (•), rural (*) and remote (+).

to characterise the sampling sites and potential contamination sources of OHPs. Sampling sites were located both in (sub-) urban, rural and remote areas (Fig. 1; Table 1). Urban sampling locations were closely located to a city or densely populated area. Rural sampling locations were characterised by agricultural activities (e.g. crop cultivation, fruit trees). Remote locations were not in the vicinity of a city, industrial sites or agricultural activities.

A homogenised sample of approximately 0.5 g whole egg was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (ε-HCH, CB 46 and 143, BDE 77 and 128). Extraction was carried out with 100 ml hexane/acetone (3:1, *v*/*v*) in an automated Soxhlet extractor (Büchi, Flawil, Switzerland) in hot extraction mode for 2 h. The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while 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. In all samples, concentrations of 22 PCB congeners (CB 28, 31, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 163, 170, 180, 183, 187, 194, 196 and 199), 7 PBDE congeners (BDE 47, 49, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p,p′*- and *o,p′*-DDT) and metabolites (*p,p′*-DDE and *p,p′*-DDD), hexachlorocyclohexanes (HCHs; α-, β- and γ-HCHs), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)), and hexachlorobenzene (HCB) were determined.

For the PCB analysis, an Agilent 6890 gas chromatograph (GC) connected to an Agilent 5973 mass spectrometer (MS) operated in electron ionisation (EI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with two ions monitored for each PCB homologue group. One µl of the cleaned extract was injected onto the column using the cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C at 700 °C/min), pressure pulse 25 psi, pulse time 1.5 min. The splitless time was 1.5 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.5 min, then increased to 180 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 12 min.

For the analysis of the OCPs and PBDEs, an Agilent 6890 GC connected to an Agilent 5973 MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 25 m × 0.22 mm × 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). Methane was used as moderating gas and the ion source, quadrupole and interface temperatures were set at 160, 150 and 300 °C, respectively. The MS was used in the SIM mode with two ions monitored for each pesticide in specific windows, while ions $m/z = 79$ and 81 were monitored for PBDEs during the entire run. One µl of the cleaned extract was injected onto the column using the cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C at 720 °C/min), pressure pulse 30 psi, pulse time 1.5 min. The splitless time was 1.5 min. Helium was used as carrier gas at constant flow (1 ml/min). The temperature of the HT-8 column was held at 90 °C for 1.5 min, then increased to 220 °C at a rate of 15 °C/min (held for 2.0 min), further increased to 242 °C at a rate of 3 °C/min and finally raised to 300 °C at a rate of 40 °C/min, held for 15 min.

Multi-level calibration curves in the linear response interval of the detector were created for the quantification, and good correlation ($r^2 > 0.999$) was achieved. The identification of OHPs was based on the relative retention times to the internal standard used for quantification, ion chromatograms and intensity ratios of the monitored ions. A deviation of the ion intensity ratios within 20% of the mean values obtained for calibration standards was considered acceptable. The quality control was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs, PBDEs and OCPs in whale blubber) was used to test the method accuracy. Determined concentrations were within 10% of the certified values. The quality control scheme is also assessed through regular

Table 1
Characteristics of the sampling locations

	Country	Classification	Number of analysed eggs	Habitat type	Industrial and/or agricultural activities
B	Belgium	Rural	6	Coniferous forest	/
CZ	Czech Republic	Rural	7	Deciduous forest	Metallic and chemical industry, intensive agriculture
D1	Germany	Rural	7	Mixed forest	Agricultural activities
D2	Germany	Rural	7	Mixed forest	/
D3	Germany	Rural	7	Coniferous forest	Chemical industry, nuclear power station, petroleum refinery
E1	Spain	Rural	7	Orange plantation	Intensive agriculture
E2	Spain	Rural	7	High altitude plateau with sparse vegetation	/
E3	Spain	Suburban	7	Natural forest close to Barcelona	Intensive industrial activities
E4	Spain	Remote	7	Deciduous forest and pine plantations	/
EST	Estonia	Remote	7	Coniferous and deciduous forest	Extensive agriculture
F	France	Suburban	7	Forest and garden near to Toulouse	Intensive agriculture
FIN1	Finland	Rural	7	Pine dominated mixed forests	Copper melter and related industry (heavy metals and sulphuric oxides)
FIN2	Finland	Suburban	5	Mixed forest near Oulu	Chemical industry, wood and paper industry, agriculture
H	Hungary	Remote	7	Oak woodland	/
I1	Italy	Suburban	7	Oak wood and small forest patches close to Rome	Intensive agriculture
I2	Italy (Sicily)	Remote	6	Deciduous forest	Extensive agriculture
N	Norway	Suburban	7	Woodland plots close to Oslo	/
NL	The Netherlands	Suburban	7	Mixed woodland	Gas station, motorway
P	Portugal	Suburban	7	Mixed forest	Agricultural activities
PL1	Poland	Rural	8	Coastal pine wood	Refinery, agricultural activities
PL2	Poland	Rural	7	Deciduous forest	Extensive agriculture
S	Sweden	Urban	7	Pine forest close to Lund	/

participation in interlaboratory comparison exercises organized by the Arctic Monitoring and Assessment Programme (AMAP) and the National Institute of Standards and Technology (NIST). For each analyte, the mean procedural blank value was used for subtraction. BDE 47 and 99 had blank levels which were lower than 5% of the values found in the samples. Nevertheless, the blank levels were subtracted from the sample values. After blank subtraction, the limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. LOQs for the analysed compounds ranged between 0.1 and 7.5 ng/g lipid weight (lw).

Statistical calculations were performed using Statistica for Windows (Statsoft, 1997). The level of significance was set at $\alpha = 0.05$ throughout this study. Data were normally distributed (Kolmogorov–Smirnov test: $p > 0.05$ for all cases) and therefore parametric tests were used. One-way ANOVAs were used to investigate whether there were geographical contamination patterns among the sampling locations in Northern (Estonia, Finland, Norway and Sweden), Eastern (Czech Republic, Hungary and Poland), Southern (Italy, Portugal and Spain) and Western (Belgium, France, Germany and The Netherlands) Europe and to study the differences in contamination levels among the sampling locations. We performed nested ANOVAs, in which sampling locations were nested within the different types, to investigate whether there was a difference in contamination levels among the urban, rural and remote sampling locations. Post hoc tests (Tukey HSD) were performed if there were significant differences among the sampling locations. Only the most notable significant differences among the sampling locations were presented in the results. Pearson correlations were performed to investigate if there were correlations among the PCB, PBDE and OCP concentrations. Variance (σ^2) in OHP levels among the sampling locations was assessed using F tests. After standardization, we conducted a principal component analysis (PCA) to compare the congener profiles among the sampling sites. Principal components (PCs) with eigenvalues above 1 were considered to account for a significant contribution to the total variance according to the latent root criterion (Hair et al., 1998). Factor loadings and factor scores were determined and used in interpreting PC patterns. Compounds with factor loadings greater than 0.65 on any PC were considered significant. The first two PCs were used for the statistical analyses.

3. Results

3.1. Levels of PCBs, PBDEs and OCPs

The mean proportion of lipids in the analysed eggs was 8.9 ± 0.4%. The environmentally most important congeners/compounds of PCBs, PBDEs and OCPs were detectable at all sampling locations. A one-way ANOVA revealed no geographical contamination patterns for either PCBs, PBDEs or OCPs ($F_{3,18} < 1.40$, $p > 0.28$ for all cases).

The concentrations of sum 22 PCB congeners ranged from 143 ± 19 ng/g lw in E4 (Spain) to 3656 ± 793 ng/g lw in CZ (Czech Republic; Fig. 2). Sum PCB concentrations differed significantly among the sampling locations (One-way ANOVA: $F_{21,129} = 13.74$, $p < 0.001$). Levels in eggs from CZ and E3 (Spain) were significantly higher than all the other sampling locations (Tukey HSD: CZ: $p < 0.001$ for all cases; E3: $0.00002 < p < 0.02$ for all cases; Fig. 2). There were no significant differences in sum PCB concentrations between the urban and rural sampling locations (Tukey HSD: $p = 0.36$), but both location types showed significantly higher concentrations compared to the remote locations (urban = rural > remote; Nested ANOVA: $F_{2,129} = 18.63$, $p < 0.001$; Tukey HSD: $p < 0.001$ for all cases).

The concentrations of sum 7 PBDE congeners ranged from 4.0 ± 0.7 ng/g lw at E1 (Spain) to 136 ± 19 ng/g lw at E3 (Spain; Fig. 2). Sum PBDE concentrations differed significantly among the sampling locations (One-way ANOVA: $F_{21,129} = 16.67$, $p < 0.001$). Levels in E3 were significantly higher compared to the other locations (Tukey HSD: $p < 0.001$ for all cases; Fig. 2). Sum PBDE concentrations were significantly higher in the urban sampling locations compared to the rural and remote locations (urban > rural > remote; Nested ANOVA: $F_{2,129} = 34.69$, $p < 0.001$; Tukey HSD: $p < 0.001$ for all cases). Eggs from the rural sampling locations showed significantly higher sum PBDE levels compared to the eggs from remote sampling locations (Tukey HSD: $p < 0.001$ for all cases). When E3 was excluded from the statistical analysis, the urban sampling locations still showed significantly higher concentrations compared to the remote locations (Nested ANOVA: $F_{2,123} = 20.66$, $p < 0.001$; Tukey HSD: $p < 0.001$), but not compared to the rural locations (Tukey HSD: $p = 0.23$).

The concentrations of sum OCPs ranged from 191 ± 32 ng/g lw in D1 (Germany) to 7829 ± 1489 ng/g lw in E1 (Spain; Fig. 2). Sum OCP concentrations differed significantly among the sampling locations (One-way ANOVA: $F_{21,129} = 17.80$, $p < 0.001$). Concentrations at E1 were significantly higher than in the other sampling locations (Tukey HSD: $p < 0.001$ for all cases). Sum OCP levels were significantly higher in the rural sampling locations compared to the remote and urban sampling locations (rural > remote > urban; Nested ANOVA: $F_{2,129} = 26.04$, $p < 0.001$; Tukey HSD: $p < 0.02$). Sum OCP levels in eggs from remote sampling locations tended to be higher compared to the urban sampling locations (Tukey HSD: $p = 0.06$).

Sum PBDE concentrations were significantly, positively correlated with the sum PCB concentrations (Pearson correlation: $n = 23$, $r = 0.59$, $p = 0.004$). Sum OCPs were not correlated with either the sum PCBs (Pearson correlation: $n = 23$, $r = -0.04$, $p = 0.86$) or the sum PBDEs (Pearson correlation: $n = 23$, $r = -0.27$, $p = 0.22$). Variance among sampling locations was highest for the OCPs, followed by the PCBs and PBDEs (F-test: σ^2(OCPs) > σ^2(PCBs): $p = 0.006$; σ^2(PCBs) > σ^2(PBDEs): $p < 0.001$; σ^2(OCPs) > σ^2(PBDEs): $p < 0.001$).

3.2. Profiles of PCBs, PBDEs and OCPs

CB 153, CB 180 and CB 138 were the most abundant PCB congeners and accounted for 28%, 19% and 12% of the sum PCBs, respectively. PCA revealed two PCs which accounted for 36% and 23% of the variance among the selected PCB congeners, respectively (Fig. 3a). There were significant differences in congener profile among the sampling locations for both PC1 and PC2 (One-way ANOVA: $F_{21,129} > 9.26$, $p < 0.001$ for all cases). CZ (Czech Republic) and E2 (Spain) showed a higher contribution of the higher chlorinated CB 180 and CB 187, and a lower contribution of CB 101, CB 105 and CB 118 compared to most of the other sampling sites (except E4, P, PL2 and S; Tukey HSD: CZ: $0.00002 < p < 0.03$; E2: $0.00002 < p < 0.07$; Fig. 3a).

BDE 99, BDE 47, BDE 153 and BDE 100 were the most abundant PBDE congeners and accounted for 36%, 30%, 12% and 11% of the sum PBDEs, respectively. PCA revealed two PCs which accounted for 48% and 22% of the total variance, respectively (Fig. 3b). With the exception of samples from NL (The Netherlands) and H (Hungary), profiles did not differ much among the sampling sites (Fig. 3b). However, we found significant differences in congener profile among the sampling locations for both PC1 and PC2

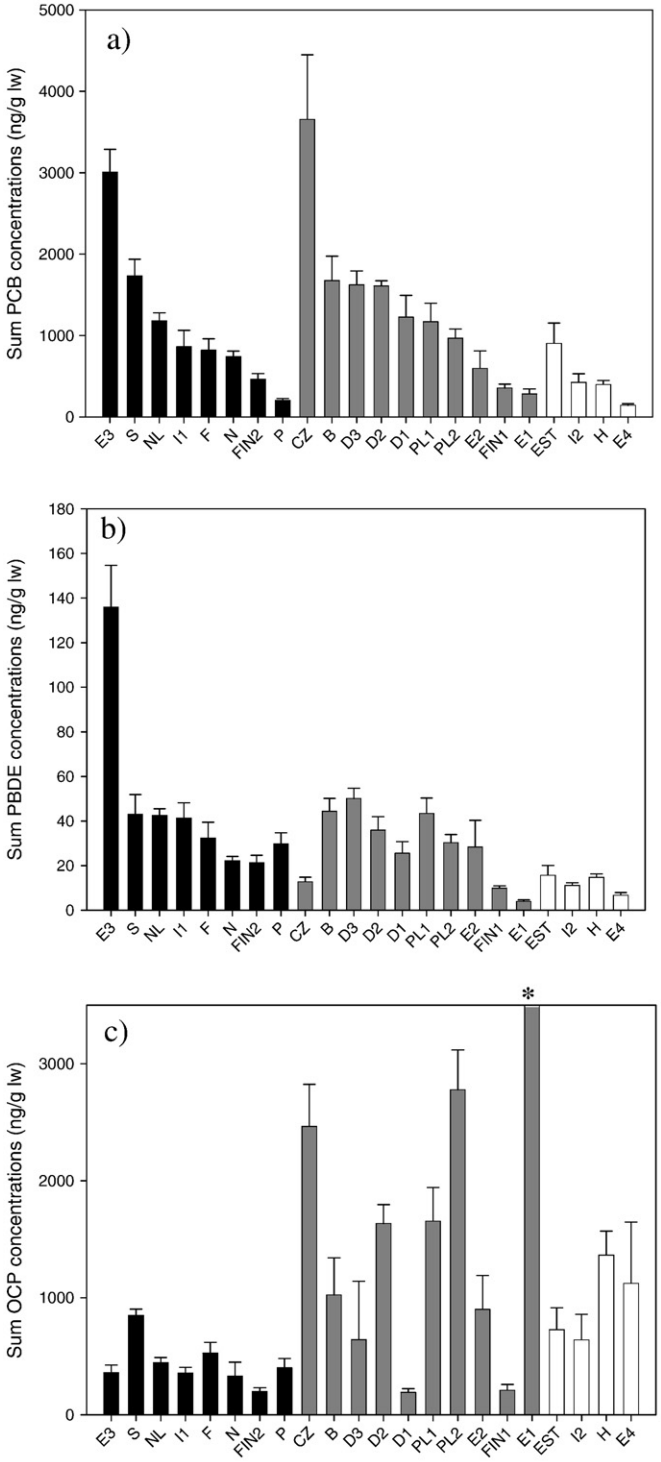

Fig. 2. Levels of sum PCBs (a), sum PBDEs (b) and sum OCPs (c): concentrations are expressed per gram lipid weight (lw). Black, grey and white bars represent the (sub-) urban, rural and remote locations, respectively. * 7830 ± 1490 ng/g lw.

(One-way ANOVA: $F_{21,129}>2.17$, $p<0.0044$ for all cases). For PC1, NL had a significantly higher contribution of BDE 49, BDE 100 and BDE 154 compared to other sampling locations (Tukey HSD: $0.002<p<0.02$; Fig. 3b). For PC2, H showed a significantly higher contribution of BDE 153 and BDE 183 compared to other locations (Tukey HSD: $0.0004<p<0.02$; Fig. 3b).

p,p′-DDE was the most abundant OCP and accounted for almost 90% of the sum OCPs. *p,p′*-DDT was detectable in all egg samples, except in one sample from each of France (F), Finland (FIN1) and Portugal (P). PCA revealed two PCs which accounted for 39% and 15% of the total variance, respectively (Fig. 3c). There were significant differences among the sampling locations for PC1 (One-way ANOVA: PC1: $F_{21,129}=17.05$, $p<0.001$; PC2: $F_{21,129}=1.00$, $p=0.46$). *p,p′*-DDE contributed less to the profile of the sampling locations in FIN2 (Finland) and N (Norway), while OxC, HCB and γ-HCH were more abundant in FIN2 and N compared to the other locations (Tukey HSD: $0.00002<p<0.007$ for all cases).

4. Discussion

To the best of our knowledge, this is the first study in which (song) bird eggs have been used as a biomonitoring tool for OHPs on such a large geographical scale. In this study, no clear geographical contamination pattern was found for the analysed OHPs. In addition to geographical variation, we also investigated the influence of the type of sampling locations on the presence of OHPs. The definition of the type of sampling location is inevitably somewhat subjective and not always straightforward. Moreover, potential contamination sources, which can have influenced the results, may have been overlooked. Nevertheless, the residue levels largely reflected the expected emission patterns. In the present study, PCBs and PBDEs were positively associated with urbanization and industrialization, while OCPs were highest in the rural sampling locations and were thus related to agricultural activities. However, PCB and PBDE concentrations were also high at the rural sampling locations, which might be due to the presence of local or more distant contamination sources. In addition, differences in diet and food availability may also be responsible for the differences in contamination levels among the sampling locations.

4.1. Levels of PCBs, PBDEs and OCPs

For PCBs, PBDEs and OCPs, concentrations differed significantly among the sampling locations. Sum PCB levels were significantly higher at the urban and rural sampling locations compared to the remote locations. PCBs have previously been linked to industrialization and urbanization (Jaward et al., 2004; Lovett et al., 1998; Van den Steen et al., 2008). Local contamination sources may be responsible for the unexpected relatively high PCB levels in some of the rural sampling locations. For example, PCBs levels were highest in the rural sampling location CZ (Czech Republic), which may be explained by metal and chemical industries in the neighbourhood (17–30 km) of this sampling location (personal observation P. Podzemny). Furthermore, higher concentrations in countries from Eastern Europe could reflect the fact that PCB production and use stopped somewhat later in this region compared to the rest of Europe (Breivik et al., 2002). However, PCB levels in Poland (PL1 and PL2), Estonia (EST) and Hungary (H) were not found to have particularly high concentrations (Fig. 2). Sum PCB levels in CZ (Czech Republic) and E3 (Spain) were significantly higher compared to the other sampling locations. As in CZ, heavy industrial activities were also reported in E3 (personal observation J.C. Senar), which is located near Barcelona (Spain, ca. 2 000 000 inhabitants).

Sum PBDE concentrations were significantly higher in the urban sampling locations compared to the rural and remote locations. As for the PCBs, PBDEs have also been linked to urbanization (Harrad and Hunter, 2004; Jaward et al., 2004). Sum PBDE levels were significantly higher in E3 (Spain) compared to the other sampling locations. As mentioned before, E3 is characterised by heavy industrial activities and localized close to a densely populated city (personal observation J.C. Senar). When excluding E3 from the statistical analysis, there was no significant difference between the PBDE concentrations in the urban and rural locations. As for PCBs, this is probably due to contamination sources in the immediate or distant vicinity of the rural sampling locations. To date, few data exist on the levels of PBDEs in passerine eggs. Previous studies with great tit eggs from different sampling sites in Belgium showed PBDE levels in the same range as in the present study (Dauwe et al., 2006; Van den Steen et al., 2008). Some of the highest sum PBDE concentrations (39 000 ng/g lw) in wildlife so far have been detected in peregrine falcon (*Falco peregrinus*) eggs from Sweden (Lindberg et al., 2004). Mean sum PBDE concentration in the peregrine falcon eggs was about 70 times higher than the levels in the

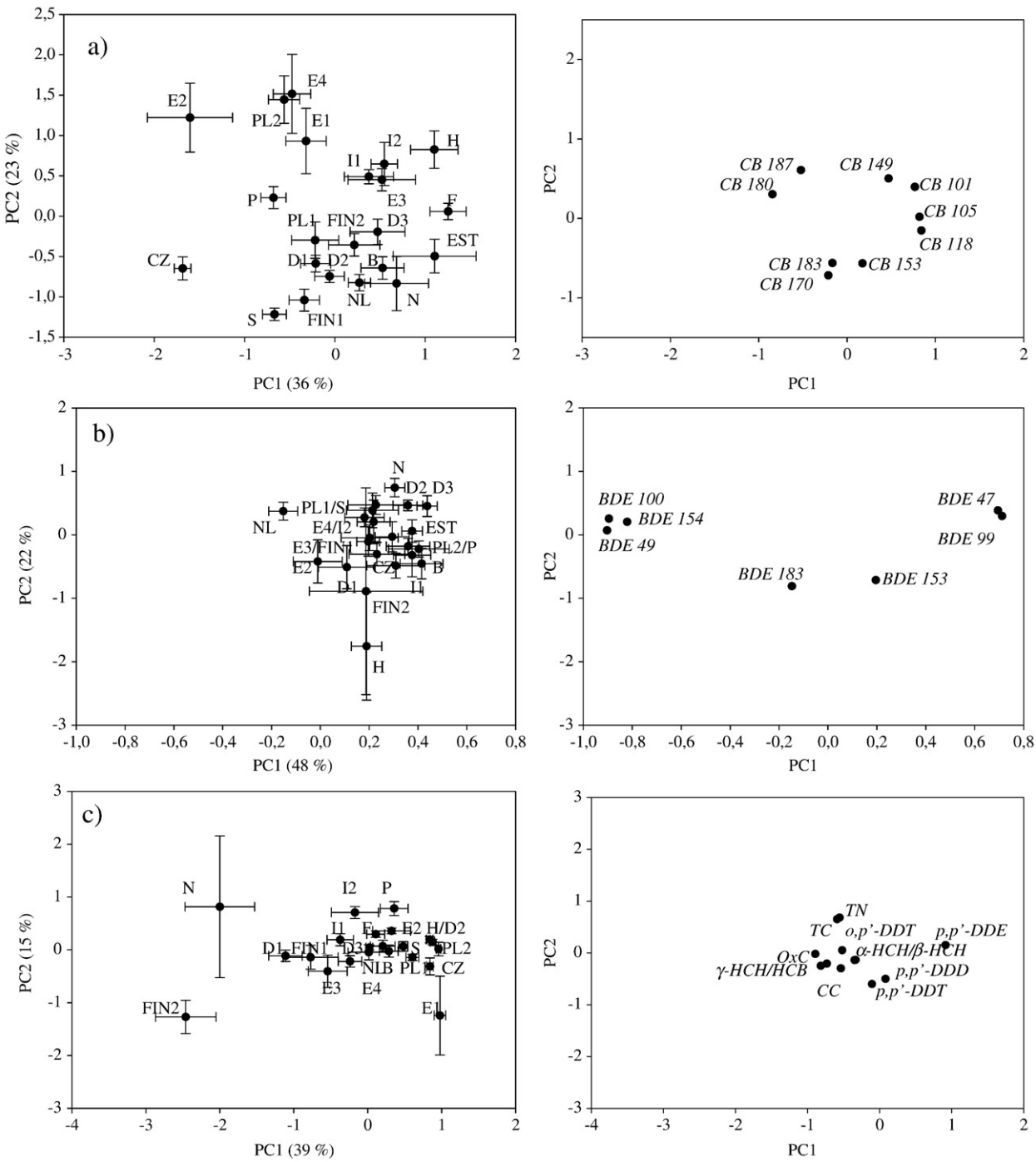

Fig. 3. Plots of factor scores with standard errors (left) and factor loadings (right) from the Principal Component Analysis (PCA) for the sum PCBs (a), sum PBDEs (b) and sum OCPs (c). Compounds with factor loadings greater than 0.65 on any PC were considered significant.

great tit eggs from Sweden in this study. However, peregrine falcons feed at a higher level in the food chain than great tits. Overall, PBDE levels were relatively low in all sampling sites of the present study. Different studies in biota and humans have shown that the North American environment is more contaminated with PBDEs compared to Europe (Hites, 2004). In addition, declines in PBDE levels have been reported for different matrices since the ban on the Octa- and Penta-BDE commercial mixtures in Europe (Johansson et al., 2006; Sellström et al., 2003).

As in the study of Van den Steen et al. (2008), OCP levels were highest in the great tit eggs from rural sampling locations. Studies using air samplers have also reported higher levels of different OCPs in rural locations (Jaward et al., 2004; Harner et al., 2004). Sum OCP levels in remote locations tended to be higher than in urban locations, which might be due to the vicinity of agricultural activities or the historical usage of OCPs in these areas. The highest concentration OCPs was observed in E1 (Spain), which is located in an extensive orange plantation in Eastern Spain. The large-scale use of pesticides is probably responsible for the observed levels.

The significant positive correlation between PCBs and PBDEs suggests similar spatial exposure and/or mechanisms of accumulation for these compounds. The higher variance in PCB and OCP levels suggests that local sources are more important for contamination with these compounds. On the other hand, the low variance in PBDE levels

suggests a lower contribution of local contamination sources for PBDEs in the sampled locations. PBDE levels in the present study are probably a good indicator of background contamination with PBDEs in Europe.

4.2. Profiles of PCBs, PBDEs and OCPs

CB 153, CB 180 and CB 138 were the most abundant PCB congeners. Previous studies with great tit eggs showed a similar congener profile (Van den Steen et al., 2006, 2008). However, we found significant differences in congener profile among the sampling locations. CZ (Czech Republic) and E2 (Spain) showed a higher contribution of the higher chlorinated PCBs (CB 180 and CB 187) and a lower contribution of lower chlorinated PCBs (CB 101, CB 105 and CB 118) compared to most of the other sampling sites. The PCB profile in eggs from CZ and E2 is probably due to contamination with Aroclor 1260, while in the other locations a mixture of Aroclor 1254 and Aroclor 1260 may be responsible for the observed patterns. Aroclor 1260 has high concentrations of CB 180, while Aroclor 1254 contains high concentrations of CB 118 (Frame et al., 1996). In addition, local contamination sources may be responsible for the different profiles among the sampling locations (Ormerod et al., 2000).

BDE 99, BDE 47, BDE 153 and BDE 100 were the most abundant PBDE congeners in all the sampling locations. Similar profiles have been observed in previous studies with great tits (Dauwe et al., 2006; Van den Steen et al., 2006, 2008). BDE 47 and BDE 99 are the major congeners in the Penta-BDE commercial mixture (WHO, 1994). Although the Penta-BDE mixture was 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). Except from NL (The Netherlands) and H (Hungary), profiles did not differ much among the sampling sites. The higher contribution of BDE 153 and BDE 183 in H compared to the other sampling locations suggests that, in addition to the Penta-BDE mixture, the Octa-BDE mixture may also be responsible for the observed contamination profile.

p,p′-DDE was the most abundant OCP and accounted for almost 90% of the sum OCPs. Since DDE is the major breakdown product of DDT, the accumulation profile of DDTs suggests a historical input rather than contribution from recent sources. DDT and its metabolites can still be found in the environment and in biota, although it has been banned in Europe for more than 25 years. *p,p′*-DDT, the principal compound of the technical DDT mixture, was detectable in most egg samples. The highly bioaccumulative *p,p′*-DDE contributed less to the profile of FIN2 (Finland) and N (Norway), while OxC, HCB and γ-HCH were more abundant at these sites compared to the other sampling locations. The local historical usage and specific applications of different OCPs may be responsible for the different profiles of FIN2 and N. In addition, other factors, such as differences in diet and variation in long-range transport, can also contribute to different profiles among sampling locations.

4.3. Toxicological significance

Different laboratory and field studies have documented reproductive and developmental problems in mammals and birds exposed to PCBs (Barron et al., 1995; Fernie et al., 2003; Gilbertson et al., 1991; Rice et al., 2003). Levels similar to the highest PCB concentrations reported in the present study have been shown to be responsible for reduced adult provisioning behaviour and decreased chick survival in European starlings (*Sturnus vulgaris*) from a PCB contaminated site (Arenal et al., 2004). On the other hand, similar PCB concentrations were not found to cause effects on breeding performance and survival in dippers (*Cinclus cinclus*; Ormerod et al., 2000). Although there is only a limited number of studies that have investigated the toxicity of PCBs in passerines, it seems that passerine species are less sensitive to the effects of PCB exposure compared to other species (Custer et al., 2003; Gill et al., 2003; Ormerod et al., 2000), which might be an advantage with regard to monitoring purposes.

Darnerud (2003) has reviewed the available toxicity data on PBDEs, mostly originating from studies performed on rats and mice. He concluded that exposure to PBDEs gives rise to adverse effects in experimental and in vivo models depending on the type of product. The effects of the Penta-PBDE mixture on neurobehavioural development are of particular concern (Darnerud, 2003). At present, a limited number of studies on toxicological levels and effects of PBDEs in birds have been performed. Developmental and immunomodulatory effects have been suggested in nestling American kestrels (*Falco sparverius*) which were exposed in ovo and after hatching to PBDEs (Fernie et al., 2005). Concentrations in the kestrel eggs were, however, two to three orders of magnitude higher compared to our results. Therefore, the low contamination levels of PBDEs in this study are not expected to cause adverse effects in the sampled populations. However, interactions with other environmental pollutants may occur and can be responsible for detrimental health effects. For example, it has been shown that PBDEs can interact with PCBs to cause developmental neurotoxic effects in mice when exposed during a critical period of neonatal brain development (Eriksson et al., 2006).

Severe population declines in different bird species have been reported in Europe during the 1950s and 1960s through the bioaccumulation of OCPs such as DDT (Douthwaite and Tingle, 1992; Lincer et al., 1975; Ratcliffe, 1970; Stickel et al., 1984). This caused the birds to lay thin-shelled eggs that broke during incubation. Following successive restrictions on its agricultural use, populations have now recovered, but the environmental persistence of DDT requires continuous monitoring. Based on previous studies, DDT concentrations in the present study are not expected to cause egg shell thinning and other reproductive effects (WHO, 1979). However, as mentioned before, interactions with other pollutants can be responsible for adverse effects.

In conclusion, our results illustrate the usefulness of great tit eggs as a biomonitoring tool on a relatively large geographical scale. Given that the North American environment is more contaminated with PBDEs than the European environment (Hites, 2004), it might be interesting to measure pollutant levels in eggs of the North American tit counterpart, the black-capped chickadee (*Poecile atricapillus*). In addition, it would be interesting to study and to evaluate the usefulness of other common bird species to map the distribution of OHPs on a regional and continental scale (Golden and Rattner, 2003). Cosmopolitan bird species, such as the peregrine falcon, might be suitable as a monitoring tool even on a global scale. Another bird species with a worldwide distribution which might be used for worldwide monitoring purposes is the European starling. The European starling is one of the most widely used bird species in fundamental biological research and it has increasingly been used in ecotoxicological studies (Arenal et al., 2004; Van den Steen et al., 2007a,b).

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