Brominated flame retardants and organochlorine pollutants in eggs of little owl (Athene noctua) from Belgium

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Abstract

Residues of brominated diphenylethers (PBDEs), organochlorinated pesticides (OCPs) and polychlorinated biphenyls (PCBs) were measured in 40 eggs of little owls (Athene noctua), a terrestrial top predator from Belgium. The major organohalogens detected were PCBs (median 2600 ng/g lipid, range 786 - 23204 ng/g lipid). PCB 153,138/163, 170, 180 and 187 were the predominant congeners and constituted 71% of total sum PCBs. PBDEs were measurable in all samples, but their concentrations were much lower than for PCBs, with a range from 29 - 572 ng/g lipid (median 108 ng/g lipid). The most prevalent PBDE congeners in little owl egg samples were BDE 99, 153 and 47. This profile differs from the profile in marine bird species, for which BDE 47 was the dominant congener, suggesting that terrestrial birds may be more exposed to higher brominated BDE congeners than marine birds. The fully brominated BDE 209 could be detected in one egg sample (17 ng/g lipid), which provides evidence that higher brominated BDEs may accumulate in terrestrial food chains. Brominated biphenyl (BB) 153 was determined in all egg samples, with levels ranging from 0.6 - 5.6 ng/g lipid (median 1.3 ng/g lipid). Additionally, hexabromocyclododecane (HBCD) could be identified and quantified in only two eggs at levels of 20 and 50 ng/g lipid. OCPs were present at relatively low concentrations, suggesting a relatively low contamination of the Belgian environment with OCPs (median concentrations of sum DDTs: 826 ng/g lipid, sum chlordanes: 1016 ng/g lipid, sum HCHs: 273 ng/g lipid). Hexachlorobenzene (HCB) and octachlorostyrene (OCS) were also found at low median levels of 134 and 3.4 ng/g lipid, respectively. Concentrations of most analytes were significantly higher in eggs from deserted nests in comparison to addled eggs, while eggshell thickness did not differ between deserted and addled eggs. No significant correlations were found between eggshell thickness and the analysed POPs.

Capsule - PBDEs are measurable in Belgian little owl eggs, but at relatively low concentrations compared to other birds of prey.

Keywords: brominated flame retardants, polychlorinated biphenyls, organochlorine pesticides, little owl, eggs, biomonitoring

Introduction

Ever rising population numbers and technological development have resulted in the increasing production of new chemicals and subsequently release into the environment. The presence of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and p,p'dichlorodiphenyltrichloroethane (p,p'-DDT) and its metabolites, in the environment is of great importance due to their bioaccumulative potential and chronic adverse effects on both humans and wildlife (Hoffman et al., 2000; Kunisue et al., 2003). In the 1970s these pollutants were banned throughout most of Europe and North America, but they are still produced and used in some developing countries (Kunisue et al., 2003). Due to their persistence, concentrations in the environment remain high and monitoring these chemicals is of great importance. Since the 1960s, polybrominated diphenylethers (PBDEs), a class of brominated flame retardants, have become widely used in textiles, plastics, electronic equipment and other materials. Intensive use has led to their ubiquitous presence in the environment and in biota, in which PBDE levels seem to increase rapidly (de Wit, 2002). High concentrations of some congeners may cause adverse effects in both wildlife and human populations (Darnerud, 2003). This has led to the growing concern of scientists over the last decade and to the need for more data on environmental levels of PBDEs (de Wit, 2002).

Birds have been used successfully as biomonitors in several studies (Burger and Gochfeld, 1995; Eens et al., 1999; Ratcliffe, 1993; Walker et al., 2001). Since they are visible, sensitive to environmental changes and highly positioned on the food chain, birds are very suitable to study bioaccumulation (Furness, 1993). Moreover, their physiology, ecology and behaviour are well studied and they are of interest to the public. Because practical and ethical reasons impede the sacrifice of free-living birds, methods for non-destructive biomonitoring have been developed (Furness, 1993). Eggs have been used successfully as a non-destructive monitoring tool for POPs in numerous studies (Becker, 1989; Becker et al., 2001; Burger and Gochfeld, 1993, Dauwe et al., 1999). Although the analysis of POPs in deserted or addled eggs has clear limitations, these can be partially avoided by analysing only highly persistent components, for which the original composition will not change due to 'post-hatching' microbiological degradation (Herzke et al., 2002).

A great number of studies have detected the presence of p,p'-DDT and its metabolite p,p'-DDE in bird species and their eggs (Becker, 1989; de Wit, 2002; Elliott et al., 2000). Levels of these contaminants in the egg content have frequently been linked to eggshell thinning and reduced reproductive success (Clark et al., 2001; Cooke et al., 1976; Ormerod and Tyler, 1992; Mañosa et al., 2003; Walker et al., 2001). Besides, other persistent organochlorine residues such as PCBs have also been linked to reproductive abnormalities and population declines in birds (Gilbertson et al., 1991; Walker et al., 2001). Consequently, in addition to measurements of contaminant levels, eggshell thickness can also be an indicator of organochlorine pollution and an important predictor of effects on reproduction and survival.

The little owl (*Athene noctua*) is a small sedentary predator, which makes it a very suitable biomonitoring species (Burger, 1993; Zaccaroni et al., 2003). This owl species feeds on a variety of preys, including insects, earthworms, small mammals, occasionally small passerines and sometimes even plant material, depending on the season and the local circumstances (Cramp and Perrins, 1993). Because very limited information is available about contamination levels in the little owl, a study was conducted to determine the concentrations of PBDEs, polybrominated biphenyls (PBBs), PCBs and organochlorine pesticides (OCPs) in

40 deserted or addled eggs of little owls in Belgium. The eggshell thickness of these eggs was also measured and related, when possible, to the contaminant load of the egg contents.

Methods and Materials

Between 1998 and 2000, 17 deserted and 23 addled eggs from different nests of the little owl were collected in the surroundings of Charleroi (Belgium) during the breeding season. The eggs were stored at -20°C until sample preparation. Before analysis, the egg yolk and white were mixed and a homogenised sample of approximately 2 g was weighed, mixed with anhydrous Na₂SO₄ and spiked with internal standards (ϵ -HCH, PCB 46 & 143 and brominated bihenyls (BB) 103 & 155). Further sample treatment and analysis were performed accordingly to previously described methods (Dauwe et al., 2003; Voorspoels et al., 2003). Briefly, extraction was carried out with 100 ml hexane/acetone (3:1, ν/ν) in a hot Soxhlet extractor (Büchi, Flawil, Switzerland) for 2h. The lipid content was determined on an aliquot of the extract (1h, 105°C), while the rest of the extract was cleaned up on a column filled with ~8g acidified silica and eluted with 15 ml hexane and 10 ml dichloromethane. The eluate was concentrated to 100µl under a gentle nitrogen stream, and transferred to an injection vial.

For PBDEs and OCPs, analysis was done with an 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 μ m). For PCBs, a GC/MS in electron ionisation (EI) mode, equipped with a DB-1 capillary column (30 m x 0.25 mm x 0.25 μ m) was used. In all samples 7 BDE congeners (28, 47, 100, 99, 154, 153, 183), BB 153, 27 PCB congeners, hexachlorobenzene (HCB), octachlorostyrene (OCS), chlordanes (*cis*-chlordane, *trans*-nonachlor and oxychlordane, expressed here as CHLs), hexachlorocyclohexanes (α -, β - and γ -HCHs) and DDTs (p,p'-DDE, p,p'-DDD, o,p'-DDT) were analysed. Limits of detection for the analysed compounds ranged between 0.1 and 0.5 ng/g lipid weight. Additionally, BDE 209 and hexabromocyclododecane (HBCD) were investigated in a limited number of samples.

Eggshell thickness was measured using a mechanical dial thickness gauge (Peacock, Japan) to the nearest 0.001 mm. All eggshells were measured by the same investigator, after separation of the membranes from the eggshells. Three measurements were made around the equator of the eggs and each measurement was repeated three times. The mean of these measurements for each egg was used in further statistical analysis.

The distribution of the organohalogenated compounds was not normal. Except for PCBs, one sample was found to be an outlier (Dixon's test) for all classes of POPs and was further omitted from statistical analysis. Correlations were carried out using non-parametric Spearman rank correlation (Statistica for Windows 5.0, StatSoft Inc., 1995). The α value was adjusted with sequential bonferroni correction to correct for increasing risk to type I errors. Comparison of the concentrations in deserted and addled eggs was performed, using non-parametric Mann-Whitney U tests. Since the eggshell thickness data were normally distributed, eggshell thickness between these two groups of eggs was compared by performing parametric Student t tests. The level of significance was set at p = 0.05 throughout the manuscript. Considering the eggs were sampled for no specific cause at the time and collection was merely related to opportunity, we were not able to investigate comparisons between regions or years.

Results

Our results revealed that PCBs constitute the major organohalogenated pollutants in the eggs of the Belgian little owl, with concentrations ranging from 786-23204 ng/g lipid and a median concentration of 2600 ng/g lipid. The predominantly congeners were PCB 153,138/163, 170, 180 and 187, constituting 71% of sum PCBs (Table 1).

Sum DDTs levels in our egg samples were relatively low, ranging from 200-7257 ng/g lipid. The most important compound was p,p'-DDE, with a median concentration of 826 ng/g lipid. Concentrations of p,p'-DDT were low (median concentration 1.7 ng/g lipid) and the median concentrations of o,p'-DDD, o,p'-DDT and p,p'-DDD (range: nd-86 ng/g lipid) were below the level of detection. HCB was detected in all samples, but concentrations showed a high variability (Table 1). Further, HCHs were detected at relatively low concentrations, with a median concentration of 27.3 ng/g lipid. The median concentrations of α -, β - and γ -HCHs were respectively 0.4 , 9.4 and 16.7 ng/g lipid. Levels of α -HCH were very low in our samples with a large number of non-detectable concentrations. Next, *trans*-nonachlor and oxychlordane were the most important chlordanes detected in the eggs, with a median concentration of 526 and 464 ng/g lipid respectively. Finally, OCS was found in low concentrations (median concentration of 3.4 ng/g lipid) in all the analysed eggs (Table 1).

PBDEs were detected in all analysed samples (Table 2), but the concentrations were much lower than for PCBs (levels of sum PBDEs were ~ 4% of levels of sum PCBs) with PBDEs levels ranging from 29-2181 ng/g lipid (this maximum value was an outlier and excluded from further calculations – see above). The most prevalent BDE congeners in little owl egg samples were BDE 99, BDE 47 and BDE 153 (Table 2, Figure 1). Furthermore, BDE 183 and BB 153 were detected in all little owl eggs (Table 2). In contrast, BDE 209 could be detected in only one egg sample at a level of 17 ng/g lipid (detection limit was 8 ng/g lipid). This sample contained also the highest levels of PBDEs (2180 ng/g lipid) and was the outlier eliminated during the statistical analysis. HBCD could be identified and quantified in only 2 egg samples, at levels of 20 and 50 ng/g lipid (detection limit was 5 ng/g lipid).

Few significant correlations were established among the analysed organohalogens (Table 3). Levels of sum BDEs were 70% correlated with levels of sum PCBs (p<0.05). All other correlations had lower correlation coefficients (Table 3). No significant correlations could be established between eggshell thickness and egg content of the different POPs (p>0.05 for all samples).

The concentrations of all PCB congeners (except PCB 18 and 132), 4 BDE congeners (BDE 99, 153, 154 and 183), BB153, HCHs and HCB were significantly higher (p<0.05) in the deserted eggs compared to the addled eggs. No significant differences were found for CHLs, OCS or for individual DDT analogues (p>0.05). Shell thickness did not differ significantly between deserted and addled eggs ($t_{25} = -0.214$: p=0.83).

Discussion

Our results indicated that PCBs constitute the major organohalogenated pollutants in the eggs of Belgian little owls. However, these results may not supply information about contamination levels in the entire country of Belgium. Samples were collected in a limited area around Charleroi in Wallonie. The landscape in Wallonie is dominated by agriculture, while industry is more important in Flanders. Therefore this study does not provide general

concentrations of the Belgian environment and future studies should take this into account. The measured PCB concentrations are in agreement with levels reported by Van den Brink et al. (2003) in little owls from Dutch river floodplains. As indicated by these authors, current PCB levels in little owl eggs may pose a risk on the functioning and the condition of little owls in these floodplains (Van den Brink et al., 2003). In general, concentrations of PCBs in Belgian little owl eggs are in line with levels reported for other birds of prey (Herzke et al., 2003). PCB levels were also within the same range as reported by Dauwe et al. (*in press*) for fat tissue of great tits. Furthermore, the predominantly congeners in the Belgian little owl eggs were PCB 153, 138/163, 170, 180 and 187, which is similar to the results of Dauwe et al. (*in press*), who found that the hexachlorobiphenyls were the most abundant congeners in Belgian great tits (*Parus major*) and thus probably the most abundant congeners in the Belgian environment.

Levels of p,p'-DDE in our egg samples were relatively low, compared with p,p'-DDE concentrations reported by Van den Brink et al. (2003) for the little owl from the Geldertse Poort, The Netherlands (range: 0.8 – 17.9 μg/g lipid). This variation in concentrations may originate from differences in pesticides use (i.e. land-use) and from differences in deposition patterns between Charleroi and Dutch river floodplains. However, concentrations were in the same range as reported for p,p'-DDE in little owls from Achterhoek, The Netherlands (Van den Brink et al., 2003). Similar concentrations were also established in burrowing owls (*Athene cunicularia*) eggs collected in California from 1998-2001 (Gervais and Anthony, 2003). The rather low HCHs levels detected in our samples are in accordance with low HCH concentrations found previously in Belgian great tit nestlings (Dauwe et al., 2003), which may suggest a relatively low contamination of the Belgian environment with HCHs. Also, OCS was found in low concentrations in all the analysed eggs.

PBDE concentrations in our egg samples were much lower than levels detected in Swedish peregrine falcon (Falco peregrinus) eggs (geometric mean up to 2000 ng/g lipid) (Lindberg et al., 2004) and Great Lakes Herring gull (Larus argentatus) eggs (up to 7000 ng/g lipid) (Norstrom et al., 2002). This is probably due to the specific diet of the little owl that constitutes primarily of insects, earthworms and mice's, while passerines are only hunted occasionally. Consequently, little owls have a lower position in the food chain compared with these predatory birds. However, PBDEs levels were in the same range as reported by Herzke et al. (2003) for the Goshawk (Accipiter gentilis) and the Merlin (Falco columbarius). The main food of these birds consists of terrestrial birds and small mammals (Goshawk) and passerines (Merlin) (Herzke et al., 2003), which is closer to the little owl's diet. Further, BDE 183 was detected in all little owl eggs, which points to an additional source of the Octa-BDE mixture. Similarly, BDE 183 was measured (at higher levels) in all eggs of wild falcons from Sweden (Lindberg et al., 2004). However, these results contrast with the lack of BDE 183 in the North Sea food web (Boon et al., 2002; Voorspoels et al., 2003). It has been suggested that the Octa-BDE mixture is not or only in relatively low levels present in aquatic ecosystems, compared to the Penta-mix (Law et al., 2003). Consequently, our data confirm the assumption that the Octa-mix may be more widespread in some terrestrial food webs than in the aquatic environment. As BDE 209 could be detected in one egg sample, this provides further evidence that higher brominated BDEs, including BDE 209, may accumulate in terrestrial food webs, as already shown by Lindberg et al. (2004). The levels of BDE 209 in eggs of the Swedish Peregrine falcon varied between < 7 and 430 ng/g lipid (Lindberg et al., 2004). Lower concentrations were found in the eggs of the little owl, probably due to differences in diet as discussed above. BB 153 was detected in all egg samples, but concentrations were lower than levels in the eggs of wild peregrine falcons from Sweden (Lindberg et al., 2004). HBCD could be identified and quantified only in 2 egg samples of the little owl. HBCD could not be detected in eggs of captive Swedish peregrine falcons (< 8 ng/g lipid) as well, but it was present in all egg samples from wild Swedish peregrine falcons (up to 2400 ng/g lipid; Lindberg et al., 2004).

Our results revealed the presence of a very specific BDE profile in the eggs of the little owl dominated by BDE 99, BDE 153 and BDE 47. The ratios of these congeners in the egg samples were different from the proportions of these congeners in the commercial mixtures, which suggests transformation of the commercial BDE mixtures in biota (Hites, 2004). Similar profiles were reported for eggs of peregrine falcons, merlins (*Falco columbarius*) and Gyrfalcons (*Falco rusticolus*) from Norway (Law et al., 2003), which displayed highest concentrations for BDE 99 and BDE 153. However, the BDE profile was markedly different in Great Lakes Herring Gull eggs (Norstrom et al., 2002), which was dominated by BDE 47, followed by BDE 99, BDE 100 and BDE 153. This discrepancy between terrestrial and marine species has recently been documented by Law et al. (2003), who suggested that birds feeding in terrestrial environments may be more exposed to the higher brominated BDE congeners than marine birds. However, metabolic capacity and degradation may differ between species as well. Nevertheless, Zimmermann et al. (1997) have suggested for PCBs that PCB patterns are influenced more by individual dietary factors than by species differences in metabolic capacity.

The low correlation coefficients determined among organohalogen compounds in little owl eggs may indicate separate sources of contamination, differences in exposure time or different persistency in the Belgian little owl. No significant correlation was found between concentrations of DDTs and eggshell thickness, indicating that levels of DDTs in the Belgian environment may only exert minor effects on little owl survival and reproduction.

Concentrations of most organic pollutants were found to be significantly higher in deserted compared to addled eggs. Generally, it is expected that addled eggs have higher concentrations of POPs, since these POPs levels may be a factor explaining the failure of eggs to hatch (through increased embryo mortality). However, if the contamination is low, it is not likely that this affects hatching success of eggs, implying that addled eggs do not have higher concentrations than deserted eggs. At the moment we have no substantial explanation why higher concentrations were found in the deserted eggs. It may be possible that parental behaviour is altered at high concentrations of organohalogenated compounds. Even low chronic exposure may be detrimental when combined with other stressors (Gervais and Anthony, 2003), leading to reduced productivity through increased risk of abandoning nests. Such a relationship between organochlorine contamination and reproductive behaviour has formerly been suggested by Bustnes et al (2001), who found increased absence from the nest site in individual glaucous gulls with high concentrations of organochlorines in the blood. Finally, shell thickness did not differ significantly between deserted and addled eggs, suggesting that POPs concentrations in our little owl eggs do not cause changes in eggshell thickness.

Conclusion

In conclusion, PCBs were the principal organohalogenated contaminants in our Belgian little owl eggs. Furthermore, the results showed that PBDEs were measurable in all eggs, although concentrations were low compared to levels of other POPs. The PBDE profile, dominated by BDE 99, 153 and 47, differed from the profile observed in marine birds, which suggests that

birds living in a terrestrial environment may be more exposed to higher brominated BDE congeners than marine birds. In addition, the fully brominated BDE 209 could be detected in one egg sample, which provides further evidence that higher brominated BDEs may accumulate in terrestrial food chains. The low levels of OCPs suggest a relatively low contamination of the Belgian environment.

Our results also revealed that concentrations of most POPs were significantly higher in eggs from deserted nests in comparison to addled eggs, even though there was no difference in eggshell thickness between deserted and addled eggs. Further, no significant correlation has been found between levels of DDTs and eggshell thickness, indicating that concentrations of DDTs in the Belgian environment are of little importance on little owl survival and reproduction.

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Table 1: Median concentration (ng/g lipid) and range (minimum - maximum) of different organochlorine pollutants in eggs of Belgian little owls. Contribution of different PCB congeners to sum PCBs (% total PCBs) and contribution of each class of compounds to total organohalogen load (% total POPs) are also presented.

Compound	N	Median	Minimum	Maximum	% total PCBs	% total POPs
НСВ	39	134	35.3	1073	-	2.8
OCS	39	3.4	0.5	7.3	-	0.07
Sum HCHs	39	27.3	5.5	356	-	0.6
Sum CHLs	39	1016	23.9	7113	-	21.6
Sum DDTs	39	826	200	7257	-	17.5
Sum BDEs	39	108	29	572	-	2.3
PCB 18	40	0.1	ND	11.0	0.01	0.01
PCB 31/28	40	23.4	4.7	554	0.9	0.5
PCB 52	40	0.9	ND	68.3	0.04	0.02
PCB 74	40	29.9	6.7	374	1.1	0.6
PCB 95	40	2.9	0.5	24.2	0.1	0.06
PCB 101	40	17.9	5.5	186	0.7	0.4
PCB 99	40	72.6	20.8	502	2.8	1.5
PCB 110	40	5.7	1.6	87.2	0.2	0.1
PCB 149	40	33.9	9.7	339	1.3	0.7
PCB 118	40	125	35.8	480	4.8	2.7
PCB 105	40	43.5	11.8	205	1.7	0.9
PCB 132	40	2.4	1.2	7.3	0.09	0.05
PCB 153	40	590	189	6486	22.7	12.5
PCB 138/163	40	484	136	4430	18.6	10.3
PCB 187	40	303	92.6	1805	11.6	6.4
PCB 128	40	46.0	13.3	369	1.8	1.0
PCB 183	40	43.8	11.5	406	1.7	0.9
PCB 167	40	18.6	5.7	115	0.7	0.4
PCB 177	40	19.7	4.7	148	0.8	0.4
PCB 156	40	36.5	12.4	302	1.4	0.8
PCB 180	40	338	115	5188	13.0	7.1
PCB 170	40	126	40.5	2568	4.8	2.7
PCB 199	40	45.3	12.0	236	1.7	1.0
PCB 196	40	40.4	14.0	236	1.6	0.9
PCB 194	40	47.0	19.4	566	1.8	1.0
Sum PCBs	40	2600	786	23204	100	55.2
Lipids	40	8.0	5.2	13.7	-	-

Table 2: Median concentration (ng/g lipid) and range (minimum - maximum) of organobrominated pollutants in eggs of Belgian little owls (N=39). Contribution of different BDE congeners to sum PBDEs (% total PBDEs) and contribution to total organohalogen load (% total POPs) are also presented.

Compound	Median	Minimum	Maximum	% total BDEs	% total POPs
BDE 28	0.9	ND	1.7	0.8	0.02
BDE 47	24.4	3.4	171	22.6	0.5
BDE 100	3.3	0.5	30.8	3.0	0.07
BDE 99	33.7	9.2	270	31.2	0.7
BDE 154	3.9	0.7	20.5	3.6	0.08
BDE 153	24.9	9.5	94.4	23.0	0.5
BDE 183	10.2	4.1	31.0	9.4	0.2
Sum BDEs	108	29	572	100	2.3
BB 153	1.3	0.6	5.6	-	0.03
Lipids	7.9	5.2	13.7	-	-

Table 3: Results of the Spearman Rank correlation, r- and p-value (in brackets), among the different organohalogen contaminants in eggs of Belgian little owls are presented.

	Sum HCHs	Sum BDEs	Sum DDTs	Sum CHLs
Sum PCBs	0.62*	0.71*	0.42*	0.50*
Sum HCHs		0.46*	0.34*	0.36
Sum BDEs			0.33*	0.40
Sum DDTs				0.55*

^{* -} p<0.05

Figure 1: Contribution of selected BDE congeners to the total PBDE load (% total PBDEs) in eggs of Belgian little owls.

