LEVELS AND PROFILES OF ORGANOCHLORINES AND BROMINATED FLAME RETARDANTS IN FEMALE BLUE TITS (*CYANISTES CAERULEUS)* AND THEIR EGGS

Van den Steen E[1], Jaspers VLB[1], Covaci A[2,3], Pinxten R[1], Neels H[2], Eens M[1]
[1]Laboratory of Ethology, Department of Biology, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[2]Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[3]Laboratory of Ecophysiology, Biochemistry and Toxicology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

Abstract
Maternal transfer of organohalogenated pollutants (OHPs) was investigated through comparisons of the levels and profiles of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) between female blue tits (*Cyanistes caeruleus*) and their eggs. Mean egg concentrations decreased significantly in relation to the laying order from 342 ± 24 ng/g lw to 235 ± 17 ng/g lw for the sum OCPs, from 1623 ± 148 ng/g lw to 1040 ± 47 ng/g lw for the sum PCBs and from 49 ± 5 ng/g lw to 27 ± 5 ng/g lw for the sum PBDEs. The egg/female lipid concentration ratios for the sum OCPs, sum PCBs and sum PBDEs decreased significantly in function of the laying order from 1.1 ± 0.1 to 0.7 ± 0.02, 1.0 ± 0.09 to 0.7 ± 0.003 and 1.2 ± 0.14 to 0.8 ± 0.05, respectively. Furthermore, our results suggest that maternal transfer in blue tits seems to be selective for the more bioaccumulative and persistent congeners/compounds. Differences in reproductive strategies among species are suggested to be important in the processes of maternal transfer of OHPs.

Introduction

Although eggs have been frequently used as a biomonitoring tool for contamination with organohalogenated pollutants (OHPs)[1,2], only a limited number of studies has investigated the processes of maternal transfer[3-5]. Maternal mobilisation and deposition of lipids into the eggs, along with organic contaminants, is believed to be controlled by both biological and physico-chemical factors. A biological factor which may be important for the maternal transfer of contaminants is the reproductive strategy of a bird species[4]. The amount of lipids mobilised and deposited to the egg may depend on the size of the clutch, size of the eggs and the developmental strategy of the embryo (altricial versus precocial)[3-5]. Depending on the reproductive strategy, lipids deposited to eggs may be from maternal or dietary sources at the time of yolk formation[4]. On the other hand, physico-chemical properties of the contaminants, such as molecular structure and degree of halogenation, may also influence the excretion of chemicals into the egg[3].

The objective of the present study was to investigate maternal transfer of OHPs in a passerine species, the blue tit (*Cyanistes caereleus*). Blue tits are small, insectivorous passerine birds that are widely distributed throughout Europe. They have previously been used as a biomonitor for heavy metals[6]. Their ubiquity allows sampling of almost any wooden area in Europe. Because blue tits are cavity-nesting birds and nest sites are often a limiting resource, they will readily nest in man-made boxes. Therefore, breeding populations can be rapidly established and monitored, and samples can be easily collected. Moreover, blue tits are resident or non-migratory in many populations and they have small home ranges, making them useful as biomonitors for local contamination in terrestrial ecosystems. Because of the large number of eggs in a clutch (up to 16), blue tits are very suitable to study the maternal transfer of pollutants. Maternal transfer of OHPs was investigated through comparisons of the concentrations and profiles between the females and their eggs. In addition, we investigated if there was an effect of laying order on the OHP concentrations.

Materials and Methods

Ten female blue tits (4 first-years and 6 adult birds) and their complete clutches were collected in 2006 from two sites near Antwerp. Eggs were numerically marked according to laying order. When the clutch was complete and the female initiated incubation, the complete clutch was collected and the female was sacrificed with CO_2. In total, 117 eggs from ten complete clutches (10-14 eggs per clutch, mean clutch size ± SE: 11.7 ± 0.3,) were

gathered. Two eggs laid on consecutive days were pooled. Samples were stored at –20°C until further treatment. A homogenised sample of approximately 0.5 g whole egg and 2 g whole female was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (ε-HCH, PCB 46 and 143, BDE 77 and 128). Extraction was carried out with 100 ml hexane/acetone (3:1, *v/v*) in an automat Soxhlet extractor 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, 22 PCB congeners (PCB 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-HCH), chlordanes (CHLs; *cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN) and oxychlordane (OxC)), and hexachlorobenzene (HCB) were analysed.

For the PCB analysis, an Agilent 6890 gas chromatograph (GC) connected with an Agilent 5973 mass spectrometer (MS) operated in electron ionisation (EI) mode was equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). For the analysis of the OCPs and PBDEs, an Agilent 6890 GC connected with an Agilent 5973 MS operated in electron capture negative ionisation (ECNI) mode was equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). Limits of quantification (LOQs) for the analysed compounds ranged between 0.1 and 3.0 ng/g lipid weight (lw) for the eggs and between 0.1 and 7.5 ng/g lw for the females.

Statistical calculations were performed using SPPS 14.0 for Windows on lipid-normalized concentrations. The level of significance was set at $\alpha = 0.05$ throughout this study. Before data analysis, samples with levels below LOQ were assigned a value of ½*LOQ. Contaminants were excluded from the analysis if more than 50 % of the samples had levels below LOQ. The data met the assumptions of normality and therefore parametric tests were performed. The presence of laying order effects on the sum OCP, sum PCB and sum PBDE concentrations was investigated using repeated measures ANOVAs. Laying order effects were examined only for the first five pooled egg samples (egg 1-2 to egg 9-10) of each clutch. Post hoc tests were performed when there were significant effects.

Results and Discussion

Contaminant levels

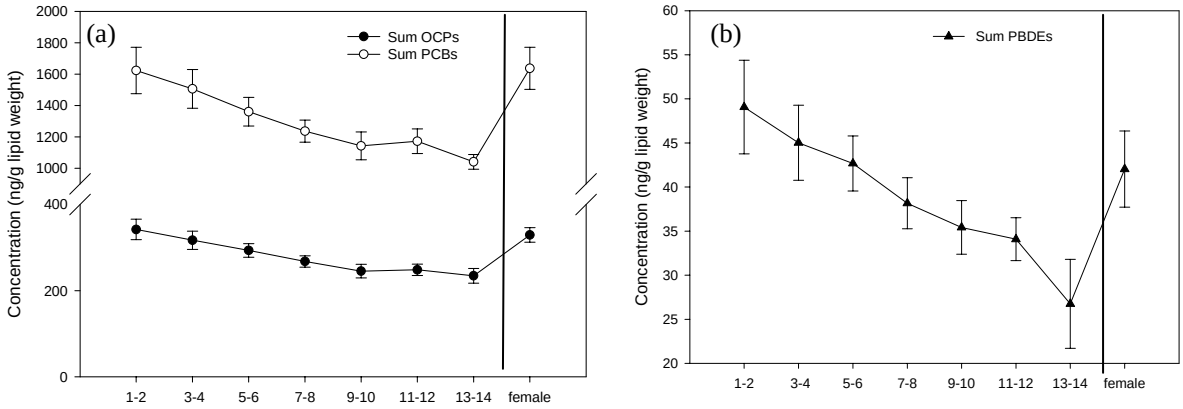

Figure 1: Mean concentration with standard error (ng/g lipid weight) of sum OCPs (a), sum PCBs (a) and sum PBDEs (b) in female blue tits (n=10) and their eggs (egg 1-2, egg 3-4, egg 5-6, egg 7-8, egg 9-10: n = 10; egg 11-12: n = 9; egg 13-14: n = 2)

PCBs were the most abundant contaminants in the analysed eggs and female blue tits, followed by OCPs and PBDEs (Figure 1). Among the OCPs, sum DDTs were the most dominant compounds and accounted for almost 90 % of the sum OCPs. Mean egg concentrations decreased significantly in relation to the laying order from 342

± 24 ng/g lw to 235 ± 17 ng/g lw for the sum OCPs, from 1623 ± 148 ng/g lw to 1040 ± 47 ng/g lw for the sum PCBs and from 49 ± 5 ng/g lw to 27 ± 5 ng/g lw for the sum PBDEs (Repeated measures ANOVA: sum OCP: $F_{4,36} = 20.49$, $p < 0.001$; sum PCBs: $F_{4,36} = 21.84$, $p < 0.001$; sum PBDEs: $F_{4,36} = 9.40$, $p < 0.001$; Figure 1). For both sum OCPs and sum PCBs, concentrations of the first pooled egg sample were significantly higher than these of the thirth, fourth and fifth egg samples (Tukey HSD: sum OCPs: $p < 0.002$ for all cases; sum PCBs: $p < 0.001$ for all cases; Figure 1). Sum PBDE concentrations of the first egg sample were higher than the concentrations of the fourth and fifth egg samples (Tukey HSD: $p < 0.001$ for all cases; Figure 1). This contrasts with a previous study in which within- and among clutch variation in great tits (*Parus major)* was investigated[2]. It may be that no laying order effects were detected in the great tit eggs because only the first six eggs of each clutch were included in that study. In addition, species differences may also be responsible for the divergent results.

Concentrations of sum OCPs, sum PCBs and sum PBDEs in female blue tits were comparable to the concentrations in the first laid eggs, with a mean concentration of 329 ±17 ng/g lw, 1636 ± 134 ng/g lw and 42.0 ± 4.3 ng/g lw, respectively (Figure 1). The egg/female lipid concentration ratios for sum OCPs, sum PCBs and sum PBDEs decreased significantly from 1.1 ± 0.1 to 0.7 ± 0.02, 1.0 ± 0.09 to 0.7 ± 0.003 and 1.2 ± 0.14 to 0.8 ± 0.05, respectively (Repeated measures ANOVA: $F_{4,36} < 20.88$, $p < 0.001$ for all cases). Our results agree with the model of Russell et al.[7] predicting that lipid normalized egg/female ratios of organochlorines aproximate one for all oviparous species. Sum OCP and sum PBDE concentrations in the female were not correlated with the concentrations in the eggs (Pearson correlation: sum OCPs: $0.06 < r < 0.37$, $0.29 < p < 0.89$; sum PBDEs: $0.41 < r < 0.49$, $0.15 < p < 0.24$). For the sum PCBs, concentrations in the female were correlated with the concentrations in the pooled samples of eggs 1-2, eggs 7-8 and eggs 11-12 (Pearson correlation; $0.64 < r < 0.74$, $0.02 < p < 0.05$), and tended to be correlated with the other egg samples (Pearson correlation; $0.53 < r < 0.62$, $0.06 < p < 0.12$).

Contaminant patterns

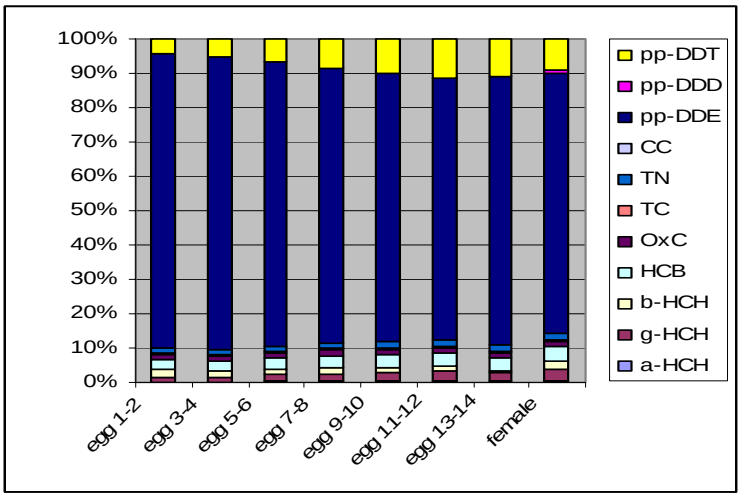

Figure 2: Contribution of the different OCPs to the sum OCPs (%)

A higher contribution of less persistent and/or low K_{ow} (octanol-water partition coefficient) OCPs (*p,p'*-DDD, CC, TN, HCB, α-HCH and γ-HCH) was found in the females compared to their eggs (Figure 2). On the other hand, the highly bioaccumulative DDT metabolite *p,p'*-DDE and TC contributed less to the profile of the females compared to the eggs (Figure 2). This is in contrast with the study of Verreault et al.[5], which reported a higher contribution of *p,p'*-DDE in the glaucous gull (*Larus hyperboreus*) female plasma compared to their eggs. The contribution of *p,p'*-DDE and OxC decreased in relation to the laying order, whereas the contribution of *p,p'*-DDT increased in relation to the laying order. A possible explanation for these results is that in blue tits lipids from maternal sources may be more important in the beginning of the egg laying period, while dietary sources will become more important during the course of egg laying. In the beginning of egg laying, the highly

bioaccumulative compounds are passed to the eggs and females are consequently able to loose a substantial portion of the highly bioaccumulative *p,p'*-DDE.

CB 153, CB 180 and CB 138 were the most abundant PCB congeners. The lower chlorinated PCB congeners, CB 28, CB 52, CB 74, CB 95, CB 99, CB 101 and CB 110, contributed more to the females than to the eggs' profile. These results are also in contrast to the study of Verreault et al.[5] in which the authors suggested that a proportion of metabolically resistant and heavily chlorinated PCBs is preferentially retained in the female. Bargar et al.[3] also found that excretion to eggs of CB 105, 156 and 189 injected into white leghorn chickens (*Gallus domesticus*) was inversely related to chlorination. These divergent results might also be due to species differences in reproductive strategies and clutch size. However, PCB congeners 170, 194 and 199 contributed also more to the profile of the females.

BDE 47, BDE 99 and BDE 153 were the most abundant PBDE congeners. The contribution of BDE 47 increased in function of the laying order, except for the last egg sample (egg 13-14; Figure 3). The divergent profile of egg 13-14 might be due to the fact that there were only two clutches with eggs 13-14. There were no apparent differences between the PBDE profiles of the females and their eggs (Figure 3).

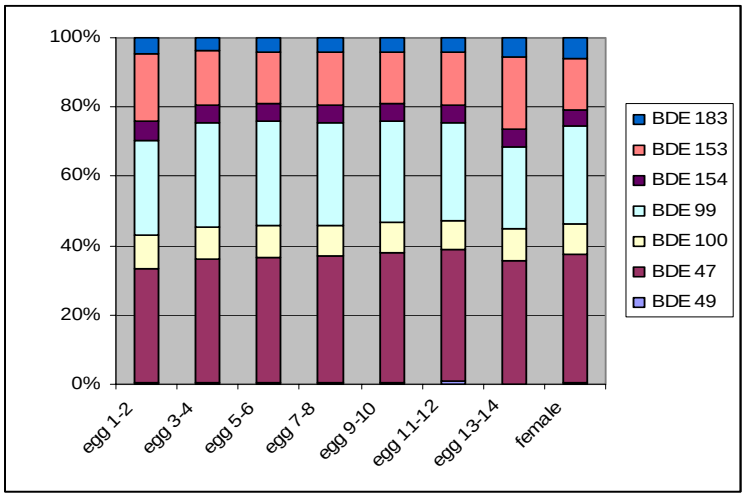

Figure 3: Contribution of analysed BDE congeners to the sum PBDEs (%)

In conclusion, it seems that there is a selective transfer of persistent congeners/compounds to the eggs of blue tits. Differences in reproductive strategies may be responsible for the diffferences between species and may be important in the processes of maternal transfer.

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