NEW DATA ON THE VARIATION OF ORGANIC POLLUTANTS WITHIN AND AMONG CLUTCHES OF A TERRESTRIAL SONGBIRD SPECIES, THE GREAT TIT (*PARUS MAJOR)*

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Introduction

Eggs have been used successfully as a non-destructive biomonitor in several studies[1,2]. Organic pollutants can be found at high concentrations in bird eggs because of their high lipid content. In addition, eggs are easy to collect and the collection of one egg from a clutch is only expected to have a minor influence on the population level in many species. However, for monitoring strategies it is of great interest to investigate whether levels in eggs change with the laying sequence. Although laying order effects have been reported in some bird species[3,4], most studies measuring organic pollutants in eggs have shown similar levels of organic pollutants in eggs from the same clutch[5,6]. However, most of these studies were conducted on piscivorous species and birds of prey with a small clutch size, making it difficult to study laying order effects. Therefore, recently studies have been performed on passerine bird species with a large clutch size[7,8].

The objective of the present study was to investigate the variation of different organic pollutants (PCBs, DDTs and PBDEs) within and among clutches of great tits (*Parus major*). In addition, the presence of laying order effects was investigated for the analysed pollutants. To investigate the impact of laying large numbers of eggs on the levels of organic pollutants, initiation of replacement clutches was experimentally induced by removing first clutches at the start of incubation. Great tits are small insectivorous passerine birds, which are distributed throughout Europe. They are cavity nesting birds and also make use of man made nest boxes, which makes it easy to establish breeding populations and to take samples. Furthermore, great tits are resident birds with small home ranges, which make them particularly useful as biomonitors of local contamination. Great tits are very suitable to study the variation in concentrations within and among clutches because of the large number of eggs in a clutch (up to 12).

Materials and Methods

Eight complete first clutches (mean clutch size ± standard error: 9.8 ± 0.4, range: 8 – 12 eggs per clutch) with known laying order were collected in 2006 from two sites near Antwerp. When the clutch was completed and the female initiated incubation, eggs were collected in order to induce the female to lay a replacement clutch (8.3 ± 0.3 eggs per clutch, range: 8 – 10 eggs per clutch), which was the case for four females. Two eggs laid on consecutive days were pooled, resulting in 39 egg samples from first clutches and 17 egg samples from replacement clutches. Samples were stored at –20°C until further treatment. A homogenised sample of approximately 0.5 g egg content was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (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, 19 PCB congeners (PCB 52, 74, 95, 99, 101, 105, 110, 118, 128, 138, 149, 153, 156, 170, 180, 183, 187, 194 and 199), 7 PBDE congeners (BDE 28, 47, 99, 100, 153, 154 and 183), dichlorodiphenyltrichloroethane (*p,p'*-DDT) and metabolites (*p,p'*-DDE and *p,p'*-DDD), 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 5.0 ng/g lipid weight (lw).

Statistical calculations were performed using SPPS 14.0 for Windows on lipid-normalised concentrations of pollutants in the eggs. 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. For all measured contaminants levels were above LOQ in more than 50 % of the samples. 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 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. To study the variation within and among clutches, nest was considered a random factor and variance components were estimated using the restricted maximum likelihood estimation (REML) method.

Results and discussion

General contamination levels

PCBs were the most abundant pollutants in the analysed eggs, followed by DDTs and PBDEs (Table 1). All analysed PCB congeners were detectable in all 38 pooled egg samples. PCB congeners 153, 180 and 138 were the most abundant congeners found in the investigated egg samples, which is in accordance with the distribution found previously in eggs and tissues of great tits from Belgium[7,9]. Sum DDT levels were lower than the sum PCB levels in our egg samples (Table 1). The most important DDT compound was *p,p'*-DDE, which accounted for more than 90% of the sum DDTs. PBDEs were found at much lower concentrations than the PCBs (Table 1). BDE 47, BDE 99 and BDE 153 were the most prevalent PBDE congeners (Table 1) and contributed for 32%, 30% and 17% to the sum PBDEs, respectively, which is similar to the PBDE profile previously found in great tit eggs from Belgium[7].

Table 1: Mean concentrations (ng/g lw) and range (minimum – maximum) of PBDEs, sum PCBs and sum DDTs of pooled egg samples.

Compound	**Mean**	**Minimum**	**Maximum**
BDE 28	0.3	0.1	0.7
BDE 47	17	5.8	39
BDE 99	16	4.2	44
BDE 100	5.9	1.5	15
BDE 153	9.2	2.3	21
BDE 154	1.8	0.5	4.6
BDE 183	3.6	1.3	13
sum PBDEs	55	16	114
sum PCBs	2550	822	4900
sum DDTs	756	224	3290

Variation within and among clutches

For the first clutches, mean egg concentrations decreased significantly in relation to the laying order from 3025 ± 416 ng/g lw to 2270 ± 386 ng/g lw for the sum PCBs (Repeated measures ANOVA: $F_{4,20} = 2.81$, $p = 0.05$; Figure 1a) and from 989 ± 339 ng/g lw to 695 ± 320 ng/g lw for the sum DDTs (Repeated measures ANOVA: $F_{4,20} = 4.13$, $p = 0.01$; Figure 1a). For the sum PBDEs, concentrations in the first clutches tended to decrease in relation with the laying order from 68 ± 10 ng/g lw to 53 ± 11 ng/g lw (Repeated measures ANOVA: $F_{4,20} = 2.62$, $p = 0.07$; Figure 1b). These results are in contrast with a previous study in which no laying order effects were found for the concentrations of sum PCBs, sum DDTs and sum PBDEs in great tit eggs[7]. It might be that laying order effects could not be detected in the latter study, because only the first six eggs, which were 3 pools of consecutive egg samples, were included in the statistical analysis of this study. Different factors, such as food quality and availability, may vary among years and can also be responsible for the observed differences between the studies in great tits. Winter and Streit (1992) found higher concentrations of PCBs and DDTs in the first six eggs of a great tit clutch compared to the last six eggs[10]. Although the results of that study also suggest an effect of laying order in the eggs of great tits, only one clutch with 12 eggs was analysed.

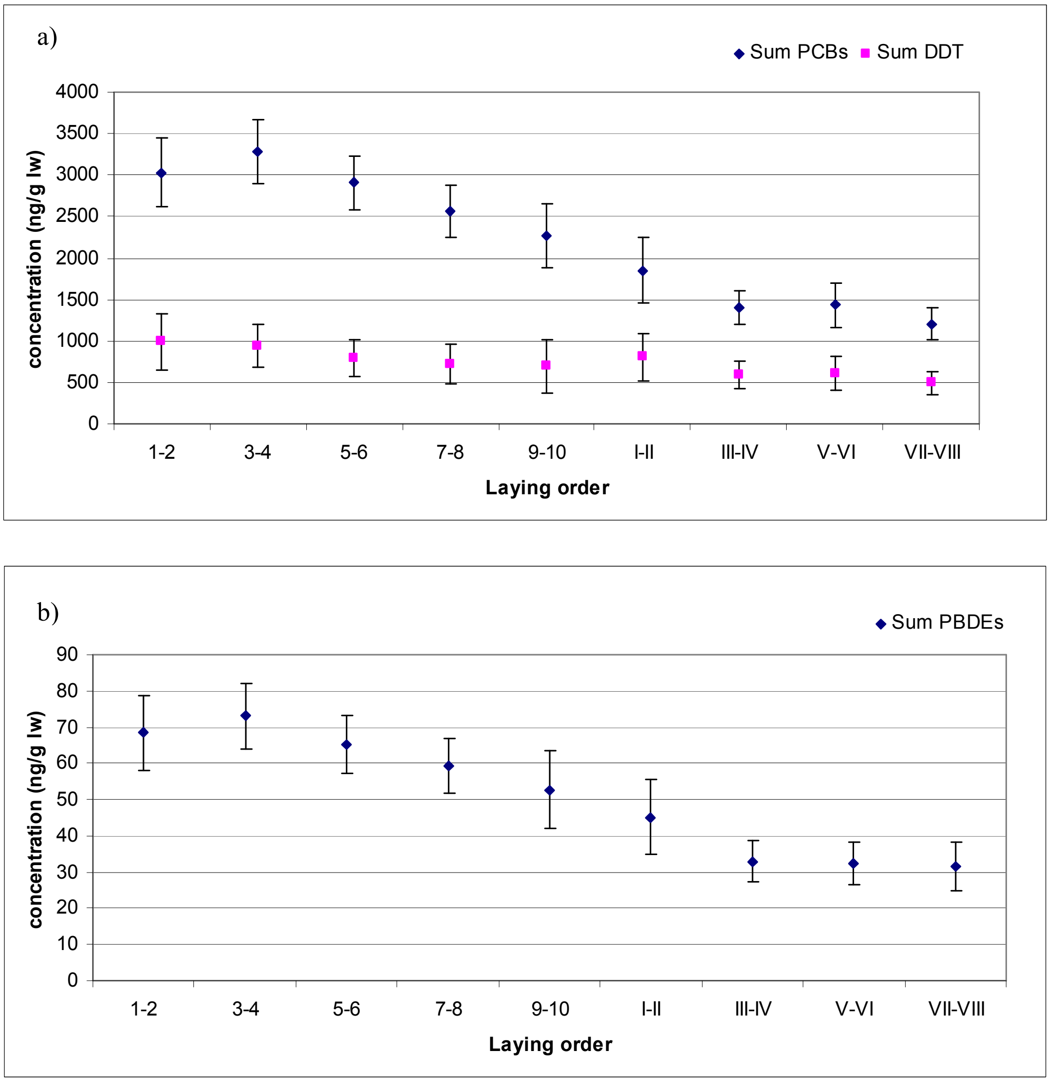

Figure 1: Mean concentrations of sum PCBs, sum DDTs and sum PBDEs (ng/g lw) in first (eggs 1-2 to eggs 9-10) and replacement clutches (eggs I-II to eggs VII-VIII) of great tits.

The females in which both first and replacement clutches were initiated, also showed significant laying order effects for the PCBs, DDTs and PBDEs (Repeated measures ANOVA: $F_{8,8} > 3.34$, $p < 0.05$, for all cases). For both PCBs and PBDEs, concentrations in eggs III-IV, V-VI and VII-VIII of the experimentally induced replacement clutches were significantly lower than these of egg 1-2 from the first clutches (Tukey HSD: $p < 0.04$, for all cases). Sum DDT concentrations tended to be lower in egg VII-VIII from the replacement clutches compared to the first egg sample of the first clutches (Tukey HSD: $p = 0.07$). Our results suggest that concentrations of organic pollutants decrease in great tit eggs in relation with the laying order and that this decrease continues after initiation of a replacement clutch.

According to the variance estimates, the variance in concentrations of PCBs, DDTs and PBDEs was larger among clutches than within clutches. The among-nest component of the variance estimates for PCBs, DDTs, and PBDEs accounted for 92%, 91%, and 64% of the total variance, compared with 8%, 9% and 36% attributed to the within-nest component. Despite the presence of laying order effects within the clutches, our results showed that variation among clutches was much larger than the variation within a clutch. This is in agreement with our previous study in great tits[7] and other studies reporting variability in organochlorine contaminants within and among clutches[11,12]. The present study shows that small within-clutch variability can also occur in species with a large number of eggs in a clutch and in which it has been shown that the nutritional requirements of egg production rely almost exclusively on dietary intake. A possible explanation for the absence of large within-clutch variability in great tit eggs is the fact that great tits have small home ranges and foraging areas. Other factors, such as the spatial distribution of pollutants, may also have an effect on the variation of pollutants within a clutch.

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