Within- and among-clutch variation of organohalogenated pollutants in eggs of great tits (Parus major)

Evi Van den Steen, Tom Dauwe, Adrian Covaci, Veerle Jaspers, Rianne Pinxten, Marcel Eens

Introduction

The high levels of various organohalogenated contaminants that are observed in avian eggs[1,2] suggest that egg laying may be an important excretory mechanism for females. If female birds are able to eliminate a substantial portion of their body burden during egg laying, levels in internal tissues might drop as contaminants are sequestered in the eggs. Whether contaminants in eggs within a clutch follow a laying order effect or not is of considerable interest because the presence of a laying order effect can have great impact on the sampling strategy. Although laying order effects have been reported for some avian species[3,4], most studies measuring organochlorine contaminants showed that eggs within a clutch have similar contaminant levels, and that a single egg statistically represents the entire clutch.[5-7] Most of previous studies were, however, conducted on piscivorous species and birds of prey. The relatively small clutch size of these species may make it particularly difficult to detect laying order effects. In comparison, passerine species have in general a large number of eggs in a clutch and may be more suitable to study the effect of laying order on contaminant concentrations.

The objective of this study was to assess the need to collect eggs of a specific laying order versus collecting eggs randomly for the purpose of monitoring organohalogenated contaminants in eggs from great tits (*Parus major*). Great tits are small insectivorous passerines that are increasingly being used in ecotoxicological studies.[8,9] The chief asset of great tits as biomonitors is their ubiquity, which permits sampling of almost any wooded area across Europe.[8] 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 easily monitored, and eggs can be easily collected. To achieve our objective we assessed the variation of different organohalogenated compounds, such as polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and DDTs, within and among clutches of great tits and we determined if there was an effect of laying order on the concentrations of these contaminants.

Materials and Methods

Ten clutches with known laying sequence were collected immediately after clutch completion during the breeding season of 2000 at several sites near Antwerp (Belgium). In total, 77 eggs were gathered and stored at -20°C until further analysis. Since half of the content of the eggs had previously been used in a study of heavy metal contamination[10], two eggs laid on consecutive days were pooled to obtain a sufficient amount of sample. This resulted in a total of 38 pooled egg samples. A homogenised sample of approximately 0.5 g was weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (PCB 46 and 143, BDE 77 and 128). Further sample treatment and analysis were performed according to previously described methods.[11] 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.

PCBs were analysed using a gas chromatograph coupled with a mass spectrometer (GC/MS) in electron ionisation (EI) mode, equipped with DB-1 capillary column (30 m x 0.25 m x 0.25 µm). For PBDEs and DDTs, a 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) was used. In all samples, 23 PCB congeners, 10 PBDE congeners, DDTs (p,p'-DDE, p,p'-DDD, p,p'-DDT and o,p'-DDT) were analysed. Limits of quantification (LOQ) for the analysed compounds ranged between 1.0 and 2.25 ng/g lipid weight (lw).

Data of all 38 pooled egg samples were used to determine the general contamination levels. To study the variation in PCB, PBDE and DDT concentrations within and among clutches of great tits, only the first six eggs in each clutch were used in the statistical analysis. One clutch contained only 4 eggs and was therefore omitted from further analysis. First, we conducted a principal component analysis (Statistica for Windows, Statsoft 1997) on the levels of the different PCB and PBDE congeners and used the principal component scores as dependent variables. The presence of a laying order effect for the different principal component scores and the sum PCBs, sum PBDEs and DDTs was examined using a non-parametric Friedman ANOVA (GraphPad Instat 3.06 for Windows). 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 (SPSS 11.0 for Windows).

Results and Discussion

General contaminant level

Our results revealed that PCBs constitute the major organohalogenated pollutants in the eggs of great tits (Table 1). All analysed PCB congeners were detectable in all 38 pooled egg samples, except PCB congener 74 which was not detectable in 2 samples. PCB congeners 101, 118, 138/163, 153, 180 and 187 were the most abundant congeners found in the investigated egg samples, which is in accordance with the distribution found in tissues of great tits from Belgium.[9] Sum DDTs levels in our egg samples were lower than the sum PCBs levels (Table 1). The most important compound was p,p'-DDE, with a median concentration of 562 ng/g lw. Concentrations of p,p'-DDT were low (median concentration 39 ng/g lw) and concentrations of o,p'-DDT and p,p'-DDD were below the LOQ in all samples. Winter and Streit[12] reported concentrations of four PCB congeners (101, 138, 158, and 180) and p,p'-DDE in eggs of great tits from an oak wood near Schlüchtern in Germany. The mean concentrations of these PCB congeners were much lower (about 15 to 50 times) compared with the results of our study. Concentrations of p,p'-DDE were also considerably lower than the concentrations in our egg samples. However, concentrations of PCBs and p,p'-DDE in the eggs of tree swallows (*Tachycineta bicolor*) from the Great Lakes were much higher than described in our study.[1]

Table 1: Mean concentrations (ng/g lw) and range (minimum – maximum) of PBDEs, sum PCBs and sum DDTs in all analysed samples of pooled eggs (n = 38).

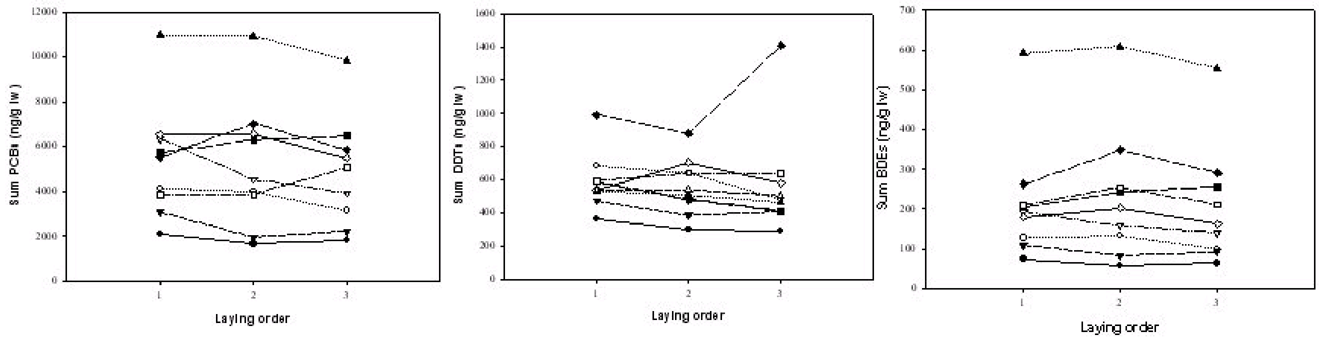
Compound	Mean	Minimum	Maximum
BDE 47	71	28	196
BDE 85	1	ND	4
BDE 99	61	16	206
BDE 100	10	2	29
BDE 153	34	6	92
BDE 154	5	ND	18
BDE 183	22	5	65
sum PBDEs	204	57	609
sum PCBs	4777	1660	10 965
sum DDTs	602	291	1409

PBDEs were found at much lower concentrations than PCBs (Table 1). BDE 47, BDE 99 and BDE 153 were the most prevalent PBDE congeners (Table 1) and contributed each between 17 and 35% to the total sum of PBDEs. To our knowledge, this is the first study reporting PBDE concentrations in passerine eggs. PBDE concentrations in our egg samples were much lower than the levels detected in the eggs of most predatory birds.[2] This is probably due to the lower trophic position of passerines. Nevertheless, the measured PBDE concentrations are comparable with levels reported in eggs of little owls from Belgium.[11]

Contamination variation within and among clutches

We conducted a principal component analysis on the concentrations of the different PCB and PBDE congeners. According to the results of the eigenvalues, only the first principal component for PCBs is considered which accounts for 76% of the total variance. One principal component covered the profile of PBDE congeners. This component accounted for 96% of the total variance. For both PCBs and PBDEs, there was no significant difference among the factor scores of consecutive egg samples within a clutch (PCBs: $F = 2.1$, $p = 0.35$; PBDEs: $F = 2.9$, $p = 0.28$). There was also no significant difference among sum PCB and sum PBDE levels of consecutive egg samples (sum PCBs: $F = 0.9$, $p = 0.69$; sum PBDEs: $F = 2.0$, $p = 0.40$). Levels of p,p'-DDE, p,p'-DDT and sum DDTs in consecutive egg samples also showed no significant differences ($F < 4.7$, $p > 0.11$). These results suggest no effect of laying order on the concentrations of PCBs, PBDEs and DDTs in the eggs of great tits (Figure 1). Winter and Streit[12] found higher concentrations of PCBs and DDTs in the first 6 eggs of a great tit clutch compared to the last 6 eggs. While these results suggest an effect of laying order in the eggs of great tits, only one clutch with 12 eggs was studied making statistical analyses impossible. Our results are in agreement with studies in which clutches of different bird species were reported to contain statistically similar concentrations of organochlorine contaminants among eggs of the same clutch.[5-7] There was also no laying order effect found for DDE concentrations in 2 passerine species, prothonotary warblers (*Protonotaria citrea*) and European starlings (*Sturnus vulgaris*).[13]

Figure 1: Concentrations (ng/g lw) of sum PCBs, sum DDTs, and sum BDEs in pooled egg samples of nine clutches of great tits. The X axis represents the laying order in a clutch, where 1 represents the first and the second egg of a clutch, 2 the third and the fourth and 3 the fifth and sixth.



According to the variance estimates and the log likelihood values, the variance in concentrations of PCBs, PBDEs and DDTs is larger between clutches than within clutches (Figure 1). The between-nest component of variance in the concentrations of PCBs, PBDEs and DDTs accounted for 93%, 97% and 78% of the total variance, compared with 7%, 3% and 22% attributed to the within-nest component. This is in agreement with other studies reporting variability in organochlorine contaminants within and among clutches.[5,7] These studies were, however, conducted on species from other bird taxa with relatively small clutch sizes compared to passerines. The present study shows that a small within-clutch variability can also occur in species where the eggs represent a large percentage of a female's body mass and in which it has been shown that the nutritional requirements of egg production rely almost exclusively on dietary intake.[14] In contrast, two studies measuring within-clutch variation in DDE concentrations in passerine eggs reported a large amount of variability among individual eggs of the same clutch.[13,15] A possible explanation for the absence of a large within-clutch variability in our study may be the fact that great tits have smaller home ranges and that pollution in our study sites presumably showed lower spatial heterogeneity compared to the study of Reynolds et al.[13]

All together, our results suggest that no special sampling strategies (e.g. sampling the first egg or pooling eggs from one clutch) are required for monitoring organohalogenated contaminants in great tits. Therefore, random egg samples of insectivorous passerines with a limited home range, such as great tits, are useful biomonitors for terrestrial organohalogenated contamination.

References

1. Bishop C.A., Koster M.D., Chek A.A., Hussell D.J.T. and Jock K. (1995) *Environ Toxicol Chem* 14: 491-501

2. Lindberg P., Sellström U., Häggberg L. and de Wit C.A. (2004) *Environ Sci Technol* 38: 93-96

3. Nisbet I.C.T. (1982) *Colonial Waterbirds* 5: 139-143

4. Mineau P. (1982) *Chemosphere* 11: 679-685

5. Custer T.W., Pendleton G. and Ohlendorf H.M. (1990) *Environ Monit Assess* 15: 83-89

6. Helander B., Olsson M. and Reutergardh L. (1982) *Holarctic Ecol* 5: 349-366

7. Newton I. and Bogan A. (1978) *J Appl Ecol* 15: 105-116

8. Eens M., Pinxten R., Verheyen R.F., Blust R. and Bervoets L. (1999) *Ecotoxicol Environ Saf* 44: 81-85

9. Dauwe T., Chu S.G., Covaci A. Schepens P. and Eens M. (2003) *Arch Environ Contam Toxicol* 44: 89-96

10. Dauwe T., Janssens E., Bervoets L., Blust R. and Eens M. (2005) *Arch Environ Contam Toxicol* (in press)

11. Jaspers V., Covaci A., Maervoet J., Dauwe T., Voorspoels S., Schepens P. and Eens M. (2005) *Environ Pollut* 136: 81-88

12. Winter S. and Streit B. (1992) *Chemosphere* 24: 1765-1774

13. Reynolds K.D., Skipper S.L., Cobb G.P. and McMurry S.T. (2004) *Arch Environ Contam Toxicol* 47: 396-401

14. Houston D.C., Donnan D. and Jones P.J. (1995) *J Zool* 235: 469-483

15. Ormerod S.J. and Tyler S.J. (1990) *Bird Study* 37: 171-176