ORGANOCHLORINES IN WHITE STORK (*Ciconia ciconia*): A COMPARISON OF LEVELS IN EGGS AND NESTLINGS

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Introduction

Over the past decades wildlife has received much attention as good indicator of ecosystems health. Due to the widespread distribution of some xenoestrogens such as organochlorines, there is a need for screening and risk evaluation of these endocrine disrupters in living organisms from the global point of view of ecosystems health[1]. Depending on the ecosystem under study, different species are selected for the purpose of evaluating levels, trends, behaviour and fate of chemicals in a selected environment. Different studies have used birds as bioindicators of environmental contamination[2]. Trophic level plays an important role when a bird species is selected as "environmental indicator". Consequently, top predators are more exposed to bioaccumulative contaminants resulting in higher concentrations and more impact. Kubiak et al. [3] showed that birds may suffer significant effects on reproductive success due to organochlorines, not only in hatching success, but also in chick status.

White Stork (*Ciconia ciconia*) is a colonial species that feeds mainly on wildlife preys; however recently rubbish dumps have become an important source of nutrients[4]. They may also be exposed to contamination via dietary intake of contaminated species through the food web. Females can transfer contaminants stored in their body fat into their eggs. Similarly, contaminant levels in White Stork nestlings are entirely dependent on their parents' diet, indicating the presence of contaminants within the local environment of the colony.

The main goal of this research programme was to establish an integrated evaluation of contamination in White Stork through the region of Madrid (Central Spain). For this purpose White Stork has been monitored for the last four years. Since eggs are known to reflect the accumulation of lipophilic contaminants in birds, the study focused on the usage of failed eggs obtained from White Storks which represent a good measure of the body burden in the laying hen. In addition, samples from nestlings from the same nests as failed eggs were also obtained to evaluate the transfer and bioaccumulation of selected organochlorine compounds in the species. In this study the preliminary results obtained from monitoring of organochlorine compounds in laying hens and their nestlings sampled from different colonies with know feeding strategies are presented.

Material and methods

Study area and sampling

Four areas were selected within the Province of Madrid (Spain) based on the trophic segregation of individuals and the vicinity to urban solid waste rubbish dumps, ranging from mainly natural feeders, to almost exclusively rubbish dump feeders. Eight complete clutches, selected for the present study, were monitored during the breeding season of 2005 at the four sites identified in our study area.

At least seven days after the expected hatch date, failed eggs were collected. A total of 10 unhatched eggs were collected. Eggs were transported to the laboratory and stored at –80 ºC until analysis. It was assumed that all eggs had the same water loss. Before residue analysis, eggs were examined, and none of them were embryonated.

A few days before fledging, when chicks were feathered and had completely stabilised in the growth of their skeletal structures (37 ± 5.25 days of age from hatching) they were banded and weighed to the nearest 5 g with electronic balances. Peak length was measured to the nearest 1mm with rule. The peak length was used to assign the age and therefore hatching order of a particular nestling using the formula proposed by Aguirre[5].

Age= -5.649+0,455*Peak length

Blood samples were collected from a total of 17 nestling. Blood (3 ml. each) was extracted with syringes from the brachial vein of each chick and transferred to heparinized vials. Samples were transported in a cooler to the laboratory within the day of collection. An aliquot of about 1ml of whole blood was centrifuged at 4000 rpm for 10 minutes; plasma was collected and stored at -80 ºC until analysis.

Chemical analysis

The following organochlorine compounds (OCs) were analyzed: *ortho* PCB congeners #28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #170, #189, #194 and DDTs, including DDT and its metabolites, TDE and DDE.

For PCB and DDT analysis in plasma samples, about 300 microliters were extracted with 3 ml of n-hexane and 2 ml of concentrated sulphuric acid. The tube was vortex stirred for 30 s and centrifuged at 4000 rpm for 10 mintures. The supernatant n-hexane phase was removed and the remaining sulphuric acid solution was re-extracted twice more with 2 ml of n-hexane. Then the n-hexane phase was concentrated under a gentle stream of nitrogen. Egg content was lyophilized and quantities of approximately 2 grams were used for organochlorine analysis. The extraction of DDTs and PCBs involved a matrix solid phase dispersion (MSPD) procedure. Further clean-up was performed by using acid and basic silica gel multilayer columns. A final fractionation of the different families of organochlorine compounds and other possible interferences was achieved by using Supelclean™ Supelco ENVI™-Carb tubes as described elsewhere[6]. The bulk of *ortho*-PCBs and DDTs were collected in a single fraction.

Identification and quantification of the organochlorine compounds was carried out using a Hewlett Packard 6890 HRGC equipped with a ^{63}Ni µ-electron capture detector. A DB-5 fused silica capillary column (60m x 250µm and 0.25µm film thickness) was used. The carrier gas was nitrogen at a head pressure of 192.2 Kpa. Detector and injector temperatures were 300ºC and 270ºC, respectively. Organochlorine compounds were identified on the basis of their relative retention time on the chromatographic column. Quantification was done within the linear range of the detector's seven-level calibration curve using HP ChemStation Plus program (Hewlett-Packard Co., Palo Alto, CA, USA). Those compounds which were under the limit of detection (LOD) were assigned as LOD/2.

Statistical analysis

General linear mixed models (GLMMs) using SAS statistical software (SAS Institute Inc., Cary, NC, USA) were constructed. Such models allow the use of the nest as a sample unit avoiding problems of pseudoreplication due to the use of the same factor within the analysis. Prior to analysis, all contaminant variables were $\log_{10}$ (x + 0.5)-transformed, because the distribution of original values was highly skewed. Including the random value 0.5 in the transformation resulted in an improved model fit compared to the standard log x transformation[7].

Differences in contaminant concentrations between eggs and nestlings in terms of total PCBs, DDT and DDE were evaluated. As explanatory variables we included sampling area and total number of fledglings (fixed factors). Nest was included as random factor. We used $\log_{10}$ (x + 0.5) transformed contaminant values because this transformation resulted in the best model fit. All the analyses were two tailed. The statistical significance of differences between categories of the same variable was computed using the LSMEANS statement of SAS.

Results and discussion

Table 1 shows average values (wet weight) of OCs found in all the failed eggs and nestlings plasma analyzed. Average PCB concentrations in failed eggs were 246.63 ng/g. This value is two orders of magnitude higher than concentrations found in nestlings with average values ranging from 2.91 to 3.51 ng/g for total PCBs. In the case of DDT and DDE, levels are one order of magnitude lower than values found for total PCBs but for those compounds it can be appreciated the differences among failed eggs and nestlings in terms or concentrations.

The plasma DDE concentrations for nestlings found in this study (0.06–0.14 ng/g) are well below egg concentrations demonstrated to have an effect as reported by Bowerman et al[8] from the study of Fleming et al[9] with Wood Stork.

Statistical analysis revealed significant differences between eggs and nestlings for all OCs under study: total PCBs ($F_{3,16}$=184.99, P<0.0001), DDE ($F_{3,16}$=93.22, P<0.0001) and DDT ($F_{3,16}$=50.33, P<0.0001). *Post-hoc* comparisons showed that there were significant differences between all eggs and nestlings (all P < 0.0001) but there were no differences among hatching order for any of the variables under study (all P > 0.7).

Table 1. Mean values (±SE) of OCs (ng/g wet weight) found in failed eggs and nestling plasma. Sample sizes are indicated.

		Hatching order		
	Egg (n = 10)	**1 (n = 7)**	**2 (n = 6)**	**3 (n = 4)**
Total PCBs (ng/g)	246.63 (±41.20)	3.29 (±0.08)	2.91 (±0.04)	3.59 (±0.80)
DDT (ng/g)	25.38 (±7.74)	0.03 (±0.007)	0.02 (±0.007)	0.02 (±0.003)
DDE (ng/g)	59.13 (±12.01)	0.06 (±0.01)	0.14 (±0.04)	0.09 (±0.03)

In the case of PCB pattern it is interesting to highlight some differences when comparing failed eggs and nestlings' plasma, as it can be seen in Figure 1. The most remarkable finding would be the case of PCB congeners 153, 138 and 180 which were the most abundant in failed eggs. Nestlings showed higher percentage contributions from less recalcitrant congeners such as PCB 118, 114, 132 and 105.

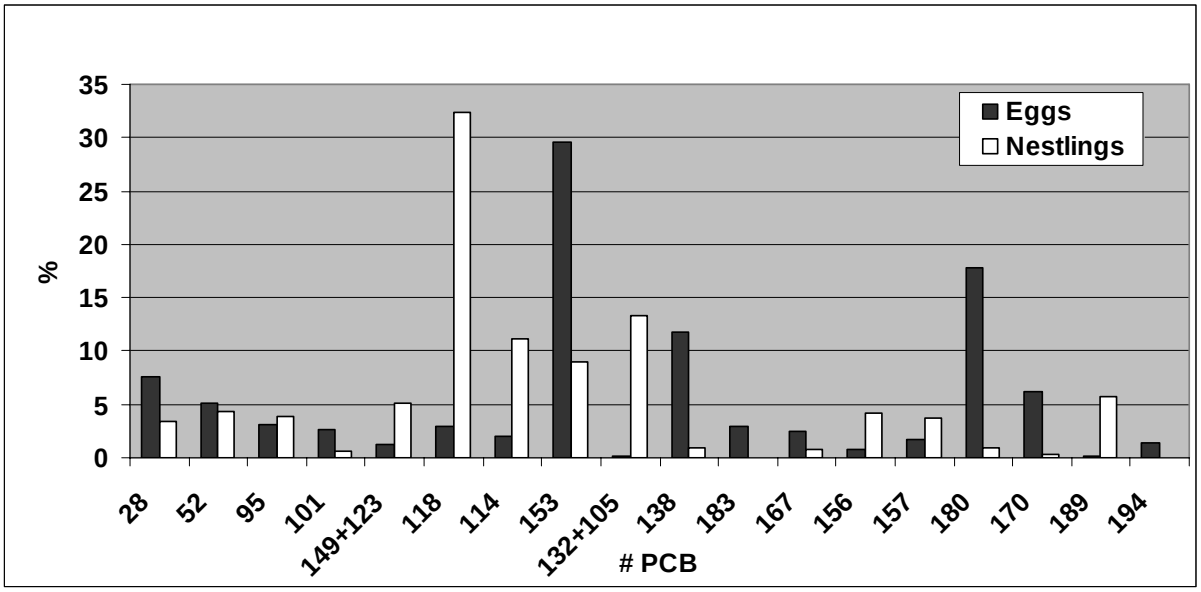

Figure 1. Average contribution of PCB congeners in eggs and nestlings.

An explanation for the differences found in our study may be that plasma levels in nestlings is mostly reflecting the current contamination via food exposure more than eggs which reflects the body burden of the hen[10]. In addition, it should be noted that PCB patterns were similar in eggs while in nestling showed significant variability. This variability was not found between siblings but found within nestlings from the same colony. This reinforces the hypothesis that exposure via food intake could greatly influence the levels and patterns in nestlings. The level of expertise of parents in foraging food or in this case, finding food at the rubbish dump, determines the contaminant intake of their nestlings.

Bowerman et al.[8] observed that the concentrations in nestlings would be appropriate to be used to estimate relative adult exposure during the nesting period, and not for the post-breeding period, due to the differences in foraging areas and parental expertise.

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