THE USE OF WHITE STORK (*Ciconia ciconia*) NESTLINGS IN A BIOMONITORING PROGRAMME FOR ORGANOCHLORINES THROUGH THE REGION OF MADRID (SPAIN)

Blázquez E[1], Aguirre JI[1], Martínez-Haro M[2], Mateo R[2], Jiménez B[1]

1Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC. Juan de la Cierva 3, 28006 Madrid, SPAIN. e-mail: bjimenez@iqog.csic.es

2Laboratory of Ecotoxicology, Unit of Animal Health, Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, Rda. de Toledo s/n, 13071 Ciudad Real, SPAIN.

Introduction

White stork (*Ciconia ciconia*) is a colonial species that feeds mainly on wildlife preys but rubbish dumps have become in the later years an important source of nutrients. They may suffer contamination by dietary intake of contaminants that accumulate through the food chain. Contaminant levels in White stork nestlings, which are dependent on their parents for food provision, indicate the presence of contaminants within a colony's local environment. Population of White Stork showed an increase in pair numbers in Spain, ranging from 16,000 breeding pairs in 1994 to 33,000 in 2004. This fact is mainly due to the use of rubbish dumps as feeding areas, which also increases the risk of contamination.

This study constitutes part of a more extended research project aiming to evaluate the presence and the effects of environmental contaminants such as trace elements (Hg, Cd, Pb, and As) and organochlorines (PCBs and DDTs) in White stork, selected as bioindicator species, in the province of Madrid, covering a gradient of potential contamination. Organochlorine compounds constitutes a class of compounds among the most studied because of their worldwide distribution and their implications in the decline of some wildlife populations[1].

The present work presents results concerning organochlorine (PCBs and DDTs) levels in plasma from White stork nestlings. The relationship between the biochemistry parameters and the organochlorine concentrations in plasma was explored. Information on baseline serum chemistry was unavailable for nestling white storks and this study constitutes the first report in the literature in which the effects of organochlorine compounds in White stork are evaluated in relation to biochemistry parameters.

Material and Methods

Study area and sampling

During the breeding season of 2004, White stork nestlings were monitored in the region of Madrid (SPAIN), in four different areas covering a gradient of potential contamination. The areas were selected based on the trophic segregation of individuals and the vicinity to urban solid waste rubbish dumps, ranging from mainly natural feeders in the Northern area, to almost exclusively rubbish dump feeders in the South-eastern.

Blood samples (about 3 ml. for each) were collected from 200 nestlings at ages between 30 to 55 days. All the nestlings were metal and PVC ringed in order to be able to identify them afterwards. An aliquot of whole blood was centrifuged at 3000 rpm for 10 minutes; plasma was collected and stored at -80 ºC until analysis.

Chemical analysis

Organochlorine (PCBs and DDTs) analysis were performed in plasma. 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* about 300 microliters of plasma were extracted with 3 ml of n-hexane and 2 ml of concentrated sulphuric acid. The tube was vortex stirred for 30 s. The supernatant n-hexane phase was removed and the remaining sulphuric acid solution was re-extracted twice more with 2 ml of n-hexane. All extracts were collected together and the resulting 7 ml of n-hexane were purified by vortex stirring with 2 ml of sulphuric acid. Then the n-hexane phase was concentrated under a gentle stream of nitrogen. Separation of the organochlorine compounds was carried out using a Hewlett Packard 6890 HRGC equipped with a 63Ni 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. The OCs were identified on the basis of their relative

retention time on the HRGC 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.

Total antioxidant status (TAS) in mmol/l was measured in plasma on an A25 Bio-Systems analyzer with a commercial kit purchased from Randox Laboratories Ltd. The method is based upon ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) incubation with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^{\cdot+}$. This has a stable blue-green colour at the wavelength of 600 nm. Antioxidants present in the sample cause suppression of this colour production to a degree which is proportional to their concentration.
Other parameters of plasma biochemistry (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CK), gamma-glutamyltransferase(γ-GT), lactate dehydrogenase (LDH), albumin, total protein, calcium, magnesium, phosphorus, triglycerides, creatinine, uric acid and urea) were measured with kits provided by BioSystems with the A25 analyzer.

The relationships between the biochemistry parameters and the organochlorine concentrations in plasma were studied with GLMs, considering the location as a factor and the sum of DDTs and the sum of PCBs as covariants.

Results and discussion
Table 1 shows average values of OCs found in white stork nestlings at different zones from the Region of Madrid, Spain. Total PCBs ranged from 1.09 to 52.80 ng/ml, being average values in the order of low ppb, with zone central exhibiting the lowest average values.

Table 1. Average levels (ng/ml, ww) of organochlorine compounds (in brackets minimum and maximum) in plasma from white stork nestlings from Madrid, Spain.

Zone	Total PCBs	DDE	TDE	DDT	Ratio DDE/DDT
Alcalá (NE)	**2.99** (1.09-8.69)	**0.16** (0.0.2-0.97)	**0.01** (0.01-0.09)	**0.04** (0.01-0.09)	**6.12**
Casa Campo (SE)	**2.48** (1.72-4.11)	**0.07** (0.03-0.13)	**0.01** (0.01-0.02)	**0.10** (0.03-0.30)	**1.60**
Casa Eulogio (SE)	**6.81** (4.30-10.97)	**0.20** (0.07-0.30)	**0.02** (0.01-0.04)	**0.04** (0.01-0.08)	**20.57**
La Torrecilla (SE)	**6.43** (2.27-28.60)	**0.11** (0.02-0.27)	**0.04** (0.01-0.16)	**0.04** (0.01-0.09)	**3.75**
Casa Frailes (SE)	**6.54** (4.68-8.80)	**0.08** (0.01-0.16)	**0.04** (0.01-0.08)	**0.06** (0.01-0.12)	**2.14**
Prado Herrero (C)	**2.23** (1.21-5.48)	**0.06** (0.01-0.36)	**0.01** (0.01-0.03)	**0.03** (0.01-0.13)	**3.49**
Pinilla (N)	**3.30** (1.89-4.68)	**0.15** (0.03-0.84)	**0.03** (0.01-0.19)	**0.03** (0.01-0.08)	**5.88**
Lozoya (N)	**3.38** (2.24-4.86)	**0.31** (0.03-0.90)	**0.02** (0.01-0.05)	**0.03** (0.01-0.05)	**13.29**
La Granjilla (N)	**15.42** (3.96-52.80)	**0.11** (0.01-0.23)	**0.01** (0.01-0.03)	**0.06** (0.01-0.20)	**4.50**

The relative contribution of each PCB congener to total PCBs in presented in Figure 1. It can be observed that, in general, the most abundant congeners were 118, 114, 153, 132 and 105 in all the zones studied. This pattern is consistent with results found in previous studies conducted in the same area with a raptor species such as the black kite[2].
Regarding DDT and their main metabolites, DDE was the most abundant, being average concentrations in the range 0.07 - 0.31 ng/ml, while average values for TDE and DDT were almost negligible. The ratio DDE/DDT found in all cases, indicates that this banned insecticide was not recently used in the studied areas.

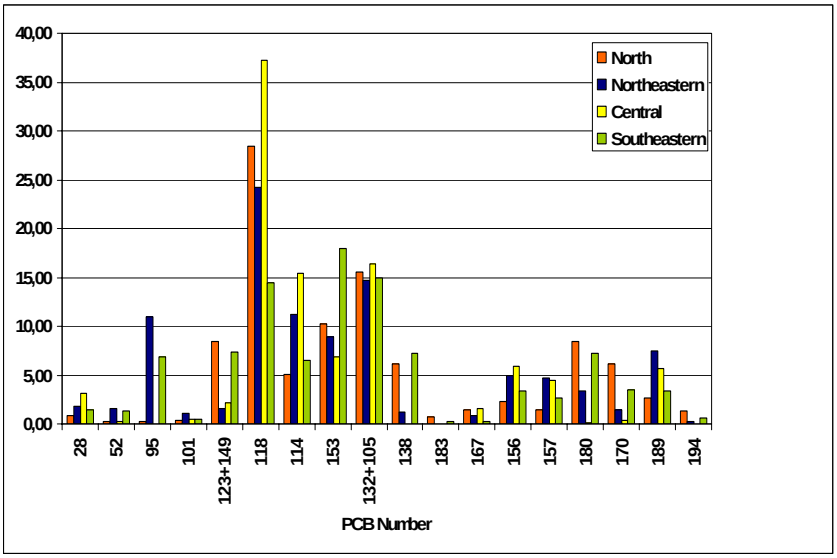

Figure 1. Percentage contribution of each PCB congener to total PCBs in White stork nestlings from all the zones studied.

Several parameters of plasma biochemistry (Table 2) were positively related with the plasmatic concentration of DDTs; triglycerides ($p<0.001$), total protein ($p=0.009$), magnesium ($p=0.001$) and total antioxidant status ($p=0.013$, Figure 2). However, the relationship was negative with the sum of PCBs for triglycerides ($p=0.011$), total protein ($p=0.031$), magnesium ($p=0.003$) and calcium ($p=0.019$). The positive relationship of DDTs with lipids like triglycerides may reflect the importance of quantification on a lipid basis in monitoring programs of organochlorines with plasma samples. Total antioxidant status is a measure of antioxidants in plasma that in part corresponds to fat-soluble vitamins. In consequence, their increase with DDTs can be due to the higher presence of lipids in plasma. However, it may be interesting to notice that the highest values were found in storks from the northern colonies located in the mountains of Madrid (Lozoya, Pinilla, La Granjilla), and the lowest in a colony closer to the city of Madrid (Prado Herrero) ($p<0.001$). This trend seems to be related to Pb blood levels (data not shown here).

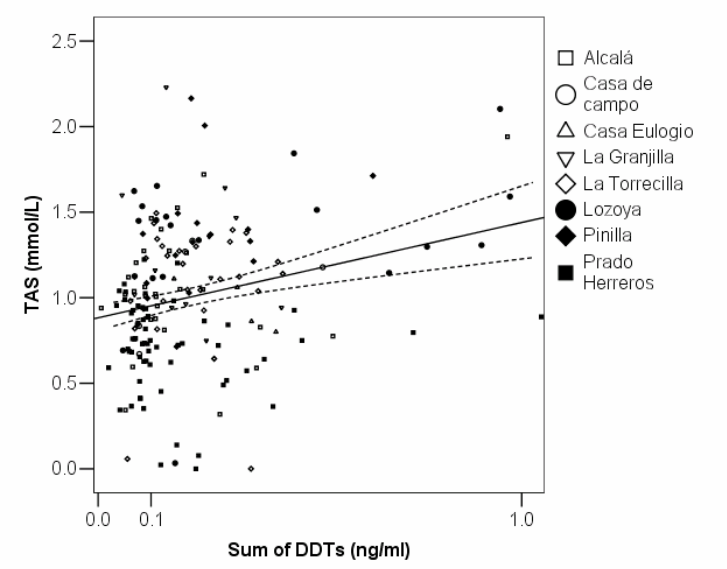

Figure 2. Plasmatic concentrations of DDTs versus Total Antioxidant Status (TAS).

Table 2. Plasma biochemistry values of white stork nestlings form Madrid, Spain.

Parameter	n	Mean	SD	Min.	Max.
TAS (mmol/l)	178	1.01	0.41	0.00	2.23
ALP (U/l)	175	697.48	248.79	120.43	1387.03
ALT (U/l)	174	51.62	15.16	23.16	129.63
AST (U/l)	174	206.77	61.42	98.00	581.21
CK(U/l)	173	818.40	323.55	254.76	1792.59
γ-GT (U/l)	174	3.07	4.57	0.00	30.54
LDH (U/l)	175	1630.56	493.75	811.70	3138.76
Albumin (g/l)	165	27.86	8.41	5.73	68.49
Protein total(g/l)	169	55.15	16.39	31.39	127.82
Triglycerides(mg/dl)	171	229.63	197.46	68.42	1296.86
Cholesterol (mg/dl)	170	288.18	79.70	176.40	648.50
Glucose (mg/dl)	169	343.14	96.54	175.48	722.93
Phosphorus(mg/dl)	167	9.04	3.18	0.00	24.32
Calcium (mg/dl)	168	17.03	4.25	9.14	34.69
Magnesium(mg/dl)	168	2.30	1.05	0.89	5.99
Creatinine(mg/dl)	93	0.67	0.28	0.16	1.76
Urea (mg/dl)	74	17.34	17.15	2.15	89.39
Uric acid(mg/dl)	108	17.92	11.28	1.26	37.72

Our preliminary results confirm the applicability of White stork as bioindicator organism. Organochlorine concentrations found in nestlings were low although some slight differences could be appreciated depending on the breeding zone where nestlings were sampled. The total antioxidant status is a measure of the antioxidant capacity of plasma and consequently a marker of oxidative stress. Organochlorines did not affect negatively TAS, but heavy metals like Pb may explain differences among colonies of storks as it has been recognised to induce oxidative stress in birds[3]. Further research is ongoing with adult individuals in order to establish a field validation of the biomagnification phenomena in this species associated to urban solid waste rubbish dumps.

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