# DIOXINS AND RELATED COMPOUNDS IN WHITE STORKS (*Ciconia ciconia*) FROM DOÑANA NATIONAL PARK, SOUTHWESTERN SPAIN.

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## Introduction

Nowadays contamination by organochlorine (OC) compounds continues to be an element of concern in top predators. Because of their ubiquitous presence, despite the regulatory measures established in the Stockholm convention that lead to the ban of some of them, the study of contamination in different levels of the food web becomes necessary in order to gain a more solid knowledge about their chemical fate and their influence on the ecosystems.

In the field of wildlife toxicology, a wide variety of species has been proposed as bioindicators, or sentinels of environmental health. The species used should provide an "early warning system" for toxic contaminants in the environment, and top predators represent the most suitable species. These species are positioned at the top of food chains and thereby negatively impacted by bioaccumulation of contaminants.

A recent study[1] in the breeding Red Kite population of Doñana National Park (DNP) reported unexpected high levels of some organochlorine compounds (e.g. PCBs and DDTs). At this point, ecologically sensitive areas present a particular interest since, in general, they represent important refuges for threatened wildlife species. Therefore, special attention should be paid to the sources and fate of these chemicals in DNP. In this regard, further investigations have been undertaken to find out whether or not organochlorine contamination exists in other species in the area, as well as their potential health effects on individuals and/or populations. Here we are focusing on the White Stork (*Ciconia ciconia*). For this purpose, archived failed eggs were used for the analysis of OC compounds, including PCDDs, PCDFs, PCBs and DDTs.

## Materials and methods

### Sampling

A total of 16 failed eggs of White Stork were obtained during the breeding seasons of 1999-2001. Samples were stored at -20° C until analysis. Eggs content was used for chemical analysis and the remaining eggshell was kept for further structural analysis.

### Analytical determination

Egg content was lyophilized and quantities of approximately 2 grams were used for residue analysis. The extraction of DDTs, PCBs and PCDD/Fs involved a solid phase matrix dispersion (SPMD) procedure. Further clean-up was performed by using acid and basic silica gel multilayer columns. A final fractionation of the studied compounds and other possible interferences was achieved by using SupelcleanTM Supelco ENVI$^{TM}$-Carb tubes as described elsewhere[2]. Three fractions were eluted: the first fraction contained the bulk of *ortho*-PCBs and DDTs while the second and third fractions contained non *ortho* substituted PCBs and PCDD/Fs, respectively.

Resolution and quantification of mono-*ortho* PCBs and DDTs were carried out by HRGC-ECD on a Hewlett Packard 6890 GC equipped with a $^{63}$Ni μ-electron capture detector. A BPX5 fused silica capillary column (60m

x 0.25mm and 0.25µm film thickness) purchased from SGE (Melbourne, Australia) was used. The carrier gas was nitrogen at a head pressure of 192.2 Kpa. Detector and injector temperatures were 300ºC and 270ºC, respectively.

Resolution and quantification of non-ortho PCBs, PCDDs and PCDFs were performed by high resolution gas chromatography coupled with high resolution mass spectrometry (HRGC-HRMS) on a GC 8000 series gas chromatograph (Carlo Erba Instruments, Milan, Italy) equipped with a CTC A 200S auto sampler (Water Instruments, Manchester, UK) and coupled to an Autospec Ultima mass spectrometer (Micromass, Manchester, UK), using a positive electron ionization source and operating in the selected ion monitoring mode at 10000 resolving power (10% valley definition), as previously described by Merino et al.[2]. Quantification of non-*ortho* PCBs and PCDD/Fs was carried out by the isotopic dilution technique following procedures from EPA[3]
Quality assurance criteria were based on the application of the quality control and quality assurance measures, which included the analysis of blank samples covering the complete analytical procedure. Concentrations are expressed on a wet weight (ww) basis. 2,3,7,8-TCDD equivalents (TEQs) were estimated for PCDD/F congeners and dioxin-like PCBs with an assigned TEF value, based on the bird toxic equivalency factors (TEFs) reported in 1998 by the World Health Organization [4].

**Results and Discussion**
Average total level of *ortho* PCBs was 848 ng.g$^{-1}$ (fresh weight basis) ranging from 76.1 to 3180 ng.g$^{-1}$ with a major contribution (>5%) of congeners #138, 153, 170, 180 as it is shown in figure 1. This profile is similar to that reported previously for the same species and area[5], and similar with the profile of White Stork inhabiting the region of Madrid (Central Spain) [6]. However, in spite of the similarities, an important difference must be mentioned. This is regarding the total PCB concentrations of both colonies, since the Madrid colony inhabiting a much higher contaminated area presents an average total level of ortho PCBs of 246 ng.g$^{-1}$, which is about four times lower than the value found in the White Stork colony from Doñana National Park .

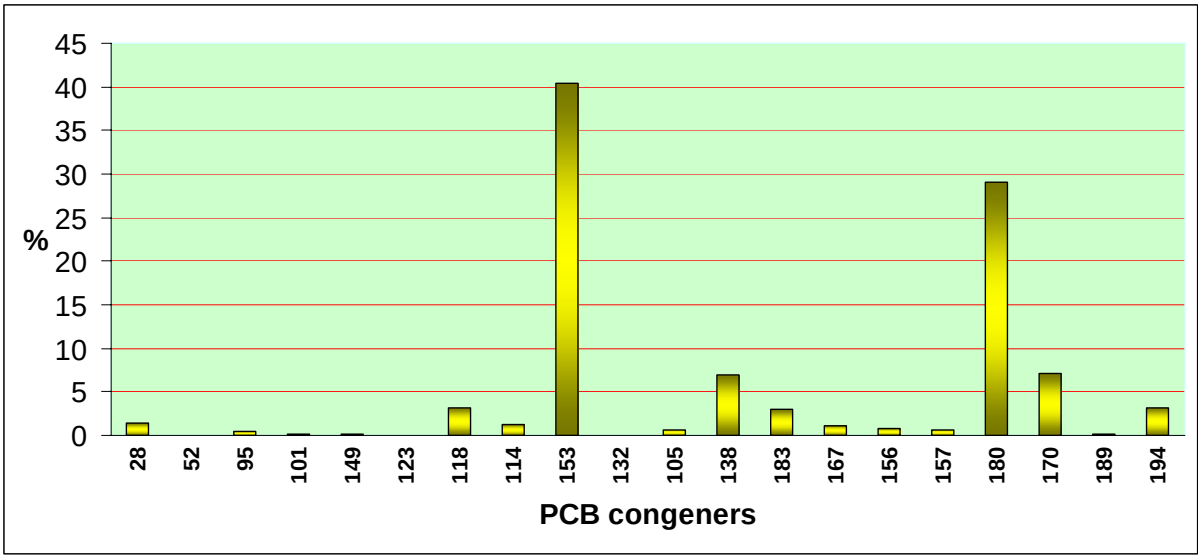

Figure 1. Relative contribution of individual congeners of *ortho* PCBs to total *ortho* PCB levels in White Stork eggs from Doñana, Spain.

Regarding *non-ortho* PCBs, the average total concentration determined (as sum of congeners #77,126 and 169) was 159 pg.g$^{-1}$ (fresh weight basis), ranging from 31.4 to 274 pg.g$^{-1}$. Although slightly higher, the magnitude of these results is also in consonance with the values found previously for the same species in the same area[5].

Total levels of PCDD/Fs in the analyzed samples were, in average, 17.7 pg.g$^{-1}$, ranging from 3.00 to 76.1 pg.g$^{-1}$ on a fresh weight basis. In all samples, the content of PCDFs was lower than that of PCDDs. Additionally, in all samples the content of OCDD was the highest among PCDD/Fs, and in most of them by one or two orders of magnitude. This, along with the fact that some 2,3,7,8 substituted PCDFs in several eggs were under the limit of detection achieved represent a remarkable difference in comparison to previous studies performed on White Stork and other bird species (Red Kite and Black kite) breeding in DNP[1,5].

p,p'-DDT and its main metabolite p,p'-DDE were found in all White Stork eggs whereas the content of p,p'-DDD was under the limit of detection in more than 30% of them. In the rest of the samples analyzed, the mean content of DDD was 0.39 ng.g$^{-1}$ (fresh weight). Conversely, p,p'-DDE was the most abundant among DDTs with an average concentration of 985 ng.g$^{-1}$ (range from 68.4 to 3156 ng.g$^{-1}$ ) followed by p,p'-DDT with an average content of 16 ng.g$^{-1}$ (range from 3.35 to 62.6 ng.g$^{-1}$ ), both on a fresh weight basis. The fact that p,p'-DDE was by far the most abundant species among DDTs, along with an average ratio [DDE]/[DDT] of 76, seem to indicate the absence of a recent usage of the pesticide. The level of DDE found is under the limit considered for reproductive impairment[7]; nevertheless, this limit was established for a different species, which should be born in mind since species-sensitivity may play a very important role. Finally, these DDT concentrations highly contrast with those found for white storks inhabiting the region of Madrid, where average total contents of p,p'-DDT and p,p'-DDE of 25.4 ng.g$^{-1}$ and 59.1 ng.g$^{-1}$ respectively have been reported[6].

Calculation of total TEQs taking into account the combined contribution of PCDD/Fs, *non-ortho* and *ortho* PCBs showed a consistent trend for all samples analyzed, where the major contribution came from the content primarily of *ortho* PCBs and secondly of *non-ortho* PCBs as it is represented in figure 2.

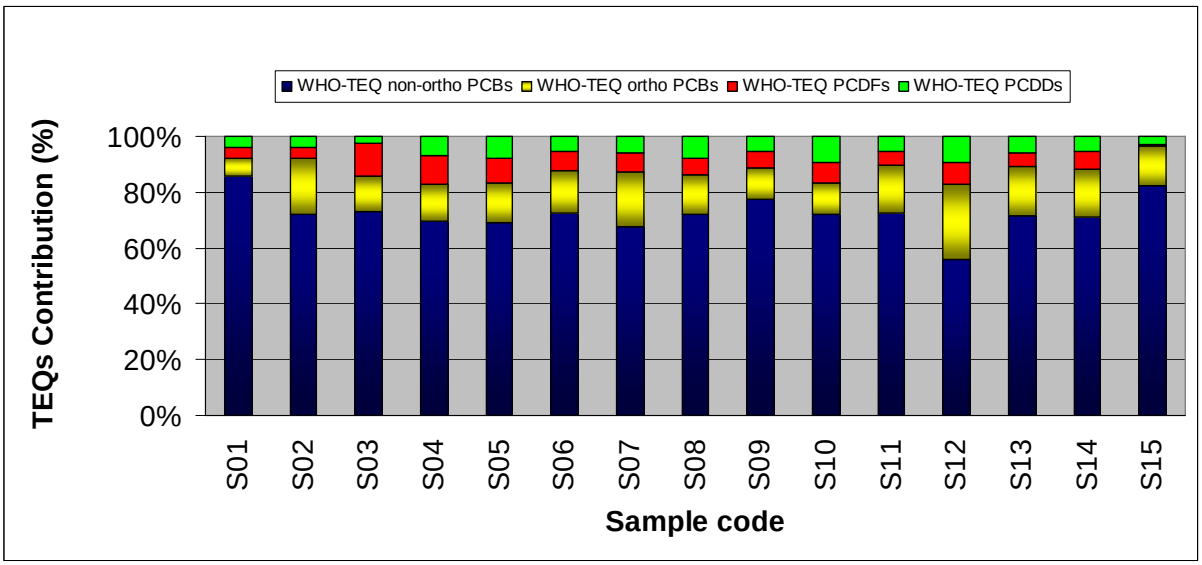


Figure 2. Relative contribution of the sum of *ortho* PCBs, *non-ortho* PCBs, PCDFs and PCDDs to total toxic equivalent quantities (TEQs) in White Stork eggs from Doñana, Spain.

**Acknowledgments**
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