AN STUDY OF THE TOXIC EQUIVALENTS DERIVED FROM PCDDs, PCDFs AND DIOXIN-LIKE PCBs IN TWO BIRD SPECIES (*Ciconia ciconia* and *Milvus migrans*) NESTING IN A PROTECTED AREA (DOÑANA NATIONAL PARK, SPAIN).

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Introduction

There is growing evidence that PCDDs, PCDFs and PCBs are extremely harmful to living organisms, especially when they bioaccumulate through foodwebs[1]. In recent years there has been increasing concern regarding environmental estrogens. Environmental chemicals with known estrogenic effects include: polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). Reported adverse effects in wildlife are declines in populations, increases in cancers, reduced reproductive function and disrupted development of immune and nervous systems. These chemicals have been shown to mimic or antagonise the action of endogenous hormones of particular interest. Due to the widespread distribution of these xenoestrogens, there is a need for screening and risk evaluation of these endocrine disrupters [2].

Aspects of black kite (*Milvus milvus*) and stork (*Ciconia ciconia*) life history make them a useful species for contaminant monitoring. They are long-lived birds and breeding pairs show a high degree of nest site fidelity, thereby permitting long-term monitoring of individualnesting territories. Effects of chlorinated pollutants has not been widely studied in this species. Previous studies[3] at Doñana National Park (PND) have demonstrated that PCBs were already present in eggs collected during the period 1980-1990.

The present study was designed in order to determine organochlorine levels (PCBs,PCDDs and PCDFs) in eggs from black kites (*Milvus milvus*) and storks (*Ciconia ciconia*) breeding near the marshes in Doñana National Park. Therefore, the isomer specific analysis of these substances in eggs samples of two bird species from Doñana National Park (PND) was performed. The goal of this study was to determine the levels of PCDDs and PCDFs and to compare these samples to samples collected in other areas. The toxicological significance of these levels and a comparison of our data to those reported for other birds populations are discussed.

MATERIAL and METHODS

Sampling

Unhatched eggs of white stork specimens (*Ciconia ciconia*) and black kites (Milvus migrans) were obtained from nests located in the area of Doñana National Park (PND) during the breading season

of 1998. Eggs samples were frozen and stored at -20° C until use. Samples for residue analysis were lyophilised and quantities of approximately 1.5 grams of lyophilised eggs were used for analysis.

Analytical determination. ***Extraction and clean up.***
Extraction and clean up were performed as previously described in detail elsewhere[4]. Basically this consisted of low pressure chromatography on neutral and base-modified silica gel and activated carbon dispersed on glass fibres. Three fractions were eluted from the carbon column for each sample. These contained ortho-substituted PCBs, non-ortho-substituted PCBs and PCDD/Fs, respectively. Further cleanup was done using silica gel impregnated with sulphuric acid, and Florisil®. Prior to the initial extraction of samples, a mixture of $^{13}C_{12}$ PCDD/Fs and non-ortho substituted PCBs internal standards, was added.

Quantification. Resolution and quantification of PCDDs, PCDFs and co-planar PCBs were performed by HRGC-HRMS using a VG AutoSpec Ultima (VG Analytical, Manchester, UK) coupled to a Fisons Series 8000 (8060) gas chromatograph. A minimum resolution of 10,000 was used when operating with the HRMS instrument. Methods blanks were routinely analysed, and no contributions were detected. Resolution and quantification of mono-ortho PCBs was carried out by HRGC-ECD using a Varian GC Instrument CX Series, STAR 3400. A fused silica capillary DB-5 column (60m, 0.25 mm id., 0.25μm film thickness,J&W Scientific, USA) and a DB-DIOXIN column were used. The carrier gas was Helium at a column head pressure of 175 Kpa.

RESULTS AND DISCUSSION

Total PCDDs and PCDFs.
All 2,3,7,8-substituted PCDDs and PCDFs were detected in all the samples studied. Total PCDD/F levels ranged from 4.56 ppt to 69.56 pg/g on a wet weight basis in all the white storks (*Ciconia ciconia*) studied. Regarding the black kites studied (*Milvus migrans*) total levels ranged from 10.40 to 13.46 pg/g on a wet weight basis. Regarding the contribution of PCDDs and PCDFs to total levels, in general a higher percentage contribution was found from PCDDs, which in some cases contributed up to 88% while in the case of PCDFs their contribution was always lower than that of PCDDs. This situation was observed for both all white storks and black kites studied.

Several tendencies were observed depending on the individual studied:
- Individuals with the highest contribution to total levels coming from 2,3,4,7,8-PnCDF with a contribution ranging from 37 to 27%. Also an important contribution comes from 2,3,7,8-TCDD and 2,3,4,7,8-PnCDD.
- Individuals with the highest contribution to total levels coming from OCDD with a percentage contribution ranging from 31 to 67%.
- Important contributions coming from 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, OCDD and 2,3,4,7,8-PnCDF with similar contribution percentages ranging from 14 to 21% respectively.

Co-planar PCBs and Non-coplanar PCBs.
Total levels for co-planar PCBs (#77, 126 and 169) found in white storks ranged from 14 to 78.77 pg/g on a wet weight basis. Levels found in black kites ranged from 39 to 52 pg/g on a wet weight basis. In general it was always found that PCB #126 was the most abundant followed by PCB #77 and PCB #169.

Average total non-coplanar PCBs exhibited 1.38μ/g (wet basis) in *C. ciconia* and 1.15 μ/g in *M. migrans*. It can be seen an important contribution of congeners PCB # 153, 180, 170 and 194. It can be seen that PCB levels corresponding to the period of 1998 are lower than levels found in previous years in Spain[18-20] and also much lower than levels found in other countries [6-17].

Calculated TEQs for PCDDs and PCDFs.
2,3,7,8-TCDD equivalents (TEQs) were estimated for PCDD/F congeners based on the Bird Toxic Equivalency Factors (TEFs) reported by the World Health Organisation[5]. Total calculated TEQs ranged from 1.66 to 2.17 pg/g (wet weight basis) in black kites specimens and from 1.14 to 6.21 pg/g (wet basis) in white storks. There were always some congeners which contributed markedly to total calculated TEQs. In the case of PCDDs the congener 2,3,7,8-TCDD always contributed with a percentage ranging from 9 to 17 %. The congener 1,2,3,7,8-PnCDD contributed to total calculated TEQs with a percentage ranging from 20 to 32% and the toxic 2,3,4,7,8-PnCDF contributed to total TEQs with a percentage ranging from 21 to 28%. Regarding the remaining congeners their contribution was always lower that the previously mentioned congeners, being their contribution in general under 1%, except in the case of congeners 1,2,3,6,7,8-HxCDD which in some cases reached contributions up to 5%.

Dioxin Toxic Equivalents (TEQs) of biochemically active planar PCBs.
Calculated TEQs for co-planar PCBs in white storks ranged from 1.15 to 6.30 pg/g on a wet basis, while in black kites the range was found to be from 3.12 to 4.02. PCB # 126 was the most important contributor to total TEQs followed by PCB # 77 and PCB # 169.

When the contribution from PCDD/Fs to total toxicity is compared with that of PCBs it can be seen that it is lower than that coming from PCBs (non-ortho and mono-ortho). It should be noted that TEQs in this study were calculated based on new TEFs reported by WHO in 1998[5]. In some individuals it was found that the largest percentage contribution to total toxicity came from mono-ortho PCBs, with percentages ranging from 44 up to 58%. Another case is that of individuals in which the highest contribution came from non-ortho PCBs with percentages ranging from 39 to 46%. The last situation is that of individuals in whichthe most important contribution came from PCDDs and PCDFs with percentages ranging from 40% to 66%.

The situation find in the birds studied is quite different from that found in the aquatic environment. For example in marine mammals regarding PCDD/Fs toxicities it has been reported that in general their contribution to overall toxicity was always lower than that coming from mono- and non-ortho PCBs [21].

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