PRESENCE OF ORGANOCHLORINE POLLUTANTS (PCDDs, PCDFs, PCBs AND DDTs) IN EGGS OF PREDATORY BIRDS FROM DOÑANA NATIONAL PARK, SPAIN

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Introduction

Organochlorine compounds such as PCDDs, PCDFs, PCBs as well as DDTs are well known as toxic and persistent contaminants that accumulate in the upper trophic levels of food chains, apparently associated with the habitat and dietary habits. In recent years there has been an increasing concern for these chemicals mainly due to their estrogenic properties. Lowered reproductive success in numerous bird species has been associated with egg-shell thinning and reduced production caused by these type of xenobiotics [1,2].

The Doñana National Park (DNP) is a protected nature reserve that serves as a wild life sanctuary or refuge for thousands of sedentary and migratory birds which nest and, in some cases, reside there temporarily. These birds offer an abundance of suitable biotopes for many species, including predatory birds and mammals. Predatory birds are top-level predators, excellent accumulators of persistent environmental pollutants and they are long-lived birds. Because of these characteristics they are very useful as monitoring species in the terrestrial food chain.

This study presents the levels of the most persistent and toxic POPs (PCDDs, PCDFs, PCBs and DDTs) found in infertile eggs of four species of the avian Falconiforme order, red kite (*Milvus milvus*), imperial eagle (*Aquila heliaca*), booted eagle (*Hieraetus pennatus*) and buzzard (*Buteo buteo*) collected at Doñana National Park, DNP (Spain). Data will be compared with that found in similar species from Doñana National Park from previous breading seasons as well as from other selected ecosystems.

Material and Methods

Sampling

Infertile eggs of red kites, imperial eagles, booted eagles and buzzards were collected at Doñana National Park (DNP) in 1999 and 2000 breading season. Egg samples were lyophilized and stored at -20 ºC until analysis.

Extraction and clean-up

Extraction was carried out by matrix solid phase dispersion as previously described in detail elsewhere [3]. Lyophilised egg sample was homogenised with 1:1 (w/w) silica gel:anhydrous sodium sulphate powder. The mixture was ground to become a fine powder, loaded into a column and spiked with a mixture containing 15 $^{13}C_{12}$ labelled 2,3,7,8-substituted PCDDs and PCDFs and $^{13}C_{12}$ labelled PCB # 77, 126 and 169. Extraction was carried out with 400 ml of 1:1 (v:v) acetone:hexane mixture. Clean-up was carried out using a multilayer column filled with neutral silica, silica modified with sulfuric acid (44 %) and silica modified with KOH. The final fractionation was achieved by using Supelclean™ ENVI™-Carb SPE cartridges as described elsewhere [4]. Three fractions were eluted containing non-*ortho* PCBs, PCDD/Fs and the rest of the organochlorine compounds (bulk of PCBs and DDTs), respectively.

Analysis of ortho-PCBs and DDTs

The analysis were performed by GC-mECD (Agilent 6890 Series II, PA, California, USA) as was described elsewere [5]. The following organochlorines were analysed: *p,p'*-DDT, *p,p*'-DDE, *p,p'*-TDE; and PCBs # 28, 52 , 95, 101, 123, 149, 118, 114, 153, 132, 105, 138, 183, 167, 156, 157, 180, 170, 189 and 194.

Analysis of PCDD/Fs and non-ortho PCBs

Purified extract were analysed by HRGC-HRMS/EI(+)-SIM on a GC 8000 series GC (Carlo Erba Instrument, Milan, Italy) coupled to an Autospec Ultima mass spectrometer (MS) (Micromass, Manchester, UK). Chromatography was run on a DB5 (J&W Scientific, USA) 60 m longitude, 0.25 µm i.d., 0.25 mm film thickness column. A 2 µl sample was injected in the splitless mode. The two major ions of the molecular ion cluster were monitored for each compound. Quantification and quality dioxin analysis such a blanks, recoveries, parallel analysis and participation in interlaboratories studies has been detailed elsewhere [6]. The results are expressed in WHO_{98}-TEQs [7] for birds. TEQs values were calculated in the upper bound determination levels meaning that the non-detectable congeners were considered as the limit of detection.

Results and Discussion

PCBs and DDTs

The sum of 23 the PCB congeners analysed (on a fresh weight basis, f.w.) showed significant variations among the birds species investigated. Thus, red kites exhibited the highest mean value (± SD) (46.1 ± 35.2 µg/g f.w.), followed by buzzard (9.6 ± 11.1 µg/g f.w.), imperial eagle (2.1± 1.2 µg/g f.w.) and booted eagle (1.57 ± 0.07 µg/g f.w.),. These average values were higher than those found in eggs from predatory birds species collected in Doñana National Park in 1998 [8] and in the period 1980-83 [9], where PCBs ranged from 0.42 to 5.89 µg/g f.w. They were also above the 4.7 mg/g f.w. level, which has been associated with eggs-shell thinning (between 9 and 20 %) and reduction in mean production [1,2,10].

PCBs # 101, 118, 138, 153, 170 and 180 were the most abundant PCB congeners, each accounting for more than 7 % over the total PCBs investigated. Non-*ortho* PCBs # 77, 126 and 169, mono-*ortho* PCB congeners # 156, 157 and 167, and PCBs # 28, 52, 95, 132, and 189 showed minor contributions. PCB #77 was the dominant non-*ortho* PCB followed by 126 and 169, except for red kite species for which the pattern was 126 > 77 > 169.

Regarding DDTs, the compound which has been given special attention was *p,p'*-DDE due to the well-established inverse relationship between *p,p'*-DDE content and egg-shell thickness having consequences on the breeding productivity of different bird species [11]. *p,p*'-DDT and its two main metabolites *p,p'*-DDE and *p,p'*-TDE were detected in all eggs samples analysed. Mean levels of *p,p'*-DDE ranged between 0.68 and 2.9 µg/g f.w. These values were lower than those associated with deleterious effects on bird's health [10]. Values of *p,p'*-DDE were always higher than *p,p'*-TDE and *p,p'*-DDT. In all cases, *p,p'*-DDE contributed with more than 95 % to the total DDTs content. These results were similar to those found in eggs from predatory birds gathered in DNP in the 1980-83 period [9] (in the range from 0.04 to 1.54 mg/g f.w.), and in other Spanish ecosystems [12,13]. These *p,p'*-DDE levels indicated that this insecticide was used in the studied area after being banned in Spain (in the 70's). However the ratio *p,p'*-DDE/*p,p'*-DDT proved that *p,p'*-DDT has not recently used in DNP.

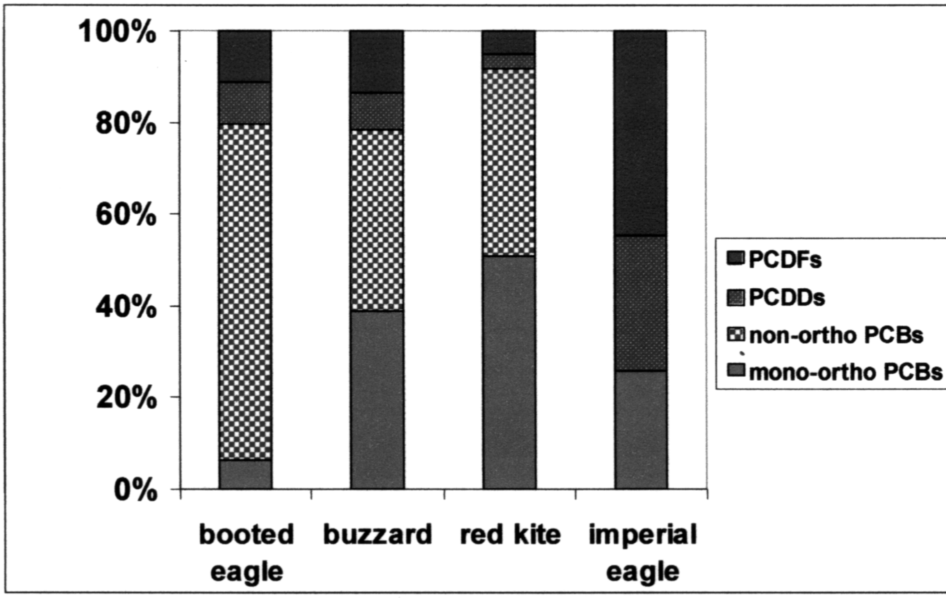

Figure 1. Toxic equivalent percentage contribution of PCDDs, PCDFs, mono- and non-*ortho* PCBs.

PCDD/Fs

Exposure to PCDDs and PCDFs relative to PCBs was low in all of predatory species studied. The mean concentration of 2,3,7,8-chlorine substituted PCDD/Fs in the studied species ranged from 14.8 to 22.8 pg/g f. w. basis, the highest values corresponding to red kite species.

The PCDD/F concentrations found in the studied eggs were much lower than those reported in eggs from birds breeding near PCDD/F emission industrial sites such as pulp mill and chlorophenol point sources [14,15].

Toxic equivalent contribution

Toxic equivalents of PCDD/Fs and dioxin like PCBs (non-*ortho* and mono-*ortho* PCBs) in terms of WHO_{98}-TEQs determined in eggs of predatory birds are shown in Figure 1. It is worth mentioning the high contribution for the total toxic equivalent of coplanar PCBs in all studied samples (always higher than 50 %), except for imperial eagle samples, for which non-*ortho* PCBs were not analysed. The highest PCB contribution was found in red kite (94 %) species. PCB #126 has the highest contribution to the toxicity in red kite species, while PCB #77 was the prevalent in the rest of the predatory bird species studied. As regards mono-*ortho* PCBs, the most important contributors to the WHO_{98}-TEQs corresponded to PCBs # 105, 156 and 157. Toxicity due to PCDFs was found to be higher than that of PCDDs. 1,2,3,7,8-PeCDD/F congeners were the most important contributors to the PCDD/F toxicity, followed by 2,3,7,8-TCDD/F congeners.

Acknowledgement

The authors would like to thank Junta de Andalucía and projects INIA (CAL00-041) and CAM (07G/0057/2000) for financial support.

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