Higher Brominated Diphenyl Ethers and Organochlorines (PCDDs, PCDFs, PCBs, DDTs) in Peregrine Falcon (Falco peregrinus) Breeding in Spain

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Introduction

Peregrine falcons (*Falco peregrinus*) are top predators that feed almost exclusively on other birds. Most populations of the species were previously endangered in the northern hemisphere because of the bioaccumulation of high concentrations of several organochlorine pesticides and mercury which affected both reproduction and survival[1]. Adverse effects of organochlorine compounds (OCs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and organochlorine insecticides in wildlife has been widely documented in the literature in past decades[2,3]. It is well documented that the peregrine falcon represents a species particularly sensitive to these contaminants[4,5]. During the past few years some studies have indicated that concentrations of organochlorine compounds have declined significantly in peregrine falcons (e.g. Sweden, California), and that the populations are now recovering[6, 7]. However, the increasing temporal trends of polybrominated diphenylethers (PBDEs) in the environment could represent a potential new threat to this species and their presence was reported recently by Lindberg et al. in peregrines from Sweden[6]. This was the first time that BDE-183 and –209 were reported in high trophic level wildlife. This has led to the need for more data on the environmental fate and levels of PBDEs.
In Spain, peregrine falcon populations have shown a decline over the last decade in the provinces of Madrid and Guadalajara (Central Spain). Therefore, in 2000 we initiated studies to evaluate the presence of organochlorines (PCDDs, PCDFs, PCBs and DDTs) in this species to determine to what extent these contaminants are involved in the peregrines decline. In addition, the recent finding of higher brominated diphenyl ethers in peregrines from Sweden[6] constitutes a good reason for exploring these new emerging contaminants in Spanish peregrines and as such compare the situation in both populations. To our knowledge, this is the first study on PBDEs conducted in the peregrine falcon in Spain.

Materials and methods

Sampling

Between 2000 and 2001 a peregrine falcon population was closely monitored in the provinces of Madrid and Guadalajara, Spain. Reproductive success and population numbers of nesting falcons were monitored. Eight unhatched eggs from six females, obtained in the study area were analyzed for OCs and PBDEs.

Analytical determination

The extraction and cleanup methods have been described in detail elsewhere[8] and can in short be described as follows: The extraction involved solid phase matrix dispersion (SPMD) procedure. Fractionation among the studied compounds and other possible interferences was achieved by using Supelclean^{TM} Supelco ENVI^{TM}-Carb tubes. Three fractions were eluted: the first fraction contained the bulk of *ortho*-PCBs and DDTs; the second and third fractions contained non *ortho* substituted PCBs and PCDD/Fs, respectively. Separation and quantification of mono-*ortho* PCBs and DDTs was carried out by HRGC-ECD using a Hewlett Packard 6890 GC equipped with a ^{63}Ni µ-electron capture detector. Separation 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 low contributions were detected. The second fraction was used for qualitative analysis of PBDEs. High resolution GC/MS analyses of PBDEs was carried out on a Micromass AutoSpec Ultima mass spectrometer connected to a Hewlett-Packard 6890 GC equipped with a CTC A200s autosampler. The GC injection port was configured for 1 uL splitless injections, at constant temperature of 275 ^{o}C. For lower brominated

PBDEs, gas chromatographic separation prior to MS was achieved using a 60 m X 0.25 mm X 0.25 μm Restek Rt_x5 capillary column. The GC column was maintained at 110°C for 1 min, then ramped at 15 °C/min to 180°C, further ramped at 2°C/min to 280°C and held there for 60 minutes. Total run time was 90.7 min. Separation of higher brominated compounds was accomplished using a 15m X 0.25 mm X 0.25 μm Restek Rt_x5 capillary column. The GC column was maintained at 110°C for 0.5 min, then ramped at 10 °C/min to 300°C , held for 20 minutes, further ramped at 20°C/min to 310°C and held there for 5 minutes. Total run time was 45 minutes. Sample ionization was performed by electron ionization (EI) at an electron voltage ranging from 30 to 40 eV depending on the optimization parameters of the instrument. Source temperature was 270 °C and the resolving power of the analyzer was 10,000. The mass spectrometer was operated in SIM mode using a total of 8 descriptors to analyze the lower PBDE congeners, and single descriptor for higher brominated PBDEs.

Results and Discussion

PCDDs, PCDFs, and PCBs and DDTs in eggs.

Total PCDD/F concentrations ranged between 6.67 and 19.10 pg/g on a wet weight basis (WW). It was remarkable that the 2,3,4,7,8-PeCDF was the most abundant congener with percentages ranging from 23% to a 40%. Non-*ortho* PCB concentrations ranged from 131.68 to 694.18 pg/g (WW), the most abundant congener PCB #126 was followed by PCB #77 and PCB #169. In the case of *ortho*-PCBs, concentrations ranged between 202.56 and 3335.16 ng/g (WW). In all eggs the most abundant PCBs were #180 and # 153, accounting with an 80% to the total. Concentrations of total PCBs in all the eggs analysed were lower than levels (> 4,000 ng/g) shown to cause reduced hatching, embryo mortality, and deformities in birds.[9] However some of them exhibited concentrations near the threshold value and this should be a cause of concern. DDE levels ranged between 228.8 and 1911.9 ng/g (WW). Some of the eggs exceed the levels associated with reproductive impairement.[9] These results clearly indicate that DDE is still present in the study area at high concentrations as reported in previous studies conducted in this area[10,11] and suggest a possible risk for the peregrine falcon populations breeding in the study area. 2,3,7,8-TCDD equivalents (TEQs) were estimated for PCDD/Fs 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 Organisation[12]. Total TEQs in all eggs analysed ranged between 12.1 and 53.9 pg/g (WW). The highest contribution to the total TEQs corresponded to non-*ortho* PCBs, with a percentage contribution between a 63 and a 78 % followed by the PCDFs, which ranged between 10 and 22%.

PBDEs.

Qualitative GC/MS analysis of PBDEs indicated the presence of BDE-99, BDE-153, BDE-154, BDE-100, BDE-47. Dominant congeners in falcon eggs were BDE-153 and BDE-99, as compared to BDE-47 dominant in piscivorous birds[13]. Similar results were reported by Herzke et al.[15], with eggs from terrestrial species such as peregrine falcons, merlins (*Falco columbarius*) and gyrfalcons (*Falco rusticolus*) from Norway showing a higher proportion of BDE-99 and BDE-153 than of BDE-47. In general, BDE-47, -99, and -100 bioaccumulate in predatory fish, birds, and mammals from aquatic food webs[14]. The higher brominated BDE-183, BDE-197, BDE-196, BDE-207, BDE-206 and BDE-209 as well as several unidentified octa- and nona- congeners were detected almost in all the samples analyzed (Figure 1). Most data produced are for the components of the penta-BDE product with very few data reported for BDE-183 or -209 in the environment[14]. Sellström et al.[16] found that the pattern of BDE congeners observed in peregrine falcon eggs is different to that seen in other biota samples; the falcon eggs contained several of the more highly brominated congeners. This agrees with the study from Lindberg[6] and with our results.

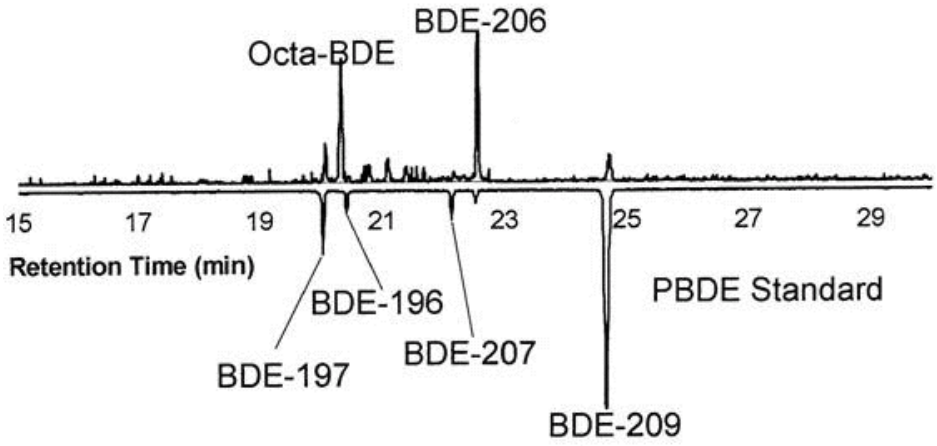

Figure 1. Example of higher BDEs in an egg from peregrine falcon.

Initially, these results seem to indicate the influence of habitat and feeding habits on the BDE congener patterns in birds of prey, perhaps suggesting that birds feeding in terrestrial environments and on other birds may be more highly exposed to the higher brominated BDE congeners than marine species. However, the differences in BDE congener patterns[14, 17] observed between Accipiter (*A. nisus, A. gentiles*) and Falco (*F. rusticolus, F. columbarius, F. peregrinus*) species, all feeding on small- and medium-sized birds, cannot be adequately explained. Perhaps, differences of xenobiotic metabolizing systems among different species could explain the differences.

Only preliminary analyses have so far been reported for terrestrial birds-of-prey[16,17] and none for predatory mammals in the terrestrial food web. Results from Lindberg et al.[6] indicate that organisms in the terrestrial environment may be more highly exposed to the higher brominated BDEs, in comparison with aquatic organisms. Our results support also this assumption.

Two important questions regarding the environmental fate of decabromodiphenylether (BDE-209) remain to be definitively answered: Firstly, what is its real potential for bioaccumulation? Secondly, are there circumstances under which it can debrominate in the environment to yield less highly brominated congeners?

In conclusion, the peregrine falcon population breeding in Spain seems still affected by organochlorine compounds which could compromise population stability. In particular, PCBs represented the major organohalogenated contaminants in the eggs studied here. In addition, the new finding of highly brominated diphenyl ethers provides new insights regarding a toxicological evaluation in wildlife and the peregrine falcon species in particular.

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