TOXICITY DERIVED FROM PCDDs, PCDFs AND DIOXIN-LIKE PCBs IN BLACK KITES (*MILVUS MIGRANS)* NESTING NEAR A MUNICIPAL SOLID WASTE INCINERATOR. PRELIMINARY RESULTS: POPULATION EFFECTS

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Introduction

During the last decade there has been increasing concern regarding environmental estrogens, especially dealing with environmental chemicals with known estrogenic effects including Polychlorinated Biphenyls (PCBs), Polychlorinated Dibenzo-p-Dioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs). In wildlife, reported adverse effects include: declines in populations, 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. Kubiak at al. (1989) showed that there was significant effect on reproductive success by organochlorines, not only in hatching success, but also in parent attentiveness and chick health[1]. Due to the widespread distribution of these xenoestrogens, there is a need for screening and risk evaluation of these endocrine disrupters in living organisms from the global point of view of ecosystems health[2]. Wildlife has received special attention during recent last decades as an indicator of ecosystem health. Many different Monitoring Assessment Programmes are very well known (e.g. the Great Lakes Herring Gull Monitoring Program)[3]. Different bioindicator species are selected in order to assess adverse effects of contaminants in wildlife depending on their characteristics and the conditions in the area of study.

Aspects of the life history of the black kites (*Milvus migrans*) 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 individual nesting territories. Black kites adapt well to human areas and many feeds at dumps, garbage tips, etc. where a wide range of contaminants can be found[4]. Effects of chlorinated pollutants have not been widely studied in this species. This study provides a good opportunity to evaluate if the group of toxic organochlorines including PCDDs, PCDFs, PCBs and some organochlorine pesticides could be involved in some of the adverse effects observed in the study population. Recently (1998-2002), we initiated a monitoring program to evaluate the health of a population of black kites nesting in the Regional Park of the Southwest of Madrid (RPSM), Spain. Several reproductive parameters were recorded as part of this monitoring program.

This study is part of a larger research investigation of the influence of a Municipal Solid Waste Incinerator (MSWI) on the kites'surroundings. In 1995, just prior to the start up of MSWI, a preliminary study found low levels of PCDD/Fs in the milk of cows grazing in the area[5].

Data sets of contaminant residues in wildlife living near a municipal solid-waste incinerator are scarce. Recently Rumbold and Mihalik[6] published the results of a comprehensive study to monitor chemical residues in eggs and chicks from different bird species. The present study was designed to

determine the significance of organochlorine levels (PCBs, PCDDs and PCDFs) and their toxic equivalents in a population of black kite breeding near a MSWI in order to study the suitability of this species as a bioindicator organism. Bird eggs are known to be good accumulators of lipophilic contaminants in the environment. Therefore, the isomer specific analysis of these substances in unhatched eggs of this species was performed. The toxicological significance of these levels and a comparison of our data to those reported for other birds populations are discussed. These kind of studies are important since they will aid in determining the range of levels of these contaminants which could represent a risk for the species, combining this information with the possible biological impact.

Material and Methods

Study area

The Municipal Solid Waste Incinerator was located in an area called Valdemingómez in the Regional Park of southeastern Madrid, in the Regional Community of Madrid, SPAIN. This area has received agricultural, municipal and some industrial developments. Several studies have indicated that the levels of contaminants such as p-p'-DDE and PCBs are elevated in the soils, sediments and biota at some of these sites[7]. The black kite colony was located in the area surrounding the MSWI which began operating in 1995. In close proximity there is a busy highway, and a secondary road driving to the MSWI area with a heavy traffic. The nesting sites of black kites were 3-15 km distant from the MSWI.

Sampling

15 unhatched eggs of black kites from 13 nests were obtained during the breeding season of 2001 from nests located in study area. Samples were stored at -80 °C until analysis. Eggs content was used for chemical analysis and the remaining eggshell was kept for structural analysis. Eggs content for residue analysis was lyophilised and quantities of approximately 3 grams of lyophilised eggs were used for residue analysis.

Analytical determination

The extraction of PCDD/Fs, PCBs and DDTs involved a solid phase matrix dispersion (SPMD) procedure. Fractionation among the studied compounds and other possible interferences was achieved by using SupelcleanTM Supelco ENVITM-Carb tubes as described elsewhere[8]. Three fractions were eluted. The first fraction contained the bulk of PCBs and DDTs. The second and third fractions contained non-ortho substituted PCBs and PCDD/Fs, respectively.

Resolution 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. A DB-5 fused silica capillary column (60 m x 250 µm and 0.25 µm film thickness) was used. The carrier gas was nitrogen at a head pressure of 192.2 Kpa. Detector and injector temperatures were 300 ºC and 270 ºC, respectively. 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 low contributions were detected. A fused silica capillary DB-5 column (60 m, 0.25 mm id., 0.25 mm 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, PCDFs, PCBs and organochlorine pesticides

Almost all 2,3,7,8-substituted PCDDs and PCDFs were detected in all the samples analysed. Total PCDD/F levels ranged from 8.9 to 85.9 pg/g on a wet weight basis (WW). Regarding the contribution of PCDDs and PCDFs to total PCDD/F levels, in general a higher percentage contribution was found from PCDDs, which in some cases contributed up to 94 % while in the case of PCDFs their contribution was always between 45 and 6%. This situation was observed in a previous study on black kites from the Doñana National Park, south Spain[9]. A clear general tendency was observed in all the individuals studied: in most cases it was observed that OCDD contributed the most to total PCDD/F levels, with a percentage contribution ranging from 17 to 82 %. In one case, 2,3,4,7,8-PnCDF contributed the most with 32%. In general, the minimum levels were found for 2,3,7,8-PnCDF or 1,2,3,7,8,9-HxCDF.

Total levels for co-planar PCBs (#77, 126 and 169) found in black kites ranged from 103.49 to 1174.20 pg/g (WW). In general PCB #126 was always the most abundant followed by PCB #77 and PCB #169. Total levels of the remaining PCBs (# 28, 52, 95, 101, 123, 149, 118, 114, 153, 132, 105, 138, 183, 167, 156, 157, 180, 170, 189 and 194) ranged from 383.3 to 13,620.20 ng/g (WW). There were significant contributions from congeners PCB # 153, 180, 170 and 194. Concentrations of total PCBs in eggs of some (50 %) of the specimens studied were much higher than levels (> 4,000 ng/g) shown to cause reduced hatching, embryo mortality, and deformities in birds[10]. PCB values in this study suggested that industrial inputs of PCBs in the PRSM ecosystem were important and should be considered as contaminants of concern.

DDT and its main metabolite (DDE) were found in all the specimens studied. DDT levels ranged between 5.54 and 63.45 ng/g (WW) while DDE levels ranged between 29.10 and 409.84 ng/g (WW). In all cases, DDE levels in Black Kites were below those that are associated with reproductive impairment[11] (11 ug/g of DDE are near or above the threshold for reproductive impairment). Potential sources of DDE in bird eggs at the site studied are probably local from heavy use of DDT in the past. This suggests that DDE is still present in the environment at high concentrations. No previous studies on contaminants and reproduction in birds nesting in the area studied are available; thus it is difficult to ascertain if DDE concentrations in breeding birds occupying high trophic levels in this area have declined.

Calculated TEQs for PCDDs and PCDFs and PCBs

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 Organisation[12]. Total calculated TEQs ranged from 11.18 to 149.26 pg/g (WW) in black kites eggs. Values found in this study were higher than those found in a previous study on black kites nesting in a protected area in Spain[9]. In general, specific PCDD/F congeners contributed markedly to total calculated TEQs. In the case of PCDDs the congener 2,3,7,8-TCDD always contributed a percentage ranging from 9 to 17 %. The congener 1,2,3,7,8-PnCDD contributed 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 %. The contribution of the remaining congeners was always lower than the previously mentioned congeners, being in general under 1 %. Calculated TEQs for co-planar PCBs in black kites indicated that PCB # 126 was the most important contributor to total TEQs followed by PCB # 77 and PCB # 169. PCDD/Fs contributed less to the total toxicity than that which came from PCBs (non-ortho and mono-ortho). In general it was found that the largest percentage contribution to total toxicity came from coplanar PCBs, with minimum percentage contribution of 60 %. However, PCDD and PCDF TEQ contributions were found to be between 5 and 10 % and mono-ortho PCBs percentage contributions were 13-31 %

The situation found in these birds is quite different from that found in the aquatic environment. For example, in marine mammals PCDD/F toxicities have been reported where, in general, the contribution

to overall toxicity coming from mono-ortho PCBs was higher than that coming from non-ortho PCBs and PCDD/Fs[13].

The lack of published data on PCDD/F and PCB concentrations in this species in other areas does not allow comparisons but levels found in this study appear to be higher than levels reported in the study of Rumbold et al[6].

Reproductive Success

We compared breeding success in this study area with that in an area with, a priori, lower levels of environmental contamination with residues from industrial or incineration activities (i.e. Doñana National Park). The main conclusions from this comparison is that hatching success in black kites nests was lower in the PRSM (72.5 %, n = 38) than in Doñana National Park[14] (84.9%). A mean number of 1.92 ± 1.15 hatched chicks was found in nests from the PRSM, which was similar to that found in Doñana, despite the lower clutch sizes in the latter population[14]. The association of a lower hatching success and a higher level of contaminants in failed eggs in the PRSM compared with that from Doñana suggests a causal effects which need to be further investigated to assess their consequences for the viability of the studied population. We are currently investigating potential effects as hormonal disrupters of pollutant content from black kite eggs, as well as the relationships between pollutant content and eggshell structure.

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