Assessment of Organochlorine Contamination In Wild Prefledging Black Kites. Effects on Thyroid Hormone Status

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Introduction

Ecotoxicological studies in birds aim to evaluate the negative effects, at individual and population level, resulting from their exposure to chemical contaminants. Especial emphasis has been done on those synthetic compounds that interfere with the endocrine systems of individuals, having negative repercussions on populations' stability. These compounds are identified as endocrine disrupting chemicals (EDCs), and organochlorines are one of the most studied because of their worldwide distribution and their implications in the decline of some wildlife populations[1]. Considering this problem, the European black kites (*Milvus migrans*) results an interesting species to be evaluated since their populations have undergone a large decline.

At present, European black kite populations are concentrated in Spain, France and Switzerland[2], and never before have been evaluated from an ecotoxicological point of view, despite of being classified as vulnerable. Some of kite characteristics make them susceptible of contaminant hazards. They are opportunistic raptors, scavengers and feed on carrion; and they are located at the top of food web suffering from the biomagnification processes of contaminants, especially from those lipophilic and recalcitrant like the organochlorines. With this idea in mind, a monitoring project on the black kites breeding in central Spain was initiated in order to evaluate the presence of chemical contaminants (PCBs, DDTs, PCDDs, PCDFs) in this population and whether they could be involved in the decrease of their number of breeding pairs. In a first stage, the presence of those contaminants, detected previously in water, sediments and biota of the breeding area, were confirmed in all unhatched eggs collected during the period 2001-2003[3]. Among the contaminants, *ortho*-PCBs presented the highest concentrations, exhibiting some eggs levels above those reported to affect reproductive success in other populations of raptors. In parallel, heavy metals and arsenic were quantified in prefledging's blood from the first year, founding lead concentrations above those considered toxic[4]. These imply that kites born in central Spain are exposed *in ovo* as post-hatching to toxic compounds which could have negative repercussions on their development.

To evaluate the exposure of prefledging kites to organochlorines (PCBs and DDTs) we used the blood of the new-born kites from 2002. Possible effects of these compounds on kite's health were investigated through their circulating levels of thyroid hormones (THs) and discussed in terms of their relations with organochlorine contaminants.

Material and Methods

Study area and sampling

The present study was conducted in the Regional Park of southeastern Madrid (RPSM), Spain. Surrounding the Park, industrial and agricultural activities are developed and a Municipal Solid Waste Incinerator (MSWI) is placed north-western of the Park. During the breeding season of 2002, blood samples and biometric measures were taken from 79 prefledging black kites from 32 nests. Blood was centrifuged at 3000 rpm for 10 minutes, plasma collected and stored at -80 ºC until analysis.

Organochlorine analysis

The following organochlorine compounds (OCs) were analyzed: *ortho* PCB congeners (#28, #52, #95, #101, #123, #149, #118, #114, #153, #132, #105, #138, #167, #156, #157, #180, #170, #189, #194) and DDTs (DDT and its

metabolites, TDE and DDE). The sample treatment is based on that described by Otero et al (1997)[5] with some modifications for avian plasma. Separation of the organochlorine compounds was carried out using a Hewlett Packard 6890 HRGC equipped with a 63Ni μ-electron capture detector. A DB-5 fused silica capillary column (60m x 250μm and 0.25μm film thickness) was used. The carrier gas was nitrogen at a head pressure of 192.2 kPa. Detector and injector temperatures were 300ºC and 270ºC, respectively. The OCs were identified on the basis of their relative retention time on the HRGC column. Quantifications were done within the linear range of the detector's seven-level calibration curve using HP ChemStation Plus program (Hewlett-Packard Co., Palo Alto, CA, USA). The LODs ranged from 0.02 to 0.10 ng/g. Those compounds which were under the limit of detection (LOD) were assigned as LOD/2.

Thyroxine (T_4) and 3,5,3,triiodothyronine (T_3) analysis

For this study, we carried out specific radioimmunoassay (RIA) techniques that determined total amounts (i.e. bound plus unbound) for each hormone. T_3 and T_4 RIAs were conducted using 125I-T_3 and 125I-T_4, respectively, prepared according to Kjeld et al.[6]. Plasma samples were diluted 4- to 10-fold prior to use in the thyroid hormone assays and measured at the midrange of the standard curves. For all RIAs, the minimum detection limit was defined as the hormone concentration for which 80% of the radiolabelled hormones (125I-T_3 and 125I-T_4) are bounded to the antiserum (ED_{80}). The minimum detection limit was 70 pg/ml of plasma for the T_3 RIA and 250 pg/ml of plasma for the T_4 RIA.

Statistical analysis

Multivariate analysis (MVA) was chosen as the most appropriated tool to investigate possible patterns and possible associations between biological and chemical variables. Principal component analysis (PCA) was used to distinguish groupings in the data, and partial least squares regression (PLS) was used to explore possible associations between organochlorines and the thyroid hormones.

Results

PCB congeners #52, #167, #156, #157 as well as DDT and TDE were under the LOD in almost all samples analysed, so they were not considered in the study because of their low variability. A comparison between sexes revealed no significant differences ($p>0.05$) in organochlorine concentrations. Considering all the kites, PCBs average concentration was 10.97 ± 10.22 ng/g on a wet weight basis (ww), for DDE the average was 1.06 ± 0.88 ng/g ww. Regarding the individual contribution of each PCB congener to the total, congeners #153, #132+105, #138, #180 were the most abundant for both sexes, contributing about 65%. The remaining congeners did not contribute more than 5%, excepting PCB #189 with a 10%. A principal component analysis considering the PCB profile revealed homogeneity within kites.

2,3,7,8-TCDD equivalents were estimated for dioxin-like PCBs with an assigned TEF (Toxic Equivalency Factor) value, based on the Bird TEFs reported in 1998 by the World Health Organisation[7]. Total TEQ (Toxic Equivalent Quantity, ΣTEQs) levels were 0.14 ± 0.05 pg/g ww in males and 0.15 ± 0.06 pg/g ww. In all kites, the highest contribution to the total TEQs corresponded to PCBs #132+105.

In Table 1 are reported the thyroid hormone concentrations in the prefledging kites. Differences in hormone levels and the ratio total T_4/T_3 between sexes were not significant ($p>0.05$). Although, T_4 concentration was higher in females than in males.

Relationships between the thyroid hormones (T_3, T_4, T_4/T_3), contaminants (PCB congeners, ΣPCBs, ΣTEQs, DDE) and the biological variables (age, weight, sex) were explored with a PLS analysis ($R^2X=0.42$, $R^2Y=0.30$, $Q^2=0.03$, 2 significant components). The analysis showed that T_3 would be the best explained hormone in terms of the variables considered ($R^2=0.44$) but the low predictability ($Q^2=0.13$) indicate that the results have to be interpreted with caution and considering the other factors. T_3 would be related positively to weight, PCB #194 and negatively to PCB #132+105 and ΣTEQs. To a lesser extent, these factors would be inversely related to T_4 hormone. Regarding the

remaining PCB congeners, only PCB #183, #180, #170, and #189 showed a positive relation to T_3 and no relation to T_4.

Table 1. Thyroid hormone concentrations in the prefledging kites for both sexes.

		n	Mean ± SD (min.-max.)
Male	T_3 (ng/ml)	28	3.87 ± 1.92 (0.36-9.54)
	T_4 (ng/ml)	29	13.59 ± 7.21 (1.65-38.45)
	T_4/T_3	28	4.10 ± 2.43 (0.77-10.34)
Female	T_3 (ng/ml)	35	3.76 ± 1.91 (1.05-8.74)
	T_4 (ng/ml)	36	15.73 ± 6.71 (5.58-31.31)
	T_4/T_3	35	5.28 ± 2.91 (0.85-12.00)

Discussion

Measurements of organochlorine levels in blood plasma have been considered as indicative of total body burden in birds and have been demonstrated to be effective in monitoring contaminant residues in avian species[8]. In prefledgings, contaminant concentrations in blood are influenced by numerous factors including the level of contamination of the yolk and the post-hatching diet. In the present study, OC levels in blood found in the prefledging kites are lower than those reported in prefledging from other avian species[8,9] which would suggest a low exposure to these contaminants. However, the PCB concentrations detected in unhatched eggs of this same breeding season are high (6.62 ± 4.79 µg/g ww) and within those from high-contaminated areas[5]. An explanation to this difference may be that blood in prefledging kites is reflecting mostly contamination coming from diet more than from egg exposure. Regarding PCB profile, the most persistent, PCB #153, #138 and #180, are the most abundant in prefledging blood as it has been described in blood and eggs of other avian raptors. However, the contribution of PCBs #132+105 and #189 is also considerable, representing about 22% of the ΣPCBs. No significant differences in PCB profile within kites suggest that they are exposed to the same sources.

Previous works[1,10] showed that free living birds exposed to high levels of PCBs or breeding in colonies from high contaminated sites could be affected by hypothyroidism (low levels of circulating T_4), depletion of thyroid gland hormone stores or enlarged thyroid glands. These works concluded that birds from high-contaminated areas could be limited under stressful conditions when an increase in hormone release may be needed. This could be occurring in our population since high levels of contaminants were detected in eggs and some prefledging kites exhibited a low T_4/T_3 ratio. However it is not possible to discern if this low ratio is a consequence of a thyroid gland disorder or an increased metabolism of T_4.

In a field study recently published with breeding glaucous gulls (*Laurus hyperboreus*)[11], the authors reported an association between high blood levels of halogenated organic contaminants and alteration of circulating TH levels in one of the breeding areas and only in males (sex-specific response). They explained that due to the intercorrelations within contaminants it was not possible the demonstration of causality. Additionally, they found a trend to increase circulating T_3 levels with increasing OC levels in females, which had significant lower levels than males. This is in agreement with Quinn et al.[12], who suggested that PCBs appear to have a bimodal response on the thyroid system: low levels are stimulatory, and higher concentrations result in inhibition. In our case, no clear significant relationships (negative or positive) between circulating THs and OC levels were detected. However, it is interesting that T_3 hormones would be the best explained in terms of the contaminants and biological variables considered. In addition, we could not define a clear etiology of the organochlorines on the concentrations of the THs. Some PCB congeners exhibited a negative trend on T_3 (i.e. #132+105) while others showed a positive trend (i.e. #194), and it happened the same for thyroxine. The sum of individual PCB congeners (ΣPCBs) was a non significant factor. These results indicate that the most appropriate approach should be to consider specific patterns of PCBs, being needed more detailed studies on toxicity of specific PCB congeners on THs.

The results presented in this study show that organochlorines measured in blood of prefledging kites, which reflect mostly contaminant exposure from diet, due not explain enough the variability in circulating thyroid hormones. Some tendencies were observed but it was not possible to define an unique etiology of PCBs. According to previous works[10,13], contaminant exposure *in ovo* might affect the thyroid status in prefledging kites and compromise their response toward environmental stressors.

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