BIOMONITORING OF ORGANOCHLORINE COMPOUNDS (PCDDs, PCDFs, PCBs AND DDTs) NEAR A MUNICIPAL SOLID WASTE INCINERATOR USING BLACK KITES (Milvus migrans) AS SENTINEL ORGANISM.

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Introduction

Wildlife has received much attention during last decades as an indicator of ecosystems health. Kubiak at al. (1989) showed that there are significant effect on reproductive success due to organochlorines, not only in hatching success, but also in 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]. Aspects of the life history of the black kites (*Milvus migrans*) make them a useful species for contaminants monitoring. They are long-lived birds, adapt well to human areas and many feeds at dumps, garbage tips, etc. where a wide range of contaminants can be found[3]. Effects of chlorinated pollutants have not been widely studied in this species.

In 2001, a monitoring program was initiated in order to evaluate the health of a population of black kites nesting in the Regional Park of the Southeastern of Madrid (RPSM), Spain. This study is part of a larger research investigation of the influence of a Municipal Solid Waste Incinerator (MSWI) on the kites'surroundings. Since Municipal Solid Waste Incinerators (MSWI) are suspected to produce some highly toxic POPs (Persistent Organic Pollutants) such as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) as well as other short of toxic chemicals (e.g. PCBs and heavy metals) this study was initiated with the aim of investigating the potential toxicity of these compounds on the kites population. The purpose of this study was to conduct the evaluation in a non-destructive way. Since eggs are known to reflect the accumulation of lipophilic contaminants in birds, the study was based on the use of unhatched eggs obtained from black kites.

Materials and methods

Study area

The present study was focused on a protected area (the Regional Park of Southeastern Madrid, RSPM) located in Madrid (Spain). The Park receives contaminant inputs from industrial and agricultural activities as has been demonstrated in previous studies[4]. A Municipal Solid Waste

Incinerator (MSWI), which began operating in 1995, is located northwest of the Regional Park. The nesting sites of black kites are 3-15 km distant from the MSWI.

Sampling

A total of 32 unhatched eggs of black kites were obtained during the breeding seasons of 2001, 2002 and 2003. Samples were stored at -80° C until analysis. Eggs content was used for chemical analysis and the remaining eggshell was kept for further structural analysis.

Analytical determination.

Egg content for residue analysis was lyophilised and quantities of approximately 3 grams were used for residue analysis. 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[5]. 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.

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 (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.

Non *ortho* PCBs and 2,3,7,8-substituted PCDD/Fs were determined on a Kratos Consept IS sector HRMS. The resolution used was better than 8000. The GC was equiped with a cooled oncolumn injector coupled to a 60 m x 32mm DB5 column with a film thickness of 0.25 µm. For the non *ortho* PCBs the starting temperature was 90^{o} C for 33 min. followed by 40^{o}/min to 210^{o} C. Then 1^{o}/min until 230^{o} C, followed by 40^{o}/min to 300^{o} C. The temperature program for the determination of the PCDD/F's was as follows: starting temperature 140^{o} C for 33 min, then 30^{o}/min to 250^{o} C followed by 2^{o}/min until 300^{o} C. For the quantification the sum of the two most abundant masses of each compound was used as well as the ^{12}C as the ^{13}C.

Statistical analysis.

Statistical analyses were carried out with SPSS 11.0 (SPSS Inc., Chicago, IL., USA). Non-parametric tests were carried out since data did not adjust to a normal distribution. Significance level was established at 0.05. Mean comparisons were made using the Mann-Whitney test and correlations were tested with Spearman correlation.

Results and Discussion

PCDDs and PCDFs

Results for dioxins and furans are presented only for the eggs collected during the breeding seasons of 2001 and 2002 since eggs of 2003 have not yet been processed. Almost all 2,3,7,8-substituted PCDDs and PCDFs were detected in all the samples analysed. In 2001 (n=13), mean PCDD and PCDF levels were 23.96 ± 20.27 pg/g wet weight (ww) and 4,61 ± 3.10 pg/g ww, respectively. For 2002 (n=10), mean levels were 39.88 ± 26.38 pg/g ww for PCDDs and 4.67 ± 2.00 pg/g ww for PCDFs. Non significant ($p>0.05$) differences for PCDDs or PCDFs were found between years, although the highest PCDD levels were found in eggs from 2002, mainly in the eggs collected from nests located near the Municipal Solid Waste Incinerator (MSWI).

In both years, the main contributor to total PCDD/F levels was OCDD (50-77%), followed by 1,2,3,4,6,7,8-HpCDD and 2,3,4,7,8-PeCDF. The relatively low contribution of PCDFs could be an indication of the influence of an incineration process. Total PCDD/F levels found here are higher than those reported in eggs of peregrine falcons inhabiting in the same area in 2000 and 2001, where the main contributor to total levels was 2,3,4,7,8-PeCDF[6]. Differences among species could reflect differences in metabolism or feeding habits, since peregrine falcons prey mainly on pigeons and black kite are scavengers.

PCBs

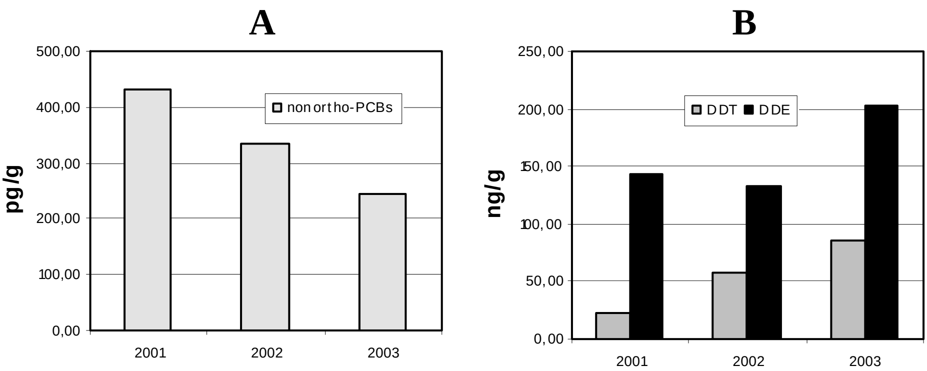
Total non *ortho*-PCB (#77, #126, #169) levels are shown in Table 1. Non significant differences among years were found, although a general tendency to decrease the levels as can be better visualized in Figure 1A. The highest levels were found in the nests located in the most affected area by industrial inputs. In all samples, the most abundant congener was PCB #126, followed by # 77 and # 169.

Table 1 shows the average of the sum of ortho-PCBs (# 28, 52, 95, 101, 123, 149, 118, 114, 153, 132, 105, 138, 183, 167, 156, 157, 180, 170, 189 and 194) during each year. Regarding *ortho*-PCBs profile, congeners #153, #180, #138 were the most abundant for each year of study. This profile is similar to those reported in previous studies conducted on raptor species[7]. Non significant differences for the sum of ortho-PCBs were found among years ($p>0.05$) but a high variability, expressed as standard deviation, is observed during all years. This fact could be explained by the distance to the industrial area surrounding the Park. The highest levels were found in eggs located near to this area and significant differences were found when levels in eggs are compared using this distance as a factor. Almost all eggs collected in the most contaminated area exhibited PCB levels higher than those reported by Hoffman *et al.* (1996) to cause reduced hatching, embryo mortality, and deformities in birds[8]. The highest level was found in an egg collected in this area in 2003, with a concentration of 29,830 ng/g on a wet weight basis. All these results suggest that PCBs represent a problem of concern in the Regional Park of South-eastern Madrid. These compounds could be associated to the low reproductive success observed on the black kite population inhabiting in this protected area.

Table 1. Organochlorine levels (average ± S.D.), expressed on a wet weight basis, in the eggs of black kite (*Milvus migrans*) collected during the period 2001-2003.

	2001 (n=13)	**2002 (n=10)**	**2003 (n=9)**
non *ortho*-PCBs (pg/g)	432.49 ± 358.85	336.28 ± 165.76	243.59 ± 223.88
***ortho*-PCBs** (ng/g)	4268.50 ± 4582.48	6621.86 ± 4786.81	5128.82 ± 9428.86
DDT (ng/g)	22.04 ± 19.96	56.95 ± 12.31	86.29 ± 25.21
DDE (ng/g)	143.60 ± 138.21	133.72 ± 63.98	202.92 ± 181.72

Figure 1. Average levels, on a wet weight basis, in eggs of black kite (*Milvus migrans*) collected during the period 2001-2003 for: A) non *ortho*-PCBs; and B) DDT and DDE.



DDTs

Regarding DDT and its main metabolite, DDE, average levels during the 3 years study (2001-2003) are presented in Table 1. As can be observed in Figure 1B, a significant tendency to increase DDT levels over the period 2001-2003 is detected, although this is not so clear for DDE. These results suggest a recent use, directly or indirectly as residue of other pesticides, of this banned compound in the area. These results are in agreement with Fernández *et al.* (2000) who previously reported the presence of DDT in sediments from the studied area[4]. In all eggs, DDE levels are well below the levels associated with reproductive impairment[9]. Although DDT levels found in this study are low, due to the different sensitivity among species and interactions among contaminants, deleterious effects of these contaminants should not be discarded. Moreover, the possibility of being used this pesticide at present is a serious problem that should be controlled and considered if the environmental health of this Park wants to be protected.

TEQs

2,3,7,8-TCDD equivalents (TEQs) were estimated based on the Bird Toxic Equivalency Factors (TEFs) reported in 1998 by the World Health Organisation[10]. In the preliminary study[11] conducted in 2001 and confirmed in 2002 it was observed that contribution to the total TEQs coming from PCDD/F congeners was low (<12%) and the main contributor were non *ortho*-PCBs (33.85 ± 29.94 pg/g ww in 2001, and 24.90 ± 14.67 pg/g ww in 2002) followed by *ortho*-PCBs. During 2003, TEQ levels for dioxin-like PCBs were 18.58 ± 17.58 pg/g ww for non *ortho*-PCBs and 5.12 ± 6.98 pg/g ww for *ortho*-PCBs. TEQ levels found in the period 2001-2003 are lower than those reported to cause reproductive dysfunction in double-crested cormorant[12]. However, deleterious effects cannot be discarded since toxicity data are not available for these compounds in black kites.

For all contaminants analysed, comparisons between fertile and infertile eggs were made but no significant differences were found. Our findings indicate that the kites born throughout these three years have been exposed *in ovo*, and probably after hatch, to organochlorine compounds, mainly PCBs. As Fernie *et al.* (2001) demonstrated in American kestrels[13], reproductive effects are seen with both direct and *in ovo* exposure. Similarly, the study from Brouwer *et al.* (1999) concluded that reproductive problems are induced following PCB exposure during prenatal as well as early postnatal periods[14]. Organochlorine compounds, mainly PCBs, could be compromising the survival of the black kite population monitored in this study.

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References

[1] Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E. and Erdman, T.C. ((1989). Arch. Environ. Contam. Toxicol. 18, 706.
[2] Jiménez, B., (1997) Trends in Analytical Chemistry, 16(10), 596.
[3] Blanco, G. (1997). J. Raptor Research 31: 71.
[4] Fernández, M., Cuesta, S. Jiménez, O., García, M.A., Hernández, L.M. Marina, M.L. and González, M.J. (2000) *Chemosphere* 41: 801.
[5] Molina, L., Cabes, M., Díaz-Ferrero, J., Coll, M., Martí, R., Broto-Puig, F., Comellas, L., Rodríguez-Larena, M.C. (2000) *Chemosphere* 40: 921.
[6] Merino, R.; Bordajandi, L.R.; Abad, E.; Rivera, J. and Jiménez, B., 2002. Organohalogen Compounds 57, 459.
[7] Giesy, J.P.; Ludwig, J.P. and Tillit, D.E. 1994. *Dioxins, dibenzofurans, PCBs and similar chlorinated diaromatic hydrocarbons and their effects on birds*. In: Schecter A. (ed) *Dioxins and health*. Plenum Press, N.Y., 249.
[8] Hoffman, D.J., Rice, C.P., Kubiak, T.J. (1996). In Beyer, W.N., Heinz, G.H., and Redmon-Norwood, A.W. eds. Lewis Publishers, Boca Raton, FL.
[9] White, D.H., Fleming, W.J. and Ensor, K.L. (1988). J. Wildl. Manag. 52: 724.
[10] Van den Berg, M.; Birnbaum, L.; Bosveld, A.T.; Brunstrom, B.; Cook, P.; Feeley, M.; Giesy, J.P.; Hanberg, A.; Hasegawa, R.; Kennedy, S.W.; Kubiak, T.; Larsen, J.C.; van Leeuwen, F.X.; Liem, A.K.; Nolt, C.; Peterson, R.E.; Poellinger, L.; Safe, S.; Schrenk, D.; Tillitt, D.; Tysklind, M.; Younes, M.; Waern, F. and Zacharewski, T. 1998. *Environ Health Perspect*. 106(12): 775-792.
[11] Merino, R.; Blanco, G.; Abad, E.; Rivera, J. and Jiménez, B., 2002. Organohalogen Compounds 57, 435.
[12] Yamashita, N.; Tanabe, S.; Ludwig, J.P.; Kurita, H.; Ludwig, M.E. and Tatsukawa, R. 1993. *Environ. Pollut.*, 79, 163.
[13] Fernie, K.J.; Smits, J.E.; Bortolotti, G.R. and Bird, D.M. 2001. *Arch. Environ. Contam. Toxicol.*, 40: 544.
[14] Brouwer, A.; Longnecker, MP.; Birnbaum, LS.; Cogliano, J.; Kostyniak, P.; Moore, J.; Schantz, S. and Winneke, G.1999. *Environ. Health Perspect.*,. 107: 639.