

Levels of polychlorinated biphenyls and organochlorine pesticides in serum samples of Egyptian Vulture (*Neophron percnopterus*) from Spain

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Abstract

Concentrations of 23 polychlorinated biphenyls (PCBs), *p,p'*-DDT and two of its metabolites, *p,p'*-DDE and *p,p'*-TDE have been measured in serum samples of up to 1 ml of Egyptian Vulture (*Neophron percnopterus*) gathered from five populations in Spain. \sum PCB concentrations were found to be in the range 3.2–97 ng/ml, while those of \sum DDTs ranged from 0.93 to 38 ng/ml. *p,p'*-DDT/*p,p'*-DDE ratios higher than one were only found in the Segovia population, which could be an indication of recent use of *p,p'*-DDT in the area. In all cases, PCB profiles were dominated by congeners 52, 132+105, 138, 153 and 180. However, some differences among the five populations studied became evident when their profiles were compared with those of technical PCB mixtures by principal components analysis. The DDT and PCB levels detected in the serums analysed were lower than those previously reported for similar avian species and those reported to have deleterious effects on survival or reproduction of birds.

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1. Introduction

Polychlorinated biphenyls (PCBs) are well-known environmental contaminants found world-wide and able to concentrate in living organisms, in particular at upper levels in both terrestrial and marine trophic chains (Tanabe and Tatsukawa, 1991; Moessner and Ballschmiter, 1997). Among the 209 possible PCB congeners, attention was initially focused in those characterised by their relative high abundance in the environment. However, in recent years, attention has also been extended to a few less abundant PCBs showing toxicity

similar to that of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (Ballschmiter and Zell, 1980; van den Berg et al., 1998) and for which the World Health Organisation (WHO) has established toxic equivalency factors (TEFs) for risk assessment (van den Berg et al., 1998) in humans/mammals, fish and birds. In the latter, the presence of PCBs has been found responsible for embryonic deformities and reduction in reproductive success in a variety of species (Hoffman et al., 1998; Fernie et al., 2000).

2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethane (*p,p'*-DDT) and its two main metabolites, 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE) and 2,2-bis-(4-chlorophenyl)-1,1-dichloroethane (*p,p'*-TDE) are persistent and toxic organochlorine pesticides showing endocrine disruption activity and causing eggshell thinning and embryo deaths in several species of birds.

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Among them, raptor species such as Egyptian Vulture (*Neophron percnopterus*) have been reported to be particularly sensitive to this group of pollutants (WHO, 1989).

Despite its wide use in the past, environmental concentration of *p,p'*-DDT in Spain have declined since this chemical was banned in 1977. Nevertheless, levels of DDTs at higher concentrations than those associated with their past use have recently been detected in various ecosystems in Spain (Fernández et al., 2000; Gómara et al., 2002a). These findings could be associated with occasional use of *p,p'*-DDT after banning.

Egyptian Vulture is a small predatory vulture species in danger of extinction, which inhabits open landscapes in arid and rugged regions where it exploits carcasses of small and medium-size animals. Egyptian Vulture populations have decreased sharply during the last century being considered the Iberian Peninsula populations among the most important in the world (Donázar et al., 2002).

In this paper, an analytical method previously validated (Gómara et al., 2002b) for the determination of PCB levels in serum samples of up to 1 ml has been used to measure the concentration of 23 PCBs (including the most toxic and abundant congeners) as well as *p,p'*-DDT, *p,p'*-DDE and *p,p'*-TDE in serum of 27 individuals of Egyptian Vulture gathered from five populations placed in different regions in Spain. The PCB profiles found in the Egyptian Vulture serum samples investigated were compared to those of technical PCB mixtures by using principal components analysis (PCA) in an attempts to identify possible differences in the sources of the PCB levels detected.

2. Materials and methods

2.1. Chemicals

All solvents were pestipur quality and were purchased from SDS (Peypin, France), except hexane (Merck, Darmstadt, Germany) and acetonitrile (Fisher, Pittsburgh, PA, USA). Formic and sulphuric acids (analysis quality) and silica gel 60 were from Merck, anhydrous sodium sulphate from J.T. Baker (Deverter, The Netherlands) and cartridges of Oasis HLB (60 mg) used for solid phase extraction (SPE) from Waters (Milford, USA). Individual standards of the selected analytes were purchased from Ehrenstorfer (Augsburg, Germany). Aroclors 1242, 1254 and 1260 were used for statistical comparison of the PCB profiles found in environmental samples.

2.2. Samples

The 27 serum samples analysed were gathered from live Egyptian Vulture (*N. percnopterus*) individuals from



Fig. 1. Geographical location of the population studied: (1) Navarra, (2) Segovia, (3) Cádiz, (4) Menorca Island, and (5) Fuerteventura Island.

five populations in Spain between 1999 and 2001 (see Fig. 1 for location). Navarra (station 1, six samples), corresponding to an industrial area, and Segovia (station 2, three samples) and Cádiz (station 3, three samples), located in rural areas, were sampled during the spring–summer season as Egyptian Vultures inhabiting these areas migrate to Africa for wintering (Donázar et al., 2002). Sedentary populations in Menorca Island (station 4, two samples) and Fuerteventura Island (station 5, 13 samples) inhabit zones close to residential and rural areas and were sampled in May and September, respectively. In each station, samples were taken the same day and from controlled individuals to prevent duplicated sampling of the same animal (Donázar et al., 2002).

2.3. Experimental

Blood samples were taken from live Egyptian Vulture individuals, collected in glass tubes, kept at 4 °C for 30 min and centrifuged (Microfuge 11, Beckman, CA, USA) at 3000 rpm for 10 min to yield serum samples of 0.18–1.3 ml. Individual samples were conserved at –20 °C until processed according to a previously validated SPE-based method described elsewhere (Gómara et al., 2002b) which has also been tested by participation in the AMAP interlaboratory exercise (Ring test for PCBs and OCs, National Institute of Public Health, Quebec, Canada). Briefly, the analytical procedure consisted on SPE of the target compounds using an Oasis cartridge (Waters, HLB 60mg/3ml) previously conditioned with 2 ml of dichloromethane, 2 ml of methanol and 2 ml of Milli-Q water. And subsequent on-line fat removal by elution of the extraction solvent (3 ml of toluene) through a multilayer column containing 60 mg of silica

gel activated at 140 °C for 48 h, 240 mg of silica modified with sulphuric acid (44%, w/w) and anhydrous sodium sulphate. Quantification was carried out by gas chromatography (GC) with micro-electron capture detection (micro-ECD) using PCB 209 and 1,2,3,4-tetrachloronaphthalene (TCN) as internal standards.

Blank samples were analysed to identify any contamination throughout the analytical procedure. No background interference was found to be introduced by the methodology proposed. The limits of detection (LODs) calculated for real-life serum samples were in the range 0.01–0.30 ng/ml of serum and the relative standard deviations of the complete method were better than 18%, irrespective of PCB concentration levels (Gómara et al., 2002b).

Determination of p,p' -DDT, p,p' -DDE, p,p' -TDE and PCB congener levels in the final extracts was performed by GC-micro-ECD (HP 6890 Series, Hewlett-Packard, Palo Alto, CA). Samples were injected in the hot splitless mode (1 μ l, 270 °C, splitless time 1.0 min) on a capillary DB-5 column (5% phenyl 95% methyl silicone, 60 m, 0.25 mm i.d., 0.25 μ m film thickness) purchased from J&W Scientific (USA). The column temperature was programmed from 80 °C (2 min) to 185 °C (3 min) at a rate of 30 °C/min, then to 230 °C (15 min) at 1.5 °C/min, and then to 270 °C (15 min) at 5 °C/min. Nitrogen was used as carrier gas (constant flow, 1.5 ml/min) and as make-up gas (30 ml/min). The detector temperature was set at 300 °C.

2.4. Multivariate analysis

A database containing the normalised PCB concentration of the congeners studied in the 27 serum samples and those found in three PCB technical mixtures (Aroclor 1242, 1254 and 1260) was prepared. Normalisation of data was carried out by expressing the concentration of individual congeners (variables) as a percentage of the sum of PCBs to minimise any statistical bias associated with order of magnitude differences in the concentrations of the analytes. The statistical analysis was carried out using the Statgraphics 5.0 program (STSC, Inc., USA).

3. Results and discussion

3.1. DDT and PCB levels in serum samples

Table 1 summarises the geometric means and range of concentrations calculated of DDTs and PCBs (as ng/ml of serum) found in the serum of individuals collected from the five Egyptian Vulture populations.

The highest levels of the sum of DDTs (i.e. concentrations of p,p' -DDE plus p,p' -TDE plus p,p' -DDT,

\sum DDT) were detected in individuals from station 5, Fuerteventura Island (mean = 6.2 ng/ml; max = 38 ng/ml). The \sum DDT geometric mean calculated for serums from this population was rather similar to that found in the other sedentary population studied, station 4 in Menorca Island. The other three populations investigated showed lower \sum DDT than those found in the island populations; the range of geometric means was 4.9 ng/ml for individuals trapped at station 3 (Cadiz), and 2.0 ng/ml for those captured in station 2 (Segovia).

The differences in the \sum DDT levels detected in the five Egyptian Vulture populations could probably be related to the characteristics of their habitats and migratory habits. Vultures with the highest values of \sum DDT (Menorca and Fuerteventura populations) are sedentary, while the other three populations, which exhibited lower \sum DDT levels, winter in the African Sahel region after nesting on the Iberian Peninsula.

As it is well known, p,p' -DDE is greatly associated with eggshell thinning in many bird species (Dirksen et al., 1995; Konstantinou et al., 2000). Among the three DDTs, p,p' -DDE was found to be the most abundant in the bird serums analysed, with concentrations ranging from 0.47 to 38 ng/ml (Table 1). However, these levels are far below the concentration in eggs that has been correlated with toxic effects in different bird species (\approx 5 μ g/g fresh weight) (Hoffman et al., 1998). According to the results obtained by Iseki et al. (2001) the concentration of POPs in eggs are similar to those found in blood samples.

Except for station 2, the DDT profile found in the serum samples from all Egyptian Vulture populations studied was that expected from areas where p,p' -DDT has not been recently used (Solé et al., 1994; van Wyk et al., 2001), i.e. p,p' -DDE levels were higher than those of p,p' -DDT and both higher than those of p,p' -TDE. The calculated p,p' -DDT/ p,p' -DDE mean ratio was lower than one at all stations, except in samples from station 2. At this sampling point, a mean ratio of 1.2 was calculated, a value that could be associated with recent exposure to p,p' -DDT in the area (Sericano et al., 1990; Solé et al., 1994). Despite the large amount of data reported related to DDT levels in eggs of raptor species, studies on the determination of DDT levels in blood or serum samples of avian species are scarce in the literature (van Wyk et al., 1993a,b, 2001). DDT concentrations found in the serum analysed in the present study are in the range of those previously detected in blood samples of vultures from different locations in South Africa in 2001 (range = 1.4–56 ng/ml) (van Wyk et al., 2001), although they are lower than those found in vultures from the same country before 1993 (average concentration = 89 ng/ml) (van Wyk et al., 1993a,b).

As can be seen from the sum of the 23 PCB congeners analysed (\sum PCBs, Table 1), some differences existed among the five populations investigated. Egyptian Vulture

Table 1
Geometric mean and range of concentrations of PCBs and DDTs in serum of Egyptian Vulture from five Spanish populations (ng/ml)

Compound	Station 1 (<i>n</i> = 6) (Navarra)	Station 2 (<i>n</i> = 3) (Segovia)	Station 3 (<i>n</i> = 3) (Cádiz)	Station 4 (<i>n</i> = 2) (Menorca)	Station 5 (<i>n</i> = 13) (Fuerteventura)
PCB 28	0.61 (ND–5.4)	0.06 (ND–0.06)	ND	ND	0.61 (0.24–1.5)
PCB 52	3.3 (1.0–6.3)	2.7 (2.2–4.1)	1.2 (ND–3.8)	0.46 (0.44–0.49)	0.60 (0.18–2.4)
PCB 95	1.0 (0.45–3.1)	0.30 (0.19–0.47)	0.25 (ND–0.50)	0.23 (0.19–0.27)	0.91 (0.28–3.0)
PCB 101	1.3 (0.55–5.1)	0.29 (0.23–0.35)	0.32 (ND–0.60)	0.34 (0.33–0.35)	1.3 (0.52–7.3)
PCB 77 + 110	1.4 (0.73–4.1)	0.08 (ND–0.15)	ND	0.14 (ND–0.14)	0.58 (0.03–3.0)
PCB 123 + 149	0.43 (0.13–2.1)	0.04 (0.03–0.09)	0.20 (ND–0.20)	0.03 (ND–0.03)	0.26 (ND–0.86)
PCB 118	0.42 (0.15–2.4)	0.08 (0.04–0.17)	0.18 (ND–0.62)	0.38 (0.22–0.65)	0.37 (0.04–2.4)
PCB 114	0.14 (0.05–0.6)	ND	ND	ND	ND
PCB 153	1.8 (0.50–20)	0.54 (0.32–1.2)	1.6 (0.59–5.6)	4.1 (1.7–10)	2.7 (0.66–17)
PCB 132 + 105	6.0 (2.0–36)	0.28 (0.20–0.40)	0.36 (0.09–1.0)	0.92 (0.73–1.2)	7.3 (1.8–27)
PCB 138	1.1 (0.27–11)	0.26 (0.15–0.66)	0.73 (0.30–2.7)	2.4 (0.95–5.8)	1.7 (0.40–11)
PCB 126 + 129 + 178	0.25 (0.14–0.70)	0.12 (ND–0.65)	0.06 (ND–0.06)	0.05 (0.02–0.18)	0.17 (ND–0.43)
PCB 183	0.11 (0.02–2.6)	ND	0.20 (ND–0.63)	0.20 (0.04–1.1)	0.11 (ND–1.6)
PCB 167	0.16 (0.02–0.70)	ND	0.15 (ND–0.15)	0.19 (0.05–0.67)	0.11 (ND–0.87)
PCB 156	0.22 (0.06–1.8)	0.06 (ND–0.06)	0.20 (ND–0.20)	0.12 (0.03–0.45)	0.11 (0.02–0.44)
PCB 157	0.30 (0.10–1.4)	0.09 (ND–0.13)	0.28 (ND–0.28)	0.09 (0.02–0.39)	0.11 (ND–1.1)
PCB 180	1.8 (0.21–18)	0.40 (0.28–0.78)	1.8 (0.77–6.6)	2.8 (1.1–6.7)	2.0 (0.47–11)
PCB 169	0.42 (ND–1.3)	ND	ND	0.08 (ND–0.08)	ND
PCB 170	0.30 (0.05–3.7)	0.10 (0.07–0.12)	0.43 (0.15–1.7)	0.94 (0.35–2.6)	0.44 (ND–8.1)
PCB 189	0.46 (0.10–2.6)	0.31 (ND–0.53)	0.01 (ND–0.01)	0.05 (ND–0.05)	0.52 (ND–1.4)
PCB 194	0.34 (0.04–3.5)	0.12 (0.09–0.19)	0.49 (0.20–1.9)	0.70 (0.24–2.0)	0.37 (0.03–1.7)
DDE	2.1 (0.47–12)	0.82 (0.52–1.0)	3.8 (1.4–8.5)	3.9 (2.5–6.0)	6.0 (0.47–38)
TDE	0.17 (ND–0.24)	0.16 (0.15–0.18)	0.15 (0.10–0.18)	0.17 (0.17–0.18)	ND
DDT	1.9 (0.76–5.0)	1.0 (0.82–1.3)	0.95 (0.92–0.97)	1.2 (0.99–1.3)	0.22 (0.14–0.46)
∑DDTs	4.12 (2.2–17)	2.0 (1.6–2.5)	4.9 (2.4–9.7)	5.2 (4.1–7.1)	6.2 (0.93–38)

serum samples from station 1 (North of Spain) showed the highest values with a geometric mean of 22 ng/ml serum and a maximum value of 97 ng/ml serum, followed by serum samples collected from populations inhabiting the islands (station 5 in the Northwest African Atlantic coast, mean = 20 ng/ml, max = 61 ng/ml; and station 4 in the Mediterranean coast of Spain, mean = 14 ng/ml, max = 33 ng/ml). The lowest PCB levels were found in serum gathered from migratory populations in the South (station 3, mean = 8.5 ng/ml, max = 26 ng/ml) and in the centre (station 2, mean = 5.8 ng/ml, max = 7.0 ng/ml) of Spain.

Despite the differences observed in PCB concentrations found in the five populations investigated, in general, rather similar average PCB profiles were obtained in all cases (Fig. 2). Not unexpectedly, PCB 52, 132 + 105, 138, 153 and 180 were found to be the most abundant congeners among those studied, and PCB 114 and 169 were the least abundant. Nevertheless, some differences existed among the relative contributions of the rest of PCBs investigated for the five populations: samples from stations 3 and 4 showed lower contributions for PCB 28, 95, 101, 77, 126 and 189 than those calculated for the rest of the sampling points, while the opposite trend was observed for PCB 118, 183, 167, 170, and 194.

Data reported concerning PCB levels in serum are very scarce in the literature. Kumar et al. (2002) reported concentrations of PCBs in serum of black and turkey vultures from South Carolina ranging from 1.6 to 15 µg/ml. Meanwhile, much higher levels have been reported for black eagles from Michigan, in the range 46–67 ng/g wet weight (Kannan et al., 2002). Total PCB levels found in our study are lower than those found in black eagles and far below those detected in vultures from South Carolina. More importantly, PCB levels detected in the samples collected in Spain are much lower than those reported for eggs (4.7 µg/g, Hoffman et al., 1993) and that have been associated with toxic effects in birds. As it was mentioning previously, POPs concentrations in eggs are similar to those found in blood samples (Iseki et al., 2001).

3.2. Multivariate analysis

Visual observation of the geometric mean values did not allow proper classification of the individuals investigated according to their differences in the PCB profiles in order to determine whether they were subjected to similar or different sources of contamination. Therefore, chemometric techniques, such as Multivariate Analysis, were used in an attempt to reveal possible similarities

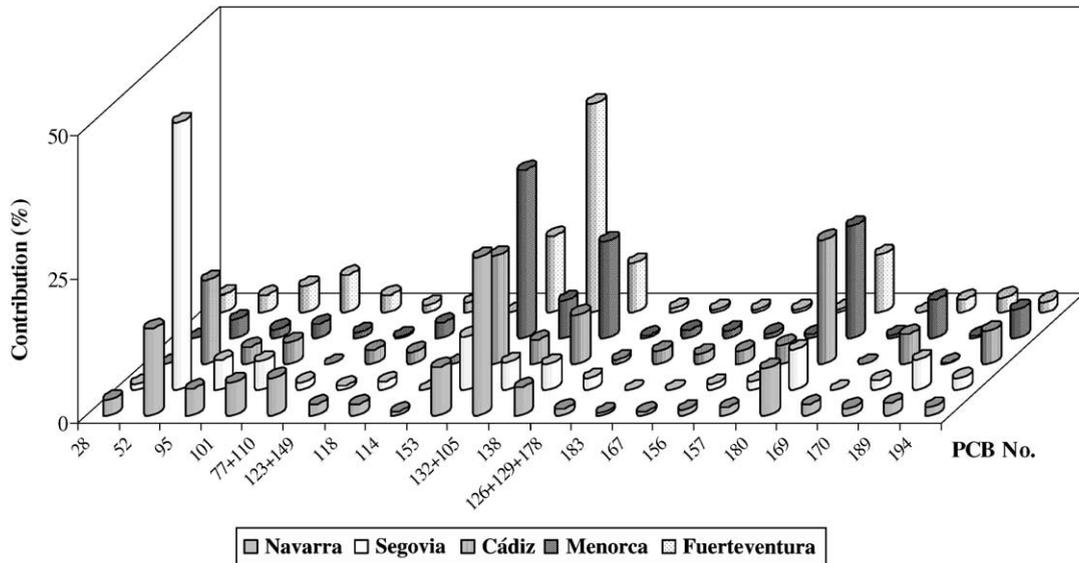


Fig. 2. Normalised PCB profiles in serum samples of Egyptian Vultures from five populations in Spain.

and differences in the PCB profiles of the serum investigated (Jiménez et al., 1998; Serrano et al., 2000).

PCA was selected to carry out the comparison. PCA is a Multivariate Analysis technique for dimension reduction (Wold et al., 1984), which allows pattern recognition in complex data sets (de Boer et al., 1993). In this study, PCA was applied to compare the PCB profiles of the 27 Egyptian Vulture serums collected in the five populations sampled with PCB profiles calculated for the technical mixtures most frequently used in industry, i.e. Aroclor 1224, 1254 and 1260. The analysed data set corresponded to the normalised contribution of the 23 PCB congeners and the Aroclor mixtures.

PCA calculation from this normalised data set indicated that the first two principal components accounted for the 81.6% of the total variability. The first and predominant PCA, PC1 contained 51.7% of the total variance and was a combination of PCB 52 and 132 + 105 (Fig. 3(a)). The second principal component, PC2, contained 29.8% of the total variance and was mainly a combination of PCB 132 + 105, 153 and 180 (Fig. 3(a)). The plot of the scores is shown in Fig. 3(b) (see Table 1 for numbering). The studied samples distributed in the two-dimensional space according to geographical location where they were gathered. Vulture individuals from station 2 (class I) and station 5 (class II) formed well-defined classes clearly separated from each other and from the other colonies. Samples from stations 3 and 4 (class III) showed PCB profiles close to that of Aroclor 1260. Meanwhile, serum PCB profiles from stations 1 (class IV) and 2 were somewhat close to A-1254 and A-1242, respectively. Finally, samples from station 5 were far from all PCB technical mixtures included in the

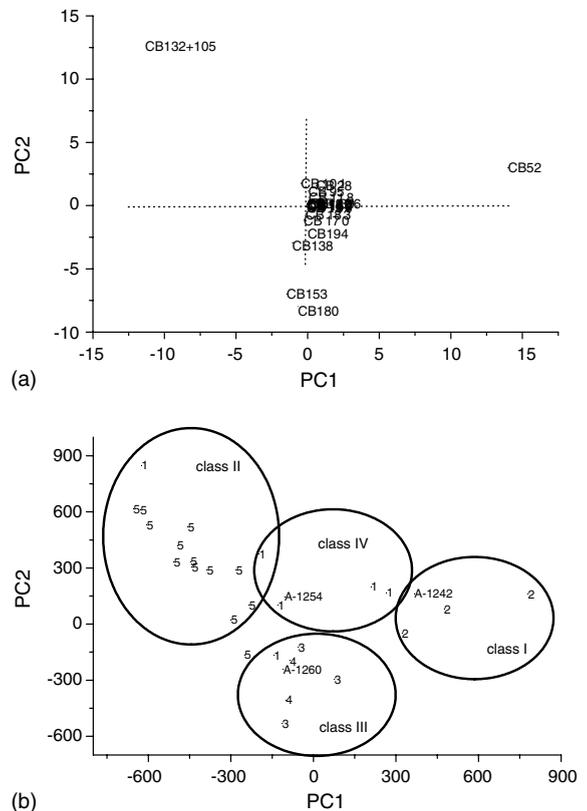


Fig. 3. Two-dimensional principal components loading (a) and score (b) plots of normalised PCB congeners in vulture serum from five Spanish populations.

study. These results show that some differences can be observed in the individuals depending on sampling area: Fuerteventura Island (station 5) is a residential area located in the North African Atlantic Ocean, far from the rest of the sampling points, and without any industrial activity. Thereby, the PCB levels and profile found in these samples could be associated with long-range atmospheric transport and environmental degradation of these pollutants. This fate has been observed by other authors in different avian species (Elliott et al., 2000). The differences in the PCB profiles could be also associated with differences in metabolic activities caused by the presence of other contaminants. In addition to PCBs, other compounds could affect the PCB metabolism as other author suggested (van den Brink and Bosveld, 2001).

4. Conclusions

An SPE-based method has been used for the analysis of PCBs and DDTs in serum of Egyptian Vulture. The small amount of sample required, ≈ 1 ml, has allowed monitoring of the concentration of these pollutants in live individuals of this endangered species. Levels detected in the five populations sampled are far below those considered to be a risk for the health of the populations. However, a *p,p'*-DDT/*p,p'*-DDE ratio higher than one was obtained for one of the colonies, which could be considered as a possible indicator of recent use of this banned pesticide. The levels of DDTs found in the vultures appear to be related with their migratory habits. Meanwhile, the PCB profiles look to depend on the area where the birds live. Finally, the application of PCA uncovered the similarity of samples in relation to different technical PCB mixtures and demonstrated differences in PCB residue profiles for the five bird populations studied.

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