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A First Record of Organochlorine Pesticides in Barn Owls (*Tyto alba*) from Portugal: Assessing Trends from Variation in Feather and Liver Concentrations

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Abstract

We evaluated feathers as a non-destructive biomonitoring tool documenting organochlorine pesticides (OCP) in liver and checked possible trends in pesticide use in two areas based on OCP concentrations in barn owls (*Tyto alba*). We measured the concentrations of 16 OCP in 15 primary feathers and 15 livers from barn owl carcasses collected on roadsides in Tagus Valley and Évora regions, south Portugal. Total OCP mean concentration was 8 120 ng g^{-1} in feathers and 178 ng g^{-1} in livers. All compounds were detected in feathers while in livers δ-HCH, endosulfan sulphate, *p,p′*-DDT and *p,p′*-DDD were not detected. The high β-HCH and heptachlor concentrations in feathers most likely derived from external endogenous contamination. *P,p′*-DDE was the OCP with the highest hepatic concentration. Both matrices indicated an exposure to recently released heptachlor. The differing OCP concentrations between Tagus Valley and Évora seem to reflect differences in land-use and pesticide use histories of the two locations, and/or faster degradation of OCP in the Tagus area.

Keywords Organochlorine pesticides · Barn owl · Feathers · Liver · Portugal

Organochlorine compounds include the most prevalent synthetic pesticides that have been broadly used in agriculture in the second half of the 20th century (Barr 2008). These organochlorine pesticides (OCP) can be classified into three groups: (1) *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT) and related compounds, (2) cyclodiene insecticides (aldrin, dieldrin, endrin, heptachlor and endosulfan) and (3) isomers of hexachlorocyclohexane (HCH) (Mitra et al. 2011). The known impact of these substances on humans includes neurotoxic, endocrine disruptive and carcinogenic effects (Wasi et al. 2013; Ritter et al. 1995; Jaga and Dharmani 2003). Despite the fact that OCP concentrations detected in wildlife are infrequently considered to be a direct cause of death, these are often reported as a cause of immunosuppression, hormone disruption and disorder of the nervous and reproductive systems (Denneman and Douben 1993).

In Portugal, the use of OCP was regulated for the first time in 1988 (Decree-Law 347/88), after an agreement between the government and the chemical companies restricting the trade of dieldrin, heptachlor and DDT in 1974, and of aldrin, endrin, hexachlorobenzene and toxaphene in 1986 (APA 2010). In concurrence with the European Directive 79/117/CEE, nine OCP were banned by decree, including DDT, the ‘drins’ (aldrin, dieldrin and endrin), heptachlor, and HCH (Ordinance 660/88). However, these substances are still detected decades later in the physical environment (Cardoso et al. 2009; Carvalho et al. 2009), in food products

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(Correia-Sá et al. 2012) and in humans (Lino and da Silveira 2006; Lopes et al. 2014).

Wildlife biomonitoring studies are useful to assess spatial and temporal trends in concentrations of environmental contaminants and to investigate related effects on populations; consequently, they can provide early warning of potential impacts in humans and protected wildlife species (Burger and Gochfeld 2004; Gómez-Ramírez et al. 2014; Espín et al. 2016; García-Fernández et al. 2020). Birds of prey are susceptible to contaminant bioaccumulation and biomagnification due to their position at the top of the food chain, often with complex trophic links connecting aquatic and terrestrial ecosystems (van Drooge et al. 2008; Mateo et al. 2012; García-Fernández 2014). As a result, measurable concentrations of OCP in tissues can be used to evaluate exposure and effects (García-Fernández et al. 2008; Martínez-López et al. 2009).

The barn owl (*Tyto alba*) is a prime candidate as an environmental bioindicator which has high probability of being associated with contamination from agricultural sources, including OCP. The species is a widespread and common resident bird, and an opportunistic meso-predator that hunts in open farmland (Bunn et al. 1982). Barn owls often use man-made structures and are known for their fidelity to nest sites (Roulin 2002), allowing for monitoring the same territories for a long time using minimally invasive methods (e.g., feathers, blood). Moreover, several carcasses can be collected on roadsides as a result of vehicle collisions (Silva et al. 2008), also allowing for access to internal tissues that otherwise would be unattainable for ethical and legal reasons. Also, feathers have great potential as minimally invasive biomonitoring tools, since OCP bind to keratin structure during the feather growth period, after which vascular connections undergo atrophy and compound concentrations remain stable (García-Fernández et al. 2013). However, comparisons with internal OCP levels may be affected by (1) differences in blood concentrations at the time of feather formation and at the time of internal tissue sampling, (2) individual or compound-related variations in metabolism, and (3) potential external contamination (see García-Fernández et al. 2013). Consequently, there is ambiguous evidence in the literature documenting strong (Eulaers et al. 2011; Jaspers et al. 2007) and weak significant correlations (Acampora et al. 2017; Dauwe et al. 2005) between OCP levels in feathers and internal tissues. Nevertheless, feathers are considered suitable for monitoring legacy contaminants such as OCP (Jaspers et al. 2019).

Considering the scarcity of data on predator contamination in Portugal, the easy access to barn owl feathers and internal tissue samples, and the lack of clear information in the literature on the relationship between contaminant concentrations in those matrices, we conducted a study aiming at: (1) evaluating the suitability of the barn owl, a widespread raptor associated with agricultural uses, as a biomonitor for OCP; (2) evaluating barn owl feathers as a non-destructive biomonitoring tool comparing OCP concentrations with those measured in livers; (3) exploring trends in pesticide use in two distinct regions in Portugal, in light of OCP concentrations in barn owl feathers and liver.

Materials and Methods

Road-killed barn owls were collected in two areas in centre-south Portugal with different farmland uses: lower Tagus River Valley (hereafter Tagus; 38°56’N–8°55’W) and Évora (38°33’N–7°54’W), with a mean distance between them of 82 km. Mixing between sample populations is very unlikely, since mean barn owl post-natal dispersal distances are 21,0 ± 12,4 km (maximum ~ 60 km; Roque et al. 2021). The climate is Mediterranean, with rains concentrated in winter, and characterized by hot, dry summers and mild winters. The Tagus area encloses the left bank of the Tagus River in the vicinity of its estuary, located in the metropolitan area of Lisbon. Based on the land uses of CORINE Land Cover 2006 (European Environment Agency 2007), farmland and forest uses each make up roughly half of the area, both in Tagus and Évora (farmland: 47% in Tagus and 46% in Évora; forest: 43% in Tagus and 40% in Évora). In Tagus, dominant land use is forest stands (mainly pines and eucalyptus; 32%), followed by complex cultivation patterns (15%) and irrigated lands, including rice fields (13%). Vineyards and olive groves occupy ca. 12% of the area. In Évora, dominant land use is non-irrigated arable land (34%) followed by agro-silvo-pastoral systems (oak forests integrating livestock or agriculture, 29%). Here, forest stands occupy ca. 18% of the area.

Barn owl feathers and livers were collected from 15 road-killed birds found on roadsides between 2009 and 2012 following the protocol described by Espín et al. (2021). Six individuals were found in the Tagus area and nine in Évora. According to plumage moult (Martínez et al. 2002), the sampled owls were one (n = 6), two (n = 6) and three or more years old (n = 3). A primary feather was randomly plucked from each bird, resulting in 15 samples with different positions in the wing. Only the feathers collected from the two older individuals were moulted flight feathers. Liver was excised from each owl. Feather and liver samples were stored in individual transparent plastic bags and in aluminium foil, respectively, and kept frozen at − 20°C until analysis.

Primary feather and liver samples were analysed for 16 OCP: four HCH isomers (α-HCH, β-HCH, γ-HCH—or lindane—and δ-HCH), three endosulfan-related compounds (endosulfan I, endosulfan II and endosulfan sulphate), four ‘drins’ (aldrin, dieldrin, endrin and endrin aldehyde), three DDT-related compounds (*p,p′*-DDT, *p,p′*-DDD and *p,p'*-DDE) and two heptachlor-related compounds (heptachlor and heptachlor-epoxide). The analytical procedures were based on the methods described by Espín et al. (2010b; 2012) and Aver et al. (2020) for feathers, and Espín et al. (2010a) for liver samples, using a gas chromatograph/mass spectrometer (GC/MS Shimadzu QP 2010 Plus). To remove external contamination from the feather surface, prior to the analytical determination, a brief washing process was performed with tap water, distilled water and Milli-Q water, and two pairs of tweezers were used to separate the barbs of the vane. Both feather parts (barbs and vane) were included in the analysis. Identification and quantification were based on an external standard (EPA Pesticide Mix 48,858, Supelco, USA), and methoxychlor. In order to compare results and check the repeatability in the chromatograms, a volume of 10 µL of methoxychlor (1 mg mL^{-1}) supplied by PolyScience® was added to samples and standards used as an internal standard. Spiked sample mean recoveries ranged from 46 to 146% in feathers and 86% to 146% in liver, depending on the compound. Detection limits ranged from 0.03 to 0.54 ng g^{-1}. OCP concentrations were expressed as ng g^{-1}.

Reported OCP values represent the mean concentration ± standard deviation (for comparison with existing literature), median and range, and frequency of detection. Total concentrations of OCP groups (ΣOCP) were calculated as the sum of individual compound concentrations: DDT and metabolites (ΣDDT) corresponded to the sum of *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT; hexaclorocyclohexanes (ΣHCH) incorporated α, β, δ and γ-isomers; heptachlor group (Σheptachlor) included heptachlor and its epoxide; Σdrins represented the sum of endrin, endrin aldehyde, aldrin and dieldrin; and Σendosulfan included endosulfan I and II, and endosulfan sulfphate. The percentage of individual compound concentrations in their group was also calculated for feathers and liver.

Since our data were not normally distributed even after several transformation trials, non-parametric, paired sample Wilcoxon tests were used to detect differences between sampling matrices, and Spearman correlations between feather and liver concentrations were also performed. The Mann–Whitney test was used to detect differences between areas in both feather and liver concentrations. The level of significance (two-tailed) for these tests was set at $p < 0.05$. All statistical analyses were conducted using R software 3.1.1.

Results and Discussion

All monitored OCP were detected in barn owl feathers while, in livers, four OCP were not detected (δ-HCH, endosulfan sulphate, *p,p'*-DDT and *p,p'*-DDD). Total OCP mean concentration was 8 120 ± 6 432 ng g^{-1} in feathers and 178 ± 112 ng g^{-1} in livers. ΣHCH (5 274 ± 4 484 ng g^{-1}) and Σheptachlor (2 555 ± 2 439 ng g^{-1}) were measured in feathers in particularly high concentrations when compared with the other OCP groups, representing together 96% of the ΣOCP in feathers. This difference was not observed in livers, in which ΣHCH (30%), ΣDrins (26%) and ΣDDT (26%) represented a similar burden (see also Table S1 suppl. mat.).

Mean concentrations of ΣHCH and heptachlor in barn owl feathers were, respectively, 19.8 and 9.6 times higher than the maximum value reported to date in feathers of European raptors (266 ng g^{-1} $\Sigma HCH_{max,}$ in western marsh harrier (*Circus aeruginosus*) from Greece, and 263 ng g^{-1} $heptachlor_{max}$ in European honey buzzard (*Pernis apivorus*) from Spain; van Drooge et al. 2008, Hela et al. 2006). Liver concentration of lindane (γ-HCH) doubled that of β-HCH, suggesting recent exposure, while elevated β-HCH concentrations in barn owl feathers could be due to a greater persistence or stability (Alvarez et al. 2005). Lindane, including technical HCH containing β-HCH, was legally used up to three years before our sampling (Regulation CE 850/2004). On the other hand, heptachlor was banned decades before (Directive 79/117/CEE; Ordinance 660/88); the great relative percentage of heptachlor in feathers and liver, and its extremely high concentration in feathers suggest recent exposure. Because the epoxide is more stable than the parent compound (Purnomo et al. 2013), our results seem to reflect current heptachlor contamination. Attention should be given to this OCP, which has been excluded from many studies assuming it was no longer permitted in Europe (van der Gon et al. 2007).

Present contamination of Portuguese barn owls with DDT and derivates is most likely due to their great persistence in the environment, since a half-life of up to 35 years in agricultural soils (Nash and Woolson 1967) makes bioavailability of DDT in soil possible beyond the interval elapsed between its restriction and our sampling. Drins were also prevalent legacy contaminants in Portuguese barn owls. Because aldrin generally is quickly degraded to endrin and dieldrin through epoxidation (Ritter et al. 1995), aldrin bioaccumulates and biomagnifies mainly in the form of its conversion products (WHO 1989). Since aldrin has been restricted in Portugal ca. 20 years before our sampling, the prevalence of the parent compound in both liver and feathers raises concern. A study conducted 30 years ago in Spain revealed higher *p,p'*-DDE (2.9 times) and aldrin (150 times) hepatic concentrations in the barn owl (Sierra and Santiago 1987), but our results show higher hepatic concentrations of endrin (1.6 times) and dieldrin (15.8 times). Nonetheless, maximum Σdrins concentration found in Portuguese barn owl livers represents only 0.03 of the minimum range of dieldrin liver residues associated with lethality in the species (6 000 ng g^{-1}; Newton et al. 1991). Maximum hepatic *p,p'*-DDE concentration in Portuguese barn owls was 12 times lower than the minimum concentration associated with lethality in birds (2 000 ng g^{-1}; Beyer and Meador 2011). Nevertheless, our values are in the range of possible behavioural effects, considering these can be observed at levels 10 to 100 times lower than those associated with bird lethality (Peakall et al. 1985 in Hellou et al. 2013).

The compounds with the highest mean concentration in feathers were β-HCH (4 587 ± 4 375 ng g^{-1}) and heptachlor (2 531 ± 2 417 ng g^{-1}; Fig. 1A), while *p,p′*-DDE (45.4 ± 48.7 ng g^{-1}) was the compound with the highest mean concentration in liver (Fig. 1B). The four HCH isomers and heptachlor were the most frequently detected compounds in feather samples (all individuals), and lindane, α-HCH, *p,p′*-DDE and heptachlor were the most frequently detected in liver samples (80–100%). ΣOCP concentration was much higher in feathers than in liver (mean = 46; median: 29 times higher). Significant positive correlation between feather and liver concentrations was found only for heptachlor epoxide (ρ = 0.719; p = 0.002). While β-HCH represented the highest percentage among the ΣHCH in feathers (84%, SE = 0.028), lindane was the isomer most accumulated in liver (67%, SE = 0.094; Fig. 2). Heptachlor

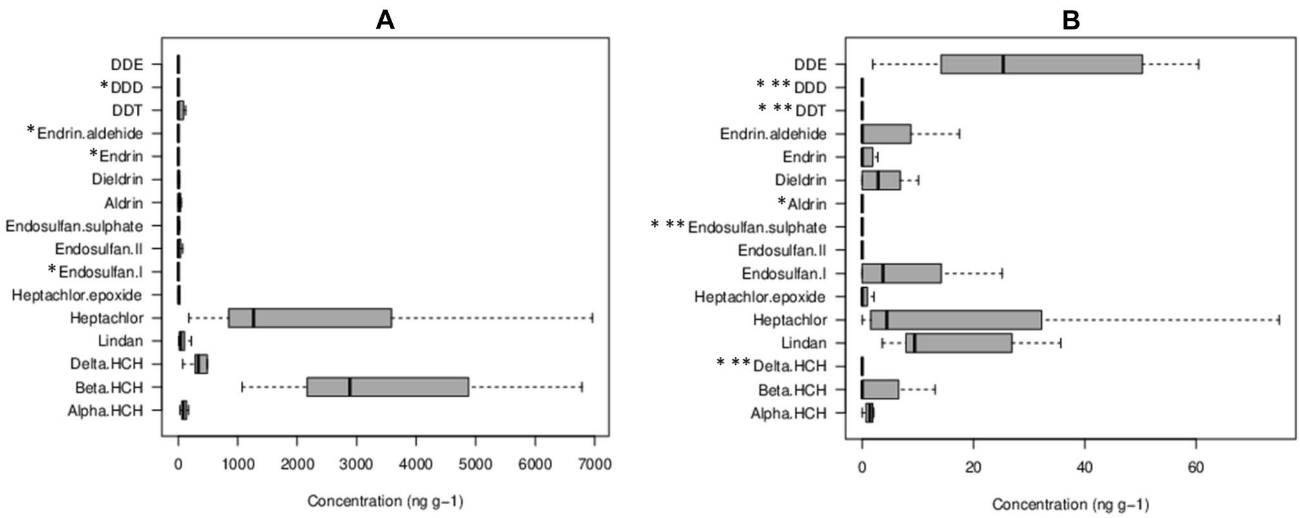
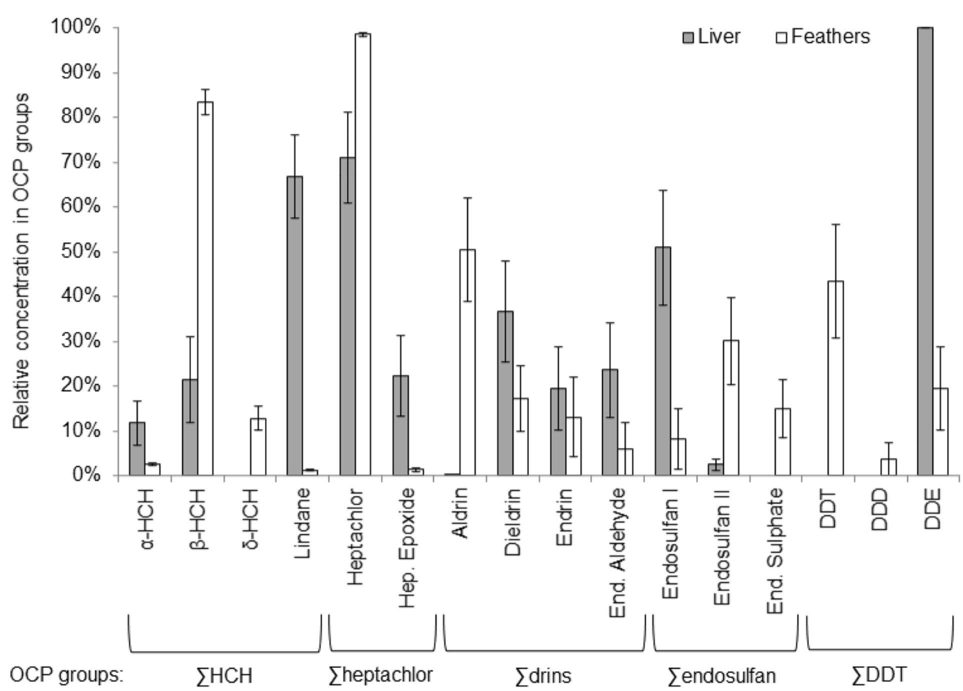


Fig. 1 Concentration (ng g^{-1}) of 16 organochlorine pesticides (OCP) in barn owl primary feathers (**A**) and liver (**B**). Box and whisker plots show the median, 25% quartiles and range. Outliers not represented. * OCP not detected in Tagus area; **OCP not detected in Évora area

Fig. 2 Relative concentrations of 16 organochlorine pesticides (OCP) expressed as percentage of total concentrations of OCP groups (ΣHCH, Σheptachlor, Σdrins, Σendosulfan and ΣDDT) in barn owl primary feathers and liver from Portugal in 2009–2012. Whiskers represent standard error



represented 99% (SE = 0.004) and 71% (SE = 0.102) of its family in feathers and liver, while its epoxide represented only 1% (SE = 0.004) and 22% (SE = 0.128; Fig. 2). Among Σdrins, aldrin was the most represented in feathers (50%, SE = 0.116), followed by dieldrin (17%, SE = 0.074), while in liver dieldrin (37%, SE = 0.112) and endrin aldehyde (24%, SE = 0.105) prevailed and aldrin was not detected (Fig. 2). Endosulfan II was the most abundant compound of its family in feathers (30%, SE = 0.098), while in liver, endosulfan I (51%, SE = 0.128) was the most represented (Fig. 2). Among ΣDDT, *p,p′*-DDT had the highest percentage in feathers (44%, SE = 0.127) while *p,p′*-DDE prevailed in liver (100%, SE = 0.00; Fig. 2).

External endogenous contamination could explain feather concentrations of β-HCH and heptachlor being so high and not reflecting liver concentrations. Preen oil is the most probable source of β-HCH and heptachlor in feathers because, unlike loose dust and airborne particles, preen oil secretions are not removed with water (Jaspers et al. 2008). *P,p′*-DDT and derivates, and drins may also highly contribute to OCP burden in the preen gland (Acampora et al. 2017). In other bird species, preen gland oil accumulates contaminants that have had little opportunity to be metabolized, thus reflecting recent exposure (Yamashita et al. 2007). Further studies are needed to understand how barn owls eliminate OCP in preen oil. A great within-species variation in the degree of metabolism of contaminants can contribute to reducing the correlation between preen oil and internal tissue contamination (Yamashita et al. 2007). For this reason, when preen oil affects feather concentrations, feather-liver concentrations might only be achieved with larger sample sizes. Additionally, variations occurred during the period elapsed between feather growth (1–2 months old), and the date of internal tissue sampling (3–36 months old) may also have affected the lack of association between OCP concentrations in feathers and internal tissues.

The compounds with the highest mean concentration in feathers were the same in Évora and Tagus: β-HCH (3 873 ± 2 940 ng g^{-1} and 5 658 ± 6 426 ng g^{-1}, respectively; Table S2 suppl.) and heptachlor (2 831 ± 2 383 ng g^{-1} and 2 081 ± 2 835 ng g^{-1}, respectively). In livers, the compound with the highest mean concentration in both areas was *p,p'*-DDE (49.3 ± 55.6 ng g^{-1} and 39.5 ± 45.8 ng g^{-1}, respectively, in Évora and Tagus). The compound with the second highest concentration differed between areas: lindane in Évora (47.9 ± 72.7 ng g^{-1}) and endrin aldehyde in Tagus (29.3 ± 45.6 ng g^{-1}). Five compounds were found in samples from Évora but not in Tagus: four compounds in feathers (endosulfan I, endrin, endrin aldehyde and *p,p'*-DDD), and one in livers (aldrin) (Fig. 1; Table S2 in suppl.). The four HCH isomers and heptachlor were the most frequently detected compounds in feather samples from both areas (in all individuals, except α-HCH; Table S2 suppl.). Lindane was detected in all liver samples from both areas, but in Tagus, dieldrin also had 100% frequency of detection, while in Évora its prevalence was 44% (Table S2 suppl.). There was a general trend for mean OCP concentrations to be higher in Évora, except for β-HCH in feathers and livers, and heptachlor epoxide and endrin aldehyde in livers. However, none of the isolated OCP mean concentrations differed significantly between study areas, and Σdrins in feathers was significantly higher in Évora than in Tagus (Table S2 suppl.).

The high concentrations and ubiquity of HCHs in feathers (mainly β-HCH) in Évora and Tagus is most likely associated with the generalized recent application of lindane in agriculture. The presence of lindane in Portuguese sediments has been linked to agricultural areas where historical land-use has been rice, wheat or grape crops, and its maximum value (450 ng g^{-1}) has been reported in coastal sediments in an estuary close to our study areas (Villaverde et al. 2008). Additionally, concentrations of HCH in terrestrial environments may also be increased by atmospheric transport after volatilization from oceans (Newton et al. 2014). Heptachlor is also more abundant in agricultural than forest soils (ATSDR 2007), but since it was banned decades earlier than lindane, the elevated concentrations of the parent compound in the barn owl may not result from historical agricultural use. Our results suggest similar temporal use and dosage in Évora and Tagus. Nevertheless, the absence of some OCP in Tagus which are present in Évora, along with a lower trend for OCP concentrations, may reflect different land-use and pesticide use histories of these two locations, and/or faster degradation of OCP in the Tagus area. These differences are not expected to derive from dietary effects, since the diet of barn owls is very similar in both sampling areas, being mostly composed of omnivore (Évora-Tagus: 30–32%), granivore (Tagus-Évora: 23–26%) and herbivore (Tagus-Évora: 22–25%) small mammals (I. Roque unpublished data). Our hypothesis of differences between areas in OCP degradation is supported by the dominance of the parent compound in the ratio *p,p'*-DDT: *p,p'*-DDE found in estuarine sediments from Sado River (partly draining agricultural lands from Évora; 0.21) compared to Tagus (0.18; calculated from Gil and Vale 1999). Some compounds (such as DDT related compounds) may degrade faster in Tagus, due to anaerobic conditions such as those encountered in soils that are either periodically or permanently flooded (Wang et al. 2007; Hao et al. 2008). Moreover, degradation of the ubiquitous HCH isomers is not improved by flooded conditions, and specially β-HCH is apparently not degraded by farming activity (Rubinos et al. 2007), which may have contributed to the observed high concentrations.

Among the OCP detected in Évora, but not in Tagus, only Σdrins concentrations in feathers differed significantly from zero. One individual from Évora showed a very high concentration of endrin (1 907 ng g^{-1}) in feathers and another individual of endrin aldehyde (234 ng g^{-1}). These high values most likely contributed to the significantly higher Σdrins in Évora. Since these individuals were not contaminated with the same compounds in the liver, they do not appear to have been exposed to these contaminants through diet. Our results suggest a current episodic exposure, possibly resulting from external contamination, most likely caused by obsolete endrin stocks remaining in the area, which represent potential sources of contamination for both wildlife and humans.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00128-022-03576-6.

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