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Concentrations of chlorinated pollutants in adipose tissue of yellow-legged gulls (*Larus michahellis*) from Spain: Role of gender and age

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ABSTRACT

Concentrations of 7 different polychlorinated biphenyl (PCB) congeners, and eleven organochlorine pesticides (OCPs) and metabolites, including DDTs (dichlorodiphenyltrichloroethane), HCHs (hexachlorocyclohexane isomers), Endosulfan, Endosulfan sulfate, Endrin, Dieldrin and HCB (hexachlorobenzene), were determined in adipose tissue of 57 yellow-legged gulls collected from NW and N Spain. Furthermore, the possible differences due to two endogenous factors, age and gender, were determined. All the analyzed PCBs were detected in over 66% of the samples, with levels of 291.9 (PCB 180), 34.5 (PCB 118), 0.7 (PCB 28), 432.6 (PCB 153), 225.5 (PCB 138), 1.3 (PCB 101) and 0.4 (PCB 52) μg/kg of adipose tissue. With respect to the OCPs and metabolites, only 4,4′-DDE and HCB were detected in more than 50% of the samples, with means of 360.6 and 2.5 μg/kg of adipose tissue, respectively. From all the considered contaminants, only 4,4′-DDE levels presented significant differences depending on the gender, with females showing higher values than males ($p < 0.01$). Significant differences ($p < 0.001$) were also found related to age for the levels of PCBs 180, 138, 101, 28 and 153, as well as 4,4′-DDE, with adult levels being higher than those in young birds. The results of the present study constitute a baseline to better assess the environmental impacts of PCB and OCP contamination at other coastal sites for future biomonitoring studies, with particular emphasis on gender- and age-related differences.

1. Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are included within the group of persistent organic pollutants (POPs). POPs are widely used chemical compounds of environmental concern, and feature long-range transport, resistance to metabolism and potential toxicity (Ashraf, 2017). The presence of these compounds are generally a result of industrial, commercial and agricultural activities. Moreover, they have become ubiquitous in the environment where, in recent decades, they have been found to cause adverse effects on humans and wildlife. Several of these compounds have been implicated, for example, in decreased reproductive success in fish-eating water-bird populations in contaminated areas (Choi et al., 2001a) and it has been shown that they can affect oxidative stress levels (Fenstad et al., 2016). These contaminants are fat soluble and not readily degradable in the environment. Furthermore, they have the potential to biomagnify and to accumulate in high concentrations in animals at the top of the food chain, considered at risk. Aquatic organisms, and those exploiting aquatic resources, are particularly exposed to increasing levels of pollutants since aquatic systems are usually the ultimate pollutant sink, either due to diffuse sources, or direct discharges from the environment (Ramos et al., 2013). Both OCPs and PCBs are a cause for concern for nearshore marine ecosystems already threatened by a variety of human activities and pressures (Good et al., 2014).

Coastal and estuarine areas from Galicia (29,575 km^2) and Asturias (10,603.57 km^2), two regions located along the northern

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Spanish coastline, are characterized by touristic, industrial, fishing, shipping, dredging and aquaculture activities, and/or contamination events. Even if there are well-preserved natural areas in both regions, there are also several important cities and industrial plants (for example, close to the cities of Vigo, A Coruña, Ferrol and Gijón). The NW/N Atlantic coast of Iberia is known to host over 80,000 yellow-legged gull (*Larus michahellis*) breeding pairs, as well as a wintering population comprising several gull species, most of which are yellow-legged gulls travelling from the Mediterranean (Arizaga et al., 2013). These seabirds, considered an integral part of aquatic ecosystems, have become sensitive biomonitors of the changes occurring due to both natural and anthropogenic factors in similar areas of Europe (Morales et al., 2016). Indeed, since the condition and reproductive success of seabirds are influenced both by the conditions of breeding areas and in remote places where they live outside the breeding season, they can be used as proxies to assess the impact of many variables affecting their environment in temporal and spatial terms (Falkowska et al., 2016). Seabirds are generally high consumers and subject to accumulation of marine pollution, and are commonly used as sentinel species for exposure to persistent contaminants. In addition, *Larus michahellis* is markedly adaptable when choosing habitats, often in the vicinity of coastal population centers. In those habitats, they can breed successfully in buildings, feeding on residues from both dumps and discards from local fishing activities. Fledglings, which have not yet been subjected to pollutant bioaccumulation, are useful for monitoring temporal and spatial changes in pollution levels around the breeding area (Abdennadher et al., 2010). Thus evaluation and measure of the effects of contaminants in living organisms and their environment are also influenced by endogenous factors such as gender and age (Burger, 2007). However, many endogenous factors have received considerable attention in wildlife, being gender one of them. The sex of a bird can affect exposure and accumulation of pollutants. One conventional explanation for differences in chemical burden suggests their transfer from breeding females to the eggs. However, results from studies on the effect of gender on toxic burden in birds are not consistent nor established for every chemical.

The aim of the present study was to evaluate the levels of different POPs in adipose tissue of gulls from different regions of the Atlantic coast of Spain, in order to determine whether organic contaminant exposure poses a threat to the environment under study. In addition, with the interest of determining the suitability of this seabird species as a bioindicator, the possible differences related to two endogenous factors, age and gender, on POPs levels was also investigated.

2. Material and methods

2.1. Study areas and sampling

Gulls were collected during the period of 2014–2016 in the regions of Galicia and Asturias (respectively situated in the NW and N of Spain) (Fig. 1). Collected animals were found dead or had died after being injured and referred to the Wildlife Recovery Centers situated in the study areas. Recovered birds suffered mainly from physical injuries, including electrocution, fall from the nest due to inexperience in flying, and others of unknown origin. Injured birds included in the study were those that had not been held at the Recovery Centre for more than 5 days before dying (the average stay was approximately 2 days). Diet during recovery was likely free of environmental contaminants. Different species of fresh fish were bought in the fish market and was for human consumption. The species were chosen depending on the size (preferable small), the protein/fat ratio, and the price.

During necropsy, several parameters such as mass measurements (g), organ weights (g), bill development and physical condition were registered. Age was determined based on the color plumage, as there is a significant color range to pure white adults, and 1-year-old juvenile gulls can easily be discerned from adult conspecifics using plumage characteristics (Grant, 1986). Gender was determined through observation of the gonads during necropsy. Twelve female juveniles, 16 female adults, 13 male juveniles and 16 male adults were identified. After sampling, the remains were destroyed hygienically by incineration, under current European legislation.

After necropsy, all specimens ($n = 57$) were immediately frozen and stored at −20 °C until samples were prepared for analysis. From each corpse, a portion of approximately 3 g of subcutaneous adipose tissue was taken, placed individually in plastic bags, and stored at −20 °C. The complete data set included 25 juveniles and 32 adults. In terms of gender, there were 29 males and 28 females.

2.2. Reagents and quantification of chlorinated compounds by GC/ MS analysis

POPs were analyzed in adipose tissue. Eleven OCPs (including metabolites) were assayed: isomer mixture of hexachlorocyclohexane (HCH) consisting of β and γ-HCH; DDT and its metabolites (namely 4,4′-DDD and 4,4′-DDE); hexachlorobenzene (HCB); and the cyclodiene insecticides heptachlor epoxide, dieldrin, endrin, endosulfan, and endosulfan sulfate. Similarly, 7 indicator PCBs (CBs 28, 52, 101, 118, 138, 153, and 180) were targeted, as they are predominantly present in biotic and abiotic matrices and have been recognized as compounds representative of the whole group of PCBs by the Agency for Toxic Substances and Disease Registry (ATSDR, 2000). Reference materials supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany) with a purity of 97–99.7% were used for OCPs standard preparation, with concentrations ranging from 10 ppb to 10 ppm. Similarly, a commercial mix of 7 PCBs from SpexCertiPrep (Stanmore, UK) (10 μg/ml in isooctane) was used for single quantification of PCB congeners IUPAC 28, 52, 101, 118, 138, 153, and 180. Stock solutions (500 μg/ml) were prepared by dissolving reference standards in acetone (Panreac) and stored at −20 °C. Working solutions for sample fortification and for injection in the GC systems were prepared by diluting stock solutions in n-hexane (Panreac®).

The protocol followed to perform the PCB and OCP extraction was adapted from a procedure used by Mateo et al. (2012). Briefly, samples were thawed at room temperature and 0.7 g of the tissue was chopped and mixed with 7 ml of n-hexane. The mixture was homogenized and frozen overnight, allowing the fat to precipitate. Five ml of the supernatant were added with 2 ml of H_2SO_4, the tubes were subsequently shaken in an orbital shaker for 10 min, sonicated for 5 min and centrifuged at 1000 × *g* for 5 min, and the acid-containing phase discarded. The above procedure was repeated until the acidic phase was completely clear. The resulted extract was evaporated, re-suspended in 200 μl n-hexane and then used for OCPs and PCBs concentration measurements.

A Bruker Scion 456 triple quadrupole gas chromatograph mass spectrometer was used to analyze the samples. Analyte separation was achieved on an Rxi-5 Sil MS column (30 m x 0.25 mm, i.d. x 0.25 film thickness). The results were analyzed using specific GCMS software. The multiple-ramp temperature program used involved a first step of 3.5 min at 70 °C, then the temperature was raised to 180 °C at a rate of 25 °C/min. This was followed by an increase to 300 °C at a rate of 15 °C/min and a final increase to 325 °C at a rate of 50 °C/min, and maintained for 5 min. The vaporized samples were injected in splitless mode at a column flow rate of 1.20 ml/min. The temperatures of the injection port, detector and interface were 280 °C, 280 °C and 300 °C, respectively. PCB and OCP residues were quantitatively evaluated carrying out the internal standard method (with 25 μg/l of PCB180 added at the beginning of the extraction process). The calibration curves were obtained by determining the relationship between the peak area and the concentration of the different standards. Solvent blanks (consisting of 500 μl n-hexane instead of tissue) were processed in parallel to the samples to assure the quality of the analyses.

To verify the suitability and performance of the procedure, the