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# Organochlorine pesticide contamination in three bird species of the Embalse La Florida water reservoir in the semiarid midwest of Argentina

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**Abstract**

Organochlorine pesticides (OCs) have a variety of acute and chronic pathological effects on animals, are persistent in the environment and are accumulated in adipose tissue of animals. In Argentina there are few studies reporting the OC contamination in the fauna. Moreover, no data are available for an ecologically relevant region, the arid-semiarid midwest region of Argentina. Recently, it was reported OC contamination in the water of an important artificial water reservoir of this area, the Embalse La Florida in the San Luis province. The present study aims to provide OC baseline data for birds of Embalse La Florida and to evaluate the potential risk of OC contamination for the local avifauna. We selected two fish-eating species, *Podiceps major* (great grebe) and *Phalacrocorax brasilianus* (neotropic cormorant) and one omnivore species, *Pitangus sulphuratus* (great kiskadee) to evaluate OC contamination. α-, β-, δ- and γ-hexachlorocyclohexane (ΣHCH), *p,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE and methoxychlor (ΣDDT), aldrin, dieldrin, endrin and endosulfan (ΣALD) and, *cis*-chlordane, *trans*-chlordane, heptachlor, heptachlor epoxide(ΣCHL) were measured in adipose tissue of two male great grebes, six neotropic cormorant (3 of each sex) and four great kiskadees (2 of each sex). We detected all OC pesticides assayed [ΣHCH range: ND to 3168.41 ng/g fat, ΣCHL range: ND to 4961.66 ng/g fat, ΣALD range: 287.07 to 9161.70 ng/g fat, ΣDDT range: 1068.98 to 6479.84 ng/g fat], with the exception of *p,p′*-DDT. Summed OC concentration in all bird species ranged from 2684.91 to 19231.91 ng/g fat. The omnivore had significantly greater concentrations of ΣCHLs than fish-eating species. Females of the neotropic cormorant had significantly higher amounts of ΣHCH and ΣCHL than males. The OC concentrations detected in birds were lower than those reported in the literature that are associated with deleterious effects on survival or reproduction in others species of birds. This study is the first report of OC contamination in birds of the midwest region of Argentina and constitutes a starting point for future studies that evaluate temporal changes of OCs in birds in this region.


*Keywords:* Organochlorine pesticides; Birds; Artificial lake; Risk evaluation; Argentina

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## 1. Introduction

Organochlorine pesticides (OCs) are important environmental contaminants that adversely affect organisms. These compounds, with their high persistence in the environment and their liposolubility, are accumulated primarily in adipose tissue and are biomagnified along food webs. Several studies in fish-eating birds and raptors have reported that OCs are associated with thinning and weakening of eggshells (Anderson and Hickey, 1972; Lundholm, 1997), feminization of males (Fry and Toone, 1981), inhibition of egg laying and a decrease in the size of nest clutch (Larson et al., 1996), a decrease in effective hatching success (King and Krynitsky, 1986) and an increase in the frequency of deformities in embryos (Fry and Toone, 1981; Larson et al., 1996). Therefore, monitoring the presence of OCs in avian species can yield information about the bioavailability, magnification and biotransference of pollutants and may prevent potential risks to birds, animals and possibly humans (Swaileh and Sansur, 2006).

Even though most OCs have been banned in Argentina, they were intensively used for several years until relatively recent times (Barra et al., 2002). Regrettably, in Argentina as in many other regions of the world, few studies have monitored OCs in animals (Menone et al., 2000a; Menone et al., 2000b; Lajmanovich et al., 2002; Lajmanovich et al., 2005). An especially critical absence of data for an ecologically relevant region, the arid-semiarid midwest, prompted the present study of OC contamination in the avifauna of this region. Since aquatic ecosystems tend to concentrate avifauna as well as contaminants (Weller, 1988; Paillisson et al., 2002; Xue et al., 2005), we performed our study in an artificial water reservoir, the "Embalse La Florida".

The Embalse La Florida (33°07′S–66°02′W; 1030 m a.s.l.) (Fig. 1) is situated in a sierras system in the geographical center of the province of San Luis (Argentina) and was built more than 50 years ago. Its surface area is 651.86 ha with a perimeter of 36 km and a water capacity of 100.97 $hm^3$. The lake provides drinking water to around 70% of the human population of the San Luis province and irrigation water to one-fifth of the territory of this province. The shoreline of the Embalse La Florida includes a wide variety of habitats, such as forests, shrubs, natural pastures and an area of high environmental value (a natural reserve, see below), where around 70 avian species are year-round residents and more than 10 migrant species nest during spring (Cid and Caviedes-Vidal, 2005). During 1992, a nature preserve (the "Reserva Natural La Florida") was created along the northern shoreline by the state government of San Luis to protect an area with native wildlife (Fig. 1). However, recent studies have reported OC contamination in the waters of Embalse La Florida above the maximum contaminant concentrations accepted for drinking water for the following OCs (mg/l): lindane 0.01, heptachlor 0.0065, heptachlor epoxide 0.0028, aldrin 0.0032, endrin 0.0024, clordanes 0.0031, *p,p′*-DDT 0.0037, *p,p′*-DDD 0.0057 (Antón et al., 2003; EPA, 2003; WHO, 2006). Therefore, the aims of our study were (A) to assess the levels of OCs in birds of Embalse La Florida, (B) to evaluate OC contamination in bird species

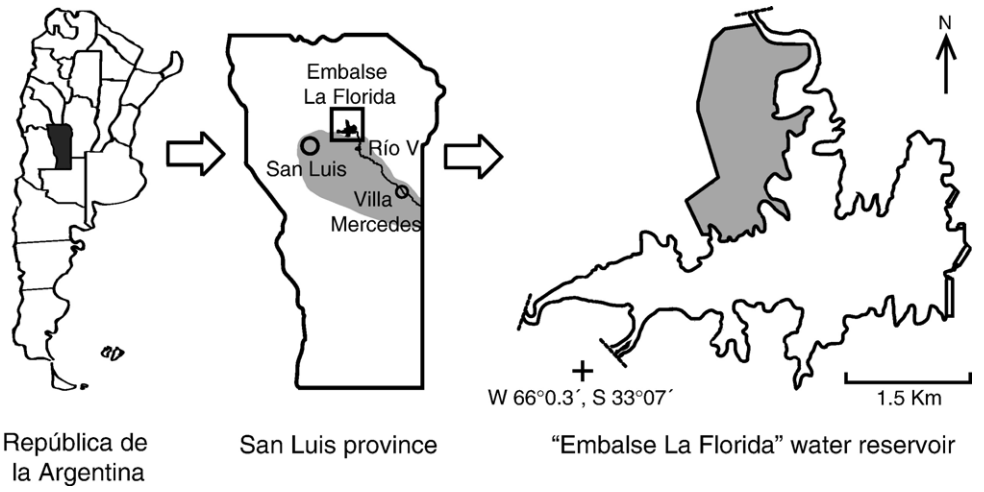


Fig. 1. Geographic localization of the "Embalse La Florida" water reservoir. This artificial lake provides drinkable water to around 70% of the human population of San Luis province and irrigation water to approximately one-fifth of the territory of this province (gray-shaded area in the center map). The gray area in the right map represents the natural reserve "Reserva Natural La Florida" created in 1992 along the northern shoreline of the Embalse La Florida water reservoir to protect the natural flora and fauna.

with different dietary habits, (C) to compare the OC burden between sexes, and (D) to discuss the potential risk posed by these contaminants for the avifauna of the study site.

## 2. Materials and methods

### 2.1. Species selection

Neotropic cormorants (*Phalacrocorax brasilianus*), great grebes (*Podiceps major*) and great kiskadees (*Pitangus sulphuratus*) were selected as receptors for this study based on the following criteria: (A) Dietary habits. We chose three species with different degrees of piscivory: neotropic cormorants and great grebes as strict fish-eaters, and great kiskadees as an omnivore that also predates on fish but not exclusively (Table 1). (B) Rank in the food chain. We selected birds ranked at or near the top of the food chain of this ecosystem to maximize the likelihood of observing bioaccumulation and biomagnification of OCs. (C) Geographical distribution. We included species with a wide geographical distribution in Argentina and the Americas in order to have baseline data for future monitoring. The distribution of neotropic cormorants and great kiskadees covers all South and Central America up to the south of the USA. The great grebe is distributed from the south of Argentina to the north of Peru and Brazil (Narosky and Yzurieta, 2003). (D) Migration behavior. We chose year-round residents and non-migrants so that most of the contamination could be attributed to the water of the reservoir itself. (E) Abundance. We selected species with a high or relatively high abundance in the Embalse La Florida (Cid and Caviedes-Vidal, 2005).

### 2.2. Sampling

During spring 2002, we captured by shooting (Walther KK300 Universal .22, Amsberg, Denmark) twelve adult birds (>1 year old): four great kiskadees (two of each sex), six neotropic cormorants (three of each sex) and two male great grebes. We also collected one great grebe egg. All captures were performed under an authorization of the Government of the Province of San Luis, Ministry of Human and Social Development, Program of Planning and Environmental Management (Permission No. 027, 01/11/2001).

Immediately after retrieval, we measured beak length, wing length, wing breadth, and beak-to-tail total length, and examined each animal for external abnormalities in beaks, eyes, wings and legs. Birds were stored individually in bags, refrigerated and sent immediately to the laboratory. On arrival, the animals were weighed and sexed, and the abdominal cavity opened for evaluation of the external appearance of internal organs by direct observation. In the species with a large body size (i.e. neotropic cormorant and great grebe), we collected samples of subcutaneous fat to assess OC contents. The internal organs (pectoral muscles, brain, gonads, liver and pectoral bone) of great kiskadees were excised, weighed and individually stored for other analyses; the remaining plucked carcasses were milled and homogenized in a commercial blender (Osterizer, model 890-48, Oster®, Milwaukee, Wl, USA). Sub-samples of the homogenate were used for OC analysis and the rest was stored for quantification of heavy metal concentrations. Great grebe egg were homogenized without the shell for OC determination.

### 2.3. Solvents and chemicals

All solvents were gas chromatography (GC) grade and obtained from Aldrich Chemical USA. Florisil, anhydrous sodium sulfate, and glass wool were pre-extracted with hexane and acetone at 2:1. The Florisil was heated overnight at 300 °C, and both the anhydrous sodium sulfate and the glass wool were heated at 100 °C. Pesticide standards and Florisil (60–80 mesh) were procured from Supelco USA. All glassware was rinsed several times with acetone and hexane.

### 2.4. Chemical analyses

We assayed all OCs detected in previous surveys (Luco et al., 1992; Antón et al., 2003). The determination

Table 1
Diet of the three species selected for this study (dietary reports of Argentina were prioritized)

| Species | Dietary items | References |
|---|---|---|
| *Phalacrocorax brasilianus* (neotropic cormorant) | Fish (*Odontesthes bonariensis*[a], *Cichlasoma facetum*[a], *Hoplias* sp., *Astyanax* sp., *Pimelodus* sp., *Oligosarcus* sp.) | Regidor and Terroba (2001) |
| *Podiceps major* (great grebe) | Fish (Odontesthes bonariensis[a], *Lycengraulis* sp., *Roeboides bonariensis*, *Triportheus* sp. and *Pimelodus* sp.), mollusks and crustaceous | Beltzer (1983b) |
| *Pitangus sulphuratus* (great kiskadee) | Fruits (*Solanum sisymbriifolium*, *Schinus longifolia*, *Morus alba*, *Ligustrum sinensis*), seeds, insects, arachnids, crustaceous, mollusks, amphibians and fish | Beltzer (1983a); Latino and Beltzer (1999); De La Peña (2001) |

[a] Fish species found in the stomach of birds captured in our study.

was carried out by the methods recommended by the AOAC 983.21-10.2.01 (AOAC, 1995) with slight modifications. This protocol was validated by performing part 186 of EPA method 608 (EPA, 1984).

Samples were homogenized in a blender with anhydrous sodium sulfate. Each sample was extracted with ether of petroleum. Sub-samples of the lipid extracts were used to gravimetrically determine the lipid content. The remaining lipid extract was concentrated to 10 ml in a Kuderna Danish system. The cleanup of the concentrate was performed in a 4-inch Florisil column (about 15 g deactivated Florisil with water 2% v/w), bottomed and topped with 1 in. of anhydrous sodium sulfate. The extract was run into the column and eluted with 100 ml 16% diethyl ether in hexane and concentrated to 5 ml by using a Kuderna Danish system.

The pesticides analyzed were α-HCH, β-HCH, δ-HCH, lindane, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, methoxychlor, endosulfan, and *cis*- and *trans*-chlordane. Gas chromatographic analysis was performed on a GC Hewlett-Packard 6890 plus equipped with a 63Ni-μEC detector and split-splitless injector in splitless mode, and Chemstation software. This employed a fused silica capillary analytical column HP-5 of 35 m × 0.32 mm i.d. with film thickness 0.25 μm and, for confirmation, a DB-1701 column (J&W Scientific Inc.) 30 m × 0.32 mm i.d. with film thickness 0.25 μm. The temperature for each column was programmed from 130° to 250 °C at 4 °C/min and a hold at 250 °C for 10 min. Injector and detector temperatures were 250° and 300 °C, respectively. Helium carrier and nitrogen make-up gas flow were 2.1 ml/min (at 130 °C) and 30 ml/min, respectively.

For the quantification of the pesticides, external standard calibration was used. Standard solutions for each compound, individually and in mixture, were prepared by dissolving the reference substances (Supelco, USA) in *n*-hexane at the following concentrations: 5 ng/ml for α-HCH, β-HCH, δ-HCH, lindane, aldrin, dieldrin, heptachlor, heptachlor epoxide, and endrin; and 10 ng/ml for *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT, methoxychlor, endosulfan, and *cis*- and *trans*-chlordane. With these standard solutions, seven-concentration calibration curves (*r*: 0.98 to 0.99) were regressed for each compound. Recoveries from fortified samples ranged from 83% to 109%. The minimum limit of quantification (MLQ) was 0.3 to 3 ng/ml and the detection limit (ng/g) was: α-HCH 0.025, β-HCH 0.01, δ-HCH 0.01, lindane 0.015, heptachlor 0.01, heptachlor epoxide 0.015, *trans*-chlordane 0.0015, *cis*-chlordane 0.0015, aldrin 0.075, dieldrin 0.02, endrin 0.025, endosulfan 0.015, *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE 0.0025 and methoxychlor 0.05. Concentrations of OCs were expressed as ng/g fat. For comparative purposes, the proportion of fat (% w/w) of the analyzed tissues of neotropic cormorants, great kiskadees and great grebes is reported in Table 2.

### 2.5. Statistical analyses

Reported OC values represent the mean ± 1 SEM of 3 individual measurements. For calculation purposes we assigned one-half the detection limit to any non-detected value (ND) (EPA, 1991; Singh and Nocerino, 2002). OC values of each compound were summed and grouped by families: (1) Diphenyl aliphatics (ΣDDT): *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD and methoxychlor; (2) hexachlorocyclohexanes (ΣHCH): α-HCH, β-HCH, δ-HCH, and lindane; (3) Cyclodienes I (ΣCHL): *cis*-chlordane, *trans*-chlordane, heptachlor, and heptachlor epoxide summed; and (4) Cyclodienes II (ΣALD): aldrin, dieldrin, endrin and endosulfan.

The normality of the data was tested by the Shapiro–Wilk test (*W* test) (Shapiro et al., 1968) and the homogeneity of the variance by Brown and Forsythe's test (Brown and Forsythe, 1974). To meet the normality assumption, concentrations by families of OC were square-root transformed and used in parametric statistics.

Variables were correlated by use of the Pearson correlation coefficient (*r*). Paired Student's *t*-tests were used to contrast OC families. The intra- and inter-species OC family frequencies were also evaluated.

An inter-specific contrast of all OC families between piscivores and omnivore was assessed by the $T^2$ Hotelling multivariate test (Hotelling, 1947). We also compared each family of OCs between species using Student's *t*-tests for independent samples (Student, 1908).

We evaluated OC accumulation differences between sexes by the *U*-Mann Whitney test (Mann and Whitney, 1947), since the data did not meet the assumptions of the parametric tests even after square-root transformation.

All data were used to perform the statistical analyses, except for those of great grebes in the intra-species and by-sex comparisons, owing to the small sample size ($N=2$).

The level of significance we used for all statistical tests was $P<0.05$.

## 3. Results and discussion

### 3.1. Status of the contamination

In all three species analyzed we found OCs. With the exception of *p,p′*-DDT (Table 2) which was not detected in any bird, all other OCs assayed were present in variable concentrations in the studied species. For simplicity, we will refer hereinafter to "all OCs" to denote all the pesticides

Table 2
OC concentration (mean and range in ng/g fat) per contaminants assayed and total OC load in bird species collected from Embalse La Florida, San Luis (Argentina)

| Species | *Phalacrocorax brasilianus* | *Podiceps major* | | *Pitangus sulphuratus* |
|---|---|---|---|---|
| Growth stage | Adults | Adults | Egg | Adults |
| Number | 6 | 2 | 1 | 4 |
| Sex | 3♀♀, 3♂♂ | 2♂♂ | – | 2♀♀, 2♂♂ |
| Body mass (g) [a] | 1443.67±66.63 | 1327.67±48.43 | 36.17 | 66.39±2.54 |
| Tissues assayed | Subcutaneous fat | Subcutaneous fat | Content | Carcass |
| Fat (% w/w) [b] | 56.32±3.38 | 69.19±5.64 | 6.89 | 5.93±2.38 |
| α-HCH | 29.38 [c] (ND [d]-171.23) [e] | ND | 199.52 | 56.15 (ND-221.54) |
| β-HCH | 473.36 (ND-1249.36) | ND | ND | 82.19 (ND-325.75) |
| δ-HCH | 139.34 (ND-480.06) | 197.99 (ND-394.99) | ND | ND |
| Lindane | 902.95 (ND-1799.28) | 371.71 (ND-742.41) | ND | 1544.23 (ND-3106.84) |
| Heptachlor | 273.54 (ND-1327.42) | ND | ND | ND |
| Heptachlor epoxide | 307.65 (1–673.16) | 200.83 (ND-400.65) | 1099.72 | 1275.04 (ND-2646.37) |
| *Trans*-chlordane | 369.10 (ND-772.92) | 328.46 (ND-655.93) | 322.87 | 1091.76 (252.93–1793.15) |
| *Cis*-chlordane | 429.16 (ND-1532.54) | 591.54 (ND-1182.09) | 2970.85 | 1075.41 (ND-2661.57) |
| Aldrin | 644.17 (ND-2497.45) | ND | 956.03 | 1993.52 (ND-5245.44) |
| Dieldrin | 227.58 (ND-1360.46) | 175.97 (ND-350.93) | 440.28 | 586.60 (ND-1154.30) |
| Endrin | 524.29 (ND-1115.98) | 1079.77 (131.73–2027.82) | 295.23 | 661.04 (ND-1467.77) |
| Endosulfan | 1709.43 (284.01–4276.23) | 500.55 (421.81–579.29) | 226.01 | 863.85 (ND-1444.79) |
| *p,p′*-DDT | ND | ND | ND | ND |
| *p,p′*-DDE | 1111.81 (215.20–1898.85) | 1077.69 (1047.06–1108.32) | 335.12 | 1299.13 (432.17–2313.84) |
| *p,p′*-DDD | 500.76 (ND-1373.50) | 1492.40 (288.36–2696.44) | 558.77 | 1433.88 (242.29–3022.26) |
| Methoxychlor | 763.97 (ND-1629.62) | 496.65 (234.46–758.84) | 309.57 | 977.74 (367.12–1769.60) |
| ∑OC [f] | 8407.52 (2684.91–16,433.26) | 6518.64 (5066.97–7970.32) | 7713.98 (ND-2970.85) | 12,943.53 (9211.72–19,231.91) |

[a] Body mass (mean±1 SEM).
[b] Proportion of fat of the samples assayed (mean±1 SEM).
[c] OC mean concentration.
[d] ND: <detection limit. Detection limits (ng/g): α-HCH 0.025, β-HCH 0.01, δ-HCH 0.01, lindane 0.015, heptachlor 0.01, heptachlor epoxide 0.015, *trans*-chlordane 0.0015, *cis*-chlordane 0.0015, aldrin 0.075, dieldrin 0.02, endrin 0.025, endosulfan 0.015, *p,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE 0.0025 and methoxychlor 0.05.
[e] OC range.
[f] Total OC load.

analyzed except *p,p′*-DDT. Neotropic cormorant tissues contained all OCs determined, whereas in great kiskadees there were no detectable amounts of heptachlor or δ-HCH, and in great grebes no α-HCH, β-HCH, heptachlor or aldrin.

Relationships among OC families were analyzed. We first tested whether OCs grouped by family were correlated when all bird samples were included. Only ΣALD and ΣCHL were significantly correlated ($r=0.73$; $P<0.05$). Secondly, we contrasted the magnitude of the contamination load among all birds and pesticide families. This analysis revealed that ΣALD and ΣDDT were present at the highest concentrations (Fig. 2; ΣALD > ΣHCH = ΣCHL and ΣDDT > ΣHCH; paired t-test, $P<0.05$).

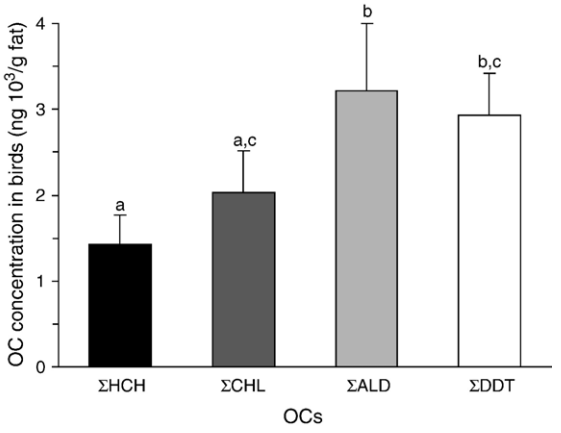


Fig. 2. Mean concentrations (±1 SEM in ng/g fat) of OCs grouped by family [(a) hexachlorocyclohexanes (ΣHCH): α-HCH, β-HCH, δ-HCH, and lindane; (b) Cyclodienes I (ΣCHL): *cis*-chlordane, *trans*-chlordane, heptachlor, and heptachlor epoxide; (c) Cyclodienes II (ΣALD): aldrin, dieldrin, endrin and endosulfan; (d) Diphenyl aliphatics (ΣDDT): *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD and methoxychlor] of birds from the Embalse La Florida. Means for each variable not sharing the same letter among OC families are significantly different (paired Student's *t*-test, $P<0.05$).

The highest concentration of a single contaminant was measured in one of the great kiskadees (aldrin at 5245.44 ng/g fat), and this was followed by endosulfan (4276.23 ng/g fat) in a neotropic cormorant.

In agreement with previous studies (King and Krynitsky, 1986; King, 1989; Choi et al., 2001; Naso et al., 2003), *p,p′*-DDE occurred with the highest frequency in our samples. This contaminant was detected in all 12 birds analyzed and its mean concentration was 1168.56±173.55 ng/g fat (range 215.20 to 2313.84 ng/g fat), the second highest mean concentration of all OCs. It has been suggested that the high chemical stability of *p,p′*-DDE and its consequent persistence in the environment may explain this (Guruge et al., 1997; Naso et al., 2003). The second most frequent contaminant was methoxychlor, present in 92% of the samples with a concentration ranging between ND and 1769.60 ng/g fat. *p,p′*-DDD was apparent in 75% of the samples (ND to 3022.26 ng/g fat) and *p,p′*-DDT, as mentioned above, was not detectable.

The high frequencies and concentrations of *p,p′*-DDD and *p,p′*-DDE measured in birds and the complete absence of *p,p′*-DDT may be interpreted as the result of a non-recent contamination (Naso et al., 2003). In our system this hypothesis may not apply, since a recent assessment of water quality in the Embalse La Florida revealed detectable amounts of *p,p′*-DDT and *p,p′*-DDD but not *p,p′*-DDE (Antón et al., 2003). Thus, the presence of *p,p′*-DDE may be the result of the metabolism of its precursors (e.g. *p,p′*-DDT and *p,p′*-DDD) by the birds and/or organisms belonging to the previous trophic level (fishes, amphibians and insects).

The occurrence of *p,p′*-DDT in this ecosystem may be attributed to long-range atmospheric transport (LRAT) from the Pampas or other regions where it was applied intensively in agriculture during the 1960s and 1970s (for further discussion see below). In Argentina the use of *p,p′*-DDT was gradually prohibited from 1968 onwards, and was finally totally banned in 1998 (SENASA, 2005). Even though the application of *p,p′*-DDT is prohibited, products such as Dicofol that are used intensively in gardening and citrus and cotton culture may contain it as contamination (Barra et al., 2002); these may be helping to maintain the contaminant loads in the ecosystems. The presence of methoxychlor, another member of the ΣDDT family, may also be attributed to LRAT, although unlike *p,p′*-DDT its use in agriculture was banned only recently, in 2000 (SENASA, 2005).

Endosulfan, in the ΣALD family, occurred at a frequency of 92% in the assayed samples and its average concentration was 1226.09 ng/g fat (range: ND to 4276.23 ng/g fat), the highest observed for all OCs determined. This high frequency of incidence and concentration may be due to its intensive and continual use since 1970 as an insecticide in a variety of crops such as tea, vegetables, fruits, and tobacco (Barra et al., 2002). Endrin was detected in 75% of the samples with an average concentration of 662.46 ng/g fat (range ND to 2027.82 ng/g fat). Dieldrin and aldrin occurred at low frequencies, 42% and 50% respectively, yet aldrin displayed a high mean concentration at 986.76 ng/g fat. The higher frequency of occurrence of endrin compared with that of aldrin is perplexing since endrin and aldrin are present in the water at similar concentrations (Antón et al., 2003). One possible explanation is that part of the detected aldrin is transformed to dieldrin by the metabolism of animals (Gallego-Iniesta and Pertierra-Rimada, 1987). An additional support for this hypothesis is that dieldrin was not detected in the water (Antón et al., 2003).

In the ΣCHL family, heptachlor occurred at the lowest frequency (17%) and the lowest average concentration (137.27 ng/g fat, range ND to 1327.42 ng/g fat). The other OCs in this family had frequencies ranging from 42% to 75% and average concentrations from 603.21 to 671.64 ng/g fat. The low frequency of heptachlor may be associated with its short persistence in the environment and conversion to heptachlor epoxide in the soil, animals and plants (Ritter et al., 1995). *Trans*- and *cis*-chlordane were synthesized and used in Argentina until 1998, when they

were banned (Barra et al., 2002; Giorgio and Digón, 2004).

The ΣHCH family was detected in lower concentrations than other OC families, although Lindane occurred at a high frequency (75%) and with an average concentration of 1028.17 ng/g fat (range: ND to 3106.84 ng/g fat). The other ΣHCH congeners exhibited frequencies from 17% to 33%. The high frequency of Lindane is in line with its extensive use all over the world including Argentina (Barra et al., 2002). The use of this product in Argentina was banned in 1998 (Giorgio and Digón, 2004).

### 3.2. Comparison of trophic levels

The total OC load (∑OC), calculated as the sum of all OC assayed for each individual, exhibited high intraspecific variation (see Table 2), and no significant differences were found between piscivores (neotropic cormorant and great grebe) and the omnivore (great kiskadee) ($T^2$ Hotelling, $P>0.05$). Naso et al. (2003), too, found no significant differences for ∑OC in birds with different dietary habits in Campina, Italy.

The magnitude of pollutant bioaccumulation has been associated with the level of contamination of dietary items, the trophic level to which the organism belongs, the ability to metabolize or eliminate contaminants, and migrations of birds (King, 1989; Walker et al., 2001; Naso et al., 2003). The status of an organism in the food chain may influence the load of contaminants in its body. As a general rule, carnivorous birds accumulate higher concentrations of pollutants than omnivores (Burger, 2002). However, in the present study, even though great kiskadees include significant amounts of plant material in their diet (see Table 1) whereas neotropic cormorants eat only fish (see Table 1), the two species had similar ∑OC values. Therefore, the status of the organism in the food chain does not seem to be the determinant for the ∑OC of the species in this study. Unfortunately, incomplete data in some cases (e.g. contamination of food items) and a lack of them in others (e.g. metabolism and detoxification assays) prevent further discussion of the reasons for the ∑OC pattern observed.

OCs grouped by families. When OCs were grouped by family, differences in contamination load between piscivores and omnivores were significantly different only for ΣCHL members (piscivores<omnivores; Student's *t*-test, $P<0.05$), whereas ΣHCH, ΣALD and ΣDDT concentrations were similar between the two trophic groups (Fig. 3). The different diets of these birds (see Table 1) may account for the differential contamination with ΣCHL, but no firm conclusion can be drawn since the level of contamination of all the exploited resource items has not been studied. The differential migration pattern might be put forward to explain the difference in ΣCHL contamination; the colony of neotropic cormorants, the strictly fish-eating group, displays a seasonal mobility (the winter population decreases to ~12.5% of the spring–summer population, Cid and Caviedes-Vidal personal observations), whereas the omnivorous great kiskadee is a year-round resident. However, the situation is more complex, since great grebes combine strict piscivory with year-round residence.

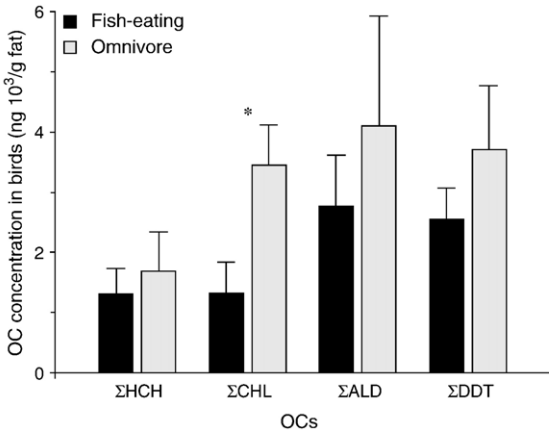


Fig. 3. Comparison of OC burdens (mean±1 SEM in ng/g fat) grouped by family (for references see Fig. 2) between fish-eating birds ($N=8$) and omnivores ($N=4$) of the Embalse La Florida. The asterisk denotes significant difference between dietary categories within the same OC family (Student's *t*-test, $P<0.05$).

### 3.3. Sex comparison

Differences between the sexes in OC accumulation were analyzed only in neotropic cormorants because the numbers of great grebes and great kiskadees were too low. The three female cormorants showed a 4.78-fold higher ΣHCH burden and 9.56-fold higher ΣCHL burden than males (Fig. 4), with these differences being significant (Mann Whitney *U* test, $P<0.05$). Of the ΣHCH family, only Lindane was present at detectable concentrations in males, whereas Lindane, α-, β- and δ-HCH were detectable in 1, 3 and 2, respectively, of the females. The sex differences in ΣCHL loads are also attributable to non-detectable concentrations of most OC family members (i.e. heptachlor, heptachlor epoxide and *cis*-chlordane) in males (with the exception of *trans*-chlordane observed in one of the three males),

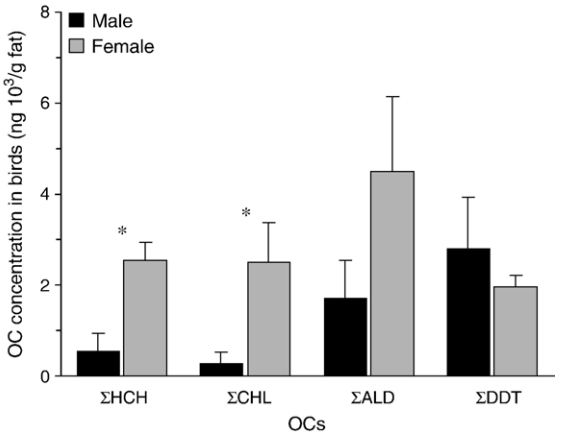


Fig. 4. Sex comparison of OC burdens (mean±1 SEM in ng/g fat) grouped by families (for references see Fig. 2) in neotropic cormorants of the Embalse La Florida. The asterisks denote significant differences between sex within the same OC family (*U*-Mann Whitney test, $P<0.05$).

while all three females exhibited measurable amounts of heptachlor epoxide and *trans*-chlordane, and two showed heptachlor and *cis*-chlordane.

Our results for the ΣDDT family agree with those reported by King and Krynitsky (1986), who found no male–female differences in *p*,*p*′-DDE in neotropic cormorants of Galveston Bay (Texas). The higher concentrations of ΣHCH and ΣCHL in females than in males found in the present study suggest that further research should investigate whether there are sex differences in prey selection or in the use of foraging areas with dissimilar degrees of contamination or in the seasonal mobility pattern. Our results conflict with those of other authors. For instance, Donaldson and Braune (1999) reported for American white pelicans (Lake Winnipeg, Canada) concentrations of *p*,*p*′-DDT, chlordane and dieldrin were significantly higher in males than in females, while no inter-sex differences were found for ΣHCH. Olafsdottir et al. (2001) found higher OC contamination in males for five avian species in Iceland and hypothesized that this may be the result of transference of contaminants from females to eggs.

### 3.4. Organochlorines in eggs

One way the females may excrete contaminants is by transferring them to eggs. Thus, eggs may contain a combination of toxicants that may be a proxy for dietary and somatic contamination of the mother (Glaser and Connolly, 2002; Burger et al., 2004). Unfortunately, the small sample size of the great grebe material (2 adult males and 1 egg) permitted to collect by the authorities precluded comparisons and statistical treatment, so we here simply present the concentrations of the OCs found. The egg of the great grebe contained most of the OCs assayed, but *p*,*p*′-DDT, aldrin, lindane, β- and δ-HCH were not detected. OC concentrations were lower in the adult males (6518.64 ng/g fat) than in the egg (7713.98 ng/g fat). The ΣHCH family (199.52 ng/g fat) and ΣDDT family (1203.46 ng/g fat) in the egg were lower than in the adult males (see Table 2), yet ΣCHL in the egg (4393.45 ng/g fat) was 4 fold higher than in the adult males. Concentrations of the ΣALD family (1917.55 ng/g fat) were comparable in the egg and the adult males.

The *p*,*p*′-DDE contamination of the great grebe egg (335.12 ng/g fat) was lower than reported for several other grebe species. De Smet (1987) found 7400.26 ng DDE/g fat in eggs of red-necked grebe (*Podiceps grisegena*) from Turtle Provincial Park, Manitoba, Canada. Lindvall and Low (1980) reported 7650 ng DDE/g fat for eggs of western grebe from Utah, and Scharenberg and Ebeling (1998) also detected high concentrations of *p*,*p*′-DDE (2604.8 ng/g fat) in the yolk of eggs of the great crested grebe (*Podiceps cristatus*) from Lake Belau (Germany), although the concentration of *p*,*p*′-DDD (138.22 ng/g fat) in eggs of these birds was lower than those (558.77 ng *p*,*p*′-DDD/g fat) found in our study for great grebe.

### 3.5. Risk evaluation

The chemicals assessed in this study are toxic, persistent, bioaccumulated in organisms and biomagnified along the food chain. Their presence may adversely affect the health, survival and reproduction of birds (Blus et al., 1985; Dirksen et al., 1995; Powell et al., 1997; Taylor and Harrison, 1999; Walker et al., 2001). Several studies have reported that high concentrations of *p*,*p*′-DDT and especially *p*,*p*′-DDE affect reproduction in birds, such as by feminization in males, eggshell thinning, embryotoxicity and diminishing or even impeding reproduction (Forsyth et al., 1994; Lundholm, 1997; Walker et al., 2001). *p*,*p*′-DDE concentrations in eggs higher than 500 to 6000 ng/g wet weight have been associated with a reduction in the reproduction of several bird species (Muñoz Cifuentes et al., 2003). The concentrations of *p*,*p*′-DDE in a great grebe egg and in adult individuals of the three species evaluated were well below those found to generate adverse effects in birds. King and Krynitsky (1986) reported that amounts of *p*,*p*′-DDE of 2600 ng/g wet weight in the carcass of neotropic cormorants were below the limit associated with critical poisoning and reproductive disorders in

birds. Additionally, there has been a reported thinning by 2.3% to 6.5% of the eggshell in the western grebe (*Aechmopharus occidentalis*) of Utah and in the red-necked grebe (*P. grisegena*) of southwestern Manitoba, with mean *p*,*p*′-DDE concentrations of 5400 and 6680 ng/g wet weight respectively, although red-necked grebes also had elevated PCB concentrations (Lindvall and Low, 1980; De Smet, 1987). No apparent adverse effects of *p*,*p*′-DDE concentrations in the range of μg/g wet weight in passerine eggs on reproductive success have been observed (reviewed by Klemens et al., 2000).

Lindane, aldrin, dieldrin, endosulfan, heptachlor epoxide and methoxychlor were also found at detectable amounts in this study. The concentration of dieldrin detected in our great grebe egg (30.34 ng/g wet weight) was below those concentrations reported to produce alterations in reproduction for in eggs of *Aguila chrisaetus* (900 ng/g wet weight), suggested as a highly sensitive species among birds (Naso et al., 2003). However, the maximum dieldrin concentration detected in an adult neotropic cormorant (901.98 ng/g wet weight) was at the level of the *A. chrisaetus* eggs, suggesting that reproductive alterations in the first species need further research. The maximum concentrations of heptachlor epoxide for all adult birds (398.51 ng/g wet weight) and the great grebe egg (75.77 ng/g wet weight) were below those values reported (1500 ng/g wet weight) to adversely affect reproduction in the American kestrel, *Falco sparverius* (Henny et al., 1983; Henny et al., 1984). Methoxychlor, lindane and endosulfan contaminations were also under the concentrations reported to harm birds (WHO, 1984, 1991; Nebeker et al., 1992; Sang et al., 1999; Eroschenko et al., 2002; EXTOXNET, 2006).

Even though we did not find external macroscopic abnormalities in animals, organs or eggs, and most of the pesticide concentrations measured in this work were below the values reputed in the literature to be harmful to birds, it is difficult to estimate the potential risk of these compounds for birds of the Embalse La Florida and to evaluate the temporal dynamics of these contaminants in the region. First, even low concentrations of some toxicants may harm organisms by interacting or synergizing with other compounds (Walker et al., 2001; Muñoz Cifuentes et al., 2003). Second, there are no species-specific toxicity assays for the species studied. Third, in Argentina there have been few studies that have used birds as bioindicators of environmental contamination by OC, but these have been specific to the humid pampas and mesopotamic zone, which is ecologically different from our study site. Fourth, there are no reports of which OCs have been applied in mid-western Argentina, nor the amounts and frequencies of their use. The lack of all the above-mentioned information stresses the urgent need to continue monitoring the avifauna of this ecosystem and to perform species-specific toxicity studies to properly evaluate the potential risk of OCs for birds of this region.

Finally, an important question is how these pesticides enter the Embalse La Florida. At least four processes may be hypothesized for our system: discharge of tributary rivers carrying these compounds, LRAT (Walker et al., 2001; Leip and Lammel, 2004), rainfall and runoff. Unfortunately, owing to the lack of data, we cannot evaluate the relative contribution of these mechanisms. Since pesticides can travel long distances, the remote sources of contamination may be any of the many agricultural regions of Argentina where these chemicals have been used extensively and intensively (Barra et al., 2002). However, local sources of contamination may also be important, since the reservoir receives tributary rivers that flow past small towns and agricultural land. Certainly, this important issue requires further investigation and must be included in future research agendas.

## 4. Conclusions

All three bird species of the Embalse La Florida that were tested (neotropic cormorants, great grebes and great kiskadees) exhibited detectable concentrations of all OCs assayed except for *p*,*p*′-DDT, which was, however, present in the water of the reservoir.

Total OC burden did not differ among the three species.

The inter-specific comparison by the OC family revealed that concentrations of contaminants in the omnivore, the great kiskadee, were higher than, or similar to, those in the piscivores (neotropic cormorant and great grebe). These results do not agree with the observations of other researchers for similar species in other aquatic ecosystems. The cause of the different patterns is unknown, although differing capacities to metabolize OCs may be involved.

OC contaminations measured in avian species of the Embalse La Florida were near or below those reported to affect reproduction.

The status of avian contamination by OC presented in this study constitutes a starting point and an invitation for future research into species-specific effects of toxicants, and for continued monitoring to manage avian populations to evaluate temporal changes in this important aquatic ecosystem.

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