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Organochlorine Contamination in Anuran Amphibians of an Artificial Lake in the Semiarid Midwest of Argentina

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Abstract Artificial water reservoirs are important for fauna in arid-semiarid regions, because they provide suitable habitats for species that depend on water, such as amphibians. Organochlorine pesticides (OCPs) are toxic, persistent compounds that tend to bioaccumulate and bioconcentrate. We evaluated contaminant levels in anurans from an artificial lake (Embalse La Florida) in a semiarid region of the Midwest Argentina. This lake is one of the few sources of permanent water in the area. OCPs were detected in all individuals. Levels ranged from 2.34 ± 0.62 ng/g wet mass of heptachlors to 9.76 ± 1.76 ng/g wet mass of hexachlorocyclohexanes. The distribution pattern of OCP was $\sum$HCH > $\sum$DDT > endosulfan > $\sum$chlordane > metoxichlor > $\sum$aldrin > $\sum$heptachlor. Contaminant levels in individuals were positively correlated with contaminant levels in the water. Burden differed between species, but not among sites differing in water contaminant levels. Results suggest that anurans may concentrate OCPs and thus provide an important source of exposure for amphibian predators. This study provides important information for potential risk assessment of amphibians in the region and contributes to our understanding of the extent of OCP contamination.

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Water availability and environmental temperature influence the physiology and behavior of anuran amphibians. Arid environments, where high ambient temperatures and low humidity occur in combination, represent an important challenge to the thermal and water balance physiology of these organisms (Wilmer et al. 2000).

The construction of reservoirs is a mechanism routinely used by humans to maintain available water in arid and semiarid regions where annual water input (e.g., rainfall) is discontinuous and unpredictable. Intrigued by the consequences of this water management strategy, several researchers investigated the importance of these artificial water bodies for amphibian communities. Artificial water reservoirs play an uncertain role in influencing the native species diversity of semiarid environments. Amphibians tolerate droughts and maintain widely distributed populations without permanent water (Burkett and Thompson 1994); conversely, artificial water reservoirs may be very important for a variety native wildlife species that rely on water for drinking or as a habitat during part or all of their life cycle (Landsberg et al. 1997). The availability of permanent water for native species of amphibians may encourage large, more stable, and more widespread populations in previously uninhabitable areas (Landsberg et al. 1997). Sources of permanent water may be important for amphibians even when temporary ponds are available, because temporary water bodies experience wide fluctuations in chemical and physical characteristics restricting growth and reproduction to short and irregularly wet periods (Lahr 1997). Therefore, qualitative and quantitative studies of contaminant levels in natural and artificial water sources found in these environments are necessary to adequately conserve amphibian communities.

The central-west region of Argentina, including the Monte desert and the arid Chaco vegetation formation, are good examples of arid rangelands with different degrees of anthropic alteration. They have dry climates, being warmer in the north and gradually becoming cooler toward the south. The northern region is a continuous shrubland and dry forest, representing a typical landscape of intermountain depressions, valleys, and slopes. Rain is the primary water source and occurs mainly during summer (50 to 600 mm/year). Even though the mean annual air temperature is between 15 and 19°C, seasonal temperatures are highly variable. Summers are hot, with high temperatures ranging from 40 to 45°C, and winter low temperatures range from −15 to −20°C (Fernández and Busso 1997).

The Embalse La Florida is an artificial lake located in the central zone of San Luis province at the beginning of the Río Quinto basin (Fig. 1). In this typical semidesert area, water availability fluctuates dramatically throughout the year and depends on yearly rainfall patterns. This reservoir provides irrigation and drinking water to one-third of the San Luis province population. The Embalse La Florida ecosystem has a diverse animal community including eight anurans that breed in the reservoir (Jofré 2004).

The reservoir receives water from two tributaries, the El Trapiche and the Río Grande rivers. These tributaries transport pollution from upstream human activities into the reservoir. The input of suspended materials, organic matter, and wastes has a marked seasonal cycle. During summers, when the tourist population in the watershed increases by at least three times compared to the rest of the year and the tributary rivers flood and water runoffs occur, the entrance of contaminated material to the reservoir increases. Based on water quality results, we considered the north shore (almost void of anthropogenic use) as the low contamination test zone and the south shore (heavily used for recreational activities) as the high contamination test zone (Antón and Caviedes-Vidal 2004).

Among the contaminants assayed in the reservoir are heavy metals (e.g., cadmium, lead) and organochlorine pesticides (OCPs) (Antón et al. 2003) (Table 1). OCPs are an important group of ambient contaminants that are highly stable, are persistent, and bioaccumulate in fat of animals (Peakall 1992). They affect reproduction by disrupting the endocrine system (Colborn et al. 1993) and elicit several harmful effects on amphibians (Berril et al. 1998; Cooke 1970, 1972, 1973; Schuytema et al. 1991). Amphibians are good models for monitoring levels of contaminants in aquatic ecosystems and are an important link in the transference of contaminants across trophic webs (Blaustein and Wake 1990; Roe et al. 2005; Unrine et al. 2007; Vitt et al. 1990). Contaminants that affect amphibian reproduction and survival may affect the structure and integrity of ecosystems and have been implicated as one potential cause of amphibian declines (Collins and Storfer 2003; Stuart et al. 2004).

The aims of this study are (1) to assess OCP burdens in amphibians from La Florida, (2) to estimate differences

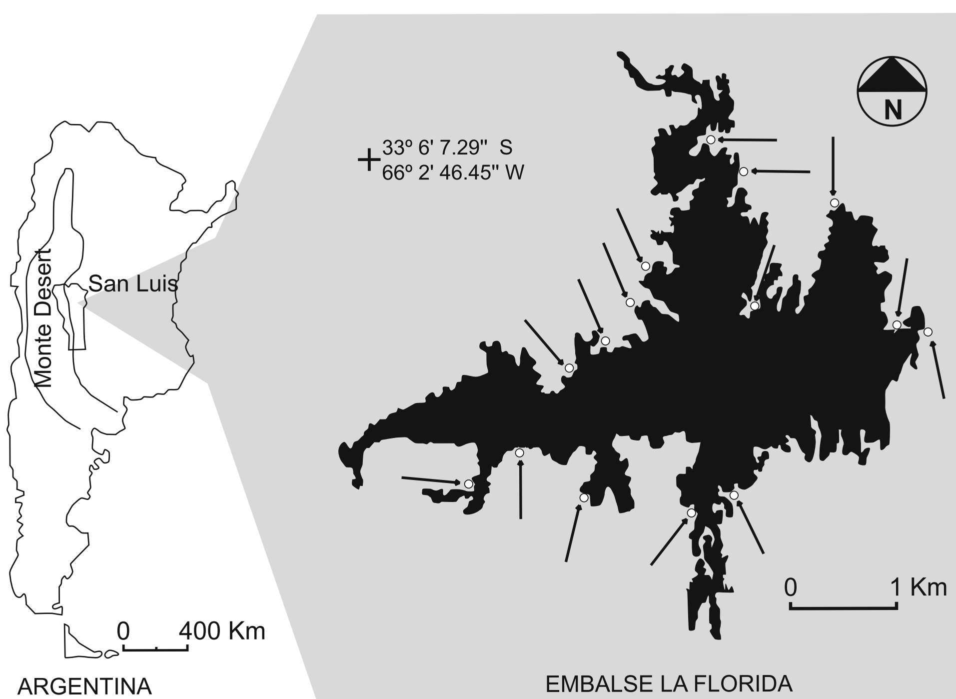

Fig. 1 Location of the Embalse La Florida, San Luis, Argentina. Arrows point to collecting sites (open circles)

Table 1 Levels of organochlorine pesticides (OCPs) in water from Embalse La Florida

OCP	Concentration (ppb)[a]	
	Luco et al. (1992)	Antón et al. (2003)
α-HCH	2.080	2.377
β-HCH	<2.680	3.390
δ-HCH	ND	10.606
Lindane	23.490	6.901
Heptachlor	0.890	4.199
Heptachlor epoxide	4.830	2.225
trans-Chlordane	ND	3.181
cis-Chlordane	ND	3.322
Aldrin	<2.670	3.062
Dieldrin	<5.570	1.702
Endrin	ND	3.247
4,4-DDE	6.250	1.334
4,4-DDD	7.460	7.708
2,4-DDT	10.880	5.283
Endosulfan	ND	8.971
Metoxichlor	ND	14.531

Note. ND, not detected

[a] Values in each column are from different sites at the Embalse La Florida

between species and compounds, and (3) to compare these levels among amphibians collected in shores with different degrees of disturbance. We tested three predictions. First, adult amphibians from la Florida accumulate OCPs. Second, there are differences in accumulation between species and compounds. Third, individuals collected in the more contaminated south shore have higher burden levels than individuals from the north shore of the dam. Data about levels of persistent organic pollutants (POPs) in South American biota and ecosystems are scarce (Programa de las Naciones Unidas para el Medio Ambiente 2002). Therefore, in addition to estimating the quantity of OCPs accumulated by amphibians, this work will contribute to our understanding of the regional extent of POPs contamination.

Materials and Methods

Adult amphibians ($n = 26$) of five species—*Chaunus arenarun* ($n = 9$), *Leptodactylus mystacinus* ($n = 4$), *Hypsiboas cordobae* ($n = 2$), *Odontophrynus occidentalis* ($n = 2$), *Melanophryniscus stelzneri* ($n = 4$), and *Pleurodema tucumanum* ($n = 5$)—were trapped using nets or by hand on the shores of the dam, and in streams and temporary ponds, between October and December of 2001 (3 sampling nights) and during November and December 2002 (2 sampling nights). Sampling sites, on the north and south shores (Fig. 1), were located on places where habitat structure appeared to be suitable for amphibian breeding. Sites located on the south shore represented potentially high exposure to contaminants and poor water quality; sites located on the north shore represented lower contaminant exposure and better water quality. At each location air and water temperature (50 cm aboveground and 5 cm below the surface, respectively) were recorded. Individuals were identified to species and transported to our laboratory, where the weight and morphometrics measurements were recorded. After euthanization amphibians were dissected and sex was determined by observation of gonads.

The AOAC 983.21 method was used to extract and clean up samples (AOAC 1995). Individual whole bodies were homogenized, weighed, mixed with Na_2SO_4, and extracted three times with petroleum ether. When individual body masses were below the minimum amount of sample required for extraction (5 g), individuals (four or five) were pooled (Table 2). The extracts were filtered and taken to a final volume of 250 ml. A sample of 25 ml from each extract was used to estimate the fat content. The volume of extract that contained 0.2 g of fat was concentrated in Kuderna Danish, then transferred to a Florisil column and eluted twice, first with a mix of petroleum ether/ethyl ether (94:6) and then with the same mix at a 85:15 proportion. The resulting fractions were evaporated to dryness in Kuderna Danish, and then the volume was adjusted to 5 ml with *n*-hexane. We assayed hexachlorocyclohexanes (isomers α-, β-, and γ-HCH and lindane), heptachlor, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, aldrin, dieldrin, endrin, 2,2-bis(4-chlorophenyl)-1,1-dichloroethane (*p,p*′-DDD), 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p*′-DDE), 2,2-bis(4-chlorophenyl)-1,1-trichloroethane (*p,p*′-DDT), endosulfan (sum of the α- and β- isomers), and methoxychlor.

Gas chromatographic analysis was performed on a GC Hewlett-Packard 6890 plus, equipped with a 63Ni-µEC detector, split-splitless injector in splitless mode, and Chemstation software. Analytical column fused silica capillary HP-5 (35 m × 0.32-mm i.d.) with film thickness of 0.25 µm was used. Helium carrier and nitrogen makeup gas flow were 2.1 (at 130°C) and 30 ml/min, respectively. For confirmation, a DB-1701 column (J&W Scientific Inc.; 30 m × 0.32-mm i.d.) with a film thickness of 0.25 µm was used. The temperature for both columns was programmed from 130 to 250°C at 4°C/min and held at 250°C for 10 min. Injector and detector temperatures were 250 and 300°C, respectively. Chromatograph response was evaluated using a solution of chlorotalonil (50 ppb), clorpyrifos (2 ppb), and δ-BHC (40 ppb) in methyl-terbutyl-ether (MTBE). Percentage recovery, evaluated using 4-4'-dichlorobiphenyl, was 87% (acceptable, 70–130%). The external standard calibration method was used for the

Table 2 Collection site, sex, and body masses of amphibians in the study

Species	Common name	Collection site	N	Body mass (g)
Chaunus arenarun	Common toad	South shore	1 F, 4 M	59.12 ± 9.10
		North shore	1 F, 3 M	53.67 ± 9.38
Leptodactylus mystacinus	Moustached frog	North shore	4 F	12.73 ± 1.10
Hypsiboas cordobae	Córdoba tree frog	South shore	1 F, 1 M	8.08 ± 1.69
Odontophrynus occidentalis	Lesser escuerzo	North shore	1 M	11.93
		South shore	1 F	31.57
Melanophryniscus stelzneri	Redbelly toad	North shore	4[a] (sex ND)	1.85 ± 0.08
Pleurodema tucumanum	Spotted-flank four-eyed frog	North shore	5[a] (sex ND)	1.94 ± 0.31

Note. F, female; M, male; ND, not determined

[a] Individual body masses below the minimum amount of sample required for extraction; four or five individuals pooled into a single sample for extraction and quantification of OCP levels

quantification of compounds. Standard solutions for each pesticide, individually and in mixtures, were prepared by dissolving the reference substances (Supelco, USA) in *n*-hexane at the following concentration levels: 20 ng/ml for α-, β-, δ-, and γ-HCH, aldrin, dieldrin, heptachlor, heptachlor epoxide, and endrin; 30 ng/ml for *p*,*p*-DDE, *p*,*p*-DDD, and *p*,*p*-DDT, methoxychlor, endosulfan, and *cis*- and *trans*-chlordane. Using these standard solutions, four-level calibration curves, showing correlation coefficients (*r*) from 0.98 to 0.99, were created for each compound. Recoveries from fortified samples ranged from 83 to 109%. Quantification levels of the method were estimated from 0.3 to 3 ng/ml, for each one of the organochlorine pesticides. The detection limits (ng/g) were as follows: 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.

The 16 pesticides were grouped into seven families to more easily visualize and analyze data. The concentration values of each compound were summed ($\sum$) in order to obtain a total value for each family (one-half the detection limit was assigned to nondetected values): $\sum$HCH includes α-HCH, β-HCH, δ-HCH, and lindane; $\sum$heptachlor includes heptachlor and heptachlor epoxide; $\sum$chlordane includes *trans*-chlordane and *cis*-chlordane; $\sum$aldrin includes aldrin, dieldrin, and endrin; $\sum$DDT includes DDE, DDD, and DDT; and, finally, endosulfan and metoxichlor are each considered a family.

One-way ANOVAs were performed to assess differences in the levels of pesticide contamination for all individuals collected, expressed as burden (ng/animal), nanograms per gram of fat, and nanograms per gram of wet mass, among the assayed pesticides, including body mass as a covariate. One-way ANOVA was also used to test differences in the levels of pesticides (same as above) of both species with enough data points to perform statistics, *C. arenarun* ($n = 9$) and *L. mystacinus* ($n = 4$). The other four species lacked enough data ($n \leq 2$) to be included in a species comparison. Total levels of OCPs and amounts per pesticide family (burden, ng/g fat, and ng/wet mass) were compared between *C. arenarun* and *L. mystacinus* using an independent-sample *t*-test. Comparisons between total levels of OCPs and amounts per family in all the individuals collected were compared between shores (north and south) by a *t*-test for independent samples. The same analysis was performed to compare OCP levels in *C. arenarum* collected on the north vs. the south shore. A logarithmic transformation was carried out before statistical analyses to achieve normality assumption. OCP values are expressed as mean ± 1 standard error of the mean. Spearman's rank correlation test was used to assess correlations between individual whole-body pesticide levels (not grouped by families) and concentrations of pesticides in water at the location of collection (Antón, unpublished data) and body mass.

Bioconcentration factors (BCF = concentration of OCPs in amphibian whole body/concentration of OCP in water) were calculated.

Results

Fifteen of the sixteen OCPs examined were detected in our screening (Table 3). All individuals captured and assayed had detectable levels of at least three of the seven pesticide families.

Average total OCP burdens of all species ranged from 38.73 ± 12.12 ng ($\sum$heptachlor) to 188.45 ± 30.16 ng ($\sum$HCH) (Fig. 2). Concentration of OCP per gram of fat varied between 250.01 ± 67.97 ng/g ($\sum$aldrin) and 1056.42 ± 314.16 ng/g ($\sum$HCH) (Fig. 2), and between 2.35 ± 0.62 ng/g ($\sum$heptachlors) and 9.76 ± 1.76 ng/g

Table 3 Concentration of organochlorine pesticides in amphibians from the Embalse La Florida

Compound	Species					
	C. arenarum	*H. cordobae*	*L. mystacinus*	*M. stelzneri*	*O. occidentalis*	*P. tucumanum*
α-HCH	1.5 (ND–5.6)[a]	4.7 (3.9–5.4)	4.82 (3.53–6.98)	6.99	2.7 (1.3–4.1)	4.7
	165.5 (ND–552.0)[b]	707.0 (214.0–1200.0)	168.62 (54.0–329.9)	138.8	458.1 (23.5–892.6)	858.4
β-HCH	0.9 (ND–2.3)	5.2 (3.0–7.3)	3.5 (0–8.9)	ND	1.9 (1.0–2.7)	4.15
	155.3 (ND–685.2)	898.6 (165–1631)	141.7 (0–419.2)	ND	308.5 (18.2–598.8)	749.7
δ-HCH	1.1 (ND–2.7)	6.1 (4.9–7.2)	2.2 (ND–5.7)	4.7	2.2 (1.5–3.0)	4.4
	186.4 (ND–783.1)	937.9 (267.9–1608.0)	105.3 (ND–271.2)	93.8	342.9 (26.9–658.9)	799.5
Lindane	0.9 (ND–2.2)	3.9 (3.2–4.6)	4.1 (2.0–6.0)	3.4	1.7 (0.9–2.6)	2.4
	121.6 (ND–299.3)	599.4 (1022.0–176.5)	131.0 (42.5–284.5)	67.4	290.8 (17.2–564.3)	433.4
Heptachlor	0.9 (ND–7.1)	1.6 (0–3.1)	2.1 (ND–6.8)	3.0	0.9 (0.6–1.1)	2.0
	251.7 (ND–2090.7)	349.5 (ND–698.9)	86.1 (ND–320.4)	60.0	127.9 (11.2–244.6)	358.8
Heptachlor epoxide	0.3 (ND–1.4)	2.4 (1.6–3.2)	0.7 (ND–3.0)	3.2	1.4 (0.8–2.0)	1.7
	31.9 (ND–160.7)	398.9 (89.1–708.8)	11.5 (ND–45.9)	63.8	230.8 (14.1–447.4)	306.1
T-Chlordane	0.7 (ND–5.7)	2.9 (2.5–3.4)	2.5 (ND–4.4)	3.3	1.4 (0.8–2.0)	19.2
	201.8 (ND–1691.0)	442.1 (136.1–748.0)	92.2 (ND–206.3)	65.6	228.6 (14.3–442.9)	2.5
C-Chlordane	1.1 (ND–5.6)	2.7 (2.0–3.4)	3.2 (0–6.8)	4.3	2.6 (1.3–3.8)	ND
	234.4 (ND–1650.9)	434.9 (110.4–759.4)	108.1 (0–321.9)	84.8	430.8 (25.2–836.4)	ND
Aldrin	0.9 (ND–2.8)	2.1 (1.7–2.5)	3.11 (2.1–4.7)	2.8	0.7 (ND–1.4)	2.9
	147.4 (ND–745.4)	262.1 (134.3–390.0)	111.6 (32.9–220.0)	55.5	155.4 (ND–310.8)	522.4
Dieldrin	0.2 (ND–1.4)	ND	1.1 (ND–1.8)	ND	ND	ND
	16.3 (ND–146.9)	ND	33.8 (ND–87.0)	ND	ND	ND
Endrin	0.6 (ND–1.8)	0.33 (ND–0.7)	2.7 (0.4–7.9)	ND	0.1 (ND–0.3)	ND
	88.2 (ND–404.9)	18.2 (ND–36.5)	111.6 (6.9–374.0)	ND	2.5 (ND–5.0)	ND
DDE	1.5 (ND–4.5)	1.4 (1.1–1.7)	2.3 (ND–6.0)	10.6	1.3 (0.8–1.8)	ND
	252.4 (ND–1329.9)	166.8 (90.3–243.3)	86.9 (ND–291.3)	209.6	200.68 (14.8–386.4)	ND
DDD	1.6 (ND–6.6)	3.8 (2.5–5.1)	5.5 (1.0–11.9)	11.4	1.6 (1.0–2.3)	3.2
	314.8 (ND–1939.8)	640.8 (137.4–1144.1)	224.2 (15.2–562.3)	226.2	258.3 (18.4–498.1)	578.7
DDT	ND	ND	ND	ND	ND	ND
	ND	ND	ND	ND	ND	ND
Endosulfan	2.5 (0.5–2.4)	2.4 (1.5–3.4)	12.1 (1.2–24.4)	24.4	1.9 (0.6–3.2)	4.5
	344.0 (79.2–1406.8)	418.0 (81.6–754.4)	403.3 (18.1–982.3)	518.1	358.8 (11.0–706.7)	820.2
Metoxichlor	1.7 (0.5–4.4)	4.9 (3.5–6.3)	6.1 (2.5–14.4)	5.5	2.4 (1.3–3.4)	3.6
	284.5 (77.2–1292.7)	796.0 (192.1–1399.9)	236.1 (50.8–681.0)	110.0	387.7 (24.9–750.5)	656.6

Note. ND, not detected

[a] For each OCP, first row is average ng/g wet mass (range)

[b] For each OCP, second row is average ng/g fat (range)

($\sum$HCH), when values were expressed as nanograms per gram wet mass. The distribution pattern was $\sum$HCH > $\sum$DDT > endosulfan > $\sum$chlordane > metoxichlor > $\sum$aldrin > $\sum$heptachlor. Differences in levels between the OCPs detected in amphibians were statistically significant for values expressed as burden (ng/animal), per gram fat, and per gram wet mass ($F_{6,125} = 4.72, p < 0.001$; $F_{6,125} = 3.50,\ p < 0.05$; and $F_{6,125} = 8.00,\ p < 0.001$, respectively) (Fig. 2). Body mass was a significant covariate (all p's < 0.001), except for the comparison with burden values ($p = 0.36$). Significant differences were also observed when family/pesticide levels were compared for *C. arenarum* ($F_{6,55} = 3.56$, $p < 0.05$, for burden; $F_{6,55} = 3.33$, $p < 0.05$, for ng/g fat; and $F_{6,55} = 3.56$, $p < 0.05$, for ng/g wet mass) and *L. mystacinus* ($F_{6,20} = 3.41$, $p < 0.05$, for burden; $F_{6,20} = 2.82$, $p < 0.05$, for ng/g fat; and $F_{6,20} = 3.41$, $p < 0.05$, for ng/g wet mass).

Chaunus arenarum had the highest average burden of total OCPs (112.30 ± 17.02 ng/animal) and *P. tucumanum* had the lowest average burden (10.02 ± 3.72 ng/animal) (Fig. 3). Average concentration value per gram of fat was lowest for *M. stelzneri* (184.00 ± 54.95 ng/g fat) and

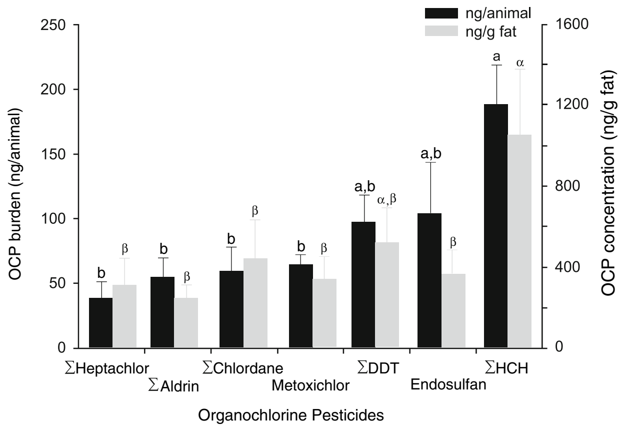

Fig. 2 Average (±standard error) burden and concentration per gram fat of OCPs detected in anurans from Embalse La Florida. Each bar represents the average for all individuals captured. Bars that share the same letters are not significantly different

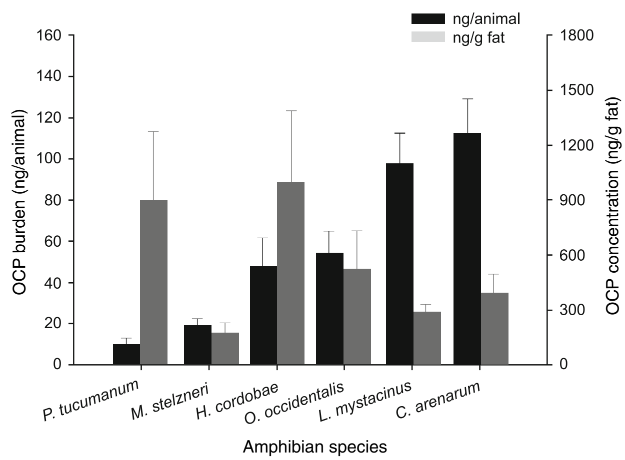

Fig. 3 Average (±standard error) burden and concentration per gram fat of OCPs in anuran species from Embalse La Florida. Each bar represents the average for all OCPs detected. Values corresponding to bars in the shaded area were compared statistically

highest for *H. cordobae* (1010.05 ± 386.78 ng/g fat) (Fig. 3). Average concentration of pesticides per gram wet mass was lowest for *C. arenarum* (2.32 ± 0.36 ng/g wet mass) and highest for *M. stelzneri* (9.27 ± 2.77 ng/g wet mass). The percentage fat content in whole bodies was Ms > Lm > Oo > Hp > Ba > Pt. *L. mystacinus* had significantly higher total OPC and metoxichlor concentrations per gram wet mass than *C. arenarum* (both p's < 0.05). Burdens and concentrations per gram fat for total OCPs and pesticide family were not significantly different between the species.

Levels of OCPs (total and per individual family/pesticide) did not differ significantly in amphibians (all species

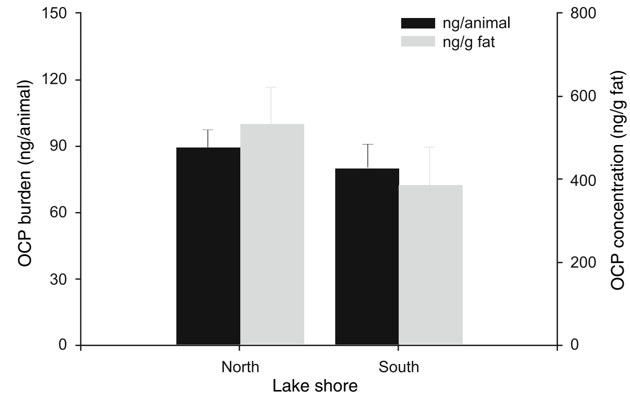

Fig. 4 Total OCP burden and concentration per gram fat in amphibians (all species together) collected on the south and north shores of Embalse La Florida. Error bars represent standard errors of means

together) collected on the north vs. the south shores of the reservoir (all p's > 0.05), except for endosulfan, which was significantly more concentrated per gram wet mass in amphibians collected in the north shore ($p < 0.05$) (Fig. 4). The same pattern of no significant differences between shores was observed for *C. arenarum*.

OCP burden (ng/animal) of all species was positively correlated with the concentration in the reservoir at the location where frogs were collected (Spearman correlation coefficient $[r] = 0.65$, $p < 0.05$) (Fig. 5) and with body mass ($r = 0.184$, $p < 0.05$). BCFs of amphibians from the Embalse La Florida were between 6.59 ($\sum$aldrin) and 15.70 ($\sum$endosulfan).

We failed to observe external or internal apparent malformations. *C. arenarum* (ID 15) lacked the left hind leg, but the missing limb could be the result of a predation attack and not to a developmental malformation.

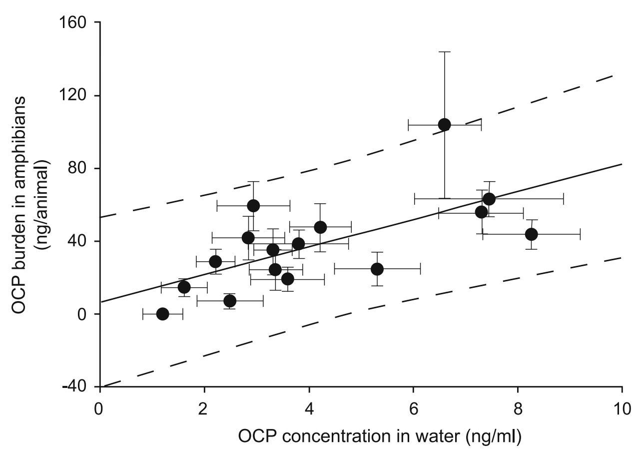

Fig. 5 Relationship between OCP levels in water and those in the whole bodies of amphibians. Error bars represents standard errors of means

Discussion

OCPs in Amphibians

Our first prediction that amphibians dwelling in the Embalse La Florida accumulate OCPs was supported. All five species, *C. arenarum*, *H. cordobae*, *L. mystacinus*, *M. stelzneri*, *O. occidentalis*, and *P. tucumanum*, captured at the study site contained detectable levels of OCPs. Of the 16 compounds assayed, DDT was the only OCP not detected.

Our study is the first to report OCP levels in amphibians from the Argentinian Midwest. Lajmanovich et al. (2005) reported organochlorine pesticides in adult *Bufo paracnemis*, *B. arenarum*, *Leptodactylus ocellatus*, and *Hyla pulchella* from farmlands of the Entre Ríos and Santa Fe provinces (Argentina); heptachlor levels ranged from 5.6 to 46 ng/g fat, chlordane levels reached as high as 24 ng/g fat, and endosulfan ranged from 5 to 19 ng/g fat. Although amphibians from that study were collected in agricultural lands, the amount of OCP (i.e., same pesticides) on their bodies was lower than the levels found in the amphibians we collected from Embalse La Florida (Table 3).

The values detected in our study are also higher than the body loads (HCHs, heptachlor, chlordane, and DDT metabolites, between 0.05 and 3.64 ng/g wet mass) of *Rana* individuals collected in farmlands and wetlands from other regions (Gillilland et al. 2001; Albanis et al. 1996). Levels detected in this study are comparable to OCP concentrations found in amphibians from protected areas. In the conservation area of Guanacaste (Costa Rica) concentrations of heptachlor, aldrin, and *p,p*́-DDE ranged from 3.7 to 54.6 ng/g wet mass (Klemens et al. 2003). Russell et al. (1995, 1997) detected concentrations of *p,p*́-DDE between 0.58 and 1001 ng/g wet mass in amphibians from Point Pelee National Park (Canada) and nearby locations. Likewise, *Rana muscosa* from Kings Canyon and Sequoia National Parks (USA) showed concentrations between 0.17 (*trans*-chlordane) and 45.69 ng/g wet weight (*p,p*́-DDE) (Fellers et al. 2004).

Interestingly, only one study reports OCP levels in amphibians higher than levels found in this study. *R. clamitans* from wetlands located close to orchards in southern Ontario containded ΣDDT and metabolites (mainly *p,p*́-DDE) that reached concentrations of 3480 ng/g fat (Harris et al. 1998).

OCP concentrations in amphibians from the Embalse La Florida are below those levels that directly affect survival when tested under laboratory conditions (Cooke 1970, 1972, 1973). Nevertheless, since amphibians are not on the top of food chains, they may transfer OCPs to higher trophic levels, such as birds, mammals, and carnivorous reptiles. Cid et al. (2007) reported contamination by OCP in avian species of the Embalse La Florida that reach concentrations of 9161.7 ng/g fat (sum of aldrin, dieldrin, endrin, and endosulfan).

Levels of OCP that these amphibians are exposed to may also cause sublethal effects such as the alteration of sexual development or enzymes activities. Another risk for amphibians in this ecosystem is the possibility of synergistic effects due to the exposure to multiple contaminants, since Embalse la Florida is also contaminated with heavy metals (cadmium and lead [Antón et al. 2003]).

Comparison Between OCPs

The pattern of OCP accumulation found in amphibians from Embalse La Florida (higher body levels of ∑HCH, DDT metabolites, and endosulfan) is consistent with recent patterns of use or the high persistence (DDT) of these compounds in the region. Use of OCP pesticides has been restricted since 1968, but Argentina banned dieldrin and HCHs in 1980 and DDT, aldrin, and endrin in 1990. Although methoxychlor, lindane, and chlordane have been banned since 1972, the latter two were still in use in 1995 (Menone et al. 2000). The high proportion of endosulfan found in the species studied may be related to its unrestricted use for the control of a large spectrum of insect pests on fruits and vegetables (Menone et al. 2000).

Most studies of OCP body burdens in amphibians detect a significantly higher proportion of DDE and DDD relative to DDT (Fellers et al. 2004; Gillan et al. 1998; Gillilland et al. 2001). We did not detect DDT in amphibians collected in La Florida, but ΣDDE and DDD was the third most abundant family of OCPs found. This result is consistent with the broad use of this pesticide ceasing a relatively long time ago (Menone et al. 2000). However, some studies report that other pesticides currently in use (e.g., Dicofol) may result in significant amounts of DDT and ratios DDT/DDE >1 in the soil, which suggests that new DDT continues to be transported from agricultural and remote areas via the atmosphere (Miglioranza et al. 1999, 2003). The absence of the parent DDT congener in the bodies of anurans present in the reservoir also suggests that anurans may metabolize the parent material to DDD and DDE forms (Fellers et al. 2004).

Comparison Among Species

Relevant factors for explaining differences in OCP concentration and patterns among species include seasonality (e.g., changes in lipid content, bioavailability, feeding preferences, migration), reproductive status (e.g., transfer of lipids to offspring), body size, trophic position, age, sex, life cycle, habitat, and feeding ecology. In addition, the body burden of a species depends on the ability of individuals to metabolize and eliminate the contaminants (Borga et al. 2004; Klemens et al. 2003).

C. arenarum, the species with the highest per-animal body accumulation in our study, also had the largest body size. Large species may also be longer-lived than small species, thus individuals of a large species may be more likely to have more exposition time and to accumulate OCPs. Additionally, aquatic species may absorb OCPs directly from the water and consume foods with high concentrations of OCP compounds (Klemens et al. 2003). Differences in OCP levels per gram of wet mass may be explained by differences in body fat content; *L. mystacinus* had a significantly higher ($t = -12.58$, $p < 0.001$) fat percentage (3.858%) than *C. arenarum* (0.794%).

Although different species may have different routes and rates for uptake and detoxification of OCPs, the general pattern of high concentrations of OCPs in amphibians from highly contaminated water and low concentrations in amphibians from less contaminated water suggests that OCP concentrations in amphibians reflect the pollution levels in the environment from which they are collected and confirm that they are suitable bioindicators for monitoring these contaminants.

Bioconcentration

The more concentrated contaminants found in water from the Embalse La Florida were the same contaminants found at high concentrations in amphibian bodies. Despite the differences in accumulation and elimination patterns that may exist among species and/or pesticides, levels of OCPs in amphibians directly reflect the level of each compound in the environment.

Comparison Between Shores with Different Contamination Levels

Our second prediction, that amphibians dwelling on the more contaminated shore would exhibit higher OCP body loads than individuals captured on the less contaminated shore, was not supported. Amphibians from both shores contained similar total OCP body loads (expressed as either burden, ng/g fat, or ng/g wet mass). Only one pesticide, endosulfan, exhibited differences (in ng/g wet mass accumulated), but in the opposite direction. Amphibians collected from the north shore (less polluted) contained higher average levels (7.12 ng/g wet mass) of this compound than amphibians from the more polluted south shore (1.41 ng/g wet mass). Accumulation of any contaminant into an organism is influenced by a variety of factors and may occur via many routes. Amphibians acquire contaminants through food and their semipermeable skin during eggs and larvae development, from the aquatic environment (Koponen et al. 2004). Most amphibians breed and metamorphose in aquatic habitats, however, terrestrial environments can also be a major source of pollutants, given that many species are terrestrial the majority of their lives. Contaminant uptake in terrestrial environments may occur by oral, pulmonary, and dermal exposure from ingesting contaminated prey, receiving aerial deposition, and occupying polluted soil (Storm et al. 1994; Johnson et al. 1999). Therefore we may conjecture another source of contamination, besides aquatic environment, in amphibians from La Florida.

Conclusion

Amphibians that reproduce in the shallow waters near or on the shores of Embalse La Florida are using the only source of permanent water available in the area and they accumulate OCPs. Levels detected were comparable to OCP concentrations found in amphibians from preserve areas and may be not high enough to elicit lethal direct effects. Nevertheless, amphibians represent a source of OCPs to individuals of higher trophic levels of this reservoir.

High body levels of $\sum$HCH, DDT metabolites, and $\sum$endosulfan are consistent with the recent patterns of use and/or the high persistence (DDT) of these compounds in the region and are directly related to concentrations in the water. Differences in OCP accumulation among species may be a consequence of differences in size, diet, metabolism, and habitat.

Data regarding concentrations of widespread organochlorine insecticides in animals are essential to understanding the extent of environmental contamination in the past and to predicting future trends (Albanis et al. 1996; Programa de las Naciones Unidas para el Medio Ambiente 2002). Results of this study will augment the limited and heterogeneous database available on levels of OCPs in South America ecosystems, will provide a baseline for future temporal trend assessments, and may prevent further biological impacts of toxic chemicals.

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References

Albanis TA, Hela D, Papakostas G, Gouter V (1996) Concentration and bioaccumulation of organochlorine pesticides residues in herons and their prey in wetlands of Thermaikos Gulf, Macedonia, Greece. Sci Total Environ 182:11–19

Antón RI, Caviedes-Vidal E (2004) Organoclorados en diques de la zona Centro de San Luis, Argentina. In: Abstracts II Reunión Binacional de Ecología, XXI Reunión Argentina de Ecología y XI Reunión de la Sociedad de Ecología de Chile

Antón RI, Caviedes-Vidal E, Cid F, Jofré M, Navarro Becerra N, Rodríguez N (2003) Ecotoxicología de los embalses de la zona centro de la provincia de San Luis. Organoclorados. Acta Toxicol Argent 11:69–70

AOAC International (1995) In: Cunniff P (ed). Official methods of analysis of AOAC International. 16th edn. AOAC International, Arlington, VA

Berril M, Coulson D, McGuillivray L, Pauli B (1998) Toxicity of endosulfan to aquatic stages of anuran amphibians. Environ Toxicol Chem 17:1738–1744

Blaustein AR, Wake DB (1990) Declining amphibian populations: a global phenomenon. Trends Ecol Evol 5:203–204

Borga K, Fisk AT, Hoekstra PF, Muir DCG (2004) Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. Environ Toxicol Chem 23:2367–2385

Burkett DW, Thompson BC (1994) Wildlife association with human-altered water sources in semiarid vegetation communities. Conserv Biol 8:682

Cid FD, Antón RI, Caviedes-Vidal E (2007) Organochlorine pesticide contamination in three bird species of the Embalse La Florida water reservoir in the semiarid midwest of Argentina. Sci Total Environ 385:86–96

Colborn T, vom Saal FS, Soto AM (1993) Developmental effects of endocrine disrupting chemicals in wildlife and humans. Environ Health Persp 101:78–384

Collins JP, Storfer A (2003) Global amphibian declines: sorting the hypotheses. Divers Distrib 9:88–98

Cooke AS (1970) The effect of p,ṕ-DDT on tadpoles of the common frog (*Rana temporaria*). Environ Pollut 1:57–71

Cooke AS (1972) The effects of DDT, dieldrin and 2,4-D on amphibian spawn and tadpoles. Environ Pollut 3:51–68

Cooke AS (1973) Response of *Rana temporaria* tadpoles to chronic doses of p,ṕ-DDT. Copeia 4:647–652

Fellers GM, McConnell LL, Pratt D, Datta S (2004) Pesticides in mountain yellow-legged frogs (*Rana muscosa*) from the Sierra Nevada Mountains of California, USA. Environ Toxicol Chem 23:2170–2177

Fernández OA, Busso CA (1997) Arid and semi-arid rangelands: two thirds of Argentina. Rala Rep 200:41–60

Gillan KA, Hasspieler BM, Russell RW, Adeli K, Haffner GD (1998) Ecotoxicological studies in amphibian populations of southern Ontario. J Great Lakes Res 24:45–54

Gillilland CD, Summer CL, Gillilland MC, Kannan K, Villeneuve DL, Coady K., Muzzall P, Mehne C, Giesy JP (2001) Organochlorine insecticides, polychlorinated biphenyls and metals in water, sediment and green frogs from southwestern Michigan. Chemosphere 44:327–339

Harris ML, Bishop CA, Struger J, Van Den Heuvel MR, Van Den Kraak MR, Dixon GJ, Ripley B, Bogart JP (1998) The functional integrity of northern leopard frog (*Rana pipiens*) populations in orchard wetlands. I. Genetics, physiology, and biochemistry of breeding adults and young-of-the-year. Environ Toxicol Chem 17:1338–1350

Jofré MB (2004) Ecotoxicología de anfibios en ambientes contaminados: aproximaciones ecológicas y morfo-funcionales. Dos casos de estudio. Tesis doctoral. Facultad de Química Bioquímica y Farmacia, Universidad Nacional de San Luis

Johnson MS, Franke LS, Lee RB, Holladay SD (1999) Bioaccumulation of 2,4,6-trinitrotoluene and polychlorinated biphenyls through two routes of exposure in a terrestrial amphibian: is the dermal route significant? Environ Toxicol Chem 18:873–878

Klemens JA, Wieland ML, Flanagin VJ, Frick JA, Harper RG (2003) A cross-taxa survey of organochlorine pesticide contamination in a Costa Rican wildland. Environ Pollut 122:245–251

Koponen PS, Nieminen P, Mustonen AM, Kukkonen JVK (2004) Postmetamorphic *Xenopus laevis* shows decreased plasma triiodothyronine concentrations and phosphorylase activity due to subacute phytosterol exposure. Chemosphere 57:1683–1689

Lahr J (1997) Ecotoxicology of organisms adapted to life in temporary freshwater ponds in arid and semi-arid regions. Arch Environ Cont Tox 32:50–57

Lajmanovich RC, de la Sierra P, Marino F, Peltzer P, Lenardón A, Lorenzatti E (2005) Determinación de residuos de organoclorados en vertebrados silvestres del Litoral Fluvial de Argentina. Temas de la Biodiversidad del Litoral Fluvial Argentino II, INSUGEO. Miscelánea 14:389–398

Landsberg J, James CD, Morton SR, Hobbs TJ, Stol J, Drew A, Tongway H (1997) The effects of artificial sources of water on rangeland biodiversity. Final report to the Biodiversity Convention and Strategy Section of the Biodiversity Group, Environment Australia. CSIRO Division of Wildlife and Ecology and Australian Government, Department of the Environment and Heritage. Available at: http://www.deh.gov.au/biodiversity/publications/technical/artificial-water/app1.html

Luco JM, Aguilar EG, Silva PG, Baudino OM, Gonzalez DM (1992) Niveles de pesticidas organoclorados en aguas de la provincia de San Luis (República Argentina). Acta Farm Bonaerense 11:121–128

Menone ML, Aizpún de Moreno JE, Moreno VJ (2000) PCBs and Organochlorines in tissues of Silverside (*Odontesthes bonariensis*) from a coastal lagoon in Argentina. Arch Environ Contam Toxicol 38:202–208

Miglioranza KSB, Aizpún de Moreno JE, Moreno VJ, Osterrieth ML, Escalante AH (1999) Fate of organochlorine pesticides in soils and terrestrial biota of "Los Padres" pond watershed, Argentina. Environ Pollut 105:91–99

Miglioranza KSB, Aizpún de Moreno JE, Moreno VJ (2003) Trends in soil science: organochlorine pesticides in Argentinean soils. J Soils Sediments 4:264–265

Peakall D (1992) Animal biomarkers as pollution indicators. Chapman and Hall, London

Programa de las Naciones Unidas para el Medio Ambiente (2002) Evaluación regional sobre sustancias tóxicas persistentes. Sudamérica oriental y occidental. Informe Regional. Fondo para el medio Ambiente Mundial, Ginebra Suiza

Russell RW, Hecnar SJ, Haffner GD (1995) Organochlorine pesticide residues in southern Ontario spring peppers. Environ Toxicol Chem 14:815–817

Russell RW, Gillan KA, Haffner GD (1997) Polychlorinated biphenyls and chlorinated pesticides in southern Ontario, Canada. Green frogs. Environ Toxicol Chem 11:2258–2263

Roe JH, Hopkins WA, Jackson BP (2005) Species- and stage-specific differences in trace element tissue concentrations in amphibians: implications for the disposal of coal-combustion wastes. Environ Pollut 136:353–363

Schuytema GS, Nebeker AV, Griffis WL, Wilson KN (1991) Teratogenesis, toxicity and bioconcentration in frogs exposed to Dieldrin. Arch Environ Cont Tox 21:332–350

Storm GL, Fosmire GJ, Bellis ED (1994) Persistence of metals in soil and selected vertebrates in the vicinity of the Palmerton zinc smelters. J Environ Qual 23:508–514

Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL (2004) Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786

Unrine JM, Hopkins WA, Romanek CS, Jackson BP (2007) Bioaccumulation of trace elements in omnivorous amphibian larvae: implications for amphibian health and contaminant transport. Environ Pollut 149:182–192

Vitt LJ, Caldwel JP, Wilbur HM, Smith DC (1990) Amphibians as harbingers of decay. Bioscience 40:418

Wilmer P, Stone G, Johnston I (2000) Environmental physiology of animals. Blackwell Science, Oxford