Levels of organochlorine pesticides and polychlorinated biphenyls in the critically endangered Iberian lynx and other sympatric carnivores in Spain

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a b s t r a c t

Accumulation of organochlorine compounds is well studied in aquatic food chains whereas little information is available from terrestrial food chains. This study presents data of organochlorine levels in tissue and plasma samples of 15 critically endangered Iberian lynx (*Lynx pardinus*) and other 55 wild carnivores belonging to five species from three natural areas of Spain (Doñana National Park, Sierra Morena and Lozoya River) and explores their relationship with species diet. The Iberian lynx, with a diet based on the consumption of rabbit, had lower PCB levels (geometric means, plasma: <0.01 ng mL^{-1}, liver: 0.4 ng g^{-1} wet weight, fat: 87 ng g^{-1} lipid weight) than other carnivores with more anthropic and opportunistic foraging behavior, such as the red fox (*Vulpes vulpes*; plasma: 1.11 ng mL^{-1}, liver: 459 ng g^{-1}, fat: 1984 ng g^{-1}), or with diets including reptiles at higher proportion, such as the Egyptian mongoose (*Herpestes ichneumon*; plasma: 7.15 ng mL^{-1}, liver: 216 ng g^{-1}, fat: 540 ng g^{-1}), or the common genet (*Genetta genetta*; liver: 466 ng g^{-1}, fat: 3854 ng g^{-1}). Chlorinated pesticides showed interspecific variations similar to PCBs. Organochlorine levels have declined since the 80s in carnivores from Doñana National Park, but PCB levels are still of concern in Eurasian otters (*Lutra lutra*; liver: 3873–5426 ng g^{-1}) from the industrialized region of Madrid.

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1. Introduction

The accumulation of persistent organic pollutants such as organochlorine pesticides and polychlorinated biphenyls (PCBs) in terrestrial food chains is less studied than in aquatic environments, largely because levels tend to be lower and concern for exposure is less in terrestrial than for aquatic predators (Larsson et al., 1990; Mason and Wren, 2001; Rice et al., 2003). However, terrestrial predators can also be on the top of aquatic food chains due to their foraging plasticity and to the interfaces between terrestrial and aquatic environments. Many species of carnivores are highly opportunistic in their choice of food, taking what is most readily available at any particular season (Mason and Wren, 2001). As an example, Christensen et al. (2005) estimated that salmon deliver 70% of all organochlorine pesticides and 90% of PCBs found in salmon-eating grizzly bears (*Ursus arctos horribilis*), thereby inextricably linking these terrestrial predators to contaminants from the North Pacific Ocean.

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But the dependence on aquatic food chains is not the only factor representing a high risk of exposure to organochlorine compounds. In birds of prey, the biomass of birds in their diet greatly determines the accumulation of organochlorine compounds in their eggs and tissues, at intraspecific (Mañosa et al., 2003) and interspecific level (van Drooge et al., 2008). Similar to raptorial birds, carnivore mammals show a high diversity of diets, ranging from those more specialized on chasing birds, mammals, fish, reptiles or other animals to the most opportunistic ones that, depending on the abundance of each resource, can show temporal or spatial variations on their use (Dip et al., 2003; Christensen et al., 2005).

Organochlorine pollutants, including dieldrin and PCBs, have played a role in the decline of some species of aquatic carnivores such as American mink (*Neovison vison*), American otter (*Lutra canadensis*) and Eurasian otter (*Lutra lutra*) (Giesy et al., 1994; Heaton et al., 1995; Murk et al., 1998; Harding et al., 1999; Mason and Wren, 2001). Furthermore, the decrease of the concentrations of these contaminants in biota (Castrillon et al., 2010) has been accompanied with a recovery of the populations of these predators (Mason, 1998) and the success of some reintroductions has been only accomplished after assessing PCBs levels in potential prey to minimize predator exposure (Mateo et al., 1999). Other species at risk of the adverse effects of PCBs have been mustelids inhabiting aquatic ecosystems or the border of these with the terrestrial habitats. These include European mink (Mustela lutreola) and polecat (Mustela putorius) (Leonards et al., 1994; López-Martín et al., 1994). Terrestrial mustelids have been less affected by PCBs because they are exposed to lower concentrations through their diet than aquatic carnivores (Mason and Wren, 2001), although their sensitivity can be similar (Leonards et al., 1998). Other families of terrestrial carnivores such as canids and felids have received little attention (Hernández et al., 1985; González-Barros et al., 1997; Shore et al., 2001; Dip et al., 2003).

The Iberian lynx (Lynx pardinus) is, according to the World Conservation Union (IUCN), the most endangered felid species in the world, with only two isolated populations inhabiting two areas in southern Spain (namely Doñana and Sierra Morena), where only 40–50 and 150–200 individuals, respectively, are estimated to remain (Ferreras et al., 2010). The Iberian lynx apparently experienced one or several Pleistocene demographic bottlenecks which acted to reduce levels of mitochondrial sequence variation and levels of microsatellite size variation in comparison to most other felid species (Johnson et al., 2004). Further, inbreeding was proposed as a cause of the loss of effectiveness of the immune system observed in lynx (Peña et al., 2006). Thus, remnant populations of this species might be particularly vulnerable to the effect of environmental stressors such as chemical contaminants. Pollutants may have either direct effects on carnivore populations due to an increase in mortality or a decrease in the reproductive success, or indirect effects due to reduction on food supplies (Mason and Wren, 2001).

This study presents data of organochlorine levels in tissues of Iberian lynx and other five species of wild carnivores sharing habitat with this endangered felid in the wetlands and surrounding woodlands of the Doñana National Park. Moreover, the analysis of some Iberian lynx from the Mediterranean woodlands of Sierra Morena permitted us to study differences associated with the habitat (wetland vs. mountain). Finally, the observed accumulation of organochlorine compounds in the different species have been related to their diet in order to test the hypothesis that diets based on birds would lead to higher accumulation of these contaminants than diets based on mammals as observed in birds of prey.

2. Materials and methods

2.1. Study areas

The Doñana area (South-western Andalusia, Southern Spain, 37°0′N, 6°30′W) comprises about 870 km^2, mostly within the Doñana National and Natural Parks. It is a humid area limited by the Atlantic Ocean to the West, the Guadalquivir River to the East, and crops extending to the North for several kilometers. The area is flat and mostly close to sea level, and the climate is Mediterranean subhumid. Three ecosystem types are predominant: fixed dunes, mobile dunes and marshes. Vegetation in the fixed dunes is a mixture of autochthonous Mediterranean shrubland, pine woodland, and has Eucalyptus camaldulensis plantations in some areas. The Sierra Morena area (Northern Andalusia, 38°13′N, 4°10′W) includes two contiguous protected Natural Parks and several private hunting estates, and comprises an area of 1125 km^2 with elevations between 500 and 1300 m. The climate is also Mediterranean sub-humid with marked seasons, and Mediterranean shrubland is dominant.

2.2. Sampling

From 2004 to 2006, blood samples were obtained from of 6 Iberian lynx, 6 Egyptian mongooses (Herpestes ichneumon) and 12 red foxes (Vulpes vulpes) trapped alive in Doñana. Blood samples were also obtained from another five Iberian lynx live-trapped in Sierra Morena. Blood was collected from the cephalic or the jugular vein into heparinized tubes. Then, the sample was stored at 4 °C until centrifugation to separate the plasma, where organochlorine concentrations were determined. Plasma was stored at −20 °C until analysis.

From 2004 to 2006, we necropsied 51 free-living carnivores: 2 feral cats (Felis silvestris catus), 9 Iberian lynx, 18 red foxes, 14 Egyptian mongooses, 4 common genets (Genetta genetta), 1 European badger (Meles meles) and 3 Eurasian otters (L. lutra). Most of the animals were road-killed, with the exception of 14 foxes that were hunted. The lynx came from Sierra Morena (n = 3) and Doñana (n = 6). All individuals of the other studied species came from Doñana, with the exception of two otters from Lozoya River (Madrid, Central Spain). Animals were sexed, and the age was estimated by tooth wear and an evaluation of facial, body, and pelt features. Only one lynx was younger than 1 year old (about 6 months); for foxes, we sampled nine adults and seven cubs (61 month); all mongooses and genets were adult individuals; and the badger was a juvenile. At necropsy, we carefully took samples of liver and adipose tissue, and stored them at −20 °C until laboratory analyzes. For some animals, adipose tissue was not available. Most of the animals included in this study were analyzed before for heavy metals and metalloids in liver, muscle and bone (Millán et al., 2008).

2.3. Laboratory analysis

Solvents and reagents used were analytical grade or equivalent high purity grade and purchased form Merck (Darmstad, Germany), and Panreac (Montcada i Reixac, Spain). Individual organochlorine standards and mixtures of pesticides and PCBs (Aroclor® 1260, Pesticide-Mix 13 and PCB-Mix 20) and PCB #209 were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Pesticide-Mix 13 includes aldrin, cis-chlordane, trans-chlordane, o,p′-DDE, p,p′-DDE, o,p′-DDD, p,p′-DDD, o,p′-DDT, p,p′-DDT, dieldrin, a-endosulfan, b-endosulfan, endrin, HCB, a-HCH, b-HCH, c-HCH, d-HCH, e-HCH, heptachlor, heptachlor-exo-epoxide, isodrin, methoxychlor, mirex and PCBs #28, #52, #101, #138, #153 and #180. PCB-Mix 20 includes PCBs #28, #31, #52, #77, #101, #105, #118, #126, #128, #138, #153, #156, #169, #170 and #180.

Sample preparation, based on the sulfuric acid clean-up of an n-hexane extraction procedure, has been described and validated previously (Guitart et al., 1990; Mateo et al., 1998, 2004; Mañosa et al., 2003). Briefly, 1 g of liver was homogenized with 9 g of sodium anhydrous sulfate in a mortar. The samples were transferred to TFT screw-capped glass tubes and 20 mL of n-hexane were added. Then, the capped tubes were horizontally shaken for 10 min, sonicated during 5 min and centrifuged at 1000g for 5 min. The extract was transferred to another screw-capped tube and the extraction process was repeated with another 10 mL of n-hexane. Both extracts were pooled in the same tube and 2 mL of sulfuric acid were added, and then slowly shaken for 10 min and centrifuged for 5 min at 1000g. The extract was cleaned-up again 2–4 additional times with 2 mL of sulfuric acid to obtain a clean extract free of fat. Lipid content (%) was estimated as the dry residue of the n-hexane extract obtained with a second aliquot of liver (0.2–1 g). Liver concentrations have been calculated in $ng\ g^{-1}$ of wet weight (w.w.) and lipid weight (l.w.). Mean lipid content of the species was used to calculate concentrations in l.w. for 14 samples without the sufficient amount to calculate lipid content. Adipose tissue (0.4 g) was directly extracted in a glass homogenizer three times with 5 mL of n-hexane and cleaned-up four times with 3 mL of sulfuric acid. The mass of non-extracted connective tissue left in the glass homogenizer was weighed and subtracted to give concentrations in lipid weight (l.w.). In the case of plasma, 0.2 mL were directly extracted with 1.5 mL of n-hexane twice, and the clean-up was done twice with 0.4 mL of sulfuric acid. The internal standard (10 μL of PCBs #209 at working solutions of 1 ng μL^{-1} for adipose tissue, 50 pg μL^{-1} for liver and 25 pg μL^{-1} for plasma) was added at the beginning of the extraction process. Blanks were processed among samples to assure quality of analyzes. The cleaned-up extracts were concentrated with a rotary evaporator and adjusted to a final volume of 0.5 mL of n-hexane.

Chromatographic analyzes were carried out on an Agilent Technologies 6890 N series equipped with a 30 m fused silica capillary column of 0.32 mm ID and 0.25 μm of film thickness (HP-5 from J&W Scientific, USA), coupled to an electron capture detector (ECD), in conditions optimized for the analytes. The oven initial temperature was 145 °C, then raised to 275 °C at a rate of 2.5 °C min^{-1}. Injector and detector temperature were 290 °C and 310 °C, respectively. Carrier gas (He) was set at an average velocity of 52 cm s^{-1}. Make-up gas (N_2) was adjusted at a flow of 30 mL min^{-1}.

Organochlorine pesticides and PCB congeners were identified by their retention time and using the internal standard (PCB #209) to localize and quantify the peaks. Quantification of organochlorine pesticides was done with calibration curves prepared with Pesticide-Mix 13. Individual PCBs with the corrected (Guitart et al., 1993) numbering of the Ballsmitter and Zell system were quantified with calibration curves of the individual congeners present in PCB-Mix 20. Moreover, other PCB congeners present in the commercial mixture Aroclor 1260 were also quantified according to the mass composition reported by Schultz et al. (1989). Concentrations of individual PCB congeners was given for those present in PCB-Mix 20. Sum of PCBs was reported for those present in PCB-Mix 20 (ΣPCB_{20}) and Aroclor 1260 mixtures (ΣPCB_{1260}). Composition in percentage was also reported for PCBs present in Aroclor 1260. The recovery of the method was calculated with plasma samples spiked with Pesticide-Mix 13 at four different concentrations (n = 3 per concentration level). Except for cyclodienes such as endrin and dieldrin that are completely lost in the clean-up step, the recovery of all analyzed compounds ranged from 79.1% to 107.5%. Recoveries with liver and adipose tissue samples with this method were >74% (Mateo et al., 1998). Corrections based on recovery data were not taken into account for quantification. Detection limits of pesticides and PCB congeners, calculated as 3SD of peak integrations obtained from blanks, were all <0.01 ng mL^{-1} or ng g^{-1}.

As an approach to estimate potential toxicity of accumulated PCBs in liver and adipose tissue, we converted tissue concentrations of dioxin-like PCBs present in PCB-Mix 20 into toxic equivalents (TEQs). These dioxin-like PCBs were #105, #118, #126, #156 and #169. Toxic equivalency factors (TEFs) were retrieved from Van den Berg et al. (2006) and multiplied by the concentration of each corresponding congener to obtain TEQs. These TEFs were 0.1 for #126, 0.03 for #169 and 0.00003 for #105, #118 and #156.

2.4. Statistical analysis

Organochlorine concentrations and TEQs were $\log_{10}$-transformed to approach a normal distribution. Then, differences among species were studied with generalized linear models with normal distribution and identity link function. Sex and age were initially included as factors in the model and later removed from the final model if they were non-significant. The effect of location in the case of Iberian lynx was studied and it was not considered if it was non-significant. The residuals of the final model were tested for normality with Kolmogorov–Smirnov test. Tukey test was used to establish differences between pairs of species. Composition of the mixture of PCB congeners expressed in terms of percentage of the total concentration was also compared between the species with largest sample size (i.e., red fox and mongoose). This comparison was performed with a multivariate analysis of the variance (Wilk's lambda value) of all the PCB congeners with detectable concentrations as dependent variables, species as a factor, and inter-subject tests to detect the significance of each specific congener. This multivariate analysis was also used to detect differences in PCB composition between liver and adipose tissue in Egyptian mongoose or fox. The relationships between the most abundant organochlorine compound (p,p′-DDE and PCBs) concentrations in different tissues or between different compounds in each tissue were studied by Pearson's correlations with all the data and separately with the species with largest sample size. Pearson's correlations were also used to test for relationships between organochlorine compound concentrations (w.w. and l.w.) and percent biomass in diet of each group of prey (i.e., mammals, birds, reptiles, amphibians, fishes, invertebrates and plants). The information on diet composition was retrieved from the literature (Fedriani, 1996; Fedriani et al., 1998; Revilla and Palomares, 2002; Blanco-Garrido et al., 2008; Medina et al., 2008; Millán, 2010; Ferreras et al., 2011). Statistical significance was set at $p < 0.05$. Statistical analyzes were performed with the software IBM SPSS Statistics version 19.0.0.

3. Results

3.1. Differences among species and locations

Organochlorine levels in plasma, liver and adipose tissue of Iberian lynx did not differ between Doñana and Sierra Morena, so the location was not considered for further comparisons with the other carnivore species. Sex and age had no effect on the organochlorine concentrations in the studied samples. Plasma levels of p,p′-DDE and ΣPCB_{20} differed among species ($p = 0.046$ and $p = 0.003$; respectively). Egyptian mongoose showed the highest levels of both types of organochlorine compounds in plasma (Table 1). With the exception of mirex, liver levels of all the detected pesticides, ΣPCB_{20} and ΣPCB_{1260} differed among species (all $p \leq 0.004$). Common genet and Eurasian otter showed the highest levels of different organochlorine compounds in liver (Table 2). Differences among species were also detected for these compounds when liver concentrations were expressed in l.w. (all $p \leq 0.041$). Differences

Table 1
Arithmetic mean (±SE) and geometric mean (maximum) concentrations (ng mL^{-1}) of organochlorine compounds in plasma of carnivores from Spain.

Compound	Iberian lynx		Red fox	Egyptian mongoose
	Doñana n = 6	S. Morena n = 5	Doñana n = 12	Doñana n = 6
p,p′-DDE	1.33 ± 0.48 0.03^{b} (2.83)	0.31 ± 0.31	<0.01 <0.01^{a}	4.23 ± 3.17 0.02^{ab} (19.2)
PCB 52	<0.01 <0.01 (2.67)	0.53 ± 0.53	0.86 ± 0.50 <0.01 (5.41)	<0.01 <0.01
PCB 101	<0.01 <0.01	<0.01	0.26 ± 0.18 <0.01 (1.62)	<0.01 <0.01
PCB 153	<0.01 <0.01	<0.01	2.55 ± 2.14 0.01 (25.9)	7.77 ± 3.60 0.39 (21.5)
PCB 138	<0.01 <0.01	<0.01	0.41 ± 0.41 <0.01 (4.97)	1.43 ± 0.92 0.02 (4.93)
PCB 180	0.42 ± 0.27 0.01 (1.45)	0.29 ± 0.29	3.60 ± 1.93 0.36 (24.0)	6.22 ± 2.35 3.90 (15.2)
ΣPCB_{20}	0.42 ± 0.27 0.01^{a} (4.11)	0.82 ± 0.82	7.68 ± 4.36 1.11^{b} (54.8)	15.42 ± 6.42 7.15^{b} (35.7)

Geometric means with the same superscript letter do not differ significantly. Differences not shown for individual congeners of PCBs. Geometric means for lynx are shown for all the animals, because no differences were found for location and statistical analyzes were conducted with these values.

Table 2
Arithmetic mean (±SE) and geometric mean (maximum) concentrations (ng g^{-1} w.w.) of organochlorine compounds in liver of carnivores from Spain.

Compound	Iberian lynx		Feral cat	Red fox	Egyptian mongoose	Common genet	Eurasian otter	European badger
	Doñana n = 3	S. Morena n = 2	Doñana n = 2	Doñana n = 18	Doñana n = 14	Doñana n = 4	Spain n = 3	Doñana n = 1
Lipid%[a]	4.70 ± 1.80 (2)		4.37 ± 0.43 (2)	3.97 ± 0.47 (12)	3.62 ± 0.97 (11)	3.14 ± 1.86 (3)	10.84 ± 2.27 (2)	2.11 (1)
HCB	0.04 ± 0.04	<0.01	0.43 ± 0.31	0.79 ± 0.36	0.08 ± 0.03	0.43 ± 0.11	28.6 ± 18.1	<0.01
	<0.01[a] (0.13)		0.30[ab](0.74)	0.16[ab] (6.62)	0.01[a] (0.41)	0.38[ab] (0.66)	14.9[ab] (63.4)	
b-HCH	0.27 ± 0.14	1.12 ± 0.52	0.23 ± 0.23	0.42 ± 0.16	<0.01	1.06 ± 0.47	1.13 ± 0.57	0.48
	0.17[b] (1.64)		0.02[ab] (0.46)	0.08[ab] (2.43)	<0.01[a]	0.72[b] (2.36)	0.14[b] (1.81)	
a-Heptachlor epoxide	<0.01	<0.01	<0.01	<0.01[a]	<0.01	<0.01	1.45 ± 1.12	<0.01
	<0.01[a]		<0.01[a]	<0.01[a]	<0.01[a]	<0.01[ab]	0.14 [b]	
b-Heptachlor epoxide	<0.01	0.24 ± 0.24	<0.01	<0.01	<0.01	0.12 ± 0.12	0.51 ± 0.33	<0.01
	<0.01[a] (0.48)		<0.01[a]	<0.01[a]	<0.01[a]	<0.01[ab] (0.48)	0.08[b] (1.14)	
p,p′-DDE	55.1 ± 29.6	25.9 ± 18.4	37.3 ± 37.3	4.1 ± 1.327	19.3 ± 6.9	754 ± 661	130 ± 66	36.3
	28.6[ab] (112)		0.27[ab] (74.6)	0.14[a] (16.5)	1.99[ab] (78.2)	216[b] (2736)	47.5[ab] (226)	5
p,p′-DDD	<0.01	<0.01	0.93 ± 0.93	1.43 ± 0.50	7.72 ± 5.37	33.9 ± 15.5	24.5 ± 11.2	84.2
	<0.01[a]		0.04[ab] (1.86)	0.07[ab] (7.95)	0.06[a] (72.3)	2.72[ab] (64.1)	15.8[b] (41.3)	
Mirex	0.56 ± 0.56	2.37 ± 2.37	1.79 ± 1.79	1.40 ± 0.36	1.70 ± 0.65	0.07 ± 0.07	<0.01	<0.01
	0.02 (4.75)		0.06 (3.58)	0.14 (4.28)	0.08 (7.64)	<0.01 (0.27)	<0.01	
PCB 28–31	2.17 ± 1.31	1.58 ± 0.46	3.55 ± 1.97	3.31 ± 0.95	3.08 ± 0.83	2.40 ± 1.04	0.55 ± 0.24	1.87
	1.51 (4.79)		2.95 (5.52)	1.52 (17.2)	1.72 (9.66)	1.64 (4.85)	0.43 (0.97)	
PCB 101	<0.01	<0.01	2.18 ± 1.27	2.01 ± 0.55	0.92 ± 0.36	3.55 ± 2.96	3.99 ± 3.36	<0.01
	<0.01		1.77 (3.45)	0.19 (8.7)	0.23 (4.71)	0.27 (13.4)	0.24 (10.7)	
PCB 118	1.62 ± 0.94	7.04 ± 3.11	14.1 ± 13.8	6.60 ± 2.11	4.69 ± 1.49	3.13 ± 2.17	127 ± 46	1.09
	0.73 (10.2)		3.09 (27.9)	1.10 (30.5)	2.94 (20.5)	1.51 (9.59)	103 (188)	
PCB 153	18.9 ± 7.8	4.5 ± 3.3	42.5 ± 32.6	251.5 ± 83.8	85.4 ± 45.6	535 ± 504	453 ± 108	12.3
	8.1 (32.8)		27.2 (75.1)	61.7 (978)	34.5 (658)	60.7 (2047)	421 (566)	
PCB 105	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	25.2 ± 14.0	<0.01
	<0.01		<0.01	<0.01	<0.01	<0.01	1.09 (48.5)	
PCB 138	6.7 ± 2.6	2.1 ± 1.5	21.2 ± 16.9	117 ± 36.9	25.9 ± 17.2	627 ± 613	444 ± 139	23.5
	3.1 (10.3)		12.7 (38.1)	32.5 (546)	7.9 (248)	44.4 (2467)	392 (671)	
PCB 126	<0.01	<0.01	<0.01	0.10 ± 0.10	<0.01	<0.01	2.17 ± 1.69	<0.01
	<0.01		<0.01	<0.01 (1.75)	<0.01	<0.01	0.18 (5.49)	
PCB 128	0.21 ± 0.21	<0.01	0.21 ± 0.21	4.90 ± 2.20	0.45 ± 0.33	0.96 ± 0.62	18.9 ± 9.5	0.21
	<0.01 (0.63)		0.02 (0.41)	0.11 (35.7)	<0.01 (4.42)	0.04 (2.60)	12.4 (35.9)	
PCB 156	0.68 ± 0.41	0.38 ± 0.15	3.25 ± 3.01	9.99 ± 3.76	5.51 ± 2.96	25.7 ± 24.0	58.6 ± 21.7	0.87
	0.16 (1.41)		1.23 (6.27)	0.49 (47.5)	2.06 (43.0)	2.79 (97.6)	49.3 (97.0)	
PCB 180	23.9 ± 8.7	4.5 ± 2.9	33.0 ± 16.7	643.0 ± 189	127 ± 60	579 ± 5343	492 ± 237	25.1
	10.2 (41.1)		28.5 (49.7)	206.6 (2574)	59.8 (878)	81.4 (2179)	395 (964)	
PCB 169	<0.01	<0.01	<0.01	0.23 ± 0.16	<0.01	<0.01	<0.01	<0.01
	<0.01		<0.01	<0.01 (2.87)	<0.01	<0.01	<0.01	
PCB 170	4.2 ± 4.2	4.7 ± 4.7	16.4 ± 10.6	224 ± 79	14.8 ± 11.2	224 ± 209	288 ± 165	<0.01
	0.1 (12.5)		12.5 (27.0)	35.2 (1150)	0.1 (159)	0.5 (849)	210 (616)	
RPCB$_{20}$	58 ± 22	25 ± 15	136 ± 93	1262 ± 378	267 ± 138	2001 ± 1890	1914 ± 666	65
	34[a] (101)		100[ab] (229)	466[ab] (4801)	117[ab] (2026)	219[ab] (7667)	1662[b] (3113)	
RTEQ$_{20}$[b]	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.5	17.2 ± 11.4	0.3 ± 0.1	0.9 ± 0.8	223 ± 169	<0.1
	0.1[b] (0.3)		0.4[b] (1.0)	1.6[b] (193)	0.2[b] (1.9)	0.5[b] (3.2)	82[a] (557)	
RPCB$_{1260}$	101 ± 44	37 ± 28	221 ± 173	1321 ± 402	486 ± 256	2874 ± 2685	3324 ± 1399	213
	51.2[a] (188)		137[ab] (395)	459[ab] (5176)	216[ab] (3765)	466[ab] (10928)	2418[b] (5426)	

Geometric means with the same superscript letter do not differ significantly. Differences not shown for individual congeners of PCBs. Geometric means for lynx are shown for all the animals, because no differences were found for location and statistical analyzes were conducted with these values.
[a] Lipid content (%) estimated as the percentage of hexane-extracted lipid was not determined in all the samples, n is shown between parentheses.
[b] RTEQ$_{20}$ expressed as pg g^{-1} w.w.

among species in adipose tissue levels were found for b-heptachlor epoxide, p,p′-DDE, p,p′-DDT, mirex, RPCB$_{20}$ and RPCB$_{1260}$ (all p 6 0.008). Common genet showed the highest levels of several organochlorine compounds in adipose tissue (Table 3). Eurasian otter showed higher RTEQ$_{20}$ liver levels expressed in w.w. or l.w. than the other carnivores ($p < 0.001$ and $p = 0.009$, respectively). No differences were found in RTEQ$_{20}$ levels in adipose tissue of the terrestrial carnivores.

Differences in PCB composition were observed between the two species with largest sample size (red fox and Egyptian mongoose) and between tissues of these species (Fig. 1). Levels of PCB congeners expressed in percentage showed differences between species in adipose tissue ($p = 0.046$) and liver ($p = 0.015$). Among these differences between species in adipose tissue (Fig. 1), it was remarkable a higher percentage of #153–132 (co-eluted congeners in order of abundance in Aroclor 1260) in Egyptian mongoose and a higher percentage of #190–170 in red fox. In liver, a higher value of #187 was observed in Egyptian mongoose ($p < 0.001$) and higher values of #180 ($p = 0.006$) and #190–170 ($p < 0.001$) were found in red fox. Comparing tissues within species, differences in overall PCB composition were only marginally significant in Egyptian mongoose ($p = 0.06$), especially due to the higher percentage of #153–132 in adipose tissue and the higher percentage of #187 in liver (both $p < 0.001$; Fig. 1).

3.2. Relationships among tissues and compounds

Organochlorine concentrations in liver (w.w.) and adipose tissue (l.w.) were correlated for p,p′-DDE ($r = 0.45$, $p = 0.006$), RPCB$_{20}$ ($r = 0.84$, $p < 0.001$) and RPCB$_{1260}$ ($r = 0.75$, $p < 0.001$) in the total sample of carnivores. When liver concentrations were expressed in l.w., these correlations were also significant ($r = 0.41$, $p = 0.013$; $r = 0.84$, $p < 0.001$; $r = 0.72$, $p < 0.001$; respectively). On the contrary, concentrations of p,p′-DDE and RPCB were not correlated

Table 3
Arithmetic mean (±SE) and geometric mean (maximum) concentrations (ng g^{-1} l.w.) of organochlorine compounds in adipose tissue of carnivores from Spain.

	Iberian lynx		Feral cat	Red fox	Egyptian mongoose	Common genet	European badger
	Doñana n = 3	S. Morena n = 3	Doñana n = 2	Doñana n = 16	Doñana n = 10	Doñana n = 4	Doñana n = 1
HCB	1.61 ± 0.43	2.29 ± 0.87	5.13 ± 2.98	5.51 ± 1.34	2.91 ± 0.34	6.80 ± 1.65	1.27
	1.71 (3.92)		4.18 (8.12)	2.67 (23.2)	2.67 (4.19)	6.07 (10.6)	
a-HCH	<0.01	<0.01	<0.01	0.28 ± 0.17	<0.01	0.80 ± 0.80	<0.01
	<0.01		<0.01	<0.01 (2.56)	<0.01	<0.01 (3.20)	
b-HCH	4.29 ± 1.19	6.34 ± 4.71	3.13 ± 0.65	27.40 ± 11.39	2.55 ± 0.51	25.61 ± 15.87	17.1
	1.23 (15.5)		3.06 (3.78)	5.97 (136)	0.61 (4.51)	11.52 (70.5)	
c-HCH	<0.01	<0.01	0.60 ± 0.60	0.60 ± 0.22	1.05 ± 0.36	1.27 ± 1.05	<0.01
	<0.01		0.03 (1.20)	0.02 (2.86)	0.08 (3.15)	0.04 (4.36)	
a-Heptachlor epoxide	0.45 ± 0.45	<0.01	0.68 ± 0.68	0.44 ± 0.29	0.58 ± 0.27	1.21 ± 1.21	<0.01
	<0.01 (1.34)		0.04 (1.36)	<0.01 (4.05)	0.02 (2.45)	0.01 (4.83)	
b-Heptachlor epoxide	2.89 ± 1.47	4.57 ± 2.39	<0.01	0.37 ± 0.37	<0.01	2.62 ± 0.55	<0.01
	0.31[ab] (8.05)		<0.01[ab]	<0.01[ab] (5.90)	<0.01[ab]	2.42[ab] (3.93)	
p,p′-DDE	455 ± 229	740 ± 234	1079 ± 922	100 ± 31	276 ± 125	17 531 ± 15 260	738
	483[ab] (1096)		560[ab] (2001)	34[ab] (506)	171[ab] (1394)	4969[ab] (63 297)	
p,p′-DDD	<0.01	<0.01	3.35 ± 3.35	0.05 ± 0.05	1.67 ± 1.67	3.48 ± 3.48	13.18
	<0.01		0.08 (6.70)	<0.01 (0.88)	<0.01 (16.7)	0.01 (13.9)	
p,p′-DDT	<0.01	<0.01	16.31 ± 13.77	0.47 ± 0.33	3.29 ± 0.61	4.20 ± 4.20	9.86
	<0.01[ab]		8.76[ab] (30.1)	<0.01[ab] (4.66)	1.43[ab] (6.83)	0.01[ab] (16.8)	
Mirex	<0.01	<0.01	2.89 ± 2.89	0.81 ± 0.35	7.36 ± 1.70	1.55 ± 0.92	<0.01
	<0.01[ab]		0.08[ab] (5.79)	0.02[ab] (4.76)	5.91[ab] (17.3)	0.23[ab] (4.11)	
PCB 28–31	0.43 ± 0.07	2.11 ± 1.71	2.46 ± 2.17	1.59 ± 1.02	0.73 ± 0.16	1.48 ± 1.10	4.76
	0.64 (5.52)		1.16 (4.63)	0.40 (16.8)	0.37 (1.58)	0.18 (4.70)	
PCB 101	1.9 ± 1.9	9.4 ± 9.4	72.0 ± 35.3	18.8 ± 4.7	13.9 ± 5.3	20.8 ± 16.0	<0.01
	0.1 (28.2)		62.8 (107)	2.6 (67.8)	0.6 (35.5)	0.9 (68)	
PCB 118	12.2 ± 5.2	16.1 ± 1.4	23.6 ± 11.9	44.8 ± 13.1	22.4 ± 5.6	12.7 ± 10.7	37
	12.8 (22.5)		20.4 (35.5)	31.1 (228)	18.8 (70.2)	4.2 (44.9)	
PCB 153	124 ± 90	62 ± 3	323 ± 262	1215 ± 411	340 ± 104	3240 ± 2827	354
	65 (305)		188 (585)	426 (5376)	264 (1239)	904 (11 717)	
PCB 105	<0.01	<0.01	2.76 ± 2.76	<0.01	<0.01	<0.01	<0.01
	<0.01		0.07 (5.52)	<0.01)	<0.01	<0.01	
PCB 138	50.9 ± 39.0	24.0 ± 5.8	158.6 ± 129.6	313.6 ± 111.8	56.9 ± 21.0	1980.8 ± 1850.0	40.7
	24.2 (129)		91.5 (288)	94.8 (1508)	41.0 (242)	343.6 (7530)	
PCB 126	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01		<0.01	<0.01	<0.01	<0.01	
PCB 128	0.23 ± 0.23	0.14 ± 0.14	11.53 ± 8.63	17.71 ± 6.91	1.10 ± 0.53	0.07 ± 0.07	1.56
	0.01 (0.68)		7.65 (20.2)	3.22 (93.5)	0.11 (4.34)	<0.01 (0.29)	
PCB 156	2.87 ± 1.41	3.19 ± 1.61	10.69 ± 8.25	23.15 ± 9.05	13.30 ± 4.17	189.80 ± 161.27	29.73
	2.28 (6.41)		6.80 (18.9)	1.74 (110)	10.02 (48.4)	57.54 (673)	
PCB 180	113.1 ± 85.5	62.3 ± 3.6	238.3 ± 187.4	1719.0 ± 486.1	214.7 ± 49.0	3343.1 ± 2902.8	261.2
	60.1 (284)		147.3 (426)	664.9 (5731)	179.9 (612)	483.8 (12 037)	
PCB 169	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	<0.01		<0.01	<0.01	<0.01	<0.01	
PCB 170	53.5 ± 42.6	25.4 ± 2.8	130.2 ± 88.4	994.3 ± 281.8	53.3 ± 17.0	1358.9 ± 1166.6	131
	25.2 (139)		95.7 (219)	444.5 (3487)	40.1 (198)	392.8 (4856)	
RPCB$_{20}$	282 ± 266	202 ± 17	973 ± 736	4348 ± 1273	716 ± 202	10 147 ± 8932	860
	199[a] (891)		636[ab] (1709)	1941[ab] (16 010)	571 [ab] (2446)	2506[b] (36 927)	
RTEQ$_{20}$[a]	0.5 ± 0.3	0.6 ± 0.1	1.1 ± 0.7	2.0 ± 0.5	1.1 ± 0.3	6.1 ± 5.2	2.0
	0.5 (0.8)		1.0 (1.8)	1.6 (6.8)	0.9 (3.6)	2.5 (21.5)	
RPCB$_{1260}$	510 ± 398	259 ± 29	1055 ± 917	4579 ± 1318	789 ± 292	17 326 ± 15 632	924
	253[a] (1307)		520[ab] (1972)	1984[b] (16 144)	540[ab] (3314)	3854[b] (64 209)	

Geometric means with the same superscript letter do not differ significantly. Differences not shown for individual congeners of PCBs. Geometric means for lynx are shown for all the animals, because no differences were found for location and statistical analyzes were conducted with these values.
[a] RTEQ$_{20}$ expressed as pg g^{-1} l.w.

within any of the studied tissues. However, a detailed study of the relationship between p,p′-DDE and RPCB levels in adipose tissue of the different species revealed a significant correlation if we exclude red fox from the analysis (p,p′-DDE with RPCB$_{20}$: $r = 0.73$, $p < 0.001$; p,p′-DDE with RPCB$_{1260}$: $r = 0.66$, $p = 0.001$; Fig. 2). These correlations between p,p′-DDE and RPCB were not observed in liver or plasma.

3.3. Relationship between organochlorine levels and diet

The percentages of biomass of mammals, birds, amphibians, fishes, invertebrates or plants in the diet (Table 4) were not significantly correlated with the mean level of organochlorine compounds in liver or adipose tissue of the different carnivore species. Only the percentage of biomass of reptiles was associated with the arithmetic mean level of RPCB$_{1260}$ in liver expressed in both w.w. and l.w. (w.w.: $n = 7$, $r = 0.794$, $p = 0.033$; l.w.: $r = 0.851$, $p = 0.015$; Fig. 3). This relationship was marginally significant for p,p′-DDE in liver expressed in w.w. ($n = 7$, $r = 0.705$, $p = 0.077$), RPCB$_{1260}$ in adipose tissue ($n = 6$, $r = 791$, $p = 0.061$) and p,p′-DDE in adipose tissue ($n = 6$, $r = 0.8$, $p = 0.056$).

4. Discussion

Organochlorine concentrations in Iberian lynx were much lower than in other carnivore species from the same locations. Although the population from Doñana National Park could be more at risk because it inhabits in the border of an aquatic ecosystem, Iberian

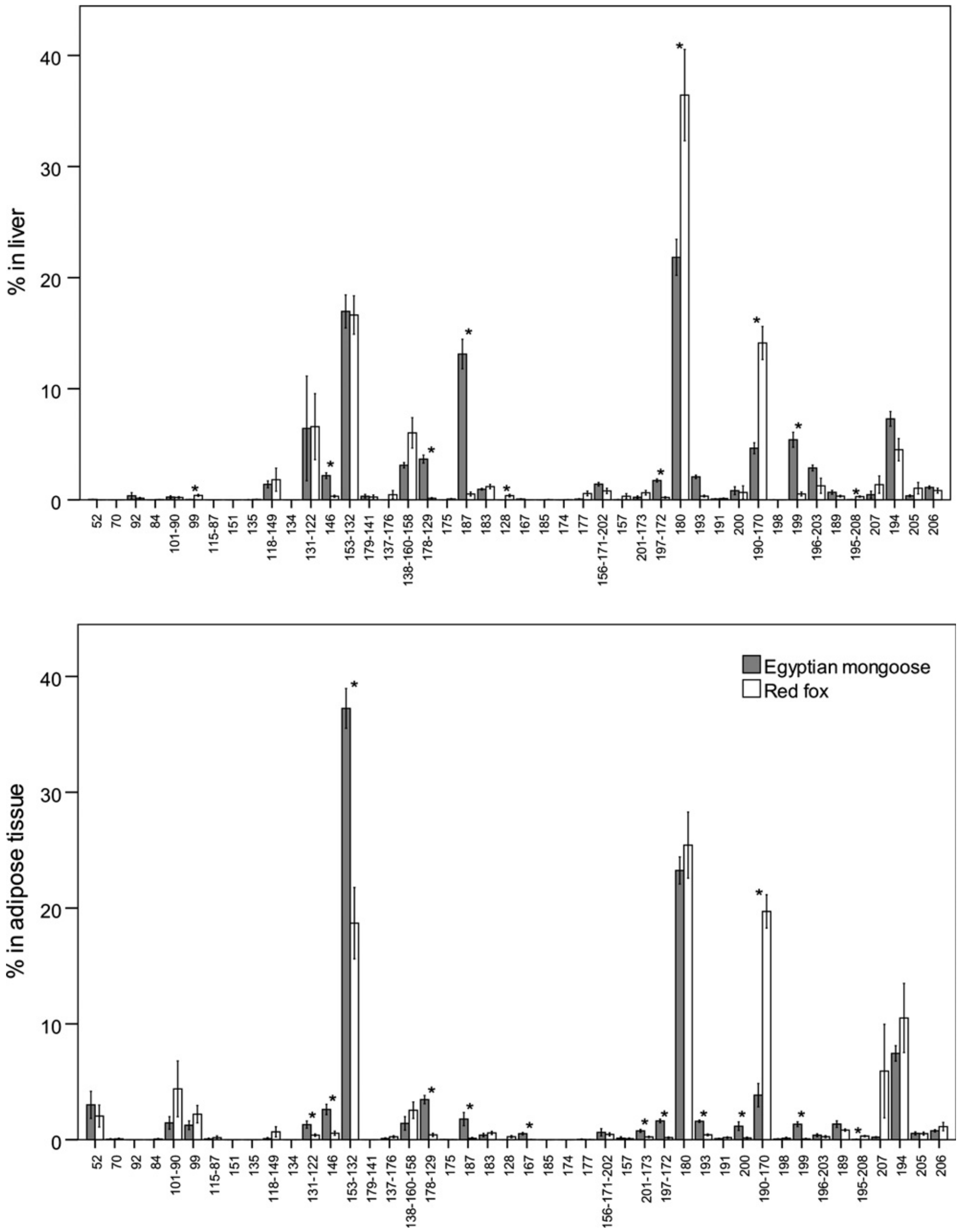

Fig. 1. Comparison of PCB pattern (mean % ±SE) in liver and adipose tissue of Egyptian mongoose and red fox from Doñana National park. Asterisks denote differences in specific PCB congeners.

lynx was less exposed to organochlorine compounds than aquatic carnivores such as the Eurasian otter and levels were similar than in Sierra Morena.

Aquatic carnivores have been among the species more affected by PCBs. Laboratory studies with American mink exposed to commercial mixtures of PCBs or contaminated fish have produced reproductive failure and lethality in animals with 0.87–1.2 and >4 μg g^{-1} w.w. of ΣPCBs in liver, respectively (Ringer et al., 1972; Aulerich et al., 1973; Platonow and Karstad, 1973). In other experimental studies, reproductive impairment has been observed in mink with ΣPCBs in adipose tissue at 13.3 μg g^{-1} w.w. (Hornshaw et al., 1983) or 86 μg g^{-1} l.w. (Jensen et al., 1977). In reference to this information, Kamrin and Ringer (1996) concluded that liver ΣPCB levels >4 μg g^{-1} w.w. were associated with lethality in mink and that reproductive impairment occurred when ΣPCB concentration was >10 μg g^{-1} w.w. in fat and 1 μg g^{-1} w.w. in liver. Based on different experimental studies, Zwiernik et al. (2008) have considered 50–78 pg TEQ g^{-1} w.w. in liver as the no-observable-adverse effect concentration (NOAEC) and 190 pg TEQ g^{-1} w.w. in liver as the lowest-observable-adverse-effect concentration (LOAEC). If we assume a similar sensitivity in other carnivore species, values of reproductive impairment and lethality were exceeded in Eurasian otters in the present study (geometric mean ΣPCB_{1260}: 2.4 μg g^{-1} w.w. in liver). Two of these otters were from the industrialized region of Madrid (Lozoya River) and contained elevated ΣPCB_{1260} levels in liver (3.87 and 5.43 μg g^{-1} w.w.), while the otter from Doñana showed a lower concentration (0.67 μg g^{-1} w.w.). One of the otters from Madrid showed 557 pg g^{-1} w.w. ΣTEQ_{20} in liver, which is above the cited LOAEL even though we have not determined levels of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofuranes (PCDFs). However, it is known that American mink may be more sensitive to PCBs than other wild carnivores and laboratory results with this species may not be extrapolated to others (Mason and Wren, 2001; Beckett et al., 2008;

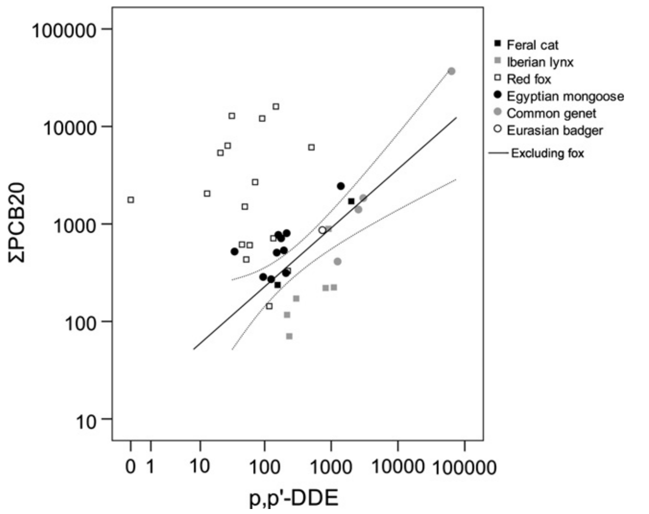

Fig. 2. Relationship between p,p'-DDE and RPCB$_{20}$ in adipose tissue of carnivores from Doñana National Park and Sierra Morena. The correlation was significant when red fox was excluded from the analysis ($r = 0.73$, $p < 0.001$).

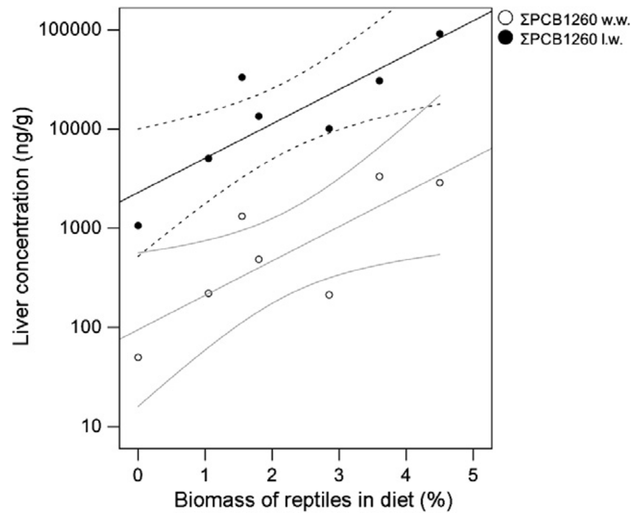

Fig. 3. Relationship between liver RPCB$_{1260}$ levels (arithmetic mean) and the presence of reptiles in the diet of seven species of carnivores from Spain. The correlations were significant for RPCB$_{1260}$ concentrations expressed in w.w. ($r = 0.794$, $p = 0.033$) and l.w. ($r = 0.851$, $p = 0.015$).

Guertin et al., 2010). In the particular case of the Eurasian otter reproductive impairment has been established at 50 lg g^{-1} l.w., independently of the tissue (Mason and Wren, 2001). Otters from Madrid showed 20 and 230 lg g^{-1} l.w. for RPCB$_{1260}$, and therefore one of the individuals was above this level of concern for PCBs. Apart from the otters, the level of 1 lg g^{-1} w.w. in liver was exceeded in some individuals of red fox (7/18), Egyptian mongoose (1/14) and common genet (1/4). Furthermore, the level of 4 lg g^{-1} w.w. in liver was exceeded in some individuals of red fox (2/18) and common genet (1/4). The RPCB level in fat of 10 lg g^{-1} w.w. was exceeded in red fox (3/16) and common genet (1/4).

Organochlorine levels detected in Iberian lynx were lower than those detected by Hernández et al. (1985) in samples collected in Doñana between 1982 and 1983. Then, RPCBs in liver of lynx showed a geometric mean and maximum value of 1.73 and 11.4 lg g^{-1} w.w., respectively. Levels of pesticides were also much higher in the 80s than now with geometric means for p,p'-DDE, p,p'-DDT, a-HCH and c-HCH of 1.53, 0.589, 0.010 and 0.016 lg g^{-1} w.w., respectively. PCB levels in otter have also declined in Doñana, although levels in Madrid were similar to those detected in Doñana in the 80s (geometric mean: 2.45 lg g^{-1} w.w.). The decline of organochlorine levels, including PCBs, has been also observed in top predator marine mammals form the Mediterranean Sea and this may represent the overall trend in Western Europe (Borrell and Aguilar, 2007; Castrillon et al., 2010). American mink from Maryland and Oregon showed PCB levels similar or higher (mean values: 1.4–9.3 lg g^{-1} w.w.; Henny et al., 1981; O'Shea et al., 1981) than the otters from Madrid, and these values were similar to those known to cause reproductive problems in controlled experiments (Mason and Wren, 2001). Other studies of organochlorine burdens in mustelids have expressed concentrations in l.w. of tissue. European mink from northern Spain have shown RPCB levels of 118.3 lg g^{-1} l.w. in muscle and 122.5 lg g^{-1} l.w. in liver (López-Martín et al., 1994) and the levels in Eurasian otters from northeastern Spain ranged from 7.21 to 64.28 lg g^{-1} l.w. (López-Martín and Ruiz-Olmo, 1996).

The terrestrial species with higher PCB levels in the present study was the red fox, exceeding those detected in Germany by Georgii et al. (1994) and Switzerland by Dip et al. (2003), but within the range of levels found in Italy by Corsolini et al. (2000). Although for the rest of the terrestrial species there are no data to compare in the literature, the available information might indicate that Iberian lynx and other carnivores from Doñana were less exposed to RPCBs than other carnivores from industrialized areas in the center and north of Spain, and that there is also a decline in this contamination.

Differences among species for TEQs were limited to liver levels in otters respect to the other species. The comparison of reported TEQs with other published studies is limited by the fact that only PCBs were measured to calculate TEQs. It is known that there is considerable site specific variation in the contribution of various PCB, PCDD and PCDF congeners to measure TEQs depending on pollution sources (Zwiernik et al., 2008). Studies with American mink from Louisiana and South Carolina have estimated that coplanar PCBs ac-

Table 4
Diet of carnivore species in Doñana National Park, expressed in percentage of consumed biomass of each prey group.

Common name	Biomass (%)							References
	Mammal	Bird	Reptile	Amphibian	Fish	Invertebrate	Plant	
Feral cat	91.86	6.29	1.05	0.00	0.00	0.77	0.00	Medina et al. (2008), Millán (2010)
Common genet	63.60	15.70	4.50	5.70	0.00	10.50	0.00	Ferreras et al. (2011)
Iberian lynx	83.80	16.20	0.00	0.00	0.00	0.00	0.00	Ferreras et al. (2011)
Egyptian mongoose	72.80	8.80	1.80	5.70	0.00	11.10	0.00	Ferreras et al. (2011)
Eurasian otter	0.80	1.10	3.60	5.20	81.10	8.20	0.00	Blanco-Garrido et al. (2008)
European badger	39.18	5.87	2.85	4.43	0.00	27.77	19.30	Revilla and Palomares (2002), Fedriani et al. (1998), Ferreras et al. (2011)
Red fox	68.50	13.95	1.55	0.00	0.00	10.10	5.85	Fedriani (1996), Ferreras et al. (2011)

count only for 20–25% of total TEQs (Tansy et al., 2003). Despite of such limitations, the comparison of TEQs between liver and adipose tissues signals the role of liver as a depository organ for coplanar PCBs in wild carnivores. Kunisue et al. (2006) found for Japanese raccoon dogs (Nyctereutes procyonoides) that mean levels of coplanar PCB-TEQs were 0.370 ng g^{-1} l.w. in liver and 0.051 ng g^{-1} l.w. in adipose tissue. Similarly, we have observed higher $RTEQ_{20}$ levels in liver of red fox and common genet (geometric mean: 0.014 ng g^{-1} l.w. both) than in adipose tissue (<0.003 ng g^{-1} l.w., Table 3).

As can be expected for these bioaccumulative non-polar contaminants, organochlorine concentrations were significantly correlated between tissues and the highest levels in liver were found in animals with the highest levels in adipose tissue. However, the correlation between the concentrations of the two major types of residues (p,p′-DDE and RPCBs) was only significant in adipose tissue after excluding red fox from the analysis. This shows some divergence in red fox from the expected association between these two types of global contaminants usually observed in Spanish biota (Guitart et al., 1996; Mateo et al., 1998, 1999, 2004; Mañosa et al., 2003; Piqué et al., 2006). As can be observed in Fig. 2, higher RPCB levels occur in red fox than expected for the correlation between p,p′-DDE and RPCBs observed in the rest of the species. The most feasible explanation for this observation is the higher opportunistic and anthropic foraging behavior of red fox that could increase the risk of exposure to sources of PCBs (Dip et al., 2003). On the other hand, the higher accumulation in red fox of PCB congeners with higher chlorination degree (i.e. #170–190, #180) in comparison with Egyptian mongoose (Fig. 1) may support the hypothesis of a difference on PCB metabolism in red fox and other canids (Georgii et al., 1994).

The study of the relationship between the diet and the organochlorine burden of carnivore species has shown some differences with that observed in birds of prey. Whereas the biomass of birds in the diet of raptors has been a significant determinant of the organochlorine exposure and accumulation in tissues (van Drooge et al., 2008), this relationship was not observed in the carnivore mammals. On the contrary, the biomass of reptiles in their diet was the only item significantly associated with the concentration of organochlorine compounds in their liver expressed both in w.w. or l.w. of tissue (Fig. 3). This relationship suggests that reptiles should accumulate large amounts of organochlorine compounds, especially if we take into account how underrepresented reptiles are in the diet of most of the carnivores (<5%, Table 4), whereas birds may represent up to 100% of raptor diets. Unfortunately, little information exists on organochlorine levels in reptiles from the study area. As far as we are aware, only the common chameleon (Chamaeleo chamaeleon) out of all the reptiles inhabiting the study area has been studied for organochlorine accumulation. Gómara et al. (2007) reported RPCB concentrations in chameleon eggs ranging from 32 to 52 ng g^{-1} w.w., showing a twofold increase in RPCBs with respect to samples collected 4 years before (Díaz-Paniagua et al., 2002). Although the correlations in Fig. 3 were based on a few species and other factors such as interspecific differences in organochlorine metabolism may explain these relationships, other studies have detected very high levels of organochlorine compounds in reptiles and may support the hypothesis of a significant contribution of reptiles in the organochlorine exposure of carnivores. Clark et al. (2000) detected as much as 3 μg g^{-1} w.w. (geometric mean: 0.733 μg g^{-1} w.w.) of p,p′-DDE in blood of diamondback water snake (Nerodia rhombifer) at Old River Slough (Texas, USA), a level equivalent to the maximum concentration found in plasma of peregrine falcons (Falco peregrinus) in 1978–1979 when p,p′-DDE peaked in this sensitive species. Other water snake species like cottonmouth (Agkistrodon piscivorus) showed similar p,p′-DDE levels in blood (0.759 μg g^{-1} w.w.) (Clark et al., 2000). In Spain, another species of water snake like viperine snake (Natrix maura) from rice farming areas in the Ebro delta showed mean levels of RDDTs (96% was p,p′-DDE) in carcass (removing viscera, head, tail and skin) of 1795.44 ng g^{-1} l.w. (1.7–5.8% lipid) (Santos et al., 1999). Lower p,p′-DDE levels have been detected in small mammals such as Algerian mouse (Mus spretus) and European wild rabbit (Oryctolagus cuniculus) from central Portugal, with 31 and 87 ng g^{-1} l.w. in liver, respectively (Mathias et al., 2007). Therefore, some increase in the diet of reptiles such as water snakes may represent a significant increase in the exposure to organochlorine compounds, especially in wetlands like Doñana where water snakes are common. Based on the data given by Santos et al. (1999) and Mathias et al. (2007) for potential prey of small-medium carnivores in Spain and Portugal, the change from a diet fully based on rabbit (100%) to a diet with 5% of viperine snake and 95% of rabbit may double p,p′-DDE exposure from 87 to 172.4 ng g^{-1} l.w. of diet. Differences in trophic position may affect dietary exposure to persistent lipohilic contaminants, as higher-trophic-level animals generally feed on animals with greater contaminant body burdens (Hebert and Weseloh, 2006). Therefore, the higher trophic position of reptiles in the area compared to other prey of the studied carnivores (i.e., rabbits, rodents, partridges, small passerines) could explain the major contribution of reptilian prey to the organochlorine burden in carnivores. In this line, an important question to elucidate would be the taxonomical composition of the reptilian portion of the diet. Given the high ecological differences in terms of habitat, diet or seasonality among the different reptile groups (e.g. turtles, lizards, snakes), the potential to accumulate organochlorine compounds from reptilian prey might vary depending on which group is consumed more frequently. In the study area, data on diet composition are only available for the common genet and the Egyptian mongoose, being lizards the major portion of the reptilian diet in both cases (Palomares and Delibes, 1991). Unfortunately, the available information is too limited to get any conclusion other than the convenience to evaluate organchlorine accumulation by reptiles in further studies.

On the other hand, declines of prey populations such as European wild rabbit by diseases can force predators to consume other type of prey such as birds or reptiles with the consequent increase in the biomagnification level of organochlorine compounds in the predators (Mañosa et al., 2003). Intraspecific differences in the diet can lead to important differences in the exposure to bioaccumulative contaminants. As an example, Hoekstra et al. (2003) found that Arctic foxes with a predominantly marine-based foraging strategy occupied a higher trophic level than individuals mostly feeding from a terrestrial-based diet. In the case of Iberian lynx from Doñana, the collapse of the Spanish rabbit populations with the rabbit hemorrhagic disease in the late 80s (Villafuerte et al., 1995) has not been accompanied by a maintenance or an increase in its organochlorine burdens as occurred with northern goshawks (Accipiter gentilis) in northeastern Spain due to the shift towards a diet based on birds (Mañosa et al., 2003). This could be explained by the highly specialized diet of Iberian lynx on this lagomorph (Delibes et al., 2000) in comparison with birds of prey that show a higher plasticity (Monleón et al., 2009).

5. Conclusions

The studied individuals from the two populations of Iberian lynx in southern Spain had a lower organochlorine burden in plasma and tissues than other carnivores with more anthropic and opportunistic foraging behavior, such as red fox, or with diets including reptiles at a higher proportion, such as Egyptian mongoose or common genet. Moreover, organochlorine levels have declined since the 80s in carnivores form Doñana National Park, but

PCB levels are still of concern in Eurasian otters from the industrialized region of Madrid.

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