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Persistent organic pollutants in juvenile Magellan penguins (*Spheniscus magellanicus*) found on the northern shore of the state of São Paulo and southern shore of the state of Rio de Janeiro, Brazil

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ABSTRACT

In the present study, persistent organic pollutants (POPs) were determined in 25 livers from Magellanic penguins, *Spheniscus magellanicus*, found on the state of São Paulo and Rio de Janeiro (Brazil). The following concentrations of POPs (wet weight) were found: $\sum$ PCBs: 18.9–775.8 ng g^{-1}; $\sum$ DDTs: 2.3–275 ng g^{-1}; and $\sum$ HCHs: 1.0–11.8 ng g^{-1}. Among the PCBs, there was a predominance of hexachlorobiphenyls (138 and 153) and heptachlorobiphenyls (180 and 187). Among the organochlorines, DDT predominated. Through histopathologic exams a positive correlation was found between HCB and cardiovascular failure ($p = 0.012$), as HCB values (wet weight) were 6.33–96.49 ng g^{-1} for positive animals and 2.45–19.63 ng g^{-1} for negative animals. Studies on POPs in Magellan penguins contribute to a better understanding of the distribution and trends of these pollutants in the western South Atlantic Ocean.

1. Introduction

The presence of penguins of the beaches of southern and southeastern Brazil is a natural phenomenon occurring in winter (Petry et al., 2004; García-Borboroglu et al., 2010). Archeological studies on middens along the Brazilian coast reveal layers of shells and fragments of arrowheads, axes, pottery, human skeletons and animal skeletons, including the bones of penguins, indicating that penguins were coming to the Brazilian coast long before colonization by the Portuguese (Madu, 2000).

Every year, Magellan penguins (*Spheniscus magellanicus*) leave their colony in Patagonia, Argentina and head to sea for months in the search for food (Fig. 1). For juveniles, this is the first pelagic migration. At specific feeding grounds, these penguins come into contact with human activities, such as fishing and tourism (Pütz et al., 2007).

A number of hypotheses have been raised to explain the increase in the appearance of penguins on the coast of Brazil (Xavier et al., 2007). It is believed that these birds may experience numerous stressful situations during migration, such as abrupt climate changes, food deprivation, infestation by parasites against which the species has no natural defense, physical harm, human actions, xenobiotic agents, predation and disease (Gandini et al., 1994; Fowler et al., 1995; Petry and Fonseca, 2002; Petry et al., 2004). Thus, many penguins arrive at Brazilian beaches in a state of cachexia, dehydration, hypothermia and trauma or covered in oil and are sent to rehabilitation centers if they are found on the cost (Xavier et al., 2007).

The presence of persistent organic pollutants (POPs) in the environment in which these penguins migrate may be another factor affecting their biology and population decline. POPs are a group of organic compounds that exhibit a combination of characteristics, such as persistence, bioaccumulation and toxicity, and can be transported over long distances in the environment (PNUMA, 2002). Organochlorines may have seasonal variations, with seasonal metabolic changes occurring between sexes and age groups, which requires particular attention regarding collection strategies and the amount of samples (Fryer and Nicholson, 1993).

This aim of the present study was to analyze the occurrence of organochlorine compounds in the liver of juvenile specimens of *S. magellanicus* found beached on the shores of southeastern Brazil and determine a possible association with cardiovascular failure.

2. Materials and methods

Twenty-five juvenile specimens were found dead on the beach or died at the *Instituto Argonauta e Aquário de Ubatuba* (municipality of Ubatuba, state of São Paulo, Brazil) in 2008. Histopathological

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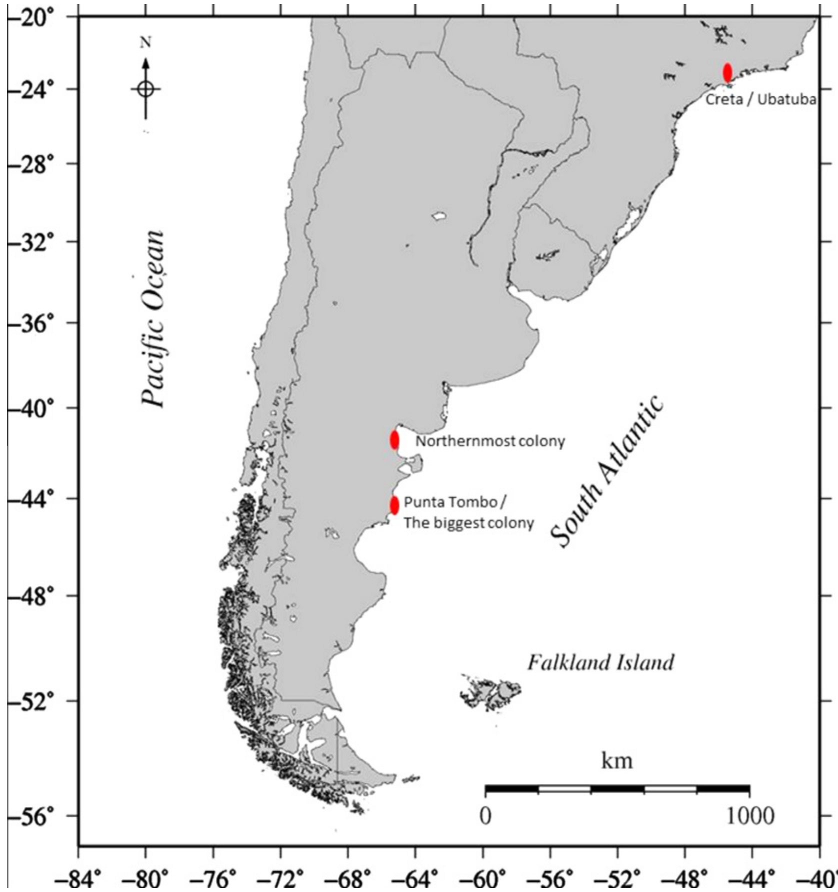

Fig. 1. *Spheniscus magellanicus* colonies and CRETA localization.

samples were collected from the lungs, heart, liver and kidneys for histological analysis, following the procedures described by Hocken (2002) and the results are shown in Carvalho et al. (2012). The liver samples for the analysis of organochlorines were wrapped separately in aluminum foil and frozen, following the method described by Ohlendorf et al. (1978).

The analysis of organochlorines (OCs) was performed at the Marine Organic Chemistry Laboratory of the Oceanographic Institute of the *Universidade de São Paulo* (Brazil). The method described by MacLeod et al. (1986) and used at the laboratory was employed for the analysis of OCs in the liver specimens.

Either the Student's *t*-test or the Mann–Whitney test was used for the comparison of concentrations of OCs in penguins with and without cardiovascular failure. Statistical analysis was performed using the SigmaStat program (Jandel Scientific Corporation), with the level of significance set to 5% ($p < 0.05$).

3. Results and discussion

The following concentrations of POPs (wet weight) were found: $\sum$ PCBs: 18.9–775.8 ng g^{-1}; $\sum$ DDTs: 2.3–275 ng g^{-1}; and $\sum$ HCHs: 1.1–11.8 ng g^{-1}. Among the organochlorines, *p*,*p*′-DDE and HCB predominated (Fig. 2). The congeners 153, 180 and 187 predominated among the polychlorinated biphenyls (Fig. 3).

For comparison purposes, data on the penguins that occur in Antarctica were used as reference (Table 1), as no data are found on POPs in penguins that inhabit the sub-Antarctic region of the South Atlantic. The concentrations of POPs in *S. magellanicus* were generally of an order of magnitude 10–10^2 greater than those found in gentoo (*Pygoscelis papua*) or adelie (*Pygoscelis adeliae*) penguins on islands of the Antarctic Peninsula (Subramanian et al., 1986; de Boer and Wester, 1991; Inomata et al., 1996).

However, it is necessary to take the distribution and biology of these species into account, especially feeding, as similarities are found between *P. papua* and the Magellan penguin. *S. magellanicus* feeds on fish, squid and crustaceans (Pinto et al., 2007; Baldassin et al., 2010) and the Atlantic breeding population reproduces along the coast of Patagonia from Complejo Islote Lobos (41°25′S, 65°2′W) south to the Isla Magdalena (52°55′S, 70°34′W). Individuals migrate from Patagonian colonies and reach northern Argentina, Uruguay and southern Brazil on their winter migration from March to September, but rarely get as far north as the state of Rio de Janeiro (21°S) (García-Borboroglu et al., 2010). *P. papua* feeds on crustaceans, neritic fish and squid (Libertelli et al., 2004; Lescroël and Bost, 2005). The Malvinas/Falklands, South Georgia and Kerguelen islands hold about 75% of this penguin population and only around 13% are found south of the ice pack limit (Quintana and Cirelli, 2000). *P. adelie* feeds on fish and krill (Ainley et al., 2004; Polito et al., 2009). This species has circum-Antarctic distribution and breeds at high latitudes around the Antarctic continent and along the Antarctic Peninsula (Polito et al., 2009).

In the present study, higher predominance indices were found for some PCB congeners (PCB 101, PCB 153 and PCB 180) in comparison to those reported in a study carried out by Inomata et al. (1996), in which only PCBs 52 and 44 surpassed the values found in the present study (Fig. 3). According to de Boer and Wester (1991), low concentrations of PCBs in *P. papua* may be due to the diet of squid, as the authors found no presence of these pollutants in the analysis of these cephalopods.

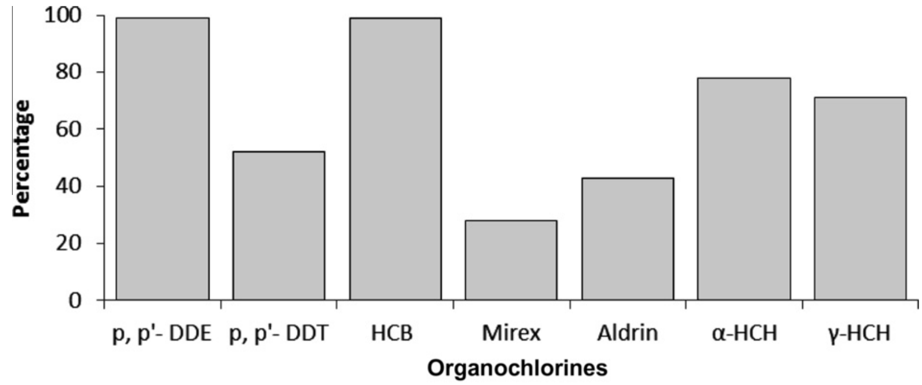

Fig. 2. Occurrence (%) of organochlorines in liver samples from *Spheniscus magellanicus*.

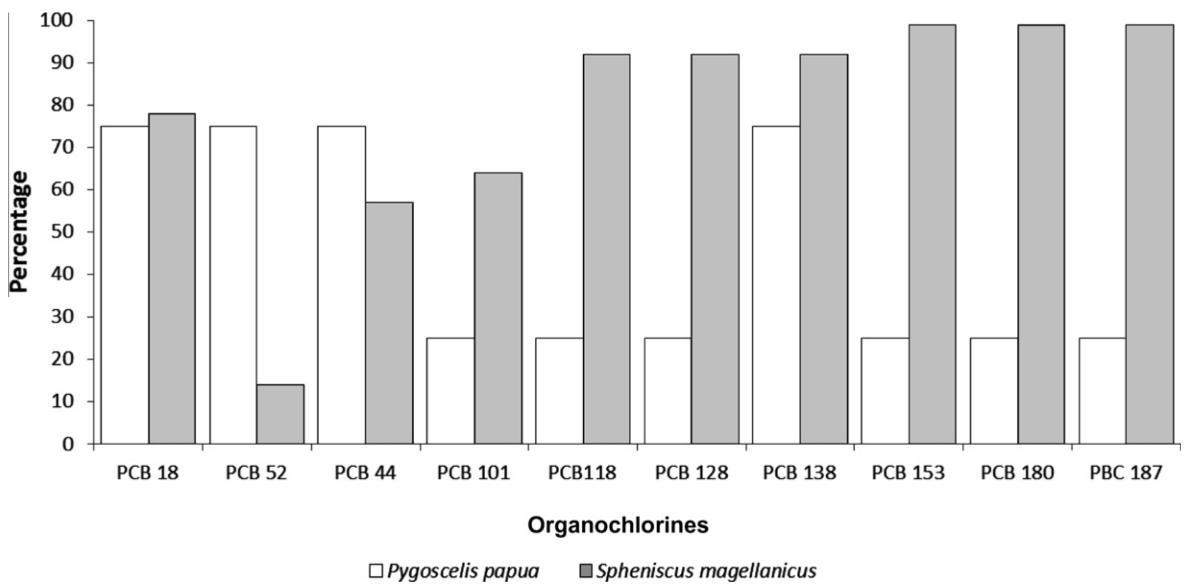

Fig. 3. Comparison of % of PCB congeners in liver samples from *Spheniscus magellanicus* and *Pygoscelis papua* (Inomata et al., 1996).

PCBs 18, 44 and 138 were the only pollutants with percentage of occurrence values similar to those reported by Inomata et al. (1996). The predominance of PCB 101, PCB 153 and PCB 180 in the present study is related to the number of chlorines and their structure, as PCBs 138, 153 and 180 have a low excretion rate in birds and mammals and tend to accumulate in the organism (Covaci et al., 2002; Maervoet et al., 2004). Most birds are capable of eliminating PCBs with low molecular weight, which results in the accumulation of a similar group of compounds (Norstrom et al., 1988). The accumulation of PCBs with a greater molecular weight is due more to difficulty in eliminating these compounds than variations in absorption capacity (Drouillard et al., 2001).

The compound with the greatest concentration in the penguins was *p,p′*-DDE. According to Ohlendorf et al. (1978), this compound causes the thinning of eggshells, resulting in broken shells and a consequent decline in the population. In *P. adeliae*, Subramanian et al. (1986) and de Boer and Wester (1991) report a similar order of magnitude, whereas Conroy and French (1974) report a 10^{-2} order of magnitude and Inomata et al. (1996) did not detect this compound.

Among the HCHs, wet weight α-HCH values ranged from 1.1 to 4.6 ng g^{-1} in *S. magellanicus* in comparison to 0.1 ng g^{-1} in *P. adeliae* (Inomata et al., 1996) and *P. papua* <0.4 ng g^{-1} (de Boer and Wester, 1991). These compounds have greater solubility in water in comparison to the majority of the other OCs determined, with a small bioconcentration factor and, consequently, low residual levels (Wells et al., 1994). However, the results reveal a prevalence value of 78% of the samples in comparison to 71% for γ-HCH. This may be explained by the possible photo- and bio-isomerization of γ-HCH into α-HCH, as indicated by laboratory studies. In the environment, however, there is indication that this conversion is the main reason for the elimination of γ-HCH (Walker et al., 1999). According to the author cited, when there is a large transformation of these compounds, the southern hemisphere is the region in which there will be a greater concentration of α-HCH, as γ-HCH the dominant isomers. It should be borne in mind that α-HCH has the greatest carcinogenic activity of the different isomers of HCH.

The most frequent anatomopathologic findings in these animals were related to vascular lesions in the lungs and liver, such as pulmonary hypertension and passive hepatic congestion (Carvalho et al., 2012). These lesions are found in birds experiencing a metabolic overload associated to environmental adversity (Jaenisch et al., 2005). During necropsy, fat stores were absent and pectoral muscles were concave in 83.3% of the specimens. According to Hocken (2002), such findings indicate a greater degree of protein malnutrition. All specimens had a dark reddish-brown material (blood altered by the digestive process) in the stomach, which, according to the same author, indicates death by starvation.

Table 1
Persistent organic pollutants in liver samples from different species of penguin (ng g^{-1}).

	S. magellanicus[a]	*P. papua*[b]	*P. papua*[c]	*P. adeliae*[c]	*P. adeliae*[d]	*P. adeliae*[e]
PCBs[f]	6.4–1983.72	1.1–4.18	1.1	n.d.		
o,p'-DDD	5.6–6.7					
o,p'-DDE	5.5–9					
o,p'-DDT	4.1–15.4					20
p,p'-DDD	2.8–7.1	0.4–0.5				
p,p'-DDE	2.3–255.19	0.6–3.4			0.5–5.6	10–80
p,p'-DDT	n.d.	0.4–1.5				
α-HCH	1.1–4.6	<0.4	n.d.–0.1	0.1		
δ-HCH	n.d.					
γ-HCH	2.9–11.8	<0.4	n.d.	0.2		
β-HCH	n.d.	<1	n.d.–0.1	0.5		
Aldrin	1.3–3.07					
Dieldrin	2.3–19.3	1–3	0.1–0.5	0.1		
Endrin	n.d.	<0.5	n.d.– 0.2	n.d.		
α-Chlordane	n.d.					
γ-Chlordane	n.d.					
Heptachlorine	n.d.					
Heptoxide A	8.0–10.7					
Heptoxide B	4.6–8.1					
HCB	2.4–108	6.5	0.1–1	1.1		
Mirex	5.9–21.6					

[a] Present study.
[b] de Boer and Wester (1991).
[c] Inomata et al. (1996).
[d] Subramanian et al. (1986).
[e] Conroy and French (1974).
[f] PCBs = ∑ congeners 18, 44, 52, 101, 118, 128, 138, 153, 180 and 187; ∑ congeners except PCB 18 in de Boer and Wester (1991).

Lung congestion was the most frequent histopathologic lesion, occurring in 75% of the cases and characterized by dilatation in circulation, pulmonary microcirculation, the filling of the vascular bed with red blood cells and consequent thickening of the walls of the parabronchi. Another important histopathologic finding was passive hepatic congestion, found in 50% of the specimens.

Sixty percent of the penguins died of cardiovascular failure and the cause of death among the other 40% was associated with anthropogenic detritus and fungal infection, as described in Carvalho et al. (2012). Correlating both groups with OCs, HCB has the lowest concentration but was the only compound found with a positive correlation ($p = 0.012$) with cardiovascular failure even though we cannot affirm that it is responsible for the deaths (see Fig. 4).

The concentrations of HCB in the present investigation differ from those reported in previous studies, notwithstanding differences in the breeding grounds and migration areas of the penguins analyzed (Table 1). While the wet weight values ranged from 2.45 to 108 ng g^{-1} in *S. magellanicus*, 72% of the samples had values of up to 50 ng g^{-1}. Inomata et al. (1996) found HCB in all tissues studied, with wet weight values ranging from 0.1 and 1 ng g^{-1} in *P. papua* and a value of 1.1 ng g^{-1} in *P. adeliae*.

HCB is a relatively volatile compound that can be transported to cold regions, incorporated into the food chain and accumulated by top species, with concentrations comparable to those encountered in areas of application (Calamari et al., 1991; Aono et al., 1997; Fuoco et al., 2009). According to Barber et al. (2005), the amount of HCB currently emitted is very low, but only a small quantity re-emitted from the soil to the air is sufficient to maintain the scenario of primary emission, which was hundreds to thousands of tons during the height of use.

According to Fowler and Fowler (2001), hepatitis encountered in microscopic analyses with characteristics of a toxic origin may be related to the buildup of heavy metals, pesticides in the fish consumed, disinfectants and diverse pollutants found in the environment. Michielsen et al. (1999) states that porphyria is regarded as the major potential toxic manifestation of HCB in experimental animals and man; moreover, HCB-induced porphyria in birds is also comparable to the disease in mammals and is characterized by a deficiency of the enzyme uroporphyrinogen decarboxylase and increased urinary excretion of highly carboxylated porphyrins. Symptoms in rats exposed to HCB include hyper-excitability, tremors, weak legs and paresis, but the author found no neuro-histopathologic changes (Michielsen et al., 1999). Thus, further investigation is needed, as there is a possibility of discovering the specific etiology in cases of dead penguins diagnosed with inconclusive neurologic symptoms. Another important point is that veterinarian protocols in Brazil use iron to treat animals for anemia; however, iron potentiates the effects of HCB through lipid peroxi-

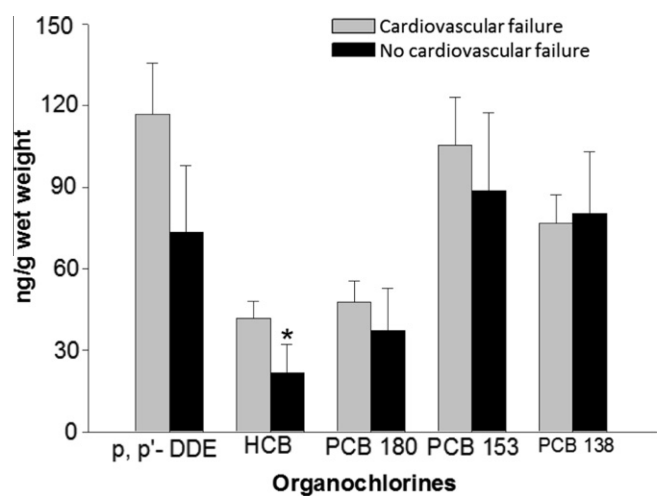

Fig. 4. Organochlorine compounds in liver samples from *Spheniscus magellanicus* (ng g^{-1}, wet weight); *statistically significant difference ($p = 0.012$).

dation (Alleman et al., 1985). It is therefore essential for rehabilitation centers to have the ability to diagnosis and treat conditions of a toxicological origin.

According to Subramanian et al. (1986), tissues such as the liver, muscle, bone and brain have low concentrations of OCs in comparison to subcutaneous and abdominal fat; however, these contaminants are redistributed among other tissues in "starving" penguins. In the present study, it was not possible to examine fat tissue due to the high degree of cachexia among the specimens.

Becker (1989) found that the variability in the concentration of OCs in the eggs of seabirds is lower than that found in fish and marine mammals. However, in a situation of inanition and cachexia, as in the penguins examined in the present study, mobilization of soluble lipid-pollutants from fat stores occurs, thereby reducing the mass of internal organs, such as the liver (Bogan and Newton, 1977). This could explain the low amounts of lipids found in the samples analyzed, as the penguins had approximately 50% lower low body mass in comparison to normal individuals (mean: 1.5–2 kg versus 4.5–5 kg).

According to Boersma (2008) and Becker (1989), penguins are sentinels of the marine environment through which scientists can learn about the rates and nature of changes occurring in the South Atlantic. Penguins face changes in the environment, whether climatic changes or negative factors stemming from human activities. According to Pütz et al. (2007), *S. magellanicus* is increasingly threatened by human activities in coastal areas along its migration path. Although penguins demonstrate adaptations to the variability in the environment, environmental disturbances constitute a threat to their populations.

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