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Persistent organic pollutants in juvenile Magellanic Penguins (*Spheniscus magellanicus*) in South America

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HIGHLIGHTS

- Decline of Polychlorinated biphenyls (PCBs) concentration in the liver of Magellanic penguins from 2008 to 2012 in South America.
- Prevalent organochlorine pesticides concentration in the liver of Magellanic penguins from 2008 to 2012 in South America.
- Magellanic Penguin POPs comparison from 3 countries, Chile, Uruguay and Brazil.

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ABSTRACT

Magellanic penguins, *Spheniscus magellanicus*, are the most abundant penguins living in temperate regions of South America and are good indicators of environmental pollution in the region. Persistent organic pollutants (POPs) were detected in the liver of Magellanic penguins found debilitated or dead on the beaches of Brazil (states of Rio de Janeiro, Sao Paulo, Santa Catarina and Rio Grande do Sul) between 2008 and 2012 as well as in Uruguay and Chile in 2011. Polychlorinated biphenyls (PCBs) were more prevalent than organochlorine pesticides (DDTs ~ HCB ~ Drins) and polybrominated diphenyl ethers (PBDEs). Among PCBs, penta-, hexa- and hepta-chlorinated congeners were predominant. Concentrations of POPs were similar between the Pacific and Atlantic penguin populations, except for PCBs, which were relatively higher in the Pacific population. During the study years (2008–2012), large variations were found in organochlorine pesticides and PCBs tended to decline. Overall, the southern portion of South America has low concentrations of POPs, with either a constant trend or evidence of decline.

1. Introduction

Magellanic penguins that reach coastal areas during the migration season are increasingly threatened by human activities, such as commercial fishing activities, tourism, oil pollution, climate change and the ingestion of plastic (Boersma and Rebstock, 2014; Brandão et al., 2011). Moreover, seabirds can be used as indicators of the marine environment (Becker, 1989; Boersma, 2008) by demonstrating the rates and nature of changes in South America. Although these birds are adaptable to change, environmental disturbances constitute a threat to the stability of their populations.

Among diverse factors, persistent organic pollutants (POPs) affect penguins in their environment and constitute a group of

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organic compounds that exhibit the combined features of persistence in the environment, bioaccumulation and toxicity. Toxic effects may occur during activities that involve a high energy cost, such as migration and breeding, due to lipid mobilization and its redistribution among tissues (Tanaka and Schroit, 1986). Moreover, these compounds can be transported over long distances (Pnuma, 2002). As organochlorines have been banned or had their use restricted in some parts of the world, such measures will eventually lead to the reduction in the amount of the chemical products released into the environment over time. However, even when banned, POPs will persist in the environment for centuries to come (Larsson and Okla, 1989).

Few studies on pollutants have been conducted on Magellanic penguins (Baldassin et al., 2012; Brandão et al., 2011). The majority of investigations have addressed organic pollutants found in Antarctic penguins (*Pygoscelis Antarctica*, *Eudyptes chrysocome*, *Pygoscelis adèliae*, *Aptenodytes forsteri*). Moreover, eggs (Cipro et al., 2010; Van den Steen et al., 2011, Corsolini et al., 2011) and blood (Jara-Carrasco et al., 2015) have been used as the main matrices.

The aims of the present study were to analyze POP concentrations in the liver of juvenile Magellanic penguins found on the Pacific and Atlantic coasts of South America and assess the current state of these pollutants in the marine environment in the region.

2. Methods

Spheniscus magellanicus is the most abundant penguin living in temperate regions. Inhabitant of the Falkland Islands as well as the Patagonia region of Argentina and Chile, the Magellanic penguin is also found from the Lobos Island Complex (41°25′S, 65°01′W) to Grande Island in Tierra del Fuego (54º54′S, 67º23′W) in Argentina and northwards to Pajaro Niño Island (33°21′S, 71°41′W) in Chile (Simeone et al., 2003). The population exceeds a million breeding pairs in reproductive age (Gandini et al., 1996; Pazos et al., 2003; Boersma, 2008). The breeding season is from September to January/February, when adults only enter the open ocean for short periods to feed, remaining most of the time in Argentine and Chilean waters (Frere et al., 1996; Gandini and Frere, 1999; Boersma et al., 2013). The molting period is February to April.

During the oceanic period, which spans from April to September, this species migrates northward, wintering on the continental shelf off the coasts of Uruguay, Brazil (Stokes et al., 1998; Pazos et al., 2003; Putz et al., 2007) and Chile (Skewgar et al., 2014). The largest Magellanic penguin colony (Punta Tombo) is in Argentina, but Chile has also colonies. Punta Arenas (Fig. 1) has the two largest colonies in Chile: Isla Magdalena (52°55′47.49″S 70°35′07.34″W) and Seno Otway (52°58′32.72″S 71°12′52.44″W). There are two small colonies in Concepcion: one on Península de Tumbes (Talcahuano) (36°36′54.25″S 73°07′06.71″W) and the another on Isla Quiriquina (36°38′05.69″S 73°03′29.67″W.

The penguin specimens (n = 116) were collected from six areas located in South America (Fig. 1). In Brazil, three different collection sites were used: two institutions in the state of São Paulo provided samples [northern limit (23°22′29.00″S 44°43′23.40″W) to the southern limit (24°11′13.77″S 46°46′51.64″W)], one institution in the state of Santa Catarina [northern limit (27°22′50.55″S 48°25′00.22″W) to the southern limit (27°50′33.98″S 48°33′46.43″W)] and one institution in the state of Rio Grande do Sul [northern limit (31°24′40.68″S 51°05′56.99″W) to the southern limit (29°20′57.27″S 49°43′01.58″W)]. In Uruguay, one institution from state of Maldonado provided samples [northern limit (34°48′58.28″S 54°31′33.01″W) to the southern limit (34°49′14.85″S 55°23′21.78″W)]. In Chile, two institutions provided samples: one from Isla Magdalena in the Magellanic region

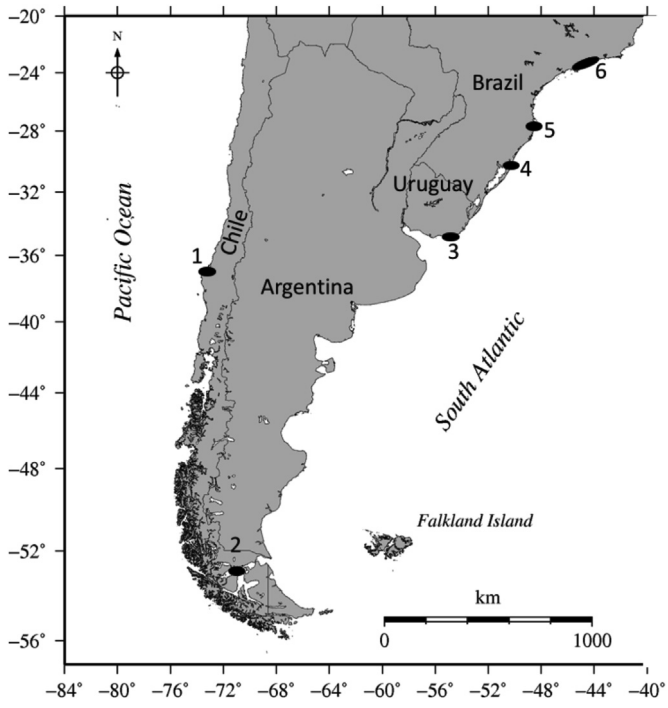

Fig. 1. Location of sampling sites: 1 Concepcion/Biobio Region/Chile; 2 Magellanic region/Chile; 3 Maldonado State/Uruguay; 4 Rio Grande do Sul State/Brazil; 5 State of Santa Catarina/Brazil; 6 State of São Paulo State/Brazil.

(52°55′47.49″S 70°35′07.34″W) and another from Concepcion in the Biobio Region [northern limit (36°27′03.96″S 72°53′32.37″W) to the southern limit (37°09′17.36″S 73°10′37.99″W)].

The animals were either found dead during beach monitoring procedures or died at rehabilitation centers between 2008 and 2012, except in 2009 (Table 1). In care treatment, only animals that remained 48 h in rehabilitation were used. These animals were in a poor body condition. Most were considered cachectic, with no fat and a decrease in chest muscle mass. Most died from hypothermia and starvation. The conditions of the stranded specimens were the same and only dead specimens with fresh tissue (24 h) were used in this study.

Liver samples were used for the analysis of organochlorines (OCs). Necropsy was performed based on Hocken (2002). The biological samples were stored separately in aluminum foil and frozen at −20 °C, following the method described by Ohlendorf et al. (1978). The analysis of OCs was carried out at the Marine Organic Chemistry Laboratory of the Oceanographic Institute of the University of São Paulo (Brazil).

Liver samples were analyzed based on procedures described by MacLeod et al. (1986) and Cascaes et al. (2014). Approximately 0.25 g of liver samples were dried with Na_2SO_4 and extracted with 80 mL hexane:dichloromethane (1:1, v:v) for 8 h using a Soxhlet

Table 1
Study years and liver samples from Magellanic penguins analyzed.

Penguins per year					
	2008	2010	2011	2012	Total
Atlantic ocean					
Brazil	37	18	27	20	**102**
Uruguay			10		**10**
Pacific Ocean					
Chile			4		**4**
Total	**37**	**18**	**41**	**20**	**116**

Table 2
Method detection limits (MDL) of PCBs, POCs and PBDEs.

PCBs	LDM	PCBs	LDM	POCs	LDM	PBDEs	LDM
PCB 8	0,38	**PCB 153**	0,38	**α-HCH**	0,31	**PBDE 28**	0,86
PCB 18	0,43	**PCB 132**	0,58	**HCB**	0,30	**PBDE 47**	0,82
PCB 31	0,48	**PCB 105**	0,51	**β-HCH**	0,43	**PBDE 100**	0,81
PCB 28	0,49	**PCB 141**	0,44	**γ-HCH**	0,36	**PBDE 99**	0,76
PCB 33	0,41	**PCB 138**	0,46	**δ-HCH**	0,41	**PBDE 154**	0,80
PCB 52	0,54	**PCB 158**	0,53	**Heptacloro**	0,22	**PBDE 153**	0,85
PCB 49	0,48	**PCB 126**	0,66	**Aldrin**	0,22	**PBDE 183**	1,06
PCB 44	0,58	**PCB 187**	0,77	**Isodrin**	0,33		
PCB 74	0,49	**PCB 183**	0,75	**Heptacloro Ep.A**	0,42		
PCB 70	0,52	**PCB 128**	0,45	**Oxiclordana**	0,47		
PCB 66	0,57	**PCB 167**	0,72	**Heptacloro Ep.B**	0,41		
PCB 95	0,54	**PCB 174**	0,71	**g-Clordana**	0,34		
PCB 56/60	0,41	**PCB 177**	0,56	**op′-DDE**	0,19		
PCB 101	0,30	**PCB 156**	0,59	**Endossulfan I**	0,27		
PCB 99	0,58	**PCB 157**	0,73	**a-Clordana**	0,45		
PCB 97	0,46	**PCB 180**	0,62	**Dieldrin**	0,23		
PCB 81	0,52	**PCB 169**	0,53	**pp′-DDE**	0,27		
PCB 87	0,41	**PCB 170**	0,53	**op′-DDD**	0,20		
PCB 77	0,51	**PCB 199**	0,58	**Endrin**	0,28		
PCB 110	0,48	**PCB 203**	0,63	**Endossulfan II**	0,42		
PCB 151	0,60	**PCB 189**	0,60	**pp′-DDD**	0,29		
PCB 123	0,50	**PCB 195**	1,01	**op′-DDT**	0,31		
PCB 149	0,58	**PCB 194**	1,09	**pp′-DDT**	0,28		
PCB 118	0,56	**PCB 206**	1,02	**Metoxicloro**	0,32		
PCB 114	0,43	**PCB 209**	0,74	**Mirex**	0,41		

extractor. Prior to extraction, PCB 103 and PCB 198 were added to all samples, blanks and reference material as surrogates. The extracts were purified using partially deactivated (5%) silica:alumina column chromatography with a 1:1 mixture of n-hexane and dichloromethane. The tissue fraction was further purified using high-performance liquid chromatography with gel permeation columns to remove lipids, which were gravimetrically determined, and the samples were then concentrated to a volume of 0.5 mL in hexane. Tetrachlorometaxylene (TCMX) was added prior to the gas chromatographic analysis as the internal standard.

Organochlorine pesticides and PCBs were analyzed using an Agilent 6890N Network gas chromatograph (GC) with a Ni-63 electron capture detector. The GC system was equipped with a 5% phenyl methyl siloxane HP-5MS capillary column measuring 30 m in length, 0.25 mm in inner diameter and 0.25 μm in thickness. The column temperature was programmed at 70 °C for 1 min, increasing at 40 °C min^{-1} to 170 °C, then increasing at 1.5 °C min^{-1} to 240 °C, held for 2 min and increasing at 15 °C min^{-1} to 300 °C, with a final hold of 5 min. The injector and detector temperatures were set to 280 °C and 320 °C, respectively. Hydrogen and nitrogen were used as the carrier and make-up gases, respectively.

PBDEs were analyzed using an Agilent 6890 Series gas chromatograph coupled to an Agilent 5973 Network Mass Selective Detector with electron impact at 70 eV. The chromatograph was equipped with a similar column as that used for the organochlorine analysis, with helium as the carrier gas. The column temperature was programmed at 70 °C for 1 min, increasing at 12 °C min^{-1} to 154 °C, then increasing at 2 °C min^{-1} to 210 °C and increasing at 3 °C min^{-1} to 300 °C, with a final hold of 5 min. The injector and interface temperatures were set to 270 °C and 300 °C, respectively. Acquisition was performed in selected ion mode.

Quality assurance and quality control were based on Wade and Cantillo (1994) and included the analysis of the procedural blank, blank spike, matrix spike, matrix duplicate and standard reference material (SRM 1945 – Organics in Whale Blubber from the National Institute of Standards and Technology), which were processed with the samples.

The method detection limits (MDL) of PCBs, POCs and PBDEs were in Table 2.

The analysis involved 51 individual PCB congeners (8, 18, 28, 31, 33, 44, 49, 52, 56, 60, 66, 70, 74, 77, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128, 132, 138, 141, 149, 151, 153, 156, 157, 158, 167, 169, 170, 174, 177, 180, 183, 187, 189, 194, 195, 199, 201, 203, 206 and 209), seven PBDE congeners (28, 47, 99, 100, 153, 154 and 183), DDTs (o,p'-DDT, p,p' -DDT, o,p' -DDD, p,p' -DDD, o,p' -DDE and p,p' -DDE), chlordanes (α- and γ-chlordane, oxychlordane, heptachlor and heptachlor epoxide), HCB, HCHs (α-, β-, γ-HCH), drins (aldrin, dieldrin, isodrin and endrin) and mirex.

Statistical analysis was performed using the SigmaStat program (Jandel Scientific Corporation) and the nonparametric Kruskal–Wallis test was used, with the level of significance set to 5% ($p < 0.05$).

The licenses issued by governmental agencies in each country were SISBIO 25671-4 for the collection and CITES for transport of the Brazilian specimens, 11BR006762/DF and 11BR007364/DF for Chile and 11BR007365/DF for Uruguay. The license for the sample removal from Chile (nº 31994) was issued on January 17, 2012 and the license for the sample removal from Uruguay was issued on January 20, 2012.

3. Results and discussion

The results were divided between Atlantic Ocean (Brazil, Uruguay) and Pacific Ocean (Chile) and only it was possible to collect samples from both areas in 2011 (Table 1). POPs were found in 100% of the livers. The highest concentrations were PCBs > DDTs ~ HCB ~ Drins > PBDEs. As these animals were in poor nutritional condition, the average lipids found on the Atlantic and Pacific coasts were 4% (0–17%) and 6% (4–8%), respectively.

3.1. Polychlorinated biphenyls (PCBs) in liver of magellanic penguins

Among the penguins found on the Brazilian coast, PCB concentrations (ng g^{-1} of wet weight) in the livers ranged from 9.9 to

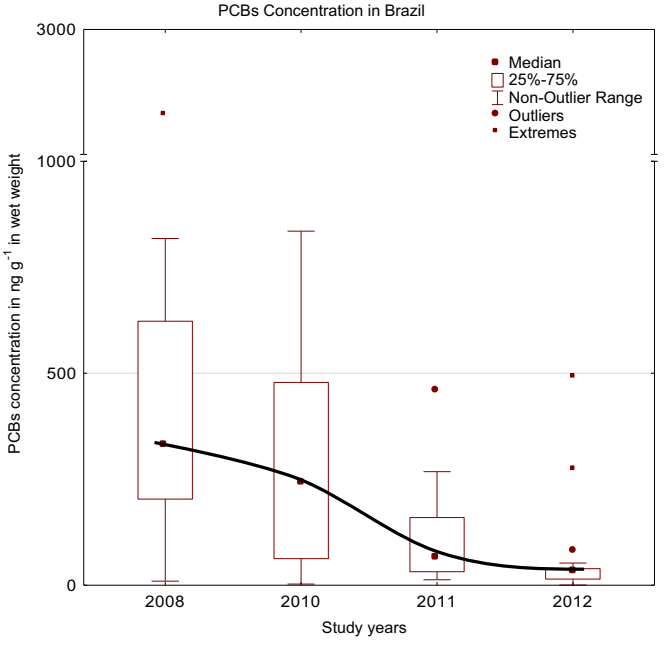

Fig. 2. ∑ PCB concentrations (ng g^{-1}) in liver of *Spheniscus magellanicus* from Brazilian coast in 2008 (n = 37), 2010 (n = 18), 2011 (n = 27) and 2012 (n = 20).

818 in 2008, 203 to 835 in 2010,13.3 to 456 in 2011 and 0.5 to 492 in 2012 (Fig. 2). Significant differences in PCB concentrations were found between 2008 and 2011 ($p < 0.001$), 2008 to 2012 ($p < 0.001$), 2010 to 2011 ($p = 0.018$) and 2010 to 2012 ($p = 0.012$), as concentrations in 2011 and 2012 were one order of magnitude lower in comparison to the previous years. There were no significant differences in PCB concentrations from 2008 to 2010 or from 2011 to 2012

Considering the penguins from the Pacific Ocean (Chile) and Atlantic Ocean (Brazil and Uruguay) in 2011, PCB concentrations found in penguins on the Chilean coast were significantly higher ($p = 0.014$) in comparison to specimens from the other areas (Fig. 3). This may be related to the fact that all penguins on the Chilean coast were found near Concepción, which is considered the most industrialized region in the country (Pérez, 1999). In penguins on the Atlantic coast, mean PCB concentrations were lower than 100 ng g^{-1}.

The relatively lower mean PCB concentrations in penguins on the Atlantic coast may have been due to the extremely poor body condition found during migration, as the scarcity of fish leads to a diet based on cephalopods, which have lower protein and fat contents in comparison to fish. Moreover, penguins expend a large amount of energy during migration. However, while cephalopod beaks were found in the stomachs of the penguins from Brazil, where this is one of their main food sources (Pinto et al., 2007; Baldassin et al., 2010), no cephalopod beaks were found in the stomachs of penguins from Uruguay. De Boer and Wester (1991) report that lower PCB concentrations in the liver of Antarctic penguins, such as the Gentoo (*Pygoscelis papua*), may be due to the diet of squids, but the low concentrations of these contaminants were found in cephalopods (less than 0.3 μg/kg of wet weight) in the feeding area of the Magellanic penguin, Falkland Island, which likely diminishes the PCB concentrations in the tissues of these penguins.

De Boer and Wester (1991) found PCB concentrations ranging from <0.4 to 0.9 ng g^{-1} of wet weight (ww) in the livers of *P. papua*. Inomata et al. (1996) found concentrations ranging from 0.1 to 0.9 ng g^{-1} ww in the livers of *P. papua* and 0.2–0.3 ng g^{-1} ww in the livers of *Pygoscelis adéliae*. Subramanian et al. (1986) found 0.28–0.83 ng g^{-1} ww in *P. adéliae*. The maximum PCB concentrations for *S. magellanicus* reached nearly three orders of magnitude higher than the concentrations reported in the studies cited. As the penguins in the present study use South America as a colony region and constitute a different species in comparison to those analyzed in the studies cited, both *P. papua* and *S. magellanicus* feed on small fish, crustaceans and cephalopods, whereas crustaceans, such as krill, account for 70% of the diet of *P. adéliae*. Table 3 displays the ΣPCB concentrations in the tissues of different penguins, including those analyzed in the present study in the 2011 and only those found in Brazil and Chile had liver concentrations one order of magnitude higher than the other tissues, as expected based on the distance from the pollutant emission sources.

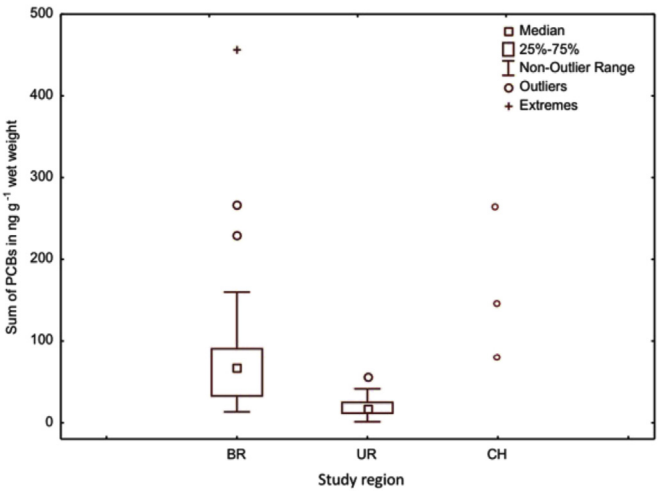

Fig. 3. ∑ PCB concentrations (ng g^{-1} wet weight) in liver of *Spheniscus magellanicus* from coastal regions in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

Fig. 4 displays the PCB congeners found in the present study and those reported by Inomata et al. (1996), in which only the concentration of PCB 52 surpassed that found in the present investigation. Most birds are capable of eliminating PCB congeners with a low molecular weight, which results in the accumulation of a similar group of compounds (Norstrom et al., 1988). Therefore, the accumulation of PCB congeners with a higher molecular weight is due more to elimination capacity than differences in absorption capacity (Drouillard et al., 2001).

Yamashita et al. (1993) found PCB concentrations three and four orders of magnitude higher than those in the present investigation in the eggs of *Phalacrocorax auritus* and *Hydroprogne cáspia*, respectively, in the Great Lakes region of North America. These animals are also piscivorous (most of the diet) and live in a very contaminated area. The sub-lethal effects of PCBs on waterbirds include changes in enzyme activity, a reduced resistance to pathogenic agents, reduced weight and mobility, deficient growth, impaired osmoregulation and impaired calcium metabolism, which results in thin eggs and therefore a decline in breeding success.

3.2. Organochlorinated pesticides in liver of magellanic penguins

Different concentrations of OCs were found in the liver of specimens of *S. magellanicus* from Brazil. HCB was the most prevalent and was found in 93% of the samples, ranging from 0.89 to 419 ng g^{-1} ww between 2008 and 2012 (Fig. 5). Statistically significant differences in HCB were found from 2008 to 2010 ($p = 0.012$), 2010 to 2012 ($p = 0.001$) and 2011 to 2012 ($p = 0.013$). In 2012, HCB concentrations in the penguins found were one order of magnitude higher in comparison to previous years. In the southern region of Brazil, one of the seven penguins analyzed exhibited a concentration one order of magnitude higher (134 ng g^{-1} ww) than the mean concentration of the other penguins. Concentrations on this order of magnitude were also found in Chile and Uruguay in 2011 (Fig.6), but with no significant difference ($p = 0.263$). These values are higher than those reported in previous studies. De Boer and Wester (1991) found a mean HCB concentration of 6.5 ng g^{-1} ww in the liver of specimens of *P. papua*. Inomata et al. (1996) found HCB in all tissues studied, with concentrations ranging from 0.1 to 1.0 ng g^{-1} ww in the liver of *P. papua* and a mean concentration of 1.1 ng g^{-1} ww in the liver of *P. adéliae*.

The penguins from the Atlantic coast exhibited similar mean HCB concentrations, Chile (20.5 ng g^{-1} ww), Brazil (13.5 ng g^{-1} ww) and Uruguay (5.05 ng g^{-1} of wet weight). This may be attributed to the dispersal of HCB over long distances (Calamari et al., 1991; Aono et al., 1997).

According to Barber et al. (2005), the amount of HCB released into the environment is currently much lower than in the past, but only a small amount released from the soil to the air is sufficient to maintain a situation of primary emission, which constituted hundreds of thousands of tons during peak production. The same authors found HCB concentrations ranging from 87 to 600 ng g^{-1} ww in fat tissue from *P. papua* in 1979. These figures are similar to those found in the liver of *S. magellanicus* (0.89–419 ng g^{-1} ww) in the present study.

Table 3
Concentration of Σ PCBs, Σ DDTs, HCB and Σ PBDEs in penguins liver, egg and blood. All papers are in ng g^{-1} wet weight except Van den Steen, 2011 that is in ng g^{-1} lipid weight.

	Places	*Tissue*	*Penguins species*	*PCBs*	*DDTs*	*HCB*	*PBDEs*
Present study (2011)[a]	Brazil	Liver	*Spheniscus magellanicus*	103.9 (13.25–456)	70.10 (5.04–223)	20.37 (2.22–61.05)	3.94 (1.18–9.69)
Present study (2011)[a]	Uruguay	Liver	*Spheniscus magellanicus*	21.5 (1.27–55.76)	160.9 (2.84–1039)	13.52 (4.10–28.03)	9.5 (1.01–35.12)
Present study (2011)[a]	Chile	Liver	*Spheniscus magellanicus*	177.68 (70.43–352)	46.22 (2.32–99.74)	5.05 (2.05–10.47)	8.52 (5.79–11.31)
Baldassin et al., 2012	Brazil	Liver	*Spheniscus magellanicus*	6.4–1983	2.3–275	2.4–108	
de Boer and Western, 1991	Antartic	Liver	*Pygoscelis papua*	1.1–4.18		6.5	
Inomata et al., 1996	Antartic	Liver	*Pygoscelis papua*	1.1–4.18		0.1–1	
Inomata et al., 1996	Antartic	Liver	*Pygoscelis adeliae*	nd		1.1	
Cipro et al., 2010	Antartic	Egg	*Pygoscelis adeliae*	32.5 (2.53–41.9)	6.29 (2.07–8.93)	22.1 (12.9–33.5)	
Cipro et al., 2010	Antartic	Egg	*Pygoscelis antarctica*	37.3 (3.11–78.7)	15.8 (2.67–38.0)	18.91 (4.99–39.1)	
Cipro et al., 2010	Antartic	Egg	*Pygoscelis papua*	26 (4.58–42.2)	5.47 (3.10–9.95)	16.2 (14.2–19.3)	
Van den Steen et al., 2011	Falklands	Egg	*Eudyptes chrysocome*	27.55 (+/− 0.70)		31.09 (+/− 1.11)	0.98 (+/− 0.04)
Corsolini et al., 2011	Antartic	Egg	*Aptenodytes forsteri*	3.59 (2.52–7.69)	7.10 (3.86–10.82)		
Corsolini et al., 2011	Antartic	Egg	*Pygoscelis adeliae*	11.28 (7.26–16.81)	0.83 (+/− 0.31)		
Corsolini et al., 2006	Antartic	Egg	*Pygoscelis adeliae*		23.0 (+/−12.0)		0.29 (+/− 0.31)
Jara-carrasco et al., 2015	Antartic	Blood	*Pygoscelis antarctica*	8.04 (+/− 1.24)	7.34 (+/− 0.9)	0.9 (+/− 0.12)	

[a] The present study only put the 2011 results. It is the year all the countries studied have samples collected.

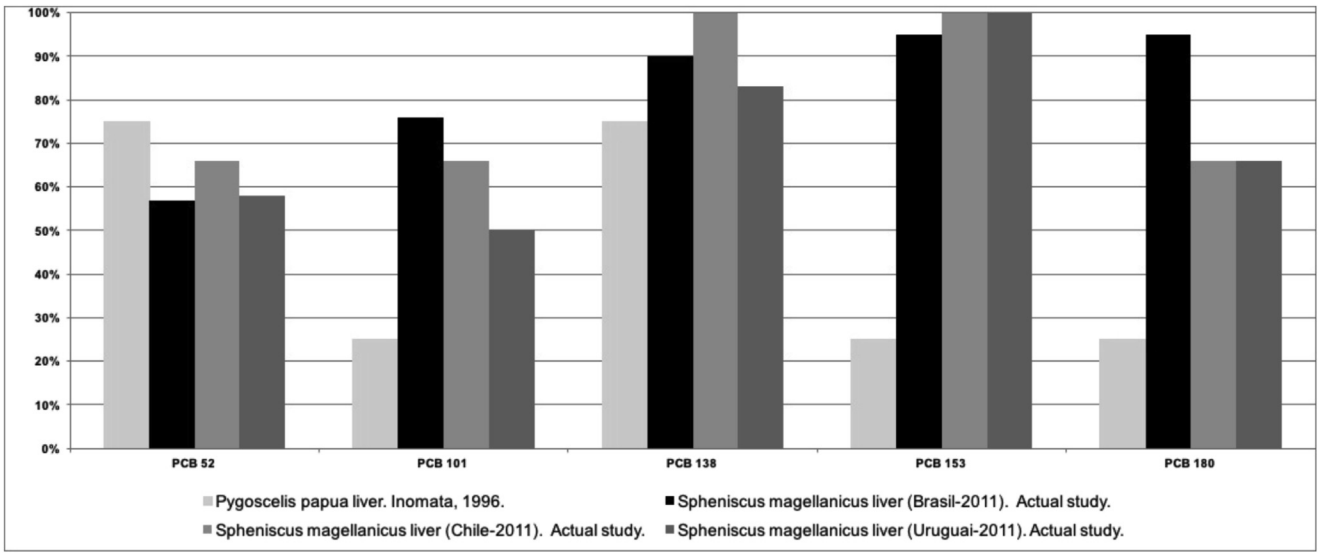

Fig. 4. Percentage of PCB congeners found in Inomata et al. (1996) and present study.

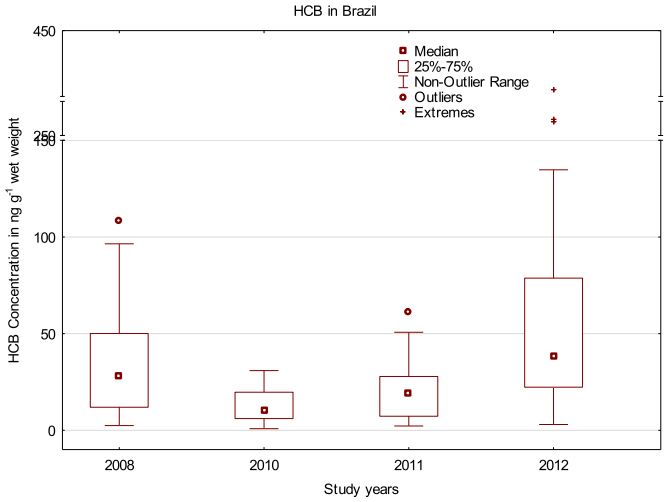

Fig. 5. HCB concentration (ng g^{-1} ww) in liver of *Spheniscus magellanicus* in 2008 (n = 37), 2010 (n = 18), 2011 (n = 27) and 2012 (n = 20) on Brazilian coast.

Baldassin et al. (2012) found a positive correlation between HCB and cardiovascular failure (p = 0.03) in 24 Magellanic penguins submitted to necropsy in the city of Ubatuba (state of São Paulo, southeastern Brazil) (Carvalho et al., 2012), but it was not possible to establish this association as the cause of death. However, a large number of penguins die with inconclusive neurological diagnoses, the etiology of which may be specified if investigated, as HCB can cause excitability, tremors and paresis in rats, with no changes in tissues submitted to histopathological analysis (Michielsen et al., 1999). Veterinary protocols for the rehabilitation of Magellanic penguins beached on the shore of Brazil employ iron dextran for the treatment of anemia. However, this compound is capable of potentiating the effects of HCB through lipid peroxidation (Alleman et al., 1985). It is therefore essential for rehabilitation centers to develop the ability to diagnose and treat diseases of a toxicological origin.

Among the DDT metabolites, *p,p'* DDE exhibited the highest concentrations. In the analysis of maximum concentrations in Brazil, the highest concentration (441 ng g^{-1} ww) was found in 2012, differing from the previous years (Fig. 7). However, considering the dataset as a whole, significant differences were found from 2008 to 2010 (p = 0.004) and 2010 to 2011 (p = 0.011).

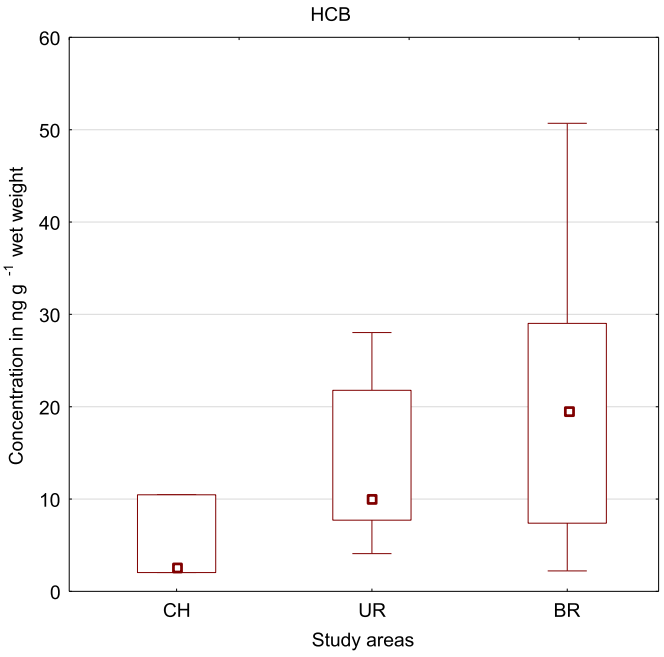

Fig. 6. HCB concentration in liver of *Spheniscus magellanicus* from coastal regions in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

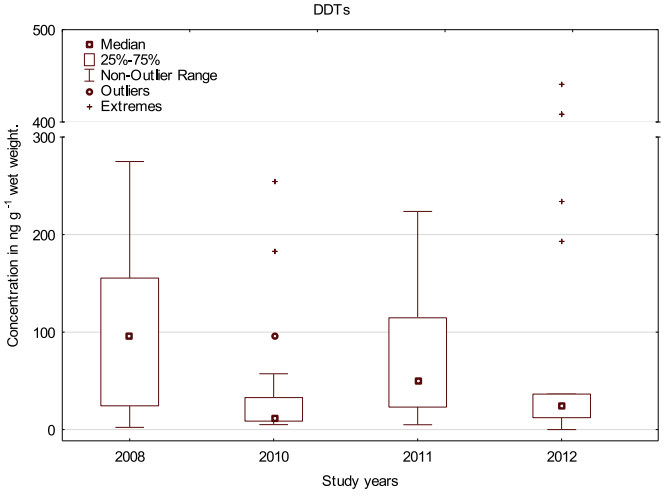

Fig. 7. Sum of DDT concentrations in ng g^{-1} ww in 2008 (n = 37), 2010 (n = 18), 2011 (n = 27) and 2012 (n = 20) in liver of *Spheniscus magellanicus* on Brazilian coast.

Moreover, no significant differences in mean concentrations were found in the comparison of the Atlantic and Pacific regions (Fig. 8).

Inomata et al. (1996) did not detect *p,p'*-DDE in the liver of specimens of *P. papua*. However, Subramanian et al. (1986) and Conroy and French (1974) found concentrations of 0.52–5.56 and 0.01–0.83 ng g^{-1} ww in *P. adéliae*, respectively, and De Boer and Wester (1991) found concentrations ranging from 0.0034 to 0.00021 ng g^{-1} of wet weight in the same species. The most abundant metabolite of *p,p'*-DDT found in the tissues and eggs of birds was *p,p'*- DDE (Ohlendorf et al., 1978). This metabolite can cause the thinning of egg shells, resulting in broken eggs and a consequent population decline. Auman et al. (1997) found more than a 5% increase in the rate of broken albatross eggs in the North Pacific Ocean in 1993/1994 and suggest that contamination by DDT was the cause. Dirksen et al. (1995) found that approximately

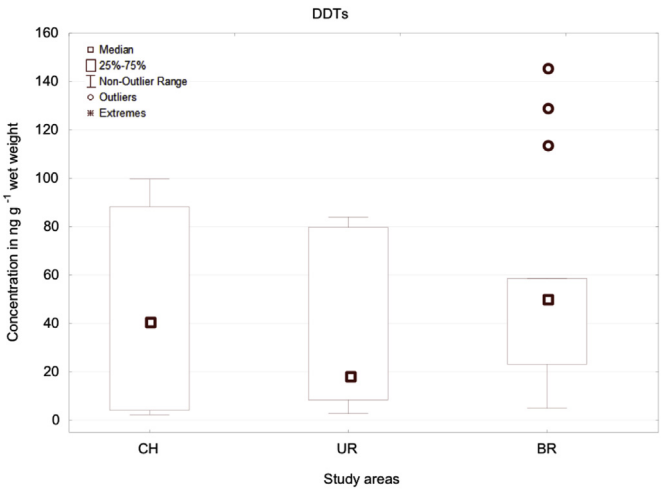

Fig. 8. Sum of the DDT concentrations (ng g^{-1} ww) in liver of *Spheniscus magellanicus* from coastal region in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

4000 ng g^{-1} ww of DDE in the eggs of the Great Cormorant (*Phalacrocorax carbo sinensis*) can lead to a 5% increase in the rate of broken eggs. DDE concentrations ranging from 3000 to 6000 ng g^{-1} in the eggs of the bald eagle (*Haliaeetus leucocephalus*) and 3000 ng g^{-1} in the eggs of the brown pelican (*Pelicanus occidentalis*) have been associated with harmful effects on the formation of egg shells (Blus, 1982; Wiemeyer et al., 1993). The concentration of DDE in the present study was three orders of magnitude lower than that reported in the studies cited. In penguins, the concentration of DDTs may vary among species and tissues (Table 3).

According to Barra et al. (2005), dicofol is a secondary source of DDT. This compound is registered with the Brazilian Ministry of Agriculture and Livestock (nº 00428705) as a non-systemic class of acaricide from the OC group. The authors also state that this insecticide used in South America may contain DDT as an impurity. In 2000, Brazil produced and imported 209 tons of dicofol, which is mainly used in the cultivation of apples, cotton and citrus fruit in the state of Paraná.

Analyzing individual cyclodienes, dieldrin accounted for the highest concentration found in Brazil (566 ng g^{-1} ww), with significant differences from 2008 to 2012 ($p < 0.001$), 2010 to 2012 ($p = 0.042$) and 2012 to 2011 ($p = 0.004$). This finding is likely due to the fact that aldrin and mirex were were banned only in 1999 with the Pesticides Law and Brazil signed the International Treaty of the Stockholm Convention only in 2001, which was enacted only in 2005.

In the comparison of different countries, an increase of one order of magnitude was found in the mean concentration of cyclodienes in the tissues of penguins found in Uruguay in comparison to those found in Chile and Brazil in 2011 (Fig. 9). This finding may be attributed to the fact that Uruguay is a smaller country that relies more heavily on agriculture, with plantations located closer to the ocean (Mañay et al., 2004; Boroukhovitch, 1998).

In the present study, significant differences in chlordane concentrations were found in the liver of specimens of *S magellanicus* from the coasts of Uruguay and Chile ($p = 0.040$), with means lower than 50 ng g^{-1} ww throughout the study period. Oxychlordane occurred in 39% of the penguins and at a higher concentration in comparison to γ-chlordane. Oxychlordane was detected at a concentration of 4.61 ng g^{-1} ww in only one penguin from the Chilean coast. Similarities were found among chlordanes found in the penguins from the coasts of Brazil and Uruguay (2.5–284 and

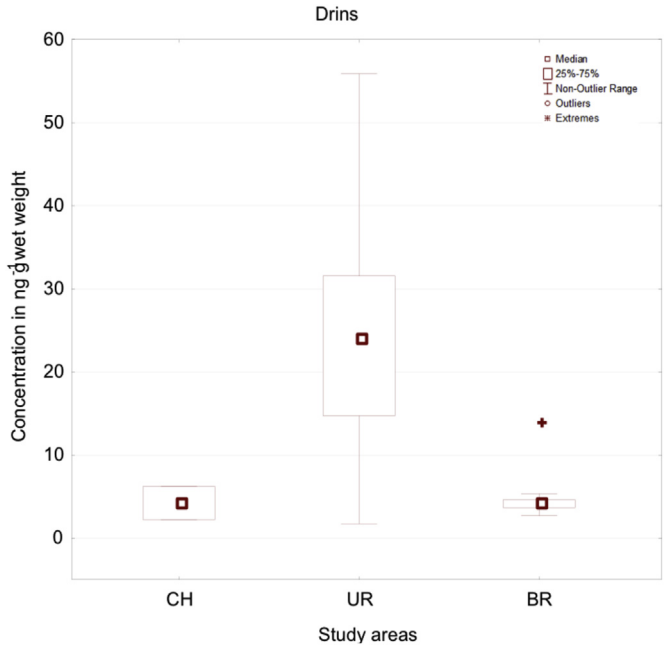

Fig. 9. Sum of the Drin concentration (ng g^{-1} wet weight) in liver of *Spheniscus magellanicus* from coastal regions in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

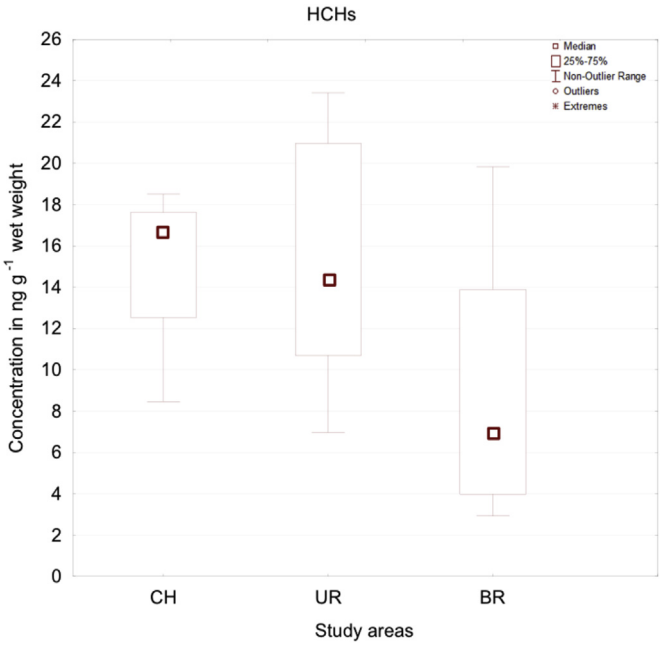

Fig. 10. Sum of HCH concentrations (ng g^{-1} ww) in liver of *Spheniscus magellanicus* from coastal regions in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

3.5–284 ng g^{-1} ww, respectively) and significant differences were found between 2008 and 2012 (p = 0.004) as well as between 2010 and 2012 (p = 0.005). Oxychlordane is the most persistent in the Atlantic environment (Wells et al., 1994), which may explain the relatively higher concentration of these compounds in the comparison of the tissue samples analyzed.

HCHs were found in 90% of the samples from Brazil and 100% of those from Chile and Uruguay. The most prevalent of HCH isomer was γ-HCH, found in 45% of the samples analyzed. In penguins from the Brazilian coast, concentrations ranged from 0.11 to 59.5 ng g^{-1} ww. Significant differences in these compounds were found in Brazil from 2008 to 2010 (p = 0.008), 2008 to 2011 (p = 0.002), 2008 to 2012 (p = 0.001) and 2010 to 2012 (p = 0.001). The concentrations were higher than those found by Inomata et al. (1996) in the liver of specimens of *P. adéliae* (0.2 ng g^{-1} ww) and by De Boer and Wester (1991) in the liver of specimens of *P. papua* (<0.0004 ng g^{-1} ww). These compounds have higher solubility in water in comparison to the majority of other OCs studied, which likely explains the lower bioconcentration and, consequently, lower residual levels (Wells et al., 1994). Moreover, significant differences in these compounds were found between the penguins from the Atlantic and Pacific coastal regions (p = 0.043) (Fig.10).

3.3. Polybrominated diphenyl ether (PBDEs) in the liver of Magellanic Penguins

A total of 59.7% of the liver samples from penguins found on the Brazilian coast exhibited measurable concentrations of PBDEs. The compound with the highest concentration was PBDE 183 (ranging from 2.6 to 25.1 ng g^{-1} of ww). Regarding the penguins found in the other regions, no concentrations of PBDEs were found above the method detection limit in 2011. The most frequent compound in the penguins from Chile was PBDE 154 (75%), ranging from 0.82 to 5.79 ng g^{-1} ww. In the penguins from Uruguay, PBDE 153 and 183 occurred in 38% of the samples (n = 5), ranging from 1.01 to 15.3 and 2.23 to 17.2, respectively. Statistically significant differences in

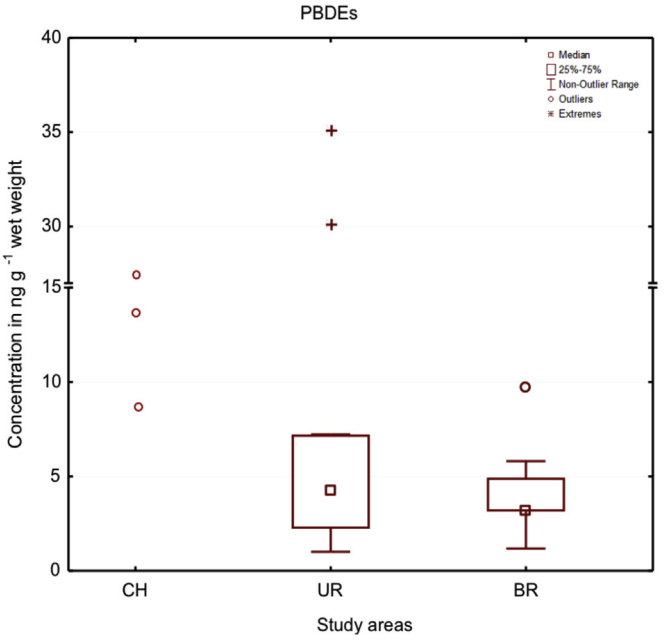

Fig. 11. Sum of the PBDE concentrations (ng g^{-1} wet weight) in liver of *Spheniscus magellanicus* from coastal regions in Chile (CH) (n = 4), Uruguay (UR) (n = 10) and Brazil (BR) (n = 27) in 2011.

PBDE concentrations were found only between the coastal regions of Chile and Brazil (p = 0.017) (Fig. 11).

Despite being the most persistent compound in this PBDE group (Albina et al., 2010), PBDE 99 occurred in only 8% of the penguins from Brazil, 25% of those from Chile and 0% of those from Uruguay. Another potentially persistent compound, PBDE 47 (Eriksson et al., 2001), was detected in 10% of the penguins from Brazil, 7% of those from Uruguay and 0% of those from Chile.

Despite the large number of experiments, the toxicological mechanism of action of PBDEs remains unclear and there is no concrete explanation for the carcinogenic effects and endocrine disorders associated with these compounds (Albina et al., 2010). The main concerns regarding the potential health risks of PBDEs are found in studies on the development of neurotoxicity in laboratory rats, in which diffusion throughout the organism has been observed following absorption in the intestine, with high concentrations found in the plasma and tissues and approximately 10% of metabolites with five to seven bromine atoms eliminated through bile.

According to Vagula and Konieczko, 2012, low molecular weight PBDEs are more toxic, more accumulative and have an affinity for lipids, which explains the higher concentration of these compounds in comparison to higher brominated congeners. The same authors report that, in three decades of studies, the concentration of these contaminants in the United States increased from 10 to 100 times more than in Europe and Asia.

4. Conclusion

Concentrations of POPs were similar between the Pacific and Atlantic penguin populations, except for PCBs, which were relatively higher in the Pacific population, most likely related to the proximity to an industrial area. During the study years (2008–2012), large variations were found in organochlorine pesticides and PCBs tended to decline. HCB was the most prevalent pesticide encountered, especially in penguins from the Atlantic coast. Among the DDTs, *p,p*' DDE was the most prevalent metabolite and cyclodienes demonstrated an increase of one order of magnitude in penguins from the Atlantic coast between 2011 and 2012. Some significant differences in the concentrations of other pesticides and PBDEs were found among the different years analyzed. Overall, the southern portion of South America has low concentrations of POPs, with either a constant trend or evidence of decline.

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