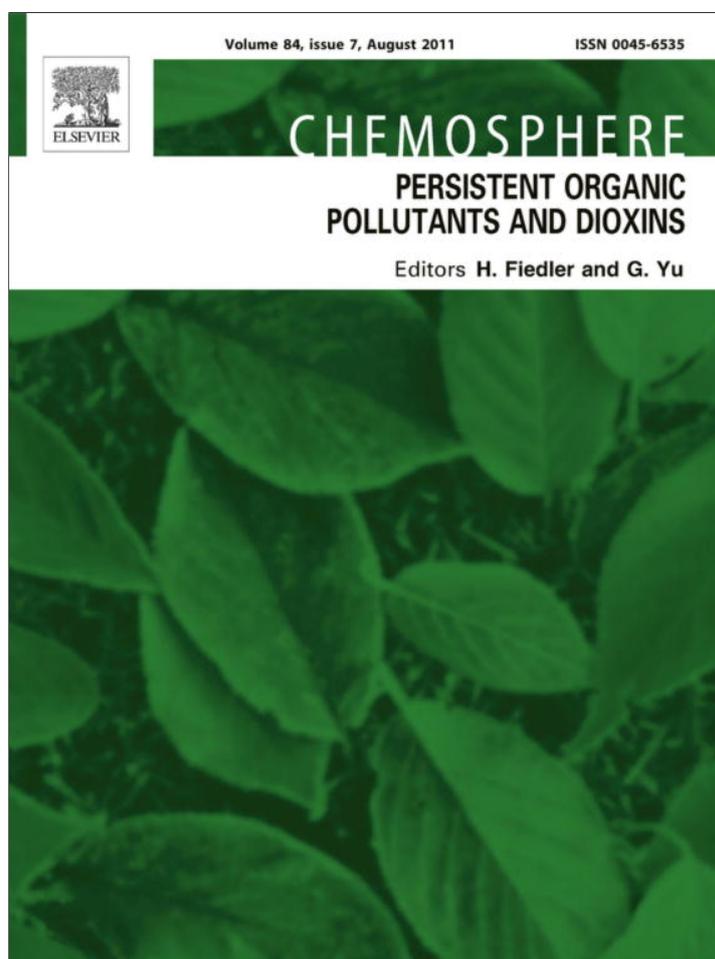


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## Organochlorine concentrations in franciscana dolphins, *Pontoporia blainvillei*, from Brazilian waters

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### ABSTRACT

Blubber samples were collected from ten franciscana dolphins either incidentally captured in fishing operations or stranded on São Paulo (SP) and Paraná (PR) states littoral, Southeastern and Southern Brazilian coast, respectively. Determination of PCB, DDT and HCB concentrations were performed by capillary gas chromatograph coupled to electron capture detector (ECD).  $\Sigma$ DDT,  $\Sigma$ PCB and HCB concentrations ranged from 264 ng g<sup>-1</sup> to 5811 ng g<sup>-1</sup> lipid, from 909 ng g<sup>-1</sup> to 5849 ng g<sup>-1</sup> lipid and from 10 ng g<sup>-1</sup> to 61 ng g<sup>-1</sup> lipid, respectively. Regarding DDTs, the distribution of the mean percentages decreased in the following order: p,p'-DDE > p,p'-DDD > p,p'-DDT. The  $\Sigma$ DDT/ $\Sigma$ PCB ratio varied between 0.27 and 0.42 in Northern and Central SP coast, while in Southern SP and PR coast the values were 1.6 and 1.9, respectively. Dissimilarities in  $\Sigma$ DDT/ $\Sigma$ PCB ratios point to different sources of organochlorine compounds to franciscana dolphins in the study area. Considering the endocrine disruptive action of organochlorine compounds, the concentrations found in franciscana dolphins from Brazilian waters may represent an additional obstacle to the conservation of this endangered cetacean species.

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### 1. Introduction

Few studies have been conducted on environmental contamination by persistent bioaccumulative toxicants in Southwest Atlantic Ocean (Aguilar et al., 1999; Dorneles et al., 2008a,b; 2010 Lailson-Brito et al., 2010). It is often possible to detect pollutant compounds in marine organisms when concentrations in water are too low to be determined with confidence by routine chemical analyses. Therefore, using cetaceans to screen the neritic environment in search for micropollutants has an obvious attraction (Dorneles et al., 2007a,b). Cetaceans are long-lived top marine predators, presenting large lipid reserves in proportion to their body size, making them the ideal repository for high concentrations of lipophilic chlorinated hydrocarbons. Hence, whales and dolphins are regarded as valuable indicators of organochlorine accumulation in marine environment (Lailson-Brito et al., 2010).

Franciscana dolphins are endemic to the western South Atlantic, ranging from Espírito Santo State (~18°S), Brazil to Chubut

Province (~42°S), Argentina, occurring in shallow waters roughly within 55 km of shore (Bastida et al., 2007). Based on morphological differences, Pinedo (1991) proposed two different forms of franciscana dolphin, one found to the north and the other to the south of Santa Catarina State. Sequence differences in the displacement loop (D-loop) region of the mtDNA between these geographic forms of franciscana dolphin were found, suggesting the existence of at least two genetic subpopulations or management stocks (Secchi et al., 1998). Applying the phylogeographic concept of stock using available genotypic, phenotypic, life history and distributional data, Secchi et al. (2003) proposed that franciscana dolphins should be splitted into four stocks for management purposes. Secchi et al. (2003) emphasized that the arguments presented in their study for the proposed divisions are not immutable and they recommended, for further improvement, studies on contaminant load, among other surveys. Therefore, one of the objectives of the present study was to provide supplementary contribution for future studies in which contaminant profiles can be used as auxiliary tools for differentiating franciscana dolphin populations.

Due to its nearshore distribution, the franciscana dolphin may be vulnerable to the effects of human activities and high

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mortalities are reported due to incidental catches. For these reasons *Pontoporia blainvillei* is the most endangered cetacean species from South Atlantic Ocean, and it is listed as 'vulnerable' by IUCN – the World Conservation Union (Bastida et al., 2007). Considering this vulnerability, it is of great interest to investigate if the accumulation of persistent bioaccumulative toxicants can pose an additional threat to this dolphin species. Consequently, the present study aimed to evaluate the organochlorine compound bioaccumulation in franciscana dolphins from Southeastern and Southern Brazilian coast.

## 2. Materials and methods

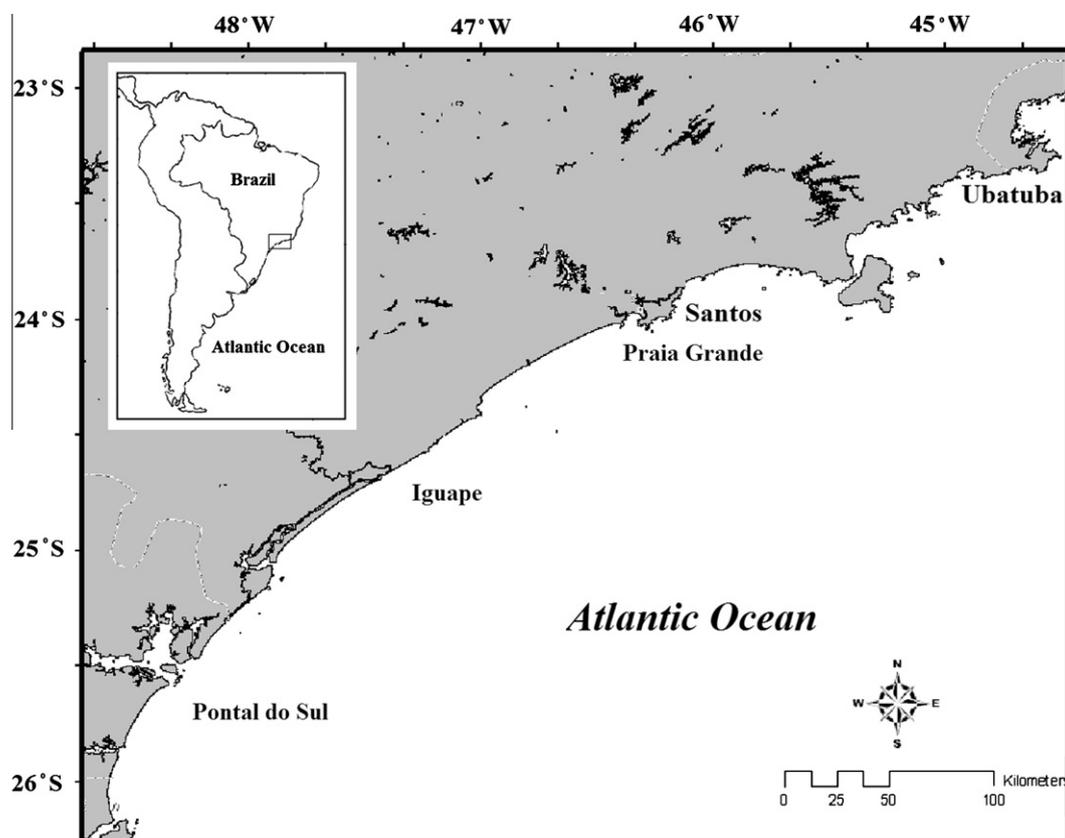
Blubber samples were collected from eight male and two female franciscana dolphins either incidentally captured in fishing operations or stranded on the beaches of São Paulo (SP = 8) and Parana (PR = 2) states, Southeastern and Southern Brazilian coast, respectively (Fig. 1). Most of the SP sampling area constitutes a region strongly influenced by pollution discharge from the industrial complex of Cubatão City, one of the largest of Brazil. Besides, it is a highly populated area that contains the major port structure of Brazil (Lamparelli et al., 2001).

After dissection, samples were wrapped in aluminum foil and kept frozen ( $-20\text{ }^{\circ}\text{C}$ ) until analyses. The adopted extraction method was a variation from those proposed by Azevedo-Silva et al. (2007) and Lailson-Brito et al. (2010). Aliquots of approximately 1.0 g of blubber were homogenized with anhydrous sodium sulfate and extracted by continuous Soxhlet apparatus, for 8 h using a mixture of hexane:dichloromethane (1:1). An aliquot (1 mL) was mixed with sulphuric acid for the clean-up. After centrifugation and phase separation, an internal standard (octachloronaphthalene)

was added for the quantification. The lipid content was measured gravimetrically.

Analyses were performed in a gas chromatographer with an electron capture detector (GC-ECD), coupled to an Automatic Injector (Shimadzu Co.). Injector and detector temperatures were kept at  $250\text{ }^{\circ}\text{C}$  and  $310\text{ }^{\circ}\text{C}$ , respectively. A fused silica DB-5 capillary column (J&W Scientific Inc.,  $30\text{ m} \times 0.25\text{ i.d. mm}$  and film thickness  $0.25\text{ }\mu\text{m}$ ) was used. The column temperature was programmed from  $100\text{ }^{\circ}\text{C}$ , held for 4 min, and increased from  $100$  to  $205\text{ }^{\circ}\text{C}$  at a rate of  $5\text{ }^{\circ}\text{C}/\text{min}$ . The temperature was held for 15 min at  $205\text{ }^{\circ}\text{C}$  and increased at a rate of  $2\text{ }^{\circ}\text{C}/\text{min}$  to the final temperature of  $290\text{ }^{\circ}\text{C}$  (Azevedo-Silva et al., 2007). The acquisition, integration, and calculation of data were performed with *Borwin 2.0* Software System.

The standard solutions used for PCBs (PCB congener mix # 6 – Food & Human Tissue Analysis, Accustandard<sup>®</sup> Laboratory) as well as for organochlorine pesticides (o,p'-DDT, p,p'-DDT, p,p'-DDD, p,p'-DDE and HCB) were from Dr. S. Ehrenstorfer – Prochem Laboratory. The analytical method was validated using a standard certified material (Cod Liver Oil – SRM-1588, National Institute of Standards and Technology – NIST). The accuracy of the method was strengthened by an intercalibration exercise performed with Tuna muscle from International Atomic Energy Agency (IAEA), since it has produced satisfactory results as well. The recovery of all compounds used in this paper ranged from 70% to 130%. The organochlorine pesticides determined were: HCB, o,p'-DDT, p,p'-DDT, p,p'-DDD and p,p'-DDE. Concerning PCBs, the following 27 congeners (IUPAC numbers) were targeted for analysis: 08, 28, 37, 44, 49, 52, 60, 66, 70, 74, 87, 99, 101, 105, 118, 128, 138, 153, 156, 158, 166, 169, 170, 179, 180, 183, 187. In addition to the quality control measures above mentioned, regular analyses of procedural blanks were performed (Relative Standard Deviation – RSD < 20% for eight replicates).



**Fig. 1.** South America map stressing Brazil and amplifying the study area, i.e., the region that extends from Pontal do Sul (Paraná State) to Ubatuba (São Paulo State), Southern and Southeastern Brazilian coast, respectively.

Descriptive statistics was employed on the determination of measures of central tendency to the replications of each sample, as well on the determination of standard deviation (STATISTICA 6.0 – Statistical Software System). Shapiro–Wilk's W test was used in order to test for normality of the data. The tests pointed that the data generated by this study did not present normal distribution. Considering this information, Spearman (rS) test was used for investigating the occurrence of correlation between the concentration of each compound and the variable total length, as well as for investigating the occurrence of correlation between the concentrations of the different compounds.

### 3. Results and discussion

PCB, DDT and HCB concentrations, as well as  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratios, in blubber of franciscana dolphins are summarized in Table 1. All concentrations presented herein are expressed in  $\text{ng g}^{-1}$  lipid weight basis since variation in lipid content among organisms or among tissue types can affect the organochlorine compound concentrations. No correlation was found between total length and DDT, PCB or HCB concentrations. However, significant relationships between DDT vs. HCB as well as between PCB vs. HCB were verified (Spearman correlations  $r = 0.63$  and  $r = 0.91$ , respectively;  $p < 0.05$ ). These findings indicate that those compounds behave similarly considering both the bioaccumulation process and the uptake form.

#### 3.1. HCB

Among the different organochlorines determined, HCB was the compound that rendered the lowest concentrations, with similar values among individuals from different areas of São Paulo State. Therefore, the present study corroborates results from previous investigations, since Yogui et al. (2003) and Kajiwara et al. (2004) reported low HCB concentrations in coastal cetaceans from Paraná and Southern São Paulo. The low HCB values can be a consequence of its high volatility and hence high dispersion capacity from source area due to high atmospheric mobility (Wania and Mackay, 1996).

#### 3.2. DDT

The commercial DDT mixture is composed of p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-DDE and o,p'-DDE (WHO, 1979), but we have only determined the concentrations of four compounds: o,p'-DDT, p,p'-DDT, p,p'-DDD and p,p'-DDE. The p,p'-DDE predominated in all samples, which contrasts with commercial formulas, considering that there is predominance of p,p'-DDT in those products. When released to the environment, p,p'-DDT degrades

into p,p'-DDE and p,p'-DDD. Therefore, the ratio between p,p'-DDE and the sum of p,p'-DDT, p,p'-DDE and p,p'-DDD ( $\text{p,p'-DDE}/\text{p,p'-DDT} + \text{p,p'-DDE} + \text{p,p'-DDD}$ ) was used for determination of the recentness of DDT entrance in the ecosystem (Aguilar, 1984). The distribution of the mean percentages decreased in the following order: p,p'-DDE > p,p'-DDD > p,p'-DDT (Fig. 2). Since the higher the percentage of p,p'-DDE the less recent is the DDT release in the environment, the results pointed to a not recent usage of DDT in the region. The same pattern was also reported for other cetaceans from Brazilian coast (Yogui et al., 2003; Lailson-Brito, 2007; Lailson-Brito et al., 2010).

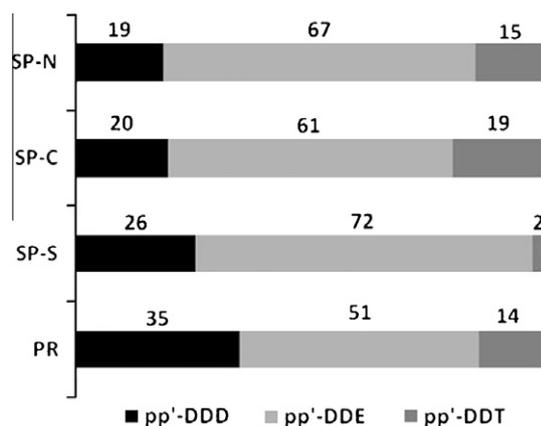
#### 3.3. PCB

The PCB concentrations verified in the present study were in the same range as levels previously reported for franciscana dolphins from Southern São Paulo and Northern Paraná (Kajiwara et al., 2004; Table 2). However, it is interesting to highlight that a higher number of PCBs (101 congeners) contributed to the  $\Sigma\text{PCB}$  in Kajiwara et al. (2004) than in the present study (27 congeners). Therefore, it is likely that the franciscana dolphins from the Central São Paulo Littoral (Santos metropolitan area) present higher PCB concentrations than individuals of the same species inhabiting Southern São Paulo and Northern Paraná. This statement is also based on the fact that the Central São Paulo Littoral finds itself under greater industrial influence. However, this would be only known for sure if the same number of congeners had been analyzed and compared.

Considering the organochlorine concentrations in coastal cetaceans from Brazilian waters (Table 2), apparently, higher levels were observed in Guiana dolphins (Yogui et al., 2003; Kajiwara et al., 2004; Lailson-Brito et al., 2010). As mentioned, Kajiwara et al. (2004) determined a higher number of PCB congeners (101), but the other two investigations dealt with the same number of congeners than the present study (27). The PCBs levels found in franciscana dolphins were also lower when compared to those detected in delphinid species from Mediterranean Sea (Borrell et al., 2001, 2006; Aguilar et al., 2002; Borrell and Aguilar, 2005). The latter area is well-known as a highly impacted ecosystem (Aguilar et al., 2002) and this could explain the higher PCB levels in Mediterranean delphinids. However, PCB concentrations in Guiana dolphins from Guanabara Bay (Rio de Janeiro state, Brazil) were in similar range of those observed in the quoted delphinids (Lailson-Brito et al., 2010). A possible explanation for lower organochlorine concentrations in franciscana dolphins is related to the fact this species is smaller than the delphinid species, including Guiana dolphins (Bastida et al., 2007). This means that these delphinids own anatomical characteristics that enable them to

**Table 1**  
Organochlorine ( $\Sigma\text{DDT}$ ,  $\Sigma\text{PCB}$  and HCB) concentrations ( $\text{ng g}^{-1}$ , on a lipid basis); sex (M = male and F = female); Total Length (TL); Lipid content (%);  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratio of blubber of franciscana dolphins from Southeastern and South Brazilian coast.

	Sex	TL (cm)	Lipid Content (%)	$\Sigma\text{DDT}$	$\Sigma\text{PCB}$	HCB	$\Sigma\text{DDT}/\Sigma\text{PCB}$	Area
Pb01	M	117	96	1364	4832	52	0.28	Praia Grande, SP
Pb02	M	110	91	1087	4033	38	0.27	Praia Grande, SP
Pb03	M	111	80	445	1421	18	0.31	Praia Grande, SP
Pb04	M	113	70	1680	5849	61	0.29	Praia Grande, SP
Pb05	M	80	66	264	909	10	0.29	Praia Grande, SP
Pb06	M	106	86	1554	3709	48	0.42	Ubatuba, SP
Pb07	M	117	85	900	3306	30	0.27	Praia Grande, SP
Pb08	F	109	96	460	1480	15	0.31	Praia Grande, SP
Pb09	M	110	17	5811	3641	49	1.60	Iguape, SP
Pb10	F	80	65	1890	996	16	1.90	Pontal do Sul, PR
Min–max values			17–96	264–5811	909–5849	10–61	0.27–1.9	
Mean $\pm$ sd			82.5 $\pm$ 23.46	1545.5 $\pm$ 1599.4	3017.6 $\pm$ 1723.56	33.7 $\pm$ 18.32	0.59 $\pm$ 0.61	
Median			82.5	1225.5	3473.5	34	0.3	



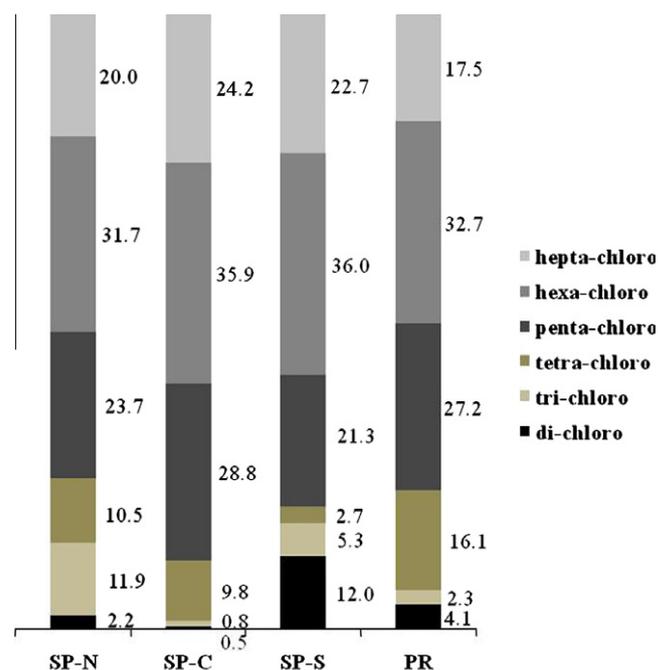
**Fig. 2.** Percentages of p,p'-DDT, p,p'-DDE and p,p'-DDD in blubber of franciscana dolphins (*Pontoporia blainvillei*) from Southeastern and Southern Brazilian coast. SP-N: Northern São Paulo coast; SP-C: Central São Paulo coast; SP-S: Southern São Paulo coast; PR: Paraná.

feed on larger fish, i.e., on prey individuals that had a longer exposure time to pollutants than those preyed by franciscana dolphins.

Concerning PCBs specifically, there was predominance of PCB-153, followed by PCB-138, as observed in previous studies dealing with other cetacean species from Brazil (Yogui et al., 2003; Lailson-Brito et al., 2010). The mean concentration of the sum of the seven indicator PCBs (congeners #28, #52, #101, #118, #138, #153 and #180) was  $1691.02 \pm 946.3 \text{ ng g}^{-1}$ . The sum of the indicator PCBs represented in average 56% of the total PCB concentration verified in this study. There was predominance of hexa- and heptachlorinated congeners (#138, #153, #180) compared to the lower chlorinated indicator PCBs (#28, #52, #101). With regard to composition of distinct congeners, it is interesting to verify that there is a tendency of increased contribution of the low-chlorinated PCBs, both northward and southward from the central area (Fig. 3). This finding seems to reflect the dispersion from industrial complex in Santos region (Central São Paulo coast), since low-chlorinated PCBs are believed to be more efficiently dispersed due to their lower vapor pressure (Wania and Mackay, 1996).

### 3.4. DDT/PCB ratio

The ratio between DDTs and PCBs ( $\Sigma\text{DDT}/\Sigma\text{PCB}$ ) has been used for characterizing the magnitude of the contributions from agricultural and industrial sources to marine mammal contamination (Aguilar et al., 1999), independently of the number of congeners



**Fig. 3.** Percentage of the PCB congener groups to the  $\Sigma\text{PCB}$  in blubber of franciscana dolphin (*Pontoporia blainvillei*) from Northern (SP-N), Central (SP-C) and Southern (SP-S) São Paulo and Paraná (PR) coasts.

determined. The  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratio varied between 0.27 and 0.42 in Northern and Central region of São Paulo State, which points to a greater influence of pollution by organochlorines of industrial origin. Contrastingly, analyzing Guiana dolphins from Southern São Paulo State, Yogui et al. (2003) verified a higher  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratio (6.5). Similarly, the two analyzed franciscana dolphins from this latter region (Pb09 and Pb10) rendered  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratios as high as 1.60 and 1.90 (Table 1). This corroborates data generated by Kajiwara et al. (2004), analyzing franciscana dolphins from Southern São Paulo and Northern Paraná, since  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratios higher than 1.0 were verified, which points to a greater influence of pollution by organochlorines of agricultural origin. Despite the low number of individuals, which does not allow the drawing of conclusions, the organochlorine profile verified in these two franciscana dolphins (Pb09 and Pb10) from Paraná and Southern São Paulo corroborates the agricultural contaminant signature observed in the same area by Kajiwara et al. (2004), analyzing franciscana dolphins, as well as by Yogui et al. (2003), investigating

**Table 2**

Minimum, maximum, mean ( $\pm\text{SD}$ ) and median values of adipose tissue  $\Sigma\text{PCB}$  and  $\Sigma\text{DDT}$  concentrations ( $\text{mg kg}^{-1}$ , on a lipid basis) of coastal cetaceans (franciscana dolphins, *Pontoporia blainvillei*, and Guiana dolphins, *Sotalia guianensis*) from Brazilian coast, including number of analyzed individuals (N) and sampling area, comprising data generated by the present study as well as information from literature.

Species/Reference	N	Area	$\Sigma\text{DDT}$ $\text{mg kg}^{-1}$ lipid	$\Sigma\text{PCB}$ $\text{mg kg}^{-1}$ lipid
<i>Pontoporia blainvillei</i>				
<sup>a</sup> Present study	10	São Paulo and Paraná, Brazil	0.26–5.81 1.55 ( $\pm 1.59$ ) 1.22	0.91–5.85 3.02 ( $\pm 1.72$ ) 3.47
Kajiwara et al. (2004) <sup>b</sup>	26	São Paulo and Paraná, Brazil	0.58–35.0	0.32–12.0
<i>Sotalia guianensis</i>				
Yogui et al. (2003) <sup>c</sup>	9	São Paulo, Brazil	0.54–125.0 35.96	0.20–9.22 4.61
Kajiwara et al. (2004) <sup>b</sup>	26	São Paulo and Paraná, Brazil	1.0–150.0	1.3–79.0
Lailson-Brito et al. (2010) <sup>a</sup>	12	Rio de Janeiro, Brazil	2.08–21.5 8.37	6.66–99.2 21.62

<sup>a</sup> PCBs = (27 congeners).

<sup>b</sup> PCBs = (101 congeners).

<sup>c</sup> PCBs = (27 congeners, but not the same from present study).

Guiana dolphins. This organochlorine profile seems to contrast with the industrial contaminant signature verified in franciscana dolphins from Northern and Central São Paulo coast. This apparent difference strengthens the need for studies that comprise the use of contaminant profiles as an auxiliary tool for discriminating distinct populations or management stocks of franciscana dolphins.

It is important to highlight that all franciscana dolphins analyzed in the present study, as well as in Kajiwaru et al. (2004), inhabited the same Franciscana Management Area (FMA II) among the FMAs proposed by Secchi et al. (2003). It is essential to bear in mind that the four FMAs proposed by the latter authors are related to genetic populations, whereas contaminant profiles can be used for differentiating ecological populations. Therefore, the data generated by the present study, as well as by other investigations (Kajiwaru et al., 2004), suggest being possible to find distinct ecological populations of franciscana dolphins within the same FMA. This hypothesis is also supported by an investigation on radio tracking carried out at Bahía Samborombón and Bahía Anegada (Argentina), since the study revealed limited movement patterns and a small home range for franciscana dolphins (Bordino et al., 2007).

Pollutant determination can help discriminating ecological populations of marine mammals regardless of the age of the analyzed individuals, since the important aspect to be considered in this approach is the proportion among the different compounds rather than the levels (Lailson-Brito et al., 2010). The predominance of PCB over DDT in marine mammal tissues, as observed in most of the individuals analyzed in the present study, had already been reported for waters under strong industrial influence in Rio de Janeiro, Southeastern Brazil (Lailson-Brito, 2007; Lailson-Brito et al., 2010). With regard to the agricultural contaminant signature, considering the important contribution of DDTs, the fight against malaria is a possible source of environmental contamination by this class of organochlorine pesticide (Lailson-Brito et al., 2008).

Considering the endocrine disruptive action of organochlorine compounds, the concentrations found in franciscana dolphins from Brazilian waters may represent an additional obstacle to the conservation of this endangered cetacean species. This apprehension is augmented if recently published information on concentrations of organobrominated (Leonel et al., 2007) and perfluoroalkyl compounds (Leonel et al., 2008) in franciscana dolphins from Brazilian waters is taken into account, since a number of reproductive disorders observed in mammals have been attributed to environmental exposure to a cocktail of persistent bioaccumulative toxicants (Sonne, 2004, 2010; Letcher et al., 2010).

#### 4. Conclusions

The results reported in the present study show new insights about contamination by organochlorine compounds in franciscana dolphins. The difference in  $\Sigma\text{DDT}/\Sigma\text{PCB}$  ratio between individuals from Northern and Central region of São Paulo State and from Paraná and Southern São Paulo points to different sources to marine mammal contamination in the study area. The concentrations of organochlorine compounds found in franciscana dolphins may be considered an additional obstacle to the conservation of this endangered cetacean species. However, more studies are necessary for a better understanding of this contamination and its impact to the conservation of franciscana dolphins.

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