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Levels of persistent organic pollutants and residual pattern of DDTs in small cetaceans from the coast of São Paulo, Brazil

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ABSTRACT

The State of São Paulo is the most developed area in Brazil and was impacted by persistent organic pollutants for several decades. This study investigated organochlorines in five species of small cetaceans (*Pontoporia blainvillei*, *Stenella frontalis*, *Sotalia guianensis*, *Tursiops truncatus* and *Steno bredanensis*) found dead along the coast of São Paulo between 1997 and 2003. DDTs ($15.9 \mu\text{g g}^{-1}$ lipid; mean for all pooled individuals) and PCBs ($8.08 \mu\text{g g}^{-1}$) exhibited the highest concentrations in the animals, reflecting large amounts formerly used in Brazil. Lower levels of mirex ($0.149 \mu\text{g g}^{-1}$), HCB ($0.051 \mu\text{g g}^{-1}$), CHLs ($0.008 \mu\text{g g}^{-1}$) and HCHs ($0.007 \mu\text{g g}^{-1}$) were detected in all species. Residual pattern of DDTs in dolphins suggests that *o,p'*-DDT is more recalcitrant than *p,p'*-DDT in the body of the animals and/or the environment. In contrast to *p,p'*-DDT, residues of *o,p'*-DDT seem to be preferentially converted into *o,p'*-DDD rather than *o,p'*-DDE.

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Limited information has been published on the use of persistent organic pollutants (POPs) in developing countries and their residual pattern in marine mammals inhabiting waters of the southern hemisphere. In Brazil, the first use of organochlorines (OCs) was recorded in 1946 when insecticides such as HCH and DDT were applied to local crops (MMA, 2006). Large amounts of chlorinated pesticides and polychlorinated biphenyls (PCBs) were used in agriculture, public health and industry for decades. In the 1980s, regulations were issued prohibiting PCBs (Directive MIC/MI/MME No. 19/1981) and restricting chlorinated pesticides in agriculture (Directives MAPA No. 329/1985 and No. 153/1988). In the 1990s,

another regulation prohibited the use of OCs in public health campaigns (Directive MS No. 11/1998). At present, OCs such as lindane are still permitted to be used as wood preservatives (Almeida et al., 2007).

The State of São Paulo is located on the southeastern coast of Brazil (Fig. 1) and is one of the most developed areas in South America. The coast of São Paulo has been historically impacted by human activities such as industry, agriculture and tourism. A significant fraction of the Brazilian inventory of POPs (Table 1) was used in São Paulo State. This study presents levels of OCs in the blubber of 13 small cetaceans belonging to five species: *Pontoporia blainvillei* ($n = 8$), *Stenella frontalis* ($n = 2$), *Sotalia guianensis* ($n = 1$), *Steno bredanensis* ($n = 1$) and *Tursiops truncatus* ($n = 1$).

Animals investigated in this study were either incidentally caught in drift nets or found stranded on beaches along the coast of São Paulo (ca. 600 km). All carcasses were fresh and sampling

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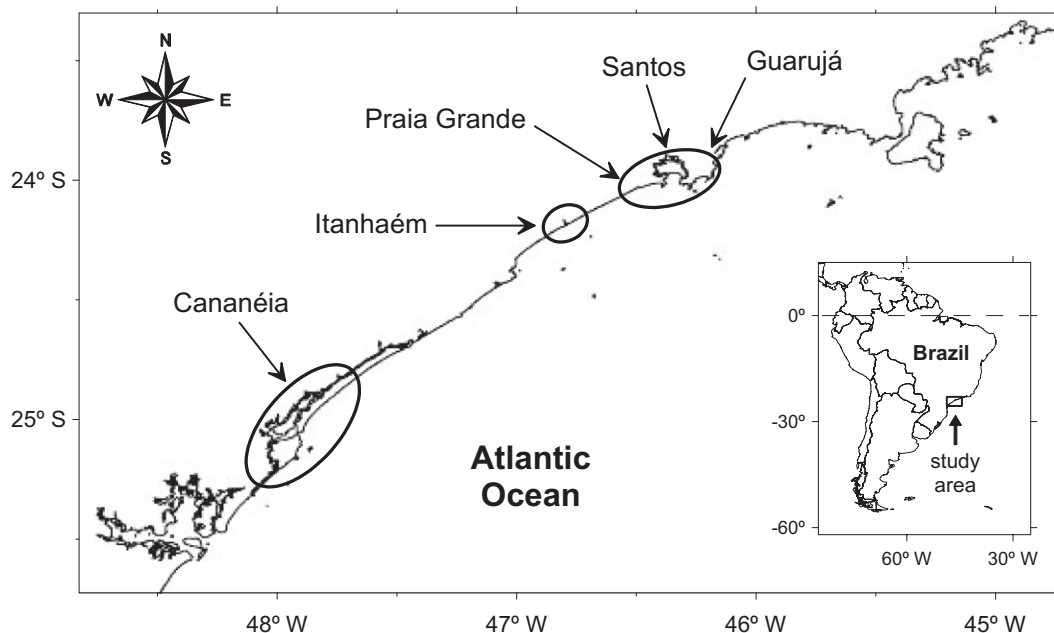


Fig. 1. Geographical setting and location of the coast of São Paulo, southeastern Brazil. Animals analyzed in this study were reported in the circled areas.

Table 1

Estimated inventory of persistent organic pollutants (POPs) used in Brazil. Source: MMA (2006) and Almeida et al. (2007).

Chemical	Estimated amount (metric tons)	Origin
DDTs	110,000	Local production (69%), import (31%)
HCHs	26,400	Local production (70%), import (30%)
PCBs	26,000	Import
Aldrin	17,300	Import
Endrin	10,600	Import
Heptachlor	6400	Import
Mirex	300	Import

procedures followed international standards for the analysis of trace organic contaminants (see Aguilar, 1985; Borrell and Aguilar, 1990; UNEP/ICES/IOC, 1991).

OC analyses were performed following procedures described by Yogui et al. (2003). Briefly, 1 g of blubber was extracted in Soxhlet apparatus for 8 h using 70 mL of *n*-hexane and dichloromethane (1:1, v/v). The extracts were then treated with sulfuric acid for clean up and concentrated to ~0.5 mL prior to injection. Lipids were gravimetrically determined using an aliquot of the original extract. GC-ECD analyses were performed in a Hewlett Packard 5890 series II gas chromatograph using a DB-5 capillary column (25 m × 0.32 mm × 0.52 μm). Hydrogen was used as carrier gas under constant pressure (40 kPa at 100 °C), whereas nitrogen was used as make-up at a rate of 30 mL min⁻¹. The oven temperature was programmed as follows: 100 °C for 1 min, ramped at 5 °C min⁻¹ to 140 °C (holding at this temperature for 1 min), ramped at 1.5 °C min⁻¹ to 250 °C (holding for 1 min), and ramped at 10 °C min⁻¹ to 300 °C with a final hold time of 10 min.

Quality assurance/quality control (QA/QC) criteria were based on Wade and Cantillo (1994). Quantification of target analytes was based on internal standards (dibromooctafluorobiphenyl, 2,2',4,5',6-pentachlorobiphenyl and 2,2',3,3',4,5,5',6-octachlorobiphenyl). The average recovery of internal standards was 89 ± 3.4% (95% level of confidence). Two blubbers were spiked with OC standards and analyzed in duplicate. The mean recovery of standards in the spiked matrices was 105 ± 4.7% (95% level of confidence). The

relative percent difference (RPD) between the spiked duplicates was 13 ± 4.3% (95% level of confidence). The NIST SRM 1588a (organics in cod liver oil) was also analyzed. Recovery of certified and reference analytes in the SRM averaged 95 ± 8.0% (95% level of confidence) of the certificate values. Analytes in laboratory blanks were subtracted from samples. Laboratory check solutions were routinely injected into the GC-ECD to confirm instrument accuracy and precision. Calibration of the instrument was performed using a six-level calibration curve. The mean limits of detection for HCHs, DDTs, PCBs, HCB, chlordanes and mirex were 1.5, 4.4, 2.1, 0.5, 0.5 and 0.1 ng g⁻¹ lipid, respectively. All analytes detected below the limits of detection were set as zero for calculation purposes.

DDTs and PCBs exhibited the highest levels of OCs in the blubber of small cetaceans from the coast of São Paulo (Table 2), reflecting large amounts used in Brazil, persistence in the environment and biomagnification in marine food webs. Historically, DDTs and PCBs are among the most used POPs in Brazil (see Table 1). HCHs were also extensively employed in both agriculture and public health campaigns. HCB was not used as a pesticide although it was produced as a by-product of industrial processes (MMA, 2006). In the vicinities of the Santos estuary there are two sites known to be contaminated with PCBs and another one contaminated with HCB (MMA, 2006). Despite their historical inventory, HCHs and HCB were found at lower levels in all analyzed animals. Such findings are in agreement with other studies carried out in Brazil (see Fillmann et al., 2007; Kajiwara et al., 2004). In contrast to other POPs, lower levels of HCHs and HCB may be a consequence of their high volatility in tropical areas (Tanabe et al., 1993). Alternatively, HCB might not be biomagnified in the local food web. This has been observed in the marine environment surrounding the Antarctic Peninsula (Goerke et al., 2004).

Low concentrations of chlordane-related compounds (CHLs) in the investigated animals support their limited use in Brazil. However, CHL levels are expected to be somewhat higher than those presented in this study since only α- and γ-chlordane were targeted in our analyses. According to Kawano et al. (1988), *trans*-nonachlor is the constituent most retained by marine mammals. In addition, α- and γ-chlordane are converted into *oxy*-chlordane

Table 2
Biological information and concentration of organochlorines ($\mu\text{g g}^{-1}$ lipid) in small cetaceans from the coast of São Paulo, Brazil.

Species	Field no.	Location	Length (cm)	Growth stage	Sex	Lipid (%)	Σ PCB ^a ($\mu\text{g g}^{-1}$ lipid)	Σ DDT ^b ($\mu\text{g g}^{-1}$ lipid)	Σ HCH ^c ($\mu\text{g g}^{-1}$ lipid)	Σ CHL ^d ($\mu\text{g g}^{-1}$ lipid)	HCb ($\mu\text{g g}^{-1}$ lipid)	Mirex ($\mu\text{g g}^{-1}$ lipid)
<i>Pontoporia blainvillei</i>	BP 010	Praia Grande	106	Juvenile	M	81.1	6.14	2.89	0.004	0.005	0.033	0.055
	BP 024	Praia Grande	120	Adult	M	87.6	4.54	1.38	<LD	0.007	0.022	0.049
	BP 031	Guaruja	93	Juvenile	M	83.7	1.95	1.18	<LD	<LD	0.053	0.046
	BP 027	Itanhaém	120	Adult	M	82.5	9.71	6.89	0.007	<LD	0.108	0.100
	BP 011	Praia Grande	134	Adult	n = 4	83.7 ± 2.8	5.59 ± 3.24	3.08 ± 2.65	0.003 ± 0.003	0.006	0.054 ± 0.038	0.063 ± 0.025
	BP 012	Praia Grande	130	Adult	F	92.2	3.98	1.02	<LD	0.003	0.014	0.040
	BP 026	Praia Grande	64	Newborn	F	89.5	3.63	0.79	<LD	0.001	0.011	0.066
<i>Stenella frontalis</i>	PA-132	Cananéia	130	Juvenile	F	81.4	1.29	2.28	<LD	0.005	0.015	0.053
	BP 029	Santos	187	Adult	n = 4	85.2 ± 6.8	2.35 ± 1.72	1.10 ± 0.84	<LD	0.003 ± 0.003	0.013 ± 0.002	0.041 ± 0.026
	BP 030	Santos	208	Adult	M	47.7	19.8	35.8	0.020	0.005	0.118	0.399
<i>Sotalia guianensis</i>	BP 033	Cananéia	196	Adult	n = 2	54.8 ± 10.0	19.3 ± 0.7	31.7 ± 5.7	0.023 ± 0.002	0.005 ± 0.001	0.113 ± 0.007	0.393 ± 0.008
	BP 025	Praia Grande	263	Adult	M	68.6	1.97	5.87	0.011	0.014	0.067	0.046
	QOM-001	Itanhaém	163	Adult	M	70.2	26.8	118	0.014	0.013	0.018	0.600
<i>Tursiops truncatus</i>	QOM-001	Itanhaém	163	Adult	M	73.6	5.91	2.42	0.008	0.038	0.080	0.094

^a Sum of PCBs 8, 18, 44, 49, 50, 52, 66, 87, 101, 105, 110, 118, 128, 138, 149, 151, 153, 157, 160, 169, 170, 173, 180, 194, 195, 206 and 209.

^b Sum of *o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, *p,p'*-DDE and *p,p'*-DDE.

^c Sum of α -HCH, β -HCH, γ -HCH and δ -HCH.

^d Sum of α -chlordane and γ -chlordane.

in the environment and the latter is more persistent than its precursors (Wells et al., 1994). Kajiwara et al. (2004) analyzed CHLs including *trans*-nonachlor and *oxy*-chlordane in cetaceans from southeastern Brazil. These authors' results reinforce the hypothesis of our underestimated chlordane values since they found total chlordanes an order of magnitude higher. Mirex was detected at higher levels than HCB and HCHs in all analyzed dolphins. Such a result is likely associated with the higher chlorination level of mirex which may contribute to its persistence in the environment. Mirex was used as both pesticide and flame retardant in the State of São Paulo.

Among the investigated species, *S. guianensis* and *P. blainvillei* have been commonly registered in inshore, turbid waters of the São Paulo coast (Bertozzi and Zerbini, 2002; Rosas et al., 2002; Santos et al., 2001a). Keeping in mind that DDTs and PCBs may be used as chemical markers for agricultural and industrial activities, the DDTs/PCBs ratio in *S. guianensis* (3.0) and *P. blainvillei* (0.46) reflects the major use of their habitats – Cananéia and Santos estuaries, respectively. The Cananéia estuary is an agricultural area while the Santos estuary is highly industrialized. Interestingly, the only specimen of *P. blainvillei* collected in coastal waters off Cananéia exhibited DDTs/PCBs ratio of 1.8. Based on data published by Kajiwara et al. (2004), mean DDTs/PCBs ratio for *P. blainvillei* caught in the vicinities of the Cananéia estuary is around 1.1. This is in agreement with our findings and might suggest spatial segregation of *P. blainvillei* along the coast of São Paulo.

A comparison of the residual pattern of DDTs in the small cetaceans from the coast of São Paulo and the technical formulation used in Brazil is shown in Fig. 2a. Active ingredients (i.e., *p,p'*-DDT and *o,p'*-DDT) are predominant in the commercial mixture. In contrast, the metabolite *p,p'*-DDE was found to be the predominant compound in all investigated animals. Similar residual pattern has been observed in small cetaceans from several parts of the world (e.g., Corcuera et al., 1995; Karuppiyah et al., 2005; Minh et al., 1999; Tanabe et al., 1993).

An interesting residual pattern was observed in the animals when separating the compositions of *o,p'*-DDT and *p,p'*-DDT. An inverse distribution is observed between the commercial mixture and small cetaceans for *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE (Fig. 2b). The precursor compound (*p,p'*-DDT) is found at higher proportion in the technical mixture while its metabolites were detected at higher percentages in the animals. This sort of distribution has been observed in other marine mammals (e.g., Fillmann et al., 2007; Kajiwara et al., 2004). In contrast, similar trends were not observed for *o,p'*-DDT and its metabolites (*o,p'*-DDD and *o,p'*-DDE) (Fig. 2c). In this case, *o,p'*-DDT is found at major proportions in both the technical formulation and the animals. To our knowledge the latter trend is a novel finding that has not been addressed by other authors (probably because *p,p'*-DDT is environmentally more important than *o,p'*-DDT – see Fig. 2a) and might suggest that *o,p'*-DDT is more stable than *p,p'*-DDT in the animals and/or environment. Furthermore, *o,p'*-DDD occurs at compositionally higher percentages than *o,p'*-DDE in the small cetaceans, suggesting that *o,p'*-DDT is preferentially converted to *o,p'*-DDD in the cetacean's body.

The contamination status of the investigated dolphins is compared in Table 3. Overall, OCs concentration in small cetaceans from the coast of São Paulo is lower than in dolphins from industrialized regions (Corsolini et al., 1995; Johnson-Restrepo et al., 2005; Kuehl and Haebler, 1995; Morris et al., 1989; Tuerk et al., 2005). The specimen of *S. bredanensis* is an exception, though. Little is known about the ecology of this species in Brazilian waters, making difficult further interpretation of contamination data. *S. guianensis* from the Cananéia estuary revealed concentration of DDTs similar to or higher than small cetaceans from India – a country highly impacted by these pesticides (see Kajiwara et al., 2006;

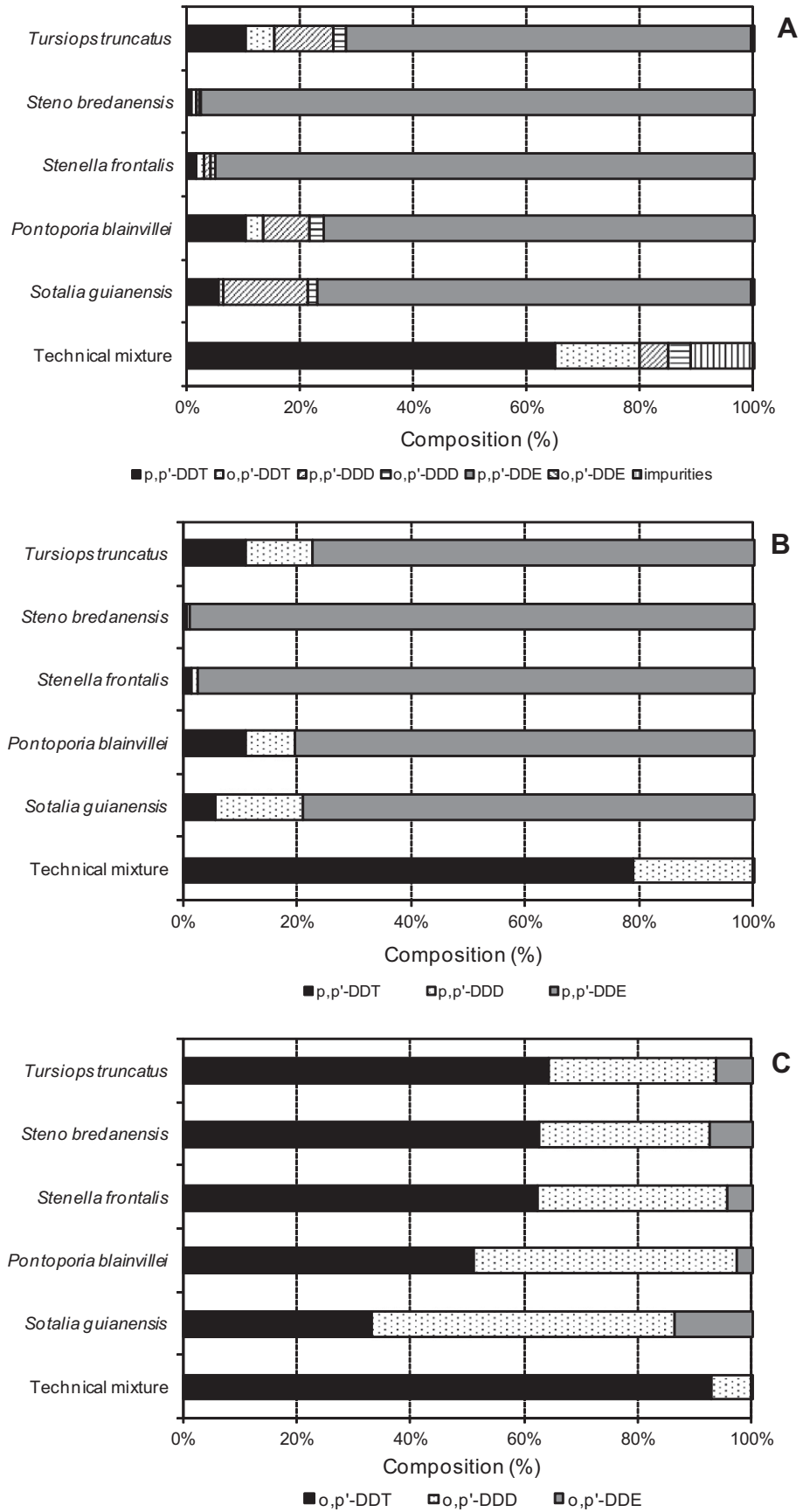


Fig. 2. Distribution of DDT-related compounds in five small cetacean species from the coast of São Paulo and the technical formulation used in Brazil (based on Larini (1993) and Santos et al. (2001b)).

Table 3
Levels of persistent organic pollutants ($\mu\text{g g}^{-1}$ lipid) in the blubber of small cetaceans from all over the world.

Species	Location	Survey years	Sex	n	PCBs ($\mu\text{g g}^{-1}$ lipid)	DDT ($\mu\text{g g}^{-1}$ lipid)	HCHs ($\mu\text{g g}^{-1}$ lipid)	CHLs ($\mu\text{g g}^{-1}$ lipid)	HCB ($\mu\text{g g}^{-1}$ lipid)	Reference
<i>Pontoporia blainvillei</i>	South Atlantic, Brazil	1999–2001	M	4	5.59	3.08	0.003	0.003	0.054	This study
	South Atlantic, Brazil	1999–2001	F	4	2.35	1.10	<LD	0.003	0.013	This study
	South Atlantic, Brazil	1997–1999	M	16	3.10	4.26	0.003	0.048	0.011	Kajiwara et al. (2004)
	South Atlantic, Brazil	1997–1999	F	10	2.22	2.48	0.003	0.038	0.010	Kajiwara et al. (2004)
<i>Stenella frontalis</i>	South Atlantic, Brazil	2001	M	2	19.3	31.7	0.022	0.005	0.113	This study
	South Atlantic, Brazil	1997–1999	M	2	59.0	36.5	0.039	0.675	0.078	Kajiwara et al. (2004)
<i>Stenella longirostris</i>	Bay of Bengal, India	1990–1991	M	3	1.31	37.3	0.744		0.030	Tanabe et al. (1993)
	Bay of Bengal, India	1990–1991	F	2	1.27	42.1	0.966		0.015	Tanabe et al. (1993)
	Bay of Bengal, India	1990–1992		3	1.60	48.0	0.220	0.160	0.028	Kajiwara et al. (2006)
	Indo-Pacific, Philippines	1996		3	3.60	16.0	0.110	0.540	0.220	Kajiwara et al. (2006)
<i>Stenella coeruleoalba</i>	Gulf of Mexico, USA	1994	F	1	51.7					Johnson-Restrepo et al. (2005)
<i>Sotalia guianensis</i>	South Atlantic, Brazil	2003	M	1	1.97	5.87	0.011	0.014	0.067	This study
	South Atlantic, Brazil	1996–2001	M	4	5.70	72.3	0.027	0.033	0.018	Yogui et al. (2003)
	South Atlantic, Brazil	1996–2001	F	5	3.74	6.81	0.006	0.016	0.013	Yogui et al. (2003)
	South Atlantic, Brazil	1997–1999	M	17	21.1	36.1	0.017	0.277	0.040	Kajiwara et al. (2004)
	South Atlantic, Brazil	1997–1999	F	9	11.4	10.4	0.007	0.163	0.022	Kajiwara et al. (2004)
<i>Steno bredanensis</i>	South Atlantic, Brazil	2000	M	1	26.8	118	0.014	0.013	0.018	This study
<i>Tursiops truncatus</i>	Gulf of Mexico, USA	1997	F	6	10.9	5.41	0.020	1.82	0.020	Tuerk et al. (2005) ^a
	South Atlantic, Brazil	1997	M	1	5.91	2.42	0.008	0.038	0.080	This study
	Cardigan Bay, Wales		F	1	760	391	2.07		1.69	Morris et al. (1989)
	Bay of Bengal, India	1990–1991	M	2	1.19	9.28	0.213		0.028	Tanabe et al. (1993)
	Bay of Bengal, India	1990–1991	F	2	0.753	14.9	0.238		0.600	Tanabe et al. (1993)
	Gulf of Mexico, USA	1990–1991	M	9	93.0	37.9		1.05	0.292	Kuehl and Haebler (1995)
	Gulf of Mexico, USA	1990–1991	F	6	7.20	3.71		0.156	0.254	Kuehl and Haebler (1995)
	Mediterranean Sea, Italy		M	5	1192	394				Corsolini et al. (1995)
	Mediterranean Sea, Italy		F	2	587	138				Corsolini et al. (1995)

^a Values expressed on a wet weight basis.

Tanabe et al., 1993). Contamination levels found in this study are in agreement with other studies on marine mammals from the Brazilian coast (see Fillmann et al., 2007; Kajiwara et al., 2004; Silva et al., 2003; Yogui et al., 2003). A review of chlorinated compounds in marine mammals from Central and South America was published by Borrell and Aguilar (1999). A comparison with results from this study shows that animals from the coast of São Paulo are among the most contaminated marine mammals in South America. This emphasizes the importance of continued efforts to investigate POPs in the Brazilian marine environment including the coast of São Paulo.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marpolbul.2010.07.022.

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