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Levels and profiles of chlorinated and brominated contaminants in Southern Hemisphere humpback whales, *Megaptera novaeangliae*

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ABSTRACT

The study documents the levels and profiles of selected contaminants [polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and methoxylated PBDEs (MeO-PBDEs)] in blubber biopsy samples collected from humpback whales (*Megaptera novaeangliae*) in Antarctic Peninsula waters. In addition, we investigated year-to-year and sex-related differences in the bioaccumulation patterns. Except for hexachlorobenzene (HCB), whose concentrations were in the same range as those found in whales from the Northern Hemisphere, levels of all other compounds were lower in Southern Hemisphere whales compared to literature data on animals from the Arctic and subarctic region. The mean contribution to the sum of all anthropogenic organohalogen compounds (ΣOHC) decreased in the following order ΣPCBs (44%) > HCB (31%) > ΣDDXs (13%) > ΣCHLs (4.6%) > ΣHCHs (4.4%) > ΣPBDEs (0.9%). The predominant compounds within each chemical class were: PCBs 153, 149, 101 and 138; *p,p′*-DDE; γ-HCH; *trans*-nonachlor; PBDEs 99 and 47. The most dominant MeO-PBDE congener was 6-MeO-BDE 47. As samples were collected during three consecutive summer seasons, year-to-year trends could be assessed indicating a significant decrease from 2000 to 2003 for ΣCHL levels. Higher ΣPBDE concentrations and higher values of the ΣPBDE / ΣMeO-PBDE ratio, as well as higher ratios between the two MeO-BDEs (2′-MeO-BDE 68/6-MeO-BDE 47) were found in females compared to males. Higher ΣMeO-PBDE concentrations and higher values of the ratios between the lower chlorinated and the higher chlorinated PCBs were found in males than in females. In addition, five out of six significant differences found through discriminant function analysis were gender-related. The literature reports both feeding in mid- to low-latitudes and sex-related differences in migration patterns for humpback whales from the Southern Hemisphere, indicating that the hypothesis of dietary differences between males and females cannot be excluded. Nevertheless, additional studies are required for further investigation of this hypothesis.

1. Introduction

Antarctica remains relatively protected from widespread human disturbance, with the exception of small areas of significant environmental pollution from military and scientific activities (Conlan et al., 2004). This relative protection is a consequence of its remoteness and extreme climate conditions, which makes the whole continent a region of high ecotoxicological interest. Taking

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into account the absence of industrial or agricultural activities in Antarctica, the continent was considered to be free of contamination by anthropogenically-produced organohalogen compounds (OHCs) until the late 1960s, when such contamination was first scientifically recorded (Reisebrough et al., 1968). Since then, a number of investigations have shown trace levels of these pollutants (Corsolini, 2009).

Environmental contamination by OHCs has received considerable attention due to their toxicity, persistency and bioaccumulative nature (Covaci et al., 2003; Fabre et al., 2005; Guo et al., 2009; Sonne, 2010). Environmental OHC pollution is caused by their past widespread use as dielectric fluid and in heat exchange systems (the case of polychlorinated biphenyls-PCBs), as well as their use as insecticides for fighting agricultural pests or controlling insect-borne diseases. This latter was the case for organochlorine pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (HCHs) and chlordanes (Fabre et al., 2005; Guo et al., 2009; Newman and Unger, 2002; Sonne, 2010). Environmental concern has also been raised by the global utilization of polybrominated diphenyl ethers (PBDEs) as flame retardants, since they present similarities with the abovementioned organochlorine pollutants regarding toxicity, persistency and bioaccumulation (Covaci et al., 2003; de Wit, 2002; Law et al., 2006). The high long-range transport potential of OHCs enabled them even to reach remote polar areas (Sonne, 2010).

Methoxylated PBDEs (MeO-PBDEs) have been evidenced in marine environments, with the tetrabrominated 2'-MeO-BDE 68 and 6-MeO-BDE 47 being the most abundant compounds (Vetter et al., 2002). MeO-PBDEs have been reported as having a natural origin (Teuten and Reddy, 2007; Teuten et al., 2005), being formed by sponges (Vetter et al., 2002) or algae (Malmvärn et al., 2005). However, biotransformation of hydroxylated PBDEs (OH-PBDEs) to MeO-PBDEs has also been demonstrated (Wan et al., 2010). These naturally-produced compounds have been detected in marine mammals at concentrations comparable or even higher than those of organobrominated compounds of anthropogenic origin, such as PBDEs (Alonso et al., 2012; Dorneles et al., 2010; Rotander et al., 2012b).

OHCs have been detected in Antarctic marine biota since various species have been used for monitoring purposes (Bengtson Nash, 2011; Corsolini, 2009). The Southern Ocean constitutes feeding ground for several marine mammal species, including large whales. These animals are long-lived upper trophic level predators that are regarded as ideal repository for lipophilic compounds due to their large lipid reserves (O'Shea and Tanabe, 2003). The humpback whale (*Megaptera novaeangliae*) migrates from low latitude breeding regions to high latitude feeding areas (Clapham, 1996). During the end of spring through early fall, Southern Hemisphere humpback whales forage in Southern Ocean waters (Dalla Rosa et al., 2008). Humpback whales are among the most abundant cetaceans in Antarctic Peninsula waters (Secchi et al., 2001; Thiele et al., 2004) and photo-identification studies have shown that individuals of the species have strong site fidelity to the region (Dalla Rosa et al., 2001). These facts turn the humpback whale into the ideal species for ecotoxicological studies in the near-shore waters of the Western Antarctic Peninsula, the operation area of the Brazilian Antarctic Program.

This study reports on organohalogen compound concentrations in humpback whales that feed in nearshore Antarctic Peninsula waters, investigating possible year-to-year and sex-related differences in bioaccumulation patterns of these pollutants.

2. Materials and methods

2.1. Samples

Samples were obtained during research cruises conducted by the Brazilian Antarctic Program (PROANTAR) in the summers of 2000/2001, 2001/2002 and 2002/2003, corresponding to the Brazilian Antarctic Operations (AOs) XIX, XX and XXI, respectively. During these surveys, 65 blubber biopsy samples were obtained from humpback whales in the western Antarctic Peninsula waters. Samples were collected from a 4 m long inflatable boat, using a 120–150 lb crossbow with arrows and tips M8 × 40 mm modified for large cetaceans (Larsen, 1998). In order to reduce the possibility of analysing biopsy samples from the same individual, the sampled whales were photo-identified whenever possible. For sex determination, DNA was extracted from skin samples using the standard phenol–chloroform protocol (Sambrook and Russell, 2001). Subsequently, DNA was used for PCR amplification of the sex-specific ZFY/ZFX genes, as detailed elsewhere (Bérubé and Palsbøll, 1996; Cunha and Solé-Cava, 2007).

2.2. Targeted analytes

Thirty PCB congeners (IUPAC numbers: CB 18, 28, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, and 209), seven PBDEs (IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183), six DDXs (*o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT), chlordanes and metabolites (CHLs), such as oxychlordane (OxC), *trans*-nonachlor (TN), *cis*-nonachlor (CN), *cis*-chlordane (CC), and *trans*-chlordane (TC), three hexachlorocyclohexane (HCH) isomers (*α*-, *β*- and *γ*-HCH), as well as hexachlorobenzene (HCB) were targeted. Two MeO-PBDEs (2'-MeO-BDE 68 and 6-MeO-BDE 47) were also determined.

2.3. Sample preparation

Individual standards for PBDEs and MeO-PBDEs (Wellington Laboratories, Guelph, ON, Canada), as well as for PCBs and OCPs (Dr. Ehrenstorfer Laboratories, Augsburg, Germany) were used for identification and quantification. All solvents used for the analysis (*n*-hexane, acetone, dichloromethane, *iso*-octane) were of pesticide-grade (Merck, Darmstadt, Germany). Sodium sulfate and silica were pre-washed with *n*-hexane before use and dried at 150 °C overnight. Extraction thimbles were pre-extracted for 1 h with the extraction mixture used for the samples and dried at 100 °C overnight. The method used for the clean-up of the samples has been previously described and validated (Covaci et al., 2002; Voorspoels et al., 2003), and is briefly presented below. Between 0.04 and 0.24 g of subcutaneous adipose tissue was accurately weighted, homogenized with approximately 8 g anhydrous sodium sulfate, spiked with internal standards BDE 77/BDE 128 (25 ng) and PCB 46/ PCB 143 (75 ng) and extracted for 2 h by hot Soxhlet with 100 mL hexane/acetone (3/1; v/v). After lipid determination (performed gravimetrically on an aliquot of the extract), the extract was cleaned-up on 8 g of acidified silica. After elution with 15 mL hexane and 10 mL dichloromethane, the cleaned extract was evaporated to near dryness and further redissolved in 200 μL of *iso*-octane.

2.4. Instrumental analyses

PBDEs, MeO-PBDEs, CHLs, and HCHs were measured with an Agilent 6890 GC connected to an Agilent 5973 mass spectrometer (MS) operated in electron capture negative ionization (ECNI) mode. The GC was equipped with a 20 m × 0.18 mm × 0.20 μm AT-5

capillary column (Alltech, Lokeren, Belgium). Methane was used as moderating gas and the ion source, quadrupole and interface temperatures were set at 250, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with ions $m/z=79$ and 81 (for tri- to hepta-BDEs and MeO-PBDEs) monitored during the entire run. For CHLs and HCHs, the two most intense ions were monitored for each compound. Dwell times were set at 40 ms. One microlitre of the cleaned extract was injected in solvent vent mode (injector temperature: 90 °C, held for 0.05 min, then with 700 °C/min to 305 °C and kept for 25 min; vent flow was set at 75 mL/min and the purge vent opened at 1.5 min). Helium was used as carrier gas at constant flow (0.8 mL/min). The temperature of the AT-5 column was kept at 90 °C for 1.50 min, then increased to 200 °C at a rate of 20 °C/min, further increased to 300 °C at a rate of 5 °C/min, kept for 15 min.

PCBs, DDXs and HCB were measured with an Agilent 6890 GC connected to an Agilent 5973 mass spectrometer (MS) operated in electron ionization (EI) mode. The GC was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The mass spectrometer was used in the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homolog group or for each pesticide. One microlitre of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C with 700 °C/min), pressure pulse 25 psi, pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, kept for 12 min.

2.5. Quality assurance/quality control

Multi-level calibration curves in the linear response interval of the detector were created for the quantification and good correlation for intervals that included the expected range of concentrations ($r^2 > 0.999$) was achieved. The identification of OHCs was based on their relative retention times (RRTs) to the internal standards used for quantification, ion chromatograms and intensity ratios of the monitored ions for quantification. A deviation of the ion intensity ratios within 20% of the mean values in the calibration standards was considered acceptable. Recoveries for individual PCB and PBDE congeners ranged between 82% and 108% (RSD ≤ 12%) during the method validation.

For each analyte, the mean procedural blank value was used for subtraction. After blank subtraction, the method limit of quantification (LOQ) was set at 3 times the standard deviation of the procedural blank, which ensures > 99% certainty that the reported value is originating from the sample and not from the blank. Therefore, LOQ depended on the sample intake and on the analyte. For analytes that were not detected in procedural blanks, LOQs were calculated for a signal-to-noise ratio equal to 10. Method LOQs ranged between 0.1 and 0.2 ng/g lipid weight (lw) for the individual compounds.

The quality control was performed by regular analyses of procedural blanks, by random injection of standards and solvent blanks. A standard reference material SRM 1945 (PCBs, OCPs and PBDEs in whale blubber) was also used to test the accuracy of the method. Obtained values were not deviating with more than 10% from the certified values for each analyte. The quality control scheme is also assessed through regular participation to inter-laboratory comparison exercises organized by Arctic Monitoring and Assessment Program (AMAP) and National Institute of Standards and Technology (NIST) from USA.

2.6. Statistical analysis

Depending on data normality (Shapiro–Wilk's W test), parametric (Student's t-test and Pearson's (r) correlation test) or non-parametric tests (Mann–Whitney U test and Spearman's (Rs) correlation test) were used. A discriminant function analysis was performed using the measured pollutants to look for effects from sex and sampling year (Antarctic Operations XIX, XX and XXI correspond to 2000/2001, 2001/2002 and 2002/2003 summers, respectively). From the seven compound class sums (ΣPCB, HCB, ΣDDX, ΣCHL, ΣHCH, ΣPBDE and ΣMeO-PBDE), only ΣPCB was not used in the test, since it did not fulfil the requirements of the tolerance limits (0.01) of the model (discriminant function analysis). Statistical analysis of the data was performed using Statistica® software (Statsoft Inc., version 7.0).

3. Results and discussion

Concentrations of OHCs and MeO-PBDEs measured in blubber samples from humpback whales biopsied in nearshore waters of the Antarctic Peninsula during three consecutive summer seasons are given in Table 1. The percentage contribution (median values) of each compound class to the sum of all anthropogenic OHCs (ΣOHCs) decreased in the following order: ΣPCBs (44%) > HCB (31%) > ΣDDXs (13%) > ΣCHLs (4.6%) > ΣHCHs (4.4%) > ΣPBDEs (0.9%). Blubber ΣOHC levels varied from 32.1 to 825 ng g^{-1} lw (median = 145 ng g^{-1} lw). ΣMeO-PBDE concentrations varied from < 0.20 to 10.4 ng g^{-1} lw (median = 0.19 ng g^{-1} lw).

3.1. Brominated compounds

PBDEs 28, 47, 99, 100, 153, 154 and 183 were detected in 8%, 83%, 100%, 38%, 29%, 50% and 13% of the samples from males, as well as in 15%, 95%, 100%, 66%, 54%, 78% and 20% of the samples from females. The ΣPBDE concentrations varied between 0.15 and 10.9, with the median value of 0.89 ng g^{-1} l.w. for males; as well as from 0.27 to 50.8, with the median value of 1.49 ng g^{-1} l.w. for females. PBDE concentrations found in the present study seemed to be lower than those in minke and fin whales from the Arctic and other regions in the Northern Hemisphere (Moon et al., 2010; Rotander et al., 2012a). In a global pollution monitoring study on PBDE levels in skipjack tuna (Ueno et al., 2004), higher concentrations in samples from the Northern Hemisphere compared to the Southern Hemisphere were also found. This is probably due to larger usage of brominated flame retardants in the northern half of the planet (Ueno et al., 2004). PBDEs comprised the compound class of lowest contribution to the ΣOHC measured in the present study (Table 1). BDE 99 was the predominant PBDE congener for both sexes and the three sampling seasons, followed by BDE 47 (Fig. S1). The summed contribution of BDE 47 and BDE 99 to ΣPBDEs varied from 28.7% to 100% (median of 87.4%) for males and from 26.7% to 100% (median of 78.5%) for females. A similar PBDE profile was found in krill (*Euphausia superba*) sampled in 2006 from the eastern Antarctic (Bengtson Nash et al., 2008), as well as in 2001–2002 from the western Antarctic Peninsula (Chiuchiolo et al., 2004). The arithmetic and the geometric mean of the BDE-99 levels were 0.68 and 0.23 ng g^{-1} l.w. in krill (Bengtson Nash et al., 2008) which were lower than the levels found in the present study (0.75 and 0.54 ng g^{-1} l.w. in male and 1.57 and 0.88 ng g^{-1} l.w. in female humpback whales). These concentrations suggest biomagnification of BDE-99 and the similar profiles point to a specific PBDE bioaccumulation in humpback whale food web around the Antarctic Peninsula. The idea of a specific bioaccumulation is supported when looking at PBDEs in Antarctic fur seals (*Arctocephalus gazella*). Only BDE 47 and 66

Table 1
Concentrations (Mean (Median) ± SD; Min–Max) of PCBs, HCB, DDXs, chlordanes, HCHs, PBDEs and MeO-PBDEs in blubber samples from male (M) and female (F) humpback whales biopsied in the Southern Ocean during the Brazilian Antarctic Operations XIX, XX and XXI (2000/2001, 2001/2002 and 2002/2003 summers, respectively) in ng g^{-1} l.w. The lipid percentages of samples, as well as the mean percentage contribution to ΣOHC [%] of each anthropogenically-produced pollutant class are also shown.

	AO XIX		AO XX		AO XXI	
	M (n=7)	F (n=15)	M (n=9)	F (n=10)	M (n=8)	F (n=16)
Lipid %	47.7 (54.8) ± 16.8 11.2–60.7	39.2 (43.6) ± 16.3 11.7–64.2	52.2 (49.5) ± 15.2 30.8–83.3	51.2 (54.9) ± 12.8 22.5–65.2	52.8 (56.9) ± 15.9 23.1–76.6	45.4 (43.3) ± 12.8 22.5–63.1
ΣPCBs	122 (53.6) ± 207 11.2–590 [39%]	131 (83.3) ± 192 4.4–761 [49%]	46.6 (32.8) ± 39.6 8.8–111 [24%]	71.2 (57.6) ± 63.4 2.6–178 [43%]	53.7 (37.6) ± 48.9 5.8–143 [31%]	82.9 (48.1) ± 103 10.8–420 [51%]
HCB	63.0 (52.1) ± 46.9 15.0–130 [34%]	35.4 (33.1) ± 20.0 6.8–74.5 [24%]	76.2 (72.5) ± 20.3 48.5–101 [44%]	35.8 (38.6) ± 16.0 7.8–62.1 [32%]	59.2 (54.2) ± 28.7 31.0–124 [43%]	24.9 (20.2) ± 12.5 10.4–43.4 [26%]
ΣDDXs	22.9 (22.4) ± 11.7 8.2–39.6 [14%]	21.2 (13.2) ± 34.0 4.0–143 [13%]	37.5 (29.6) ± 22.3 17.5–85.3 [20%]	17.7 (11.0) ± 17.0 2.6–58.9 [14%]	23.9 (18.0) ± 16.5 8.0–56.8 [16%]	11.1 (10.6) ± 5.8 3.9–23.8 [10%]
ΣCHLs	8.24 (7.8) ± 4.79 3.1–15.2 [4.8%]	5.86 (5.7) ± 3.3 1.9–14.4 [4.2%]	11.1 (11.7) ± 3.34 7.4–16.3 [6.3%]	4.95 (5.1) ± 1.84 1.7–7.9 [4.3%]	7.19 (6.5) ± 3.06 3.3–13.7 [5.2%]	3.71 (3.6) ± 1.27 1.6–6.0 [3.9%]
ΣHCHs	13.7 (8.8) ± 16.2 2.6–49.5 [7.6%]	11.5 (9.2) ± 11.0 2.2–43.7 [6.7%]	10.5 (6.3) ± 11.8 3.6–41.1 [5.3%]	6.96 (4.3) ± 5.03 2.2–14.9 [5.5%]	5.23 (4.75) ± 2.4 2.8–10.0 [4.2%]	6.57 (4.55) ± 5.36 1.6–19.0 [6.7%]
ΣPBDEs	2.49 (1.16) ± 3.76 0.45–10.9 [0.8%]	5.77 (1.51) ± 12.6 0.36–50.8 [3.0%]	0.97 (0.79) ± 0.49 0.41–1.78 [0.7%]	1.65 (1.34) ± 1.36 0.27–4.82 [1.2%]	1.32 (0.89) ± 1.38 0.15–4.49 [0.9%]	1.84 (1.57) ± 1.11 0.88–5.35 [1.7%]
ΣMeO-PBDEs	0.34 (0.25) ± 0.48 < LOQ–1.36	0.21 (0.14) ± 0.34 < LOQ–1.37	1.64 (0.4) ± 3.32 0.14–10.4	0.23 (0.18) ± 0.26 < LOQ–0.74	0.3 (0.3) ± 0.27 < LOQ–0.86	0.16 (0.11) ± 0.21 < LOQ–0.79

were found in blubber of Antarctic fur seal pups sampled around the Antarctic Peninsula in 2004 (Schiavone et al., 2009) and levels of BDE 47 in those seals were in the same range as those found in the present study. Since BDE 99 was found in Antarctic krill, which is at the bottom level of the Antarctic food web, one can only conclude that BDE 99 is metabolized before it can reach the top (e.g. Antarctic fur seals) of the Antarctic food chain. The presence of BDE 183 in Antarctic Peninsula humpback whales is noteworthy, since the congener present in the Octa-BDE mixture was not detected in dolphins inhabiting waters around Rio de Janeiro state, a region of high urban and industrial activity in South America (Dorneles et al., 2010). An explanation for this is the possible existence of differences in metabolizing capacities of krill and fish, similar as for BDE 99. This possibility is based on the fact that BDE 183 has been detected in Antarctic krill (Bengtson Nash et al., 2008), associated with the fact that debromination of BDE 183 to BDE 154 has been observed in fish (Stapleton et al., 2004a). Therefore, the possibility that BDE 183 is more easily found in krill-eating cetaceans than in fish-eating predators should be further investigated. The sum of BDEs 47, 99 and 100 represented on average over 60% of the total PBDEs. This percentage compares favorably with those of other biological matrices from Antarctica (Corsolini, 2009).

The targeted MeO-PBDEs, 2′-MeO-BDE 68 and 6-MeO-BDE 47 were detected in 58% and 83% of the analysed males, as well as in 29% and 61% of the females. The maximum concentrations of 2′-MeO-BDE 68 and 6-MeO-BDE 47 were of 1.4 and 9.0 ng $g-^{1}$ lw for males, as well as of 0.5 and 0.9 for females, respectively. Only 58% and 28% of the samples for males and females, respectively, had measurable levels of both MeO-PBDEs. Vetter (2006) hypothesized that higher contributions of 2′-MeO-BDE 68 are caused by sponges or associated organisms, whereas higher proportions of 6-MeO-BDE 47 are an indication of the presence of algae or associated organisms. Therefore, the ratio between 2′-MeO-BDE 68 and 6-MeO-BDE 47 was calculated for the 24 individuals, generating values between 0.16 and 0.62 (median ratio of 0.34) for males (n=14), as well as values ranging from 0.23 to 0.79 (median of 0.49) for females (n=10). These values compare favorably with those found in oceanic dolphins from Southwest Atlantic, whose values ranged from 0.16 to 0.81 (Dorneles et al., 2010). Following Vetter (2006), the values found in the present study imply that Antarctic Peninsula humpback whales would be receiving MeO-BDEs predominantly from algae or associated organisms. To the authors' knowledge, there is no published data on MeO-PBDE concentrations, and hence ratios, in krill, the dominant diet of mysticetes from the Southern Hemisphere.

3.2. Polychlorinated biphenyls (PCBs)

PCBs 28, 49, 194, 205, 206 and 209 were not detected in any sample, and PCBs 172, 195 and 199 were detected in only one sample. PCBs 153, 149, 101 and 138 were the congeners with the highest concentrations and percentage contribution (PC) to ΣPCB. Summed, the PC to ΣPCBs of these four congeners was 51% (median value) for both males and females, and varied between 6 and 58% for males, as well as between 0% and 69% for females. The ΣPCB concentrations varied between 5.8 and 590 ng g^{-1} l.w., with the median value of 40.0 ng g^{-1} l.w. for males; as well as from 2.6 to 761 ng g^{-1} l.w., with the median value of 56.5 ng g^{-1} l.w. for females. PCB levels were lower in Antarctic Peninsula (present study) than in subarctic humpback whales (Metcalfe et al., 2004). In fact, PCB concentrations were lower in Antarctic Peninsula humpback whales than in mysticetes from the Northern Hemisphere in general (Hoekstra et al., 2005; Moon et al., 2010).

Considering only PCB 153 for comparison purposes, we observed that these concentrations were at least one order of magnitude lower in our study than those found in humpback whales

biopsied in Canadian waters, in 1991 (Gauthier et al., 1997). This is not surprising, given that the animals analysed by Gauthier et al. (1997) not only foraged in a highly polluted area, but also in a period of higher environmental concentrations of PCBs. Environmental levels of these compounds have been slowly decreasing since their ban in the 1970s (Fowler, 1990). PCB levels of Antarctic Peninsula humpback whales seem to be in the same range as those of other marine mammal species from Antarctica, comparing to data reviewed by Miranda-Filho et al. (2007). PCBs comprised the compound class with the highest contribution to ΣOHCs in females and the second highest in males (Table 1). Grouping PCBs by the number of chlorine atoms in the molecule, we observed that there was a greater contribution of penta- and hexa-CBs (Fig. S2) to ΣPCBs in comparison to the other measured CBs. Considering individuals that have concentrations of tri- and tetra-CBs above LOQ, the ratio between the lighter (3 and 4 chlorine atoms) and the heavier (from 6 to 7 chlorine atoms) PCBs varied from 0.03 to 1.04 (median value of 0.26) in males and from 0.02 to 0.7 (median value of 0.05) in females. These ratios are lower than those found in cetaceans inhabiting waters close to highly industrialized and urbanized areas from South America (Lailson-Brito et al., 2010). The opposite, i.e., a higher contribution of low-chlorinated PCBs in Antarctic marine mammals would be expected as a consequence of the more efficient long-range atmospheric transport (LRAT) of more volatile compounds (Wania and Mackay, 1993). The more efficient LRAT of lower chlorinated congeners was shown by PCB profiles in air samples from Antarctic Peninsula region (King George Island), as the more volatile compounds predominated in that matrix (Montone et al., 2003). The explanation for the apparently contradictory data generated by the present study and by Lailson-Brito et al. (2010) may be provided by the abundance of suspended particulate matter (SPM) in the estuarine waters inhabited by the dolphins of the quoted study. The more hydrophobic compounds present a higher affinity to SPM and end up having a shorter residence time in water, which reduces their availability (Dachs et al., 1996; Jonker and Koelmans, 2002). The ratios between the lighter and the heavier PCBs of Antarctic Peninsula humpback whales were similar to those found in oceanic dolphins from Southwest Atlantic (Lailson-Brito et al., 2012). The fact the both cetacean groups, i.e., Antarctic Peninsula humpback whales and oceanic dolphins from South America, feed far away from the source regions suggests that the LRAT of OHCs plays and important role for such PCB profiles.

3.3. Chlordanes (CHLs)

The ΣCHL concentrations varied between 3.1 and 16, with a median value of 8.0 ng g^{-1} l.w. for males; as well as from 1.6 to 14, with a median value of 4.7 ng g^{-1} l.w. for females. Lower *trans*-chlordane (TC) concentrations were found in the present study compared to those found in subarctic humpback whales (Metcalfe et al., 2004). This difference is also present when comparing ΣCHL concentrations between Antarctic Peninsula humpback whales (this study) and minke whales (*Balaenoptera acutorostrata*) from the Northern Hemisphere (Moon et al., 2010). The highest ΣCHL concentration found in the present study (14.4 ng g^{-1} l.w.) was still lower than the lowest level (113 ng/g l.w.) found in bowhead whales (*Balaena mysticetus*) from Alaska (Hoekstra et al., 2005). *Trans*-nonachlor (TN) and oxychlordane (OxC) were the major components; however, *cis*-nonachlor (CN) and TN were the only CHLs detected in all individuals. The summed contribution of TN and OxC to ΣCHLs varied between 51.1% and 79.2%, with a median percentage of 74.3 in males, as well as between 27.9% and 82.9%, with a median value of 66.4% in females. None of the eight males from the AO-XXI had detectable levels of TC. It has been demonstrated that TC degrades in air by both photolysis and oxidation more readily than *cis*-chlordane (CC) (Oehme, 1991). In addition, the half-life of TC in some fish species is shorter than that of CC (Kawano et al., 1988). Therefore, the *cis*/*trans* ratio of chlordane was calculated for generating information on this profile in a top predator from the Antarctic region. The CC/TC ratio ranged from 1.23 to 13.4, with a median value of 5.62 in males, as well as from 0.51 to 16.8, with a median value of 2.96 in females. These ratios are comparable to the range (2.41–5.67) found in humpback whales from the Northern Hemisphere (Gauthier et al., 1997).

3.4. Hexachlorocyclohexanes (HCHs)

The α-HCH and γ-HCH isomers were detected in all analysed samples, while β-HCH was below LOQ in 66% of them. The ΣHCH concentrations varied from 2.6 to 50, with a median value of 6.1 ng g^{-1} l.w. for males; as well as from 1.6 to 44, with a median value of 5.2 ng g^{-1} l.w. for females. The HCH concentrations found in the present study were one order of magnitude lower than those found in subarctic humpback whales (Metcalfe et al., 2004), being also lower than those found in other whale species from the Northern Hemisphere (Hoekstra et al., 2005; Moon et al., 2010). The latter observation is supported by the fact that the highest ΣHCH concentration (50 ng/g l.w.) found among Antarctic Peninsula humpback whales was still lower than the lowest level (100 ng/g l.w.) found among minke whales from the Korean coast (Moon et al., 2010). The same holds for comparison with bowhead whales from Alaska among which 111 ng/g l.w. was the lowest ΣHCH concentration (Hoekstra et al., 2005). Regarding the contribution of each HCH isomer to ΣHCHs, the compounds were found in the following median (Min–Max) proportions: 39.6% (13.8–77.8) α-HCH; 7.59% (4.2–19.2) β-HCH; and 53.8% (15.4–86.2) γ-HCH for males, as well as 26.5% (12.0–79.4) α-HCH; 5.16% (0.66–14.7) β-HCH; and 69.8% (13.9–86.7) γ-HCH for females. In global monitoring studies, relationships among HCH isomers have been used as indication of metabolism and sources of environmental contamination. In the present investigation, the γ-HCH/α-HCH ratio varied from 0.18 to 7.39, with a mean value of 2.24 ($\pm$1.52). In a study that analysed samples from emerald rockcod (*Trematomus bernacchii*) collected during the 2000 and 2001/02 Italian Antarctic Expeditions, the γ-HCH/α-HCH ratios found for muscle and whole body homogenate were 12 ($\pm$16) and 85, respectively (Corsolini et al., 2006). Reviewing published information on contribution of α-, β- and γ-HCH isomers to ΣHCHs in marine mammals inhabiting mid- to high-latitude areas of the Northern Hemisphere, only two out of twenty-two species/populations presented a mean percentage higher than 20% of γ-HCH contribution and a β-HCH contribution lower than 20% (Andersen et al., 2001; Kleivane et al., 2000, 1995; Kucklick et al., 2002; Mössner and Ballschmiter, 1997; Mossner et al., 1992; Oehme et al., 1995; Tanabe et al., 1996; Willett et al., 1998). For most of these species/populations, β-HCH was the HCH isomer of higher importance due to its higher environmental persistence and biomagnification capacity (Borgå et al., 2001; Senthilkumar et al., 2001). The contribution of β-HCH found in our study contrasts with previous investigations on marine mammals from Northern polar areas (Mössner and Ballschmiter, 1997; Mossner et al., 1992; Oehme et al., 1995; Tanabe et al., 1996). Studies on atmospheric HCH concentrations in South American countries have demonstrated γ-HCH to be the major isomer (Estellano et al., 2008; Meire et al., 2012; Pozo et al., 2004). These investigations emphasized the recent use of lindane (γ-HCH) in South America through the evaluation of the α/γ-HCH ratio. A α/γ-HCH ratio lower or close to 1.0 would indicate recent lindane release (Willett et al., 1998). In Brazil, Chile and Bolivia, the α/γ-HCH ratio of the atmospheric concentrations ranged from 0.4 to 1.5, from 0.6 to 0.9, and from 0.06 to 1.19, respectively (Estellano et al., 2008; Meire et al., 2012;

Pozo et al., 2004). This release in the Southern Hemisphere would explain the higher γ-HCH contribution in Antarctic than in Arctic marine mammals.

3.5. DDT and metabolites (DDXs)

In this article we have decided to use ΣDDX (instead of ΣDDT) for the summed concentrations of the six related compounds (*o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT), as it has been the adopted option in many recent publications in order to avoid possible misunderstandings (He et al., 2014; Vanden Berghe et al., 2012; Wang et al., 2014; Weijs et al., 2010a,b). Concerning DDX isomers (e.g. *p,p′*- and *o,p′*-DDT and metabolites DDE and DDD), *o,p′*-DDE and *o,p′*-DDD were detected in only 5 and 6% of the analysed samples, respectively. The ΣDDX concentrations varied from 8.0 to 85.3, with the median value of 28.7 ng g^{-1} l.w. for males; as well as from 2.64 to 143, with the median value of 11.0 ng g^{-1} l.w. for females. Concentrations of DDT and metabolites (DDXs) found in the present study were orders of magnitude lower than those found in baleen whales from the Northern Hemisphere (Hoekstra et al., 2005; Moon et al., 2010), including humpback whales (Gauthier et al., 1997; Metcalfe et al., 2004). The ratio between DDE and the sum of DDT, DDE and DDD has been used for determining the chronology of DDT entrance into a given ecosystem, in which a higher ratio indicates an earlier DDT entrance in the environment (Aguilar, 1984). Considering that *o,p′*-DDE and *o,p′*-DDD were detected in a reduced number of samples, the abovementioned ratio was calculated only for the *p,p′*-compounds. The ratio rendered the maximum value of 1.0 for both sexes, and minimum and median values of 0.53 and 0.66 for males, as well as 0.38 (minimum) and 0.61 (median) for females, respectively. With respect to the chronology (Aguilar, 1984), these calculated ratios probably reflected an early environmental contamination by DDT. The percentage contribution of the *p,p′*-DDE to ΣDDXs (the sum of 6 DDXs: *o,p′*-DDD, *o,p′*-DDT, *o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT) rendered the median values of 61 and 58% for males and females respectively, which is comparable to results from other Antarctic aquatic species, including krill, fish and penguins (Corsolini et al., 2006).

3.6. Hexachlorobenzene (HCB)

HCB concentrations varied between 15.0 and 130, with median value of 61.3 ng g^{-1} l.w. for males; as well as between 6.75 and 74.5, with the median value of 32.1 ng g^{-1} l.w. for females. The HCB concentrations found in the present study were in the same range as those found in whales from Northern Hemisphere (Hoekstra et al., 2005; Metcalfe et al., 2004; Moon et al., 2010). HCB was the predominant compound among the pesticides measured in the present study (Fig. S3). In fact, HCB comprised the compound with the highest percentage contribution to ΣOHCs in males and the second highest in females (Table 1). OHC concentrations in Guiana dolphins (*Sotalia guianensis*) from Guanabara Bay (Lailson-Brito et al., 2010), a highly contaminated environment from southeast Brazil, and from Paraná State, a less contaminated area (South Brazilian region) (Lailson-Brito et al., 2010), and in franciscana dolphins (*Pontoporia blainvillei*) from waters of Rio Grande do Sul state (southernmost Brazilian state) (Leonel et al., 2010), were statistically compared with HCB data generated by the present study. Significantly higher HCB concentrations were found for humpback whales than for both franciscana (Leonel et al., 2010) and Guiana dolphins (Lailson-Brito et al., 2010) from the Southern Brazil. The higher volatility of HCB combined with the well-known cold trap effect (Valsaraj and Thibodeaux, 2010) increases the contribution of HCB relatively to other OHCs. In fact, this combination of factors is not only reflected in the contribution, but also in the concentration itself, as exemplified by the aforementioned comparison to HCB data from dolphins.

3.7. Sex differences and year-to-year variations

For investigating possible sex-related differences in the bioaccumulation of OHCs and MeO-PBDEs by humpback whales, comparisons between sexes were performed using samples obtained during the same Antarctic Operation, regarding ΣHCHs, ΣCHLs, ΣDDXs, HCB, ΣPCBs, ΣPBDEs, BDE 99, BDE 47, ΣMeO-PBDEs and the ΣPBDEs/ΣMeO-PBDEs ratio, as well as the percentage contribution of ΣPBDEs to the sum of all anthropogenic OHCs (ΣOHCs).

Concerning whales of the AO-XXI, significantly higher ΣPBDE concentrations were found in females than in males ($p=0.04$). Within the same Antarctic Operation (AO-XXI), significantly higher ΣMeO-PBDE concentrations were found in males than in females ($p=0.02$), while significantly higher values of the ΣPBDE/ΣMeO-PBDE ratio were found in females than in males ($p=0.006$). In fact, the same finding (significantly higher values of the ΣPBDE/ΣMeO-PBDE ratio in females than in males) was also found for AO-XIX ($p=0.03$). Differences between males and females, regarding the contribution of natural (ΣMeO-PBDEs) and anthropogenic (ΣPBDEs) brominated compounds to the sum of both classes are shown in Fig. 1. The ratio between 2′-MeO-BDE 68 and 6-MeO-BDE 47 had a median value of 0.34 and ranged from 0.16 to 0.62 for males ($n=14$). For females ($n=10$), the ratio varied from 0.23 to 0.79 (median 0.49). Since only a limited number of individuals of each sex had detectable levels of both MeO-PBDEs, the sex-related statistical analysis could only be performed considering all analysed males and females, i.e., without sorting out individuals to their respective sampled years. Significantly higher values of the ratio between 2′-MeO-BDE 68 and 6-MeO-BDE 47 were found in females than in males ($p=0.01$). Differences between males and females, regarding the contribution of ΣPBDEs to the sum of all anthropogenic OHCs (ΣOHCs) were found in AO-XXI, with females presenting higher values than males ($p=0.01$).

The ratios between the lighter (3 and 4 chlorine atoms) and the heavier (from 6 to 10 chlorine atoms) PCBs were used for further investigation on possible sex differences. When individuals with tri- and tetra-CB levels below LOQ were excluded from the analyses, the small number of remaining individuals prevented

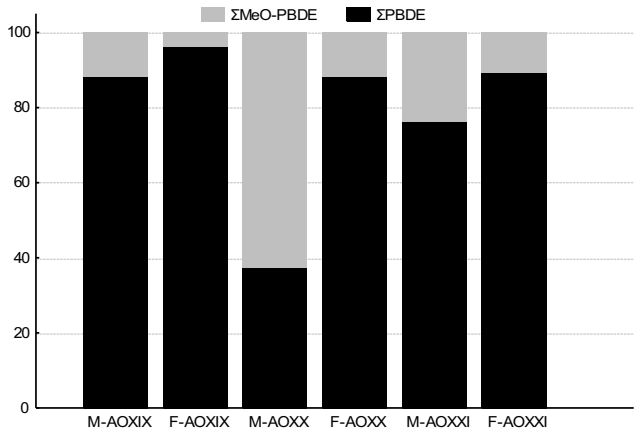

Fig. 1. Relative contribution of ΣMeO-PBDE and ΣPBDE to the sum of organobrominated compounds in blubber samples from male (M) and female (F) humpback whales biopsied in the Southern Ocean during the Brazilian Antarctic Operations XIX, XX and XXI (2000/2001, 2001/2002 and 2002/2003 summers, respectively).

comparison within the same Antarctic Operation, with the exception of the AO-XX, for which there was no significant difference. However, when males and females from all Brazilian Antarctic Operations were considered, significantly higher ratios were found in males than in females ($p=0.04$).

The sex differences in the PCB profiles can be explained by the mother-to-calf transfer ratio of pollutants, since the efficiency of OHC transfer in marine mammals decreases significantly with increasing number of halogen substituents (Dorneles et al., 2010; Haraguchi et al., 2009; Ikonomou and Addison, 2008; Vanden Berghe et al., 2012). However, the significant differences for ratios between organobrominated compounds (the ΣPBDE/ΣMeO-PBDE ratio and the 2′-MeO-BDE 68 / 6-MeO-BDE 47 ratio), as well as the significantly higher ΣPBDE concentrations in females than in males, cannot be explained by a dissimilar placental and lactational transfer efficiency for these brominated compounds. PBDEs and MeO-PBDEs seem to be transferred similarly through both placenta and milk, as suggested by Vanden Berghe et al. (2012) and Dorneles et al. (2010). Regarding the significantly higher ΣPBDE concentrations in females than in males, some studies have shown sex differences in metabolism of xenobiotics (Hall, 2002); however, even considering the possibility of a higher metabolization rate in males, PBDEs are highly persistent compounds that are efficiently transferred from mother to offspring. Therefore, both the continuous bioaccumulation by males and the higher concentrations in this gender than in females are common findings for PBDEs in marine mammals (O'Shea and Tanabe, 2003).

A recent study (Dorneles et al., 2010) raised the hypothesis of using the ΣPBDE/ΣMeO-PBDE ratio to investigate possible differences in feeding areas/prey species for small cetaceans. The ratio could be used, for instance, as an indication of inshore *versus* offshore contribution to food intake (Dorneles et al., 2010). Therefore, the observed significant differences in the ΣPBDE/ΣMeO-PBDE ratios between males and females would point out sex-related differences in feeding areas or prey species. In the present study, male and female humpback whales were biopsied at the same feeding area, where krill is their main diet (Dalla Rosa et al., 2008; Friedlaender et al., 2009; Kawamura, 1994). However, males and females generally have dissimilar migration patterns (Dawbin, 1997), which have probably caused differences in bioaccumulation over time. Indeed, it has been reported that some females remain in the feeding areas throughout winter in the Southern Hemisphere, as an explanation to the fact that the sex ratio of migrating humpback whales near breeding areas is highly skewed towards males (Brown et al., 1995). Studies demonstrated the migratory destination of Antarctic Peninsula humpback whales to be the northwest coast of South America (Acevedo et al., 2007; Engel et al., 2008; Stevick et al., 2004) and southwest coast of Central America (Rasmussen et al., 2007), and a study on population structure of South Pacific humpback whales found a significant bias towards males in those breeding grounds (Olavarria et al., 2007). These literature data strongly suggest that some females remain in the Antarctic Peninsula region during austral winter. Thus, the hypothesis that sex-related differences in migration patterns lead to dietary dissimilarities should be taken into account, since feeding in mid- to low-latitude waters can occur for some mysticete species/populations (Silva et al., 2013), including humpback whales from the Southern Hemisphere (Silva et al., 2011; Zerbini et al., 2011). Additional studies are required for shedding further light on these gender-related differences in OHC profiles, especially those comprising data on ratios of stable isotope of carbon, nitrogen and sulfur. Therefore, method optimization allowing the use of a small amount of skin sample for both stable isotope measurements and molecular sex determination would be a priority for next studies.

To investigate potential year-to-year variation in the humpback whale exposure, pairwise group comparisons were performed (AO-XIX *versus* AO-XX, AO-XX *versus* AO-XXI, AO-XXI *versus* AO-XIX), for males and females, regarding ΣCHLs, CC/TC ratio, ΣDDX, ΣHCH, HCB, ΣPCB, ΣPBDE, BDE 99, BDE 47, ΣPBDE/ΣMeO-PBDE ratio, as well as BDE 47/BDE 99 ratio. Significant differences were found for ΣCHLs between females of AO-XIX and AO-XXI, as well as between males of AO-XX and AO-XXI ($p=0.02$ in both cases), with lower concentrations in more recent periods in both scenarios. No statistically significant differences were found for the other tested ratios or compounds. A significant difference was also found for the BDE 47/BDE 99 ratio, for females of AO-XIX and AO-XX. The BDE 47/BDE 99 ratio was significantly higher in female humpback whales sampled during AO-XIX (2000/2001 summer) than in those sampled during AO-XX (2001/2002 summer) ($p=0.001$). Debromination of BDE 99 to BDE 47 was demonstrated (Stapleton et al., 2004b) and a model applied to experimental laboratory data indicated the occurrence of debromination of BDE 47 to BDE 28 (Tomy et al., 2004). The BDE 28/BDE 47 ratio was not calculated since only eight whales presented detectable levels of BDE 28. The latter finding would indicate, at first, a more intense debromination of BDE 99 to BDE 47 in Antarctic marine food webs in a previous period. However, as the efficiencies of placental and lactational transfer differ between BDE 47 and BDE 99 (Dorneles et al., 2010; Haraguchi et al., 2009; Ikonomou and Addison, 2008), a year-to-year oscillation in the reproduction rate of this humpback whale population could also be an explanation for such finding. It is worth mentioning that year-to-year oscillations in the reproduction rates have already been observed for Antarctic marine predators (Croxall, 1992), but any possible effect on debromination should be further examined. Local sources (Hale et al., 2008) and long range transport (Wania, 2003) of PBDEs are contributing to levels and profiles in Antarctic biota and year-to-year variations for the compounds originated from these different sources need additional investigation as well.

In order to clarify the differences that were found in the current study, a discriminant function analysis was performed using the OHC concentrations for investigating possible dissimilarities in the organohalogen accumulation patterns of Antarctic Peninsula humpback whales according to sex and sampling period (AOs). The results of such analysis is shown in Table 2, as well as in Fig. 2, which exhibits the graphical representation of the first two canonical variables, illustrating the grouping of male and female Southern Hemisphere humpback whales from distinct AOs. The discriminant function analysis rendered six significant differences out of 15 possible ones. Among these six, only one was observed between groups of the same sex (OA-XIX-F x OA-XX-F), i.e., only one reflected a year-to-year variation. The other five were gender-related significant differences (Table 2), which (1) highlight the need to keep males and females separate in this kind of study, (2) reinforce the hypothesis of dietary dissimilarities between

Table 2
The *F* values (upper-right), as well as the *Mahalanobis* (D^2 – lower-left) distance related to the grouping of male (M) and female (F) humpback whales biopsied in the Southern Ocean during the Brazilian Antarctic Operations XIX, XX and XXI (2000/2001, 2001/2002 and 2002/2003 summers, respectively), obtained from the discriminant analysis.

	AO-XIX-M	AO-XIX-F	AO-XX-M	AO-XX-F	AO-XXI-M	AO-XXI-F
AO-XIX-M		2.11	0.85	2.33*	0.76	3.23*
AO-XIX-F	6.07		3.12*	2.47*	2.08	2.26
AO-XX-M	2.52	5.4*		2.55*	1.32	5.16*
AO-XX-F	7.97*	5.4*	5.81*		0.65	0.78
AO-XXI-M	2.44	4.14	2.74	1.64		1.56
AO-XXI-F	9.03*	3.54	8.56*	1.65	2.98	

* Significant ($p < 0.05$).

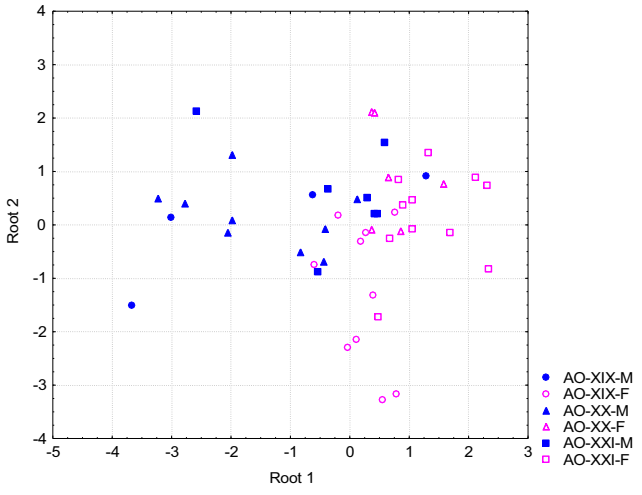

Fig. 2. Graphical representation of the first two canonical variables, illustrating the grouping of male (M) and female (F) Antarctic Peninsula humpback whales according to sex and sampling period (Antarctic Operations XIX, XX and XXI correspond to 2000/2001, 2001/2002 and 2002/2003 summers, respectively). AOs XIX, XX and XXI are represented by circle, triangle and square, respectively; as well as males and females are represented by blue filled and pink hollow geometric forms, respectively.

male and female Antarctic Peninsula humpback whales, and (3) emphasize the role of reproduction (gestational and lactational transfer) in the bioaccumulation process of OHCs in humpback whales.

4. Conclusions

Probably a wider time interval is needed for the identification of changes in OHC levels, as the short period considered in the present study (from 2000/2001 to 2002/2003) was not enough to observe decreases in concentrations, with the exception of chlordanes. The environmental persistence and the volatility of OHCs, combined with the cold trap effect, may result in concentrations of toxicological concern for Antarctic wildlife, which is best exemplified in the present study by HCB. The hypothesis that dissimilarities in food intake of male and female humpback whales from the Antarctic Peninsula region would generate sex-related differences in persistent pollutant profiles needs to be further investigated.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2015.02.007.

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