Persistent organic pollutants in blood samples of Southern Giant Petrels (*Macronectes giganteus*) from the South Shetland Islands, Antarctica

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**A B S T R A C T**

Seabirds play an important role as top consumers in the food web and can be used as biomonitors of exposure to pollutants. Contamination studies involving non-destructive sampling methods are of considerable importance, allowing better evaluation of the levels of pollutants and their toxic effects. In the present study, organohalogen contaminants were analyzed in 113 blood samples from Southern Giant Petrel (*Macronectes giganteus*) adults and chicks collected in the austral summer of 2011/2012 and 2012/2013 from colonies on Elephant and Livingston Islands, South Shetland, Antarctica. Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), pentachlorobenzene (PeCB), mirex, dichlorodiphenyltrichloroethane and derivatives (DDTs) and chlordanes were detected in all birds, whereas polybrominated diphenyl ethers (PBDEs) were not detected in any blood samples. No significant differences were found in organochlorine levels between sampling events. Adults exhibited significantly higher levels than chicks, except for PeCB. PCBs, HCB, mirex and DDTs were statistically similar in males and females from Elephant Island. Females on Livingston Island exhibited higher HCB values than males, but no sex differences were found regarding other organochlorines. The similarity in organochlorine levels between sexes in birds with very marked sexual segregation in feeding habits during the breeding season may indicate that significant amounts of contaminants are acquired during migration to lower latitudes, when the diets of males and females are similar. Birds sampled on Livingston Island exhibited significantly lower levels of PCBs, HCB, DDTs, mirex and chlordanes in comparison to those on Elephant Island, which could be the result of distinct foraging patterns between the two colonies. Organochlorine levels were similar between years in birds captured in two consecutive breeding seasons. Blood samples from Southern Giant Petrels adults and chicks proved to be useful for the comparison of intra-specific contamination levels and appear to be adequate for the long-term assessment of organohalogen contaminants in Antarctic top predators.

Organochlorine contaminants in blood samples of Southern Giant Petrels reflected intra-specific differences and suggested distinct foraging patterns between colonies.

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1. Introduction

Seabirds play an important role as top consumers in the food web and can be used as biomonitors of exposure to pollutants, revealing potential threats to the environment (Furness and Camphuysen, 1997). Several classes of contaminants, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs), are often found in the marine environment, including remote places, such as the Southern Ocean (e.g., Corsolini, 2009; Cipro et al., 2010). These contaminants are incorporated into the food web, reaching top
predators due to bioaccumulation and biomagnification processes (Fisk et al., 2001).

Organic contaminants are usually analyzed in lipid-rich tissues, such as the adipose tissue, liver and muscle, which usually limits sampling to dead specimens and constitutes an obstacle to eco-
toxico logical and long-term monitoring studies (Henriksen et al., 1998; Knudsen et al., 2007). Contamination studies using non-
destructive sampling methods, such as the analysis of pollutants in the blood, can help overcome these limitations and are of considerable importance, allowing the evaluation of the levels of pollutants and their toxic effects (Bustnes et al., 2001), and has the advantage of repeated sampling from the same individual and a more representative sampling design (Furness and Camphuysen, 1997; Keller et al., 2004; Yordy et al., 2010; Carravieri et al., 2014). Additionally, blood contaminant concentrations were significantly correlated with concentrations in lipid-rich tissues in wildlife, such as marine mammals and seabirds, and in humans (Kanja et al., 1992; Boon et al., 1994; Henriksen et al., 1998).

The Southern Giant Petrel Macronectes giganteus (Procellariiformes: Procellariidae) is an important top predator of the South Atlantic and the Southern Ocean (Hunter, 1985; Oiiformes: Procellariidae) is an important top predator of the South

Antarctica (Fig. 1). At Elephant Island, sampling of adults was conducted during two consecutive breeding seasons (November 2011 and November/December 2012), whereas chicks were

collected only in the December 2012, and only from adult

birds. Approximately 4 mL of blood from the superficial ulnar artery were collected from each bird for contaminant analysis. The whole blood was stored in glass vials (previously baked at 450 °C), con-
taining heparin (0.1 mL for each 1 mL of blood), and kept frozen at 

−20 °C until analysis.

All captured birds were banded (the ring number was recorded for birds already banded in previous years) and the sex was identi-
tified by the morphology and morphometry (body weight, bill length, wing length and tarsal length) of the individual. Southern Giant Petrels show evident sexual dimorphism by size, with males being larger and heavier than females, and also with larger and more robust culmen (Hunter, 1984; Copello et al., 2006). All samples were collected with permits (Brazilian Antarctic Authorities and Environmental Ministry, 2011 and 2012) in accordance to Annex II, Article 3 of the Protocol on Environmental Protection to the Antarctic Treaty and the regulation from the Scientific Committee on Antarctic Research (SCAR).

2.2. Sample preparation

Samples were analyzed for organic contaminants, according to the methods described by Keller et al. (2009), with minor modi-
fications. Briefly, 3 g of sample was first amended with a suite of 13C-
labeled internal standards in ethanol (mass known) and then allowed to equilibrate with the sample overnight at 4 °C. Samples were then extracted twice in a focused microwave extractor (FME) (Discover, CEM Corp.) equipped with an autosampler (Explorer, CEM Corp.). Following extraction, acidified silica and alumina clean-ups were performed on automated SPE workstations (RapidTrace, Biotage). Samples were solvent-exchanged to iso-octane and reduced to a final volume of approximately 250 μL. The sample preparation method is described in detail in Supplementary Information (SI).

Aliquots of Standard Reference Material (SRM 1958 - Organic Contaminants in Fortified Human Serum, National Institutes of Standards and Technology - NIST) and in-house control material of pooled Southern Giant Petrels blood, as well as triplicate laboratory blanks and six calibration solutions for each batch were prepared using the same techniques. Calibration solutions were subjected to the same sample preparation steps, as were samples and laboratory blanks. Results from SRM 1958 were within the ranges of the certi-
vate values (Table S1). All analyses were carried out at Hollings Marine Laboratory [National Institute of Standards and Technology (NIST) Chemical Sciences Division].

2.3. Sample analysis

Samples were analyzed on a gas chromatograph coupled to a triple quadrupole mass spectrometer (Agilent 7000 GC-MS/MS coupled to an Agilent 7890A gas chromatograph). A programmable temperature vaporization (PTV) inlet operated in the solvent vent mode was used for injections. Additional information about the chromatographic parameters is described in SI.

Eighty-one PCB congeners and 12 OCPs were determined by GC-

MS/MS in the electron ionization mode (EI) operated in multiple reaction monitoring (MRM). Compounds and transitions monitored are listed in Table S2. The guard column (5 m × 0.25 mm) was connected to a DB-XLB capillary column (Agilent Technologies) with 30 m × 0.18 mm, 0.18 μm film thickness.

Twenty-five PBDE and five chlorinated pesticides were analyzed in the negative chemical ionization mode (NCI) operated in selected ion monitoring (SIM) (Table S3) using the same instrument but not in the MS/MS mode. The guard column (5 m × 0.25 mm) was connected to a 5% phenyl methyl/polygloxane capillary column (DB-5MS, Agilent Technologies) with 30 m × 0.18 mm, 0.18 μm film thickness.
Amounts of each analyte were calculated using the slope and y-intercept of at least a three-point calibration curve. A compound was considered to be significantly above the limit of detection (LOD) if the mass of an analyte in the sample was greater than the mass of the analyte in the lowest detectable calibrant solution and also above the average mass of the analyte detected in blanks + three times the standard deviation of the blank response. Average LOD values for all analytes are showed in Table S4.

Lipid analyses were carried out using the SmartTrac II from CEM (Matthews, NC). An aliquot (approximately 250 µL) of the sample was dried in a microwave and total fat was determined by nuclear magnetic resonance (NMR). Each blood sample was analyzed in triplicate in the NMR. Replicates of the SRM 1958 were analyzed with each set of 18 samples.

2.4. Statistics

Normality and equality of variance assumptions were checked before analysis using Shapiro-Wilk and Levene’s test, respectively. Data did not meet these assumptions, even after log transformation, thus Kruskal-Wallis test followed by pairwise Wilcoxon rank sum tests, using Bonferroni adjustment to the alpha value, were used to confirm differences in organic contaminants levels between years, locations, ages and sexes. All statistical analyses were performed using the software program R 3.0.2 (R Development Core Team, 2013) with a significance level of 5%.

3. Results and discussion

Organohalogen contaminants were detected in the blood
samples of all Southern Giant Petrels adults and chicks (Table 1, Tables S5–S7). Mirex (2.56–43.1 ng g⁻¹) and DDTs (2.16–34.5 ng g⁻¹) were the predominant compounds detected in adults, followed by PCBs (2.14–21.55 ng g⁻¹) and HCB (1.85–12.1 ng g⁻¹). Chicks exhibited a different pattern |DDTs (0.61–5.00 ng g⁻¹) > HCB (0.98–3.61 ng g⁻¹) > mirex (0.28–2.63 ng g⁻¹) > PCBs (0.09–1.65 ng g⁻¹)|, which may be related to differences in exposure to these contaminants (Fig. S1).

Chicks reflect exposure from maternal transfer to the egg and food provided by parents during the rearing period (Verreault et al., 2006; Foster et al., 2010), whereas adults reflect contamination from their breeding grounds and migration areas (Tanaka et al., 1986). HCB, 4,4′-DDE and PCBs are the predominant contaminants found in Antarctic organisms, such as seals, penguins, fish and krill (Corsonioli et al., 2007; Corsonioli, 2009; Cipri et al., 2010), which are important prey for Southern Giant Petrels during the breeding season (Forero et al., 2005; Rey et al., 2012).

Mirex, which was one of the most abundant contaminant found in the blood of Southern Giant Petrel adults, has been found at higher levels in birds that migrate to lower latitudes of the Southern Hemisphere (e.g., Brown Skuas), than in resident birds (e.g., penguins) (Tanguchi et al., 2009; Cipri et al., 2013), which indicates that exposure to this compound mainly occurs in regions other than Antarctica. Mirex was used in the northern and southern hemispheres to combat ants and termites and as a flame retardant (Ritter et al., 1995). Although it has not been used in the United States since 1978 (IARC, 1979; ATSDR, 1995), mirex had continuous residual use in South America and Australia until the late 2000s (Barra et al., 2006; DEH, 2006). The abundance of mirex in blood samples of Southern Giant Petrels reflects the use of this substance in the southern hemisphere.

Table 2 shows organic contaminant levels in the blood of Southern Giant Petrels and of high trophic level seabirds from North Atlantic, Arctic, Antarctic and Subantarctic regions, reported in previous studies. Southern Giant Petrels had overall organic contaminant levels lower than birds from Northern Hemisphere. PCB, DDT and chlordane levels in Southern Giant Petrels were up to two orders of magnitude lower than those reported in Glauous Gulls (Larus hyperboreus) and Great Skuas (Catharacta skua). Glauous Gulls and Great Skuas breed in high latitudes of the Northern Hemisphere, and like Southern Giant Petrel, they have migratory habits and occupy a high trophic position in the food web (del Hoyo et al., 1996). Differences in HCB and mirex levels among these species were less pronounced. Southern Giant Petrels presented HCB and mirex values in the same magnitude range of Glauous Gulls and of Great Skuas from Iceland and Shetland (Sonne et al., 2013; Busnites et al., 2006).

Organochlorine levels in South Polar Skuas (Catharacta maccormicki), from East Antarctica (Busnites et al., 2006), were higher (but in the same magnitude order) than those found in adults of Southern Giant Petrels. Southern Giant Petrels had organochlorine levels comparable to those reported in Wandering Albatrosses (Diomedea exulans), a circumpolar migrant from the Southern Hemisphere, except for PCBs, which presented maximum values higher in Wandering Albatrosses (Carravieri et al., 2014).

PCBs have been frequently reported as one of the predominant contaminants in the Antarctic biota (e.g., Cipri et al., 2013; Corsonioli, 2009). The hexa- and hepta-congeners of PCBs were predominant in all birds (Table 1), representing 30.5%–65.7% and 21.9%–58.3% of total PCBs for each homolog group, respectively. The most representative congeners within these groups were PCBs 138, 153 – 152, 170 and 180 (Fig. S2). The prevalence of high-chlorinated PCBs is due to the accumulation of these compounds and the excretion of lower chlorinated congeners by higher trophic level organisms (Maervoet et al., 2004).
Pentachlorobenzene (PeCB) was used as a mixture in PCB products, as a fungicide, a flame retardant and is also a byproduct of a variety of industrial process (Bailey et al., 2009). In the present study, PeCB was detected in all individuals, with levels ranging from 0.06 ng g\(^{-1}\) to 0.43 ng g\(^{-1}\) (Table 1). PeCB is a persistent organic pollutant and has been found in the arctic marine food web (Norstrom et al., 1988) as well as recently reported in fishes, penguins and other birds from Antarctica (Goutte et al., 2013).

The chlordane group was represented in the blood samples of Southern Giant Petrels mainly by oxychlordane, although trans-nonachlor was also detected in blood samples. Oxychlordane, which is a metabolite of cis- and trans-chlordane and nonachlor (Eisler, 1990), is persistent and continues to be frequently detected in wildlife in remote locations (e.g., Henriksen et al., 2000; Bustnes et al., 2001). Birds can metabolize chlordanes to oxychlordane more efficiently than other organisms, usually showing higher proportion of this compound in their tissues (Kawano et al., 1988). Cis-chlordane (LOD < 0.02 ng g\(^{-1}\)), trans-chlordane (LOD < 0.005 ng g\(^{-1}\)) and cis-nonachlor (LOD < 0.005 ng g\(^{-1}\)) were not detected in blood samples.

PBDEs (LOD < 0.003 ng g\(^{-1}\)) and HCHs (LOD < 0.01 ng g\(^{-1}\)) were not detected in the blood samples of Southern Giant Petrels. PBDEs have been found in eggs, internal tissues and the plasma of antarctic and subantarctic birds, but usually at low levels (Carravieri et al., 2014; Bustnes et al., 2006; Corsolini et al., 2006). In Antarctica, HCHs are mainly detected in organisms on low trophic levels and birds that feed mainly on krill (Corsolini et al., 2006, 2011; Taniguchi et al., 2008). Moreover, HCHs are rapidly metabolized and excreted by birds, and consequently are less prone to accumulate in their tissues (Clark et al., 1987).

### 3.1. Intra-specific comparisons on organic contaminant levels

Levels of PCBs and OCs were similar between breeding seasons for males and females on Elephant Island (p > 0.05) (See SI, Table S8 for Wilcoxon comparisons and p-values). Thus, samples from different years were pooled for subsequent analyses.

Differences in contaminant levels are usually expected in species with sexual trophic segregation (González-Solís et al., 2002b; Carravieri et al., 2014; such as Southern Giant Petrels. Southern Giant Petrel males and females exhibited a high degree of similarity in contaminant levels (Table 1; Fig. 2). Statistically significant (p < 0.05) differences between sexes were found only for PeCB and chlordanes in birds on Elephant Island (higher levels in males), and only for HCB (p < 0.05) in birds on Livingston Island (higher levels in females) (Fig. 2; Table S8).

During the breeding season, males feed mainly on carrion, such as penguins and seals, while females rely greater on marine prey, such as fish and cephalopods (Hunter, 1983; Forero et al., 2005; Rey et al., 2012). However, during the migration season, the diet of Southern Giant Petrel males and females appears to be similar, with greater dependence on marine resources than carrion for both sexes (Hunter, 1983; Bugoni et al., 2010). Thus, one may infer that males and females have similar contamination levels due to the more significant uptake of contaminants at lower latitudes during the migration period, when their feeding habits are more alike.

An additional aspect to consider is that the contamination found in components in the blood is the result of 1) the recent intake through food, 2) the mobilization of compounds from lipid-rich tissues and 3) the redistribution of contaminants to blood and internal tissues (Henriksen et al., 1998; van den Brink et al., 1998; Bustnes et al., 2010). Thus, the blood samples of Southern Giant Petrels likely reflect contamination over a general period and not only recent exposure after the birds have arrived at breeding colonies.

Levels of organochlorine contaminants in chicks were clearly lower than adults (Table 1; Fig. 2). Significant differences between ages were found for PCBs, PeCB, HCB, DDTs, mirex and chlordanes (p < 0.05) (Table S8). In addition to the contamination acquired during the embryonic period, chicks are constantly exposed to these contaminants during the rearing period. Petrels feed their young with stomach oils produced from their diet, which has a high lipid content and concentrated levels of organochlorines derived from preys (Warham, 1977; Foster et al., 2010). However, chicks accumulate these compounds for a short period of time (two or three months), which partially explains the low levels in their blood in comparison to breeding adults, which are at least five to six years of age (average age at first breeding) (Marchant and Higgins, 1990). Growth dilution may be also a significant factor leading to lower organochlorine levels in chicks than in their parents (Bourgeon...
3.2. Differences between colonies - Elephant Island vs. Livingston Island

Birds on Livingston Island exhibited, in general, lower contaminant levels than those on Elephant Island, with more noticeable differences for males than females (Fig. 2). Males on Livingston Island exhibited significantly lower (p < 0.05) levels than males on Elephant Island for five classes of chlorinated contaminants (HCB, DDTs, mirex, PCBs and chlordanes) (p < 0.05) (Table S8). Significant differences were also found between the two islands for females on, where HCB and mirex levels were significantly (p < 0.05), lower on Livingston Island than on Elephant Island (Table S8).

Geographic differences in contamination levels may be caused by segregation in foraging areas among Southern Giant Petrel colonies, despite the proximity between the two breeding sites. Elephant Island is situated in the northernmost portion of the South Shetland Islands and is about 300 km from Livingston Island (Fig. 1). During the migration and/or breeding season, males on Elephant Island may forage mainly at lower latitudes and therefore in more contaminated areas than males on Livingston Island, which likely spend more time foraging at higher latitudes of the Southern Ocean. Differences in foraging strategies between females of the two colonies may be less striking than males, which are reflected in contamination levels. Different foraging patterns among Southern Giant Petrel colonies and between sexes have been reported for other locations, such as South Georgia and Patagonia (González-Solís et al., 2000; Forero et al., 2005; Quintana et al., 2010; Petry and Kruger, 2011; Rey et al., 2012). During the breeding period, males forage closer to the colony, and therefore, provisioning chicks more frequently than females (Hunter, 1983; González-Solís et al., 2000). However, there is a lack of information regarding Southern Giant Petrel foraging strategies, mainly during their non-breeding season, on Elephant and Livingston Islands and assumptions regarding differences in foraging patterns in these areas need to be investigated further.

3.3. Recaptures

Despite the small number of individuals recaptured, it was possible to notice that organohalogen concentrations in blood samples of all five birds were very similar between the two sampling years (Table 3), with no statistical differences (p > 0.05) (Table S8).

Several factors can influence the toxicokinetics of organochlorines in organisms, such as variations in diet, food resources and body condition. For example, Bustnes et al. (2001) found no significant changes in organochlorine levels among breeding seasons for Glaucous Gulls, which were in a relatively stable environment in terms of food sources (Bustnes et al., 2000). On the other hand, Great Black-backed Gulls (Larus marinus), which usually experience a considerable variation in food availability, exhibited high intra-individual variability between two consecutive breeding seasons (Bustnes et al., 2005). The effect of environmental conditions on individual contaminant levels may lead to incorrect conclusions regarding trends in contaminant levels in the environment (Bustnes et al., 2005). Thus, bird species with low short-term variability in organochlorine levels, such as that observed in the present study on the Southern Giant Petrel, may be more effective monitors in long-term assessments.

Organic contaminant levels in birds are generally monitored using eggs, liver, and blood samples from different individuals (e.g., Elliott and Shutt, 1993; Braune, 2007; Vander Pol and Becker, 2007). However, seabirds usually present high degree of intraspecific variability regarding levels of PCBs and OCs (Elliott, 2005; Colabuono et al., 2012), which may be attributed to several factors, such as diet, age and distribution (Elliott, 2005). Thus, repeated sampling from the same individuals provides a more accurate evaluation of temporal trends in environmental contamination in a long-term monitoring.
Table 3
Organochlorine contaminant levels (ng g⁻¹ wet weight) in blood samples of five recaptured birds from Elephant Islands. Birds were first sampled in November 2011, with second sampling in November 2012. 2PCBs = sum of 81 congeners; 2DDTs = sum of 2,4-DDE, 4,4’-DDE, 2,4-DDD, 2,4-DDT, 4,4’-DDT; 2Chlordanes = sum of trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor and oxychlordane.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Male</th>
<th>Female</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>V27716</td>
<td>V28122</td>
</tr>
<tr>
<td>2PCBs</td>
<td>14.9</td>
<td>16.9</td>
</tr>
<tr>
<td>Pentachlorobenzene</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>HCB</td>
<td>6.20</td>
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<tr>
<td>2DDTs</td>
<td>33.9</td>
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<tr>
<td>Mirex</td>
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<td>19.4</td>
</tr>
<tr>
<td>2Chlordanes</td>
<td>2.85</td>
<td>2.42</td>
</tr>
</tbody>
</table>

4. Conclusions

The results presented herein contribute to information on organohalogen contaminants in antarctic top predators. Blood samples from Southern Giant Petrels adults and chicks have proven useful to compare contamination levels in these birds. Mirex, DDTs, PCBs and HCB were the predominant compounds in blood samples. Organic contaminant levels were higher in adults than chicks as a consequence of bioaccumulation during breeding and wintering periods. In general, no sex differences in organochlorine levels were found, but geographic differences between colonies were detected in contamination levels, suggesting different foraging patterns by birds from Elephant and Livingston Islands. Differences in diet and foraging areas of Southern Giant Petrels in both colonies should be better investigated using stable isotopes and/or geolocators. Despite the small number of recovered birds, organohalogen levels in blood samples from breeding adults generally appeared not to change after one year. Greater efforts to recapture ringed birds would be useful for long-term monitoring to assess trends in organohalogen levels in the antarctic environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.05.041.

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