

Persistent organic pollutants and porphyrin levels in excreta of penguin colonies from the Antarctic Peninsula area

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ABSTRACT. Persistent organic pollutants (POPs) and their effects on Antarctic seabirds by using excreta as a non-destructive biomonitoring tool have received little consideration. Here we determine the levels of polychlorinated biphenyls (PCBs) and some organochlorine pesticides such as dichlorodiphenyltrichloroethanes (DDTs), hexachlorobenzene (HCB), heptachlor, and endrin aldehyde in penguin excreta. Animal exposure to these environmental contaminants was determined through porphyrins in penguin droppings. Stool samples of Adélie penguin (*Pygoscelis adeliae*), chinstrap penguin (*Pygoscelis antarctica*) and gentoo penguin (*Pygoscelis papua*) were collected on two locations of the Antarctic Peninsula area: Base O'Higgins (Antarctic Peninsula) and Ardley Island (King George Island). Despite POPs have been banned more than three decades ago, the levels (ng g⁻¹ ww) of PCBs (1.45–2.35), DDTs (1.33–1.76), HCB (0.51–1.70), endrin (0.48–0.71) and heptachlor (0.97–2.40) showed that these pollutants are still present in Antarctica. Porphyrin levels in excreta (4.6–6.7 nmol g⁻¹ dw) were significantly correlated to POPs, indicating certain chemical exposure on penguin colonies that inhabit the Antarctic Peninsula area. The levels of heptachlor found in penguin guano may be affecting some biota in terrestrial sites next to nesting places. Further studies and better understanding of POPs impact on animal performance in Antarctic biota are recommended.

Introduction

Even though the production of persistent organic pollutants (POPs) has been banned for over 35 years, their levels still remain in the environment, and because they can be transported by wind and water, their effects can be evidenced far from where they were released (Geisz and others 2008). Polychlorinated biphenyls (PCBs) and chlorinated pesticides such as DDTs are toxic chemicals that adversely affect people, wildlife and the environment (Bright and others 1995; Roosens and others 2007; Llansola and others 2010). Antarctica is the most remote continent of the planet, but despite of this it is not exempted from anthropogenic impacts (Bargagli 2008; Corsolini 2009). Since 1970s some POPs have been found in biota (Corsolini and others 2003), air (Bidleman 1993), water (Bicego and others 1996), sediments (Montone and others 2001) and soil (Roosens and others 2007).

Only 0.34 % (about 50,000 km²) of the Antarctic continent is ice-free exposed rock mostly in the Peninsula area and remote mountain regions (BAS 2004). The ice-free areas constitute the main habitat for much of the wildlife and plants of Antarctica, in addition to fauna that inhabit Antarctica seasonally. There also

concentrate most of the research stations and most human activities on the continent (Tin and others 2009). This thin coastal strip represents only 0.05% of the total land area of Antarctica or about 6,000 km² (Poland and others 2003). This habitat is globally important as the home for most of the terrestrial biota of Antarctica, including iconic Antarctic species such as *Pygoscelis* penguins, which are found nowhere else in the world (Woehler and Poncet 1993). These ice-free areas are the sites of most environmental contamination (Tin and others 2009).

Some evidence has shown that Antarctic fauna are susceptible to contaminants at lower concentrations than similar temperate species (Poland and others 2003). On the other hand, polar animals have a high content of lipids and a slow metabolism to save energy, and therefore they have a very slow process of pollutants detoxification (Chapman and Riddle 2005). This implies that Antarctic organisms may be more vulnerable to the adverse effects of persistent pollutants than animals from other regions (Bargagli 2008). Moreover, many contaminants in the polar environments may remain for decades (Poland and others 2003). Therefore, more research is necessary to investigate now baseline levels of POPs to monitor

possible future changes in Antarctica and to understand the effects of contaminants on the environment in the cold regions.

Biomonitoring can provide valuable data of chemical levels in seabirds, like penguins, because they are considered sensitive species of environmental perturbations (Boersma 2008). Moreover, seabirds have long life cycles and provide subtle responses to contaminant exposure (Joshi and others 2013). Penguins represent about 90% of the bird biomass in the Southern Ocean, where they breed on the Antarctic continent and sub-Antarctic islands (Williams and Rothery 1990; Williams 1990). Antarctic penguins usually nest in colonies on sub-Antarctic islands and the Antarctic Peninsula area (Woehler and Poncet 1993). Since 2000, this seabird species has been listed as near threatened (IUCN 2012). These animals can be excellent indicators of POPs exposure because they rely on their fat reserves to mitigate extreme cold during the winter, an energy burden that is one of the main targets for bioaccumulation of the highly lipophilic pollutants (Roosens and others 2007; Taniguchi and others 2009). Although some studies have documented the presence of POPs in Antarctic organisms (Corsolini and others 2002; Corsolini and others 2003; Cipro and others 2013), little is still known about their potential adverse effects on penguins.

Bird excreta have proven to be one of the best non-destructive matrices for measuring persistent pollutants (Sun and others 2006; Espejo and others 2014), due to minimal intervention on the animal during sampling, and provide a net result between consumed and intestinal absorbed fractions of food. The use of porphyrins in excreta as a biomarker of exposure in birds has showed a close link between the contaminant and induction of a specific porphyrin in target organs (Fossi and others 1996; Casini and others 2001). Porphyrins are a group of biomolecules generated in the synthesis of hemoglobin and cytochroms, which are metabolised and accumulated in trace amounts in erythropoietic tissues such as liver or kidney, and then disposed with urine and guano (Lim 2002). Likewise, the resulting oxide by-products such as copro-, uro- and protoporphyrins are not toxic at physiological levels, but accumulated in excess may affect liver function and bone marrow. Some contaminants can interfere with heme biosynthesis leading to altered levels of porphyrins that are accumulated or excreted (Fossi and others 1996). We predicted that the levels of organic pollutants in excreta would correlate with those of porphyrins.

Determining organic pollutant levels in penguin excreta can provide a quantitative indication of environment impact caused by POPs in the marine Antarctic fauna as well as in the terrestrial ecosystem. The aim of this research was to determine the levels of some POPs and porphyrins in excreta of penguins that inhabit the Antarctic Peninsula area. A secondary objective was to validate the use of the QuEChERS method (Asensio-Ramos and others 2010) to detect organochlorine pesti-

cides and PCBs in penguin excreta. QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is an analytical method originally developed for measuring a wide range of pesticides in fruits and vegetables (Anastassiades and others 2003). Recently, some studies have shown that this method can be used for environmental purposes, and can be adapted to other kinds of contaminants and other biological matrices, such as fish tissue (Norli and others 2011) and penguin blood samples (Jara-Carrasco and others 2015).

Material and methods

Field work

This study was carried out at two locations of the Antarctic Peninsula area (see Figure 1) during the southern summer (January-February 2011). Twenty excreta samples (5 to 10 g each) were collected from nesting sites of Adélie penguin and chinstrap penguin colonies at Base O'Higgins (63°19'01"S, 57°54'29"W) – situated in the Antarctic Peninsula, while 20 samples of gentoo penguin colonies were obtained on the Ardley Island (62°13'48"S, 58°54'34"W) – situated south of King George Island. Before collecting samples, a bioethic letter was provided by the Universidad de Concepción, which was strictly required to obtain authorization from the Chilean Antarctic Institute (INACH) to carry out the field campaign. Each sample collected was from many individuals of the breeding colony. Excreta were collected immediately after bird defecation. Careful sampling and storage procedures were observed to avoid contamination. Fresh penguin droppings were taken with stainless steel spoon from the top of the mass of excrements deposited on the ground, then placed in aluminum foil and stored at -20°C in a steel container until their analysis in the laboratory.

Laboratory work

The levels (wet weight) of PCBs (congeners 138, 153 and 180), hexachlorobenzene (HCB), heptachlor, dichlorodiphenyltrichloroethanes (*p,p'*-DDT, -DDE, -DDD) and endrin aldehyde were determined at the Environmental Chemistry Laboratory (accredited ISO 17025) of the Universidad de Concepción. The extraction of the contaminants in excreta was performed by using the QuEChERS method (Asensio-Ramos and others 2010) used for sediments, here modified. First, all the samples were thawed and then weighed. Five grams of wet sample was put in a 50 ml centrifuge tube with 10 ml of n-hexane and stirred for 1 min in a vortex mixer. Gram quantities of salts (4 g of Mg SO₄ and 2 g of NaCl) were added to each tube (to drive analytes partitioning between the aqueous residue and the solvent) by stirring (vortex mixer) for 1 min, followed by sonication (15 min) in an ultrasonic bath, and centrifugation (4°C, 20 min at 5000 rpm). After vortex mixing and centrifugation, clean-up and removal of residual water was performed using a d-SPE procedure (PSA adsorbent and anhydrous MgSO₄ were mixed with

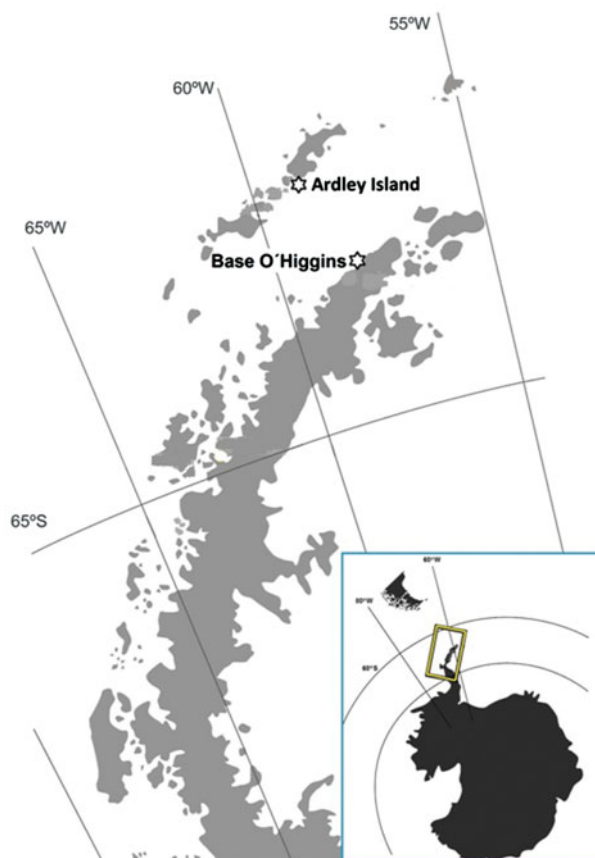


Fig. 1. Map of the Antarctic Peninsula area showing the sampling sites on Ardley Island (King George Island) and Base O'Higgins (Antarctic Peninsula).

the sample extract). After centrifugation (5000 rpm, 5 min), the volume of the extracts was reduced in a rotary evaporator (40°C). The final extract (1 ml) was stored in an amber vial, brought to dryness under a nitrogen stream. An internal standard (PCNB, 5 ng ml⁻¹) and the samples were injected into a gas chromatograph equipped with an electron capture detector (GC-ECD Perkin Elmer, series 9000). The separation of the analytes was performed in a PTE-5 capillary column (30 m × 0.25 mm inner diameter, 0.25 μm thick stationary phase) and helium as the carrier gas. The temperatures of the injector and detector were 240°C and 360°C, respectively. The temperature program of the oven was: 100°C for 10 min, then increased until 280°C at 5°C per minute, and kept for 12 min. The analytes of interest were separated within 58 min. Analysis of POPs in penguin excreta was accompanied by a quality control program, which consisted of repetitive analysis of blank samples and excreta samples spiked with known concentrations of the analytes. The detection limits were 0.002 μg l⁻¹. Surrogate recovery ranged from 68% up to 110% for PCB and 60% up to 108% for pesticides. The results were within the specified values with recovery rates between 67.9 and 108.2 % for all the analytes at the monitoring rates. Blank sample values were 0.5, 0.05, 1.0, 0.2 and 1.0 % for HCB, heptachlor, DDTs, endrin aldehyde and PCBs, respectively.

The levels (dry weight) of porphyrins (uro-, copro- and copro-) were determined at the Biomarkers Laboratory of the Universidad de Concepción. Porphyrins were determined as described by Lockwood and others (1985). Briefly, 1 ml of a 5 N HCl solution was placed into a centrifuge tube containing 100 mg of excreta and vortex-mixed for 1 min. Then, diethyl ether (3 ml) was added and mixed to obtain an emulsion. Afterwards, 3 ml of distilled water were added and the extract was again mixed and then centrifuged at 700 g for 10 min. The supernatant was removed, and the precipitant containing the porphyrins was analyzed in a spectrophotofluorimeter (Perkin Elmer model LS50). The porphyrins were detected using the following excitation/emission wavelengths: 405/595 nm for uroporphyrin, 400/595 nm for coproporphyrin, and 410/605 nm for protoporphyrin (Casini and others 2001). The quantitation of porphyrins was performed with external standards (uroporphyrin III octamethyl ester, coproporphyrin III dihydrochloride, protoporphyrin IX dimethyl ester) obtained from Porphyrin Products Inc. (Logan, UT).

The levels of POPs and porphyrins (X) were subjected to logarithmic transformation [$\ln(X+1)$] for statistical analyses. Statistical elaboration of data was performed using Statistica software (Statsoft). Comparisons of mean levels were performed using one-way ANOVAs.

Table 1. Concentration (ng g^{-1} wet weight) of persistent organic pollutants in excreta samples of different *Pygoscelis* penguins (mean \pm standard error) from the Antarctic Peninsula area.

POPs	<i>P. antarctica</i>	<i>P. adeliae</i>	<i>P. papua</i>
HCB	0.51 ± 0.27^b	0.53 ± 0.46^b	1.70 ± 1.57^a
Heptachlor	0.97 ± 0.33^b	1.09 ± 0.88^b	2.40 ± 0.81^a
DDTs	1.33 ± 0.54^a	1.61 ± 0.64^a	1.76 ± 1.31^a
Endrin aldehyde	0.48 ± 0.13^b	0.53 ± 0.18^{ab}	0.71 ± 0.18^a
PCB-138	0.49 ± 0.19^b	0.51 ± 0.14^b	0.97 ± 0.55^a
PCB-153	0.38 ± 0.27^b	0.48 ± 0.17^{ab}	0.65 ± 0.18^a
PCB-180	0.58 ± 0.28^a	0.63 ± 0.23^a	0.72 ± 0.24^a
Sum PCBs	1.45 ± 0.65^b	1.61 ± 0.47^b	2.35 ± 0.76^a

HCB = hexachlorobenzene; DDTs (dichlorodiphenyltrichloroethanes) = *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE; PCB = polychlorinated biphenyl. Different letters in a row indicate statistical significant differences (ANOVA, $p \leq 0.05$).

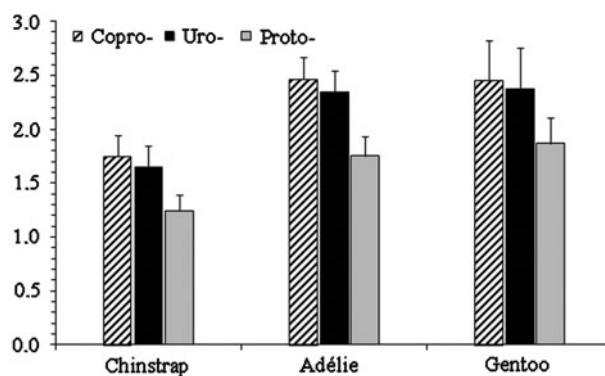


Fig. 2. Mean concentration (ng g^{-1} dry weight) and standard error of porphyrins in excreta samples of different *Pygoscelis* penguins from the Antarctic Peninsula area.

Correlations for pollutants and porphyrins were calculated through Pearson's test. Differences were considered significant at $p \leq 0.05$.

Results

Table 1 shows the mean concentrations of POPs in penguin excreta. Across the three penguin species studied here, PCBs (1.45 – 2.35 ng g^{-1} ww) and DDTs (1.33 – 1.76 ng g^{-1} ww) presented the highest levels of all analyzed POPs. On the contrary, the lowest concentrations corresponded to endrin aldehyde with 0.48 , 0.53 and 0.71 ng g^{-1} ww in excreta of *P. antarctica*, *P. adeliae* and *P. papua*, respectively. The concentrations of HCB, heptachlor and PCB-138 were significantly higher in gentoo penguin excreta than those levels found in chinstrap and Adélie penguin excreta. The levels of endrin aldehyde and PCB-153 were higher (ANOVA, $p \leq 0.05$) in gentoo penguin droppings than in chinstrap excreta. The levels of DDTs and PCB-180 showed not significant differences in excreta of the three penguin species studied. Not significant differences in the concentration of HCB, heptachlor, endrin aldehyde, PCB-153 and PCB-138 were found in chinstrap penguin excreta as compared to Adélie penguin excreta. The contaminant concentration patterns in the three species of penguins studied was: gentoo penguin >

Adélie penguin > chinstrap penguin. The levels of POPs found in excreta of the three penguin species, considering all the samples together, indicated the following relation: PCBs > DDTs > heptachlor > HCB > endrin aldehyde.

The porphyrin levels in the samples analysed are shown in Figure 2. The total porphyrins levels were 4.6 , 6.6 and 6.7 nmol g^{-1} in chinstrap, Adélie and gentoo penguin excreta, respectively. The levels of copro- ($p = 0.132$), uro- ($p = 0.121$) and protoporphyrin ($p = 0.068$) did not show significant differences among the penguin species. Copro- (1.01 – 2.81 nmol g^{-1}), uro- (0.99 – 2.76 nmol g^{-1}) and protoporphyrin (0.77 – 2.11 nmol g^{-1}) found in the excrements of chinstrap penguins were similar to the levels of copro- (1.69 – 3.66 nmol g^{-1}), uro- (1.72 – 3.26 nmol g^{-1}) and proto- (1.26 – 2.46 nmol g^{-1}) obtained in Adélie penguins excreta, and also were similar to the levels of copro- (1.09 – 3.51 nmol g^{-1}), uro- (1.05 – 3.53 nmol g^{-1}) and proto- (0.87 – 3.12 nmol g^{-1}) found in gentoo penguin excreta. Persistent PCB congeners 180 and 138 contributed more to the total PCB content in the excreta samples than congener 153.

There was a significant positive correlation between the concentrations of PCBs and DDTs with the levels of porphyrins (Table 2). The levels of PCBs found in penguin excreta were significantly correlated ($p \leq 0.05$, Pearson, $r > 0.5$) with those of porphyrins (copro-, uro-,

Table 2. Pearson correlation among POPs and porphyrins (copro-, uro-, proto-) in excrements of the three species of penguins from the Antarctic Peninsula area.

	HCB	Heptachlor	DDTs	Endrin	PCBs	Copro	Uro	Proto
HCB	1							
Heptachlor	0.27	1						
DDTs	-0.05	-0.15	1					
Endrin	0.05	0.57*	-0.15	1				
PCBs	0.19	0.42*	0.88*	0.29	1			
Copro	-0.12	-0.03	0.48*	0.04	0.69*	1		
Uro	-0.12	-0.03	0.46*	0.04	0.59*	0.99*	1	
Proto	0.06	0.01	0.71*	0.04	0.58*	0.94*	0.96*	1

*Statistically significant (ANOVA, $p \leq 0.05$).

proto-). Also, the levels of DDTs were highly correlated with the levels of protoporphyrins. There was a medium positive correlation ($p \leq 0.05$, Pearson, $r > 0.3$) between the levels of uroporphyrins and coproporphyrins with those of DDTs. Moreover, a positive significant correlation was observed in the levels of endrin/heptachlor, PCBs/heptachlor, and PCBs/DDTs, which could indicate that these contaminants all together induce porphyrin synthesis.

Discussion

Our levels detected of PCBs, DDTs, HCB, endrin and heptachlor in penguin excreta reveal that these organic pollutants are still relevant within the Antarctic food web. These organic pollutants are been incorporated into penguins mainly through diet, where are metabolised and then eliminated via excretion (Falkowska and others 2013; Joshi and others 2013). Because gentoo penguins do not travel outside the Antarctic Peninsula area (Williams 1990), these seabirds are concentrating POPs that have already been transported into the area via water or air and further redistributing them through local bird activities.

The lack of published data on POPs and porphyrins levels in penguin excreta makes it difficult to discuss these results. In the present study, the levels of DDTs (1.33–1.76 ng g⁻¹) found in excreta of penguins from the Antarctic Peninsula area were about 106–80 times lower than those reported in excreta of penguins from marine environments of lower latitudes (Falkowska and others 2013). Overall, our levels of POPs were generally lower than those reported in the same penguins species, but measured in other biological materials such as eggs (Cipro and others 2010) and lipid tissue (Taniguchi and others 2009). Our levels of HCB, DDTs, PCB-138, PCB-153 and PCB-180 found in chinstrap penguin excreta were 1.7, 5.5, 3.1, 5.8 and 3.4 times lower than those levels observed by Jara-Carrasco and others (2015) in blood of the same species, respectively. Taniguchi and others (2009) found that the concentrations of HCB, DDTs and PCBs in the fat tissue of penguins (average levels of *Pygoscelis adeliae*, *Pygoscelis antarctica* and *Pygoscelis papua*, pooled together) found at King George Island were 373, 193 and 256 ng g⁻¹, which is about 220, 110 and 109 times higher than our

highest levels in excreta, respectively. The lower levels of POPs found in penguin excreta can be explained as these pollutants tend to bioaccumulate mainly in the organism. PCB-138, 153 and 180 have a low excretion rate in birds and tend to accumulate in the animal (Maervoet and others 2004). A study found that 8.4% of PCBs ingested were excreted in the excrements of otters (*Lontra canadensis*), which revealed that PCBs of 16,000 ng/g lipid weight excreta is equivalent to 50,000 ng g⁻¹ lipid weight tissue, a critical level for otters based on reproductive toxicity (Smit and others 1994). POPs in excreta as related to internal tissue threshold levels for effects on reproduction, neurobehavioral development and immune suppression of Antarctic species is an issue that need to be more investigated.

The presence of PCBs in excreta is highly related to their molecular structure, as most birds are capable of eliminating PCBs with low molecular weight (Norstrom and others 1988). The congeners 138 and 180 are hexa- and hepta-chlorinated PCBs, respectively, which constitute most of the residues of PCBs from food ingested by penguins (Focardi and others 1995). A study performed in the Lake Ontario ecosystem stated that twelve PCB congeners (including 153, 138 and 180) constituted over 50% of the PCBs in fish and are considered to be environmentally important (Oliver and Niimi 1988). The tetra-, penta-, and hexa-chlorinated isomer groups exhibit greater toxicity than the other chlorinated forms, thus congener PCB-138 is classified in a group more potent than PCB-180 in inducing hepatic function alterations and immunotoxicity in humans (Llansola and others 2010). In the present study, only gentoo penguin excreta showed PCB-138 levels higher than those of PCB-180.

When comparing the POPs levels measured in the current study with those reported recently in other marine species and biological materials from the Northern Hemisphere, our findings appear to be much lower. A study on eggs of white-tailed sea eagles (*Haliaeetus albicilla*) from Baltic Sea, showed a depressed nest productivity related to eggshell thickness caused by PCBs (500,000 ng g⁻¹) and *p,p'*-DDE (120,000 ng g⁻¹) (Helander and others 2002). In blubber of seals from White Sea (Russia) the levels of DDTs and PCBs were about 4,000 and 5,000 ng g⁻¹ lw, respectively, whereas the levels of HCB were

62 ng g⁻¹ lw (Muir and others 2003). In some species from contaminated environments, PCBs have usually been the dominant contaminant class, whereas levels of DDTs are lower (Evenset and others 2005). Recent studies have found that organic pollutant concentrations in tissue, body compartment or egg of several Arctic marine mammal species and populations exceed a general threshold level of concern of 1,000 ng g⁻¹ ww (Letcher and others 2010).

The presence of POPs can be a serious threat to Antarctic ecosystems and their endemic animals, because they bioaccumulate and biomagnify in aquatic food webs, resulting in high concentrations in top-predatory wildlife species (Guertin and others 2010). In Antarctica, trophic chains are relatively simple and short, where seabirds at the top of the food webs, like penguins, depend mainly on the silverfish (*Pleuragramma antarcticum*) and krill (*Euphausia superba*) (Corsolini 2009). Concentrations (ng g⁻¹ ww) of DDTs (0.41), PCB-153 (0.1) and HCB (0.06) have been reported in krill (Cipro and others 2010), the main prey of Antarctic penguins, which are about 28, 5 and 4 times lower than our mean levels in penguin excreta, respectively. Levels (ng g⁻¹ ww) of HCB (0.66) and DDTs (3.04) have been found in Antarctic fish (Cipro and others 2013), which are about twice lower than our mean levels in excreta. Biomagnification is the main route of contamination at the higher levels, where the feeding habits of penguins play a crucial role in POPs intake (Corsolini 2009). Our data showed that *P. papua* excreta had the highest concentrations of HCB, heptachlor and PCB-153 as compared to *P. antarctica* and *P. adeliae*. Diet could explain the differences found in the concentration of POPs in penguin excreta (Falkowska and others 2013), because the three colonies studied are distant and this may affect the diet of penguins and consequently the contaminant and porphyrin levels. The differences found in penguin excreta could be a result of increased bioaccumulation of contaminants for these seabirds, probably associated to changes in diet through time and space. Gentoo penguin feed mainly on Antarctic fish, but its diet may be supplemented with an intake of krill and different crustaceans and cephalopods (Berón and others 2002).

Our results provide important details for interpretation of contaminant exposure. The fingerprint of the porphyrins in the excreta samples from the three localities studied showed the following pattern: coproporphyrins > uroporphyrins > protoporphyrins. This pattern observed is probably due to the same nature of chemical stress in the area studied (Casini and others 2001). Results from the present study showed that the highest total porphyrin levels (6.7 nmol g⁻¹) detected in gentoo penguin excreta are 2.24 lower than those levels reported by Fossi and others (1996) in droppings of Japanese quail (*Coturnix japonica*), which were treated with 50 mg kg⁻¹ of PCBs in the diet for 25 days. In a study performed in Talcahuano (Chile), a highly impacted area by anthropogenic activities, levels of total porphyrins (8.6 ng g⁻¹ dw) measured in excreta of kelp gulls (*Larus dominicanus*) showed that

this species is at high toxicological risk (Casini and others 2001). Japanese quail treated with 500 mg of HCB per kg body weight for 60 days was associated with 4.8 nmol g⁻¹ of total porphyrins in excreta (Casini 1997), which was similar to our levels in chinstrap penguins, and about 30% lower than our levels in Adélie and gentoo penguins. The levels of porphyrins in penguin excreta studied here are similar to those found by Celis and others (2012) in gentoo penguin excreta at Base O'Higgins. Also, our total porphyrins levels were lower than those levels (9.1 nmol g⁻¹ dw) found in Humboldt penguin (*Spheniscus humboldti*) excreta, a species of seabird that naturally inhabits the coast between Peru (5°S) and Chile (43°S), which were linked to heavy metal contamination (Celis and others 2014).

The positive correlation between porphyrins (uro-, copro-, and proto-) and POPs indicates a high affinity between them, which is linked to physiological problems in the pancreas, liver and kidneys of birds (Casini and others 2003). This means that colonies of penguins living on Base O'Higgins and Ardley Island might be suffering some negative biological effects from POPs contamination. Some local pollution (fuel combustion, waste incineration, sewage disposal, paint or accidental oil spills) or the impact of the tourism increases (6,704–36,875 tourists from 1992–1993 to 2009–2010) and scientific bases with their associated activities as plane and ship trips have been noted in the northern area of the Antarctic Peninsula (Lynch and others 2010; IAATO 2010; Barbosa and others 2013). Most Antarctic research stations are located on the King George Island within 5 km of the coast. Base O'Higgins is a permanently staffed base, where around a hundred pairs of gentoo penguins inhabit in the surrounding area, which is frequently visited by tourists. Research stations are the major land-based activity in the Antarctic, and, as a consequence, are the source of most locally derived pollutants (Poland and others 2003).

Porphyrins have a typical absorption spectrum, thus their detection levels are very sensitive and specific (Fossi and others 1996). Porphyrins are produced and accumulated in small amounts in erythropoietic tissues, liver and kidney and excreted by urine or faeces, which can be identified as coproporphyrin, uroporphyrin and protoporphyrin (Casini and others 2003). It has been noted that polycyclic aromatic hydrocarbons (PAHs), metals, and organic contaminants might interfere with heme synthesis (Fossi and others 1996). In our study, POPs and porphyrins levels indicate gentoo penguins from Base O'Higgins and Ardley Island are exposed to these pollutants at some level. Certain organic pollutants such as DDTs and PCBs can affect the immuno-haematological response of *Pygoscelis antarctica* that nest in some sites of the South Shetland Islands and Base O'Higgins (Jara-Carrasco and others 2015). As a consequence, the use of porphyrins in droppings of seabirds can be considered as a valid biomarker of exposure to chemicals, which is consistent with some other reports (Casini and others 2001; Celis and others 2012).

The correlations between POPs and porphyrins indicate despite of the low chemical concentrations at which Antarctic penguins can be exposed, compared to seabirds of the Arctic and other latitudes (Bustnes and others 2010; Letcher and others 2010; Falkowska and others 2013), the levels of POPs found in our study suggest that these contaminants could potentially be altering the synthesis of porphyrins in the species studied. There is evidence showing that even low concentrations of some toxic pollutants may have hazardous effects on penguins (Subramanian and others 1986). However, Antarctic penguins are exposed to a wide range of other contaminants, and therefore the influence of some other pollutants in Antarctica cannot be neglected (Roosens and others 2007; Corsolini 2009; Taniguchi and others 2009; Espejo and others 2014). It is possible that POPs in combination may act additively, antagonistically or synergistically. A study showed that while PCB-153 alone did not result in porphyrin accumulation in rats, co-administration of PCB-153 with dioxin revealed a strong synergistic effect (Van Birgelen and others 1996).

Long-range atmospheric transport appears to be the main mechanism for exposing cold regions to POPs (Bargagli 2008). However penguin excreta can also increase contaminant levels in some environments (Sun and others 2006). Penguins form large breeding colonies on locations where they can number in the hundreds or tens. Because they feed exclusively off shore but nest on land, penguins can act as biovectors of contaminants from marine ecosystems to terrestrial ecosystems (Roosens and others 2007). Through this transport pathway, penguins can also concentrate POPs that are biomagnified and bioaccumulated through the marine food webs. This may have serious implications for any biota and possible incorporation into the terrestrial food web. Polar invertebrates have low detoxifying activity to metabolise PCB 138, 153 and 180 (Bright and others 1995). Evidence on sediment quality shows that the incidence of benthic macroinvertebrate effects increase when concentrations of PCBs > 61.2, DDTs > 12.6, endrin > 4.7 and heptachlor > 0.11 ng g⁻¹ ww (Burton 2002). Our heptachlor levels (0.97–2.40 ng g⁻¹) detected in penguin excreta are above threshold values (defined as the concentration above which adverse effects are expected to occur) for this organic pollutant, indicating that penguins are transporting this chemical from the sea to the nesting areas to toxic levels for the surrounding biota that live on Base O'Higgins and Ardley Island, such as the Antarctic whelk (*Neobuccinum eatoni*) or the Antarctic scallop (*Adamussium colbecki*). The scallop is a filter feeder, therefore it can accumulate more POPs than be expected in relation to its tropic position in the Antarctic food web (Corsolini 2009). Some evidence from the Antarctic Peninsula indicated high heavy metal levels in coastal soils near penguin rookeries at Base O'Higgins (Celis and others 2015). The effects of POPs in freezing ground are of concern because of their potential for detrimental effects on biota (Poland and others 2003). As practically no data on POPs are

available for this area, our findings suggest the need for further studies.

Conclusions

The levels of PCBs, DDTs, endrin, heptachlor and HCB found in this study indicate these contaminants are still relevant in the Antarctic environment and can affect the food chain through bioaccumulation and biomagnification processes. Our study showed that QuEChERS is a useful tool in the detection of POPs in excreta. The detection of these POPs in excreta of the three species of penguins that inhabit the Antarctic Peninsula area, showed a clear capacity of these population of seabirds to accumulate and eliminate these pollutants, where the feeding habits of penguins play a crucial role in chemical uptake.

Porphyrins can be used as nondestructive indicators of exposure to organic pollutants of penguin species potentially at risks, especially when sequential sampling must be carried out for long-term studies. The levels of porphyrins detected in excreta indicate that these POPs may be producing some biological effects in the colonies of penguins studied. Gentoo penguin is the species that showed the highest concentrations of these contaminants in excreta, which can be attributed to their diet and trophic level.

The organic pollutants found in excreta of Antarctic penguins can be a source of contamination of terrestrial ecosystems from marine environments. The levels of heptachlor found in penguin guano may be affecting some benthonic organisms that live around nesting sites.

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References

- Anastassiades, M. S. Lehotay, D. Stajnbaher and one other. 2003. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal of AOAC International* 86: 412–431.
- Asensio-Ramos, M., J. Hernández-Borges, L. Ravelo-Pérez, and M. Rodríguez-Delgado. 2010. Evaluation of a modified QuEChERS method for the extraction of pesticides from agricultural, ornamental and forestal soils. *Analytical and Bioanalytical Chemistry* 396: 2307–2319.
- Barbosa, A., E. de Mas, J. Benzal, J. Diaz and eight others. 2013. Pollution and physiological variability in Gentoo penguins

- at two rookeries with different levels of human visitation. *Antarctic Science* 25:329–338.
- Bargagli, R. 2008. Environmental contamination in Antarctic ecosystems. *Science of the Total Environment* 400: 212–226.
- BAS (British Antarctic Survey). 2004. Antarctica: 1:10000000 scale map. BAS (Misc) 11. British Antarctic Survey, Cambridge URL: <http://www.npolar.no/en/services/maps/printed/topographic-antarctica.html> (accessed 11 December 2015).
- Berón, M.P., R. Néstor, R. Coria and one other. 2002. Monitoreo de la dieta post-reproductiva del pingüino papúa (*Pygoscelis papua*) en Isla Laurie (Orcadas del Sur, Antártida): periodo 1997–1999. *Ornitología Neotropical* 13: 413–422.
- Bidleman, T., M. Walla, R. Roura and two others. 1993. Organochlorine pesticides in the atmosphere of the Southern Ocean and Antarctica, January– March, 1990. *Marine Pollution Bulletin* 26: 258–262.
- Bícego, M., R. Weber and R. Ito. 1996. Aromatic hydrocarbons on surface waters of Admiralty Bay, King George Island, Antarctica. *Marine Pollution Bulletin* 32: 549–553.
- Boersma, P.D. 2008. Penguins as marine sentinels. *Bioscience* 58: 597–607.
- Bright, D.A., S.L. Grundy, and K.J. Reimer. 1995. Differential bioaccumulation of non-ortho-substituted and other PCB congeners in coastal Arctic invertebrates and fish. *Environmental Science and Technology* 29: 2504–2512.
- Burton, G.A. 2002. Sediment quality criteria in use around the world. *Limnology* 3: 65–75.
- Bustnes, J.O., B. Moe, D. Herzke and five others. 2010. Strongly increasing blood concentrations of lipid-soluble organochlorines in high Arctic common eiders during incubation fast. *Chemosphere* 79: 320–325.
- Casini, S. 1997. Effects of hexachlorobenzene and polychlorinated biphenyls on porphyrin metabolism: a potential application in ecotoxicological studies. *Pharmacological Research* 35: 221–222.
- Casini, S., M.C. Fossi, J.F. Gavilan and four others. 2001. Porphyrin levels in excreta of sea birds of the Chilean coasts as nondestructive biomarker of exposure to environmental pollutants. *Archives of Environmental Contamination and Toxicology* 41: 65–72.
- Casini, S., M. Fossi, C. Leonzio and one other. 2003. Review: porphyrins as biomarkers for hazard assessment of bird populations: destructive and non-destructive use. *Ecotoxicology* 12: 297–305.
- Celis, J., S. Jara, D. González-Acuña and two others. 2012. A preliminary study of trace metals and porphyrins in excreta of Gentoo penguins (*Pygoscelis papua*) at two locations of the Antarctic Peninsula. *Archivos de Medicina Veterinaria* 44: 311–316.
- Celis, J., W. Espejo, D. González-Acuña and two others. 2014. Assessment of trace metals and porphyrins in excreta of Humboldt penguins (*Spheniscus humboldti*) in different locations of the northern coast of Chile. *Environmental Monitoring and Assessment* 186: 1815–1824.
- Celis, J.E., R. Barra, W. Espejo and two others. 2015. Trace element concentrations in biotic matrices of Gentoo penguins (*Pygoscelis papua*) and coastal soils from different locations of the Antarctic Peninsula. *Water, Air and Soil Pollution* 226: 2266, doi: 10.1007/s11270-014-2266-5.
- Chapman, P.M. and M.J. Riddle. 2005. Toxic effects of contaminants in polar marine environments. *Environmental Science and Technology* 39: 200A–206A.
- Cipro, C., S. Taniguchi and R. Montone. 2010. Occurrence of organochlorine compounds in *Euphausia superba* and unhatched eggs of *Pygoscelis* genus penguins from Admiralty Bay (King George Island, Antarctica) and estimation of biomagnification factors. *Chemosphere* 78: 767–771.
- Cipro, C., F. Colabuono, S. Taniguchi and one other. 2013. Persistent organic pollutants in bird, fish and invertebrate samples from King George Island, Antarctica. *Antarctic Science* 25: 545–552.
- Corsolini, S., T. Romeo, N. Ademollo and two others. 2002. POPs in key species of marine Antarctic ecosystem. *Microchemical Journal* 73: 187–193.
- Corsolini, S., N. Ademollo, T. Romeo and two others. 2003. Persistent organic pollutants in some species of a Ross Sea pelagic trophic web. *Antarctic Science* 15: 95–104.
- Corsolini, S. 2009. Industrial contaminants in Antarctic biota. *Journal of Chromatography A* 1216: 598–612.
- Espejo, W., J. Celis, D. González-Acuña and two others. 2014. Concentration of trace metals in excrements of two species of penguins from different locations of the Antarctic Peninsula. *Polar Biology* 37: 675–683.
- Evenset, A., G.N. Christensen and R. Kallenborn. 2005. Selected chlorobornanes, polychlorinated naphthalenes and brominated flame retardants in Bjørnøya (Bear Island) freshwater biota. *Environmental Pollution* 136: 419–30.
- Falkowska, L., A.R. Reindl, E. Szumilo and five others. 2013. Mercury and chlorinated pesticides on the highest level of the food web as exemplified by herring from the Southern Baltic and African penguins from the Zoo. *Water, Air and Soil Pollution* 224: 1549.
- Focardi, S., S. Corsolini and R. Bargagli. 1995. Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in Antarctic fish, seabirds and Weddell seal from Terra Nova Bay (Ross Sea). *Antarctic Science* 7: 31–35.
- Fossi, M.C., S. Casini and L. Marsili. 1996. Porphyrins in excreta: a nondestructive biomarker for the hazard assessment of birds contaminated with PCBs. *Chemosphere* 33: 29–42.
- Geisz, H., R. Dickhut, M. Cochran and two others. 2008. Melting glaciers: A probable source of DDT to the Antarctic marine ecosystem. *Environmental Science and Technology* 42: 3958–3962.
- Guertin, D., A. Harestad, M. Ben-David and two others. 2010. Fecal genotyping and contaminant analyses reveal variation in individual river otter exposure to localized persistent contaminants. *Environmental Toxicology and Chemistry* 29: 275–284.
- Helander, B., A. Olsson, A. Bignert and two others. 2002. The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle (*Haliaeetus albicilla*) in Sweden. *Ambio* 31: 386–403.
- IAATO (International Association of Antarctic Tour Operators). 2010. 2009–2010 tourism summary. URL: http://www.iaato.org/tourism_stats.html (accessed 15 December 2015).
- IUCN (International Union for the Conservation of Nature). 2012. Red list of threatened species. URL: <http://www.iucnredlist.org/search> (accessed 22 October 2014).
- Jara-Carrasco, S., M. González, D. González-Acuña and five others. 2015. Potential immunohematological effects of persistent organic pollutants on Chinstrap penguin. *Antarctic Science* 27: 373–381.
- Joshi, M., P. Bakre and Bhatnagar. 2013. Avian guano: A non-destructive biomonitoring tool for organic pollutants in environment. *Ecological Indicators* 24: 284–286.
- Letcher, R.J., J.O. Bustnes, R. Dietz and six others. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Science of the Total Environment* 408 : 2995–3043.

- Lim, C.K. 2002. Analysis of biosynthetic intermediates, 5-amino levulinic acid to heme. In: Smith, A.G., and M. Witty (editors). *Heme, chlorophylla and bilius : methods and protocols*. Totowa: 95–109.
- Llansola, M., C. Montoliu, J. Boix and V. Felipo. 2010. Polychlorinated biphenyls PCB 52, PCB 180, and PCB 138 impair the glutamate-nitric oxide-cGMP pathway in cerebellar neurons in culture by different mechanisms. *Chemical Research in Toxicology* 23: 813–820.
- Lockwood, W.H., V. Poulus, E. Rossi and one other. 1985. Rapid procedure for fecal porphyrin assay. *Clinical Chemistry* 31: 1163–1167.
- Lynch, H. J., K. Crosbie, W.F. Fagan and one other. 2010. Spatial patterns of tour ship traffic in the Antarctic Peninsula region. *Antarctic Science* 22: 123–130.
- Maervoet, J., S.G. Chu, D. Vos and four others 2004. Accumulation and tissue distribution of selected polychlorinated biphenyl congeners in chickens. *Chemosphere* 57: 61–66.
- Montone, R., S. Taniguchi and R. Weber. 2001. Polychlorinated biphenyls in marine sediments of Admiralty Bay, King George Island, Antarctica. *Marine Pollution* 42: 611–614.
- Muir, D., T. Savinova, V. Savinov and three others. 2003. Bioaccumulation of PCBs and chlorinated pesticides in seals, fishes and invertebrates from the White Sea, Russia. *Science of the Total Environment* 306: 111–131.
- Norli, H., A. Christiansen and E. Deribe. 2011. Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. *Journal of Chromatography A* 1218: 7234–7241.
- Norstrom, R.J., M. Simon, C.G. Muir and one other. 1988. Organochlorine contaminants in Arctic marine food chains: identification, geographical distribution and temporal trends in polar bears. *Environmental Science and Technology* 22: 1063–1071.
- Oliver, B.G. and A.J. Niimi. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environmental Science and Technology* 22: 388–397.
- Poland, J.S., M.J. Riddle and B.A. Zeeb. 2003. Contaminants in the Arctic and the Antarctic: a comparison of sources, impacts, and remediation options. *Polar Record* 39 (211): 369–383.
- Roosens, L., N. van Den Brink, M. Riddle and three others. 2007. Penguin colonies as secondary sources of contamination with persistent organic pollutants. *Journal of Environmental Monitoring* 9: 822–825.
- Smit, M.D., P.E.G. Leonards, A.G.M. van Hattum and one other. 1994. *PCBs in European otter (Lutra lutra) populations*. Amsterdam: Vrije Universiteit.
- Subramanian, A., S. Tanabe, Y. Fujise and one other. 1986. Bioaccumulation of organochlorines (PCBs and *p,p'*-DDE) in Antarctic Adélie penguins (*Pygoscelis adeliae*) collected during a breeding season. *Environmental Pollution* 40: 173–189.
- Sun, L., X. Yin, X. Liu and six others. 2006. Levels of hexachlorocyclohexanes and dichloro-diphenyl-trichloroethanes in penguin droppings collected from Ardley Island, Maritime Antarctic. *Human and Ecological Risk Assessment* 12: 328–338.
- Taniguchi, S., R. Montone, M. Bicego and three others. 2009. Chlorinated pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in the fat tissue of seabirds from King George Island, Antarctica. *Marine Pollution Bulletin* 58: 129–133.
- Tin, T., Z.L. Fleming, K.A. Hughes and six others. 2009. Impacts of local human activities on the Antarctic environment. *Antarctic Science* 21: 3–33.
- Van Birgelen, A., K. Fase, J. van Der Kolk and four others. 1996. Synergistic effect of 2,2',4,4',5,5'-hexachlorobiphenyl and 2,3,7,8-tetrachlorodibenzo-p-dioxin on hepatic porphyrin levels in the rat. *Environmental Health Perspectives* 104: 550–557.
- Williams, T.D. and P. Rothery. 1990. Factors affecting variations in foraging and activity patterns of Gentoo penguins (*Pygoscelis papua*) during the breeding season at Bird Island, South Georgia. *Journal of Applied Ecology* 27: 1042–1054.
- Williams, T.D. 1990. Annual variation in breeding biology of gentoo penguin, *Pygoscelis papua*, at Bird Island, South Georgia. *Journal of Zoology* 222: 247–258.
- Woehler, E.J. and S. Poncet. 1993. *The distribution and abundance of Antarctic and Subantarctic penguins*. Cambridge: Scientific Committee on Antarctic Research.