PERFLUORINATED COMPOUNDS IN FUR SEAL PUPS AND PENGUIN EGGS FROM SOUTH SHETLAND, ANTARCTICA

Schiavone A[(1)], Corsolini S[(1)], Tao L[(2)], Kannan K[(2)], Trivelpiece W[(3)], Torres D Jr[(4)], Focardi S[(1)]
[(1)] Department of Environmental Science "G. Sarfatti", University of Siena, via P.A. Mattioli, 4, I-53100 Siena, Italy, E-mail address: schiavone4@unisi.it [(2)] Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, P.O. Box 509, Albany, New York 12201-0509 U.S.A. [(3)] U.S. Antarctic Marine Living Resources Division, Southwest Fisheries Science Center, 8604 La Jolla Shores Drive, PO Box 271, La Jolla, CA 92037, U.S.A. [(4)] Instituto Antártico Chileno (INACH), Plaza Muñoz Gamero, 1055, Punta Arenas, Chile

Abstract
In this study, the presence of perfluorochemicals (PFCs) in penguin eggs and Antarctic fur seals was reported for the first time. In fact, little has yet been reported on PFC concentrations in Antarctic organisms. A previous study reported PFOS concentrations below the limit detection in Adélie penguin eggs (< 0.1 ng/g wet wt). In our study we found a mean concentration of 0.4 ng/g wet wt of PFOS in Adélie penguin eggs. This difference could be due to the differences in the collection period, 1995/1996 and 2004 respectively. PFCs detected in bird eggs and seal pups suggested oviparous and viviparous transfer of PFOS to eggs and off-springs.

Introduction
Perfluorinated compounds (PFCs) have emerged as a new class of global environmental pollutants. Several studies have assessed them in a wide range of organisms[(1)(2)], including humans[(3)(4)], from low latitude regions to remote areas, suggesting atmospheric transport of volatile precursor compounds and/or transport in ocean currents[(5)(6)(7)]. PFCs are persistent and bioaccumulative although environmental behavior of this class of chemicals differs from other known persistent organohalogens. Because of their lipophobic nature, chemicals such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), do not accumulate in lipids but are found at greatest concentrations in blood and liver[(8)(9)(10)]. The physico-chemical structure of these compounds suggests the possibility of interactions with serum proteins[(11)]. In this study, muscle and liver samples of Antarctic fur seal (*Arctocephalus gazella*) pups and eggs of two penguins species (*Pygoscelis adeliae* and *Pygoscelis papua*) were analyzed for PFOS and related perfluorinated sulfonic acids (PFSAs) as well as a suite of perfluorinated carboxylic acids (PFCAs) ranging in carbon length from 7 to 12, to assess their presence and patterns in Antarctic biota.

Materials and methods
Collection of Samples. Twenty muscle and seventeen liver from Antarctic fur seal (*Arctocephalus gazelle*) pups were collected between January and February 2004. Antarctic fur seal pups were found dead at Livingstone Island, South Shetland, Antarctica (62°39' S, 60°30' W), and the tissue samples were taken from the carcasses at the time of necropsy. Sampling location, liver weight, gender, body weight and body length were recorded.
Unhatched eggs of Adélie penguins (*Pygoscelis adeliae*, n=13) and Gentoo penguins (*Pygoscelis papua*, n=13), were collected at King George Island, South Shetland, Antarctica (62°10' S, 58°67' W), during the 2004/05 field season. All samples were wrapped in polyethylene bags and stored at -20°C until analysis.
Analyses of perfluorinated compounds. Perfluorinated compounds were analyzed following the method described elsewhere, with some modifications[(12)(9)]. Analytes were detected and quantified using an Agilent 1100 series high-performance liquid chromatography (HPLC) coupled with an Applied Biosystems API 2000 electrospray triple-quadrupole mass spectrometer (ESI-MS/MS). The MS/MS was operated in electrospray negative ion mode. Target compounds were determined by multiple reaction monitoring (MRM). Reported concentrations were not corrected for the recoveries. Blanks were analyzed by passing water and reagents through the entire analytical procedure. Blanks contained trace levels of PFOA in penguin egg analysis and trace of PFOA and PFOS in seal samples analysis. However, the levels found in blanks were 2 to 10 fold lower than those found in samples. Concentrations reported here were subtracted from the mean value found in blanks.
The limit of quantitation (LOQ) was determined based on the linear range of the calibration curve prepared at a concentration range of 0.1 to 20 ng/mL. The tissue samples were compared to this unextracted standard calibration curve. Because of variety of matrixes analyzed and because of evolving analytical methods, LOQ was variable. The LOQ was determined as the lowest acceptable standard in the calibration curve, deemed acceptable if it was within ±30% of the theoretical value and the peak area of the standard was at least twice as great as the

matrix blanks. LOQ was 0.4 ng/g for all analytes in seal samples, and 0.1 ng/g for all analytes in penguin eggs, except for PFOA and PFHpA, for which the LOQ was 0.2 and 0.5 ng/g, respectively. Concentrations greater than the LOQ were considered valid.

Data Analysis. Statistical analyses were performed with STATISTICA 7 for Windows (Ver 7.1; Statsoft, Italia srl) at a significance level of p= 0.05. Statistically significant differences between the mean concentrations of PFCs were investigated by a single factor one-way analysis of variance and when significant differences were found, they were tested among each other by Tukey's post hoc test.

Results and discussion

Four PFSAs (PFOS, PFHS, PFDS, PFOSA) and six PFCAs (PFOA, PFNA, PFDA, PFHpA, PFUnDA, PFDoDA) were analyzed in seal samples. All of these 10 contaminants, except PFDS, were analyzed in penguin samples (Table 1). PFOS was detected in all of the samples. Concentrations of PFOS in liver and muscle of seal pups were higher than that found in penguin eggs, and seal liver samples contained significantly (ANOVA $p<0.005$) higher concentrations of PFOS. In seals, the tendency of increasing PFOS concentration was in the order liver > muscle, as found in other studies[2][13]. This may be partially attributable to the lipophobic properties of PFCs; however, PFCs also have a high affinity for plasma albumin[14], and it has been hypothesized that PFCs may bind to hepatic proteins such as fatty acid–binding proteins[15], thus explaining the high PFCs concentrations in blood and liver.

The variability in concentrations of PFOS among the penguin eggs was low ($p> 0.05$). The bioaccumulation of persistent lipophilic organic contaminants is governed by the lipid ingestion of the birds and changes in their lipid mass. The low variability of PFOS concentrations suggests that other, biological parameters influence the bioaccumulation of PFOS in birds[16].

∑PFCAs were detected in seal muscle (mean 1.3 ng/g ww) and liver (mean 6.2 ng/g ww) samples and were of the same order of magnitude as ∑PFSA (1.4, 10.4 ng/g wet wt, respectively), while in penguin eggs PFCAs (mean 1.08 ng/g wet wt in Gentoo penguin eggs and 6.6 ng/g wet wt in Adélie pengun eggs), were an order of magnitude higher than PFSAs (mean 0.29 ng/g wet wt in Gentoo penguin eggs and 0.56 ng/g wet wt in Adélie penguin eggs). Statistically significant linear correlations were only found between concentrations of some PFCAs ($p= < 0.05$; $r^2>0.6$) in the seal liver samples, as found in another study of polar bear liver samples[17], suggesting a common source.

PFCA profile is dominated by PFNA (mean 3.3 ng/g ww) in the liver of seals; the same trend was reported in marine mammals[18][19], including polar bears[17] where concentrations decreased with increasing chain length, and the predominant PFC was the PFOS (mean 9.4 ng/g wet wt). Interestingly, PFCA profile is dominated by PFUnDA (mean 0.59 ng/g wet wt) in Gentoo penguin eggs, and by PFUnDA (2.33 ng/g wet wt), and PFHpA (mean 2.53 ng/g wet wt) in Adélie penguin eggs, which concentrations were also higher than that of PFOS (mean 0.29, 0.38 ng/g wet wt, respectively) (Figs. 1). Similar results were reported in other studies on birds[18][12][20]. Birds show a preferential bioaccumulation of fluorinated long-chain or an enhanced capacity for PFOS elimination[21].

Concentration of PFCs differed significantly between seals and penguins ($p< 0.005$) and a species-specific difference was found among the two species of penguins ($p< 0.005$). Although, these organisms share the same environment, differences in diet and metabolism might explain the observed variations in concentrations[22][23]. The diet of lactating female Antarctic fur seals (*Arctocephalus gazelle*) at South Georgia was investigated by Rein and Arnould (1996). Antarctic krill *Euphausia superba* was the main prey item, followed by fishes and squid. Myctophids, *Protomyctophum choriodon*, were the most numerous prey taxon during this lactation period[24]. On the other hand, differences in the PFC concentrations between penguin species, may be related to diet, reproductive status, ecological niches, and migration. Both penguin species were from the South Shetland Islands, and feed primarily on *Euphausia superba*, although Gentoo penguins feed significantly more fish (Antarctic silverfish *Pleurogramma antarcticum*), than Adélie penguins. *E. crystallorophias*, pelagic and benthic species of amphipods were minor components of the pygoscelid diet[25]. Also their feeding ecology may influence the bioaccumulation of fluorinated compounds[26]; in fact, Gentoo penguins feed inshore and are deep divers, while Adélie penguins are shallow-diving, offshore foragers[27].

In comparison with seals and birds from the Artic, concentrations of PFOS and PFOA in penguins and seals from the South Ocean were 10-100 fold lower. Although the contamination levels of PFOS and PFOA are low in the southern hemisphere fauna, occurrence of perfluorinated compounds in these remote locations suggests

widespread global distribution of PFCs. Due to the global increasing trend of certain PFCs such as PFCAs, Antarctic organisms should be monitored in future, considering that Antarctic ecosystems are very important in governing the global ecological equilibrium.

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Table 1: Concentrations (ng/g wet wt; Mean ± SD) of perfluorinated compounds in Antarctic samples (nd = not detected).

	fur seal pup, muscle		fur seal pup, liver		Gentoo penguin egg		Adélie penguin egg	
	% detected	Mean ± SD	% detected	Mean ± SD	% detected	Mean ± SD	% detected	Mean ± SD
PFHS	0	nd	82	0.2 ± 0.2	0	nd	0	nd
PFHpA	80	0.5 ± 0.3	100	1.0 ± 1.9	15	0.1 ± 0.3	54	2.5 ± 5.5
PFOS	100	1.29 ± 0.7	100	9.4 ± 3.2	100	0.3 ± 0.1	100	0.4 ± 0.2
PFOSA	0	nd	100	0.2 ± 0.1	0	nd	54	0.2 ± 0.3
PFOA	50	0.8 ±0.8	6	0.3 ± 1.4	8	0.02 ± 0.06	23	0.1 ± 0.2
PFNA	0	nd	94	3.3 ± 1.7	0	nd	8	0.03 ±0.1
PFDA	0	nd	71	0.6 ± 0.5	31	0.1 ± 0.2	85	1.3 ± 2.9
PFDS	0	nd	6	>0.4	not analyzed		not analyzed	
PFUnDA	0	nd	71	0.9± 0.9	100	0.6 ± 0.5	100	2.3± 6.5
PFDoDA	0	nd	6	>0.4	54	0.3 ± 0.8	85	0.5 ± 0.4

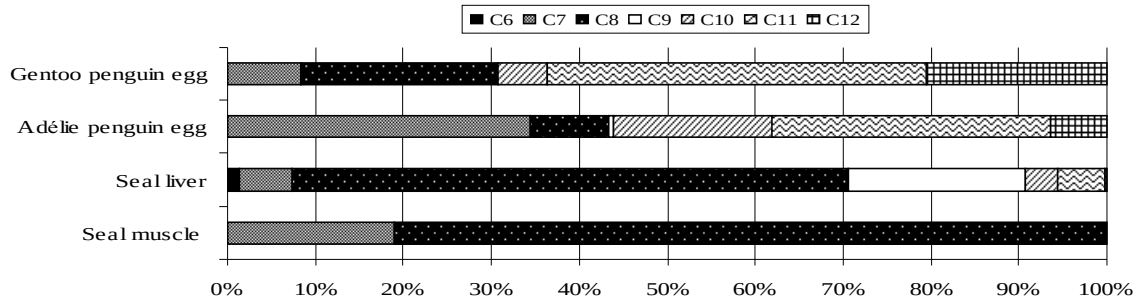

Figure 1: PFC profiles by number of carbon atoms, in fur seal and two species of penguins from Antarctica. C6:PFHxS; C7: PFHpA; C8: ∑PFOS+PFOSA+PFOA C9: PFNA; C10: ∑PFDA; C11: PFUnDA; C12: PFDoDA.

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