PAPER

Perfluoroalkyl compounds (PFCs) in wildlife from an urban estuary†

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Previous research has documented the bioaccumulation of perfluoroalkyl compounds (PFCs) in apex predators in remote locations but few studies have evaluated urban estuaries. To assess the importance of PFCs in San Francisco Bay, two apex predators in the San Francisco Bay, double-crested cormorants (*Phalacrocorax auritus*) and Pacific harbor seals (*Phoca vitulina richardii*), were sampled. Prey fish (*Atherinops affinis* and *Menidia audens*) were also evaluated to better understand potential sources of PFCs to the foodweb. Perfluorooctane sulfonate (PFOS) was the primary PFC detected in cormorant eggs, small fish and harbor seal serum. PFOS detected in San Francisco Bay seal serum was typically an order of magnitude higher than those at the reference site. PFOS concentrations were highest in seals and cormorant eggs from the highly urbanized southern portion of the Bay. PFOS in eggs from the southern part of the Bay remained relatively constant between 2006 and 2009 despite the phase-out of perfluorosulfonyl-based compounds nationally. In addition, these levels exceed the avian predicted no effects concentration of 1.0 μg mL^{-1}. Concentrations of the remaining PFCs measured were substantially lower than those of PFOS.

Introduction

Since their initial production in the late 1940s, perfluoroalkyl compounds (PFCs) have been used extensively in a number of industrial, commercial, and consumer applications such as stain and water repellants for carpets, textiles, and paper products, insecticides, aqueous film-forming foams, surfactants, fluoropolymer production, metal-plating and cleaning, and lubricants.[1] PFCs are widely used because they are chemically and thermally stable, have excellent surface tension lowering properties, and are both hydrophobic and oleophobic.

Perfluorooctane sulfonate (PFOS) and to a lesser extent, eight to 12 carbon chain perfluorocarboxylates (C8–C12 PFCAs) have been widely detected in biota around the world including marine mammals, birds, fish, reptiles, and benthic organisms.[2,3] The sources of these compounds are likely from industrial, commercial and consumer use of the PFC-containing products and from the abiotic and biotic transformation of PFC precursors. PFOS and PFCAs are the terminal transformation products of a number of precursors such as perfluorooctansulfonamides,[4] and perfluoroalkyl sulfonamide alcohols[5] and, for PFCAs, fluorotelomer alcohols.[6,7] The long range transport of these volatile precursors and their gas-phase transformation is believed to be a significant step leading to their presence in remote regions.[8] Surface waters, particularly oceans, have been identified as the major sink of PFCs.[9]

Pacific harbor seals (*Phoca vitulina richardii*) and double-crested cormorants (*Phalacrocorax auritus*) are year-round residents of San Francisco Bay. Because these seals and cormorants exhibit site fidelity, and forage, reproduce and nurture young in

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Environmental impact

This project evaluated concentrations of perfluoroalkyl compounds (PFCs) in apex predators residing in San Francisco Bay (*i.e.*, Double-crested cormorants (*Phalacrocorax auritus*) and Pacific harbor seals (*Phoca vitulina richardii*)). Prey fish (*Atherinops affinis* and *Menidia audens*) were also monitored to evaluate food web dynamics. Perfluorooctane sulfonate (PFOS) was detected in all tissues; in some instances, at quite high concentrations. In seal serum, concentrations in San Francisco Bay were typically an order of magnitude higher than those measured at the reference site. In general, higher concentrations were observed in animals residing the southern portion of the estuary. PFOS concentrations in eggs from this area exceeded the avian predicted no effects concentration of 1.0 μg mL^{-1}.

some of the more industrialized areas of the San Francisco Bay, they represent excellent indicators of Bay health and the uptake of contaminants.

Since the passage of the Marine Mammal Protection Act in 1972, harbor seal populations have dramatically increased along the California coast; the state-wide population is estimated to be 34,000.[10] In contrast, seal numbers within the Bay have not changed markedly since the 1970s especially in the South Bay (estimated at approximately 500 seals).[11] The possible causes for this lack of recovery are unclear and include: loss of habitat, disturbance, changes in prey availability, and contaminants.[11,12]

While limited data exist on the concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and pesticides in San Francisco Bay seals[11–13] and cormorants,[14] no data for PFCs are currently available. In addition, there is recent evidence suggesting that PFCs may cause deleterious health effects to marine mammals including suppression of the immune system, alteration of lipid metabolism, and induction of the proliferator-activated receptor-alpha (PRAR-alpha) pathway.[15–19] Similar adverse outcomes have been identified for avian exposure to PFCs including reduced hatching success, reduced body weight, and increased liver weight.[20–22]

The goal of this project was to evaluate whether PFCs are at levels of concern in apex predators residing in a heavily urbanized estuary. Two apex predators are commonly monitored in San Francisco Bay, cormorants and seals; these species were monitored in this study to determine whether PFCs were accumulating in the Bay Area food webs. In addition, prey species of these animals were also evaluated to determine possible sources of PFC to the food chain and whether there were areas of particular concern in the estuary. A recent study of San Francisco Bay seals suggests that they are carnivorous opportunists, which consume a variety of fish including gobies (*Gobiida*), topsmelt (*Atherinops affinis*) and anchovies (*Engraulis mordax*), and that their diets parallel the relative abundance of fish which is a function of habitat.[23] The diet of San Francisco Bay double-crested cormorant is not known; however, based on studies of cormorants in other locations, it is likely it is almost exclusively fish and will also vary according to habitat. Cormorants that reside in shallow water habitats typically consume small fish such as gobies, and sculpin; those that reside in deeper water tend to consume larger fish such as white croaker (*Micropogonias furnieri*).[24]

To assess the potential impacts of PFCs on apex predators residing in urban estuaries, 70 tissue samples were collected between 2006 and 2009 from two seal colonies and three cormorant colonies residing in the Bay and one reference seal colony. To evaluate potential sources of PFC from prey, small fish were collected from 10 sites in the Bay and one reference location.

Experimental section

Field samples

In the fall of 2009, small prey fish were sampled using beach seines from ten intertidal and subtidal sites around the margins of San Francisco Bay and one reference site in Tomales Bay

(Fig. 1). Topsmelt (*Atherinops affinis*) were collected at eight of the sites, and Mississippi silverside (*Menidia audens*) were collected at six. All fish were segregated by species and measured for total length and mass. Lengths of the fish were targeted to young of the year and the length varied between 40 and 80 mm. Composite samples of seven to fifteen fish of each species were prepared in the field. The fish were wrapped in foil and transported on ice to the laboratory where they were frozen at $-20\ ^{\circ}$C. Samples were shipped on ice by overnight courier to AXYS Analytical (Sidney, BC Canada) for homogenization and analysis.

Although seals move within and even out of the Bay, they tend to spend the majority of their time at the same locations.[11] Samples were collected from seals residing in two of the three largest haul-out sites in San Francisco Bay, Castro Rocks and Mowry Slough.[11] Haul-out sites are rocky outcroppings, tidal mudflats, and sandy shorelines that seals use to rest, molt, pup, nurse and forage. The Castro Rocks haul-out site lies in the northern portion of the Bay directly beneath the Richmond Bridge (37.9339 N, 122.4109 W); the Mowry Slough haul-out site is located in the southern portion of the Bay in the Don Edwards National Wildlife Refuge (37.493 N, 122039 W). Samples were also collected from a reference haul-out site located approximately 40 kms north of San Francisco Bay in Tomales Bay located in Point Reyes National Seashore (38.2191N, 122.9616W) (Fig. 1). Tomales Bay is an ideal reference site given the similarity of the environment of the two estuaries but the absence of any significant industrial, commercial and residential areas in the national park. All seal samples were collected under the NOAA-NMFS permit # 555-1870-00 issued to Dr James Harvey of Moss Landing Marine Laboratories, Moss Landing, California in accordance with State and Federal laws.

Thirty-four seals were captured at Castro Rocks in August of 2006, and May and June of 2007 and 2008. Another six samples of archived blood from harbor seals collected in 2004 from Mowry Slough were analyzed for PFCs. Twenty-one Tomales Bay seals were collected in May and June of 2007 and 2008. All seals were captured using tangle/seine nets[25] and released to the wild after sampling.

At the time of capture, seals were weighed and measured. Blood was drawn into sterile vials using a vacutainer system (Becton Dickinson, Franklin Lakes, NJ) and stored at $-4\ ^{\circ}$C for less than 8 h, centrifuged and the serum decanted into polypropylene tubes and frozen at $-20\ ^{\circ}$C until analysis at AXYS Analytical. Age class was assigned based on weight and time of year: weaner (1 to 12 months), yearling (1 to 2 years), subadult (2 to 5 years), and adult (see Supplemental Information for sample sizes by age).

Cormorant eggs were collected in the Spring (March–May) in 2006 and 2009 from three locations in San Francisco Bay (Fig. 1). An appropriate reference site was not identified for cormorants. The Wheeler Island site was located in the northern portion of the Bay near the confluence of the San Joaquin and Sacramento rivers (N38.08018, W121.965). The Richmond Bridge site was located directly above the Castro Rocks seal haul-out site (N37.93684, W122.427) and the Don Edwards National Wildlife refuge was near the Mowry Slough seal site (N37.44109, W122.044; N37.4505, W122.086). Eggs were collected in accordance with State and Federal laws under the following permits:
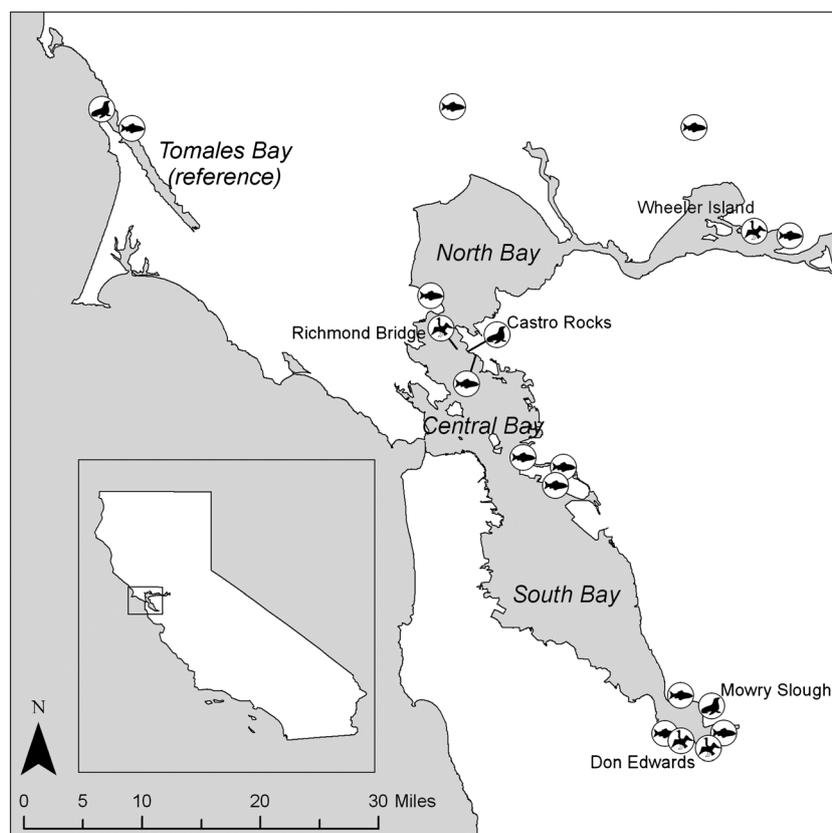
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J. Environ. Monit., 2012, **14**, 146–154 | 147

Fig. 1 Location of seal, bird, and small fish sampling sites.

California State Migratory Bird Scientific Collection permit No. MB173904-0 issued to Dr Josh Ackerman and Federal Migratory Bird Scientific Collection permits issued by California Department of Fish and Game (CDFG) permits, SC-004741 issued to Dr Collin Eagle-Smith and SC-10107 issued to Dena Spatz.

Eggs were collected by hand (one from each nest) and aged by flotation. An effort was made to collect the freshest eggs possible. Eggs were labeled, placed into polyethylene bags, set into fiber egg cartons, and stored on ice until placement in a refrigerator at 4 °C. Samples were shipped cold by overnight courier to AXYS. At the laboratory, the eggs were weighed and composited by site, with each site having three composites consisting of seven eggs each. Eggs were stored at 4 °C until analysis.

Sample extraction and instrumental analysis

Seal serum samples (0.5 g each) were spiked with isotope-labeled surrogate standards ($^{13}C_4$ PFOS, $^{13}C_2$ PFOA and $^{13}C_2$ perfluorodecanoate (PFDA)). The samples were then mixed with a 50% aqueous solution of formic acid prior to extraction and cleanup on an SPE cartridge (Waters Oasis WAX, 60 mg and 150 mg) with methanol as the eluent. Egg homogenates (2.0 g sample size) and fish tissue (2.0 g sample size) were spiked with isotope-labeled surrogate standards ($^{13}C_4$ perfluorobutanoate (PFBA), $^{13}C_2$ perfluorohexanoate (PFHxA), $^{13}C_2$PFOA, $^{13}C_5$ perfluorononanoate (PFNA), $^{13}C_2$ PFDA, $^{13}C_2$ perfluorododecanoate (PFDoA) and $^{13}C_4$ PFOS). Samples were then agitated in a basic methanol

solution and centrifuged. An aliquot of the supernatant was diluted with water and spiked with recovery standards ($^{18}C_8$ PFOA for egg homogenates and $^{13}C_2$ 2*H*-perfluoro-2-decenoic acid for fish tissue) prior to cleanup on an SPE cartridge with methanol as the eluent.

Concentrations of PFOS, PFOA, PFBA, perfluoropentanoate (PFPeA), PFHxA, perfluoroheptanoate (PFHpA); PFNA, PFDA, perfluoroundecanoate (PFUnA), PFDoA, perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS) and perfluorooctanesulfonamide (PFOSA) were measured using a high performance liquid chromatograph (Waters 2795, Milford MA) with a triple quadrupole mass spectrometer (Micromass Quattro Ultima, Manchester, UK) operating in the electrospray negative mode. Data were acquired using MassLynx v.4.1 software. A 15 μl sample was injected onto an Agilent Zorbax XDB reverse phase column (C18, 7.5 cm, 2.1mm id, 3.5 micron particle size column, Agilent, Santa Clara, CA). Acetonitrile/water and ammonium acetate/acetic acid were used as the mobile phases.

The following target compound transitions (parent - daughter) were monitored using Multiple Reaction Mode: perfluorobutanoate (PFBA, 213 to 169); pefluoropentanoate (PFPeA, 263 to 219); perfluorohexanoate (PFHxA, 313 to 269); perfluoroheptanoate (PFHpA, 363 to 319); PFOA (413, to 369/219); perfluorononanoate (PFNA, 463 to 419); perfluorodecanoate (PFDA, 513 to 469); perfluoroundecanoate (PFUnA, 563 to 519); perfluorododecanoate (PFDoA, 613 to 569); perfluorobutanesulfonate (PFBS, 299 to 80/99); perfluorohexanesulfonate (PFHxS, 399 to 80/99), PFOS (499 to 80/99), and perfluorooctanesulfonamide (PFOSA, 498 to 78). Results were

corrected for losses during extraction and cleanup through the use of internal standards. Solvents used were of HPLC grade or better and purchased from VWR International, Mississauga, ON. Isotope-labeled standards ($^{13}C_4$PFBA, $^{13}C_2$PFHxA, $^{13}C_2$PFOA, $^{13}C_5$PFNA, $^{13}C_2$PFDA, $^{13}C_2$PFDoA, $^{13}C_4$PFOS, $^{18}O_8$PFOA and $^{13}C_2$ 2H-perfluoro-2-decenoic acid) were purchased from Wellington Labs and Perkin Elmer. Native standards were purchased from Sigma Aldrich and Fluka.

Target analytes for all samples included PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFBS, PFHxS, PFOS, and PFOSA. In addition, small fish were analyzed for the following PFCs precursors: N-ethyl-perfluorooctanesulfonamide, N-ethyl-perfluorooctanesulfonamidoethanol, N-methyl-perfluorooctanesulfonamide, N-methyl-perfluorooctanesulfonamidoethanol. None of the precursors were detected. PFOSA was not detected in any seal sample and in some instances the laboratory spike for the seals exceeded the laboratory percent recovery control limits (these values were rejected). PFOSA was only detected in one bird egg sample near the MDL. As a result, the PFOSA results are not reported for seals and birds. PFOSA was detected in two small fish samples at relatively low concentrations. At the time of analysis radio-labelled PFOSA was not available; therefore this result should be viewed somewhat qualitatively.

Method detection limits (MDLs) were developed as described in the Code of Federal Regulations, Vol. 40, Part 136, Appendix B. Initial calibration occurred daily using a six-point calibration curve; a midlevel calibration standards were run after every 20th sample. All data analyses conformed to the QA/QC protocols of the laboratory and external review by SFEI.

Statistical analysis

Seal, bird, and fish data were evaluated for statistical significance using analysis of variance (ANOVA, $p < 0.05$) with fixed effects for site (all), time (birds), sex (seals) and age (seals). One half the MDL was used for values below the MDL. Data were tested for normality using the Kolmogorov-Smirnov test. Seal and bird data were log normal and were transformed prior to analysis with two exceptions: bird egg PFOS and PFNA data did not require log-transformation to achieve normality. An evaluation of interaction effects between variables was conducted and with the exception of PFDoA in seals, no significant interactions were identified (PFDoA exhibited an interaction between age and site). All tests were performed in Systat v. 11 (Systat Software, Inc. Chicago, IL).

Results

Small fish

Three PFC compounds were detected in small fish (Table 1). PFOS was detected in 9 of the 14 composited samples in concentrations ranging from 5.7 to 80 ng g^{-1} ww (see Table 1 for summary and Supplemental Information for individual results). Higher mean concentrations were observed at the South Bay sites relative to Central and North Bay sites; however, this was not statistically significant. PFOSA was detected in two fish samples; one of which also contained PFDA. None of the PFC precursors were detected in small fish. No PFCs were detected in samples from the reference site.

Seals

Eight PFCs were detected in seal serum (Table 1). PFOS, PFNA, and PFUnA were detected in all samples. Average concentrations of PFOS were generally an order of magnitude higher than those of other PFCs. The second highest observed concentrations were of PFHxS with a mean concentration of 65 ng mL^{-1} from Mowry Slough (South Bay) and 37 ng mL^{-1} from Castro Rocks (Central Bay). Concentrations of the remaining compounds were less than 20 ng mL^{-1}. Concentrations of PFCs were greater in the Mowry Slough seals than the Castro Rocks seals with one exception (PFDA). Seals from San Francisco Bay had higher concentrations than seals from the reference site. The differences among the sites (South Bay *vs.* Central Bay *vs.* Tomales Bay) were statistically significant for all compounds ($p = 0.001$) except PFHpA which was significant ($p = 0.01$) and PFUnA which was not significant.

Cormorants

Seven compounds were detected in cormorant eggs (Table 1). Average concentrations of PFOS were generally an order of magnitude higher than those of the other PFCs. Longer chain PFCAs such as PFNA, PFDoA, and PFUnA were the next most prevalent compounds, average concentrations ranged from 3.8 to 30 ng g^{-1}. PFHxS was largely only detected in the South Bay and average concentrations increased from 10.4 in 2006 to 25.2 in 2009. Concentrations of PFHxS across all sites for the two sampling events were statistically significant ($p = 0.05$). As with seals, concentrations of PFCs in eggs were always greater in the South Bay ($p = 0.001$) and PFOA was always detected in the South Bay site and sporadically detected at the remaining two sites. Concentrations of PFOS remained virtually identical between the two sampling events.

Discussion

Small fish

Consistent with previous studies,[3,26,27] PFOS was the main PFC detected in small fish in this study. The average concentration of PFOS observed in San Francisco Bay is in the range of values reported for prey fish elsewhere (Table 2). Interestingly, an order of magnitude higher concentration was observed in Lake Ontario sculpin, a benthic-dwelling fish.[27] In general, sulfonates tend to have a higher bioaccumulation potential in fish than the carboxylates and PFCs with fewer than eight carbons in the perfluoroalkyl chain tend not to bioaccumulate.[28,29] These patterns were confirmed in this study.

Higher PFOS concentrations were observed in Mississippi silversides relative to topsmelt (33.1 *vs.* 10.1 ng g^{-1}, see Supplemental information); however, this difference was not statistically significant. Silversides inhabit the shallow tidal areas while topsmelt frequent areas of open water. Higher concentrations of PFOS also were observed in Lake Ontario benthic-dwelling fish (*e.g.*, sculpin) than in pelagic fish (alewife and smelt). This difference was attributed to diet, specifically benthic-dwelling shrimp which have relatively high concentrations given their trophic status.[27]

PFOSA, which was detected in 2 of the 14 composites at concentrations of 4.3 and 9.6 ng g^{-1}, also has been reported in prey fish collected in the Great Lakes and Europe.[3,26,27] Fish are

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J. Environ. Monit., 2012, **14**, 146–154 | 149

Table 1 Average concentrations of PFCs detected in seal serum (ng mL^{-1}), bird eggs (ng g^{-1} ww), and small fish (ng g^{-1} ww) by location[a,b,d]

			PFHxS		PFOS		PFHpA		PFOA		PFNA		PFDA		PFUnA		PFDoA	
Matrix	Year	Site	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)	FD	Mean (SD)
Small fish	2009	SB	ND		3/3	43.4 (16.1)	ND		ND		ND		ND		ND		1/3	[c]3.4
		CB	ND		2/5	6.67 (5.79)	ND		ND		ND		ND		ND		ND	
		NB	ND		4/5	21.2 (33.0)	ND		ND		ND		ND		ND		ND	
		Tomales Bay - Ref	ND		ND		ND		ND		ND		ND		ND		ND	
Seal	2004	SB- Mowry	6/6	64.7 (49.2)	6/6	1044 (595)	3/6	0.58 (0.43)	6/6	7.83 (2.82)	6/6	18.0 (13.7)	6/6	6.60 (6.00)	6/6	8.77 (5.11)	6/6	6.69 (3.55)
	2006 2007 2008	CB-Castro Rocks	34/34	37.4 (26.4)	34/34	301 (273)	10/34	0.61 (0.70)	30/34	2.85 (2.69)	34/34	11.7 (8.4)	34/34	9.66 (8.30)	34/34	6.28 (4.80)	31/34	4.02 (3.84)
	2007 2008	Tomales Bay - Ref	16/21	2.40 (2.45)	21/21	35.5 (28)	ND		1/21	[c]1.07	21/21	2.56 (7.03)	20/21	1.93 (1.63)	21/21	5.08 (4.51)	14/21	0.71 (0.58)
Bird eggs	2006	SB- Don Edwards	3/3	10.4 (2.6)	3/3	1253 (327)	ND		3/3	7.91 (2.47)	3/3	13.4 (3.2)	3/3	22.0 (3.6)	3/3	8.91 (0.41)	3/3	12.8 (7.7)
		CB-Richmond Bridge	2/3	2.84 (0.14)	3/3	280 (364)	ND		ND		1/3	[c]5.19	1/3	*7.97	3/3	4.84 (1.40)	3/3	5.96 (2.05)
		NB-Wheeler Island	ND		3/3	104 (30)	ND		1/3	[c]2.70	3/3	7.04 (1.35)	3/3	8.4 (2.7)	3/3	7.15 (2.56)	3/3	6.09 (2.64)
	2009	SB- Don Edwards	3/3	25.2 (13.2)	3/3	1244 (472)	ND		3/3	24.3 (3.9)	3/3	30.5 (7.8)	3/3	22.9 (4.7)	3/3	7.84 (1.40)	3/3	7.6 (2.7)
		CB-Richmond Bridge	2/3	4.44 (1.76)	3/3	123 (32)	ND		1/3	[c]5.92	3/3	3.81 (0.87)	3/3	8.9 (4.6)	3/3	7.17 (3.55)	3/3	9.6 (5.2)
		NB-Wheeler Island	ND		3/3	84 (11)	ND		ND		3/3	5.90 (1.78)	3/3	9.52 (2.60)	3/3	4.00 (1.02)	3/3	4.05 (0.99)

[a] SB- South Bay.; CB- Central Bay; NB- North Bay; Ref- reference site north of San Francisco Bay. [b] ND - Analyte not detected above MDL. FD - Frequency of detection. [c] Indicates a single value detected. No average was calculated. [d] Non-detect concentrations are treated as one-half the MDL for purposes of calculating mean and standard deviations.

able to metabolize precursors such as N-ethyl perfluorooctanesulfonamide to PFOS, demonstrating that the mechanism to degrade PFOSA and other precursors exist in fish[4,27] and suggesting that some of the PFOS detected in fish may be due to the metabolism of precursors.

Seals

PFOS was the main PFC detected (Table 1), corroborating previous data for seals,[30–35] harbor porpoises (*Phocoena phocoena*[36]), and bottlenose dolphins (*Tursiops truncatus*,[37,38]). PFOS concentrations observed in serum in this study were higher than previously reported values for seals including ringed, gray, harbor, elephant, and Baikal (Table 3). The average PFOS

concentrations for San Francisco Bay seals (412 ng mL^{-1}) are slightly higher than concentrations observed in blood of four stranded harbor seals collected off the coast of northern Germany (349 ng g^{-1} ww,[39]). However, stranded animals are less likely to be representative of the overall population as a result of emaciation and compromised immune systems.

The highest reported PFOS concentrations in marine mammals have been observed in polar bears and dolphins.[3,27,37] Average concentrations of PFOS in blood from dolphins collected in Charleston Harbor were 1,171 ng g^{-1} ww[38] and are similar to the values observed for the South Bay seals. Because polar bears eat seals and the concentrations of PFOS in arctic seals is substantially lower, these results suggest that marine mammals residing in urban estuaries such as Charleston Harbor,

Table 2 Concentrations of PFOS observed in small fish worldwide (ng g^{-1} ww)

Species	N	Mean (std dev)	Location	Collection Date	Reference
Topsmelt (*Atherinops affinis*)/Silversides (*Medina audens*)	13	20.7(25.0)	San Francisco Bay	2009	This study
Topsmelt (*Atherinops affinis*)	1	ND	Reference	2009	This study
Herring	10	2.3	Baltic Sea	1989	51
Slimy sculpin (*Cottus cognatus*)	5	450 (98)	Lake Ontario	2002	27
Alewife (*Alosa pseudoharengus*)	6	46 (15)	Lake Ontario	2002	27
Smelt (*Osmerus mordax*)	6	110 (55)	Lake Ontario	2002	27
Round gobies (*Neogobius melanostomus*)	12	10.6 (5.3)	Rivers near Great Lakes	1998–1999	26
Pinfish (*Lagodon rhomboids*)	10	4.8 (4.5)	Florida US	2004	37
Pigfish (*Orthopristis chrysoptera*)	10	3.1 (2.5)	Florida US	2004	37

Table 3 Concentrations of PFOS observed in the blood of seals and dolphins worldwide

Species	Sample	Units	n	Mean (std dev)	Range	Location	Date	Reference
Harbor seals (*Phoca viulina*)	Serum	ng/mL	40	412 (425)	1960–16.5	San Francisco Bay, CA	2004–2008	This study
	Serum	ng/mL	21	35.5 (28)	134–13.5	Tomales Bay, CA	2007–2008	This study
	Blood	ng/g	4	349 (370)	48–887	German Bight	2007	39
Ringed seals (*Phoca hispida*)	plasma	ng/ml	18	110	16–230	Baltic Sea	1990s	2
	plasma	ng/mL	18	9	5–14	Norwegian Arctic	1990s	2
	Blood	ng/g	5		2.5–8.6	Canadian Arctic	2004	33
Gray seals	plasma	ng/ml	26	37	14–76	Baltic Sea	1990s	2
	plasma	ng/ml	12	28	11–49	Canadian Arctic	1990s	2
Baikal seals (*Pusa sibirica*) (male)	Serum	ng/g	19	5.8 (4.9)	1.4–17	Lake Baikal	2005	15
(female)	Serum	ng/g	24	4.5(3.6)	1.3–1.4	Lake Baikal	2005	15
Elephant seals (*Mirounga leonine*)	Blood	ng/mL	59	0.53	<0.08–3.52	Antarctic	2004–2005	67
Bottlenose Dolphins (*Tursiops truncates*)	plasma	ng/g	13	658	194–1715	Sarasota Bay, FL	2003	38
	plasma	ng/g	42	462	69–2019	Indian River Lagoon, FL	2003	38
	plasma	ng/g	47	1171	472–3073	Charleston, SC	2003	38
	plasma	ng/g	5	646	232–1240	Delaware Bay	2003	38
	plasma	ng/g	2	49	46–52	Bermuda	2003	38

Delaware Bay, and Guanabara Bay (Brazil) have a substantially higher PFOS burden than their counterparts in remote locations.[3,38,40,41]

Information on the toxicity of PFOS to seals is limited. Thresholds for effects from PFOS exposure for other mammals are several orders of magnitude higher than the concentrations observed in this study (*e.g.*, no adverse effect levels of 82.6 ug/g in serum for male monkeys,[42] and benchmark dose for neonatal survival for rats of 25 ug/g in serum[18]). However, a number of researchers have recently reported adverse effects at concentrations comparable to those observed in San Francisco Bay seals. For example, reduced birth weights and head circumferences in humans were observed at a median population concentration of PFOS of 5 ng mL^{-1} cord blood.[43] Suppression of the immune system in seals, rats, and sea otters has also been observed.[15,17,44,45] Guruge *et al.*[44] observed a significant increase in emaciation and mortality in a study on the effects of PFOS on rat resistance to influenza. Rats that had 670 ng ml^{-1} of PFOS in their plasma had a 17 percent survival as compared to 46 percent survival for the control (2.1 ng ml^{-1}). In a study of California sea otters, Kannan *et al.*[17] reported a significant correlation between the incidence of disease and PFOS/PFOA concentrations in livers (PFOS concentrations ranged 1 to 884 ng g^{-1} ww).

PFHxS was consistently detected at high concentrations in seals from the South Bay. This is a somewhat anomalous finding as PFNA is usually the second most frequently detected PFC in pinnipeds.[15,31,32,39,46] It was also detected in relatively high concentrations in bird eggs. PFHxS has been identified as an ingredient in aqueous film-forming foams for fire suppression (AFFFs)[47] and carpet treatment applications.[48] The prevalence of PFHxS in this study may reflect local sources such as military, aviation, and wastewater treatment facilities.

The PFCs with fewer than six carbons were not detected in any sample which is consistent with previous studies suggesting their low bioaccumulation potential.[28,29,32] The detection of PFCAs was correlated strongly with elevated concentrations of PFOS (p = 0.001). Average PFOA concentrations in the Mowry Slough and Castro Rocks seals were low, 7.8 ng mL^{-1} and 2.8 ng mL^{-1}, respectively. With one exception, PFOA was not detected in the seals from the reference site. These results are consistent with the literature[30–32,35] and the hypothesis that PFOA has a low bioaccumulation potential.[28,29]

No statistically significant difference in PFOS was observed among males and females. The average PFOS concentration detected in male seals was 299 ng mL^{-1} (n = 29); the average concentration for females was 268 ng mL^{-1} (n = 32, see Supplemental Information). No sex differences have been reported by other researchers[3,35,36,41,46] except for Ishibashi *et al.*[15] who observed statistically higher concentrations in male serum *versus* female serum for Lake Baikal seals.

PFC concentrations did not increase with age. These results are consistent with prior seal studies[32,34,35,46] and the relatively short-half lives of PFCs in marine mammals. The estimated half-life of PFOS in bottlenose dolphins is 21 weeks.[3] Although PFCs do not appear to increase with age, some researchers have observed significantly elevated concentrations in recently weaned seals.[15,46] In this study, PFOS concentrations in weaned pups were statistically higher than yearlings and subadults (p = 0.001). The higher concentration may be due to lactational or *in utero* transfers.[15] PFCs have been detected in dolphin milk[3] and elevated fetal PFC concentrations relative to the mothers have been observed in seals, dolphins and melon-headed whales.[31,40,46,49] These results suggest that both mechanisms are plausible.

Cormorant eggs

PFOS was the primary PFC detected in cormorant eggs (Table 1). This is consistent with previous bird data.[50–53] Average PFOS concentrations in the South Bay are 1,249 ng g^{-1} ww and were between 2 and 16 times higher than values reported elsewhere (Table 4). Average PFOS concentrations in Central Bay/Richmond Bridge and North Bay/Wheeler Island eggs were lower (202 and 113 ng g^{-1} ww, respectively) and are within the range reported by other researchers.[51–55]

Adverse effects associated with avian exposure to PFOS include: reduced body weight; an increase in liver weight; an increase in incidence of small testes for northern bobwhite quail (*Colinus virginianus*) and mallard (*Anas playrhynchos*);[21] and reduced hatching and pipping success in chickens

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J. Environ. Monit., 2012, **14**, 146–154 | 151

Table 4 Concentrations of PFOS observed in bird eggs worldwide (ng g^{-1} ww)

Species	n	Mean (std dev)	Range	Location	Collection Date	Reference
Double-crested cormorant (*Phalacrocorax auritus*)	6	1249	833–1760	South Bay	2006/2009	This study
	6	202	63–700	Central Bay	2006/2009	This study
	6	94	73.8–135	Delta	2006/2009	This study
	4	157	21–220	Manitoba, Canada	1995	41
	4	210	130–210	Lake Winnipeg, Canada	1990s	2
Common Guillemot (*Uria aalge*)	8	325	243–432	Baltic Sea	1989	51
	9	614	551–669	Baltic Sea	2003	57
	10	400	200–760	Baltic Sea	2003	52
	10	16	5.2–22	Iceland	2002	52
Ring-billed gull (*Larus delawarensis*)	3	67	30–126	Lake Huron, Michigan	1995	41
Herring gull (*Larus argentatus*)	13	586 (62)		Toronto Harbor, Canada	2007	55
Herring gull (*Larus argentatus*)	13	148(22)		Granite Island, Lake Superior	2007	55
Ivory gull (*Pagophila eburnea*)	10	79.2	24.2–113	Norwegian Arctic	2007	54
Night heron (*Nycticorax nycticorax*)	9	115	22.6–343	Hong Kong	2006	53

(*Gallus domesticus*).[20,22] Based on effects on offspring survival of northern bobwhite quails, Newsted *et al.*[21] developed a predicted no effect concentration (PNEC) of 1 μg mL^{-1} for eggs. Effects to hatching and pipping success ranged from 0.1 μg PFOS/g egg (lowest observed adverse effect level[20]) to 100 μg/g.[22] An additional concern is that PFCs appear to enhance the toxicity of dioxin and dioxin-related compounds in wild cormorants.[56] While there is uncertainty regarding the relative sensitivity of cormorants to PFCs, Newsted *et al.*[21] suggested that the inter-species differences may not be significant.

Conclusions

Seals and cormorant eggs collected from the South Bay contained higher average concentrations of PFOS than those reported for other sites. The elevated concentrations of PFOS in the South Bay and the absence of a decrease following the curtailment in production in 2001 in the US suggest continued sources, particularly given that PFOS has relatively short half-lives in dolphins (21 weeks,[3]) and birds (two to three weeks,[21]). The spatial trend is likely due to several factors including: the limited circulation in the southern portion of the Bay; the greater influence of stormwater runoff, wastewater effluent, and other potential sources; and the different type of habitats/prey species present in each area (tidal mudflats and shallow water *versus* rocky outcrops in relatively deep water).

The absence of a decline in PFOS concentrations in bird eggs over time is in contrast to several recent reports documenting the decline of PFCs in biota elsewhere including seals in the Arctic,[35] sea otters along the California coast,[17] birds in the Baltic Sea,[57] and humans in the US.[58] Some of these declines have been dramatic; for example, a 60 percent decrease in PFOS concentrations was observed in human blood donors from six centers across the US over the period 2000 to 2006.[58] However, decreases in PFOS in biota are by no means universal[30,49] and the absence of a decline has been attributed to on-going sources.

Sources of PFCs to the Bay remain to be elucidated. A number of land uses and pathways that have been historically associated with PFCs are located along the Bay shoreline including airports, military facilities, landfills, wastewater treatment plants, oil refineries, and stormwater discharges.[3,47,59,60] PFCs are also present in consumer products including treated textiles, carpets, and food-packaging materials, and diffuse releases from homes and commercial facilities represent yet another potential source.[3,61,62] In addition, although perfluorooctane sulfonyl fluoride (POSF) based compounds were phased out in 2001[63] and the USEPA restricted the use of perfluoroalkyl sulfonates in the 2002 Significant New Use Ruling,[64] a number of uses were exempted such as the use of perfluoroalkyl sulfonates as an anti-erosion additive used in aviation hydraulic fluid, a component of photoresist in the semi-conductor/electronics industry, and a coating for surface tension applications in analog and digital imaging films, plates, and paper. It is not known whether these uses are significant in the Bay Area. Finally, a previous study of San Francisco Bay sediments identified PFOS and PFC precursors such as 2-(*N*-ethylperfluorooctane sulfonamide) acetic acid and 2-(-*N*-methylperfluorooctane sulfonamide) acetic acid in concentrations greater than or equal to PFOS[65] suggesting that the sediments may also be a potential contribution.

The concentrations of PFOS observed in cormorant eggs and seals are within the range where adverse effects have been observed. It is not known whether these will translate into population effects. However, the seal colony at Mowry Slough has not shown a significant increase since the 1970s;[11] this is in marked contrast to similar sites in Point Reyes National Seashore where there has been an increase in population.[66] The reasons for this lack of increase are unknown; it will be important to elucidate if contaminants such as PFCs are a factor.

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J. Environ. Monit., 2012, **14**, 146–154 | 153

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