SURVEY OF PERFLUORINATED ALKYL ACIDS IN SELECTED BIOTA FROM HOMEBUSH BAY, SYDNEY AUSTRALIA

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Introduction

Perfluorinated carboxylic and sulfonic acids and their salts have been used for a variety of commercial and industrial applications since the 1950's and also incorporated into a number of consumer goods [1, 2]. The global dissemination of these compounds is now widely recognized, and some have been identified as potentially toxic and bio-accumulative [3, 4]. Perfluorooctane sulfonate (PFOS) was recognized as a persistent organic pollutant and added to Annex B of the Stockholm convention in 2009 [5].

Despite many reports on contamination in biota internationally [e.g. 6], no data to date has been published on concentrations present in Australian wildlife. This paper presents data from a number of species all collected from the an urban/industrial region of Sydney, Australia. Archived biota samples, originally collected between 2007-2008 were analysed for perfluorinated carboxylic (PFCAs) and sulfonic acids (PFSAs). Samples analysed were Sydney Rock Oysters (*Saccostrea commercialis*), Sea Mullet (*Mugil cephalus*), Silver gull (*Larus novehollandiae*) and Australian White Ibis (*Threskiornis molucca*) eggs. The study location, Homebush bay, is in the upper part of the Sydney Harbour / Parramatta River estuary.

Methods and Materials

Sea mullet were collected in January 2007, whereas Silver Gull and Australian White Ibis were collected in December 2008 and Sydney Rock Oysters were collected in January 2009. All samples were archived at -20^{o}C prior to transport to Entox and sample analysis. Oysters were stored in methanol rinsed polypropylene jars, eggs were stored in acid/DCM rinsed glass jars and Sea Mullet were stored whole and prior to transport their liver and muscle dissected stored in methanol rinsed polypropylene jars and frozen at -20^{o}C.

An ion pairing method was used to extract 1 – 2 g of Sea Mullet liver homogenate and muscle tissue, following the method published by Hansen et al. [7]. Oysters (whole animal homogenates) and eggs (homogenates without shell) were extracted by shaking with acetonitrile three times (5 ml, shaken on orbital shaker at 150 rpm for 30 minutes), following digestion with 2 ml methanolic KOH (10 mM) overnight. This was done based on modification of methods previously published [8, 9]

All extracts were concentrated under high purity nitrogen gas to approximately 1 ml, solvent exchanged into methanol, and diluted to 50 ml with milli-Q water. Following dilution extracts were cleaned using SPE following the methods of Taniyasu et al. [10]. A 50 µl solution of mass labeled internal standards at 0.08 ng/µl were added to the samples prior to extraction. Standards were obtained from Wellington laboratories and consisted of ^{13}C and/or ^{18}O labeled perfluoro- butanoic, hexanoic, octanoic, nonanoic, decanoic, undecanoic, dodecanoic PFCAs (abbreviated as PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA respectively), and perfluoro- hexane, and octane PFSAs (abbreviated as PFHxS and PFOS), all >98% purity.

All samples were analysed on a QTRAP 4000 MS/MS (ABsystems) coupled with a prominence HPLC system (Shimadzu). A column (Altima $C_{18,}$ 5 µm, 150 mm x 2 mm 100 Å)(Grace Davison) was installed between the solvent reservoirs and autosampler to separate peaks present in the system from those in the samples. Separation in the samples was achieved using a Gemini C_{18} column (3 µm, 50 x 2 mm) (Phenomenex). Both columns were held at 55^{o}C to prevent excessive back pressure. The mobile phase consisted of 10% (A) and 90% (B) methanol with 5mM ammonium acetate, and was run using a gradient. MS data was acquired in the scheduled MRM mode with two transitions monitored for all analytes except

perfluorobutanoic and pentanoic acid (PFBA and PFPeA). For PFOS the 499>99 transition was used for quantification so as to avoid interference with TDC. Five point calibration standards were injected with every 10 samples, consisting of varying concentrations of native PFCAs and PFSAs (0.1 – 100 ppb) and constant concentrations of internal (4 ppb).

Results and Discussion:

Most of the analytes were consistently absent from procedural blanks, with the exception of PFOA which was often present at 0.01 – 0.1 ng/ml. The instrumental detection limits (IDL) were based on blank contamination or variation in the lowest standards with S/N >3, and varied with extraction method/sample type. Method detection (MDL) was set at 3 x IDL, and limits of quantification (LOQ) at 2 x MDL resulting in LOQs of 0.4 – 1.8 ng/g. Reproducibility was assessed by analysis of duplicates of each sample. The agreement between duplicates varied with analyte and sample but was generally good.

Table 1 shows concentrations in ng/g wet weight for all samples analysed. Overall the results showed most Perfluorinated acids at very low concentrations and some such as PFOA and the butane sulfonate (PFBS) were not detected above LOQ in any sample. As has been observed in other studies [e.g. 11, 12], PFOS dominated the concentration profiles in egg and fish samples, accounting for 57 – 100% of reported compounds. In Gull eggs PFOS concentrations ranged from 19 – 85 ng/g ww (wet weight), in Ibis eggs concentrations ranged from 13 – 114 ng/g ww. In Sea Mullet liver concentrations of PFOS were ranged from 44 –107 ng/g ww, and muscle concentrations were approximately 20 – 60 times lower. The egg samples also contained a relatively high proportion of long chained PFCAs (C10 – 13). The oysters showed much lower total PFCA and PFSA concentrations than either fish or eggs, and concentration profiles were dominated by PFDoDA at up to 6 ng/g ww. Although lower in concentration PFOS was still detected consistently in the oysters at 0.6 – 2.3 ng/g ww. The inter-site differences in oyster concentrations did not mirror those previously reported in sediments collected from the same areas [13].

Sydney Rock Oysters are filter feeding organisms and Sea Mullet are a detritovorous species and therefore both low trophic level. The particularly low levels in oysters may be indicative that the water column is a less significant exposure pathway whereas as mullet which feed directly on fine sediments which are a source of PFCs in Sydney Harbour [13] may have a greater level of exposure. The presence of PFCs in these organisms, however, indicates that they are entering the aquatic food chain of Sydney Harbour. The presence of PFCs in Silver Gull (aquatic and terrestrial feeders) and Australian White Ibis (mostly terrestrial feeders) indicate that food chain exposure is occurring but a more detailed survey of biota would be required to properly understand the significance of the levels observed.

Table 1 Concentrations of perfluorinated acids in various biota samples (ng/g ww) (cont. overleaf)

Sample type	Site	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFTriDA	PFHxS	PFOS	PFDS
Ibis eggs	Mt. Annan 1	0.8	0.9	1.8	0.8	4.0	0.8	1.8	43	nd
		<0.8	0.9	1.7	0.7	3.5	0.5	1.6	55	nd
	Mt. Annan 2	<0.8	<0.9	3.2	1.2	4.3	<0.5	0.9	38	nd
		<0.8	<0.9	2.5	0.9	3.2	<0.5	0.7	29	nd
	Mt. Annan 3	<0.8	<0.9	2.9	1.1	4.1	<0.5	1.2	20	0.6
		<0.8	<0.9	1.9	0.8	2.8	<0.5	0.6	13	<0.6
	Mt. Annan 4	<0.8	<0.9	0.9	<0.7	3.1	<0.5	1.8	56	<0.6
		<0.8	<0.9	1.0	<0.7	3.0	<0.5	1.8	63	<0.6
	Mt. Annan 5	<0.8	<0.9	1.1	<0.7	1.5	<0.5	7.1	114	nd
		<0.8	<0.9	1.0	<0.7	1.3	<0.5	6.8	100	nd
	Home-bush 1	<0.8	<0.9	<0.6	<0.7	1.0	<0.5	1.2	44.9	nd
		<0.8	<0.9	<0.6	0.7	1.6	0.7	2.0	82.3	nd
	Home-bush 2	<0.8	<0.9	<0.6	<0.7	nd	nd	1.6	15.2	nd
		<0.8	<0.9	<0.6	<0.7	nd	nd	1.0	15.0	nd

Gull eggs	Home-bush 3	<0.8	<0.9	<0.6	<0.7	<0.7	nd	0.7	11.9	nd
		<0.8	<0.9	<0.6	<0.7	<0.7	nd	<0.6	11.7	nd
	goat island 1	<0.8	<0.9	1.5	1.9	5.2	0.7	1.2	23	0.6
		<0.8	<0.9	1.4	1.9	5.1	0.7	1.3	25	0.6
	goat island 2	<0.8	<0.9	2.1	2.5	8.2	2.6	1.6	29	0.8
		<0.8	<0.9	2.1	2.8	8.7	2.6	1.7	33	0.8
	cockatoo island	1.1	0.9	1.6	1.8	2.4	3.5	3.8	21	<0.6
		1.0	<0.9	1.5	1.7	2.4	3.9	3.3	19	<0.6
	snapper island	3.0	2.2	4.9	2.3	11	2.7	6.4	85	2.9
		2.6	1.9	4.7	2.4	9.9	2.2	6.8	80	1.5
Sea Mullet 1	Liver	<1.8	nd	2.6	3.4	3.7	<0.6	1.2	45	1.4
		<1.8	<1.2	2.7	2.7	4.2	2.3	0.6	50	<1.2
	Muscle	<1.8	<1.2	nd	<0.6	<0.6	nd	nd	2.6	nd
		nd	nd	nd	<0.6	<0.6	nd	nd	1.4	nd
Sea Mullet 2	Liver	<1.8	<1.2	1.5	1.8	2.3	0.8	0.7	73	1.4
		<1.8	<1.2	1.3	1.7	3.0	1.4	0.7	61	<1.2
	Muscle	<1.8	nd	nd	nd	<0.6	nd	nd	0.8	nd
		<1.8	nd	nd	nd	<0.6	nd	nd	1.2	nd
Sea Mullet 3	Liver	<1.8	<1.2	3.0	2.6	6.1	1.3	0.7	81	<1.2
		<1.8	<1.2	3.1	3.0	6.3	1.2	0.7	80	1.8
	Muscle	<1.8	nd	nd	nd	<0.6	nd	nd	2.1	nd
		<1.8	nd	nd	<0.6	<0.6	nd	nd	2.3	nd
Sea Mullet 4	Liver	<1.8	<1.2	1.9	0.8	<0.6	<0.6	0.8	44	<1.2
		<1.8	<1.2	2.2	1.7	2.1	1.0	0.6	50	<1.2
	Muscle	<1.8	nd	nd	<0.6	<0.6	nd	nd	2.1	<1.2
		<1.8	nd	nd	nd	nd	nd	nd	1.0	2.0
Sea Mullet 5	Liver	<1.8	<1.2	1.8	1.7	2.4	1.0	0.8	107	1.3
		<1.8	<1.2	1.7	1.4	1.9	1.1	0.8	106	<1.2
	Muscle	<1.8	<1.2	nd	nd	<0.6	nd	nd	4.9	nd
		<1.8	nd	nd	<0.6	<0.6	nd	nd	3.3	nd
Oysters	Rhodes Rd	<0.6	nd	<0.6	<0.7	3.5	0.9	nd	1.2	<1
		<0.6	nd	<0.6	<0.7	3.3	1.1	nd	1	<1
	Meadowbank	nd	nd	<0.6	<0.7	2.9	0.9	nd	1	<1
		nd	nd	<0.6	<0.7	1.3	<0.6	nd	0.7	<1
	Kissing point	<0.6	nd	<0.6	<0.7	1.5	0.6	nd	0.6	<1
		<0.6	nd	<0.6	<0.7	1.4	<0.6	nd	0.7	<1
	Home-bush	<0.6	nd	0.7	0.8	5.8	0.8	nd	2.3	<1
		<0.6	nd	0.6	0.8	6.0	1.1	nd	1.9	<1
	Brays bay	<0.6	nd	<0.6	<0.7	1.2	<0.6	nd	1.1	<1
		<0.6	nd	0.6	<0.7	2.7	1	nd	1.1	<1

Table 2 Recoveries of internal standards in samples, averages (and range)

	PFHxA	PFOA	PFNA	PFDA	PFUnDA	PFDoDA	PFHxS	PFOS
Eggs	86 (64 - 125)	80 (48 - 116)	66 (36 - 107)	52 (21 - 107)	39 (8 - 110)	24 (3 - 101)	55 (12 - 104)	41 (3 - 112)
Sea Mullet	100 (50 - 149)	120 (38 - 210)	98 (30 - 160)	72 (25 - 110)	52 (20 - 110)	38 (10 - 84)	99 (26 - 160)	68 (25 - 115)
Oysters	104 (79 - 117)	101 (81 - 124)	102 (94 - 113)	105 (90 - 118)	89 (32 - 113)	52 (43 - 71)	111 (97 - 136)	94 (66 - 109)

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