TISSUE DISTRIBUTION OF PERFLUOROALKYL COMPOUNDS IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) FROM SOUTHEAST COASTAL USA

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Abstract
Bottlenose dolphins (*Tursiops truncatus*) from the eastern U.S. coast and the Gulf of Mexico have a coastal habitat and many are year-round residents in waters surrounded by human activity. Elevated organohalogen compounds have been found in dolphins from this region. In previous work we reported elevated concentrations of PFCs in plasma of bottlenose dolphins collected in 2003 from the estuarine waters of Charleston, South Carolina (CHS), Indian River Lagoon (IRL) and Sarasota Bay, Florida. Samples collected in 2005 from dolphins in the Charleston and Indian River Lagoon area. Our objective was to compare with the 2003 data and to examine distribution in four tissues/sample types that were available from almost all animals (blubber, plasma, feces and urine). PFOS was the most prominent of the PFC of the 13 PFCs determined in dolphin samples with concentrations in plasma ranging from 491-6,250 ng/g in dolphins from CHS and 303-3,610 ng/g in samples from IRL. PFOS was the most prominent PFC in blubber, ranging in concentration from 104-1534 ng/g wet wt at CHS and from 71-790 ng/g at IRL. In general the pattern of PFCs in blubber resembled plasma and urine i.e. dominated by PFOS but with detectable levels of PFCAs, particularly, PFNA, PFDA and PFUA.

Introduction
Bottlenose dolphins (*Tursiops truncatus*) from the eastern U.S. coast and the Gulf of Mexico have a coastal habitat and many are year-round residents in waters surrounded by human activity. Elevated organohalogen compounds have been found in dolphins from this region including PCBs and organochlorine pesticides and polybrominated diphenyl ethers (PBDEs) in blubber [1-4] and perfluoro alkyl compounds (PFCs) in liver [5]. In previous work [6-8] we reported elevated concentrations of PFCs in plasma of bottlenose dolphins collected in 2003 from the estuarine waters of Charleston, South Carolina, Indian River Lagoon and Sarasota Bay, Florida. Highest concentrations were found in animals from the Charleston area.

In this study we analysed samples collected in 2005 from dolphins in the Charleston and Indian River Lagoon area. Our objective was to compare the 2003 data and to examine distribution in four tissues/sample types that were available from almost all animals (blubber, plasma, feces and urine). The analysis of PFCs in blubber, which has rarely been attempted in marine mammals due to analytical difficulties with the high lipid content, was of particular interest. The potential bias in the comparison of whole prey body loads and predator plasma/liver PFC concentrations, instead of whole body burden extrapolation, was also evaluated.

Methods
Collection: Samples were collected from free-ranging bottlenose dolphins inhabiting the Indian River Lagoon (IRL), located on the east coast of Florida, as well as waters surrounding Charleston, SC (CHS) in July and August 2005. Collections of samples for these sites were conducted by the Harbor Branch Oceanographic Institution and the National Ocean Service as part of the bottlenose dolphin health and risk assessment project (NMFS Scientific Research Permit issued to G. Bossart). Sample numbers are summarized in Table 1. Dolphin blubber and feces (0.1-0.5g) and dolphin plasma (1 to 1.5g) were extracted using the procedure described by Tomy et al [9]. In brief,

homogenized samples were extracted with methanol. This extraction was repeated and the combined extracts were centrifuged at 13,500 *rpm*. This step removed most lipid co-extractives. The methanol was reduced to a small volume under a flow of nitrogen and transferred to a LC injection vial. Urine samples (3-5 g), were diluted with MilliQ-water and extracted with a WAX solid phase extraction cartridge.

Table 1. Sample numbers and characteristics for dolphins from the Charleston area and Indian River Lagoon

Location	Males	Females	N blubber	N feces	N plasma	N urine	mean Age (yr)	range Age (yr)	mean length (cm)	range length (cm)
CHS	10	9	18	13	19	18	12	3-24	220	185-254
IRL	3	15	16	7	18	16	10	4-23	227	190-272

HPLC-MS/MS analysis: Concentrations of PFCs in samples were determined by high-performance liquid chromatography with negative electrospray tandem mass spectrometry (HPLC-MS/MS). Samples were injected with an Agilent 1,100 HPLC system. Chromatography was performed on a Luna 3µ C8 column (50 x 2 mm, Phenomenex, Torrance, CA, USA) at a temperature of 30˚C using a water/methanol (0.01M ammonium acetate) mobile phase. HPLC-MS/MS detection utilized a negative ion turboelectrospray API2000 (Applied biosystems-MDS Sciex) for fish and plasma of dolphin. Analyses of water, sediment, and zooplankton were conducted with a negative ion turboelectrospray API4000 (Applied biosystems-MDS Sciex) using a Agilent pump with the Teflon® parts removed in order to improve detection limits of PFCAs.

QA/QC and statistical analysis: Data quality assurance and quality control included field blanks (for water, sediment, and plasma), laboratory blanks, matrix spikes, and standard material injection every 10 samples in order to monitor changes in the sensitivity of the instrument. Concentrations were expressed as ng/g wet wt except for feces, which were calculated on a dry weight basis. Results were log transformed to reduce skewness and comparisons between sampling years and between tissues were made with transformed data. Nondetect concentrations were replaced with ½ MDL for calculation of means.

Results and Discussion

PFC concentrations and trends: PFOS was the most prominent of the PFC of the 13 PFCs determined in dolphin samples (Table 2). Concentrations of PFOS in plasma ranged from 491-6,250 ng/g in dolphins from CHS and 303-3,610 ng/g in samples from IRL. PFHxS and PFDS were also present at much lower concentrations. PFDA and PFNA were the major PFCAs detected in plasma. Geometric mean concentrations of PFOS in the 2005 animals were higher than those reported for samples collected in 2003 from CHS and IRL, however, the differences were not statistically significant (Students t test; P>0.05). While geomeans of PFHxS, PFOSA, PFNA, PFNA, PFDA and

Table 2. Concentrations (geometric mean and range; ng/g wet wt) of PFCs in dolphin plasma [1]

Location		PFOA	PFNA	PFDA	PFUA	PFDoA	PFDS	PFHxS	PFOS	PFOSA
CHS (n=19)	Mean	28	118	163	96	3.1	33	76	1560	42
2005	Range	<0.1-561	28-560	60-667	28-496	<0.1-30	10-267	8.5-471	491-6250	13-124
IRL (n=18)	Mean	6.9	17	18	12	0.14	11	92	1090	3.4
2005	Range	1.1-66	8.7-66	8.1-138	5.2-68	<0.1-14	2.9-51	16-766	303-3610	0.5-15
CHS (n=47)	Mean	33	51	130	50	8.2	nd	33	1171	24
2003[1]	Range	4.6-163	11-214	41-542	10-320	0.8-62		4.6-165	472-3073	7.4-102
IRL (n=42)	Mean	6.9	11	15	11	1.2	nd	27	462	0.8
2003[1]	Range	1-70	3-51	4.3-53	1.7-64	<0.5-6		2.2-332	69-2010	<0.5-6.5

[1]Results for 2003 from Houde et al [8]. PFDS was not determined in the 2003 samples

PFUA were also higher in 2005 samples the differences were found to be statistically significant only for PFHxS concentrations.

Tissue comparison: The concentration profiles for 9 major PFCs in blubber, feces, urine and plasma of bottlenose dolphins are shown in Figure 1. The methanol extraction procedure enabled routine analysis of PFCs in blubber and they were detectable in all samples. PFOS was the most prominent PFC in blubber, ranging in concentration from 104-1534 ng/g wet wt at CHS and from 71-790 ng/g at IRL. In general the pattern of PFCs in blubber resembled plasma and urine i.e. dominated by PFOS but with detectable levels of PFCAs, particularly, PFNA, PFDA and PFUA. However concentrations of PFCAs were generally in the low ng/g range especially in samples from IRL.

The pattern of PFCs in feces was different from the other three sample types. Only PFOS and PFDA were consistently above MDLs in feces (Figure 1). PFOS concentrations in feces ranged from <0.1-650 ng/g dry wt

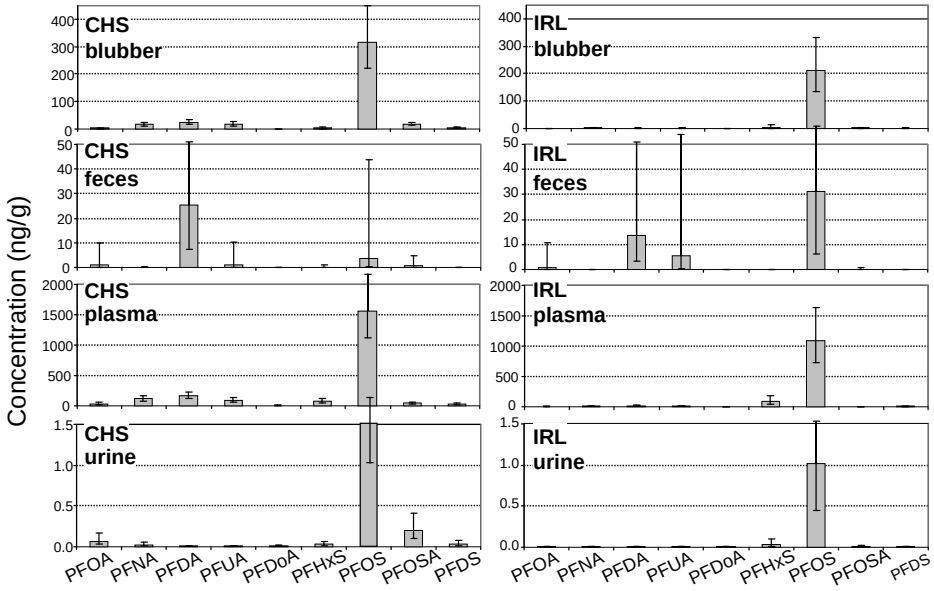


Figure 1. PFC concentrations (geomeans ± 95% confidence limits) in blubber, feces, plasma and urine from bottlenose dolphins samples from Charleston SC (CHS) and Indian River lagoon (IRL) collected in 2005

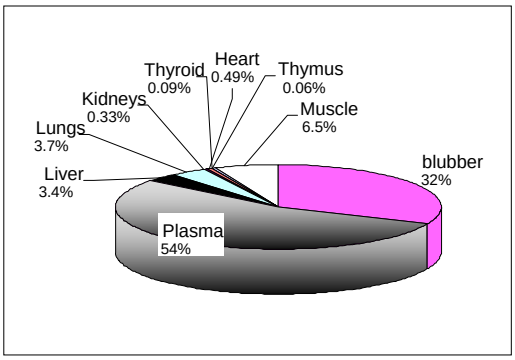
and was detectable in 70% of samples. PFDA concentrations ranged from <0.1-120 ng/g dw and was detectable in 95% of samples. There was a much larger the variance of PFCs in feces compared to blubber, plasma or urine due to the large number of non-detects. This likely was related to high variability in sample size. In urine, PFOS was detectable in 97% of samples and PFOSA in 50% of samples. PFCAs were less prominent in urine, with only PFOA and PFNA detected in 32% and 18% of samples, respectively. Interestingly PFHpA, was detected in 50% of urine samples but only in 5% of blubber samples and was undetectable in feces.

Body burden revisited: In Houde et al.[6] we estimated the whole body concentrations of PFCs based on analysis of liver, kidney, lungs, heart, thyroid, thymus, and muscle from two dead dolphins from southeastern US waters. We did not analyze blubber samples at that time. However, the detection of PFCs in blubber suggests that this whole body distribution needs to be revisited. To do this we estimated the concentrations in blubber for the dead animals using an average blubber to plasma ratio for the 2005 animals from CHS. The actual blubber weight for these animal were unknown so we assumed blubber was 20% of total body weight based on measurements of blubber mass to body mass by Struntz et al [10]. The concentrations in each tissue were then multiplied by total tissue weight to obtain the body burden in ng. The results shown in Figure 2, indicate that blubber is an important tissue for PFCs. Given the lipophobic and anionic properties of PFCs it seems likely that they are associated with the small amount of proteinaeous tissue within the blubber. The samples we analysed were generally free of skin and other tissue.

Conclusions

An increase in dolphin tissue PFCs concentrations was found to occur within a 2 year period from 2003 to 2005 with mean PFOS concentrations increasing 1.3-fold in plasma of CHS dolphins (1171-1,560ng/g) and 2.3-fold (462-1,090 ng/g) in IRL dolphin plasma. This increase in PFCs is of concern in these dolphin populations, especially given the high levels already present in some of these animals. As top-level predators, marine mammals, such as dolphins, have extensive fat stores and have been known to accumulate high levels of persistent organic lipophilic pollutants. While the concentration of PFCs found in dolphin blubber is lower compared to plasma, the fact that blubber tissue comprises 20% or more of the total body weight makes the finding of detecting relatively high levels of PFCs in dolphin blubber significant. During periods of fasting, starvation, lactation, or other physiological demands, stored blubber lipids may be mobilized which may not only potentially redistribute known chemicals such as PCBs but also the PFCs.

Figure 2. Tissue distribution of PFOS in bottlenose dolphins based on estimated or measured tissue weights in 2 animals. . Results are based on analysis of samples from two dead animals analysed by Houde et al [6] with results for blubber added from this study.



Acknowledgements

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