



## Feasibility of using the National Marine Mammal Tissue Bank for retrospective exploratory studies of perfluorinated alkyl acids☆

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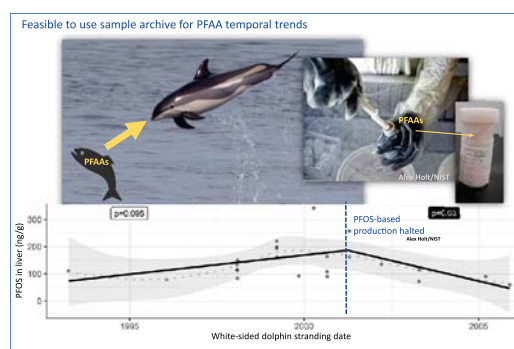
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### HIGHLIGHTS

- Perfluorinated alkyl acids (PFAAs) can leach into samples during archiving processes.
- PFAAs were measured in the U.S. National Marine Mammal Tissue Bank (NMMTB).
- Leaching of two PFAAs was negligible, while perfluorooctanoate (PFOA) was problematic.
- Southern rough-toothed dolphins had higher PFAAs than northern white-sided dolphins.
- Perfluorooctane sulfonate (PFOS) significantly decreased after 2001 in liver of both species.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Perfluorinated alkyl acids (PFAAs) have been used for 50+ years in materials such as stain-resistant treatments for paper and clothing, lubricants, and foam fire extinguishers. PFAAs are characterized by a fully fluorinated alkyl chain with a terminal acid group. Their long half-lives and ubiquitous environmental distribution create considerable concern for wildlife and human exposure. There is interest in examining temporal trends of PFAAs using the National Marine Mammal Tissue Bank (NMMTB), but NMMTB tissues are frozen and cryohomogenized in polytetrafluoroethylene (PTFE)-based materials. Because PTFE supplies may leach PFAAs into samples, this study mimicked collection, processing and storage steps of NMMTB samples and measured PFAA leaching to determine the feasibility of using this sample archive for PFAA temporal trends. We also explored concentrations in Atlantic white-sided dolphin (*Lagenorhynchus acutus*, WSDs) and rough-toothed dolphin (*Steno bredanensis*, RTDs) blubber ( $n = 3$  and  $0$ ) and liver ( $n = 48$  and  $12$ , respectively). The materials used in NMMTB protocols may add up to 0.968 ng/g perfluorooctanoic acid (PFOA), 0.090 ng/g perfluorononanoic acid (PNFA), and 0.221 ng/g perfluorooctane sulfonate (PFOS) to each archived sample. Leaching of PFNA and PFOS from supplies compared to dolphin levels was negligible, but PFOA contributions were substantially higher than levels found in most dolphin liver samples. Therefore, monitoring PFOA temporal trends from the NMMTB would require careful consideration. RTDs had significantly higher levels of PFOS and PFNA than WSDs. Both species have similar life history, trophic status, and foraging behaviors in deep pelagic waters, so differences could be from latitudinal

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variation in contamination. RTDs stranded in Florida; WSDs stranded farther north mostly in Massachusetts. Juveniles had significantly higher levels of PFOS and PFNA than adults in both species, suggesting growth dilution as they approach maturity. PFOS significantly decreased after 2001 in both species as expected based on changes in production.

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## 1. Introduction

Perfluorinated alkyl acids (PFAAs) have been manufactured for >50 years and are used in materials such as stain-resistant treatments for paper and clothing, lubricants, and in foam fire extinguishers. PFAAs are characterized by a fully fluorinated alkyl chain with a terminal acid group. Their long half-lives and ubiquitous environmental distribution create considerable concern for wildlife and human exposure. The two most commonly measured PFAAs are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) with measurements of shorter and longer chain PFAAs becoming more common, including perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnA). PFAAs appear to be the ultimate degradation products of several commercially used perfluorinated compounds, are globally distributed throughout the environment, and bioaccumulate in blood and liver rather than fatty tissues (Hekster et al., 2003; Schultz et al., 2003).

Temporal studies have shown that PFAA concentrations increased in marine biota from the 1980s to early 2000s (Sturm and Ahrens, 2010). Recent declines in some environmental samples have been noted for PFOS since 3M, a global science company, voluntarily halted production of PFOS-based chemistry in 2002 (Houde et al., 2011; Newsted et al., 2017; O'Connell et al., 2010; Riget et al., 2013; Sturm and Ahrens, 2010). Some of these studies have taken advantage of the benefits of using samples archived in long-term, formal specimen banks. Another

specimen bank that can provide samples for exploring PFAAs is the U.S. National Marine Mammal Tissue Bank (NMMTB) initiated in 1989 and maintained by the National Institute of Standards and Technology (NIST). NMMTB tissues are sampled under strict protocols, maintained frozen at  $-180^{\circ}\text{C}$  in polytetrafluoroethylene (PTFE)-based materials, and cryohomogenized in PTFE disc mills under cleanroom conditions (Becker et al., 1999). Because these PTFE materials may leach PFAAs into samples that they touch, great care must be taken during sample collection, processing and analysis if the samples are to be used for PFAA measurements (Begley et al., 2005; Flaherty et al., 2005). Thus, this study began by testing the feasibility of measuring PFAAs in NMMTB samples.

Two species were chosen from the NMMTB for analysis in this study: the Atlantic white-sided dolphin (*Lagenorhynchus acutus*) and rough-toothed dolphin (*Steno bredanensis*). The Atlantic white-sided dolphin (WSD) inhabits cooler waters from Cape Cod to the United Kingdom and the current samples were collected from animals that stranded mainly in Massachusetts, U.S.A., whereas the warmer subtropical and tropical rough-toothed dolphins (RTDs) in this study stranded in Florida. Both species are similar in their pelagic diets of mainly fish and squid and their age and length at maturity (Clarke, 1986; Craddock et al., 2009; Layne, 1965; Sergeant et al., 1980; Waring et al., 2010). WSDs mature between six and 12 years (201–210 cm) for females and seven to 12 years (220 cm) for males, and RTDs mature around 14 years (225 cm) for males and 10 years (210 cm) for females

**Table 1**  
PFAA concentrations in sampling, processing, and storage materials and estimates of PFAA additions (in bold, bottom 3 rows) to NMMTB samples from these materials.

NMMTB materials	Concentration in Materials				Estimated surface area or volume touching the sample	Estimated ng transferred to entire sample		
	PFOS	PFOA	PFNA	units		PFOS	PFOA	PFNA
Materials that touch the initial 300 g sample								
Sampling gloves								
Glove type 1 (vinyl, used 1998–2005)	21.1	13.8	12.9	ng/g	$\approx 4 \times 4$ inch, one side	6.2	4.0	3.8
Glove type 2 (vinyl, used 2003–2008)	259	178	13.0	ng/g	$\approx 4 \times 4$ inch, one side	52	35	2.6
Glove type 3 (copolymer, used 2008–present)	<3.42	<3.44	<3.28	ng/g	$\approx 4 \times 4$ inch, one side	0.52	0.53	0.50
Water from PTFE squirt bottle	0.036	0.046	<0.025	ng/mL	100 mL	3.56	4.60	2.50
FEP bags	5.86	160	0.879	ng/g	$\approx 4 \times 4$ inch, one side	5.84	159	0.88
Materials that touch the 150 g subsample								
180 mL PTFE jars	<0.0105	<0.0112	<0.0157	ng/g of water	5% of 150 g sample	0.08	0.08	0.12
Cryohomogenization gloves	3.73	1.35	1.16	ng/g	negligible	0	0	0
Cryohomogenized serum samples	2.29	0.796	0.207	ng/mL		–	–	–
Blank serum samples	2.27	0.492	0.142	ng/mL		–	–	–
Addition from cryohomogenization process	0.017	0.304	0.066	ng/mL	150 mL sample	2.50	45.7	9.83
15 mL PTFE jars	<0.00410	<0.00439	<0.00614	ng/mL of water	1 mL	0.00410	0.00439	0.00614
<b>Estimated ng/g addition to samples from material contamination 1998–2005</b>						<b>0.069</b>	<b>0.864</b>	<b>0.090</b>
<b>Estimated ng/g addition to samples from material contamination 2003–2008</b>						<b>0.221</b>	<b>0.968</b>	<b>0.086</b>
<b>Estimated ng/g addition to samples from material contamination 2008–present</b>						<b>0.050</b>	<b>0.852</b>	<b>0.079</b>

FEP = fluorinated ethylene propylene; PTFE = polytetrafluoroethylene.

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