Marine Pollution Bulletin 64 (2012) 66–74

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Assessment of perfluorinated compounds (PFCs) in plasma of bottlenose dolphins from two southeast US estuarine areas: Relationship with age, sex and geographic locations

Patricia A. Fair [a,*], Magali Houde [b], Thomas C. Hulsey [c], Gregory D. Bossart [d,e], Jeff Adams [f], Len Balthis [a], Derek C.G. Muir [g]

[a] National Oceanic and Atmospheric Administration, National Ocean Service, Center for Coastal Environmental Health & Biomolecular Research, 219 Fort Johnson Road, Charleston, SC 29412-9110, USA
[b] Environment Canada, Centre Saint-Lauremt, 105 McGill Street, Montréal, QC, Canada H2Y 2E7
[c] Medical University of South Carolina, 135 Rutledge Avenue, Charleston, SC 25056, USA
[d] Harbor Branch Oceanographic Institution at Florida Atlantic University, 5600 U.S. 1 North, Ft. Pierce, FL 34946, USA
[e] Georgia Aquarium, 225 Baker Street, NW Atlanta, GA 30313, USA
[f] National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Silver Spring, MD, USA
[g] Aquatic Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, Burlington, ON, Canada L7R 4A6

ARTICLE INFO

Keywords:
Perfluorinated compounds (PFCs)
PFOS
PFOA
Bottlenose dolphins
Tursiops truncatus

ABSTRACT

Plasma PFCs were measured in 157 bottlenose dolphins (*Tursiops truncatus*) sampled from two US southeast Atlantic sites (Charleston (CHS), SC and Indian River Lagoon (IRL), FL) during 2003–2005. ∑PFCs, perfluoroalkyl carboxylates (∑PFCAs), perfluoroalkyl sulfonates (∑PFSAs) and individual compounds were significantly higher in CHS dolphins for all age/sex categories compared to IRL dolphins. Highest ∑PFCs concentrations occurred in CHS juvenile dolphins (2340 ng/g w.w.); significantly higher than found in adults (1570 ng/g w.w. males; 1330 ng/g w.w. females). ∑PFCAs were much greater in CHS dolphins (~21%) compared to IRL dolphins (~7%); ∑PFSAs were 79% in CHS dolphins versus 93% in IRL dolphins. PFOS, the dominant compound, averaged 72% and 84%, respectively, in CHS and IRL dolphins. Decreasing PFC levels occurred with age on the bioaccumulation of PFCs in both sites. These observations suggest PFC accumulation in these two dolphin populations are influenced by site-specific exposures with significantly higher levels in CHS dolphins.

Published by Elsevier Ltd.

1. Introduction

Perfluorinated compounds (PFCs) have received increased concern due to their persistence, bioaccumulation and global distribution (Giesy and Kannan, 2001; Houde et al., 2006b, 2011). PFCs are a class of synthetic compounds characterized by chains of carbon atoms of varying length to which fluorine atoms are strongly bonded. They have been widely used as surface coatings and protectants due to their unique surfactant properties, both hydro- and lipophilic, that enhance water, grease and soil repellency (ASTDR, 2009; Kissa, 2001; Lehmler, 2005; Rayne and Forest, 2009). The chemical structure of PFCs makes them extremely stable, resistant to biodegradation, photooxidation, and hydrolysis.

PFCs have been globally detected in surface coastal and ocean waters (Ahrens et al., 2009a; Yamashita et al., 2004) in a wide variety of aquatic and terrestrial animals (Giesy and Kannan, 2001; Houde et al., 2006b; Kannan et al., 2004, 2005; Olsen et al., 2005; Taniyasu et al., 2003). The highest concentration of PFCs have been measured in fish-eating, apex predators, such as mink, bald eagles and aquatic mammals (Giesy and Kannan, 2001; Houde et al., 2005a). Some of the highest PFC levels reported in marine mammals are found in bottlenose dolphins inhabiting the estuarine waters of Charleston, South Carolina, an urban area of the southeast US (Houde et al., 2005b). Concentrations of PFCs found in the Charleston dolphins were on the same order of magnitude to that of occupationally exposed humans (Olsen et al., 2003a). Trophic biomagnification of PFCs was also reported in the dolphins' foodweb in CHS and in Sarasota Bay, Florida (Houde et al., 2006b).

Generally, there is a lack of clear trends in the relationship between age and accumulation of PFCs reported in the literature. Many studies in mammals have observed no correlation between PFCs and age (Dia et al., 2006; Kannan et al., 2002a,b, 2001; Van de Vijver et al., 2007). However, several reports have confirmed significantly higher PFC concentrations in pups and juvenile Antarctic elephant seals, harbor porpoise, dolphins and Baikal seals compared to adults (Houde et al., 2006a; Ishibashi et al., 2008a; Tao et al., 2006; Van de Vijver et al., 2003).

* Corresponding author. Tel.: +1 843 762 8533; fax: +1 843 762 8700.
E-mail address: pat.fair@noaa.gov (P.A. Fair).

0025-326X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.marpolbul.2011.10.022

PFCs frequently detected in biological samples are perfluoroalkyl sulfonates (PFSAs) and perfluoroalkyl carboxylates (PFCAs). The two PFCs used in the largest amounts in the US over the past 60 years are perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) (ASTDR, 2009). In 2000, 3 M Company voluntarily phased-out the production of perfluorooctyl sulfonyl fluoride (PFOSF) (Prevedouros et al., 2006) and since May 2009, PFOS and PFOSF have been included in Annex B (restricted) of the Stockholm Convention on persistent organic pollutants (POPs) (Stockholm Convention, 2009). As a result, significant drops in PFOS levels have occurred in some regions such as reported in Canadian Arctic ringed seals (Butt et al., 2007), sea otters along the California and Alaska coast (Hart et al., 2009; Kannan et al., 2006a) as well as in humans in the US (Olsen et al., 2008b). However, large reservoirs of PFOS and precursors in the environment, continuing use in products and continuing production in some countries (Paul et al., 2009; UNEP, 2007) contribute to persistence of these chemicals.

Because of the widespread environmental and human health concerns regarding PFC compounds, especially PFOS and PFOA, a large body of toxicological, epidemiological and environmental information has been published see reviews (Lau et al., 2004; Lau et al., 2007). Some PFCs have demonstrated developmental, reproductive, and carcinogenic toxicity in animals studies (Kennedy et al., 2004; Lau et al., 2007). PFCs are potentially harmful to marine mammals (Ishibashi et al., 2008b) and biochemical perturbations have been observed in wildlife species under field conditions as a consequence of exposure to PFOS (Hoff et al., 2004, 2005). Concern has been raised over the potential toxicity of persistent organic contaminants in marine mammals with a series of die-offs during the late 1980s and 1990s (Houde et al., 2005a; O'Shea, 1999; O'Shea and Tanabe, 2003). While the deaths that occurred during several of these epizootics were attributed primarily to morbillivirus, it was suggested that contaminants such as polychlorinated biphenyls (PCBs) and chlorinated pesticides may have been a contributing factor. A retrospective analysis of liver tissues from bottlenose dolphins that died during the high mortality epizootic along the Atlantic coast of the United States during 1987 and1988 found that concentrations of PFOS in the affected bottlenose were statistically greater than other species not affected during the epizootics, and to other bottlenose dolphin populations (Kuehl et al., 2009). Also in that study, PFOS concentrations in liver were found to be as great as, or greater than, concentrations of PCBs, chlorinated pesticides, and polybrominated diphenyl ethers (PBDEs).

Despite the ubiquitous occurrence of PFCs, very little is known regarding the impact of these contaminants on the health of wildlife populations. Exposure data is a critical component for assessing causal relationships between exposure and potential health effects and mitigating sources of exposure. The influence of biological variables such as age and sex are an important consideration in assessing contaminants and health-related data. The aim of this study was to compare the levels of PFCs in plasma of dolphins from two estuarine southeast US areas, Charleston, SC (CHS) and Indian River Lagoon (IRL), FL, for a three-year period (2003–2005) and to examine the influence of age, sex and location. This study extends the information on PFC concentrations in these dolphin populations reported by (Houde et al., 2005b) for 2003 and provides a greater sample size from which to investigate PFC concentrations as related to the above variables and to draw statistical conclusions.

2. Materials and methods

2.1. Study population

Samples were collected during bottlenose dolphin (*Tursiops truncatus*) capture–release health assessments conducted at two study sites, CHS and the IRL, during the summers of 2003–2005. Collections were conducted under NMFS Permit No. 998–1678, issued to Gregory Bossart, V.M.D., Ph.D. Detailed information pertaining to the study sites, methods for capture, sampling and release are described elsewhere (Fair et al., 2006). The CHS site (32°46′35″N, 79°55′51″W) included the Charleston Harbor, portions of the main channels and creeks of the Ashley, Cooper, and Wando Rivers, and the Stono River Estuary. For the IRL site, assessments were conducted near Titusville, FL (28°36′43″N, 80°48′27″W) and Stuart, FL (27°11′51″N, 80°15′10″W) and included portions of the Mosquito Lagoon, Indian River, Banana River, north and south forks of the St. Lucie River, and Sebastian Inlet. This study was part of the Bottlenose Dolphin Health and Risk Assessment (HERA) Project, aimed at assessing the health status of dolphins in these two areas and investigating associations between dolphin health and environmental stressors (Fair et al., 2006). Information pertaining to the study sites, methods for capture, sampling and release are described elsewhere (Fair et al., 2006). Age was determined by examining the post-natal dentine layers of an extracted tooth (Hohn et al., 1989). We evaluated PFC concentration in blood plasma samples collected from a total of 76 dolphins from CHS and 81 dolphins in the IRL.

2.2. Exposure assessment

Concentrations of PFCs in blood plasma were determined at the Environment Canada's Laboratories in Burlington Ontario. Sample extraction, analysis, and quality control procedures are detailed by Houde et al. (2005b). PFCs were quantified using high-performance liquid chromatography with negative electrospray tandem mass spectrometry (HPLC–MS/MS). PFC analytes determined are listed in Supplementary Table 1. Data quality assurance and control measures included both field and laboratory blanks, matrix spikes and standard material injection. Nondetect concentrations were replaced with ½ Minimum Detection Limit (MDL) for calculation of means.

2.3. Data analysis

Descriptive statistics for PFC concentrations presented in Table 1 includes the number of individuals, geometric mean, range, and 95% confidence intervals stratified by site, age, and sex. To meet assumptions of normality and homogeneity, concentrations of contaminants were log transformed. Significant differences between $\sum$PFCs, $\sum$PFCAs (PFOA, PFNA, PFDA, PFUA, PFDoA, PFTriA, PFTA, PFPA, see Supplementary Table 1 for acronym description), $\sum$PFSAs (PFOS, PFOSA, PFHxS), means in sex, age class, and site were assessed by analysis of variance (ANOVA) for greater than two categories and student's *t*-test for comparison of two categories using SAS (Version 9, SAS Institute Inc., Cary, NC). Additionally, the above PFC analytes were examined temporally for differences between the collection years (2003, 2004 and 2005) using ANOVA and post hoc comparisons. Interpretation of statistical significance testing should consider the small population sizes as a result of stratification by age, sex, and site. Sexual maturity in bottlenose dolphins has been categorized from 5 to 12 years for females and 10 to 13 years for males (Mead and Potter, 1990). In our study, adults were defined as females age 7 and older and males age 10 and older and juveniles categorized as less than these ages. Classification of the age/sex categories were juveniles (JUV), adult female (AF), and adult male (AM). Student's *t*-test comparison of PFC contaminants in juvenile males and females found no differences between these two groups, therefore, these were combined into one category termed 'juveniles' for a more robust comparison.

The relationships between the concentrations of different PFCs were examined using Pearson rank correlation. The relationship between $\sum$PFC and PFOS concentrations and age for CHS and IRL