High Accumulation of Perfluorooctane Sulfonate (PFOS) in Marine Tucuxi Dolphins (*Sotalia guianensis*) from the Brazilian Coast

PAULO R. DORNELES,*,†,‡ JOSÉ LAILSON-BRITO,†,‡ ALEXANDRE F. AZEVEDO,‡ JOHAN MEYER,§ LARA G. VIDAL,‡ ANA B. FRAGOSO,‡ JOÃO P. TORRES,† OLAF MALM,† RONNY BLUST,§ AND KRISHNA DAS||

Radioisotope Laboratory, Biophysics Institute, Rio de Janeiro Federal University (UFRJ), Brazil, Aquatic Mammal Laboratory (MAQUA), Oceanography Faculty, Rio de Janeiro State University (UERJ), Brazil, Laboratory for Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Belgium, and Laboratory for Oceanology, MARE Center, Liege University, Belgium

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Perfluorooctane sulfonate (PFOS) and other perfluoroalkyl compounds (PFCs) were measured in liver samples from 29 marine tucuxi dolphins from Rio de Janeiro state (RJ), Brazil. PFC measurement combined liquid chromatography and mass spectrometry, using a CapLC system connected to a Quadrupole-LIT mass spectrometer. PFOS was the only PFC detected and it was so in all samples. PFOS concentrations ($ng \cdot g^{-1}$ dw) of dolphins ($n = 23$) from the highly contaminated Guanabara Bay (in RJ) varied between 43 and 2431 as well as between 76 and 427 from areas of RJ other than the quoted bay ($n = 6$). Concentrations of three fetuses and one neonate varied between 664 and 1590. Fetus/mother ratios were calculated in two situations (2.75 and 2.62). It seems that mother-to-calf transference plays important role for relationships between PFOS and age. When a one-year-old male calf presenting 2431 $ng \cdot g^{-1}$ dw was excluded from the test, significant correlations were observed between PFOS concentrations and both age and total length. Despite the placental transference, PFOS concentrations were not significantly lower in females than in males. PFOS levels in marine tucuxi dolphins from Guanabara Bay are among the highest detected to date in cetaceans, and this may represent a threat to the small population concerned.

Introduction

Since the beginning of the industrialization process marine ecosystems have become the final destiny for pollutants. Perfluoroalkyl compounds (PFCs) have been used in a number of industrial and household products comprising fire-fighting foams, personal hygiene goods, arthropodicide formulations, and protectors to fabrics, paper, and other surfaces (*1*). Due to their environmental persistence, bioaccumulative capacity as well as their broad utilization, PFCs have been of great environmental concern since their global distribution was first demonstrated using samples from marine biota (*2*). The apprehension has been amplified by the observation of biochemical perturbations in wildlife species, under field conditions, as a consequence of exposure to perfluorooctane sulfonate (PFOS) (*3, 4*).

Despite the awareness of the scientific community about the environmental problem, the only information about PFCs in marine ecosystems from the southern hemisphere concerns measurements in biota from Antarctic and adjacent waters (*5, 6*), as well as from the southern extreme of the Brazilian littoral (*7*). There is information from South American waters other than the one generated by the latter investigation; however, it comprises data from the north coast of Colombia (*8*), which in fact constitute the northern hemisphere. Although many investigations have shown higher PFC levels in biological samples from waters close to areas of concentrated industrialization and urbanization (*5, 9–11*), there are no previous studies on PFC levels in environmental samples from the most industrialized regions of the southern half of the planet.

The Brazilian coastline is around 8500 km in length, and Guanabara Bay, in Rio de Janeiro State, Southeast Brazilian region, is the most anthropogenically disturbed area along the country shoreline. The estuary is bordered by 12 000 industries and four cities (including Rio de Janeiro metropolitan area) with a total population of about 11 million people (*12*). Despite the anthropogenic pressure, Guanabara Bay supplies food and breeding grounds for marine tucuxi dolphins (*Sotalia guianensis*). This dolphin species occurs exclusively in coastal waters of western Atlantic on South and Central America, from southern Brazil (27°35′S, 48°34′W) to Honduras (15°58′N, 79°54′W) (*13*). Regarding Guanabara Bay specifically, population assessment studies, accomplished through photoidentification, have reported a population of about 70 marine tucuxi dolphins (*14*). Important from the ecotoxicological point of view is that, through these photoid studies, it was possible to verify that this small population presents habitat fidelity, since the same individuals are found year-round in this site (*15*). The main reason for the marine tucuxi dolphin residence in Guanabara Bay seems to be the presence of food, since feeding-related activities predominate along the entire year (*16*).

Considering all the difficulties concerning the determination of PFCs in water (*17*), as well as the demonstrated biomagnification capacity of some these compounds (*11, 18*), analyses of cetacean tissues are of interest (*19*), due to their top position on the food chain and their long life span. Therefore, PFC determination was carried out in samples from marine tucuxi dolphins from Guanabara Bay and other coastal areas of Rio de Janeiro State. The main objective of the present study was to determine the current concentrations of fluorochemicals in dolphins from a region of high industrialization and urbanization in the southern hemisphere. Besides, aiming to provide an additional contribution to the knowledge on placental transfer of PFCs, hepatic PFOS concentrations were determined in three fetuses and one newborn calf. Since two of these fetuses were obtained during the necropsies of their mothers, fetus/mother ratios of hepatic PFOS concentrations were calculated in two situations and

* Corresponding author phone: +55 21 25615339; fax: +55 21 22808193; e-mail: dorneles@biof.ufrj.br, dornelespr@gmail.com.
† Rio de Janeiro Federal University (UFRJ).
‡ Rio de Janeiro State University (UERJ).
§ University of Antwerp.
|| Liege University.

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TABLE 1. Mean Hepatic PFOS Concentrations (ng·g^{-1}, Dry Weight), with Standard Deviation (±SD), Concentration Range, Median, Number of Individuals of Each Group/Area of Stranding Analyzed (*n*), Average Total Length (TL, in Centimeters), With Range of Length, As Well As Average Age (In Years), With Range of Age of the Analyzed Marine Tucuxi Dolphins and Number of Individuals (*n*, in Parenthesis) Who Had Their Age Estimated[a]

area	group	PFOS mean ± SD (Median) Min - Max	PFOA	PFNA	PFHxS	*n*	TL (cm) min−max	age (ys) min−max
GB	males	829 ± 648 (746) 43−2431	<7.3	<7.6	<3.2	12	180 148−191	7 ($n = 7$) 01−14
GB	females	481 ± 254 (436) 184−800	<7.3	<7.6	<3.2	7	183 149−198	27 ($n = 4$) 23−30
GB	fetuses	1259 ± 291 (1142) 1044−1590	<7.3	<7.6	<3.2	3	73 71−75	0
GB	neonates	664 (664)	<7.3	<7.6	<3.2	1	93	0
RJ (non-GB)	males	288 ± 93 (246) 233−427	<7.3	<7.6	<3.2	4	181 164−194	NA
RJ (non-GB)	females	203 ± 179 76−330	<7.3	<7.6	<3.2	2	182.5 180−185	NA

[a] The limits of detection were 1.5 ng·g^{-1} for PFOS; 7.3 ng·g^{-1} for PFOA; 7.6 ng·g^{-1} for PFNA; and 3.2 ng·g^{-1} for PFHxS. NA: not available; RJ: Rio de Janeiro state; GB: Guanabara Bay.

compared to the same parameter regarding published information about other marine mammal species.

Materials and Methods

Sampling of Hepatic Tissue from Cetaceans and Sample Preparation. Liver samples were collected from 29 marine tucuxi dolphins (*Sotalia guianensis*) that stranded on the beaches of Guanabara Bay ($n = 23$) and other areas ($n = 6$) of Rio de Janeiro State, Southeast Brazilian region. The cetacean specimens have had their age determination based on growth layer groups (GLGs) present in dentine and cementum of the teeth (*20*). However, not all the individuals had their age determined. The exact number of individuals of each group, who had their age estimated, along with information on sex and total length (TL) of the analyzed specimens, is shown in Table 1.

PFAS Extraction and Cleanup. PFAS extraction was carried out by solvent extraction based on the method described by Berger and Haukås (*21*) with adaptations. Briefly, the samples were freeze-dried and then ground to a powder. Internal standards (^{13}C-PFOS and ^{13}C-PFOA from Wellington Laboratories, Canada) and 9 mL of acetonitrile were added to approximately 1.0 g of the ground sample in a polypropylene (PP) centrifuge tube. The sample was thoroughly mixed using a Vortex chemical mixer and then extracted three times for 10 min in an ultrasonic bath at room temperature. Between each period of 10 min, the samples were thoroughly mixed. The samples were then centrifuged at 2500 rpm for 5 min. One mL of the final supernatant was transferred to a micro vial containing approximately 25 mg of activated carbon and 50 μL glacial acetic acid. The sample was then mixed for 1 min using a vortex mixer. After centrifugation (10 000 rpm, 10 min) 500 μL of the supernatant was transferred to a clean micro vial. Sample blanks were extracted along with every batch of samples. Spiked samples were used to determine recovery rates for the perfluorochemicals concerned.

Determination of Perfluorochemical Concentrations. Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were the PFCs determined in the present study. The concentrations of PFCs were measured combining liquid chromatography and mass spectrometry, using a CapLC system (Waters, U.S.) connected to a Quadrupole-LIT quadrupole mass spectrometer (Applied Biosystems, UK). Aliquots of 5 μL were loaded on an Optiguard C18 precolumn (10 mm × 1 mm i.d., Alltech, U.S.). The analysis was performed on a Fluophase PFP column (50 mm × 1 mm i.d., Thermo, U.S.) at a flow rate of 40 μL/min. The mobile phase was 2 mM NH4OAc (A)/Acetonitrile (B). A gradient elution was used starting at 35% B and going to 90% B in 5 min. At 5 min and 6 s the initial conditions were resumed. The PFCs were measured under (−) electrospray ionization using the transitions from mother to daughter ion to identify them. Ion mass transitions were: PFOS (499→80/99), ^{13}C-PFOS (503→80/99), PFOA (413→369), ^{13}C-PFOA (417→372), PFHxS (399→80/99), PFNA (469→419). The dwell time was 0.1 s. The ES-capillary voltage was set at −4.5 kV and the cone voltage varied according to the PFC being measured. The PFC concentrations were calculated using an unextracted calibration curve. Acetonitrile was injected after every batch of 5−10 samples in order to make sure that there were no memory effects. To determine the stability of the HPLC-MS/MS system, a standard was injected after every batch of samples. The recovery rates of the measured PFCs varied from 105 to 110% for the PFCs measured. Three spiked samples and three blanks were extracted with each batch of samples, which consisted of about 24 samples. No blank peaks were found. The calculation of the limits of detection (LOD) was based on a signal-to-noise ratio of three in the chromatogram of extracts of spiked pig liver that did not contain any detectable amounts of PFCs. This incorporates all the dilution factors occurring during the extraction method.

Statistical Analysis. First, Shapiro-Wilk's W test was used in order to test for normality of the data. The test was applied to the different groups, i.e., marine tucuxi dolphins from Guanabara Bay (the whole group, only males, only females, etc.) as well as from other areas of Rio de Janeiro State. When normal distributions were verified, differences in PFOS concentrations between groups were tested by the Student's t test, while Pearson test was used for evaluating the existence of correlations of PFOS concentrations versus age and total length. When nonnormal distributions were observed, pairwise group comparisons were made using the Mann−Whitney U test, while Spearman (r_S) test was used for investigating the occurrence of correlation between PFOS concentrations and the variables total length and age. The level of significance was set to $p \leq 0.05$.

Results and Discussion

The perfluorocarboxylates PFOA and PFNA were not detected in any of the samples. Regarding the perfluoroalkanesulfonates PFHxS and PFOS, the former was not detected in any of the samples, whereas the latter was detected in all of them. Many investigations have demonstrated that PFOS is the most abundant polyfluoroalkyl compound in tissues from

TABLE 2. Mean Hepatic PFOS Concentrations (ng·g^{-1}, Wet Weight), With Standard Deviation (± SD), Concentration Range, Median, Number of Individuals of Each Species/Area of Sampling of Cetaceans from All over the World[a]

species	area	range	median	mean ± SD	*n*	ref.
marine tucuxi dolphin	Guanabara Bay, Brazil	13−902	260	268 ± 194	23	p.s.
franciscana dolphin	Southernmost Brazil	3.6−42	NA	24 ± 11.8	13	(*7*)
harbor porpoise	North Sea	12−395	63	93	48	(*11*)
sperm whale	North Sea	19−52	NA	36	6	(*11*)
white-beaked dolphin	North Sea	14−443	NA	132	7	(*11*)
white-sided dolphin	North Sea	<10−26	NA	NA	2	(*11*)
striped dolphin	North Sea	11−11	11	11	2	(*11*)
fin whale	North Sea		<10	<10	1	(*11*)
pygmy sperm whale	NW Atlantic Ocean	7−23	15	15	2	(*5*)
clymene dolphin	NW Atlantic Ocean	79−168	NA	123 ± 36	3	(*5*)
striped dolphin	NW Atlantic Ocean	37−388	212	212	2	(*5*)
rough-toothed dolphin	NW Atlantic Ocean	43−66	54	54	2	(*5*)
bottlenose dolphin	NW Atlantic Ocean	48−1520	NA	489 ± 356	20	(*5*)
harbor porpoise	Norway	71−749	131	213 ± 195	19	(*24*)
harbor porpoise	Iceland	26−67	33	38 ± 14	8	(*24*)
harbor porpoise	Denmark	129−620	204	270 ± 171	7	(*24*)
harbor porpoise	Baltic Sea	232−1149	350	534 ± 357	7	(*24*)
harbor porpoise	Black Sea	33−1790	210	NA	31	(*29*)
bottlenose dolphin	Mediterranean Sea	170−430	NA	270	5	(*2*)
striped dolphin	Mediterranean Sea	65−160	NA	100	4	(*2*)
ganges river dolphin	Ganges River, India	<35−81	NA	NA	2	(*2*)
bottlenose dolphin	Italian Coast	<1.4−110	NA	NA	6	(*22*)
striped dolphin	Italian Coast	16−40	NA	NA	4	(*22*)
common dolphin	Italian Coast		940	940	1	(*22*)
long-finned pilot whale	Italian Coast		270	270	1	(*22*)
finless porpoise	Japanese Coast	81−631	NA	278	5	(*59*)

[a] N.A., not available; p.s., present study.

marine biota (*22–28*). Data generated by a recent study shows that PFOS can account for 90% of the PFC load in marine mammal tissues (*29*). PFOA was not detected also in analyses of human blood samples from Brazil (*30*). Although the donors of the blood samples analyzed in the latter investigation inhabited Rio Grande, a city located at the southern extreme of Brazil, this corroboration draws attention to the possibility that the pattern of PFCs detected herewith reflects the use of fluorochemicals at Rio de Janeiro state industrial park, as well as the PFCs present in the consumer products in Brazil. Information concerning the use of PFCs in domestic and industrial products in Brazil is scanty. However, it is known that PFCs have been used in fire-fighting foams in the country (*31*). Concerning the perfluoroalkanesulfonates, it is known that the insecticide Sulfluramid (*N*-ethyl perfluorooctane sulfonamide) is largely used in Brazil for agricultural control of leaf-cutting ants (*32, 33*) as well as a consumer product marketed for the domestic control of cockroaches and termites. In a mammal organism, this fluorinated insecticide is rapidly metabolized to PFOS (*34*).

Hepatic PFOS concentrations from marine tucuxi dolphins that stranded on the beaches of Rio de Janeiro state, along with age and total length (TL), are presented in the Table 1. There was a significant difference in PFOS levels between dolphins from Guanabara Bay and individuals that stranded on beaches of Rio de Janeiro state other than those within Guanabara Bay (dolphins from out of Guanabara Bay) (Mann−Whitney U test, $p = 0.04$). Since data in the present study were originally expressed in ng·g^{-1} dry weight, conversion to wet weight was carried out for comparison to data from the literature, using information on moisture content of each liver sample, obtained through the freeze-drying process. The concentrations of perfluorooctane sulfonate in marine tucuxi dolphins from Guanabara Bay are among the highest detected to date in cetaceans (Table 2). In addition to the information in Table 2, since the crude data on hepatic PFOS concentrations of bottlenose dolphins from North-Western Atlantic Ocean (*5*) as well as from Italian Coast (*22*) have been exposed in previous studies, it was possible to carry out statistical comparisons (Mann−Whitney U test) between marine tucuxi dolphins from Guanabara Bay and bottlenose dolphins from both areas mentioned. In our analysis, the dolphins from Guanabara Bay exhibited significantly higher hepatic PFOS concentrations than bottlenose dolphins from Italian Coast ($p = 0.005$). Up to our knowledge, the highest hepatic PFOS level reported for cetaceans is the concentration of 1520 ng.g^{-1} ww determined in a male bottlenose dolphin from North-Western Atlantic Ocean (*5*). Comparing the PFOS concentrations verified in males of this highly exposed population to those observed in male marine tucuxi dolphins from Guanabara Bay, it was possible to observe that there was no significant difference ($p = 0.19$).

Regarding dolphins from Guanabara Bay, at first, there were no significant correlations between PFOS and age or TL. In males, these absences of correlation were verified considering both age (Pearson Test, $p = 0.86$) and TL (Spearman, $p = 0.37$). Due to the small number of female marine tucuxi dolphins from Guanabara Bay who had their age determined ($n = 4$), correlation testing regarding specifically females was carried out concerning TL only and the Spearman test pointed to the absence of the correlation concerned ($p = 0.15$). However, when a one-year-old male calf presenting 2431 ng·g^{-1} dw (the highest PFOS concentration verified in the present study) was excluded from the test, significant correlations between PFOS concentrations and both age and TL (Pearson Test, $p = 0.03$, $p = 0.02$, respectively; Figure 1) were observed in male marine tucuxi dolphins from Guanabara Bay. The high hepatic PFOS concentration verified in a one-year-old calf could still be a consequence of a high burden of the concerned pollutant acquired from transplacental and lactational transfer. The fact that a significant correlation was only verified after exclusion of the mentioned calf from the testing suggests that mother-to-calf transference plays important role for the relationships between PFCs and age in marine tucuxi dolphins. Although there are data to the contrary, e.g., regarding polar bears (*35, 36*), published information (*6, 11*)

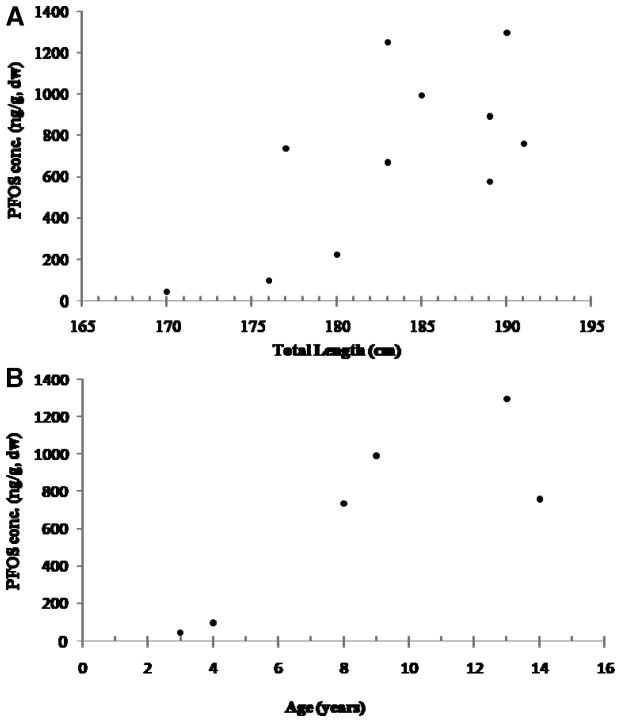

FIGURE 1. Graphic representations of the statistically significant positive correlations observed between PFOS concentrations and biological parameters concerning male marine tucuxi dolphins from Guanabara Bay when the PFOS level verified in a one-year-old male calf presenting 2431 ng·g^{-1} dw (the highest PFOS concentration verified in the present study) was excluded from the test. Figure 1A concerns PFOS concentration and total length of dolphins (Pearson Test, $p = 0.02$). Figure 1B concerns PFOS concentration and age of dolphins (Pearson Test, $p = 0.03$).

supports the idea that the latter suggestion can be extended to other marine mammal species, particularly cetaceans and pinnipeds. Analyzing blood samples from Antarctic elephant seals, Tao et al. (*6*) verified significantly higher PFOS concentrations in pups than in juveniles or adults. This observation corroborated data regarding harbor porpoises, since significantly higher hepatic PFOS concentrations in juveniles than in adults had been verified (*11*). With regard to the pollutant burden acquired during the nursing period, the presence of PFOS in milk of delphinids has been demonstrated recently (*28*). Besides the presence of a one-year-old calf in the sample set, the possibility that unknown pathophysiological conditions have acted as influencing factors on the absence of significant correlations between PFOS and age should not be dismissed. This possibility is reinforced when information from literature is taking into account regarding differences in PFOS levels related to health status. Van de Vijver et al. (*11*) verified that PFOS concentrations of harbor seals with bronchopneumonia were significantly lower than the levels determined in individuals that did not present the pathological process concerned. Kannan et al. (*37*) observed that hepatic PFOS concentrations were significantly higher in sea otters that died from infectious diseases than in those who died from trauma and other causes. These findings are especially important considering the number of investigations in which no correlation between PFOS and age has been observed in mammals (*5, 22, 29, 38, 39*), implying further that the presence of diseased animals in the sample set may hamper the occurrence of age-related significant correlations due to the interference in PFOS assimilation and metabolization that a pathological process can generate.

The PFOS concentrations of three marine tucuxi fetuses and one newborn calf (all from Guanabara Bay) varied between 664 and 1590 ng·g^{-1} dw (Table 1). Fetus/mother (F/M) ratios of hepatic PFOS concentrations in marine tucuxi dolphins from Guanabara Bay were calculated in two situations (F/M = 2.75 and F/M = 2.62). These high values suggest that placental transfer constitutes important elimination and assimilation pathway of PFOS, for females and fetuses of the species, respectively. Calculating the fetus/mother ratios (F/M) of hepatic PFOS concentrations from the exposed data on a harbor porpoise fetus−mother pair (*24*), it was possible to verify a similar value (F/M = 2.57). High PFOS concentrations in cetacean fetuses had been verified in previous studies. Among 19 analyzed harbor porpoises from North Norway/Barents Sea, the highest PFOS concentration (224 ng·g^{-1}, wet weight) was observed in a fetus liver (*24*). A similar finding was observed in a recent study, since the highest renal PFOS concentration among 31 harbor porpoises (1371 ng·g^{-1}, wet wt) was observed in a fetus (*29*).

Despite the fact that placental and lactational transfer can constitute elimination pathways for PFCs in females, there was no significant difference between sexes, regarding PFOS concentrations in dolphins from Guanabara Bay (*t* test; $p = 0.2$). However, it is important to consider that male and female marine tucuxi dolphins belong to different age groups (Table 1). Apparently contrasting information can be observed in literature concerning gender-related differences in PFOS levels. In most cases, no significant differences between males and females were observed in wild mammals (*3, 5, 22, 29, 38–41*). Significant higher PFOS concentrations have been verified in females than in males, regarding harbor porpoises and harbor seals (*11*). Concerning the porpoises, the authors have drawn attention to the fact that when the comparison was carried out within the different age categories, no gender specific relationship could be detected, but the same did not hold for harbor seals (*11*). Contrastingly, male gray seals presented higher PFOS concentrations than females concerning both liver (*22*) and blood (*5*). In addition, male sea otters presented significantly higher hepatic PFOS concentrations than females (*37*). In a recent investigation in which the exposure to organotin compounds (OTs) was evaluated through determination of total tin, the same two fetus−mother pairs of marine tucuxi dolphins were analyzed. Placental transfer of OTs was also verified, and similarly to the observed in the present study, there was no significant difference between males and females (*42*). In that case, it was hypothesized that the placental transfer of OTs in cetaceans is not intense enough to produce a statistically significant lower concentration in females than in males. However, the values of the fetus−mother (F/M) ratios of hepatic PFOS concentrations observed in the present study (F/M = 2.75 and F/M = 2.62) are higher than those verified concerning hepatic total tin (F/M = 0.096 and F/M = 0.216) levels. In fact, the values mentioned regarding the fetus/mother ratios of PFOS concentrations are even higher than those observed in literature, concerning organochlorine compound (OC) levels in other dolphin species. The fetus−mother concentration ratios of some chlorinated hydrocarbons, comprising hexachlorobenzene (HCB), hexachlorocyclohexane (HCH) isomers (α, β, γ), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and related compounds in adipose tissue of a striped dolphin (*Stenella coeruleoalba*) fetus-mother pair varied from 0.38 (PCBs) to 0.97 (HCB) (*43*). This constitutes important information when it is considered that statistically significant differences between sexes are commonly observed concerning organochlorine compound contamination in cetaceans (*44*). An explanation for these seemingly contradictory data, regarding differences in PFOS concentrations between sexes in wild mammals, may be found if it is considered that significant differences in the elimination rate of some PFC

have been observed among species and even between males and females of the same species (*45–47*).

Regarding the effects of PFCs on the fetus, a recent study has generated information of concern since PFOS concentrations in cord serum of nonoccupational exposed humans were negatively associated with birth weight and size (*48*). This latter finding highlights that PFOS levels commonly observed in environmentally exposed organisms are enough for eliciting developmental toxicity. Taking the high PFOS concentrations observed in predator marine mammals into consideration, especial concern on in uteri exposure to this pollutant is raised about the animal group mentioned.

Considering that marine tucuxi dolphins inhabit anthropogenically disrupted environments, facing a number of potential and known threats (*49*), evaluating the magnitude of the species exposure to PFCs is of great interest for conservation purposes, especially when it is taken into account that molecules of this class of pollutants have been shown to be endocrine disrupters, tumor promoters (*50*), and immunosuppressors (*34*). The immunosuppressive action has been also demonstrated regarding other pollutants of environmental concern, such as cadmium (*51*), organotins (*52*), and organochlorines (*53*). Some investigations have demonstrated that cadmium does not seem to constitute a problem for coastal cetaceans in Brazil (*54–56*); however, the same could not be stated about organotins (*42*) and organochlorines (*57*). Therefore, the immunosuppressive action and the developmental toxicity constitute subjects of importance for the conservation of a cetacean population assessed to be composed of approximately 70 individuals (*14*), particularly when the possibility of synergistic effect between PFOS and other toxic organic compounds is taken into consideration, since marine tucuxi dolphins from Guanabara Bay have been shown to be highly exposed to both organochlorines (*57*) and organotins (*42*). Taking the high concentrations observed in fetuses and young marine tucuxi dolphins into consideration, especial concern is raised on possible teratogen and developmental effects. This apprehension is augmented if recently published information on congenital malformation in environmentally exposed mammals is taken into account, since it was attributed to in uteri exposure to a cocktail of organic pollutants (*58*).

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Literature Cited

(1) Giesy, J. P.; Kannan, K. Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.* **2002**, *36*, 146A–152A.

(2) Giesy, J. P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001**, *35*, 1339–1342.

(3) Hoff, P. T.; Scheirs, J.; Van de Vijver, K.; Van Dongen, W.; Esmans, E. L.; Blust, R.; de Coen, W. Biochemical effect evaluation of perfluorooctane sulfonic acid-contaminated wood mice (*Apodemus sylvaticus*). *Environ. Health Perspect.* **2004**, *112*, 681–686.

(4) Hoff, P. T.; Van Campenhout, K.; Van de Vijver, K.; Covaci, A.; Bervoets, L.; Moens, L.; Huyskens, G.; Goemans, G.; Belpaire, C.; Blust, R.; De Coen, W. Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects. *Environ. Pollut.* **2005**, *137*, 324–333.

(5) Kannan, K.; Koistinen, J.; Beckmen, K.; Evans, T.; Gorzelany, J.; Hansen, K. J.; Jones, P. D.; Helle, E.; Nyman, M.; Giesy, J. P. Accumulation of perfluorooctane sulfonate in marine mammals. *Environ. Sci. Technol.* **2001**, *35*, 1593–1598.

(6) Tao, L.; Kannan, K.; Kajiwara, N.; Costa, M.; Fillmann, G.; Takahashi, S.; Tanabe, S. Perfluorooctanesulfonate and related fluorochemicals in albatrosses, elephant seals, penguins, and polar skuas from the Southern Ocean. *Environ. Sci. Technol.* **2006**, *40*, 7642–7648.

(7) Leonel, J.; Kannan, K.; Tao, L.; Fillmann, G.; Montone, R. C. A baseline study of perfluorochemicals in franciscana dolphin and Subantarctic fur seal from coastal waters of Southern Brazil. *Mar. Pollut. Bull.*, in press, doi: 1 0.1016/j.marpolbul. 2008.01.012.

(8) Olivero-Verbel, J.; Tao, L.; Johnson-Restrepo, B. J.; Guette-Fernández, J.; Baldiris-Avila, R.; O'byrne, I.; Kannan, K. Perfluorooctanesulfonate and related fluorochemicals in biological samples from the north coast of Colombia. *Environ. Pollut.* **2006**, *142*, 367–372.

(9) Hoff, P. T.; Van de Vijver, K. I.; Van Dongen, W.; Esmans, E. L.; Blust, R.; De Coen, W. Perfluorooctane sulfonate in bib (*Trisopterus luscus*) and plaice (*Pleuronectes platessa*) from the Western Scheldt and the Belgian North Sea: distribution and biochemical effects. *Environ. Toxicol. Chem./SETAC* **2003**, *22*, 608–614.

(10) Van de Vijver, K. I.; Hoff, P.; Van Dongen, W.; Esmans, E.; Blust, R.; De Coen, W. Exposure patterns of perfluorooctane sulfonate in aquatic invertebrates from the Western Scheldt estuary and the Southern North Sea. *Environ. Toxicol. Chem./SETAC* **2003**, *22*, 2037–2041.

(11) Van de Vijver, K. I.; Hoff, P. T.; Das, K.; Van Dongen, W.; Esmans, E. L.; Jauniaux, T.; Bouquegneau, J.-M.; Blust, R.; De Coen, W. M. Perfluorinated chemicals infiltrate ocean waters: link between exposure levels and stable isotope ratios in marine mammals. *Environ. Sci. Technol.* **2003**, *37*, 5545–5550.

(12) Kjerfve, B.; Ribeiro, C. H. A.; Dias, G. T. H.; Fillipo, A. M.; Quaresma, V.; da., S. Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. *Cont. Shelf Res.* **1997**, *17*, 1609–1643.

(13) Cunha, H. A.; da Silva, V. M. F.; Lailson-Brito, J.; Santos, M. C. O.; Flores, P. A. C.; Martin, A. R.; Azevedo, A. F.; Fragoso, A. B. L.; Zanelatto, R. C.; Solé-Cava, A. M. Riverine and marine ecotypes of *Sotalia* dolphins are different species. *Mar. Biol.* **2005**, *148*, 449–457.

(14) Azevedo, A. F.,, Comportamento e uso do espaço por *Sotalia guianensis* (Cetacea, Delphinidae) na Baía de Guanabara (RJ) e variação geográfica dos assobios da espécie ao longo da costa do Brasil, Thesis, UERJ, Rio de Janeiro, 2005.

(15) Azevedo, A. F.; Lailson-Brito, J.; Cunha, H. A.; Van Sluys, M. A note on site fidelity of marine tucuxis (*Sotalia fluviatilis*) in Guanabara Bay, southeastern Brazil. *J. Cetacean Res.Manage* **2004**, *6*, 265–268.

(16) Azevedo, A. F.; Oliveira, A. M.; Viana, S. C.; Van Sluys, M. Habitat use by marine tucuxis (*Sotalia guianensis*) (Cetacea: Delphinidae) in Guanabara Bay, south-eastern Brazil. *J. Mar. Biol. Ass. U. K.* **2007**, *87*, 201–205.

(17) Martin, J. W.; Kannan, K.; Berger, U.; de Voogt, P.; Field, J.; Franklin, J.; Giesy, J. P.; Harner, T.; Muir, D. C.; Scott, B.; Kaiser, M.; Järnberg, U.; Jones, K. C.; Mabury, S. A.; Schroeder, H.; Simcik, M.; Sottani, C.; van Bavel, B.; Kärrman, A.; Lindström, G.; van Leeuwen, S. Analytical challenges hamper perfluoroalkyl research. *Environ. Sci. Technol.* **2004**, *38*, 248A–255A.

(18) Houde, M.; Bujas, T. A. D.; Small, J.; Wells, R. S.; Fair, P. A.; Bossart, G. D.; Solomon, K. R.; Muir, D. C. G. Biomagnification of perfluoroalkylcompoundsin the bottlenose dolphin (*Tursiops truncatus*) food web. *Environ. Sci. Technol.* **2006**, *40*, 4138–4144.

(19) Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G. Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.* **2006**, *40*, 3463–3473.

(20) Dietz, R.; Heide-Jørgensen, M. P.; Teilmann, J.; Valentin, N.; Härkönen, T. Age determination in European harbor seals *Phoca vitulina*. *Sarsia* **1991**, *76*, 17–21.

(21) Berger, U.; Haukås, M. Validation of a screening method based on liquid chromatography coupled to high resolution mass spectrometry for analysis of perfluoroalkylated substances in biota. *J. Chromatogr., A* **2005**, *1081*, 210–217.

(22) Kannan, K.; Corsolini, S.; Falandysz, J.; Oehme, G.; Focardi, S.; Giesy, J. P. Perfluorooctanesulfonate and related fluorinated hydrocarbons in marine mammals, fishes, and birds from coasts of the Baltic and the Mediterranean Seas. *Environ. Sci. Technol.* **2002**, *36*, 3210–3216.

(23) Moody, C. A.; Martin, J. W.; Kwan, W. C.; Muir, D. C. G.; Mabury, S. A. Monitoring perfluorinated surfactants in biota and surface water samples following an accidental release of fire-fighting foam into Etobicoke creek. *Environ. Sci. Technol.* **2002**, *36*, 545–551.

(24) Van de Vijver, K. I.; Hoff, P. T.; Das, K.; Van Dongen, W.; Esmans, E. L.; Siebert, U.; Bouquegneau, J.-M.; Blust, R.; De Coen, W. M. Baseline study of perfluorochemicals in harbour porpoises

(*Phocoena phocoena*) from Northern Europe. *Mar. Pollut. Bull.* **2004**, *48*, 992–997.
(25) Van de Vijver, K. I.; Hoff, P.; Das, K.; Braseur, S.; Van Dongen, W.; Esmans, E.; Reijnders, P.; Blust, R.; De Coen, W. Tissue distribution of perfluorinated chemicals in harbor seals (*Phoca vitulina*) from the Dutch Wadden Sea. *Environ. Sci. Technol.* **2005**, *39*, 6978–6984.
(26) Bossi, R.; Riget, F. F.; Dietz, R.; Sonne, C.; Fauser, P.; Dam, M.; Vorkamp, K. Preliminary screening of perfluorooctane sulfonate (PFOS) and other fluorochemicals in fish, birds and marine mammals from Greenland and the Faroe Islands. *Environ. Pollut.* **2005**, *136*, 323–329.
(27) Houde, M.; Wells, R. S.; Fair, P. A.; Bossart, G. D.; Hohn, A. A.; Rowles, T. K.; Sweeney, J. C.; Solomon, K. R.; Muir, D. C. G. Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico and the Atlantic Ocean. *Environ. Sci. Technol.* **2005**, *39*, 6591–6598.
(28) Houde, M.; Balmer, B. C.; Brandsma, S.; Wells, R. S.; Rowles, T. K.; Solomon, K. R.; Muir, D. C. G. Perfluorinated alkylcompounds in relation with life-history and reproductive parameters in bottlenose dolphins (*Tursiops truncatus*) from Sarasota Bay, Florida, USA. *Environ. Toxicol. Chem./SETAC* **2006**, *25*, 2405–2412.
(29) Van de Vijver, K. I.; Holsbeek, L.; Das, K.; Blust, R.; Joiris, C.; De Coen, W. M. Occurrence of Perfluorooctane sulfonate and other perfluorinated alkylated substances in harbor porpoises from the Black Sea. *Environ. Sci. Technol.* **2007**, *41*, 315–320.
(30) Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M. Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. *Environ. Sci. Technol.* **2004**, *38*, 4489–4495.
(31) Figueredo, R. C. R.; Ribeiro, F. A. L.; Sabadini, E. Ciência de espumas - aplicação na extinção de incêndios. *Quím. Nova* **1999**, *22*, 126–130.
(32) Zanuncio, J. C.; Zanuncio, T. V.; Pereira, J. M.; Oliveira, H. N. Controle de Atta laevigata (Hymenoptera: Formicidae) com a isca landrin-f, em área anteriormente coberta com *Eucalyptus*. *Ciência Rural* **1999**, *29*, 573–576.
(33) Stape, J. L.; Binkley, D.; Jacob, W. S.; Takahashi, E. N. A twin-plot approach to determine nutrient limitation and potential productivity in *Eucalyptus* plantations at landscape scales in Brazil. *For. Ecol. Manage.* **2006**, *223*, 358–362.
(34) Peden-Adams, M. M.; EuDaly, J. G.; Dabra, S.; EuDaly, A.; Heesemann, L.; Smythe, J.; Keil, D. E. Suppression of humoral immunity following exposure to the perfluorinated insecticide sulfluramid. *J. Toxicol. Environ. Health A* **2007**, *70*, 1130–1141.
(35) Sonne, C.; Bossi, R.; Dietz, R.; Leifsson, P. S.; Rigét, F. F.; Born, E. W. The Potential Correlation between Perfluorinated Acids and Liver Morphology in East Greenland Polar Bears (*Ursus maritimus*). *Toxicol. Environ. Chem.* **2008**, *90* (2), 275–283.
(36) Dietz, R.; Bossi, R.; Rigét, F. R.; Sonne, C.; Born, E. W., Increasing perfluorinated acids in East Greenland polar bears (*Ursus maritimus*)—A new toxic threat to the Arctic bears., *Environ. Sci. Technol.*, in press, doi: 10.1021/es7025938.
(37) Kannan, K.; Perrotta, E.; Thomas, N. Association between perfluorinated compounds and pathological conditions in southern sea otters. *Environ. Sci. Technol.* **2006**, *40*, 4943–4948.
(38) Kannan, K.; Newsted, J.; Halbrook, R. S.; Giesy, J. P. Perfluorooctanesulfonate and related fluorinated hydrocarbons in mink and river otters from the United States. *Environ. Sci. Technol.* **2002**, *36*, 2566–2571.
(39) Dai, J.; Li, M.; Jin, Y.; Saito, N.; Xu, M.; Wei, F. Perfluorooctanesulfonate and perfluorooctanoate in red panda and giant panda from China. *Environ. Sci. Technol.* **2006**, *40*, 5647–5652.
(40) Bossi, R.; Riget, F. F.; Dietz, R. Temporal and spatial trends of perfluorinated compounds in ringed seal (*Phoca hispida*) from Greenland. *Environ. Sci. Technol.* **2005**, *39*, 7416–7422.
(41) Smithwick, M. M.; Muir, D. C. G.; Mabury, S. A.; Solomon, K. R.; Martin, J. W.; Sonne, C.; Born, E. W.; Letcher, R. J.; Dietz, R. Perfluoroalkyl contaminants in liver tissue from East Greenland polar bears (*Ursus maritimus*). *Environ. Toxicol. Chem./SETAC* **2005**, *24*, 981–986.
(42) Dorneles, P. R.; Lailson-Brito, J.; Fernandez, M. A. S.; Vidal, L. G.; Barbosa, L. A.; Azevedo, A. F.; Fragoso, A. B. L.; Torres, J. P. M.; Malm, O. Evaluation of cetacean exposure to organotin compounds in Brazilian waters through hepatic total tin concentrations. *Environ. Pollut.*, in press, doi: 10.1016/j.envpol.2008.03.007.
(43) Tanabe, S.; Tatsukawa, R.; Maruyama, K.; Miyazaki, N. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. *Agric. Biol. Chem.* **1982**, *46* (5), 1249–1254.
(44) Kim, G. B.; Tanabe, S.; Iwakiri, R.; Tatsukawa, R.; Amano, M.; Miyazaki, N.; Tanaka, H. Accumulation of butyltin compounds in Risso's dolphin (*Grampus griseus*) from the Pacific coast of Japan—Comparison with organochlorine residue pattern. *Environ. Sci. Technol.* **1996**, *30*, 2620–2625.
(45) Butenhoff, J. L.; Gaylor, D. W.; Moore, J. A.; Olsen, G. W.; Rodricks, J.; Mandel, J. H.; Zobel, L. R. Characterization of risk for general population exposure to perfluorooctanoate. *Reg. Toxicol. Pharmacol.* **2004**, *39*, 363–380.
(46) Kennedy, G. L., Jr.; Butenhoff, J. L.; Olsen, G. W.; O'Connor, J. C.; Seacat, A. M.; Perkins, R. G.; Biegel, L.; Murphy, S. R.; Farrar, D. The toxicology of perfluorooctanoate. *Crit. Rev. Toxicol* **2004**, *34*, 351–384.
(47) Kudo, N.; Kawashima, Y. The toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. *J. Toxicol. Sci* **2003**, *28*, 49–57.
(48) Apelberg, B.; Witter, F. R.; Herbstman, J. B.; Calafat, A. M.; Halden, R. U.; Needham, L. L.; Goldman, L. R. cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. *Environ. Health Perspect.* **2007**, *115*, 1670–1676.
(49) Azevedo, A. F.; Lailson-Brito, J.; Dorneles, P. R.; Van Sluys, M.; Cunha, H. A.; Fragoso, A. B. L., Human-induced injuries to marine tucuxis (*Sotalia guianensis*) (Cetacea: Delphinidae) in Brazil. *JMBA Biodivers. Rec*, accepted.
(50) Liu, R. C. M.; Hurtt, M. E.; Cook, J. C.; Biegel, L. B. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl:CD BR (CD) rats. *Fundam. Appl. Toxicol.* **1996**, *30*, 220–228.
(51) Simonyte, S.; Planciuniene, R.; Cherkashin, G. Influence of cadmium and selenite ions on the mice resistance to experimental bacterial infection. *Trace Elem. Electrolytes* **2006**, *23*, 277–280.
(52) Whalen, M. M.; Green, S. A.; Loganathan, B. G. Brief butyltin exposure induces irreversible inhibition of the cytotoxic function on human natural killer cells, in vitro. *Environ. Res.* **2002**, *A88*, 19–29.
(53) Sonne, C.; Dietz, R.; Larsen, H. J. S.; Loft, K. E.; Kirkegaard, M.; Letcher, R. J.; Shahmiri, S.; Møller, P. Impairment of cellular immunity in West Greenland sledge dogs (*Canis familiaris*) dietary exposed to polluted minke whale (*Balaenoptera acutorostrata*) blubber. *Environ. Sci. Technol.* **2006**, *40*, 2056–2062.
(54) Lailson-Brito, J.; Azeredo, M. A. A.; Malm, O.; Ramos, R. A.; Dibeneditto, A. P. M.; Saldanha, M. F. C. Trace metal concentrations in liver and kidney of franciscana, *Pontoporia blainvillei*, of the North coast of the Rio de Janeiro State, Brazil. *LAJAM* **2002**, *1*, 107–114.
(55) Dorneles, P. R.; Lailson-Brito, J.; Santos, R. A.; Costa, P. A. S.; Malm, O.; Azevedo, A. F.; Torres, J. P. M. Cephalopods and cetaceans as indicators of offshore bioavailability of cadmium off Central South Brazil Bight. *Environ. Pollut.* **2007**, *148*, 352–359.
(56) Dorneles, P. R., Jr; Secchi, E. R.; Bassoi, M.; Lozinski, C. P. C.; Torres, J. P. M.; Malm, O. Cadmium concentrations in franciscana dolphin (*Pontoporia blainvillei*) from south Brazilian coast. *Braz. J. Oceanogr.* **2007**, *55*, 179–186.
(57) Torres, J. P.; Lailson-Brito, J.; Dorneles, P. R.; Azevedo e Silva, C. E.; Azeredo, A.; Meire, R. O.; Vidal, L.; Lozinski, C.; Azevedo, A. F. Malm, O. Organochlorines in blubber of marine tucuxi dolphin, Sotalia guianensis, from Rio de Janeiro coastal bays, Brazil. *Organohal. Compd.* **2006**, *68*, 580–582.
(58) Sonne, C.; Dietz, R.; Born, E. W.; Leifsson, P. S.; Andersen, S. Is there a link between *hypospadias* and organochlorine exposure in East Greenland sledge dogs (*Canis familiaris*). *Ecotoxicol. Environ. Saf.* **2008**, *69*, 391–395.
(59) Nakata, H.; Kannan, K.; Nasu, T.; Cho, H.-S.; Sinclair, E.; Takemura, A. Perfluorinated contaminants in sediments and aquatic organisms collected from shallow water and tidal flat areas of the Ariake Sea, Japan: environmental fate of perfluorooctane sulfonate in aquatic ecosystems. *Environ. Sci. Technol.* **2006**, *40*, 4916–4921.

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