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Perfluorooctanesulfonate and Perfluorooctanoate in Red Panda and Giant Panda from China

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Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are important perfluorochemicals (PFCs) in various applications. Recently, it has been shown that these compounds are widespread in the environment, wildlife, and humans. The giant panda and the red panda belong to the order Carnivora, but are highly specialized as bamboo feeders. Both species are considered rare and endangered. In this study, we report for the first time on levels of PFOS and PFOA in serum of the giant panda and the red panda captured in zoos and animal parks from six provinces in China. PFOS was the predominant compound in all panda samples measured (ranging from 0.80 to 73.80 μg/L for red panda and from 0.76 to 19.00 μg/L for giant panda). The PFOA level ranged from 0.33 to 8.20 μg/L for red panda, and from 0.32 to 1.56 μg/L for giant panda. There was a positive significant correlation between concentrations of PFOS and PFOA in the serum obtained from pandas. No age- or sex- related differences were observed in concentrations of the fluorochemicals in panda sera. Greater concentrations of the fluorochemicals were found for those individuals collected from zoos near urbanized or industrialized areas than for other areas. These data combined with other reported data suggest that there are large differences in distribution of perfluorinated compounds in terrestrial animals.

Introduction

Perfluorinated compounds (PFCs) have been manufactured for over 50 years. They form a diverse group of chemicals used in a variety of specialized consumer and industrial products such as surfactants and surface protectors in carpets, textiles, leather, paper product, food containers, fire-fighting foams, cosmetics, and upholstery (reviewed in *1*, *2*). Given the energy of the carbon−fluorine bond, it is expected that perfluorinated sulfonic and carboxylic acids will be resistant to hydrolysis, photolysis, biodegradation, or metabolism (*3*). The environmental fate of fluorinated organic compounds has received little attention for many years, due to the lack of suitable analytical methods and authentic standards. It is only recently that these compounds have been found widespread in the environment (*4*). Perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and longer chain (C_8−C_{15}) perfluorocarboxylates are the most commonly measured PFCs in human blood, surface water, and freshwater and marine biota. They are found even in the Arctic, Antarctic, and remote oceanic environments far from their sources (*5*−*9*). Because of their potential for persistence and bioaccumulation (*10*), there is concern regarding the environmental distribution and toxicological impacts of PFCs. It has been reported that PFCs have toxic effects on peroxisome proliferation, endocrine function, carcinogenicity, postnatal development, and reproduction, and cause alterations in cholesterol levels, steroid levels, mitochondrial bioenergetics, and lipid metabolism *in vivo* (*11*, *12*).

Although some investigators have examined PFC levels in several wildlife species, including marine animals and sea birds, few studies have reported on the occurrence of PFCs in terrestrial mammals (*13*, *14*). The giant panda (*Ailuropoda melanoleuca*) and the red panda (*Ailurus fulgens*) are endemic to the Himalayan Hengduan Mountains in China. The giant panda is now only found in the wild in Shanxi, Gansu, and Sichuan provinces. The giant panda population totals around 1000 individuals (*15*). The red panda has a much larger distribution area than the giant panda, extending from central Nepal eastward along the Himalayas through Bhutan, India, and Burma into China (*16*). Although the total population of red pandas is estimated to be 7000−8000, it is confronted with the same environmental and genetic pressures as the giant panda population, such as habitat loss and fragmentation, and inbreeding effects (*17*). Currently, the giant panda and red panda are classified as Category I and Category II, respectively, under the Wild Animal Protection Law in China. Both of them are listed in Appendix I by Convention on International Trade in Endangered Species (CITES) and are considered rare and endangered. Both panda species belong to the order Carnivora, but bamboo forms 80% of the diet of both wild and captive pandas, with the remaining 20% being eggs, rodents, insects, and other protein sources. Monitoring the concentrations of PFCs in panda tissues is important for understanding the distribution and potential impact of these compounds in this species. It is also important to assess the potential sources by investigating PFC contamination in a terrestrial species that has little direct contact with marine sources (far removed from the marine environment, have no marine species in diet, etc.). Because some other persistent contaminants, such as organochlorine compounds, correlate with mammal health (*18*), PFCs may also be related to panda health. There is, however, little information on the distribution and degree of contamination of PFCs, particularly in wildlife, in China (*19*−*20*). The purpose of this study is to understand the geographical pattern of contamination of PFOS and PFOA in China. This was done by measuring concentrations of PFCs in the blood of captive pandas in eight cities from six different provinces. The influence of age and gender on blood PFC concentrations was also investigated.

Materials and Methods

Sample Collection. Blood samples of red pandas were collected from 27 individuals in 7 different locations, which were Chongqing (n = 12), Chengdu (n = 3), Yele (n = 3), Kunming (n = 1), Hefei (n = 5), Fuyang (n = 1), and Fuzhou (n = 2). Nine blood samples of the giant panda were collected from the Beijing Zoo during September−November 2004

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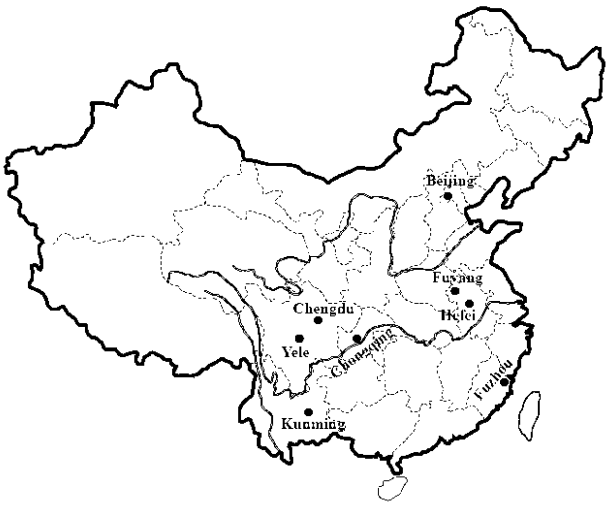

FIGURE 1. Map of China showing sampling locations for collecting blood samples from pandas.

(Figure 1). Blood (3−5 mL) was drawn from panda's left forearms after anesthetization by a veterinarian, and the samples were centrifuged after collection. Serum was transferred into polypropylene cryovials which were kept frozen at −20 °C until analysis. Age was recorded (especially since there was a lineage for the giant panda), as well as sex and sampling location (Table 1).

Extraction. The procedure for extraction of chemicals from the blood was similar to that described previously (*21*). One mL of 0.5 M tetrabutylammonium hydrogensulfate solution and 2 mL of sodium carbonate buffer (0.25 M, pH 10) were added to 0.4 mL of serum sample in a polypropylene tube and the solution was thoroughly mixed for extraction. Five mL of methyl *tert*-butyl ether (MTBE) was added to the above mixture, and the tube was shaken for 20 min at 250 rpm. The organic and the aqueous layers were separated by centrifugation at 3000 rpm for 15 min. Then, the solvent was removed and transferred to a second 15 mL polypropylene tube. The extraction was repeated twice as described above. All three extracts were combined and evaporated at room temperature under nitrogen gas, and replaced with 1.0 mL of methanol. The extract was passed through a nylon mesh filter (0.2 μm) into an HPLC vial before injection into the liquid chromatograph.

Analysis. The solution was then analyzed by LC/MS as previously reported (*22*). 1,2-^{13}C−perfluorooctanoic acid was used as the internal standard spike with 10 ng/mL of serum in this study. In brief, mass spectra were taken on an LC/MS system equipped with an orthogonal spray interface, and employing electron spray ionization in negative mode. The fragmentor and capillary voltages were 200 and 4000 V, respectively. The nebulizer pressure was 50 psig and the drying N_2 gas flow rate was 10.0 L/min. The selected ion monitoring mode employed for quantification of PFOA and PFOS was 413 and 499, respectively. Calibration curves constructed for PFOS ranged from 0.1 to 100 μg/L and were linear with a correlation value $r > 0.998$ (data not shown). Percentage recovery for PFOS was 105.86% with a coefficient of variation (CV) of 2.4% for human serum samples. Percentage recovery for PFOA was 94.77% with a coefficient of variation (CV) of 3.6% for human serum samples. Human serum with low endogenous levels of PFOA and PFOS from Japan was used for QC matrix samples. The lowest limits of detection (LOD) (μg/L) were 0.06 for PFOA and 0.04 for PFOS in the serum samples (*21*). The lowest limits of quantification (LOQ) (μg/L) were 0.1 for both chemicals in the serum samples. The mode of analysis was implemented in an international intercalibration study and the results demonstrated acceptable analysis of PFOS and PFOA (The concentrations of five different samples were PFOA, 9.6 + 0.1, 3.8 − 0.2, 11.5 + 0.2, 12.3 − 1.4, 7.7 − 0.6 ng/mL; and PFOS, 18.5 + 0.4, 5.4 + 0.6, 27.7 + 1.5, 11.5 + 0.2, 19.1 + 3.5 ng/mL, respectively).

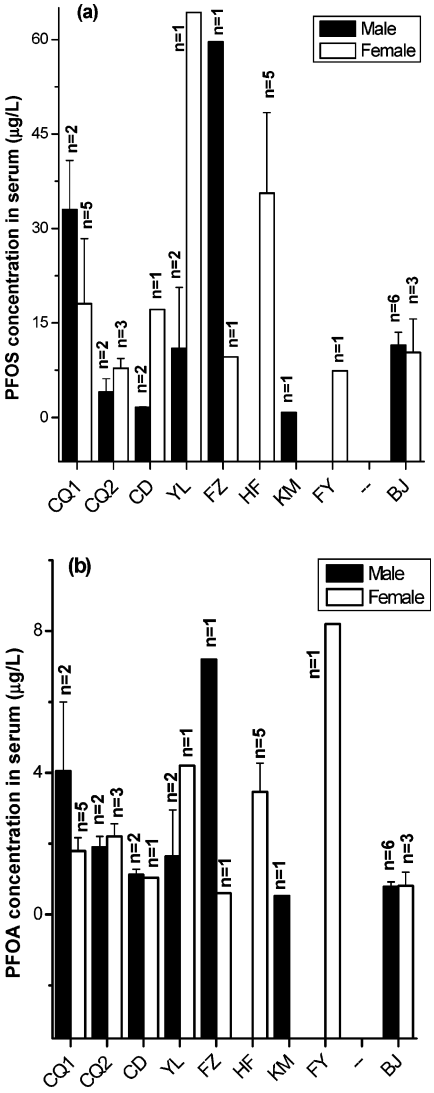

FIGURE 2. Concentrations of PFOS and PFOA in sera of pandas: (a) PFOS; (b) PFOA: CQ1, Chongqing wild animal park; CQ2, Chongqing zoo; CD, Chengdu zoo; YL, Yele zoo; FZ, Fuzhou zoo; HF, Hefei wild animal park; KM, Kunming zoo; FY, Fuyang zoo; BJ, Beijing zoo.

Statistical Methods. The normality of the data was analyzed by means of a Kolmogorov−Smirnov test. A log-transformation was done to ensure normality of the distribution for PFCs concentrations in all samples. One-way ANOVA was performed to investigate differences between genders and age, and linear regression tests were conducted to evaluate relationships among contaminants. All statistical analyses were conducted with SPSS software (Version 13.0 for windows, SPSS Incorporate, Chicago, IL).

Results and Discussion

Levels of PFCs in Red and Giant Pandas. PFOS and PFOA were detected in all serum samples, and PFOA was at much lower concentrations than PFOS. The PFOS concentration ranged from 0.80 to 73.80 μg/L for red panda and from 0.76 to 19.00 μg/L for giant panda. The PFOA level ranged from 0.33 to 8.20 μg/L for red panda and from 0.32 to 1.56 μg/L for giant panda. Concentrations of PFOS varied up to 92-fold

TABLE 1. Details of the Panda Blood Samples from China Analyzed in 2004

province	location	no. of samples(serum)	age (yrs):mean	gender distribution M	gender distribution F
		red panda			
Chongqing	Chongqing wild animal park	7	1−8	2	5
	Chongqing zoo	5	3−10	2	3
Sichuan	Chengdu zoo	3	5.6 (4−8)	2	1
	Yele zoo	3	1−8	2	1
Yunnan	Kunming zoo	1	4	1	
Fujian	Fuzhou zoo	2	3−8	1	1
Anhui	Hefei wild animal park	5	5−7		5
	Fuyang zoo	1	4		1
		giant panda			
Beijing	Beijing zoo	9	10.5 (2.5−16)	6	3

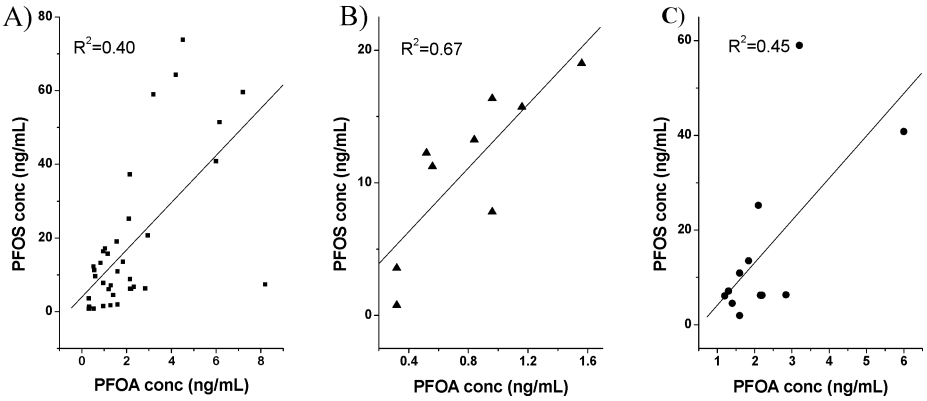

FIGURE 3. Linear regressions between concentrations of PFOS and PFOA in sera of pandas: (A) red panda and giant panda ($n = 36$), (B) giant panda at Beijing ($n = 9$), and (C) red panda at Chongqing ($n = 12$). Coefficients of variation (R^2) are shown.

among the samples, depending on the species and location (Figure 2). The greatest concentration of PFOS was found in red panda serum from Hefei while the lowest concentrations were found in red panda from Kunming. PFOS concentrations were generally high in the serum of panda obtained from Chongqing, Fuzhou, Yele, and Hefei. A spatial evaluation of PFOS concentrations in red panda showed that panda from Hefei and Yele had significantly greater concentrations than those from Chongqing and Chengdu. Furthermore, a considerable spatial difference in concentrations of PFOS was observed among different sampling locations within a city. For instance, the average concentrations of PFOS in red panda from Chongqing wild animal park (22.31 ± 7.85 μg/L, $n = 7$) were significantly greater than those from Chongqing zoo (6.31 ± 1.42 μg/L, $n = 5$). Both of them are close to urbanized and industrialized areas in the vicinity of Chongqing, in southern China, and this suggests that the higher levels of fluorochemicals may not be attributable to proximity to industrialized regions but instead to differences in zoo practice. Although there is only sparse information concerning perfluorochemical production plants in China, large end-user industries might have an influence on the total pollution burden on an animal, including the presence of PFOS.

Pandas in captivity in zoos are offered milk as a part of their diet, supplemented with cake made of rice, corn, soybeans, and honey. Dierenfeld et al. (*23*) published a study looking at the diets administered to panda in zoos in Beijing, Chengdu, Fuzhou, and Xian. The results showed that the zoo diets consisted of 20−80% bamboo and smaller amounts of grains (13−65%), with 8−25% being milk, eggs, and meat. Although the average concentrations of PFOS in red panda from Yele were significantly greater than those from Chongqing, the average concentrations of PFOA were not significantly different in these two populations. These variations in patterns could be due to differences in diet, zoo cleaning practices, and human contact at these different locations, thus suggesting that the sources of PFOS and PFOA in Yele are different from those for Chongqing. Relatively low concentrations of PFOS and PFOA in giant panda may indicate lesser exposure and/or a greater ability to metabolize and excrete precursors of PFOS and PFOA as compared to red panda (*19*).

The results on levels of PFCs in red pandas and giant pandas suggest that PFCs, especially PFOS, are widespread contaminants in panda in China. The different distribution of PFCs in the panda might be an indicator for different exposure sources of and/or pathways for PFCs in the captive animal populations. Toxic effects of PFOA and PFOS on panda are unknown and are subjects for further investigation.

Relationships Between the Two PFCs. Statistically significant positive correlations were found between PFOS and PFOA concentrations within all serum samples ($R^2 = 0.40$, SE = 15.83, $p = 0.000$, $n = 36$) (Figure 3A). If the outlier (PFOA: 8.20 μg/L) is deleted, the correlation coefficient is improved and SE is reduced ($R^2 = 0.63$, SE = 12.35, $p = 0.000$, $n = 35$). When the concentrations were grouped by province, the correlation coefficient is improved and SE is greatly reduced within locations at Beijing ($R^2 = 0.67$, SE = 3.71, $p = 0.007$, $n = 9$) and Chongqing ($R^2 = 0.45$, SE = 13.60, $p = 0.007$, $n = 12$) (Figure 3B and C). While for Figure 3C the outliers (PFOA, 8.20 μg/L; PFOS, 59.00 μg/L) were deleted, the correlation coefficient was not improved but SE is reduced ($R^2 = 0.37$, SE = 9.51, $p = 0.034$, $n = 10$). These results indicate that the exposure sources of PFOS and PFOA to panda are similar or coexist for the two compounds, or PFOA can occur as an impurity in PFOS formulations, although the exposure sources and/or pathways to PFC are unknown.

TABLE 2. Range of Concentrations of PFOS and PFOA in Wildlife for Blood Plasma (ng/mL or ng/g ww; Mean Concentrations Are Given in Parentheses)

species	PFOS	PFOA	location	ref
polar bear	52−104 (68)[a]		Beaufort Sea	*24*
ringed seal	<3−12		Canadian Arctic	*25*
ringed seal	16−230 (110)		Baltic sea	*25*
ringed seal	5−14 (9)		Norwegian Arctic	*25*
gray seal	14−76 (37)		Baltic Sea	*25*
gray seal	14−49 (28)		Canadian Arctic	*25*
bottlenose dolphins	194−1715 (780)	0.7−26 (6.3)	Sarasota Bay (U.S.)	*26*
bottlenose dolphins	46−52 (49)	0.6−0.9 (0.8)	Bermuda	*26*
bottlenose dolphins	472−3037 (1315)	4.6−163 (44)	Charleston, U.S.	*26*
bottlenose dolphins	232−1240 (751)	20−115 (72)	Delaware Bay, U.S.	*26*
loggerhead turtle	1.4−96.8 (5.45)	0.49−8.14 (2.95)	Southeastern coast of U.S.	*30*
Kemp's ridley turtle	13.8−60.2 (41.9)	2.77−4.25 (3.46)	Southeastern coast of U.S.	*30*
snapping turtle	1−170 (72)		Lake St, Clair, MI	*25*
double-crested cormorant	1−270 (170)		Lake Huron, Great Lakes	*25*
herring gull	66−79 (73)		Lake Huron, Great Lakes	*25*
black-tailed gull	2−12 (6)		Hokkaido, Japan	*25*
glaucous gull	48.1−349 (134)	<0.70−0.74	Norwegian Arctic	*31*
laysan and black-footed albatrosses	9−26 (18)		Midway Atoll, North Pacific	25
bald eagle	1−2570 (360)		Midwestern U.S.	25
carrion crow	22−300 (112) [a]		Tokyo Bay area	29
domestic duck	6−9 (7.5)		Tokyo Bay area	29

[a] Concentrations of PFCs measured in whole blood samples were converted to serum/plasma values based on the difference in volume by multiplying by a factor of 2, so as to allow a comparison across different studies.

Gender- and Age-Related Accumulation. Mean concentrations of both PFOS and PFOA for female red pandas (22.94, 2.76 μg/L) were greater than those for males (15.97, 2.52 μg/L) although the difference was not statistically significant ($p = 0.5$). Mean concentrations of PFOS and PFOA for female giant pandas were very similar to those for males. To investigate possible gender- and age-related accumulation of PFCs, data of blood samples from panda of eight cities were pooled and analyzed for possible gender- and age-related patterns. No significant difference was found for gender effect on the concentrations of perfluorinated compounds. When the data were grouped by regions, no significant difference was found for the gender effect on the concentrations of PFOS and PFOA. Similarly, it was reported that no gender-related differences in the concentrations of PFOS or PFOA were found in human blood in China (*19*). No differences between genders were observed for PFCs in plasma of free-ranging bottlenose dolphins (*26*) and in polar bear liver, and for PFOS in ringed seal liver (*27*). However, differences in concentrations of PFCs between the sexes had been observed in blood samples from Poland, Japan, and the United States (*28*). Also, no association between age and the concentrations of PFOS and PFOA was observed in the present study. This is consistent with other studies. There was no correlation between PFOS in liver of gray and ringed seals from the Baltic and Mediterranean Sea and age of the animals (*28*). No association between PFOS in the blood of Japanese donors and their ages could be found (*29*). Similar results have also been observed in human blood in China (*19*). Some studies, however, have shown that several PFCs were negatively associated with age in bottlenose dolphin (*26*) and the other showed that the concentrations of PFOS increased with age in ridley turtles (*30*). Since the accumulation of perfluorinated compounds in the biota is influenced by their binding to proteins rather than lipids, the residue levels, therefore, may be influenced by factors associated with the protein metabolism of different species.

Global Comparison of PFC Concentrations in Wild Animals. Mean concentrations of PFOS in red panda serum were 20.36 ± 4.35 μg/L ($n = 27$) and in giant panda were 11.10 ± 2.01 μg/L ($n = 9$). The value for PFOA in red panda was 2.67 ± 0.40 μg/L ($n = 27$) and in giant panda was 0.80 ± 0.14 μg/L ($n = 9$). The mean concentrations of PFOS and PFOA from related studies are summarized in Table 2. Concentrations of PFCs measured in whole blood samples in references (Table 2) were converted to serum/plasma values based on the difference in volume by multiplying by a factor of 2 (*19*, *28*, *29*), so as to allow a comparison across different studies. Concentrations of PFOS in serum of red panda and giant panda were 65 and 110-fold less, respectively, than those from bottlenose dolphins at Charleston (Table 2). The mean concentrations of PFOS in serum of red panda were slightly greater than those in loggerhead turtles from the southeastern coast of the United States, ringed seals from the Norwegian Arctic, albatrosses from Midway Atoll, North Pacific, and gulls from Hokkaido in Japan. Concentrations of PFOA in serum of red panda and giant panda were 27 and 90-fold less, respectively, than those from bottlenose dolphins at Delaware (Table 2). The mean concentrations of PFOA in serum of red panda were slightly greater than those in bottlenose dolphins from Bermuda or in glaucous gull from the Norwegian Arctic (*31*). The greater concentrations of PFOS in the panda samples may be related to their frequent exposure to PFC-containing environment. Furthermore, increased industrial and economic activities in China have led to the rapid development of various industries such as textiles, electronics, and packing products, and so to an increased exposure to PFCs. Because the sources and pathways of exposure to the panda population are still unknown, the risk could increase if the releases are not controlled.

Our study provides, for the first time, baseline data regarding the extent of exposure of pandas to PFCs, and also provides additional information to the few reports on concentrations of PFCs in terrestrial mammals. However, since the number of panda samples in our current study was small, and samples were from zoos and animal parks, the results may not reflect exposure in the wild animal population. In addition, the drawback of the paper is that only PFOS and PFOA were determined in this study considering longer-chained homologs may be important toxicologically and for source determination. Risk assessments for PFCs are not available at present because information on physiological, toxicological, and ecological effects of PFCs is rare (*32*). Although PFCs have been shown to be not toxic in cynomolgus monkeys at a serum PFOS concentration of 100

μg/L, high concentrations of PFOS resulted in decreased body weight, increased liver weight, lowered serum total cholesterol, lowered triiodothyronine concentrations, and lowered estradiol levels in the monkeys (*33*). Therefore, only when more species-specific information on PFCs becomes available will risk assessments for humans and wildlife be possible. Although our findings provide significant information on PFCs in pandas, further studies with larger numbers of samples are necessary to further understand the sources and pathways of exposure, and to evaluate the potential toxic effects to wildlife of PFCs.

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Supporting Information Available

Table S1 showing mean, standard error, median, minimum, and maximum perfluorochemical concentrations (μg/L) in sera of pandas from different cities. Table S2 describing the optimized analytical parameters. The standard curve of PFOS is given in Figure S1, and the LC/MS chromatogram of PFOS and PFOA in a deionized water is given in Figure S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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