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Accumulation of perfluorinated compounds in captive Bengal tigers (*Panthera tigris tigris*) and African lions (*Panthera leo Linnaeus*) in China

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ABSTRACT

The accumulation of perfluorinated compounds (PFCs) in the sera of captive wildlife species Bengal tigers (*Panthera tigris tigris*) and African lions (*Panthera leo Linnaeus*) from Harbin Wildlife Park, Heilongjiang Province, in China were analyzed by high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). Perfluorooctanesulfonate (PFOS) was the predominant contaminant with a mean serum concentration of 1.18 ng mL^{-1} in tigers and 2.69 ng mL^{-1} in lions. Perfluorononanoic acid (PFNA) was the second most prevalent contaminant in both species. The composition profiles of the tested PFCs differed between tigers and lions, and the percentages of perfluorooctanoic acid (PFOA) were greater in lions than in tigers, indicating different exposures and/or metabolic capabilities between the two species. Assessments of the risk of PFC contamination to the two species were obtained by comparing measured concentrations to points of departure or toxicity reference values (TRVs). Results suggest no risk of PFOS exposure or toxicity for the two species.

1. Introduction

Bengal tigers (*Panthera tigris tigris*) and African lions (*Panthera leo Linnaeus*) are protected species. The Bengal tiger is recognized as the national animal of China and renowned as the Royal Bengal Tiger. Unfortunately, three of the eight subspecies of tigers have been extinct since the 1950s, and the remaining five subspecies are listed as endangered (IUCN, 2003). The African lion is the second largest feline in the world, second to the tiger; the lion population has been reduced by 30–50% over the last three generations (IUCN Red List, 2004). Although the primary reasons for the declining numbers of these two endangered species are the extensive loss of habitat and poaching for traditional medicines (Graham et al., 2006), increases in environmental pollution may also be contributing to their extinction.

Perfluorinated compounds (PFCs) have been used in many commercial applications, such as fire-fighting foams, paper, leather, and inks, due to their insolubility in both oil and water. Several toxicological studies have shown that certain PFCs are toxic. For example, exposure to perfluorooctanesulfonate (PFOS) causes an elevation in liver enzymes, hepatic vacuolization, weight loss, and peroxisomal proliferation in rodent (Haughom and Spydevold, 1992; Sohlenius et al., 1993; Berthiaume and Wallace, 2002; Seacat et al., 2003). For this reason, PFC monitoring in the environment and in organisms has become a scientific, regulatory concern. PFCs are a global environmental contaminant and many studies have explored PFC accumulation in marine animals (Kannan et al., 2002; Taniyasu et al., 2003; Houde et al., 2005). Only a few studies have focused on PFC accumulation of terrestrial animals (Giesy and Kannan, 2001; Hoff et al., 2004; Dai et al., 2006) and human (Olsen et al., 2003; Taniyasu et al., 2003; Inoue et al., 2004; Kannan et al., 2004; Tittlemier et al., 2004; Guruge et al., 2005; Kärrman et al., 2005; Kubwabo et al., 2005; Calafat et al., 2006; Yeung et al., 2006).

The major objectives of this study were to elucidate the accumulation of PFCs in Bengal tigers and African lions in China; and assess PFC risk to the two wildlife species.

2. Materials and methods

2.1. Sample collection

The sera of Bengal tigers ($n=5$) and African lions ($n=5$) were collected in November 2006 from Harbin Wildlife Park (125°42′–130°10′W, 44°04′–46°40′N), which is located in Heilongjiang Province in Northern China. The genders and ages of all animals

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Table 1
Gender and age data for tested Bengal tigers and African lions

Species	No.	Sex	Age (years)
Bengal tiger *Panthera tigris tigris*	BT1	♂	>10
	BT 2	♂	>10
	BT 3	♂	>10
	BT 4	♀	>10
	BT 5	♀	>10
African lion *Panthera leo Linnaeus*	AL1	♂	10
	AL 2	♂	3
	AL 3	♀	4
	AL 4	♀	4
	AL 5	♀	4

tested are listed in Table 1. Blood samples were drawn from the left forearm of lions and tigers following anesthetization by a veterinarian; samples were centrifuged to obtain serum samples. Serum samples were transferred into polypropylene cryovials and kept frozen at −20 °C until analysis.

2.2. Reagents and chemicals

The potassium salts of PFOS, perfluorohexanesulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), and perfluoroheptanoic acid (PFHpA) were purchased from Wellington Laboratories, Inc. (ON, Canada). Unsaturated fluorotelomer carboxylate (8:2 FTUCA) and saturated fluorotelomer carboxylate (8:2 FTCA) were provided by the Asahi Glass Co. Ltd. (Tokyo, Japan). Perfluorohexanoic acid (PFHxA) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Perfluorodecanoic acid (PFDA) was purchased from Fluorochem Ltd. (Derbyshire, UK), and perfluorobutanesulfonate (PFBS) was purchased from Chiron (Trondheim, Norway). Purities of all analytical standards were greater than or equal to 95%. Oasis® weak anion exchange (WAX; 6 cc, 150 mg, 30 μm) solid phase extraction (SPE) cartridges were purchased from Waters (Milford, MA). Methanol (MeOH, residual pesticide and PCB analytical grade), ammonium acetate (97%), ammonium solution (25%), acetic acid (99.9%), tetra-*n*-butyl ammonium hydrogen sulfate (TBA), methyl-*tert*-butyl ether (MTBE), sodium carbonate, and sodium hydrogen carbonate were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.3. PFC analysis

Tiger and lion serum samples were analyzed for ten PFCs (PFOS, PFHxS, PFBS, PFDA, PFNA, PFOA, PFHpA, PFHxA, 8:2 FTCA, and 8:2 FTUCA) using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). After samples were thawed at room temperature, PFC extraction and analysis were performed according to the protocol described elsewhere with modifications (Hansen et al., 2001; Taniyasu et al., 2005; Yeung et al., 2006). Extraction of PFCs from blood/serum samples was achieved using an ion-pairing extraction method previously described (Hansen et al., 2001), and the final ion-pairing extract was subjected to further purification using the SPE-Oasis-WAX-method (Taniyasu et al., 2005). Briefly, sera were extracted with 1 mL of 0.5 M TBA solution, 2 mL of 0.25 M sodium carbonate buffer (pH 10), and 5 mL MTBE in a 15-mL polypropylene (PP) tube. The organic and aqueous layers were separated by shaking at 250 rpm for 20 min and centrifuged at 3000 rpm for 15 min. Four milliliters MTBE were removed and transferred to a 15-mL PP tube. This extraction procedure was repeated twice as described above, except that 5 mL MTBE were removed each time instead of 4 mL. All three extracts were combined in a separate 15-mL PP tube and concentrated to 0.5 mL under nitrogen gas after the addition of 1 mL MeOH. The final solution was diluted into 100 mL Milli-Q water for SPE cleanup. All samples were then extracted using an Oasis® WAX cartridge. The cartridge was pre-equilibrated by the addition of a sequence of 4 mL of 0.1% NH_4OH in MeOH, 4 mL MeOH, and 4 mL water at a rate of 2 drops sec^{-1}. Samples (100 mL) were then passed through these cartridges at a rate of 1–2 drops sec^{-1}. After loading all samples, cartridges were rinsed with 20 mL of 0.01% NH_4OH in H_2O and three volumes (10 mL each) Milli-Q water. The cartridges were then washed with 4 mL of 25 mM acetate buffer solution (pH 4). Any water remaining in the cartridges was removed by centrifugation at 3000 rpm for 2 min, and PFCs were eluted as two fractions. The first (Fr. 1) and second fractions (Fr. 2) were eluted by the addition of 4 mL MeOH and 4 mL of 0.1% NH_4OH in MeOH, respectively. Fr. 1 and Fr.2 were concentrated to 0.5 mL, respectively, under a stream of nitrogen.

2.4. Instrumental analysis

Perfluorinated compounds analysis was performed by HPLC–MS/MS. Analyte separation was achieved using an Agilent HP1100 liquid chromatograph (Agilent, Palo Alto, CA) interfaced with a Micromass Quattro Ultima Pt mass spectrometer (Waters Corp., Milford, MA) operated in the electro spray negative mode. Next, 10-μL extracts were injected into a guard column (XDB-C8, 2.1 mm i.d. × 12.5 mm, 5 μm; Agilent Technologies, Palo Alto, CA) connected sequentially to a Betasil C18 column (2.1 mm i.d. × 50 mm length; Thermo Hypersil-Keystone, Bellefonte, PA). The mobile phase was 2 mM ammonium acetate–MeOH, beginning with 10% MeOH. Detailed instrumental parameters have been reported elsewhere (Taniyasu et al., 2005).

2.5. Quality control

A calibration curve was prepared from a series of concentrations (0, 2, 10, 50, 200, 1000, 5000, and 20000 pg mL^{-1}), and standard deviations were less than 20%. Standard solutions of 1000 pg mL^{-1} were injected ten times during analysis to detect any deviation in the response of the system. Blanks and recoveries were assessed following the same procedure as described above with each group of extractions. The blanks were all below the limit of quantifications (LOQs), and the details regarding the removal of interfering substances are described elsewhere (Yamashita et al., 2004; So et al., 2007). All native standards were spiked into blood samples and analyzed. The resultant recoveries showed that the results sufficiently accounted for the matrix effect. Mean recoveries of PFOS, PFHxS, PFBS, PFDA, PFNA, PFOA, PFHpA, PFHxA, 8:2 FTCA, and 8:2 FTUCA ranged from 70% for 8:2 FTCA to 117% for PFHxS (Table 2). The concentrations of PFCs in the experimental samples were not corrected for their corresponding recoveries.

Table 2
Average recovery percentages, standard deviation for recovery (SD), and limits of quantification (LOQ) of the used analytical procedure for each tested compound

Compound	Average recovery (%)	SD (%)	LOQ (ng mL^{-1})
PFOS	111	3	0.05
PFHxS	117	3	0.05
PFOA	96	19	0.05
PFDA	107	5	0.05
PFNA	103	5	0.05
PFBS	114	2	0.05
PFHpA	112	10	0.25
PFHxA	112	2	0.25
8:2 FTCA	70	8	0.25
8:2 FTUCA	82	6	0.05

3. Results and discussion

3.1. Concentrations and composition profiles

Total PFC concentrations were determined to be the sums of the measured concentrations of PFOS, PFHxS, PFDA, PFNA, and PFOA. The other five PFCs (PFBS, PFHpA, PFHxA, 8:2 FTCA, and 8:2 FTUCA) analyzed were not included because their concentrations were lower than the respective LOQs for all samples (Table 3). The PFCs, 8:2 FTOH and 10:2 FTOH have been detected in the plasma dolphins (*Tursiops truncatus*) (Houde et al., 2005). However, these two compounds were not detected in any samples in this study.

Total PFC serum concentrations ranged from 1.40–2.08 ng mL^{-1} in tigers and from 3.84–7.25 ng mL^{-1} in lions (Table 3). In lions, the total PFC concentration in lion No. 2 was almost twice that in No. 1 even though both lions are the same gender and No. 2 was the youngest lion sampled. The effects of age and gender on PFC concentration are unknown and cannot be determined from the results of this study due to its small sample size. In lions, the mean serum concentrations of total PFCs were 5.55 ± 2.41 ng mL^{-1} in males (n = 2) and 4.25 ± 0.40 ng mL^{-1} in females (n = 3). Similarly, the mean serum concentrations of total PFCs were 1.82 ± 0.31 ng mL^{-1} in males (n = 3) and 1.72 ± 0.45 ng mL^{-1} in females (n = 2) in tigers. The mean serum concentration of total PFCs was approximately 3-fold less in tigers (1.78 ± 0.32 ng mL^{-1}) than in lions (4.77 ± 1.43 ng mL^{-1}). Since Bengal tigers and African lions both belong to the order Carnivora and have similar feeding habits, the observed results may suggest that bioaccumulation is species-specific. In addition, the other factors such as age, (generally >10 y in tigers, approx 4 y in lions), their history probably and so on might affect their exposure to PFCs. A detailed comparison of the total PFC concentration measurements in this study with that of previous studies is difficult because only limited studies have been conducted for terrestrial mammals, and the specific PFCs detected in the studies are different (Dai et al., 2006).

PFOS occurred at the highest concentrations among PFCs in this study (Fig. 1), consistent with results from captive pandas (Dai et al., 2006) and humans (Yeung et al., 2006) in China. PFOS serum concentrations ranged from 0.863–1.43 ng mL^{-1} in tigers and from 2.24–3.03 ng mL^{-1} in lions; the mean PFOS serum concentration in lions (2.69 ng mL^{-1}) was two times higher than in tigers (1.18 ng mL^{-1}). In tigers and lions, mean PFOS concentration was approximately 10- and 50-fold less than those in the captive pandas and human in China, respectively. The percentage of total PFCs contributed by PFOS differed between the two species, ranging from 61.6–75.0% in tigers and from 41.7–71.2% in lions. The reason

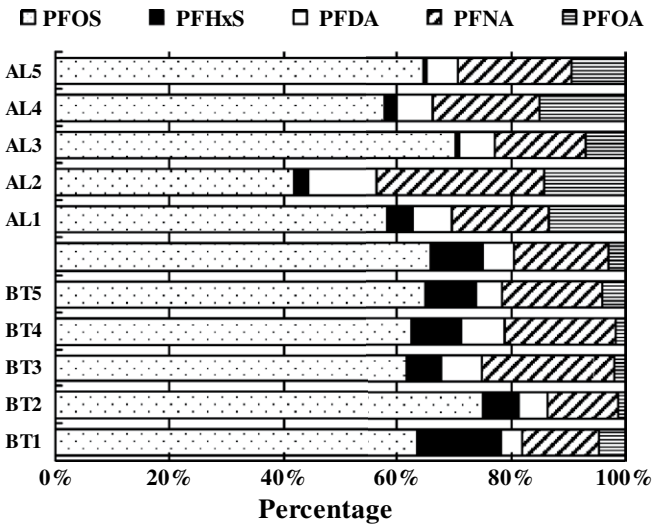

Fig. 1. PFC composition profiles in Bengal tigers and African lions in China.

for this difference may be that the two species eliminate these PFCs differently. In this study, PFOS concentrations were either comparable with or lower than those in other terrestrial mammals, such as liver samples of caribou (*Rangifer tarandus*) in Nunavut, Canada [3.8–24 ng g^{-1} wet weight (ww)] (Tittlemier et al., 2005), liver of Arctic fox (*Alopex lagopus*) in Arviat, NU (250 g g^{-1} ww), liver of mink (*Mustela vison*) from Watson Lake, Yukon (8.7 ng g^{-1} ww) (Martin et al., 2004), and liver of wood mice (*Apodemus sylvaticus*) in Belgium (140–178550 ng g^{-1} ww) (Hoff et al., 2004) and in sera of giant pandas (*Ailuropoda melanoleuca*) and red pandas (*Ailurus fulgens*) from China (0.76–73.80 ng mL^{-1}) (Dai et al., 2006). PFOS contamination of local sources of air, water, and food might contribute to PFOS concentrations in local species. For example, wood mice that inhabited an area near a fluorochemical plant had higher PFOS concentrations (Hoff et al., 2004) compared with those living far from fluorochemical plants. Carnivorous tigers and lions had lower PFOS serum concentrations than pandas (Dai et al., 2006), although tigers primarily feed on a mix of beef, ox, chicken, rabbit, sheep, goat, calf, deer, and pig, while the panda diet consists of 20–80% bamboo, small amounts of grains (13–65%), and 8–25% of milk, eggs, and meat (Dai et al., 2006). Location may be an additional compounding factor in comparing the results of this study with the investigation of PFCs in pandas. Pandas living in zoos located in heavily industrial cities would potentially be exposed

Table 3
PFC concentrations in Bengal tigers and African lions in China

Species	No.	PFOS	PFHxS	PFDA	PFNA	PFOA	Total PFCs
Bengal tiger *Panthera tigris tigris*	BT1	1.32	0.306	0.0773	0.277	0.0978	2.08
	BT 2	1.43	0.121	0.0950	0.237	nd	1.91
	BT 3	0.942	0.132	0.116	0.293	nd	1.48
	BT 4	0.863	0.858	0.0993	0.329	nd	1.40
	BT 5	1.33	0.177	0.0965	0.357	0.0828	2.04
	Average	**1.18**	**0.164**	**0.0968**	**0.298**	**0.0461**	**1.78**
	(SD)	**(0.256)**	**(0.310)**	**(0.0138)**	**(0.0464)**	**(0.0497)**	**(0.320)**
African lion *Panthera leo Linnaeus*	AL1	2.24	0.172	0.266	0.650	0.514	3.84
	AL 2	3.03	0.177	0.880	2.13	1.04	7.25
	AL 3	2.90	nd	0.256	0.661	0.286	4.10
	AL 4	2.73	0.107	0.291	0.883	0.712	4.72
	AL 5	2.56	nd	0.215	0.789	0.373	3.94
	Average	**2.69**	**0.0911**	**0.381**	**1.02**	**0.584**	**4.77**
	(SD)	**(0.308)**	**(0.0877)**	**(0.280)**	**(0.626)**	**(0.301)**	**(1.43)**

"nd" indicates analyte concentration was lower than LOQ and was treated as 0.5*LOQ in calculating mean values.

to higher sources of PFCs, whereas the tigers and lions tested in this study live in a relatively remote open area with no obvious PFC sources. Future studies are needed to investigate the effects of local PFC sources on PFC concentrations detected in tigers, lions, and pandas.

The short chain PFHxS compound is an intermediate in the production of several PFCs (Kannan et al., 2002) and was observed in sera from tigers and lions. PFHxS serum concentrations were detected in three of the five lion samples (ranging from non-detectable–0.177 ng mL^{-1}) and in all tiger samples (ranging from 0.121–0.858 ng mL^{-1}). The mean PFHxS concentrations were approximately 10-fold and 30-fold lower than PFOS concentrations in tigers and lions, respectively. In contrast to the other PFCs measured, PFHxS concentrations were higher in tigers than in lions (Table 3). PFHxS has also been detected in other wildlife species, such as in liver samples from terrestrial mink in the United States (ranging from 4–85 ng g^{-1} ww) (Kannan et al., 2002).

The mean PFOA concentrations were approximately 26-fold less and 5-fold less than the mean PFOS concentration in tigers and lions, respectively. The mean PFOA serum concentration in tigers (0.0461 ng mL^{-1}) was approximately 10-fold less than in lions (0.584 ng mL^{-1}). In tigers, mean PFOA concentration was both 20-fold less than that in the captive giant panda, 50-fold less than that in the red panda, and 34-fold less than that in human in China. In lions, mean PFOA concentration was similar with those in pandas and human in China. In this study, PFOA was the fifth highest PFC concentration in tigers and the third highest in lions (Fig. 1). PFOA also contributed to a minor percentage of total PFCs in wildlife, such as in the livers of mink and Arctic fox (less than 2 ng g^{-1} ww for both species) (Martin et al., 2004) and caribou (non-detectable to 12 ng g^{-1} ww) (Tittlemier et al., 2005). However, in human, PFOA concentrations are just lower than PFOS concentrations except in reports from Korea (Kannan et al., 2004) and China (Yeung et al., 2006), which indicated that PFC concentration patterns in wildlife and humans are different. These observed differences could be explained by potential contaminant sources. Degradation of FTOH in the atmosphere may be a source of contamination to tigers. Other expected sources include the consumption of contaminated food and exposure to detergents during cage cleaning. Higher percentages of PFOA in humans may arise from exposure to fluorotelomer-based precursors found in microwave popcorn bags and certain items of cookware.

PFNA accounted for the second greatest contribution to total PFC concentrations in both species. PFNA serum concentrations ranged from 0.237–0.357 ng mL^{-1} in tigers and from 0.650–2.13 ng mL^{-1} in lions (Fig. 1). The mean concentration of PFNA was approximately 5-fold lower in tigers than in lions. More PFNA was detected in lion No. 2 than in the other lions. PFNA concentrations were greater than PFOA concentrations in both tigers and lions. The PFC 8:2 FTOH can be degraded into PFOA and PFNA in equal amounts, but PFNA is more bioaccumulative than PFOA, which results in higher PFNA concentrations (Keller et al., 2005). This difference in bioaccumulation may also account for higher PFNA concentrations compared with PFOA concentrations in this study.

PFDA was the fourth highest contributor to total PFCs in both species. PFDA serum concentrations ranged from 0.0773–0.116 ng mL^{-1} in tigers and from 0.215–0.880 ng mL^{-1} in lions (Fig. 1). The mean concentration of PFDA was approximately 4-fold lower in tigers than in lions. In all samples, PFDA concentrations were approximately 3-fold lower than PFNA concentrations. PFNA concentrations that exceed those of PFDA have also been measured in other mammals, such as caribou (non-detectable to 26 ng g^{-1} ww for PFNA and non-detectable to 14.5 ng g^{-1} ww for PFDA) (Tittlemier et al., 2005), mink (16 ng g^{-1} ww for PFNA and 3.7 ng g^{-1} ww for PFDA), and Arctic fox (22 ng g^{-1} ww for PFNA and 14 ng g^{-1} ww for PFDA) (Martin et al., 2004). FTOHs are major sources of environmental PFCAs (Houde et al., 2006); the atmospheric oxidation of 10:2 FTOH to PFDA may also contribute to the concentrations of PFDA measured in both tigers and lions.

Table 4
Correlations (r^2) among PFCs in all samples

	PFOS	PFHxS	PFOA	PFNA	PFDA
PFOS	1				
PFHxS	0.05	1			
PFOA	0.69**	0.001	1		
PFNA	0.63**	0.07	0.75**	1	
PFDA	0.56*	0.07	0.65**	0.79**	1

* Correlation is significant at the $p < 0.05$ level (two-tailed).
** Correlation is significant at the $p < 0.01$ level (two-tailed).

3.2. Correlations among PFCs

Correlations among PFCs in all samples were assessed using a Spearman rank correlation analysis (Table 4). Significant correlations were observed between PFOS and PFNA ($r^2 = 0.63$, $p = 0.006$) and PFDA ($r^2 = 0.56$, $p = 0.013$). An additional significant positive correlation was found between PFDA and PFNA ($r^2 = 0.79$, $p = 0.005$). No correlations existed between PFHxS and the other PFCs in this study (Table 4) perhaps due to the lack of PFHxS detection in certain samples (Table 3). Numerous linear correlations between the concentrations of different PFCs have been found in humans and wildlife (Kannan et al., 2004; Martin et al., 2004; Yeung et al., 2006), suggesting that PFC exposure occurs simultaneously, probably through the same pathways, and, thus, may indicate similar origins within certain geographical locations (Houde et al., 2006).

3.3. Risk assessment

The risk of PFOS exposure to captive tigers and lions was estimated by comparing the probability of exceeding several points of departure, or toxicity reference values (TRLs) (Yeung et al., 2006). The route of exposure could affect the "points of departure" for risk characterization and the exposure duration was longer than three years. Although there were no studies reported the "points of departure" for serum of tigers and lions, some researches have reported that PFOS concentrations in blood were lower than those in liver and the two concentrations were highly correlated with each other (Kannan et al., 2001; Dauwe et al., 2007). Protective values, the benchmark internal concentrations (BMICs), were chosen as "points of departure" for risk characterization. A BMIC of 33 μg mL^{-1} was derived from the lower 95% confidence limit of the rat BMIC based on rat pup weights during lactation (3M, 2003), 44 μg mL^{-1} from the no observed adverse effect level (NOAEL) of rat liver toxicity (3M, 2003; Seacat et al., 2003), and 62 μg mL^{-1} from the lower 95% confidence limit of the BMIC (10% response) for rat liver tumor formation (Seacat et al., 2003). Acceptable lions/tigers exposures to specific chemicals are calculated by reducing the no-observed-adverse-effect levels (NOAEL) from animal experiments by uncertainty factors to account for susceptible populations and interspecies differences (Akingbemi et al., 2004). Extrapolating from our data, acceptable tiger/lion's concentrations would be 0.33, 0.44, and 0.62 μg mL^{-1}. In this study, all the measured PFOS serum concentrations in tigers and lions were less than these TRVs (0.33, 0.44, and 0.62 μg mL^{-1}), which suggested that there would be no immediate risk to the tigers and lions to PFOS exposure. It should be noted that the toxicity thresholds for tigers/lions may differ from those obtained in rats, a refined risk assessment should be undertaken with a larger sample size and more data points for the evaluation of risk. Besides, mixture effects of other PFCs were

not in consideration. In future studies, it would be instructive to examine the combined/cumulative risks of all PFCs.

4. Conclusions

In summary, PFOS was the dominant PFC observed in captive Bengal tigers and African lions in China. The presented survey will be useful in the design of more complete studies for determining PFC contamination in the general population of tigers and lions, including expanded sample sizes and enlarged geographic areas for sampling. PFOS does not currently pose a risk for these two species. However, a potential risk may emerge with the rapid development of industry and prolonged exposure times.

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