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Tissue Distribution and Maternal Transfer of Poly- and Perfluorinated Compounds in Chinese Sturgeon (*Acipenser sinensis*): Implications for Reproductive Risk

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It is critical to investigate the tissue distribution and maternal transfer of poly- and perfluorinated compounds (PFCs) in wild fish for assessing potential effects on ecosystems. Concentrations of 23 PFCs in nine organs and egg were measured in 16 17- to 25-year-old female Chinese sturgeon (*Acipenser sinensis*, an anadromous fish), that died during propagation. Three polyfluorinated amides were detected in stomach, intestine, and gills and 7:3 FTCA was specifically accumulated in liver. The greatest total concentration of PFCs in egg was 35.1 ± 10.4 ng/g ww and was predominated by perfluorooctane sulfonate (PFOS) and perfluorotridecanoate acid (PFTriDA). The longer-chain C_{11}−C_{14} and C_{16} perfluorinated carboxylates were more accumulated in Chinese sturgeon than PFOS, partly due to the increasing trends of PFCAs with fish age. Maternal transfer ratios of PFCs expressed as ratios of concentrations in the egg to those in the liver ranged from 0.79 (perfluorooctanoate) to 5.5 (PFTriDA), depending on their carbon chain lengths or protein−water coefficients. The PFOS equivalent of PFC mixtures, calculated by multiplying the relative potency factor of each PFC to PFOS by the corresponding concentration, ranged from 90.6 to 262 ng/g. The hazard quotient was 0.20, implying potential reproductive effects of PFCs on Chinese sturgeon.

Introduction

Poly- and perfluorinated compounds (PFCs) are a class of widely used, persistent, bioaccumulative compounds that are ubiquitous in the environment (*1*). PFCs have been used in many products including fire-fighting foams, inks, and water repellents, and as coatings on paper and textiles during the past 50 years (*2*). Recently, due to their global detection in different environmental matrices (atmosphere (*3*), precipitation (*4*), surface water (*5*), and biota (*6*)) and toxicity (*7*), PFCs have received greater scientific and regulatory scrutiny. Some PFCs have been associated with alternations in gap junction intercellular communication, lipid metabolism, cholesterol and steroid levels, impaired development (*8, 9*), and a significant reduction in the hatching success of chicken embryos following injection of environmentally relevant concentrations of perfluorooctane sulfonate (PFOS) into eggs (*10*).

Despite their relatively small acid-dissociation constants (pK_a) (*11*) and the fact that they preferentially partition to protein instead of lipids, PFCs have been reported to pose a potential environmental risk through accumulation by higher trophic level organisms such as in polar bears and dolphins (*12, 13*). Investigating distributions of PFCs among tissues is important for understanding their pharmacokinetic and toxic effects on organisms, and some studies have been conducted on air-respiring mammals and birds including harbor seals (*Phoca vitulina*), harbor porpoises (*Phocoena phocoena relicta*), and the common guillemot (*Uria aalge*) (*14*−*16*). Within these studies, relatively great variations in concentrations have been observed among homologues and species. As water-respiring organisms, fish exhibit different pharmacokinetics and toxicity from air-respiring mammals and birds (*17*). However, only one experimental exposure has investigated tissue distributions of perfluorinated acids (PFAs), including perfluorooctanoate (PFOA), C_{10}−C_{13} perfluorocarboxylic acids (PFCAs), perfluorohexane sulfonate (PFHxS), and PFOS in fish (*18*). While providing preliminary information on the pharmacokinetics of PFCs in fish, maternal transfer of PFCs to eggs was not determined. During embryonic development, organisms usually exhibit greater sensitivity to pollutant exposure, especially for oviparous/egg-producing organisms such as fish and birds (*19*). Accumulation of residues in eggs by maternal transfer is the predominant exposure route for embryos, which can result in reproductive and early development toxicities, and therefore contribute to population-level effects (*20*−*22*). Fish-specific developmental toxicities including pericardial edema and malformations of the tail, have been reported for zebrafish (*Danio rerio*) exposed to PFOS (*21*), indicating maternal transfer of PFCs to fish eggs is of concern.

Indirect contamination of aquatic environments with PFC precursors such as fluorotelomer alcohols (FTOHs), polyfluorinated amides, fluorotelomer iodides, olefins, acrylates, and phosphates has been proposed in previous research (*24, 25*). Previous studies have validated the transformation processes from FTOHs to the corresponding PFCAs via atmospheric oxidation, microbial degradation, and rat metabolism, in which both saturated and unsaturated fluorotelomer carboxylates (FTCAs and FTUCAs) have been observed as the intermediate metabolites (*26*−*28*). During the transformation from polyfluorinated amides to corresponding PFAs, perfluorooctane sulfonamide (PFOSA), 2-(perfluorooctanesulfonamido) acetic acid (FOSAA), and 2-(*N*-ethylperfluorooctane sulfonamido) acetic acid (N-EtFOSAA) have been reported as intermediate metabolites (*29, 30*). The detection of PFOSA and some FTCAs and FTUCAs in wildlife such as ringed seals (*Phoca hispida*) and seabirds (*31, 32*) supports the PFC-precursor hypothesis, which may be a

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significant source of PFCs in marine environments in addition to PFCs accumulated by Chinese sturgeon in the Yangtze River.

Due to the rapid decline of its population, the Chinese sturgeon (*Acipenser sinensis*) is listed as a grade I protected animal in China. This decline has been attributed to chemical contamination in addition to dam construction and the resulting loss of habitat, especially in spawning areas, and overfishing (*20, 33, 34*). In this study, concentrations of 23 PFCs including 10 PFCAs (C_6-C_{14}, and C_{16} PFCAs), 4 perfluorinated sulfonic acids (PFSAs, C_6-C_8, C_{10} PFSAs), and related chemicals including 5 FTCAs and FTUCAs (6:2 FTCA, 6:2 FTUCA, 10:2 FTCA, 10:2 FTUCA, and 7:3 FTCA) and 4 polyfluorinated amides (FOSAA, N-MeFOSAA, N-EtFOSAA, PFOSA) were measured in the egg, liver, kidney, gallbladder, intestine, stomach, muscle, heart, gill, and ovary of 16 wild-caught female Chinese sturgeon. The distribution and relative concentrations of PFCs among tissues and age-related accumulation patterns were investigated, with an emphasis on maternal transfer to eggs, and a preliminary assessment of potential effects of the Yangtze River PFC mixture on the reproduction of Chinese sturgeon by use of hazard quotients (HQs).

Materials and Methods

Chemicals and Reagents. Details are given in Supporting Information.

Sample Collection and Artificial Fertilization for Chinese Sturgeon. The Chinese sturgeon is anadromous with initial reproduction occurring at an average age of approximately 14 y (*33*). Every June or July, maturing adults leave the ocean and ascend the main channel of the Yangtze River to spawn, and stay in the river for a period of approximately 1 y before reproducing in middle October to early November, after which they return to the sea for 3 to 5 y before spawning again. The Yangtze River is the largest river in China and flows through urban and industrial areas. Concentrations of PFCs in Yangtze River (<0.005−2.1 ng/L for PFHxS, 0.29−14 ng/L for PFOS, 2.1−260 ng/L for PFOA, <0.005−10 ng/L for PFNA) are greater than those in the marine environment (<0.005−1.36 ng/L for PFHxS, <0.023−9.68 ng/L for PFOS, 0.243−15.3 ng/L for PFOA, 0.002−0.692 ng/L for PFNA) (*35, 36*). It seems that Yangtze River would have contributed large contributions to the PFCs exposure in Chinese sturgeon. Further studies on the clearance rate and food item should be conducted to investigate the exposure route to Chinese sturgeon.

Because they are listed as a grade I protected animal in China, a limited number of Chinese sturgeon were allowed to be captured (by roller hook) for propagation and scientific study, and then released back into the Yangtze River. However, during artificial propagation some of the sturgeon died. Between 2003 and 2006, eggs were collected for artificial spawning while other organs were collected from individuals who died. Samples were kept at −20 °C until analysis. Ages of sturgeons were estimated by growth layers in the cleithrum, as described in previous research (*33*). Details of the samples are shown in Table S1, Supporting Information.

Details about the artificial fertilization for Chinese sturgeon have been described by Hu et al. (*20*). From 2003 to 2006, 16 females were captured from Yangtze River for artificial fertilization. Information on reproductive performance was obtained for 7 of these females. Fecundity was calculated by dividing the number of eggs by body weight (kg). Unfertilized eggs were counted and discarded, and the fertilization was also calculated. Survival was then expressed by the percentage of the number of survival larval sturgeon in total fertilized eggs. Details are given in Table S2, Supporting Information.

Quantification of PFCs and Quality Assurance/Quality Control. Sample extraction was based on the ion pairing method as described in a previous paper (*1*), and the details are given in Supporting Information.

Data Analysis. Details are given in Supporting Information.

Results and Discussion

Concentrations of Perfluorinated Acids (PFAs) and Distributions among Tissues. Of the 14 PFAs including 10 PFCAs and 4 PFSAs, all but PFHxA and PFHpS were detected, with PFTriDA detected in all but one of the 56 samples. The total concentrations of detected PFAs (ΣPFAs) in different tissues are shown (Table 1). Representative chromatograms for each individual PFC are given in Figure S1, Supporting Information. The greatest mean concentration ($p < 0.01$) of 35.1 ± 10.4 ngΣPFAs/g ww, and 14.2 ± 5.5 ngΣPFAs/g ww were observed in egg and liver, respectively. These concentrations were 10−100 times greater than those in other organs (Figure 1a).

Ratios of concentrations in the liver to those in the muscle (liver/muscle ratios, LMR) for PFOS, PFUnDA, PFDoDA, and PFTriDA were compared with those reported for other animals (*14−16*). The LMRs of PFOS, PFUnDA, PFDoDA, and PFTriDA for Chinese sturgeon were 61.5, 63.4, 11.1, and 55.3, respectively, which were generally greater than those in harbor seal (3.0 for PFOS), harbor porpoise (more than 8.0 for PFOS), or common guillemot (8.6, 19.0, 6.4, and 5.5 for PFOS, PFUnDA, PFDoDA, PFTriDA, respectively). The relatively large variation of LMRs among species may lead to improper estimates on trophodynamic behaviors when concentrations of PFCs in liver are used (*13, 37*). Concentrations of PFOS and ΣPFCAs in Chinese sturgeon eggs were 14.6 ± 9.3 and 20.1 ± 19.6 ng/g ww, respectively, which were greater than those in the liver (5.8 ± 3.2 ng PFOS/g ww and 7.6 ± 5.8 ng ΣPFCAs/g ww). While occurrence of PFCs in livers of fish has been reported (*1, 38*), there are few reports of PFCs in fish eggs. Only PFOS was detected in lake whitefish (*Coregonus clupeaformis*) and brown trout (*Cyprinus carpio*) eggs among four target PFCs (PFHxS, PFOS, PFOSA, and PFOA) (*38*).

Patterns of relative concentrations of PFAs in different tissues varied only slightly (Figure 2a), with the pattern in the gallbladder being the most different. PFOS was the predominant PFA, accounting for 41.6%, 43.0%, and 75.7% of ΣPFAs in egg, liver, and gallbladder, respectively. Among the nine detected PFCAs, PFTriDA was the most abundant in all tissues except muscle, accounting for 27.5% (gallbladder) to 70.6% (stomach) of the total PFCAs. Accumulation of PFTriDA was even greater than that of PFOS in the gills (51.0%), heart (28.6%), stomach (60.5%), ovary (41.2%), and intestine (38.7%). PFUnDA was another predominant PFCA and accounted for 10.2% of ΣPFCAs in muscle to 23.0% in gallbladder. This PFTriDA-dominated pattern is different from those previously reported, where PFOA, PFNA, or PFUnDA were generally the most abundant (*31, 39*), a result that has previously been observed in seabirds (*40*). To further understand the patterns of relative concentrations of PFCAs among tissues of Chinese sturgeon, the most prevalent C_6-C_{10} PFCAs reported in other studies were classified as shorter-chain PFCAs, while the $C_{11}-C_{14}$ and C_{16} PFCAs were classified as longer-chain PFCAs. The shorter-chain PFCAs, longer-chain PFCAs, and PFSAs in Chinese sturgeon liver contributed 10%, 46%, and 44% of total PFAs, respectively (Figure 2b). Such a congener-specific pattern leads to the relatively great ΣPFCAs/PFOS ratio in Chinese sturgeon (1.3 in the liver and 1.4 in the egg) compared to the guillemot (0.24 in the liver and 0.10 in the egg), polar bear from Sanikiluaq (0.10 in the liver), and herring gulls from Røst (0.20 in the egg) (*16, 39, 41*).

To evaluate selective bioaccumulation of PFCs in Chinese sturgeon, regression analyses were conducted between age

TABLE 1. Mean Concentrations (ng/g ww) and Ranges of Poly- and Perfluorinated Compounds in Chinese Sturgeon

tissues	E[a]	L	I	H	St	Ov	M	Gi	Gb	K
	$n = 14$	$n = 7$	$n = 7$	$n = 5$	$n = 5$	$n = 4$	$n = 6$	$n = 6$	$n = 1$	$n = 1$
PFHxA	nd[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFHpA	nd	0.19 ± 0.16 (nd−0.56)	nd	nd	nd	nd	nd	nd	nd	nd
PFOA	0.15 ± 0.09 (nd−0.42)	0.16 ± 0.06 (nd−0.26)	0.07 ± 0.03 (nd−0.13)	0.10 ± 0.05 (0.04−0.18)	0.09 ± 0.02 (0.06−0.12)	0.07 ± 0.02 (0.05−0.09)	nd−0.17 (2/6)	0.13 ± 0.02 (nd−0.18)	0.06	nd
PFNA	0.27 ± 0.27 (nd−1.03)	0.25 ± 0.12 (0.07−0.40)	0.07 ± 0.02 (0.05−0.10)	0.21 ± 0.28 (nd−0.70)	nd	nd	0.12 ± 0.03 (nd−0.18)	nd	0.05	nd
PFDA	1.1 ± 0.48 (0.51−1.7)	0.79 ± 0.3 (0.62−1.4)	nd	0.12 ± 0.10 (nd−0.28)	nd−0.09 (2/5)	0.13 ± 0.06 (0.08−0.20)	nd	0.06 ± 0.03 (nd−0.10)	0.13	0.75
PFUnDA	3.8 ± 1.4 (1.7−5.1)	1.6 ± 0.96 (0.53−3.2)	0.15 ± 0.13 (0.05−0.44)	0.23 ± 0.18 (0.06−0.44)	0.15 ± 0.18 (nd−0.46)	0.43 ± 0.32 (0.14−0.89)	0.03 ± 0.01 (nd−0.04)	0.15 ± 0.10 (0.04−0.34)	0.14	1.3
PFDoDA	1.4 ± 0.6 (0.78−2.7)	0.38 ± 0.20 (0.17−0.65)	0.15 ± 0.16 (nd−0.50)	0.09 ± 0.05 (nd−0.16)	0.07 ± 0.08 (nd−0.21)	0.12 ± 0.07 (nd−0.21)	0.03 ± 0.02 (nd−0.07)	0.08 ± 0.02 (nd−0.11)	0.07	1.1
PFTriDA	12.4 ± 4.6 (7.4−22.6)	3.9 ± 4.3 (0.95−13.1)	0.79 ± 1.3 (0.11−3.7)	0.50 ± 0.41 (0.13−0.99)	0.87 ± 1.3 (0.11−3.1)	0.93 ± 0.68 (0.09−1.8)	0.07 ± 0.06 (nd−0.18)	0.80 ± 0.97 (0.17−2.7)	0.17	7.5
PFTeDA	0.96 ± 0.25 (0.78−1.4)	0.27 ± 0.19 (0.65−0.8)	0.09 ± 0.11 (nd−0.35)	0.08 ± 0.03 (nd−0.13)	nd	0.13 ± 0.07 (0.08−0.24)	nd	0.10 ± 0.09 (nd−0.28)	nd	1.7
PFHxDA	0.28 ± 0.10 (nd−0.47)	nd−0.07 (1/7)[c]	nd−0.15 (1/7)	nd	nd	0.07 ± 0.04 (nd−0.12)	nd	0.08 ± 0.03 (nd−0.14)	nd	nd
PFHxS	nd	0.10 ± 0.02 (nd−0.16)	nd	nd	nd	nd	nd	nd	nd	nd
PFHpS	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
PFOS	14.6 ± 9.3 (7.2−27.6)	5.8 ± 3.2 (1.6−9.2)	0.52 ± 0.38 (0.25−1.2)	0.40 ± 0.25 (nd−0.62)	0.21 ± 0.23 (0.08−0.61)	0.38 ± 0.34 (0.09−0.81)	0.09 ± 0.09 (nd −0.27)	0.16 ± 0.18 (nd−0.52)	1.9	1.8
PFDS	0.24 ± 0.24 (0.07−1.0)	0.05 ± 0.01 (0.04−0.07)	0.20 ± 0.39 (nd−1.1)	nd	nd	nd	nd	nd	nd	0.29
7:3 FTCA	nd	0.57 ± 0.45 (0.13−1.4)	nd	nd	nd	nd	nd	nd	nd	nd
6:2 FTCA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6:2 FTUCA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10:2 FTCA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10:2 FTUCA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
FOSAA	nd	nd	nd−0.06 (2/7)	nd	0.05 ± 0.02 (nd−0.12)	nd	nd	0.06 ± 0.04 (nd−0.11)	nd	nd
N-MeFOSAA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
N-EtFOSAA	nd	nd	nd	nd	nd−0.05 (1/5)	nd	nd	nd	nd	nd
PFOSA	nd	0.06 ± 0.07 (nd−0.18)	0.23 ± 0.34 (nd−0.98)	0.03 ± 0.04 (nd−0.11)	0.41 ± 0.63 (nd−1.5)	0.10 ± 0.11 (0.03−0.26)	0.04 ± 0.02 (nd−0.07)	0.27 ± 0.26 (0.08−0.77)	nd	0.98
N-EtFOSA	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
ΣPFCs	35.1 ± 10.4 (21.0−55.5)	14.2 ± 5.5 (6.7−22.7)	2.3 ± 2.4 (0.82 −7.8)	1.8 ± 0.98 (0.86−3.1)	1.9 ± 2.3 (0.64−6.1)	2.4 ± 1.1 (1.4−4.1)	0.38 ± 0.18 (0.27−0.53)	1.9 ± 1.6 (0.70−5.2)	2.5	15.5

[a] E: egg; L: liver; M: muscle; H: heart; Ov: ovary; St: stomach; I: intestine; Gi: gill; K: kidney; Gb: gallbladder. [b] Not detected. [c] 1 of 7 samples was detected above MDL.

and $\log_{10}$-transformed concentrations of PFCs in egg. Increasing age-related trends were also found for longer-chain PFCAs including PFUnDA, PFDoDA, PFTriDA, PFTeDA, and PFHxDA (slope = 0.01−0.03, $r^2 = 0.02$−0.29, $p = 0.049$−0.605). No statistically significant associations were found between shorter-chain C_6−C_{10} PFCAs or PFOS (Figure S2, Supporting Information). Possible reasons for such homologue-specific profiles in Chinese sturgeon are its status as a higher-level predator feeding on insects and fish, water-respiration, and relatively long life span. This set of characteristics and slower excretion rate of longer-chain PFCs compared to more hydrophilic shorter-chain PFAs including PFOS, might lead to a specific accumulation of the longer-chain PFCAs. This pattern was also observed in rainbow trout (*42*). Thus, it is likely that there would be greater accumulation of longer-chain PFCAs and large ΣPFCAs/PFOS, which would result in a relatively large ratio in Chinese sturgeon.

Concentrations and Distributions of Precursors in Tissues. Among the 4 target polyfluorinated amides, PFOSA, FOSAA, and N-EtFOSAA were detected. Chromatograms of these three compounds are shown in Figure S1, Supporting Information. While these precursors have been reported to occur in water of Lakes Ontario and Erie (*43*) and in human blood from Washington State, U.S. (*44*), this is the first report of FOSAA occurring in wildlife. PFOSA was mainly detected in the intestine (0.23 ± 0.34 ng/g ww), stomach (0.41 ± 0.63 ng/g ww), and gills (0.27 ± 0.26 ng/g ww) which were even greater than those of PFOS, and the concentration in liver was relatively small (0.06 ± 0.07 ng/g ww) (Figure 1b). The small accumulation of PFOSA into the liver of the Chinese sturgeon was similar to that observed for guillemot, in which PFOSA was detected in the kidney (0.53 ng/g ww) and egg (0.79 ng/g ww) but not in the muscle or liver (*16*). However, PFOSA was not detected in the eggs of Chinese sturgeon, which suggests a species-specific difference in transfer to eggs. FOSAA was detected only in absorptive organs including the intestine (0.03 and 0.06 ng/g ww in two samples), stomach (0.05 ± 0.02 ng/g ww), and gills (0.06 ± 0.04 ng/g ww). Similarly, a relatively small concentration of N-EtFOSAA was detected in one sample of stomach. These results suggest that polyfluorinated amides such as PFOSA, FOSAA, and N-EtFOSAA would be accumulated through absorptive organs and then rapidly metabolized by Chinese sturgeon. Metabolism of PFOSA has been observed in both rat and rainbow trout liver microsomes (*30, 41*), and FOSAA and N-EtFOSAA have been also reported to be rapidly metabolized and exhibited relatively short half-lives in blood (*44*). Of the 5 FTCAs and FTUCAs, only 7:3 FTCA was detected, specifically in liver (0.57 ± 0.45 ng/g ww). This result is comparable to that observed for the liver of ringed seals (0.5−2.5 ng/g ww) from Sachs Harbor (*45*). A potential cause of this pattern is that 7:3 FTCA is an intermediate metabolite during the pathway from 8:2 FTOH to PFOA and PFNA. This is consistent with the finding of 7:3 FTCA in the livers of rats exposed to

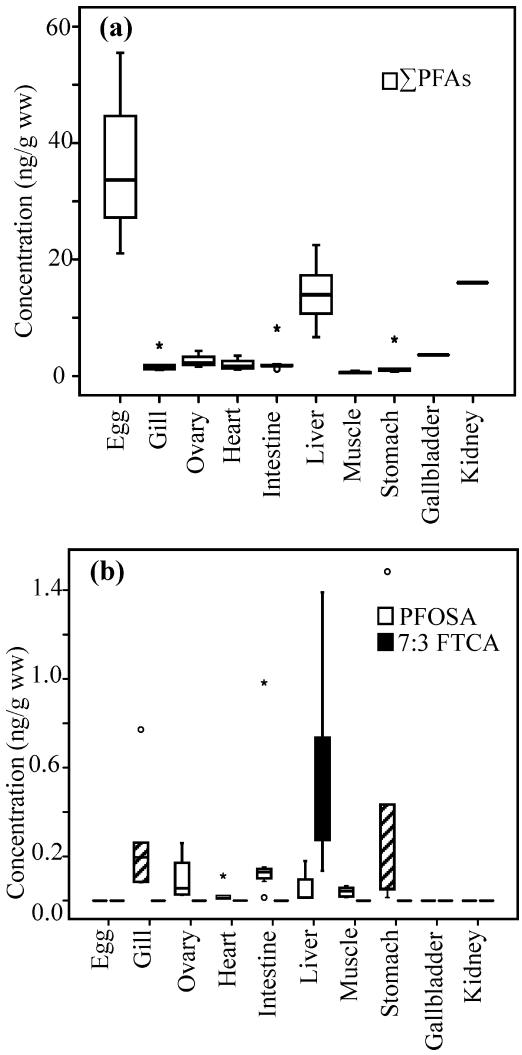

FIGURE 1. Tissue distribution of ΣPFAs, PFOSA, and 7:3 FTCA in Chinese sturgeon. The straight horizontal line represents the median concentration. The 25th and 75th percentiles define the boxes and the whiskers represent the 10th and 90th percentiles. The circular symbol represents an outlier (defined as observation >1.5 times the interquartile range from the edge of the box) and the asterisk represents an extreme (defined as observation >3 times the interquartile range from the edge of the box). The hatching boxes represent the absorbed organs.

8:2 FTOH (*25*). Alternatively, 7:3 FTCA could be absorbed from water or diet and then accumulated in liver or rapidly metabolized to PFCAs. These distribution characteristics of polyfluorinated amides and FTCAs (and FTUCAs) in Chinese sturgeon indicate a potential contribution of precursors to observed concentrations of PFAs residues in wildlife.

Accumulation of PFAs in Eggs. Concentrations of longer-chain PFCAs and PFOS detected in eggs were several times greater than those in the liver. ELRs were calculated based on individuals, and relatively large ELRs of 1.9 for PFOS and 0.79 (PFOA) to 5.5 (PFTriDA) were calculated for PFCAs. Overall, ELRs were greater than those in guillemot, which ranged from 0.39 for PFNA to 2.69 for PFOS (*16*). They were also greater than the ratios for triphenyltin (TPT, 0.34), hexachlorobenzene (HCB, 0.61), and total 1,1,1-trichloro-2,2-bis-(4′-chlorophenyl) ethanes (DDTs, 0.27) in Chinese sturgeon (*20, 33*). A significant positive correlation (slope = 0.64, $r^2 = 0.53$, $p < 0.001$) between ELRs of PFCAs and chain length was observed (Figure 3a). PFOS exhibited an even larger ELR than did the PFCAs with similar carbon chain-length. The mechanism for the preferential transfer of PFCs

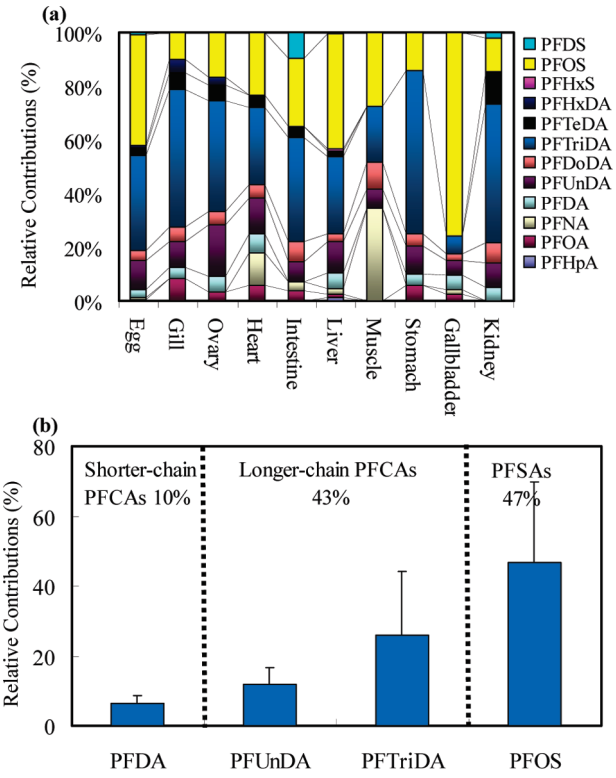

FIGURE 2. Relative contribution (percent) of different PFAs to total PFAs in (a) different tissues of Chinese sturgeon; (b) livers of Chinese sturgeon (only PFCs with >5% relative contributions are presented). The error term indicates standard deviation.

to the egg of Chinese sturgeon is unknown. However, in birds, PFOS has been suggested to be accumulated in the liver, and then transferred to egg as a protein−PFOS complex (*16*). Based on such a potential mechanism, the correlation between the protein−water partition coefficients (log K_{pw}) of PFAs (data from ref *17*) was investigated. Statistically significant, positive correlations were observed between ELRs and log K_{pw} (slope = 1.22, $r^2 = 0.69$, $p < 0.001$) (Figure 3b). This result suggests that the relatively large ELRs of some PFAs could be due to transport of protein-bound PFCAs, rather than a simple equilibrium among lipid pools as is observed for organo-chlorine compounds such as PCBs.

Preliminary Reproductive Risk Assessment. A preliminary estimation of potential effects of PFCs on reproduction of Chinese sturgeon was conducted by calculation of hazard quotients (HQs). Because Chinese sturgeon eggs contained several PFCs of different chain length and there is little information on the toxicity of these compounds, especially in fish eggs, some estimation of their relative toxic potencies (RPs) had to be made. As an initial estimate of the overall mixture toxicity, RP values were developed to normalize concentrations of each PFC to an equivalent concentration of PFOS. The total concentrations of PFOS equivalents (PFOS-EQs) were calculated as the sum of the products of the RPs and the associated concentration of each detected PFC. RP values were calculated using cytotoxicity data for PFAs as determined by in vitro assays compiled from the literature (*46, 47*). It is understood that there may be different RP values based on different end points and that the in vitro responses might not relate directly to in vivo responses, but based on previous studies, there is information to suggest that this is a reasonable initial estimation of toxic potency (*48, 40*). Thus, in this study, the RPs of PFOA, PFNA, PFDA, PFDoDA, PFTeDA, PFHxDA, and PFOS were obtained by normalizing the PFAs EC_{50} concentrations of cytotoxicity to PFOS EC_{50} (EC_{50PFOS}/EC_{50PFA}) (details are given in Supporting Informa-

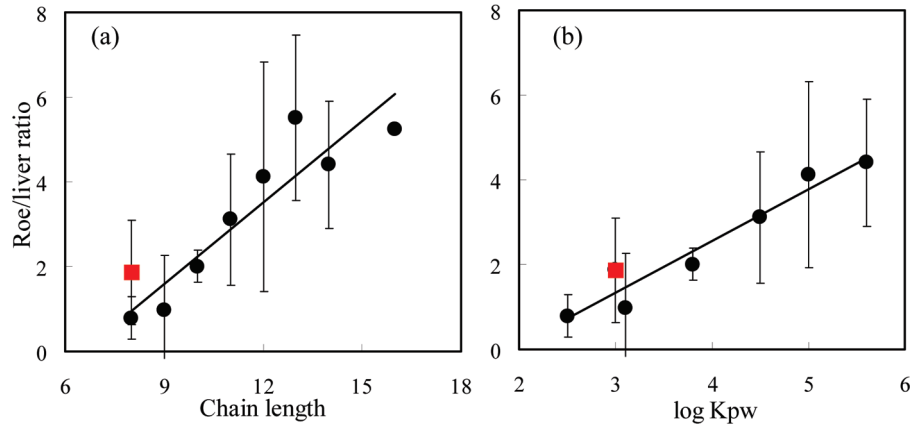

FIGURE 3. Correlations between egg to liver ratios (ELRs) of PFCAs, PFOS, and carbon chain length (a) and logarithm of the protein−water partition coefficients ($log_{10}K_{pw}$) (b). ■PFOS; ●PFCAs; PFHxDA was detected in only one liver sample, the standard deviation was not calculated); Ratio = 0.6398 × chain length − 4.1634, $r^2 = 0.53$, $p < 0.001$ (PFOS was excluded). Ratio = 1.22 × log K_{pw} − 2.34, $r^2 = 0.69$, $p < 0.001$. $log_{10}K_{pw}$ values were available from ref *17*. The error term indicates standard deviation.

TABLE 2. Correlations Among Fecundity, Fertilization, and Survival, and Log_{10}-Transformed Concentrations of PFAs and PFOS-EQs in Egg

	fecundity	fertilization	survival
ΣPFCAs	slope = 553, $r^2 = 0.03$, $p = 0.69$	slope = −29, $r^2 = 0.01$, $p = 0.82$	slope = −176, $r^2 = 0.32$, $p = 0.18$
PFOS	slope = 184, $r^2 = 0.04$, $p = 0.67$	slope = −6, $r^2 = 0.01$, $p = 0.88$	slope = 41, $r^2 = 0.18$, $p = 0.34$
PFOS-EQ	slope = 927, $r^2 = 0.07$, $p = 0.56$	slope = −37, $r^2 = 0.01$, $p = 0.81$	slope = −143, $r^2 = 0.16$, $p = 0.38$

tion). Such calculated RPs for PFAs to cause in vitro cytotoxicity were comparable to those derived from other end points, such as acyl-CoA oxidase activities and inhibition potentials of cellular communication (*8, 49*), and inhibition potentials of cellular communication have been applied to estimate ecological risk assessment of birds (*40*).

Relative contributions of different PFAs to the concentration of ΣPFOS-EQ in egg were investigated (Figure S3, Supporting Information). The longer-chain C_{13} PFCA accounted for the greatest proportion of ΣPFOS-EQ in egg. As an assessment of the sensitivity of ΣPFOS-EQ to variations in RP values based on cytotoxicity were calculated, PFTriDA and PFOS contributed to the greatest proportion of ΣPFOS-EQ. To reduce uncertainty in these sorts of assessments, additional information on the reproductive toxicity of longer-chained PFCAs and PFOS to fish is needed.

To estimate the risk associated with the protection of the 90th percentile of Chinese sturgeon, concentrations of ΣPFOS-EQ in eggs were log-transformed to more closely approximate the normal probability distribution, and then the 90th percentile ΣPFOS-EQ concentration (2.14×10^2 ng/g in eggs) was determined. The 90th percentile concentration was then divided by the benchmark dose, expressed as the toxicological reference value (TRV_{NOAEL}) (eq 1)

$$HQ = \text{90th percentile PFOS-EQ concentration} / TRV_{NOAEL} \quad (1)$$

Previous studies on trans-generational toxicities of PFCs have reported the NOAEL values using the curve between the survival decrease of F1 generation and the dose of PFOS (*50, 51*). Since the Chinese sturgeon is an endangered species, the most sensitive species for which toxicity information was available was selected for the preliminary risk assessment. The toxicity reference value (TRV_{NOAEL}) was estimated to be 1.1 μg PFOS/g ww egg based on the $NOAEL_{suvival}$ of 10 μg PFOS/L for F1 generation in zebrafish (*51*) and a bioconcentration factor (BCF_{egg}) of 115 for accumulation from water to egg of fathead minnows (*50*).

Since the HQ of 0.20 was less than 1.0, reproductive impairment would not be expected to be caused by current PFC concentrations in Chinese sturgeon eggs from the Yangtze River. However, the risk assessment for Chinese sturgeon should be interpreted with caution since no sturgeon-specific information on toxicity of any PFCs to eggs is available, uncertainty factors (UFs) should be eliminated in the further study.

To further evaluate the suspected reproductive toxicity posed by PFAs in eggs, associations among ΣPFCAs, PFOS, ΣPFOS-EQ and reproductive end points including fecundity, fertilization, and survival were investigated by correlation analyses (Table 2). Although no statistically significant associations were found, weak negative correlations between ΣPFCAs or PFOS-EQ and reproductive end points especially for survival were observed, suggesting potential effects of PFCs on the reproduction of Chinese sturgeon. However, more detailed toxicity information on reproduction of Chinese sturgeon, especially for longer-chain PFCAs, is required to reduce the uncertainty in the risk assessment.

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Supporting Information Available

Text, figures, and tables addressing (1) details of Chinese sturgeon samples; (2) 23 chemicals used in the analysis; (3) extraction methods and instrumental conditions; (4) data analysis and RPs calculations; (5) recoveries and MDLs of

PFCs in different tissue matrices; (6) chromatograms of detected PFCs; (7) correlations between concentrations of longer-chain PFCAs and PFOS in eggs and age; (8) relative contributions to PFOS-EQ. This information is available free of charge via the Internet at http://pubs.acs.org.

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