SUPPORT INFORMATION

for

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

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This supporting information provides detailed descriptions of sample collection, artificial fertilization, quantification of PFCs, quality assurance/quality control, correlation analysis between reproductive endpoints and PFAs, chromatograms of detected PFCs, and sensitivity analysis of different PFAs. Figures, and tables addressing: (Table S1) details of Chinese sturgeon samples; (Table S2) reproductive parameters of Chinese sturgeon; (Table S3) multiple reaction monitoring (MRM) of target PFCs; (Table S4) method detection limits (MDLs) and recoveries; (Figure S1) chromatograms of detected PFCs; (Figure S2) Correlations between concentrations (ng/g ww) of longer-chain PFCAs and PFOS in eggs and age; (Figure S3) relative contributions to PFOS-EQ.

Chemicals and Reagents. Standards of the 23 target compounds (detailed information is provided in Supporting Information) and three stable isotope labeled standards including 1,2,3,4-$^{13}C_4$-perfluorooctanoic acid (1,2,3,4-$^{13}C_4$-PFOA), 1,2,3,4-$^{13}C_4$-perfluorononanoic acid (1,2,3,4-$^{13}C_4$-PFNA), and 1,2,3,4-$^{13}C_4$-perfluorooctane sulfonate (1,2,3,4-$^{13}C_4$-PFOS) were purchased from Wellington Laboratories Inc. (Guelph, Ontario, Canada). All solvents, including methanol and methyl *tert*-butyl ether (MTBE), were all of HPLC grade and were purchased from Fisher Chemicals (New Jersey, USA). Water was obtained from purification of distilled water by a Milli-Q Synthesis water purification system (Millipore, Bedford, MA, USA).

Quantification of PFCs and Quality Assurance/Quality Control. In brief, approximately 0.2-0.5 g of homogenized tissue was transferred to a 15 ml polypropylene (PP) centrifuge tube. Fifty microliters (50 µl) of 20 µg/l mass-labeled internal standard 1,2,3,4-$^{13}C_4$-PFOA, 1,2,3,4-$^{13}C_4$-PFNA, and 1,2,3,4-$^{13}C_4$-PFOS, 1 ml of 0.5 M tetrabutylammonium hydrogen sulfate solution (TBAS), and 2 ml of 0.25 M sodium carbonate buffer were added for extraction. After mixing, 5 ml MTBE was added and the mixture was shaken for 20 minutes at 300 rpm and then sonicated for 10 minutes. The organic layer was separated by centrifugation at 3600 rpm for 15 min and then transferred to a second 15 ml PP tube. Extraction was repeated twice and all three extracts were combined. The final extract was blown to dryness under a gentle blow of nitrogen, and then reconstituted with 300 µl of methanol and filtered through a 0.2 µm nylon mesh filter for analysis.

Aliquots of extracts were analyzed using a Waters ACQUITY UPLC™ system (Waters, Milford, MA, USA) with a Waters Micromass Quattro Premier XE (triple-quadrapole) detector operated in electrospray negative mode (ESI^- mode). Separation of PFCs was achieved with a Waters ACUITY UPLC BEH C18 column (1.7 μm; 2.1 mm × 100 mm), preceded by a Waters ACUITY UPLC BEH C18 guard column (1.7 μm; 2.1 mm × 50 mm). The guard column displaced the peaks caused by contamination from the HPLC such that they did not interfere with the analytes in the samples. The injection volume was 5 μl. Methanol (A) and 5 mM ammonium acetate (B) were used as the mobile phases. Initially 10% A was increased to 65% in 6 min, then increased to 75% at 7 min, a further 75% methanol was increased to 100% over 4 min and kept for 2 min, followed by a decrease to initial conditions of 10% A and held for 3 min to allow for equilibration. The flow rate was 0.2 mL/min. The column and sample room temperatures were maintained at 40°C and 10°C, respectively. Data were acquired under multiple reactions monitoring (MRM) mode and the optimized parameters were described as follows: source temperature, 110°C; desolvation temperature, 350°C; capillary voltage, 2.50 kV; desolvation gas flow, 800 L/h; cone gas flow, 50 L/h; multiplier, 650 V (Table S3).

Since minor contamination of PFHxA was found during some batches, procedure blank experiments were performed along with each batch of samples. Standard injections were done among two or three sample injections, and methanol injections were done after each standard injection to monitor background contamination. As for PFHxA with detectable blank contamination, the method detection limits (MDLs) were defined to be three times the procedure blanks, which ranged from 0.11 ng/g in the intestine to 0.36 ng/g in the egg. MDLs of other PFCs were defined for each tissue matrix as three times the noise, and ranged from 0.02 ng/g for PFOSA to 1.8 ng/g for 6:2 FTCA (Table S4). The compound-specific matrix spiking recoveries were determined for each organ by duplicates, and the values ranged from 60% for 7:3 FTCA in the egg to 134% for FOSAA in the muscle. Quantification was adjusted for recoveries by use of internal standards. Concentrations of C_6-C_8 PFCAs were corrected by $^{13}C_4$-PFOA, C_9-C_{14} (and C_{16}) PFCAs by $^{13}C_4$-PFNA, 6:2 FTUCA and 10:2 FTUCA by $^{13}C_2$-6:2 FTUCA, 6:2 FTCA, 7:3 FTCA, 10:2 FTCA by $^{13}C_2$-6:2 FTCA, PFSAs and polyfluorinated amides by $^{13}C_4$-PFOS, respectively. Average recoveries for $^{13}C_4$-PFOA, $^{13}C_4$-PFNA, and $^{13}C_4$-PFOS ranged from 69 ± 14% in the liver to 87 ± 14% in the intestine, from 73 ± 16% in the egg to 98 ± 13% in the intestine, and from 77 ± 13% in the muscle to 90 ± 19% in the intestine, respectively. Average recoveries of $^{13}C_2$-6:2 FTUCA and $^{13}C_2$-6:2 FTCA in liver were 80 ± 6% and 81 ± 8%, respectively. Concentrations of target analytes were determined based on calibration curves that were generated using concentration series of 0, 20, 40, 80, 160, 320, 640, 1200, and 2400 pg/ml, which showed strong linearity (correlation coefficients > 0.99).

Data Analysis. A one-way analysis of variance (ANOVA) was used to investigate the differences in concentrations of PFCs among tissues, and the Levene's test was used to check equality of variances. Concentrations less than their respective method detection limits (MDLs) were assigned a proxy value of MDL/2. Normal distributions of concentrations of PFCs was determined by use of the Kolmogorov-Smirnov test. A log-transformation was done to ensure the normality of the data distribution. Linear regression was performed to evaluate relationships between concentrations of PFCs and age, the ratios of concentrations in the egg to those in the liver (ELRs), chain length, and protein-water partition coefficients (log K_{pw}). All data analyses were performed with SPSS 15.0.

Relative Toxic Potencies (RPs) Calculations for Preliminary Risk Assessment. 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}). The RPs of C_7 PFCA, C_8 PFCA, and C_9 PFCA showed similar values which were 0.80, 1.00, and 1.17, receptively. Greater RPs for C_{10} PFCA (6.55), C_{12} PFCA (6.68), and C_{14} PFCA (7.64) compared to shorter carbon chain length were observed, showing similar RP values, but that of C_{16} PFCA was relatively low (2.88). Such chain length-related toxicity has been suggested to be the primary determinant of some types of toxicity of PFCs (*7, 46*), while it is beyond the scope of this paper to discuss these relationships in detail. Since no QSAR data for cytotoxicty of PFUnDA or PFTriDA were available, based on the similarity of RPs for PFCA with chain length from 10 to 14, the mean (6.96) of C_{10} PFCA, C_{12} PFCA, and C_{14} PFCA was used as those of PFUnDA and PFTriDA. RPs for PFOSA, FTCA and FTUCAs, polyfluorinated amides, PFHpS and PFDS were ignored due to their low concentrations. Concentrations of PFOS-EQ were calculated as the sum of the product of the concentration of each PFC in egg multiplied by the respective RP, which ranged from 90.6 PFOS ng/g to 262 PFOS ng/g. These values were preferred to those derived from other endpoints because the endpoint in the *in vitro* assays was cell lethality and the value to be predicted in Chinese sturgeon was also lethality.

SUPPORTING INFORMATION TABLE S1. Details of Chinese Sturgeon Samples.

Sample code	Sex	Date of collection	Age (year)	Body weight (kg)	Body length (cm)	Tissue collected
A0466	F	2003	24	254	285	L, St, I, Gi, K
A0406	F	2004	18	174	245	E, M, H, Ov, St
A0410	F	2004	17	140	246	E, L, M, H, Ov, St, I, Gb
A0412	F	2004	24	230	287	E, L, M, H, St, I, Gi
A0414	F	2004	25	263	285	E, L, I, Gi
A0408	F	2004	22	230	258	E
A0447	F	2005	19	192	247	E, L, M, H, Ov, I, Gi
A0445	F	2005	18	187	237	L, M, H, Ov, I, Gi
A0403	F	2005	24	260	280	E
A0444	F	2005	23	224	270	E
A0452	F	2005	23	207	282	E
A0449	F	2005	22	252	275	E
A0500	F	2005	22	227	261	E
A0439	F	2006	21	223	262	E, L, M, H, St, I, Gi
A0440	F	2006	17	176	250	E
A0441	F	2006	24	240	300	E

E: egg; L: liver; M: muscle; H: heart; Ov: ovary[a]; St: stomach; I: intestine; Gi: gill; K: kidney; Gb: gallbladder. a. ovary where the eggs has been fully released.

SUPPORTING INFORMATION TABLE S2. PFOS-EQ, Reproductive Parameters (Fecundity[a], Fertilization[b], and Survival[c]) in 7 Individuals.

	Fecundity (kg^{-1})	Fertilization (%)	Survival (%)	PFOS-EQ (ng/g, ww)
A0444	1342	68.9	81.3	181.8
A0441	792	55.0	74.6	132.3
A0452	1266	76.3	89.0	147.5
A0403	1246	61.3	75.0	132.8
A0447	1041	19.6	34.0	169.2
A0449	940	58.3	71.9	170.3
A0500	1048	46.9	100	139.2

a. egg numbers per weight; b. percentage of fertilized eggs in the total eggs; c. percentage of the 5-day survival larval in the fertilized eggs

SUPPORTING INFORMATION TABLE S3. Multiple Reaction Monitoring (MRM) Transitions of Poly- and Per-fluorinated Compounds (PFCs)

Compound	Acronym	Parent Ion	Daughter Ion	Cone Voltage	Collision Energy
Perfluorohexanoate	PFHxA	313	269	14	22
Perfluoroheptanoate	PFHpA	363	319	17	9
Perfluorooctanoate	PFOA	413	369	15	10
Perfluorononanoate	PFNA	463	419	18	9
Perfluorodecanoate	PFDA	513	469	20	12
Perfluoroundecanoate	PFUnDA	563	519	20	13
Perfluorododecanoate	PFDoDA	613	569	23	11
Perfluorotridecanoate	PFTriDA	663	619	23	12
Perfluorotetradecanoate	PFTeDA	713	669	19	15
Perfluorohexadecanoate	PFHxDA	813	769	23	13
Perfluorohexane sulfonate	PFHxS	399	80	52	40
Perfluoroheptanesulfonate	PFHpS	449	80	50	40
Perfluorooctane sulfonate	PFOS	499	80	62	37
Perfluorodecane sulfonate	PFDS	599	80	75	45
7:3 fluorotelomer saturated carboxylate	7:3 FTCA	441	337	21	15
6:2 fluorotelomer saturated carboxylate	6:2 FTCA	377	63	12	8
6:2 fluorotelomer unsaturated carboxylate	6:2 FTUCA	357	293	16	16
10:2 fluorotelomer saturated carboxylate	10:2 FTCA	577	493	22	12
10:2 fluorotelomer unsaturated carboxylate	10:2 FTUCA	557	493	22	20
2-(perfluorooctane sulfonamido) acetic acid	FOSAA	556	498	45	24
2-(N-methylperfluorooctane sulfonamide) acetic acid	N-MeFOSAA	570	419	36	22
2-(N-ethylperfluorooctane sulfonamido) acetic acid	N-EtFOSAA	584	419	32	22
perfluorooctane sulfonamide	PFOSA	498	78	42	34

SUPPORTING INFORMATION TABLE S4. Method Detection Limits (MDLs) (ng/g ww) and Recoveries (n=2) of PFCs in Chinese Sturgeon.

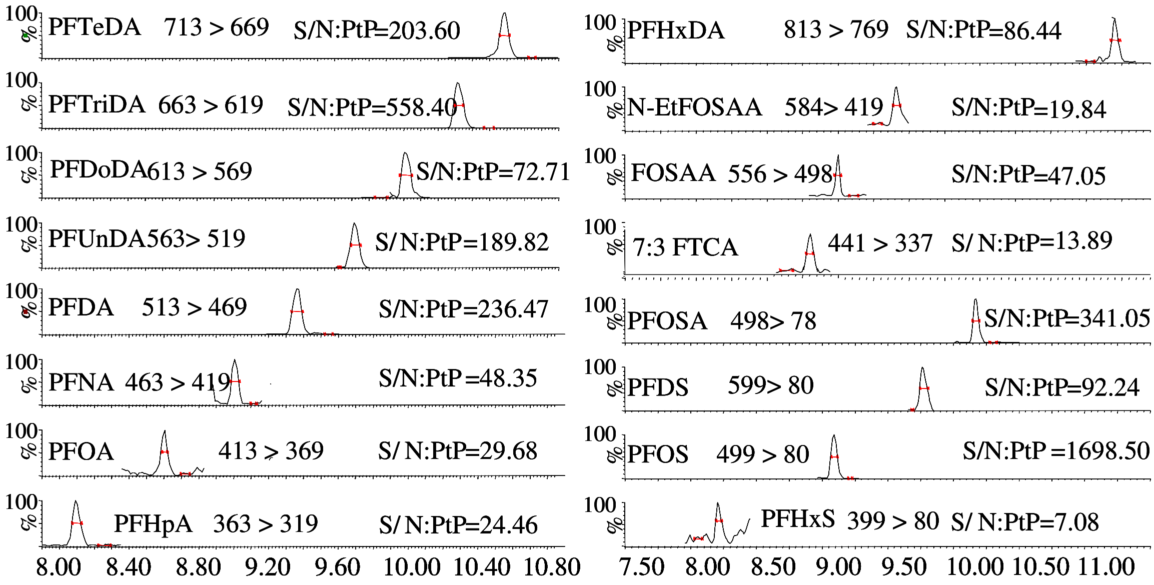
	Egg		Liver		Muscle		Ovary	
	MDLs	Recovery	MDLs	Recovery	MDLs	Recovery	MDLs	Recovery
PFHxA	0.36	73±5%	1.02	71±9%	0.32	112±10%	0.28	96±3%
PFHpA	0.08	91±4%	0.10	122±1%	0.08	108±28%	0.10	90±6%
PFOA	0.11	66±1%	0.13	88±1%	0.07	78±5%	0.05	73±5%
PFNA	0.18	79±4%	0.09	107±4%	0.05	120±5%	0.10	101±5%
PFDA	0.18	74±4%	0.15	89±15%	0.05	112±29%	0.05	101±1%
PFUnDA	0.10	87±7%	0.07	102±7%	0.02	110±22%	0.05	88±4%
PFDoDA	0.10	95±16%	0.08	102±31%	0.04	112±9%	0.05	111±7%
PFTriDA	0.10	81±2%	0.11	106±15%	0.05	96±10%	0.05	112±19%
PFTeDA	0.07	107±16%	0.12	118±2%	0.06	102±26%	0.08	71±4%
PFHxDA	0.10	89±25%	0.07	100±14%	0.06	74±9%	0.06	67±1%
PFHxS	0.05	74±3%	0.13	99±9%	0.05	83±11%	0.05	80±2%
PFHpS	0.09	81±5%	0.09	84±10%	0.05	61±5%	0.06	70±4%
PFOS	0.18	102±6%	0.33	89±7%	0.05	85±14%	0.06	92±15%
PFDS	0.07	92±2%	0.04	93±2%	0.05	74±15%	0.05	67±2%
7:3 FTCA	0.08	72±3%	0.10	72±3%	0.06	100±28%	0.07	68±5%
6:2 FTCA	1.8	60±10%	1.4	77±9%	1.1	83±8%	1.0	88±5%
6:2 FTUCA	0.35	107±2%	0.35	80±10%	0.20	75±15%	0.20	78±7%
10:2 FTCA	0.65	100±6%	0.57	83±5%	0.50	80±10%	0.53	98±8%
10:2 FTUCA	0.05	86±2%	0.10	82±7%	0.13	90±5%	0.22	91±7%
FOSAA	0.07	61±5%	0.06	85±10%	0.05	134±24%	0.05	71±2%
N-MeFOSAA	0.07	64±8%	0.13	134±13%	0.06	95±21%	0.08	77±5%
N-EtFOSAA	0.06	85±7%	0.10	110±6%	0.08	99±27%	0.05	81±3%
PFOSA	0.06	60±1%	0.05	82±1%	0.02	89±3%	0.07	79±1%
$^{13}C_4$-PFOA	/	69±14%	/	81±23%	/	77±9%	/	74±5%
$^{13}C_4$-PFNA	/	73±16%	/	91±14%	/	84±18%	/	97±10%
$^{13}C_4$-PFOS	/	88±11%	/	88±18%	/	77±13%	/	79±4%
$^{13}C_2$-6:2 FTCA	/	/	/	81±8%	/	/	/	/
$^{13}C_2$-6:2 FTUCA	/	/	/	80±6%	/	/	/	/

	Kidney		Gallbladder		Heart		Intestine	
	MDLs	Recovery	MDLs	Recovery	MDLs	Recovery	MDLs	Recovery
PFHxA	0.12	85±19%	0.19	81±26%	0.21	96±22%	0.11	96±6%
PFHpA	0.12	100±4%	0.08	91±11%	0.08	86±7%	0.07	103±6%
PFOA	0.11	73±8%	0.06	76±7%	0.04	107±5%	0.05	71±3%
PFNA	0.14	116±24%	0.04	124±18%	0.05	107±1%	0.05	108±4%
PFDA	0.11	104±14%	0.06	120±13%	0.05	108±10%	0.10	115±13%
PFUnDA	0.08	90±9%	0.05	90±11%	0.05	99±7%	0.05	99±8%
PFDoDA	0.22	96±17%	0.05	96±6%	0.06	117±14%	0.09	125±5%
PFTriDA	0.13	114±3%	0.05	112±1%	0.07	115±6%	0.05	100±6%
PFTeDA	0.24	80±3%	0.07	108±11%	0.08	115±15%	0.05	89±3%

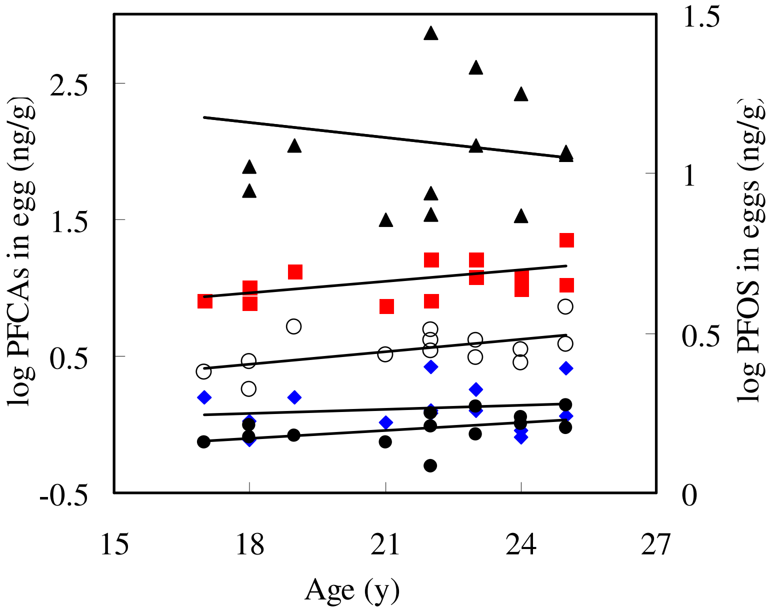
PFHxDA	0.26	73±14%	0.07	87±11%	0.15	114±21%	0.15	68±2%
PFHxS	0.10	80±2%	0.05	81±26%	0.07	78±2%	0.04	81±4%
PFHpS	0.1	74±4%	0.05	85±7%	0.10	92±4%	0.05	68±1%
PFOS	0.17	81±1%	0.05	90±8%	0.05	85±3%	0.11	81±6%
PFDS	0.08	70±8%	0.09	79±11%	0.08	75±7%	0.07	72±9%
7:3 FTCA	0.07	78±16%	0.06	80±8%	0.09	66±2%	0.07	77±1%
6:2 FTCA	1.0	76±8%	1.2	81±5%	0.95	90±10%	0.90	78±2%
6:2 FTUCA	0.23	82±6%	0.18	87±8%	0.13	88±7%	0.15	70±9%
10:2 FTCA	0.56	87±11%	0.68	77±5%	0.46	103±12%	0.55	81±2%
10:2 FTUCA	0.20	83±13%	0.10	89±10%	0.12	85±3%	0.07	86±10%
FOSAA	0.11	90±16%	0.04	63±13%	0.03	86±7%	0.03	80±1%
N-MeFOSAA	0.09	82±20%	0.04	84±18%	0.03	124±11%	0.06	112±1%
N-EtFOSAA	0.09	71±19%	0.08	61±4%	0.04	92±7%	0.08	117±1%
PFOSA	0.06	67±14%	0.02	67±9%	0.02	65±1%	0.02	74±1%
$^{13}C_4$-PFOA	/	71%	/	70%	/	82±15%	/	87±14%
$^{13}C_4$-PFNA	/	82%	/	81%	/	78±13%	/	98±13%
$^{13}C_4$-PFOS	/	79%	/	79%	/	83±7%	/	90±19%
$^{13}C_2$-6:2 FTCA	/	/	/	/	/	/	/	/
$^{13}C_2$-6:2 FTUCA	/	/	/	/	/	/	/	/

	Gill		Stomach	
	MDLs	Recovery	MDLs	Recovery
PFHxA	0.31	124±16%	0.25	102±10%
PFHpA	0.10	107±11%	0.07	85±11%
PFOA	0.08	107±2%	0.06	83±21%
PFNA	0.15	70±3%	0.12	103±1%
PFDA	0.06	117±23%	0.07	108±9%
PFUnDA	0.08	105±6%	0.04	81±9%
PFDoDA	0.08	109±4%	0.05	103±28%
PFTriDA	0.06	119±8%	0.06	79±16%
PFTeDA	0.09	100±1%	0.08	102±4%
PFHxDA	0.09	114±3%	0.10	81±10%
PFHxS	0.07	103±9%	0.05	82±10%
PFHpS	0.05	77±1%	0.05	62±1%
PFOS	0.09	78±8%	0.06	99±10%
PFDS	0.10	77±1%	0.09	70±1%
7:3 FTCA	0.07	68±0.3%	0.09	74±9%
6:2 FTCA	0.8	76±7%	1.0	82±5%
6:2 FTUCA	0.15	83±7%	0.15	73±4%
10:2 FTCA	0.59	78±8%	0.64	82±9%
10:2 FTUCA	0.27	77±5%	0.09	77±12%
FOSAA	0.03	81±4%	0.04	82±16%
N-MeFOSAA	0.06	119±10	0.04	89±1%
N-EtFOSAA	0.05	105±6%	0.04	64±11%

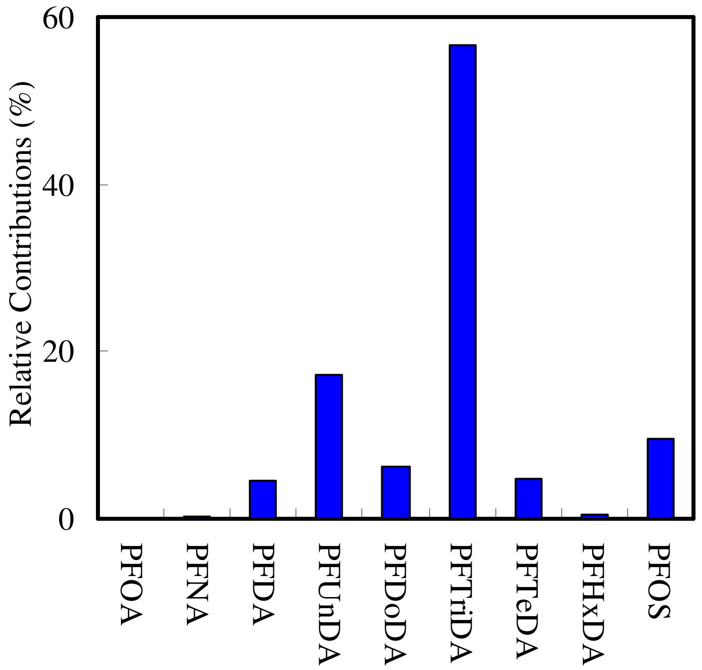
PFOSA	0.04	75±4%	0.02	64±11%
$^{13}C_4$-PFOA	/	80±16%	/	84±12%
$^{13}C_4$-PFNA	/	88±12%	/	83±15%
$^{13}C_4$-PFOS	/	89±8%	/	84±3%
$^{13}C_2$-6:2 FTCA	/	/	/	/
$^{13}C_2$-6:2 FTUCA	/	/	/	/



SUPPORTING INFORMATION FIGURE S1. Typical UPLC/MS/MS Chromatograms of Detected PFCs in Chinese Sturgeon.



SUPPORTING INFORMATION FIGURE S2. Correlations between concentrations (ng/g ww) of Longer-chain PFCAs and PFOS in eggs and age: $\log_{10}C_{PFUnDA}$=0.03×age-0.10, r^2=0.29, p=0.049, $\log_{10}C_{PFDoDA}$=0.01×age-0.09, r^2=0.02, p=0.605, $\log_{10}C_{PFTriDA}$=0.03×age+0.44, r^2=0.25, p=0.066, $\log_{10}C_{PFTeDA}$=0.02×age-0.44, r^2=0.19, p=0.124, $\log_{10}C_{PFOS}$=-0.02×age+1.44, r^2=0.03, p=0.528.



SUPPORTING INFORMATION FIGURE S3. Relative Contribution of Each PFC to PFOS-EQ in Eggs.