SUPPORTING INFORMATION:

Perfluorinated Alkyl Acids in Hawaiian Cetaceans and Potential Biomarkers of Effect: Peroxisome Proliferator-Activated Receptor Alpha and Cytochrome P450 4A

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ADDITIONAL MATERIALS AND METHODS

Quantification of PFAAs. A linear regression of the calibration curve (without forcing the intercept through zero) was used to calculate the concentrations of PFAAs in the samples and SRMs. Each PFAA was quantified utilizing a relative response ratio to

the internal standard most similar in structure. The limits of detection (LOD) were calculated as the maximum value of either 1.) the average mass of each PFAA (ng) measured in the sample plus 3 standard deviations of the blanks or 2.) the lowest calibrant detectable, all divided by the mass (g) of the sample. All concentrations include both linear and branched isomers.

The calibration curves for the PFAA calibrants had $R2 \geq 0.99$. In addition, PFOS concentrations measured in SRM 1947 samples demonstrated reproducible results when compared to values listed in the CoA. Both SRM replicates, identified as SRM 1947-1 and SRM 1947-2, were prepared in conjunction with liver samples and the SRM replicates SRM 1947-3 and SRM1947-4 were prepared in conjunction with kidney samples. PFOS values determined in the SRM 1947 replicates were similar to the values provided on the CoA (Table S2).

mRNA Quantification. Reverse transcription was performed by heating 11 μL of the purified RNA and 3 μL of random primers at 70ºC for 5 minutes. M-MLV Reverse Transcriptase and dNTPs were added to the sample and the solution incubated for 65 minutes at 37ºC. Random primers, reverse transcriptase, and dNTPs were purchased from Promega®.

A consensus sequence of four mammal species for each gene was created to design primers for a segment of the cetacean gene. Complete coding DNA sequences for PPARα and CYP4A from GenBank were aligned using MacVector to create a consensus sequence for each gene [PPARα: human (accession # L02932), cow (accession # BT020756, mouse (accession # AK035676), rat (accession # M88592); CYP4A: dog (accession # DQ138950), human (accession # L04751), pig (accession # AF384031), rat

40 (accession # M33936)]. Primer pairs were designed in conserved regions of these
41 sequences for amplicons of ~702 (PPARα) and ~423 (CYP4A) base pairs. The product of
42 the cetacean primers (i.e. a segment of the cetacean gene sequence) was used to design
43 qPCR primers for the samples (Table S3). Primer pairs for qPCR were developed from
44 these cetacean sequences for amplicons of 194 (PPARα) and 190 (CYP4A) base pairs.
45 Primer pairs were validated with cetacean samples using traditional PCR and
46 sequencing of the PCR product. The PCR cocktail consisted of 0.5 µL cDNA, 0.25 µL of
47 both forward and reverse primers, 11.5 µL of nuclease-free water, and 12.5 µL of
48 GoTaq® Hot Start Green Master Mix from Promega®. The PCR machine used was the
49 Mastercycler® pro by Eppendorf. The reaction procedure was an initial hold of 95ºC for 3
50 minutes, a three-step cycle repeated 40 times of 95ºC for 15 seconds, a temperature
51 gradient ranging from 52ºC to 60ºC for 30 seconds, and 72ºC for 30 seconds. This was
52 followed by an extended annealing step after the last cycle of 72ºC for 1 minute. The
53 product was loaded into a gel (1% agarose and 0.5µg/ml ethidium bromide in 2.5% 40X
54 TAE buffer) and separated using gel electrophoresis at ~100 V for ~1 hour. The gel was
55 viewed under ultraviolet light and appropriate bands were removed. The cDNA in the
56 bands were purified using the Wizard® SV Gel and PCR Clean-Up System from
57 Promega® and 5 µL were combined with 1 µL of each forward and reverse primer in
58 separate tubes. These samples were sequenced and the output was uploaded into
59 MacVector with Assembler and validated by known sequences through a BLAST search
60 in GenBank.
61 Real-time PCR (qPCR) was performed on the Eppendorf Mastercycler® ep
62 *realplex*. The qPCR cocktail consisted 1 µL of cDNA, 0.25 µL of forward and reverse

primers, 3.5 µL of nuclease-free water, and 5 µL GoTaq® qPCR Master Mix from Promega®. The reaction consisted of an initial hold at 95ºC for 3 minutes and a two-step cycle repeated 40 times of 1.) 95ºC for 15 seconds and 2.) 55ºC for 45 seconds. After the last reaction cycle, a melt curve was generated at 95ºC for 15 seconds, 55ºC for 15 seconds, and a ramp back up to 95ºC over 20 minutes. All samples were run in triplicate. Relative concentrations were calculated using the $\Delta\Delta C_q$ method.

$$\Delta C_{q\ (sample)} = C_{q\ (sample)} - C_{q\ (reference\ gene)}$$

$$\Delta\Delta C_{q\ (sample)} = \Delta C_{q\ (sample)} - \Delta C_{q\ (baseline)}$$

Fold change was calculated by the transformation:

$$\text{fold change} = 2^{-\Delta\Delta Cq\ (\text{sample})}$$

Since the $\Delta\Delta C_q$ value is a negative number and represents a negative relationship (i.e. values that are more negative indicate higher mRNA expression), statistical analyses was performed using the $-\Delta\Delta C_q$ to represent a positive relationship.

Protein Quantification. After electrophoresis, the gel was incubated in cold BupH™ Tris-Glycine transfer buffer from Thermo Fisher Scientific with 20% methanol for 15 minutes prior to transfer to a Whatman Westran™ Polyvinylidine Fluoride (PVDF) membrane (Sigma-Aldrich®, St. Louis, MO) pre-wetted in methanol (30 seconds) and cold transfer buffer (5 minutes). The transfer module was appropriately constructed, filled with cold transfer buffer, and placed in the miniVE tank filled with cold water. The module ran at a constant voltage of 60 V for 2 hours.

After the transfer was complete, the membrane was washed in a blocking solution of Tris-buffered saline and 0.1% Tween (TBST) and 5% non-fat milk for 1 hour on an orbital shaker. Blocking, antibody probing, and washing were performed on a SNAP i.d.®

2.0 Protein Detection System from EMD Millipore (Billerica, MA). Antibody probing used dilutions in TBST of 1:750 of CYP4A primary antibody (catalogue # ab1280 rabbit anti-rat cytochrome P450 CYP 4A1/2/3 polyclonal antibody from EMD Millipore), 1:1667 of mouse monoclonal alpha tubulin primary antibody from Novus Biologicals (Littleton, CO), and 1:500 of secondary antibody (stabilized goat anti-rabbit [CYP4A] and goat anti-mouse [alpha tubulin] IgG peroxidase conjugated from Thermo Fisher Scientific). Primary antibody probing included an extended incubation time of 30 minutes on an orbital shaker. Four 30 mL washes of TBST were performed after primary and secondary antibody probes.

Chemiluminescent visualization and band density quantification were performed using WesternSure™ Premium Substrate, a C-DiGit Blot Scanner, and Image Studio software (version 4.0.21) from LI-COR, Inc. (Lincoln, NE). The manufacturer recommendation was utilized for background subtraction (median pixel, border width of three pixels, right/left segments).

Quality Control. Application of molecular biology methods to stranded cetacean tissues often requires a higher than desired tolerance of mRNA and protein degradation. Nonetheless, quality control measurements were applied and adhered to as much as was feasible. For qPCR analysis, melt curves and C_q values were assessed to determine whether the sample was satisfactory for analysis. A satisfactory melt curve had a single peak above the threshold in specific expected temperature ranges (i.e. 80.0ºC – 82.0ºC and 85ºC – 86.5ºC for PPARα, 79.5ºC – 82.0ºC and 86.0ºC – 87.5ºC for CYP4A, 81.0ºC – 83.5ºC for YWHAZ). Consistent melt curves and C_q values with a standard deviation

<2.00 for at least two of the three triplicates must occur for a sample to be considered satisfactory.

Prior to quantifying target genes, candidates for reference genes were thoroughly investigated. Previous studies used multiple software programs to examine reference gene candidates expressed in cetacean skin biopsies.[1,2] The highest ranked reference genes for reliability and consistency included β-Actin (Act-B), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), succinate dehydrogenase complex, subunit A, flavoprotein (SDHA), and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (YWHAZ). Because there were no studies comparing expression of these reference genes in cetacean liver or kidney cells, pilot experiments were performed using cetacean liver and kidney samples from this study.

Expression of the reference genes was quantified in 38 liver and kidney samples from 14 cetaceans using qPCR. Three factors were considered when assessing reference gene candidates. First, expression of the reference gene should be as consistent as possible across tissues that may reflect different degradation levels. Next, correlation tests examining the relationship between mRNA expression and RNA quality evaluated the consistency of the reference gene across samples. To test this, RNA was separated using gel electrophoresis and RNA quality was scored 1 (low quality) through 5 (high quality) on an ordinal scale. RNA from fresh HEK cells was used as a standard for a high quality control. Lastly, melting curve profiles from the qPCR analysis assessed the reliability of the reference gene during the amplification reaction. Consistent melting curves are necessary to ensure the reaction performed appropriately for all of the samples.

The reference gene analysis suggested that YWHAZ, GAPDH, and Act-B were viable candidates for reference genes in stranded cetacean livers and kidneys. SDHA was not considered an appropriate reference gene for these samples. GAPDH had the highest mean mRNA expression (i.e. lowest mean C_q value, $C_q = 22.29$), followed by YWHAZ ($C_q = 23.80$), and Act-B ($C_q = 26.41$). GAPDH also had the most significant correlation ($\alpha = 0.10$) between mRNA expression and RNA quality ($R^2 = 0.179$, $p = 0.018$), followed by YWHAZ ($R^2 = 0.107$, $p = 0.063$), and Act-B ($R^2 = 0.151$, $p = 0.068$). YWHAZ produced the most satisfactory melting curves (33 of 38), followed by GAPDH (31 of 38), and Act-B (23 of 38). Also, YWHAZ had the most consistent melting curve profile, whereas profiles for both GAPDH and Act-B were much more variable. YWHAZ was the most appropriate reference gene candidate for this study because of its high mRNA expression, strong correlation between mRNA expression and quality, and consistent and reliable melting curve profiles. However, it is important to note that reference genes may perform differently under different annealing temperature restrictions set by the target genes.

In western blot analyses, each blot was stripped with ReBlot Plus Strong Stripping Solution 10X (Millipore) and probed for alpha tubulin in order to discern between low expression and substantial protein degradation. There were also three internal standards in each blot (50 µg of positive control, 5 µg of positive control, and 50 µg of NIST SRM QC03LH3 pygmy sperm whale liver homogenate). These standards were used to determine the efficiency of each blot relative to the standard curve blot, and sample concentrations were corrected accordingly.

References

(1) Spinsanti, G., Panti, C., Lazzeri, E., Marsili, L., Casini, S., Frati, F., & Fossi, C. M. Selection of reference genes for quantitative RT-PCR studies in striped dolphin (Stenella coeruleoalba) skin biopsies. *BMC Molecular Biology*. **2006**, *7* (1), 32.
(2) Spinsanti, G., Panti, C., Bucalossi, D., Marsili, L., Casini, S., Frati, F., & Fossi, C. M. Selection of reliable reference genes for qRT-PCR studies on cetacean fibroblast cultures exposed to OCs, PBDEs, and 17β-estradiol. *Aquatic Toxicology*. **2008**, *87* (3), 178-186.

Supporting Information – Tables

Table S1. Animal Information for PFAA Measurement Samples. *Animal ID* denotes storage identification numbers. *Stranding Date* is the date of the animal stranding, the actual necropsy date may be different. *Stranding Day Number* (*Str. Day*) corresponds with *Stranding Date*, with day 1 = 01/01/1997, day 2 = 01/02/1997, etc. Abbreviations are utilized for *Common Name* (*W.* = Whale), *Sex* (*F* = female, *M* = male) and *Age Class* (*A* = adult, *J* = Juvenile, *C* = calf).

Animal ID	Species	Common Name	Family	Stranding Date	Str. Day	Stranding Location	Sex	Age Class
12470-001	*S. coeruleoalba*	Striped Dolphin	Delphinidae	1/2/1997	2	Mokuleia, Oahu	M	A
15028-001	*S. longirostris*	Spinner Dolphin	Delphinidae	8/29/1997	241	Kailua, Oahu	M	C
15063-001	*M. novaeangliae*	Humpback Whale	Balaenopteridae	2/12/1998	408	Manale Harbor, Maui	F	C
15121-001	*T. truncatus*	Bottlenose Dolphin	Delphinidae	8/31/1998	608	Punaluu, Oahu	M	C
15320-001	*K. breviceps*	Pygmy Sperm Whale	Kogiadae	6/3/2000	1250	Na Pali, Kauai	M	J
15377-001	*K. sima*	Dwarf Sperm Whale	Kogiadae	8/31/2000	1339	Kailua, Oahu	M	A
KW2007003	*K. breviceps*	Pygmy Sperm Whale	Kogiadae	4/25/2007	3767	Kihei, Maui	M	A
KW2007004	*S. longirostris*	Spinner Dolphin	Delphinidae	6/30/2007	3833	Punaluu, Oahu	M	J
KW2007005	*S. longirostris*	Spinner Dolphin	Delphinidae	9/30/2007	3925	Waianae, Oahu	F	A
KW2008003	*M. novaeangliae*	Humpback Whale	Balaenopteridae	2/25/2008	4073	Lahaina, Maui	F	C
KW2008004	*S. longirostris*	Spinner Dolphin	Delphinidae	3/15/2008	4092	Lahaina, Maui	M	A
KW2008005	*S. attenuata*	Spotted Dolphin	Delphinidae	5/16/2008	4154	Honolulu, Oahu	M	J
KW2008006	*S. coeruleoalba*	Striped Dolphin	Delphinidae	6/21/2008	4190	Hilo, Hawaii	F	C
KW2008008	*Z. cavirostris*	Cuvier's Beaked W.	Ziphiidae	7/28/2008	4227	Kaunakakai, Molokai	M	J
KW2008009	*S. longirostris*	Spinner Dolphin	Delphinidae	10/14/2008	4305	Kailua-Kona, Hawaii	F	A
KW2008010	*O. orca*	Killer Whale	Delphinidae	10/21/2008	4312	Poipu, Kauai	M	A
KW2008011	*S. longirostris*	Spinner Dolphin	Delphinidae	11/29/2008	4351	Waikoloa, Hawaii	F	A
KW2009002	*M. novaeangliae*	Humpback Whale	Balaenopteridae	2/9/2009	4423	Kekaha, Kauai	F	C
KW2009004	*S. longirostris*	Spinner Dolphin	Delphinidae	3/13/2009	4455	Waianae, Oahu	F	C
KW2009006	*F. attenuata*	Pygmy Killer Whale	Delphinidae	5/22/2009	4525	Maalaea, Maui	M	A
KW2009008	*S. coeruleoalba*	Striped Dolphin	Delphinidae	6/4/2009	4538	Kailua, Oahu	M	J
KW2009009	*S. coeruleoalba*	Striped Dolphin	Delphinidae	6/14/2009	4548	Maalaea, Maui	F	A
KW2009011	*S. coeruleoalba*	Striped Dolphin	Delphinidae	7/5/2009	4569	Kihei, Maui	F	C
KW2009012	*K. sima*	Dwarf Sperm Whale	Kogiadae	8/27/2009	4622	Kilauea, Kauai	M	A
KW2009015	*S. attenuata*	Spotted Dolphin	Delphinidae	11/25/2009	4712	Kailua-Kona, Hawaii	M	A
KW2010005	*I. pacificus*	Longman's Beaked W.	Ziphiidae	3/22/2010	4829	Hana, Maui	M	J
KW2010006	*S. longirostris*	Spinner Dolphin	Delphinidae	4/15/2010	4853	Waimanalo, Oahu	M	J
KW2010007	*T. truncatus*	Bottlenose Dolphin	Delphinidae	4/23/2010	4861	Waikaloa, Hawaii	M	A
KW2010008	*S. coeruleoalba*	Striped Dolphin	Delphinidae	6/14/2010	4913	Milolii, Hawaii	M	A
KW2010011	*S. attenuata*	Spotted Dolphin	Delphinidae	8/6/2010	4966	Haputo, Guam	M	C
KW2010012	*M. densirostris*	Blainville's Beaked W.	Ziphiidae	8/16/2010	4976	Maalaea, Maui	M	J
KW2010019	*P. crassidens*	False Killer Whale	Delphinidae	11/27/2010	5079	Kawela, Molokai	F	A
KW2011001	*T. truncatus*	Bottlenose Dolphin	Delphinidae	2/13/2011	5157	Lydgate Park, Kauai	F	J
KW2011002	*P. electra*	Melon-headed Whale	Delphinidae	3/20/2011	5192	Kailua, Oahu	M	C
KW2011003	*T. truncatus*	Bottlenose Dolphin	Delphinidae	4/13/2011	5216	Waikaloa, Hawaii	M	C
KW2011006	*T. truncatus*	Bottlenose Dolphin	Delphinidae	4/23/2011	5226	Waikaloa, Hawaii	F	C
KW2011007	*T. truncatus*	Bottlenose Dolphin	Delphinidae	5/21/2011	5254	Lydgate Park, Kauai	M	A
KW2011008	*P. macrocephalus*	Sperm Whale	Physeteridae	5/30/2011	5263	Laie, Oahu	F	C
KW2011009	*P. electra*	Melon-headed Whale	Delphinidae	6/20/2011	5284	Kosrae, Micronesia	F	C
KW2011011	*P. electra*	Melon-headed Whale	Delphinidae	7/8/2011	5302	Waiehu, Maui	M	A
KW2011013	*S. longirostris*	Spinner Dolphin	Delphinidae	8/12/2011	5337	Kona, Hawaii	M	C
KW2011016	*Z. cavirostris*	Cuvier's Beaked W.	Ziphiidae	8/23/2011	5348	Saipan	M	A
KW2011018	*S. longirostris*	Spinner Dolphin	Delphinidae	8/30/2011	5355	Nanakuli, Oahu	F	C
KW2011021	*S. bredanensis*	Rough-toothed Dolphin	Delphinidae	9/23/2011	5379	Anaholo, Kauai	M	A
KW2012002	*S. coeruleoalba*	Striped Dolphin	Delphinidae	7/6/2012	5666	Waimanalo, Oahu	F	A
KW2012003	*P. electra*	Melon-headed Whale	Delphinidae	7/9/2012	5669	Kailua, Oahu	M	A
KW2012004	*S. longirostris*	Spinner Dolphin	Delphinidae	7/23/2012	5683	Waianae, Oahu	F	C
KW2013001	*M. novaeangliae*	Humpback Whale	Balaenopteridae	1/10/2013	5854	Kaiolohia, Lanai	F	C
KW2013002	*M. novaeangliae*	Humpback Whale	Balaenopteridae	1/15/2013	5859	Hawaii Kai, Oahu	M	C
KW2013006	*S. coeruleoalba*	Striped Dolphin	Delphinidae	3/2/2013	5905	Kailua, Oahu	M	J
KW2013007	*M. novaeangliae*	Humpback Whale	Balaenopteridae	3/3/2013	5906	Waianae, Oahu	M	C
KW2013009	*S. longirostris*	Spinner Dolphin	Delphinidae	3/8/2013	5911	Kailua-Kona, Hawaii	M	J
KW2013010	*M. novaeangliae*	Humpback Whale	Balaenopteridae	3/17/2013	5920	Lanai	F	C

Table S2. Concentrations of PFAAs (ng/g, wet weight) Measured in SRM 1947 Samples. SRM 1947-1 and SRM 1947-2 samples were prepared with liver samples and SRM 1947-3 and SRM 1947-4 were prepared with kidney samples. Values from the NIST SRM 1947 Certificate of Analysis (CoA) are listed for comparison. Concentrations shown as "<" a specific number describe the actual LOD.

Compound	SRM 1947-1	SRM 1947-2	SRM 1947-3	SRM 1947-4	CoA SRM 1947
PFNA	<2.16	<4.73	<2.90	<2.52	0.20
PFDA	<0.349	<0.765	<0.750	<0.653	0.26
PFUnA	<1.07	<2.36	<2.12	<1.85	0.28
PFDoA	<0.353	<0.772	<0.757	<0.659	0.20
PFOS	5.20	6.07	5.67	5.74	5.90 ± 0.39

Table S3. PCR Primers for PPARα, CYP4A, and YWHAZ. Primers for PPARα and CYP4A were developed using a consensus sequence of four mammal species. The primers used for qPCR analysis are the first pairs listed for PPARα (194 base pairs) and CYP4A (190 base pairs).

Gene	Forward Primer	Melting Temp. (°C)	Reverse Primer	Melting Temp. (°C)	Product Size (base pairs)
PPARα	5'- CAGGATCAGATGGCTCCGTT -3'	62.4	5'- GAAACCCTTGCAGCCTTCAC -3'	62.4	194
	5'- AGCCCCATCTGTCCCCTCT -3'	64.5	5'- GCCTCCTTGTTCTGGATGC -3'	62.3	702
CYP4A	5'- GAACGACATCATCTACAGGCTG -3'	62.7	5'- GCTCCCATTCTCCATTCTGG -3'	62.4	190
	5'-TCCTTGATGACCCTGGACAC -3'	62.4	5'- AGCCTGGAGGAAAGGTGAG -3'	62.4	423
YWHAZ*	5'- AAATGAAAGGAGACTACTACCGCTA -3'	61.3	5'- AGACCCAATCTGATAGGATGTGTTG -3'	62.9	151

* Source: Spinsanti et al. 2006

Table S4. Concentrations of All PFAAs (ng/g, ww) Measured in Liver Samples. Concentrations shown as "<" a specific number describe the actual LOD. Abbreviations are utilized for *Common Name* (*W.* = Whale, *D.* = Dolphin.).

Animal ID	Common Name	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFBS	PFHxS	PFOS	PFOSA
12470-001	Striped Dolphin	<6.50	<0.575	<0.520	<0.723	<0.509	4.31	3.52	22.5	0.850	6.64	<0.894	<0.374	<0.542	18.3	2.74
15028-001	Spinner Dolphin	<5.73	<0.506	0.796	<0.637	0.503	9.09	7.06	31.5	2.25	6.59	<0.788	<0.330	<0.477	37.4	4.32
15063-001	Humpback W.	<7.06	<0.624	<0.565	<0.785	<0.552	<3.68	<0.595	4.73	<0.600	1.66	<0.971	<0.406	<0.588	1.24	<0.700
15121-001	Bottlenose D.	<5.25	<0.464	<0.420	<0.584	<0.411	22.9	15.5	41.4	2.38	8.47	<0.723	<0.302	<0.438	33.5	1.65
15320-001	Pygmy Sperm W.	<17.8	<1.58	<1.43	<1.98	<1.39	<9.32	<1.50	<4.64	<1.52	3.9	<2.45	<1.03	<1.49	2.05	<1.77
15377-001	Dwarf Sperm W.	<8.53	<0.754	<0.683	<0.949	<0.668	<4.45	5.34	28.4	1.56	11.6	<1.17	<0.491	<0.711	14.3	8.29
KW2007003	Pygmy Sperm W.	<6.75	<0.597	<0.540	<0.751	<0.529	<3.52	<0.569	<1.75	<0.574	<0.748	<0.929	<0.389	<0.563	<0.569	2.50
KW2007004	Spinner Dolphin	<7.32	<0.647	<0.586	<0.814	1.00	34.3	39.7	134	12.4	23.9	1.68	<0.422	0.722	149	19.6
KW2007005	Spinner Dolphin	<6.50	<0.575	<0.520	<0.723	<0.509	5.85	8.20	39.9	2.30	7.66	<0.894	<0.374	<0.542	29.0	5.78
KW2008004	Spinner Dolphin	<8.75	<0.773	<0.700	<0.973	0.829	17.5	17.1	79.7	3.47	9.14	<1.20	<0.504	<0.729	38.2	<0.868
KW2008006	Striped Dolphin	<8.35	<0.737	<0.668	<0.928	3.18	99.6	57.5	149	9.93	20.3	1.75	<0.481	<0.695	106	<0.828
KW2008008	Cuvier's Beaked Whale	<8.22	<0.727	<0.658	<0.915	<0.644	15.7	14.5	83.8	8.50	25.9	2.04	<0.474	<0.685	17.8	<0.816
KW2008009	Spinner Dolphin	<8.49	<0.750	<0.679	<0.944	<0.664	<4.42	1.7	16.4	<0.721	3.41	<1.16	<0.489	<0.707	5.00	<0.842
KW2008010	Killer Whale	<9.21	<0.814	<0.737	<1.02	<0.721	<4.80	8.46	31.7	1.51	11.6	<1.26	<0.530	<0.767	18.5	25.4
KW2008011	Spinner Dolphin	<7.95	<0.702	<0.636	<0.884	<0.622	9.54	11.99	50.5	3.20	7.37	<1.09	<0.458	<0.662	30.0	<0.788
KW2009002	Humpback W.	<5.38	<0.476	<0.431	<0.599	<0.421	<2.81	<0.453	<1.40	<0.458	<0.596	<0.741	<0.310	<0.448	0.472	<0.534
KW2009004	Spinner Dolphin	<7.59	<0.671	<0.607	<0.844	0.972	16.7	21.5	69.5	5.20	9.04	<1.04	<0.437	<0.632	45.6	<0.753
KW2009006	Pygmy Killer W.	<6.99	<0.618	<0.559	<0.778	<0.547	8.33	13.8	68.9	6.08	17.8	1.08	<0.403	<0.583	26.7	15.1
KW2009008	Striped Dolphin	<9.60	<0.848	<0.768	<1.068	<0.751	16.6	23.2	85.1	5.58	22.9	1.85	<0.553	<0.800	33.7	<0.952
KW2009009	Striped Dolphin	<8.78	<0.776	<0.702	<0.977	1.37	29.3	30.4	156	8.67	28.6	1.97	<0.506	<0.732	54.1	8.90
KW2009011	Striped Dolphin	<9.85	<0.870	<0.788	<1.09	<0.771	<5.13	12.32	62.2	3.17	14.1	<1.35	<0.567	<0.820	18.2	<0.977
KW2009012	Dwarf Sperm W.	<9.42	<0.832	<0.753	<1.04	<0.737	<4.91	4.768	25.4	1.48	14.1	<1.29	<0.542	<0.785	5.67	<0.934
KW2009015	Spotted Dolphin	<8.49	<0.750	<0.679	<0.944	<0.664	<4.42	2.9	12.7	<0.721	3.20	<1.16	<0.489	<0.707	9.00	<0.842
KW2010005	Longman's Beaked Whale	<7.07	<0.625	<0.566	<0.786	<0.553	31.9	38.1	170	18.3	46.8	3.45	<0.407	<0.589	24.8	<0.701
KW2010006	Spinner Dolphin	<5.52	<0.488	<0.442	<0.614	1.07	31.6	24.7	76.6	5.17	10.8	<0.759	<0.318	<0.460	64.4	<0.548
KW2010007	Bottlenose D.	<6.36	<0.562	<0.508	<0.707	0.899	<3.31	4.34	19.3	2.23	8.51	0.877	<0.366	<0.530	15.6	45.5
KW2010008	Striped Dolphin	<8.87	<0.783	<0.709	<0.986	<0.694	4.86	7.80	29.1	0.875	6.97	<1.22	<0.511	<0.739	15.8	<0.879
KW2010011	Spotted Dolphin	<9.36	<0.827	<0.749	<1.04	<0.733	8.16	4.3	22.1	2.11	10.4	<1.28	<0.539	<0.780	6.96	<0.928
KW2010012	Blainville's Beaked Whale	<7.75	<0.684	<0.620	<0.862	<0.606	<4.04	17.4	118	9.07	23.4	1.57	<0.446	<0.645	10.8	<0.768
KW2010019	False Killer W.	<7.72	<0.682	<0.618	<0.859	<0.604	<4.02	5.22	35.3	3.18	12.8	2.09	<0.445	<0.643	7.67	21.6
KW2011001	Bottlenose D.	<9.32	<0.824	<0.746	<1.03	<0.730	20.2	30.7	115	10.8	25.7	2.23	<0.537	<0.777	30.4	<0.925
KW2011002	Melon-headed W.	<5.67	<0.501	<0.453	<0.630	0.504	55.7	74.0	174	11.5	25.7	1.72	<0.326	<0.472	86.7	2.67
KW2011003	Bottlenose D.	<6.84	<0.604	<0.547	<0.760	0.856	<3.56	1.2	6.83	<0.581	3.45	<0.940	<0.394	<0.569	6.83	4.49
KW2011006	Bottlenose D.	<8.61	<0.761	<0.689	<0.958	1.07	<4.49	1.6	10.89	1.08	4.91	<1.18	<0.496	<0.718	10.4	4.73
KW2011007	Bottlenose D.	<7.05	<0.623	<0.564	<0.784	<0.552	<3.67	4.54	29.9	16.6	40.9	24.0	<0.406	<0.587	10.3	<0.699
KW2011008	Sperm Whale	<6.82	<0.602	<0.545	<0.758	<0.533	26.0	39.0	114	9.27	36.9	2.62	<0.392	<0.568	18.8	<0.676
KW2011009	Melon-headed W.	<6.46	<0.570	<0.516	<0.718	<0.505	11.5	6.96	20.1	1.47	4.88	<0.888	<0.372	<0.538	11.4	<0.640
KW2011011	Melon-headed W.	<7.52	<0.664	<0.601	<0.836	<0.588	11.8	9.93	23.1	0.742	7.05	<1.03	<0.433	<0.626	16.9	<0.745
KW2011013	Spinner Dolphin	<7.42	<0.655	<0.593	<0.825	1.54	19.5	10.40	20.8	<0.631	3.27	<1.02	<0.427	<0.618	20.6	<0.736
KW2011016	Cuvier's Beaked Whale	<7.22	<0.638	<0.577	<0.803	<0.565	15.4	13.6	104	20.3	61.1	4.31	<0.416	<0.601	13.8	<0.716
KW2011018	Spinner Dolphin	<8.66	<0.765	<0.693	<0.964	2.07	31.7	19.4	46.4	2.11	6.21	<1.19	<0.499	<0.722	44.8	<0.859
KW2011021	Rough-toothed D.	<6.69	<0.591	<0.535	<0.744	<0.523	<3.48	1.57	10.46	<0.568	3.48	<0.920	<0.385	<0.557	3.81	0.939
KW2012002	Striped Dolphin	<4.79	<0.423	<0.383	<0.533	<0.375	6.30	14.4	43.5	1.59	5.74	<0.659	<0.276	<0.399	22.9	2.86
KW2012003	Melon-headed W.	<7.02	<0.620	<0.561	<0.780	<0.549	4.71	9.52	28.8	1.00	6.57	<0.965	<0.404	<0.584	13.4	<0.696
KW2012004	Spinner Dolphin	<6.57	<0.580	<0.525	<0.731	1.44	23.0	15.9	36.9	2.34	7.24	<0.904	<0.378	<0.547	32.2	<0.651
KW2013001	Humpback W.	<6.45	<0.570	<0.516	<0.717	<0.505	<3.36	<0.543	9.74	<0.548	3.23	<0.887	<0.371	<0.537	4.35	<0.639
KW2013002	Humpback W.	<5.56	<0.491	0.559	<0.618	<0.435	<2.89	2.762	14.2	1.35	3.41	<0.764	<0.320	<0.463	11.6	1.05
KW2013006	Striped Dolphin	<4.77	<0.421	<0.381	<0.530	1.01	27.1	25.1	80.7	3.67	10.5	0.807	<0.274	<0.397	32.8	<0.473
KW2013007	Humpback W.	<7.01	<0.619	<0.560	<0.779	<0.548	<3.65	<0.590	3.41	<0.596	<0.776	<0.964	<0.403	<0.584	4.30	<0.695
KW2013009	Spinner Dolphin	<5.26	<0.465	<0.421	<0.585	0.512	10.1	18.3	46.3	2.30	5.65	<0.723	<0.303	<0.438	795	<0.522
KW2013010	Humpback W.	<8.92	<0.788	<0.713	<0.992	<0.698	<4.65	6.54	40.2	1.80	6.59	<1.22	<0.514	<0.743	5.52	<0.885

Table S5. Concentrations of All PFAAs (ng/g, ww) Measured in Kidney Samples. Concentrations shown as "<" a specific number describe the actual LOD. Abbreviations are utilized for *Common Name* (*W.* = Whale, *D.* = Dolphin.)

Animal ID	Common Name	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFTriA	PFTA	PFBS	PFHxS	PFOS	PFOSA
12470-001	Striped Dolphin	<2.39	<0.522	<0.473	<0.657	<0.462	<1.92	2.77	9.50	<0.502	6.52	0.528	<0.340	<0.492	12.8	3.85
15063-001	Humpback W.	<3.17	<0.693	<0.627	<0.872	<0.614	<2.55	<0.660	<1.87	<0.667	<0.689	<0.651	<0.451	<0.653	1.23	<0.778
15320-001	Pygmy Sperm W.	<3.67	<0.801	<0.725	<1.00	<0.709	<2.95	<0.763	<2.16	<0.770	<0.796	<0.753	<0.522	<0.755	<0.763	1.61
KW2007004	Spinner Dolphin	<2.66	<0.581	<0.526	<0.732	<0.515	<2.14	2.24	6.28	<0.559	3.55	<0.546	<0.379	<0.548	13.2	3.72
KW2007005	Spinner Dolphin	<2.19	<0.479	<0.434	<0.603	<0.424	<1.76	2.17	5.29	<0.461	4.02	0.624	<0.312	<0.452	8.60	5.10
KW2008003	Humpback Whale	<2.99	<0.653	<0.591	<0.822	<0.579	<2.41	0.981	<1.76	<0.629	<0.650	<0.614	<0.426	<0.616	6.55	<0.733
KW2008004	Spinner Dolphin	<2.47	<0.540	<0.489	<0.679	0.884	8.71	6.36	26.3	0.906	6.50	0.592	<0.352	<0.509	17.3	3.08
KW2008005	Spotted Dolphin	<2.84	<0.620	<0.561	<0.780	0.989	5.73	5.18	15.8	<0.596	7.13	0.814	<0.404	<0.584	14.5	6.81
KW2008008	Cuvier's Beaked Whale	<2.80	<0.612	<0.554	<0.771	<0.542	2.51	4.97	21.4	2.80	24.4	2.34	<0.399	<0.577	10.6	1.25
KW2008009	Spinner Dolphin	<2.34	<0.511	<0.462	<0.643	<0.452	<1.88	0.95	<1.38	<0.492	1.81	<0.480	<0.333	<0.482	1.76	1.34
KW2008010	Killer Whale	<2.86	<0.626	<0.566	<0.788	<0.554	<2.30	4.72	9.64	<0.602	8.87	0.942	<0.408	<0.590	12.3	10.1
KW2008011	Spinner Dolphin	<3.34	<0.729	<0.660	<0.918	<0.646	<2.69	1.76	7.39	<0.702	1.72	<0.686	<0.475	<0.688	4.52	0.993
KW2009002	Humpback Whale	<1.97	<0.430	<0.389	<0.541	<0.381	<1.58	<0.410	<1.16	<0.413	<0.427	<0.404	<0.280	<0.405	0.574	<0.482
KW2009004	Spinner Dolphin	<2.87	<0.627	<0.567	<0.789	0.893	3.85	7.86	26.1	2.08	10.1	0.934	<0.408	<0.591	24.0	2.43
KW2009006	Pygmy Killer W.	<2.83	<0.618	<0.559	<0.778	<0.547	<2.28	4.88	18.8	1.10	11.5	1.14	<0.403	<0.583	9.15	10.6
KW2009008	Striped Dolphin	<3.37	<0.736	<0.666	<0.926	<0.652	<2.71	4.10	9.37	<0.708	6.60	0.933	<0.480	<0.694	8.12	<0.826
KW2009009	Striped Dolphin	<2.47	<0.540	<0.489	<0.679	0.989	6.19	5.17	17.2	1.10	13.0	1.49	<0.352	<0.509	13.2	5.44
KW2009011	Striped Dolphin	<2.65	<0.580	<0.525	<0.730	<0.513	<2.13	4.68	20.5	<0.558	10.7	1.06	<0.378	<0.546	8.18	0.755
KW2009012	Dwarf Sperm W.	<2.44	<0.533	<0.483	<0.671	<0.472	<1.96	1.19	<1.44	<0.513	4.48	0.543	<0.347	<0.503	1.29	3.41
KW2009015	Spotted Dolphin	<3.18	<0.694	<0.628	<0.874	<0.615	<2.56	0.90	<1.87	<0.668	<0.690	<0.652	<0.452	<0.654	1.78	1.00
KW2010005	Longman's Beaked Whale	<3.03	<0.661	<0.598	<0.832	<0.585	27.8	26.0	149	16.8	53.1	3.62	<0.431	<0.623	20.6	<0.742
KW2010006	Spinner Dolphin	<3.70	<0.809	<0.732	<1.01	<0.716	11.25	8.35	22.5	<0.778	6.16	0.831	<0.527	<0.763	21.8	2.71
KW2010007	Bottlenose D.	<2.54	<0.555	<0.502	<0.698	0.968	<2.04	2.45	6.19	<0.534	6.30	0.809	<0.361	<0.523	9.91	15.3
KW2010008	Striped Dolphin	<3.09	<0.674	<0.610	<0.849	<0.597	<2.48	3.54	11.70	<0.649	5.29	0.906	<0.439	<0.636	7.28	<0.757
KW2010011	Spotted Dolphin	<2.37	<0.517	<0.468	<0.651	<0.458	1.98	3.32	17.2	6.43	26.2	15.2	<0.337	<0.487	4.68	<0.580
KW2010012	Blainville's Beaked Whale	<2.08	<0.454	<0.411	<0.572	<0.402	<1.67	3.00	15.9	<0.437	8.72	1.24	<0.296	<0.428	4.34	0.945
KW2010019	False Killer W.	<2.87	<0.627	<0.567	<0.789	<0.555	<2.31	3.82	17.3	0.959	12.6	1.31	<0.408	<0.591	4.80	8.22
KW2011001	Bottlenose D.	<2.77	<0.606	<0.548	<0.763	0.546	8.28	13.2	61.2	6.63	23.3	1.64	<0.395	<0.571	20.8	4.97
KW2011002	Melon-headed W.	<2.33	<0.509	<0.461	<0.641	0.630	37.4	27.1	59.7	3.80	16.3	1.40	<0.332	<0.480	34.3	2.67
KW2011003	Bottlenose D.	<3.07	<0.670	<0.606	<0.843	0.669	<2.47	1.09	<1.81	<0.644	1.90	0.648	<0.436	<0.631	3.44	7.40
KW2011006	Bottlenose D.	<2.76	<0.603	<0.546	<0.759	0.759	<2.22	1.24	4.75	<0.580	3.03	0.617	<0.393	<0.569	5.22	3.16
KW2011007	Bottlenose D.	<2.16	<0.471	<0.427	<0.593	<0.417	<1.73	3.06	12.3	1.18	9.87	1.02	<0.307	<0.444	6.37	3.17
KW2011008	Sperm Whale	<3.01	<0.656	<0.594	<0.826	0.668	32.6	25.4	75.4	4.30	27.7	3.82	<0.428	<0.619	13.5	<0.737
KW2011009	Melon-headed W.	<3.64	<0.796	<0.721	<1.00	<0.705	<2.93	2.08	<2.15	<0.766	2.09	<0.748	<0.519	<0.750	3.70	<0.893
KW2011011	Melon-headed W.	<3.36	<0.733	<0.664	<0.923	<0.650	5.10	6.33	6.18	<0.706	4.70	0.732	<0.478	<0.691	7.83	2.09
KW2011013	Spinner Dolphin	<2.90	<0.633	<0.573	<0.796	1.19	12.45	7.27	15.0	<0.609	5.81	0.655	<0.412	<0.596	13.9	1.00
KW2011016	Cuvier's Beaked Whale	<3.82	<0.834	<0.755	<1.05	<0.739	<3.07	4.69	21.8	4.43	40.5	7.46	<0.544	<0.786	7.38	<0.936
KW2011018	Spinner Dolphin	<2.81	<0.614	<0.556	<0.773	1.64	18.6	9.23	20.1	0.915	6.69	0.969	<0.400	<0.579	19.5	0.748
KW2012002	Striped Dolphin	<2.80	<0.612	<0.554	<0.771	<0.542	2.70	7.34	17.0	<0.589	6.62	0.719	<0.399	<0.577	16.5	5.26
KW2012003	Melon-headed W.	<2.36	<0.515	<0.467	<0.649	<0.457	<1.90	2.54	1.64	<0.496	2.67	0.514	<0.336	<0.486	3.52	2.33
KW2012004	Spinner Dolphin	<3.67	<0.801	<0.725	<1.00	1.70	16.0	7.51	13.43	<0.770	4.48	<0.753	<0.522	<0.755	15.9	<0.899
KW2013001	Humpback W.	<2.82	<0.617	<0.559	<0.777	<0.547	<2.27	0.87	<1.66	<0.594	1.56	<0.580	<0.402	<0.582	2.15	<0.693
KW2013002	Humpback W.	<3.59	<0.783	<0.709	<0.986	<0.694	<2.89	2.41	3.33	<0.754	1.88	<0.736	<0.511	<0.739	8.20	1.66
KW2013006	Striped Dolphin	<2.63	<0.575	<0.521	<0.724	1.28	25.1	11.0	40.1	1.22	10.7	1.19	<0.375	<0.543	19.7	1.10
KW2013007	Humpback W.	<3.44	<0.751	<0.680	<0.946	<0.665	<2.77	0.89	<2.03	<0.723	<0.747	<0.706	<0.490	<0.708	2.42	<0.843
KW2013009	Spinner Dolphin	<3.04	<0.664	<0.601	<0.836	0.732	9.19	4.78	5.71	<0.639	2.41	<0.624	<0.433	<0.626	8.88	1.85
KW2013010	Humpback W.	<3.87	<0.844	<0.765	<1.06	<0.748	<3.11	5.85	22.3	<0.813	4.66	<0.794	<0.550	<0.796	4.76	0.995

Table S6. Descriptive Statistics for PFAA Concentrations by Phylogenetic Family for Liver and Kidney. The means, medians, standard deviations, and ranges represent all of the samples measured for liver (A.) and kidney (B.). Summation of concentrations for ΣPFCAs, ΣPFSAs, and ΣPFAAs do not include any concentrations below the LOD (< LOD). The minimum concentrations of the ranges are the lowest detected value and does not include concentrations below the LOD unless otherwise specified. Ranges with one concentration listed had only one sample with detectable concentrations. Only one animal was sampled in family Physeteridae (sperm whale KW2011008).

A. Liver

PFAA	Phylogenetic Family	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Delphinidae	16 (44.4)	0.690	< LOD	0.626	0.503 - 3.18
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	0 (0)	--	--	--	--
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	0 (0)	--	--	--	--
PFNA	Delphinidae	26 (72.2)	15.6	9.32	19.0	4.31 - 99.6
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	3 (75.0)	16.3	15.6	12.2	15.4 - 31.9
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	26
PFDA	Delphinidae	36 (100)	15.7	11.2	15.6	1.20* - 74.0
	Kogiadae	2 (50.0)	2.79	< LOD	2.63	4.77 - 5.34
	Ziphiidae	4 (100)	20.9	16.0	11.6	13.6* - 38.1
	Balaenopteridae	2 (33.3)	1.73	< LOD	2.56	2.76 - 6.54
	Physeteridae	1 (100)	--	--	--	39
PFUnA	Delphinidae	36 (100)	53.5	38.4	43.7	6.83* - 174
	Kogiadae	2 (50.0)	14.2	< LOD	14.7	25.4 - 28.4
	Ziphiidae	4 (100)	119	111	36.8	83.8* - 170
	Balaenopteridae	5 (83.2)	12.2	7.24	14.6	3.41 - 40.2
	Physeteridae	1 (100)	--	--	--	114
PFDoA	Delphinidae	31 (86.1)	3.82	2.30	3.99	0.742 - 16..6
	Kogiadae	2 (50.0)	1.02	< LOD	0.608	1.48 - 1.56
	Ziphiidae	4 (100)	14.0	13.7	6.13	8.50* - 20.3
	Balaenopteridae	2 (33.3)	0.709	< LOD	0.687	1.35 - 1.80
	Physeteridae	1 (100)	--	--	--	9.27
PFTriA	Delphinidae	36 (100)	11.4	8.07	8.75	3.20* - 40.9
	Kogiadae	3 (75.0)	7.50	7.77	6.42	3.94 - 14.1
	Ziphiidae	4 (100)	39.3	36.4	17.9	23.4* - 61.1
	Balaenopteridae	4 (66.7)	2.60	2.45	2.37	1.66 - 6.59
	Physeteridae	1 (100)	--	--	--	36.9
PFTA	Delphinidae	11 (30.6)	1.46	< LOD	3.90	0.807 - 24.0
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	4 (100)	2.84	2.75	1.26	1.57* - 4.31
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	2.62
ΣPFCAs	Delphinidae	36 (100)	101	77.1	84.5	12.3 - 343
	Kogiadae	3 (75.0)	24.1	24.8	25.7	3.94 - 46.9
	Ziphiidae	4 (100)	212	194	70.6	150 - 309
	Balaenopteridae	5 (83.2)	16.6	9.68	20.4	3.41 - 55.1
	Physeteridae	1 (100)	--	--	--	228
PFOS	Delphinidae	36 (100)	52.8	24.8	131	3.81* - 795
	Kogiadae	3 (75.0)	5.58	3.86	6.23	2.05 - 14.3
	Ziphiidae	4 (100)	16.8	15.8	6.06	10.8* - 24.8
	Balaenopteridae	6 (100)	4.58	4.33	3.96	0.472* - 11.6
	Physeteridae	1 (100)	--	--	--	18.8
PFOSA	Delphinidae	15 (41.7)	4.84	< LOD	9.49	0.939 - 45.5
	Kogiadae	2 (50.0)	3.04	< LOD	3.61	2.50 - 8.29
	Ziphiidae	0 (0)	--	--	--	--
	Balaenopteridae	1 (16.7)	--	--	--	1.05
	Physeteridae	0 (0)	--	--	--	--
ΣPFSAs	Delphinidae	36 (100)	57.4	31.3	130	4.75 - 795
	Kogiadae	3 (75.0)	8.20	4.09	9.73	2.05 - 22.59
	Ziphiidae	4 (100)	16.8	15.8	6.06	10.8 - 24.8
	Balaenopteridae	6 (100)	4.76	4.33	4.34	0.472 - 12.7
	Physeteridae	1 (100)	--	--	--	18.8
ΣPFAAs	Delphinidae	36 (100)	159	105	166	20.3 - 878
	Kogiadae	4 (100)	32.3	28.7	33.3	2.50 - 69.4
	Ziphiidae	4 (100)	228	206	75.6	168 - 333
	Balaenopteridae	6 (100)	21.4	22.6	22.6	0.472 - 60.6
	Physeteridae	1 (100)	--	--	--	247

* The concentration is the actual minimum.

B. Kidney

PFAA	Phylogenetic Family	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Delphinidae	14 (42.4)	0.579	< LOD	0.425	0.546 - 1.70
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	0 (0)	--	--	--	--
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	0.668
PFNA	Delphinidae	15 (45.5)	5.84	< LOD	8.22	1.98 - 37.4
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	2 (50.0)	8.17	2.03	13.1	2.51 - 27.8
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	32.6
PFDA	Delphinidae	33 (100)	5.42	4.68	4.90	0.901* - 27.1
	Kogiadae	1 (50.0)	--	--	--	1.19
	Ziphiidae	4 (100)	9.7	4.83	10.9	3.00* - 26.0
	Balaenopteridae	5 (71.4)	1.65	0.891	1.99	0.868 - 5.85
	Physeteridae	1 (100)	--	--	--	25.4
PFUnA	Delphinidae	29 (87.9)	15.4	12.3	14.6	1.64 - 61.2
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	4 (100)	52.0	21.6	64.7	15.9* - 149
	Balaenopteridae	2 (28.6)	4.27	< LOD	8.01	3.33 - 22.3
	Physeteridae	1 (100)	--	--	--	75.4
PFDoA	Delphinidae	11 (33.3)	1.00	< LOD	1.59	0.906 - 6.63
	Kogiadae	0 (0)	--	--	--	--
	Ziphiidae	3 (75.0)	6.06	3.62	7.37	2.80 - 16.8
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	4.30
PFTriA	Delphinidae	32 (97.0)	7.56	6.50	5.84	1.72 - 26.2
	Kogiadae	1 (50.0)	--	--	--	4.48
	Ziphiidae	4 (100)	31.7	32.5	19.3	8.72* - 53.1
	Balaenopteridae	3 (42.9)	1.34	< LOD	1.61	1.56 - 4.66
	Physeteridae	1 (100)	--	--	--	27.7
PFTA	Delphinidae	26 (78.8)	1.23	0.809	2.53	0.514 - 15.2
	Kogiadae	1 (50.0)	--	--	--	0.543
	Ziphiidae	4 (100)	3.67	2.98	2.71	1.24* - 7.46
	Balaenopteridae	0 (0)	--	--	--	--
	Physeteridae	1 (100)	--	--	--	3.82
ΣPFCAs	Delphinidae	33 (100)	35.9	27.4	32.1	0.901* - 146
	Kogiadae	1 (50.0)	--	--	--	6.22
	Ziphiidae	4 (100)	111	68.6	112	28.9* - 276
	Balaenopteridae	5 (71.4)	6.40	0.981	12.0	0.891 - 32.9
	Physeteridae	1 (100)	--	--	--	170
PFOS	Delphinidae	33 (100)	11.4	9.15	7.42	1.76* - 34.3
	Kogiadae	1 (50.0)	--	--	--	1.29
	Ziphiidae	4 (100)	10.7	8.99	7.06	4.34* - 20.6
	Balaenopteridae	7 (100)	3.70	2.42	2.87	0.574* - 8.20
	Physeteridae	1 (100)	--	--	--	13.5
PFOSA	Delphinidae	28 (84.8)	3.61	2.67	3.51	0.748 - 15.3
	Kogiadae	2 (100)	2.51	2.51	1.27	1.61* - 3.41
	Ziphiidae	2 (50.0)	0.759	0.707	0.413	0.945 - 1.25
	Balaenopteridae	2 (28.6)	0.631	< LOD	0.516	0.995 - 1.66
	Physeteridae	0 (0)	--	--	--	--
ΣPFSAs	Delphinidae	33 (100)	15.0	14.9	8.25	2.78 - 36.9
	Kogiadae	2 (100)	3.15	3.15	2.17	1.61 - 4.70
	Ziphiidae	4 (100)	11.3	9.62	6.80	5.28 - 20.6
	Balaenopteridae	7 (100)	4.07	2.42	3.40	0.574 - 9.86
	Physeteridae	1 (100)	--	--	--	13.5
ΣPFAAs	Delphinidae	33 (100)	50.8	45.9	38.3	3.68 - 183
	Kogiadae	2 (100)	6.27	6.27	6.58	1.61 - 10.9
	Ziphiidae	4 (100)	122	78.3	119	34.1 - 297
	Balaenopteridae	7 (100)	10.5	4.58	13.7	0.574 - 38.6
	Physeteridae	1 (100)	--	--	--	183

* The concentration is the actual minimum.

Table S7. Descriptive Statistics for PFAA Concentrations by Age Class for Liver and Kidney Samples. The means, medians, standard deviations, and ranges represent all of the samples measured for liver (A.) and kidney (B.). Summation of concentrations for ΣPFCAs, ΣPFSAs, and ΣPFAAs do not include any concentrations below the LOD. The minimum concentrations of the ranges are the lowest detected value and does not include concentrations below the LOD unless otherwise specified. Ranges with one concentration listed had only one sample with detectable concentrations.

A. Liver

PFAA	Age Class	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Adults	3 (14.3)	0.401	< LOD	0.283	0.829 - 1.37
	Juveniles	4 (40.0)	0.593	< LOD	0.323	0.512 - 1.07
	Calves	9 (45.0)	0.760	< LOD	0.779	0.503 - 3.18
PFNA	Adults	11 (52.4)	6.59	4.31	6.97	4.31 - 29.3
	Juveniles	8 (80.0)	19.4	18.4	11.6	10.1 - 34.3
	Calves	11 (55.0)	17.0	8.63	24.0	8.16 - 99.6
PFDA	Adults	20 (95.2)	8.55	7.80	6.87	1.57 - 30.4
	Juveniles	9 (90.0)	23.2	24.0	11.5	14.5 - 39.7
	Calves	16 (80.0)	14.9	7.01	20.1	1.25 - 74.0
PFUnA	Adults	20 (95.2)	40.8	29.1	35.8	10.5 - 156
	Juveniles	9 (90.0)	91.2	84.5	46.7	46.3 - 170
	Calves	19 (95.0)	43.9	26.8	48.8	3.41 - 174
PFDoA	Adults	17 (81.0)	3.66	1.56	5.35	0.742 - 20.3
	Juveniles	9 (90.0)	7.66	7.04	5.28	2.30 - 18.3
	Calves	14 (70.0)	2.88	1.96	3.42	1.08 - 11.5
PFTriA	Adults	20 (95.2)	13.1	7.66	14.3	3.20 - 61.1
	Juveniles	10 (100)	19.9	23.2	12.7	3.94* - 46.8
	Calves	18 (90.0)	8.85	6.40	9.18	1.66 - 36.9
PFTA	Adults	6 (28.6)	2.01	< LOD	5.12	0.877 - 24.0
	Juveniles	7 (70.0)	1.56	1.63	0.934	0.807 - 3.45
	Calves	3 (15.0)	0.726	< LOD	0.593	1.72 - 2.62
ΣPFCAs	Adults	20 (95.2)	73.3	52.6	63.9	15.5 - 256
	Juveniles	10 (100)	162	153	83.1	3.94 - 308
	Calves	19 (95.0)	87.5	55.3	102	3.41 - 344
PFOS	Adults	20 (95.2)	17.6	15.6	12.7	3.81 - 54.1
	Juveniles	10 (100)	116	31.6	242	2.05* - 795
	Calves	20 (100)	25.3	14.9	28.3	0.472* - 106
PFOSA	Adults	11 (52.4)	6.84	0.939	11.5	0.939 - 45.5
	Juveniles	1 (10.0)	2.33	< LOD	6.07	19.6
	Calves	6 (30.0)	1.21	< LOD	1.54	1.05 - 4.73
ΣPFSAs	Adults	21 (100)	24.2	21.0	17.7	2.50 - 63.0
	Juveniles	10 (100)	118	31.6	243	2.05 - 795
	Calves	20 (100)	26.3	16.7	28.5	0.472 - 106
ΣPFAAs	Adults	21 (100)	97.5	87.9	73.8	2.50 - 319
	Juveniles	10 (100)	280	202	236	5.99 - 878
	Calves	20 (100)	114	68.4	128	0.472 - 447

* The concentration is the actual minimum.

B. Kidney

PFAA	Age Class	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Adults	3 (16.7)	0.384	< LOD	0.263	0.884 - 0.989
	Juveniles	4 (36.3)	0.510	< LOD	0.349	0.546 - 1.28
	Calves	8 (44.4)	0.617	< LOD	0.467	0.630 - 1.70
PFNA	Adults	4 (22.2)	2.12	< LOD	2.23	2.70 - 8.71
	Juveniles	7 (63.6)	8.60	5.73	9.56	2.51 - 27.8
	Calves	7 (38.9)	7.59	< LOD	11.4	1.98 - 37.4
PFDA	Adults	18 (100)	3.59	3.30	1.95	0.901* - 7.34
	Juveniles	10 (90.9)	7.56	4.97	7.19	2.24 - 26.0
	Calves	16 (88.9)	6.02	2.87	7.92	0.868 - 27.1
PFUnA	Adults	15 (83.3)	10.6	9.57	7.75	1.64 - 26.3
	Juveniles	10 (90.9)	31.7	15.9	42.6	5.71 - 149
	Calves	11 (61.1)	15.8	9.08	21.0	3.33 - 75.4
PFDoA	Adults	6 (33.3)	0.730	< LOD	0.989	0.906 - 4.43
	Juveniles	4 (36.3)	2.70	< LOD	5.06	1.22 - 16.8
	Calves	5 (27.8)	1.21	< LOD	1.79	0.915 - 6.43
PFTriA	Adults	17 (94.4)	8.18	6.40	8.87	1.72 - 40.5
	Juveniles	10 (90.9)	13.3	7.13	15.3	2.41 - 53.1
	Calves	14 (77.8)	6.91	3.76	8.47	1.56 - 27.7
PFTA	Adults	15 (83.3)	1.12	0.726	1.62	0.514 - 7.46
	Juveniles	8 (72.7)	1.23	0.933	1.00	0.814 - 3.62
	Calves	9 (50.0)	1.57	< LOD	3.50	0.617 - 15.2
ΣPFCAs	Adults	18 (100)	25.2	22.2	19.6	0.901 - 78.8
	Juveniles	10 (90.9)	64.5	35.7	78.0	12.1 - 276
	Calves	16 (88.9)	37.9	21.7	49.5	0.891 - 170
PFOS	Adults	18 (100)	8.13	7.61	4.86	1.29* - 17.3
	Juveniles	10 (90.9)	13.0	13.2	7.24	4.34 - 21.8
	Calves	18 (100)	9.57	5.89	9.08	0.574* - 34.3
PFOSA	Adults	16 (88.9)	4.56	3.29	4.09	0.993 - 15.3
	Juveniles	9 (81.8)	2.34	1.61	2.05	0.945 - 6.81
	Calves	9 (50.0)	1.34	< LOD	1.76	0.748 - 7.40
ΣPFSAs	Adults	18 (100)	12.6	11.5	7.37	2.78 - 25.2
	Juveniles	11 (100)	15.2	16.9	8.13	1.61 - 25.7
	Calves	18 (100)	10.7	8.66	9.54	0.574 - 36.9
ΣPFAAs	Adults	18 (100)	37.9	36.5	23.3	3.68 - 86.2
	Juveniles	11 (100)	79.6	56.9	82.4	1.61 - 297
	Calves	18 (100)	48.6	28.7	56.2	0.574 - 183

* The concentration is the actual minimum.

Table S8. Descriptive Statistics for PFAA Concentrations by Sex for Liver and Kidney. The means, medians, standard deviations, and ranges represent all of the samples measured for liver (A.) and kidney (B.). Summation of concentrations for ΣPFCAs, ΣPFSAs, and ΣPFAAs do not include any concentrations below the LOD (< LOD). The minimum concentrations of the ranges are the lowest detected value and does not include concentrations below the LOD unless otherwise specified.

A. Liver

PFAA	Sex	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Females	6 (31.6)	0.729	< LOD	0.798	0.972 - 3.18
	Males	10 (31.3)	0.490	< LOD	0.324	0.503 - 1.54
PFNA	Females	11 (57.9)	15.6	6.30	22.8	5.85 - 99.6
	Males	19 (59.4)	11.8	6.51	12.9	4.31 - 55.7
PFDA	Females	16 (84.2)	15.0	12.0	15.3	1.64 - 57.5
	Males	29 (90.6)	13.3	8.99	15.0	1.25 - 74.0
PFUnA	Females	18 (94.7)	53.7	40.2	47.1	4.73 - 156
	Males	30 (93.8)	50.8	29.5	47.4	3.41 - 174
PFDoA	Females	15 (78.9)	3.54	2.30	3.50	1.10 - 10.8
	Males	25 (78.1)	4.50	2.17	5.61	0.700 - 20.3
PFTriA	Females	18 (94.7)	10.9	7.24	10.0	1.70 - 36.9
	Males	32 (100)	13.9	8.83	14.0	3.20* - 61.1
PFTA	Females	5 (26.3)	0.934	< LOD	0.756	1.75 - 2.62
	Males	11 (34.4)	1.71	< LOD	4.17	0.807 - 24
ΣPFCAs	Females	18 (94.7)	98.8	72.1	92.9	6.39 - 341
	Males	32 (100)	94.8	56.5	87.1	3.41 - 344
PFOS	Females	19 (100)	25.2	18.8	25.3	0.472* - 106
	Males	31 (96.9)	48.7	16.4	139	2.05 - 795
PFOSA	Females	5 (26.3)	2.59	< LOD	5.19	2.86 - 21.6
	Males	13 (40.6)	4.43	< LOD	9.56	0.939 - 45.5
ΣPFSAs	Females	19 (100)	27.5	25.8	25.5	0.472 - 107
	Males	32 (100)	52.9	19.6	139	2.05 - 795
ΣPFAAs	Females	19 (100)	126	98.7	116	0.472 - 447
	Males	32 (100)	148	97.2	172	2.50 - 878

* The concentration is the actual minimum.

B. Kidney

PFAA	Sex	# Samples Detected (%)	Mean (ng/g, ww)	Median (ng/g, ww)	St. Dev.	Range (ng/g, ww)
PFOA	Females	7 (36.8)	0.555	< LOD	0.459	0.546 - 1.70
	Males	8 (28.6)	0.467	< LOD	0.318	0.630 - 1.28
PFNA	Females	8 (42.1)	5.38	< LOD	8.37	2.70 - 32.6
	Males	10 (35.7)	5.97	< LOD	9.30	1.98 - 37.4
PFDA	Females	17 (89.5)	5.30	3.82	6.04	0.868 - 25.4
	Males	27 (96.4)	5.55	3.82	6.40	0.891 - 27.1
PFUnA	Females	13 (68.4)	16.5	13.4	20.3	4.75 - 75.4
	Males	23 (82.1)	18.2	10.7	28.8	1.64 - 149
PFDoA	Females	5 (26.3)	1.05	< LOD	1.67	0.915 - 6.63
	Males	10 (35.7)	1.59	< LOD	3.34	0.906 - 16.8
PFTriA	Females	16 (84.2)	7.10	4.48	7.69	1.56 - 27.7
	Males	25 (89.3)	10.1	6.40	12.2	1.86 - 53.1
PFTA	Females	10 (52.6)	0.844	0.617	0.845	0.617 - 3.82
	Males	22 (78.6)	1.65	0.812	3.01	0.514 - 15.2
ΣPFCAs	Females	17 (89.5)	35.1	34.9	42.5	0.981 - 170
	Males	27 (96.4)	42.1	24.8	55.6	0.891 - 277
PFOS	Females	19 (100)	9.23	6.55	7.27	0.574* - 24.0
	Males	27 (96.4)	10.2	8.54	7.52	1.29 - 34.3
PFOSA	Females	12 (63.2)	2.21	0.993	2.41	0.748 - 8.22
	Males	22 (78.6)	3.21	1.97	3.65	0.945 - 15.3
ΣPFSAs	Females	19 (100)	11.3	8.93	8.23	0.574 - 26.4
	Males	28 (100)	13.3	10.8	8.51	1.61 - 36.9
ΣPFAAs	Females	19 (100)	46.4	38.6	48.7	0.574 - 183
	Males	28 (100)	55.5	36.5	60.7	1.61 - 297

* The concentration is the actual minimum.

Table S9. Descriptive Statistics of PPARα and CYP4A mRNA Expression in Liver and Kidney. Calculations for the *Mean*, *Median*, and *St. Dev.* are listed for both $-\Delta\Delta C_q$ and fold change relative to the baseline ΔC_q value (i.e. PPARα ΔC_q baseline = 14.98, CYP4A ΔC_q baseline = 14.56).

Gene	Tissue	# Samples Detected (%)	Mean		Median		St. Dev.	Range (fold change)
			$-\Delta\Delta C_q$	fold change	$-\Delta\Delta C_q$	fold change		
PPARα	Liver	33 (67.3%)	9.67	817	10.0	1050	3.14	2.22 - 22100
	Kidney	18 (39.1%)	8.29	313	8.33	322	3.04	1.00 - 4710
CYP4A	Liver	37 (75.5%)	10.6	1560	11.5	2940	4.02	1.00 - 231000
	Kidney	31 (67.4%)	6.70	104	6.49	89.9	2.00	4.29 - 9950