SUPPLEMENTARY MATERIALS AND METHODS

Determination of PFOA and PFDA blood content by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Instrumentation. Measurement of PFOA and PFDA in blood plasma samples from rainbow trout was performed at the 3M Medical Department. A Shimadzu HPLC system (Shimadzu Scientific Instruments, Inc., Columbia, MD) was used for this study. The system consisted of two Model LC-20AD pumps, a SIL-HTc integrated auto-sampler and controller unit with a Model CTO-20AD oven for column temperature control. The auto-sampler was interfaced to the model API 5000 triple stage quadrupole mass spectrometer purchased through Sciex Applied Biosystems (Foster City, CA). The oven was held at a constant 40°C and the auto-sampler trays at 6°C. The HPLC column used was an ACE brand, base deactivated 5 µm column purchased from MAC MOD analytical (Chads Ford, PA); this column was 100 by 2.1 mm and was used with a betasil, C-18, 5-micron (10 x 2.1 mm i.d.) analytical guard column purchased from Thermo-Fisher (San Jose, CA).

Quality control. PFOA and PFDA standard materials were purchased from Sigma-Aldrich (St. Louis, MO) with listed purities of 95.3% and 99.1%, respectively. Both compounds were the free acid and appeared to be all linear material as no branched isomer peaks appeared on the LC-MS/MS chromatograms. Stock standard solutions were prepared in methanol and diluted to working concentrations of 0.1 to 10 ng/µl for spiking standard curves. After determining that a pool of "blank" trout serum was free of detectable PFOA or PFDA (quantitation limit of 1.0 ng/ml), this matrix was used for creation of standard curves and spiked recovery samples. Bovine calf serum, also free of trace levels of PFCs, was used for dilution of the unknown samples. To remove trace amounts of PFOA present in commercial reagent grade water or from standard laboratory water purification systems, water used in the preparation of all buffers, mobile phases and analytical reagents was purified on a conditioned C-18 phase HPLC column. Furthermore, all components of the HPLC system that include polytetrafluoroethylene (PTFE) based materials, which contain traces of PFOA and other fluorinated chemicals, were removed from the system and replaced with non-contaminating alternative components when feasible. A dual

HPLC column approach was used to remove residual instrument fluorochemical background contamination, as described by Powley *et al.* (2005). Internal dual ^{13}C-labeled PFOA and PFDA standards (Wellington Laboratories, Guelph, Ontario, Canada) were used for quantitation (described below). The interassay coefficients of variance were ≤ 4.4%, and the lower limit of quantitation (LLQ) was 5.0 ng/ml for both compounds. The laboratory was blinded to any information on the effect of PFOA or PFDA on Vtg expression in trout.

Sample preparation. Appropriate dual ^{13}C-labeled PFOA and PFDA internal standards were added to PFOA- and PFDA- treated samples, respectively. Every extraction set included appropriate assay controls, including an aqueous blank, a serum blank, a set of spiked controls, a set of recovery standards and a standard curve with PFOA or PFDA concentrations ranging from 5 to 1000 ng/ml. To each sample (100 µl), 1 ml of 1.0 N formic acid was added and the samples were well-mixed by vortexing. Just prior to extraction, 100 µl of saturated ammonium sulfate was added to all samples followed by vortexing. Solid phase extraction was performed using Oasis HLB cartridges (Waters Corp., Milford, MA) conditioned with 1 ml methanol, 1 ml acetonitrile (ACN) and 1 ml 0.1 N formic acid. Samples were loaded onto conditioned columns; after applying a vacuum, the column was rinsed twice with 1 ml C-18 purified water and then 1 ml of 15% ACN. The columns were then centrifuged for five minutes at 2500 x g. Finally, samples were eluted with 300 µl of 0.1 N ammonium acetate, twice with 300 µl ACN and finally with 300 µl C-18 purified water for a final volume of 1200 µl. Injection volumes for detection of PFOA and PFDA were set at 4 and 5 µl, respectively.

LC-MS-MS Conditions. The API 5000 was operated using the Turbo Ion Spray pneumatically assisted electrospray ionization source in the negative ion mode. A programmed gradient elution was used to elute the compounds of interest from the HPLC column with a constant flow rate of 250 µl/min. The buffer used was 2 mM ammonium acetate and the organic solvent used was acetonitrile according to the flowing ratios over the timed program run: (time, ratio of ACN:2 mM ammonium acetate) 0.01 min, 30:70; 1.8 min, 30:70; 3.5 min, 60:40; 4.5 min, 60:40; 5.5 min, 90:10; 7.0 min, 90:10; 7.8 min, 30:70; 10.5 min, 30:70. Transition ions were monitored as follows: PFOA, 413 to 369 amu; dual ^{13}C-labeled

PFOA internal standard, 415 to 370 amu; PFDA, 513 to 469 amu; and dual ^{13}C-labeld PFDA internal standard, 515 to 470 amu. For the internal standards, one labeled ^{13}C carbon was lost as CO_2 and was given up in Q2 region of the mass spectrometer; therefore, the product ion formed is only 1 amu different in the product ion for the internal standard when compared to the product ion of the compound.

Quantitation. Calculations for determining PFOA and PFDA plasma concentrations were based on the transition ions listed above. Peak areas were integrated and the ratio of the compound to the internal standard was then calculated. Standard curves (5 to 1000 ng/ml) were evaluated using a quadratic regression model (PFOA curve, R = 1.0; PFDA curve, R =0.99). All standards were weighted at 1/x for the regression analysis. Analyte concentrations calculated to be below the LLQ were reported as less than the LLQ, which was 5 ng/ml for both compounds (corresponding to 12 nM PFOA and 9.7 nM PFDA).

ADDITIONAL MATERIALS AND METHODS REFERENCES

Powley, C. R., George, S. W., Ryan, T. W. and Buck, R. C. (2005). Matrix effect-free analytical methods for determination of perfluorinated carboxylic acids in environmental matrixes. *Anal. Chem.* **77**, 6353-8.