Analysis of Statistically-representative Human Health Fish Tissue Sample in the Great Lakes for Perfluorinated Compounds

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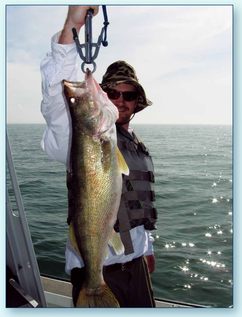
Background

EPA is conducting multiple probability-based fish contamination studies under the agency's National Coastal Condition Assessment (NCCA), including the Great Lakes Human Health Fish Tissue Study. The Office of Science and Technology (OST), The Great Lakes National Program Office (GLNPO) and the Office of Research and Development (ORD) are combining resources and expertise to conduct this study. State and contractor-led field teams collected fish samples from a representative subset of 157 of the 225 NCCA nearshore sites in the Great Lakes during 2010. Fillet tissue from each of the samples was analyzed for 13 perfluorinated compounds (PFCs) and a variety of other chemicals (i.e., mercury, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and omega-3 fatty acids). This is the first statistically representative assessment of these contaminants and omega-3 fatty acids in Great Lakes fish for human health applications.

Why Study PFCs in Fish?



Since 2000, perfluorinated compounds have emerged as contaminants of concern because they are broadly distributed and persistent in the environment. This class of synthetic compounds contains thousands of chemicals formed from carbon chains with attached fluorine molecules. The chemical structure of PFCs gives them unique properties, such as thermal stability and the ability to repel both water and oil. They are used in a wide variety of consumer and industrial products (e.g., non-stick cookware, food packaging, fabric stain protectors, lubricants, paints, and firefighting foams). Perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS) are two of the best known PFCs. People living in industrialized nations commonly have detectable concentrations of PFCs in their blood serum. Elevated concentrations of PFOS and PFOA in human blood have been linked to a number of potential health effects, including immunotoxicity, decreased sperm count, and thyroid disease. Recent studies suggest that consumption of fish from contaminated waters may be the primary source of human exposure to PFOS.

Study Design

Assessment of chemicals in Great Lakes fish under the Great Lakes Human Health Fish Tissue Study involved:

- Sampling of 157 randomly selected sites (about 30 sites per lake) in the nearshore region (depths up to 30 m or distances up to 5 km from shore) during 2010
- Collecting one fish composite sample from each site (optimally, 5 similarly sized adult fish of the same species that are consumed by humans)
- Preparing fillet samples in a single laboratory and analyzing them for PFCs and other contaminants of concern.

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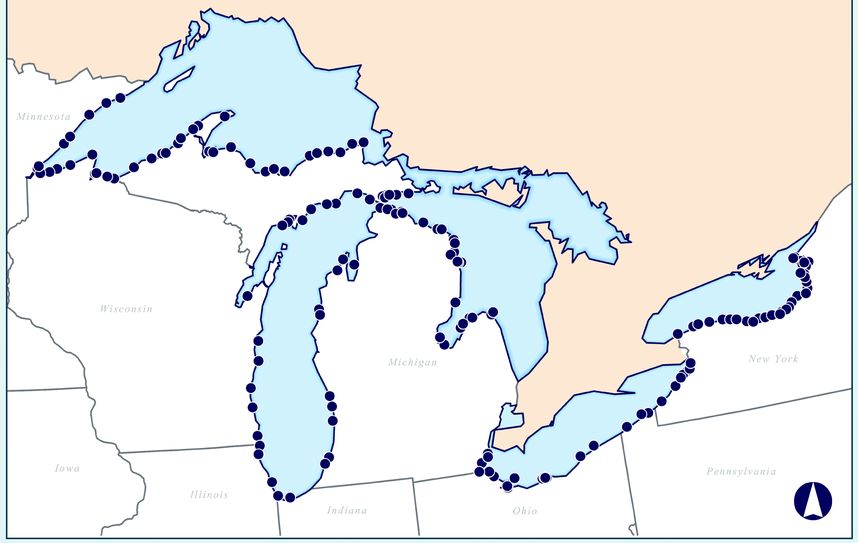
NCCA Great Lakes Human Health Fish Tissue Study Sampling Locations (157)



Analytical Methodology for PFCs

Fish tissue analyses were conducted using procedures developed by the TestAmerica laboratory in West Sacramento, CA. Their PFC procedure involves spiking each sample with a suite of 12 $^{13}C_{12}$-labeled PFCs; extracting the samples using a solution of water, methanol, and sodium hydroxide; subjecting sample extracts to clean up using solid-phase extraction (SPE); spiking PFCs eluted from the SPE cartridge with additional labeled recovery standards, and analyzing them by HPLC-MS/MS. The response for each PFC is compared to the response for a ^{13}C-labeled PFC analog using the technique known as isotope dilution.

Target Analytes

Perfluorinated Compounds				
Name	**Abbreviation**	**Formula**	**CAS Number**	**Labeled Analog**
Perfluorobutyric acid	PFBA	C_3F_7COOH	375-22-4	$^{13}C_4$-PFBA
Perfluoropentanoic acid	PFPeA	C_4F_9COOH	2706-90-3	$^{13}C_5$-PFPeA
Perfluorohexanoic acid	PFHxA	$C_5F_{11}COOH$	307-24-4	$^{13}C_2$-PFHxA
Perfluoroheptanoic acid	PFHpA	$C_6F_{13}COOH$	375-85-9	$^{13}C_4$-PFHpA
Perfluorooctanoic acid	PFOA	$C_7F_{15}COOH$	335-67-1	$^{13}C_4$-PFOA
Perfluorononanoic acid	PFNA	$C_8F_{17}COOH$	375-95-1	$^{13}C_5$-PFNA
Perfluorodecanoic acid	PFDA	$C_9F_{19}COOH$	375-76-2	$^{13}C_2$-PFDA
Perfluoroundecanoic acid	PFUnA	$C_{10}F_{21}COOH$	2058-94-8	$^{13}C_2$-PFUnA
Perfluorododecanoic acid	PFDoA	$C_{11}F_{23}COOH$	307-55-1	$^{13}C_2$-PFDoA
Perfluorobutane sulfonate	PFBS	$C_4F_9SO_3^-$	375-73-5†	*
Perfluorohexane sulfonate	PFHxS	$C_6F_{13}SO_3^-$	355-46-4†	$^{18}O_2$-PFHxS
Perfluorooctane sulfonate	PFOS	$C_8F_{17}SO_3^-$	1763-23-1†	$^{13}C_4$-PFOS
Perfluorooctanesulfonamide	PFOSA	$C_8F_{17}SO_2NH_2$	754-91-6	$^{13}C_8$-PFOSA

† CAS number for the parent acid form
* No labeled analog was available for PFBS, so this analyte was quantified using the response for $^{18}O_2$-PFHxS

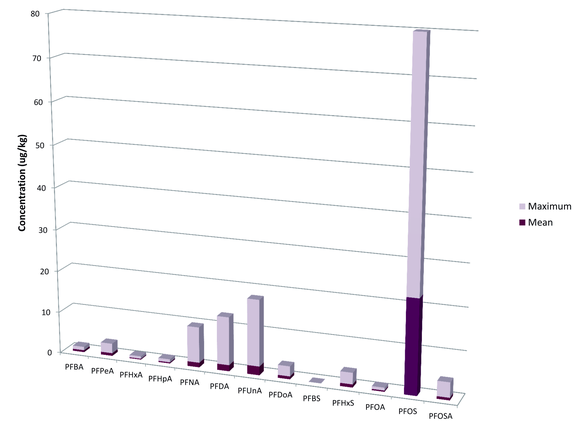
Method Implementation Issues

During tissue analysis, the laboratory noted that recoveries of the 12 labeled analogs spiked into each sample exhibited greater than expected variability. Some samples also seemed to have very low recoveries for the 12 labeled analogs, but other samples in the same batch had acceptable recoveries. The affected sample results were examined to look for correlations with lipid content, collection or preparation dates, and species. Recoveries appeared to be related only to the species of the sample, and samples of salmonid species with high lipid contents were not the samples with uniformly low labeled analog recoveries. Comparative sample extraction and cleanup tests suggested that some component of the tissue matrix extracted from certain fish species (e.g., smallmouth bass, yellow perch, walleye, and suckers) was not completely removed by the extract cleanup procedures. The comparative tests ultimately indicated that the effect was related to the mass of tissue that was extracted.

As a result, the laboratory:

- modified the procedures to extract 1 g of sample instead of the original 5-g aliquot
- concentrated the extract to a smaller final volume to preserve the reporting limits
- analyzed the remaining samples using the smaller sample size, and reanalyzed smaller aliquots of the earlier samples with unusually low recoveries
- noted that recoveries of $^{13}C_8$-PFOSA remained lower than recoveries of the other 11 labeled analogs in some samples
- confirmed that the recovery correction inherent in the use of isotope dilution quantitation yielded results for the unlabeled PFOSA that met project requirements (based on matrix spike and matrix spike duplicate samples prepared with each batch).

Mean and Maximum PFC Concentrations in Great Lakes Fish (Preliminary Unweighted Data)



Ion Current Profiles for PFOS and its Labeled Analog