Evaluation of legacy and replacement PFAS in human livers banked over the past 20 years

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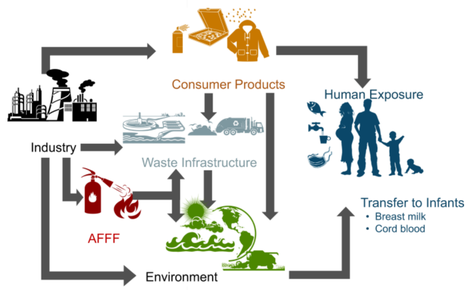
ABSTRACT

Per- and polyfluoroalkyl substances are a class of nonbiodegradable synthetic environmental toxicants with broad uses. Over the past 25 years >12,000 PFAS have been produced, but just 31 have been evaluated for toxicity. Legacy PFAS, PFOA and PFOS, are highly persistent, deposit in human liver, and are associated with hepatoxicity. Little is known whether emerging PFAS accumulate and increase toxicity to human liver. We hypothesize that legacy PFAS concentrations will lower, but other emerging PFAS concentrations will be higher and more frequently detected in adult human livers collected in recent years; and that total liver PFAS concentrations will be similar in recent years vs. livers from 20+ years ago. Herein, we have banked human livers from 2000 (n=100) versus 2018-2020 (n=100), with PFAS measurement and analysis underway. A pilot feasibility study was performed to develop a highly sensitive solid phase extraction-liquid chromatography mass spectrometry method for PFAS extraction/detection. 15 PFAS were detected at concentrations ranging from 0.05 to 10.5 ng/g of liver for specimens collected in 2000 (n=6) and 2018-2020 (n=4). A larger number and diversity of replacement PFAS was observed in livers from 2018-2020. Pilot data suggests that PFNA, PFDA, PFUdA, 7:3 FTCA and PFBS liver concentrations are increasing while legacy PFAS are slightly decreasing. The trends observed thus far appear to be concurrent with known past and current commercial PFAS production levels.

INTRODUCTION

- PFAS are synthetic environmental toxicants widely used due to their oil-, stain-, and water-repellent properties.
- 85,000 tons composed of >118 PFAS are actively produced in the United States annually (EPA report 508_2021.09.08).
- The liver is a site of deposition for numerous PFAS in many species, including human (Bassler et al. 2019), rat (Lau et al. 2007), and fish (Taniyasu et al. 2003).
- Exposure to some PFAS (i.e., PFOS and PFOA) has been associated with liver injury which includes apoptosis and altered serum adipocytokines (Bassler et al. 2019), and increased liver function biomarkers such as ALT and AST (Gallo et al. 2012).
- There is limited knowledge about whether PFAS levels are declining in the liver.
- This study aims to understand PFAS bioaccumulation patterns in human liver; and whether these are different now, than they were in the early 2000s.



Sunderland et al. J Expo Sci Environ Epidemiol. 2019,29: 131–147.

Figure 1. PFAS exposure pathways for humans. Exposure arises from the industrial production of PFAS which results in chemical exposure via drinking water, soil, agriculture, wastewater treatment plants, food contact materials, through the transfer from mother to offspring via cord blood and breast milk etc.

Table 1. Human serum elimination half-lives for PFAS

PFAS Name	abbr.	Half-life
Perfluorobutanesulfonic acid	PFBS	665 hr. (Olsen et al. 2009)
Perfluorohexanesulfonic acid	PFHxS	8.5 yr. (Olsen et al. 2007)
Perfluorooctanesulfonic acid	PFOS	5.4 yr. (Olsen et al. 2007)
Perfluorobutanoid acid	PFBA	72 - 81 hr. (Chang et al. 2008)
Perfluoro-n-pentanoid acid	PFPeA	Unknown
Perfluorohexanoic acid	PFHxA	32 d (Russell et al. 2013)
Perfluoroheptanoic acid	PFHpA	1.2 yr. (Zhang et al. 2013)
Perfluorooctanoic acid	PFOA	3.8 yr. (Olsen et al. 2007)
Perfluorononanoic acid	PFNA	4.3 yr. (Zhang et al. 2013)
Perfluorodecanoic acid	PFDA	12 yr. (Zhang et al. 2013)

HYPOTHESIS

Legacy PFAS concentrations will have decreased, but other emerging PFAS concentrations increased and are more frequently detected in human livers collected in recent years; and that the total sum of specimen PFAS concentrations will be similar in recent years versus livers from 20+ years ago

MATERIALS and METHODS

Study population. Exempt normal human livers (N=10) were acquired from the Liver Tissue Distribution System (LTDS) Center from the Department of Laboratory Medicine and Pathology at the University of Minnesota (Minneapolis, MN, USA). Portions of livers collected in the years 2000 (n=6) and 2018-2020 from de-identified liver transplant recipients during surgery were obtained and processed for PFAS tissue quantification via liquid-chromatography mass spectrometry (LC-MS).

Liver PFAS extraction, and LC-MS/MS quantification. Approximately 1 g of liver was homogenized in 3 mL of LC-MS grade water; and a 1 mL aliquot was spiked with 50 µL of a 40 ng/mL PFAS internal standard mixture and digested with 8 mL of freshly prepared 0.01 N sodium hydroxide in methanol. Next, samples were sonicated for 1 hour, centrifuged, and a 4 mL aliquot was diluted (1:9) with 36 mL of water, and processed for weak anion exchange-solid phase extraction on a Waters™ 3 cc, 50 mg (60µm) sorbent SPE Oasis WAX cartridge (Milford, MA). Next, samples were evaporated under nitrogen flow, and a 40 µL aliquot was diluted with 60 µL of 10 mM ammonium acetate buffer in an autosampler vial prior to LC-MS/MS analysis. Targeted PFAS quantification was conducted on a SCIEX ExionLC AC UHPLC system coupled to a SCIEX X500R quadrupole time-of-flight tandem mass spectrometer (Toronto, Canada) in negative ESI, MRM HR mode. The mobile phase consisted of 10 mM ammonium acetate/water (A) and 10 mM ammonium acetate/methanol(B). Data was acquired using SCIEX OS-Q Data Processing and Reporting Software version 2.0.0.45330

RESULTS

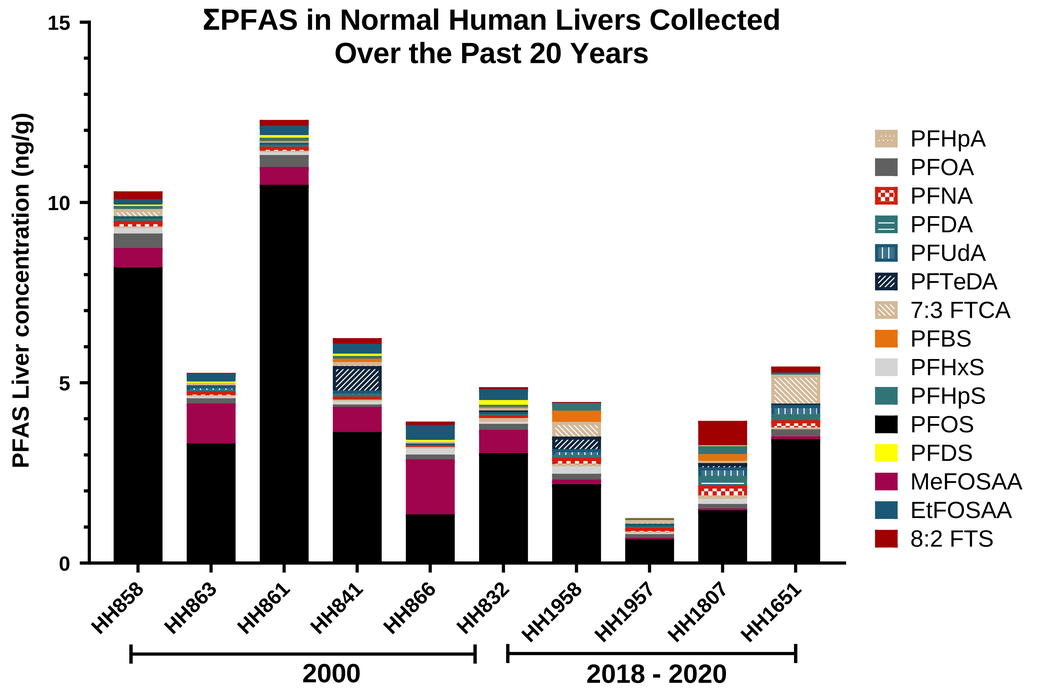


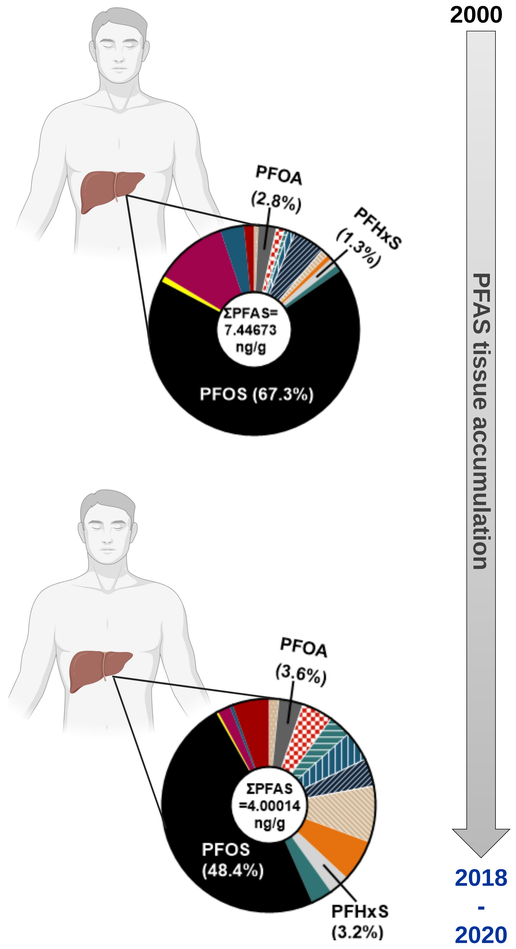
Figure 2.Targeted quantification of PFAS concentrations in adult livers. In this pilot feasibility study, banked exempt adult liver samples were acquired from the Liver Tissue Distribution System(LTDS) Center from the Department of laboratory Medicine and Pathology at the University of Minnesota (Minneapolis, MN, USA). Portions of livers collected from de-identified liver transplant recipients during surgery (n=10) were obtained and processed for PFAS tissue quantification via liquid-chromatography mass spectrometry. Data presented herein represents summary data (Mean ± SEM) of all measured PFAS in the specimen of each person – "PFAS fingerprint".

Table 1. Characteristics of per- and polyfluoroalkyl substances in adult liver (N=10)

Chemical (abbreviation)	Overall detection rate, n (%)	Detection rate in samples collected in 2000, n (%)	Liver concentration in samples collected in 2000, mean (ng/g) ± SEM	Detection rate in samples collected in 2018-2020, n (%)	Liver concentration in samples collected in 2018-2020, mean (ng/g) ± SEM
Perfluorooctanoic acid (PFOA)	10 (100)	6 (100)	0.207 ± 0.130	4 (100)	0.145 ± 0.038
Perfluoroheptanoic acid (PFHpA)	4 (40)	1 (16.67)	<LOQ	3 (75)	0.071 ± 0.020
Perfluorononanoic acid (PFNA)	9 (90)	5 (83.33)	0.106 ± 0.031	4 (100)	0.189 ± 0.074
Perfluorodecanoic acid (PFDA)	5 (50)	2 (33.33)	0.068 ± 0.006	3 (75)	0.170 ± 0.089
Perfluoroundecanoid acid (PFUdA)	10 (100)	6 (100)	0.079 ± 0.028	4 (100)	0.182 ± 0.084
Perfluorotetradecanoid acid (PFTeDA)	3 (30)	1 (16.67)	<LOQ	2 (50)	0.233 ± 0.161
Perfluorooctanesulfonic acid (PFOS)	10 (100)	6 (100)	5.009 ± 3.526	4 (100)	1.938 ± 1.180
Perfluorobutanesulfonic acid (PFBS)	3 (30)	1 (16.67)	<LOQ	2 (50)	0.252 ± 0.086
Perfluorohexanesulfonic acid (PFHxS)	8 (80)	6 (100)	0.100 ± 0.047	2 (50)	0.171 ± 0.045
Perfluoroheptanesulfonic acid (PFHpS)	6 (60)	4 (66.67)	0.073 ± 0.014	4 (100)	0.218 ± 0.001
Perfluorodecanesulfonic acid (PFDS)	4 (40)	4 (66.67)	0.080 ± 0.034	0 (0)	<LOQ
N-Methylperfluorooctanesulfonamido acetic acid (MeFOSAA)	9 (90)	6 (100)	0.835 ± 0.403	3 (75)	0.087 ± 0.038
N-ethyl perfluorooctanesulfonamido acetic acid (EtFOSAA)	6 (60)	6 (100)	0.271 ± 0.088	0 (0)	<LOQ
7:3 Fluorotelomer carboxylic acid (7:3 FTCA)	8 (80)	4 (66.67)	0.109 ± 0.066	4 (100)	0.342 ± 0.343
8:2 Fluorotelomer sulfonic acid (8:2 FTS)	7 (70)	5 (83.33)	0.135 ± 0.062	2 (50)	0.423 ± 0.365

Limit of quantification (LOQ) is defined as chemical concentrations <0.05 ng/g.

CONCLUSION



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Future Studies

- Use untargeted high-resolution mass spectrometry to characterize additional PFAS types accumulating in adult human livers over the past 20+ years as a continuation of the present study.

Acknowledgements

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