# FluoroCouncil Critical Review

## "Accumulation of Perfluoralkyl Substances In Human Tissues" *Environment International* 59 (2013) 354–362

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Pérez et al. reported levels of perfluorohexanoic acid (PFHxA) in limited numbers of autopsied samples of human tissues, including brain, liver, lung, bone and kidney, with highest levels being reported in liver and brain. When analyzing the data, individual samples do not appear internally consistent or within the range of published literature. For example, a liver sample from one individual had a relatively high concentration of PFHxA, but the brain sample from the same individual had a low concentration of PFHxA. In addition, data presented in the Pérez et al. paper on levels of PFHxA would, overall, be an outlier within current literature reporting levels of PFHxA in various human matrices. The article, as published, has several analytical method shortcomings. Specifically, the authors use an analytical method - turbulent flow chromatography - that has not been validated for human tissue analysis. This method has been used by the pharmaceutical industry to analyze human fluid samples, urine and plasma, on a large scale, but not solid tissues. In validating this method, the Perez et al. lab used pig tissue instead of the human tissue, which is the matrix of the study, and failed to report the recovery data in the publication. Recovery data in the range of 80-120% is considered of scientific value and publishable in this type of experiment, but there is no way to know whether or not this range has been respected. Overall, the data reported by Pérez et al. can be considered an anomaly and further research is needed before any definite conclusion can be made as to whether PFHxA accumulation occurs in human tissues.

### FluoroCouncil Comments

Perez et al. report for the first time PFASs accumulation in various human tissues from the same subjects. The authors present data on 21 different PFASs in 99 samples of autopsy human tissues, including brain, liver, lung, bone and kidney from 20 different individuals. All subjects were reported to have lived in Tarragona (Catalonia, Spain) for at least 10 years prior to death and ranged in age from 28 to 83 years. Sample collection was reported to occur within 24 hours of death and samples were stored at -20°C, but there is no information on the state or preservation of cadavers prior to sample collection. Further details were unknown regarding sample collection.[1] The samples were initially solvent extracted and followed by online purification by turbulent flow chromatography and were then analyzed by liquid chromatography coupled to tandem mass spectrometry. Using this method the authors most notably reported maximum median levels of perfluorobutanoic acid (PFBA) in kidney and lung at concentrations of 263 and 807 ng/g respectively, PFHxA in liver and brain at 68.3 ng/g (range: 353 – DL) and 141 ng/g (range: 486-10), respectively, while the highest levels of perfluorooctanoic acid (PFOA) were reported in bone at 20.9 (234- DL) ng/g.

[1] Because these tissues were collected post-mortem and complete autopsy details are missing, it is unclear whether the reported accumulations in the tissues actually occurred in the tissues or the result of blood in the tissues. After death the vasculature breaks down and the maintenance of blood vessels is lost. Thus, the results may simply reflect blood levels of the compounds rather than accumulation of the material. This is particularly suspect in the brain values where it is unknown whether the breakdown of the blood brain barrier occurs after death and if so, the "accumulation" of PFASs reported in the brain may be the result of breakdown of the vasculature and not the result of accumulation. Additionally the section(s) of the brain analyzed were not indicated in the paper. While the brain is a highly vascularized organ, certain regions are more vascular than others.

In regards to sample acquisition and storage conditions, the paper does not discuss specific conditions around tissue collections or storage prior to acquiring the samples. In addition, there was a discrepancy in the paper in regards to storage temperature of the samples.

In a previous publication, using the same method, the lab used two enrichment columns including the Thermo Fisher Scientific: Cyclone (50 mm×0.5 mm, 60 μm particle size, 60 Å pore size) and C18 XL (50 mm×0.5 mm, 60 μm particle size, 60 Å pore size), connected in tandem (Llorca, 2012). However, the analytical procedure published in the Pérez et al. paper indicated the use of one column.

From the details of the analytical procedure stated in the Pérez et al. paper, the accuracy and precision of the analytical method is not clear. In a previous publication by the same working group, the analytical method and recovery, along with accuracy and precision data are provided in much more detail (Llorca, 2012). The method used for the sample purification step in this publication, turbulent flow chromatography (TFC), has not previously been published as a method to analyze human tissue samples. The method has been used mainly by the pharmaceutical industry to analyze human urine and plasma samples on a large scale. The paper by Pérez et al. states that the lab used only pig tissue for standardization and recovery experiments given the small volume of human tissue available (2 g per sample, ½ gram for each analysis). No validation experiment was performed with human control tissues. Given the differences in these two matrices, using pig tissue to quantify data for human tissue samples could lead to inappropriate interpretation of data.

Typically, recovery data in the range of 80-120% is considered of scientific value and publishable in this type of experiment. The authors failed to report on recovery data in the publication.

The Principal Component Analysis (PCA) conducted using the data generated in this paper attempted to compare concentration levels of different substances. The data set had a small sample size (20 individuals) and, therefore, was insufficient and inconclusive. Also, this type of analysis is typically used on soil and sediment values, not tissue sample concentrations.

More specifically, and in regards to the actual data, the concentrations of PFHxA reported for brain and liver tissues of the same individuals in the Pérez et al paper do not reflect consistent results for PFASs accumulation in human tissues. When comparing ratios of brain- to liver- concentrations reported in the paper per individual subject, there is a 1000-2000 fold difference among individuals. The data indicate instances in which individual liver samples had a relatively high concentration of PFHxA, but the brain sample from the same individual had a low concentration of PFHxA and vice versa. Samples from individuals Nr. 5, 9, and 11 had no detectable PFHxA in liver, but had the highest levels in brain in this study. One would not expect such large differences of PFHxA concentrations in liver and brain tissues from the same individual.

The data presented in the Pérez et al. paper, specifically, levels of PFHxA-seem not to be consistent with current literature reporting levels of PFHxA in various human matrices. For example, Erisson et al (2007) published a pilot study measuring PFHxA in human blood samples of individuals living within Catalonia, Spain, the same target region of Spain within the Pérez paper, but did not find any detectable PFHxA in any sample. In a 2011 publication by Pérez, the author did not report detectable levels of PFHxA in human hair samples, and in only 3 out of 30 human urine samples was PFHxA detectable.

Pérez et al. discuss dietary exposure as a major route of human exposure to PFASs within the Catalonia region. However, Domingo et al (2012) conducted a study that calculated total intake of PFHxA from food sources in Catalonia, Spain, and showing only 2 out of 12 analyzed food groups with any detectable PFHxA level. The levels of PFHxA Pérez et al. reported in liver, kidney lung, brain and bone does not correlate with aforementioned studies of food sources in the same region.

Perez et. al. indicated that the data presented in this article "…should be beneficial for the development of theoretical PBPK models, whose validation is still incomplete"(p.258). The data presented by Perez et al., however, only shows a snapshot of PFASs in human post mortem tissue at best. In order to perform PBPK studies blood levels and data from fresh tissue are needed. Therefore, the statement by Perez et al. is misleading in that it infers that the data presented is useful in developing human models for the compounds. (See also Fabrega 2015 and FluoroCouncil's critical review of Fabrega et al.)

Overall, the data reported by Pérez et al. can, at best, be considered an anomaly. Several issues of unresolved analytical uncertainties have become obvious. Based on the small sample size, use of a technique with limited validation and inconsistent findings, and compared to the previous publications, the data for PFHxA presented in this paper are unrealistic and cannot be considered valid documentation for the potential for PFHxA to accumulate in human tissues. Further, the small volume of human tissue used in this study per sample and the lack of proper standardization and recovery data shed major doubt on the values reported. Further research is needed before any definite conclusion can be made in particular as to whether PFHxA accumulation occurs in human tissues.

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