Letter

Radiosynthesis and biological distribution of [F]-labeled perfluorinated alkyl substances.

Jennifer L. Burkemper, Tolulope A Aweda, Adam J Rosenberg, David Lunderberg, Graham F. Peaslee, and Suzanne E. Lapi

Environ. Sci. Technol. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acs.estlett.7b00042 • Publication Date (Web): 09 Mar 2017

Radiosynthesis and biological distribution of [^{18}F]-labeled perfluorinated alkyl substances.

Jennifer L. Burkemper[1], Tolulope A. Aweda[1], Adam J. Rosenberg[2,3], David M. Lunderberg[4], Graham F. Peaslee[5], Suzanne E. Lapi[1]*

[1]Department of Radiology, University of Alabama at Birmingham- School of Medicine, Birmingham, AL 35294

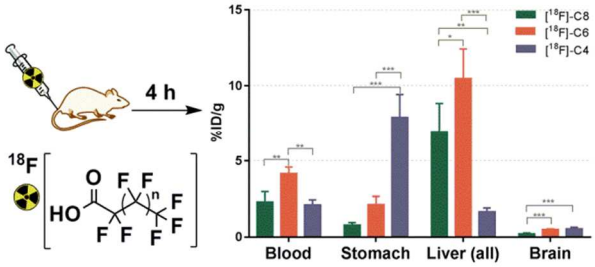
[2]Mallinckrodt Institute of Radiology, Washington University School of Medicine in St. Louis, St. Louis, MO 63110

[3]Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, TN, 37235

[4]Chemistry Department, Hope College, Holland, MI 49423

[5]Department of Physics, University of Notre Dame, Notre Dame, IN 46556

TABLE OF CONTENTS GRAPHICS.



ABSTRACT.

A novel method for radiolabeling perfluorinated alkyl substances (PFAS) with fluorine-18 has been developed, and after purification, the stability and biological distribution in healthy mice was evaluated. Three PFAS, [^{18}F]PFOA (C8), [^{18}F]PFHxA (C6), and [^{18}F]PFBA (C4) were readily labeled and isolated in average yields between 12 and 31%. The stability of each compound was monitored in 0.1% ammonium hydroxide (NH_4OH) in methanol, in saline, and in human, mouse and rat sera. The amount of intact, radiolabeled PFAS was determined by radiometric instant thin layer chromatography and was calculated by the amount of free fluorine-18 observed over time. All compounds were highly stable in 0.1% NH_4OH in methanol and saline, with <10% defluorination observed out to 4 h. Interestingly, each compound had differing affinities for the serum proteins. *In vivo* biodistribution studies in mice showed uptake in all organs examined, with the highest uptake being exhibited in the liver for both [^{18}F]PFOA and [^{18}F]PFHxA, and the stomach for [^{18}F]PFBA. The results of this initial study suggest that this method could be valuable in helping to determine the biological uptake of any PFAS in mammals.

INTRODUCTION.

While there is significant literature precedent discussing the distribution of varying chain lengths and derivatives of perfluorinated alkyl substances PFAS in tissues, both animal[1-2] and human[3-5], this is a difficult assessment to make because of the difficulty of PFAS analysis in biological matrices. PFAS can be difficult to measure analytically in biological systems, because analysis traditionally involves isolating the

tissues, digestion of the tissue, and extraction of the compounds. It subsequently involves the use of highly sensitive techniques such as HPLC-MS/MS[6-8] or combustion-IC[9] as the PFAS family of compounds have no convenient UV-visible signatures. To make these measurements in living biological systems, one common technique is the use of radioisotopic labeling, but there has been very little published using radiolabeled PFAS to assess biodistribution. To date, the only studies include [^{35}S]-labeled perfluorosulfonic acid (PFOS)[10] and [^{14}C]-labeled perfluorooctanoic acid (PFOA)[11-13] but no [^{18}F]-labeled derivatives. In this paper we present the first radiolabeling of PFAS with fluorine-18 ($t_{1/2}$ = 110 min), which allows for tracking the biodistribution of these compounds in real time using positron emission tomography (PET), and precise quantification of the amount of radiolabeled compound inside organs and tissues of interest. The ease with which any PFAS compound could be radiolabeled with [^{18}F]F^- is chemically distinct from the significant organic synthesis techniques required to correctly label PFAS with either ^{35}S or ^{14}C. This technique allows for radiolabeling and subsequent purification in less than one hour.

The work presented here focuses on the development of the [^{18}F]-labeled derivatives of perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHxA) and perfluorobutanoic acid (PFBA). While any representative PFAS could have been studied, the proof-of-concept study presented here started with three carboxylic acids of varying chain length. By inducing an $^{19}F \rightarrow {}^{18}F$ exchange, the native compound (i.e. without structural modifications) can be studied and the pharmacokinetic properties of the radiolabeled compounds are identical to the native PFAS of interest. Initial studies to

determine the *in vitro* stability of the radiolabeled compounds and *in vivo* biological distribution in healthy mice were also performed.

EXPERIMENTAL.

Radiosynthesis and isolation of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA.

The radiosynthesis and isolation of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA is described in detail in the supporting information. In summary, ^{18}O-enriched water was bombarded with a 20-MeV proton beam from the University of Alabama medical cyclotron facility at 40 μA. For the amount of starting activity needed, the target was bombarded for ~10 min. The [^{18}F]F^- produced was extracted from the water target by solid-phase extraction and purified for radiosynthesis. Purity of the [^{18}F]PFAS was determined by the amount of free [^{18}F]F^- in solution as determined by radiometric instant thin layer chromatography. Radiosynthesis and purification of each PFAS took approximately 1 hour.

Stability Studies.

The stabilities of the radiolabeled PFAS were tested under a variety of conditions, including: (1) in 0.1% NH_4OH in methanol at room temperature, (2) in saline at room temperature, (3) in mouse serum at 37^{o}C, (4) in human serum at 37^{o}C, and (5) in rat serum at 37^{o}C. For each condition the amount of intact, radiolabeled PFAS was determined at 30 min, 1 h, 2 h, and 4 h with n=3 replicates. With the exception of the 0.1% NH_4OH in methanol solution (as this was what the [^{18}F]PFAS were isolated in), 25

μL of the desired [^{18}F]PFAS was added to 225 μL of the corresponding solution (saline or sera) in a 1.5 mL Eppendorf microcentrifuge tube and incubated as the temperatures described above. The amount of activity added to tubes was dependent on final isolation yields. For these studies, 7.4 MBq (200 μCi) [^{18}F]PFOA, 7.03 MBq (190 μCi) [^{18}F]PHxA, and 3.3 MBq (90 μCi) [^{18}F]PFBA were used.

In both the NH_4OH and saline studies, a 1 μL aliquot of the solution was removed at each time point and spotted for iTLC analysis (0.1% NH_4OH in methanol mobile phase). The serum stability studies were performed for each condition twice, using two different methods of analysis. In the first method of analysis, a 1 μL aliquot of the solution was removed for iTLC analysis at each time point. In the second method of analysis, a 50 μL aliquot was taken from each tube and the serum proteins were precipitated with the addition of 150 μL of acetonitrile. The aliquots were vortexed and the proteins were pelleted via centrifugation. The supernatant, containing the non-protein bound compound, was collected and the pellet was further washed with another 150 μL of acetonitrile. The wash was combined with the original supernatant. The combined supernatants (total collected volume, ~ 300 μL) and the protein pellet were each counted using an automated Wizard^2 2480 gamma counter (Perkin Elmer). The data were analyzed and are presented as the percentage (of total) bound to serum proteins. Afterwards, the supernatant was also analyzed via iTLC in the same manner as previously outlined.

Biodistribution studies in normal mice.

All animal studies were conducted in compliance with the guidelines for the care and use of research animals established by the University of Alabama's Institutional Animal Care and Use Committee. After radiolabeling, each respective [^{18}F]PFAS was diluted into saline to a final concentration of ~ 0.074 MBq (2 μCi) /μL. Healthy, male CD1 mice (n=4 per compound) were then intravenously injected with an average of 7.22 MBq (195 μCi), approximately 100 μL, of the desired [^{18}F]PFAS. Animals were anesthetized with 0.1 % isofluorane and euthanized at 4 h post injection and organs and tissues of interest were harvested, weighed, and the radioactivity was measured using an automated gamma counter. Data were decay corrected and calculated as the percent injected dose per gram of tissue (%ID/g).

RESULTS AND DISCUSSION.

Radiosynthesis and isolation of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA.

All three PFAS tested were successfully radiolabeled with [^{18}F]F^- and isolated. Initial attempts to synthesize [^{18}F]PFOA were performed in anhydrous acetonitrile, but the radiochemical yields were not consistent. The [^{18}F]PFOA reaction mixture had to be dissolved in upwards of 500 μL of acetonitrile in order to account for solvent loss (due to evaporation) during the radiolabeling process. The larger reaction volume also resulted in decreased overall yields that varied widely. The problem with radiolabeling [^{18}F]PFOA was solved by performing the reaction in 300 μL of DMSO, at 125^{o}C and thus this method was used for the remaining two PFAS. Additionally, results from data (not shown), indicated that at lower temperatures (~100^{o}C) the exchange reaction did not proceed with PFBA or PFHxA.

Previously published work has shown that fluoride (non-radioactive) can be separated and purified from PFAS on WAX cartridges by washing the retained fluoride off with 25 mM NH_4OAc (pH 4.5), while still retaining PFAS.[6, 9] This non-radioactive method for purification should transfer easily to the radioactive samples. However, before attempting to purify the products, the retention of free [^{18}F]F- on the WAX cartridges was determined. Fluorine-18 was diluted in water and loaded directly onto a freshly prepared WAX cartridge. On average, 31 $\pm$ 8% (n=3) of the loaded activity was trapped onto the cartridge. After the cartridge was washed with 4 mL of 25 mM NH_4OAc (pH 4.5), it was determined that less than 2% of the initially loaded activity was retained. It was concluded that this was an acceptable method for purification of the [^{18}F]PFAS compounds from any unreacted [^{18}F]F- remaining in the reaction mixture.

On average, [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA were radiolabeled and isolated in decay-corrected yields of 13 $\pm$ 9% (n = 8) , 31 $\pm$ 9% (n = 3), and 12 $\pm$ 3% (n = 2) respectively. The purities of the compounds were examined via iTLC. **Figure 1**

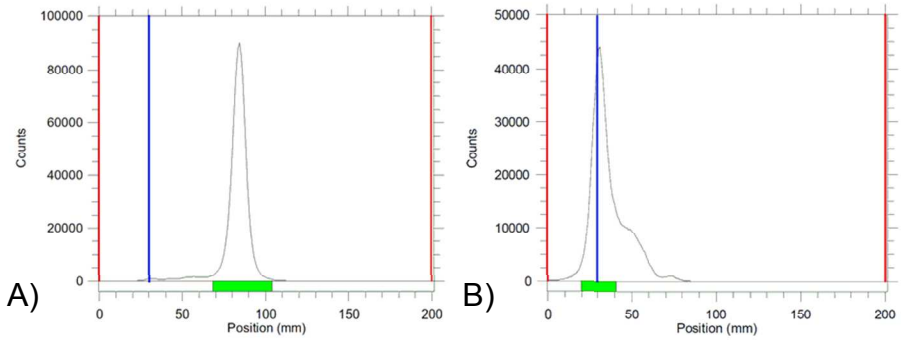


Figure 1. An example of iTLC results for (A) [^{18}F]PFHxA (Rf = 1) and (B) free [^{18}F]F$^-$ (Rf = 0). The origin is marked with a blue line. Instant TLC strips were developed in 0.1% NH_4OH in methanol.

shows iTLC comparisons between [^{18}F]PFHxA in 0.1% NH_4OH in methanol (A) and free [^{18}F]F$^-$ in the K_2CO_3 solution (aq) (B). All three [^{18}F]PFAS compounds migrate with the

mobile phase to ~85 mm (R_f = 1.0) and unreacted [^{18}F]F^- stays at the origin, ~30 mm (R_f = 0). Assuming all of the non-radioactive PFAS was co-eluted with radiolabeled product during purification, the estimated average specific activities of the final products were calculated to be 60 ± 10 MBq/μmol for [^{18}F]PFOA, 12 ± 4 MBq/μmol for [^{18}F]PFHxA and 3.4 ± 0.4 MBq/μmol for [^{18}F]PFBA. The labeling method has proven to be quite robust and could likely be applied in the future for the radiolabeling of any per- or polyfluorinated compound.

Stability Studies.

While it is known that non-radiolabled PFAS are stable in a variety of environments,[14-15] the stability of the radiofluorinated compounds needed to be assessed. On average, [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA defluorinated <10% over a 4 h period in all conditions, as determined by iTLC analysis. To assess the *in vitro* stabilities of the compounds in biologically relevant systems, the [^{18}F]PFAS were incubated in mouse serum, human serum and rat serum. Literature has shown that each of the compounds has a different biological half-life *in vivo* in these systems.[16]

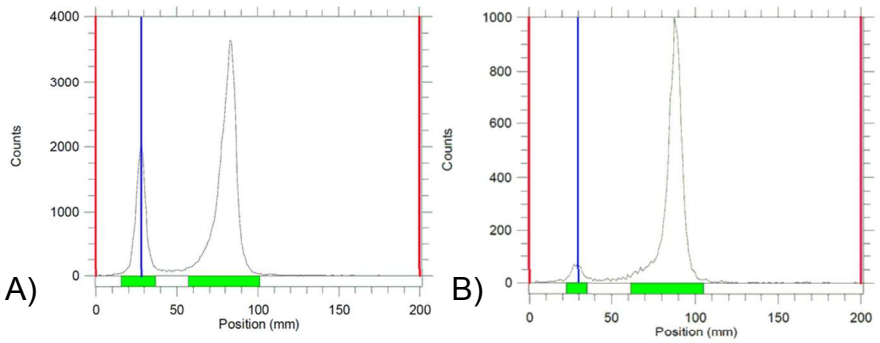


Figure 2. (A) Shows the iTLC result of the [^{18}F]PFHxA/rat serum mixture at 1 h after the end of synthesis. Rf of [^{18}F]PFHxA = 1, Rf of [^{18}F]PFHxA + serum = 0. (B) Shows the iTLC results of the acetonitrile supernatant of the same [^{18}F]PFHxA/serum mixture after the precipitation of the serum proteins. The origin is marked with a blue line.

Initially the serum stability studies were carried out in the same manner as the stability tests in saline and 0.1% NH_4OH, by removing aliquots and analyzing them directly using iTLC. However, the results were convoluted, see **Figure 2 (A),** as the protein peak observed at 30 mm had the same R_f as free $[^{18}F]F^-$. Thus, a second method of analysis (via protein precipitation) was used in order to properly determine the true serum stability of each compound. **Figure 2 (B)** illustrates the iTLC results of the acetonitrile supernatant after the proteins were precipitated and removed from the mixture. Both the supernatant and the protein pellet were assessed for radioactivity. The small peak observed at 30 mm is most likely free fluorine, however the possibility of protein bound $[^{18}F]$PFHxA cannot be ruled out as this precipitation method does not guarantee complete precipitation of the proteins.[17] The results of the serum binding studies of $[^{18}F]$PFAS with mouse serum are presented in **Figure 3**. Results of the three $[^{18}F]$PFAS in rat and human sera are included in the supporting information.

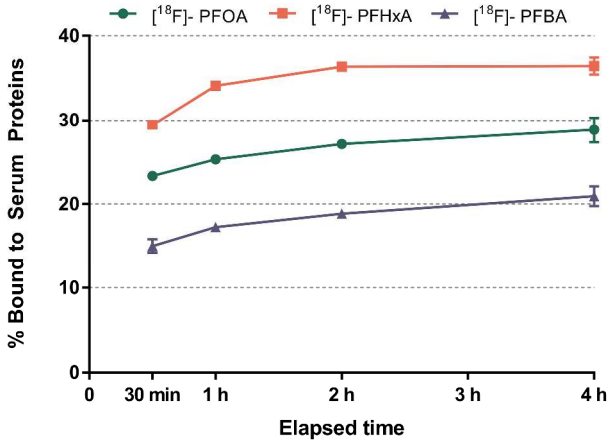


Figure 3. Results of the serum stability studies with $[^{18}F]$PFOA, $[^{18}F]$PFHxA and $[^{18}F]$PFBA in mouse serum. The data were calculated as the percent of total activity bound to the serum proteins.

Biodistribution studies in normal mice.

The biodistribution studies of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA were carried out in healthy mice. Fifteen different organs and tissues were dissected and assayed 4 h post-injection using a gamma counter to quantitatively determine the uptake of the tracer. This figure does not represent all the activity injected. Urine and feces were not collected in this study but would account for the remainder of activity not represented. A comparison of the biodistribution results of each tracer is seen in **Figure 4.**

Each of the tracers exhibited some degree of uptake in all the organs and tissues of interest that were tested, including the brain. The highest uptake of [^{18}F]PFOA observed was in the liver, with 7 $\pm$ 2 %ID/g, however similar amounts were observed in the femur (4 $\pm$ 1 %ID/g) and lungs (4 $\pm$ 2 %ID/g). Uptake of [^{18}F]PFHxA was highest in the liver and femur with 10 $\pm$ 2 %ID/g and 5 $\pm$ 1 %ID/g, respectively. It should be stated that free [^{18}F]F^- is known to accumulate in bone[18-20] however it is not known at this time if the compounds were accumulating in the bone marrow or bone matrix.[21-22] Studies are ongoing to determine the “state”, free [^{18}F]F^- or intact [^{18}F]PFAS, and location of the PFAS compounds in the femur. Unlike the other two compounds, the highest uptake of [^{18}F]PFBA was found in the stomach, with 8 $\pm$ 2 %ID/g. The liver showed a 4 to 6 fold decrease in uptake with just 1.7 $\pm$ 0.7 %ID/g when compared to [^{18}F]PFOA and [^{18}F]PFHxA.

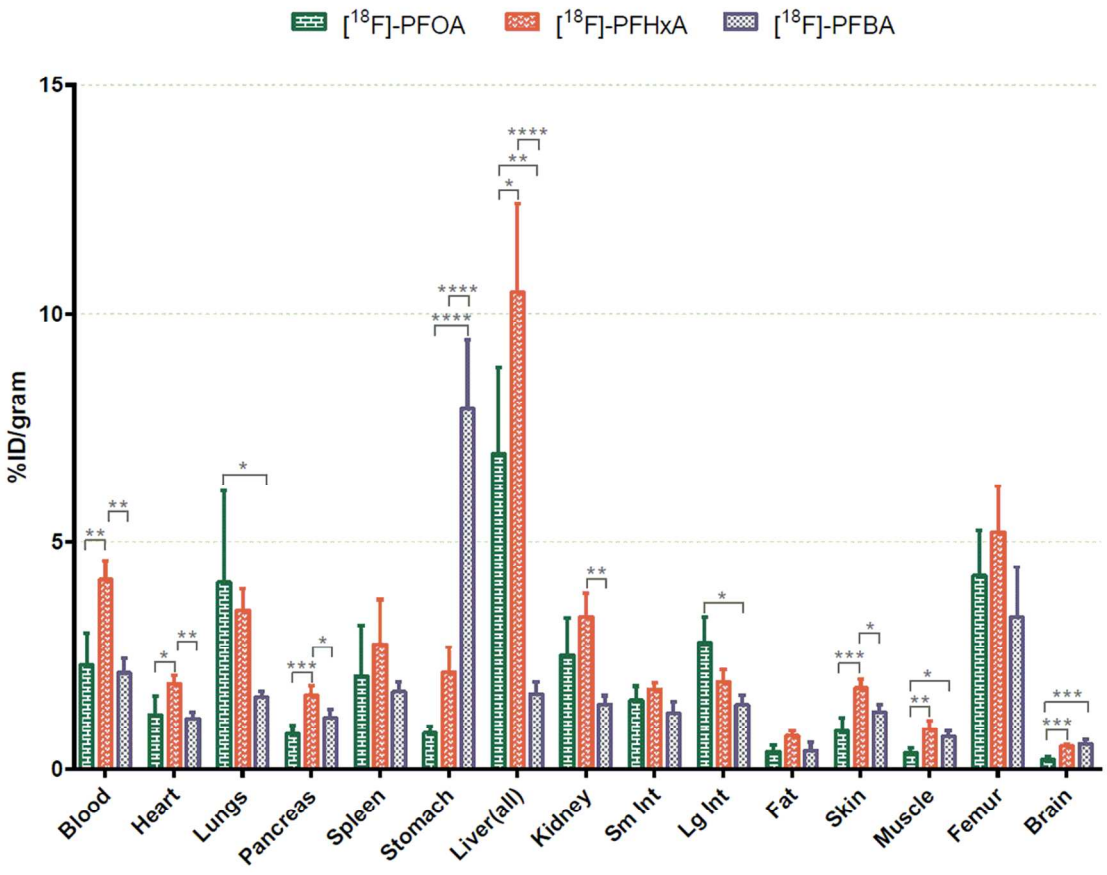


Figure 4. The complete biodistribution results of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in healthy mice, 4 hours after injection. The results are presented as the percent of injected dose per gram of tissue. Error bars are indicative of the standard deviation (n=4). Statistical analysis was performed to determine statistical differences (p values). Data are presented in table format in the supporting information, **Table S1**. * $p < 0.05$, ** $p < 0.008$, *** $p < 0.0006$, **** $p < 0.0001$.

Implications

The method development described here describes the first successful radiolabeling of three forms of PFAS with fluorine-18, its apparent stability in biological media, and its preliminary biodistribution study in mice. It appears likely that any PFAS that can be synthesized and isolated could be radiolabeled in such a manner and used to directly measure uptake and biodistribution kinetics in biological systems. Since ^{18}F has a relatively short half-life, it is less likely to be useful for elimination kinetics studies, but it

does open the possibility of directly measuring uptake in human subject volunteers, since trace amounts of the compounds are easily measurable and the radioactivity short-lived. The only previous human biodistribution study, Perez *et.al.*,[4] used cadavers, although there are some similarities found in the mouse biodistribution reported here. Similarly, this novel tool for studying PFAS behavior could be used in environmental remediation studies to measure the fate of radiolabeled compounds in environmental treatment systems. Further studies are needed for different PFAS compounds as well as different biological and environmental systems to assess the full impact of this novel radiosynthetic method.

ASSOCIATED CONTENT

Supporting Information

Radiosynthesis and isolation of [^{18}F]PFAS; serum stability study in rat and human serum (Figure S1); biodistribution results of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in healthy mice (Table S1).

AUTHOR INFORMATION.

*Corresponding Author

*E-mail: lapi@uab.edu. Telephone (205) 975-8689. Address 1924 6th Ave. S., WTI 310F, Birmingham, AL 35244 (S.E.L.)

Orcid

Suzanne E. Lapi, 0000-0003-0090-0191

Notes

The authors declare no competing financial interest.

FUNDING SOURCES.

This research was supported through funding from the Department of Radiology at the University of Alabama at Birmingham. Funding support was also provided by the National Science Foundation (RUI-PHY-1613188).

ACKNOWLEDGMENTS.

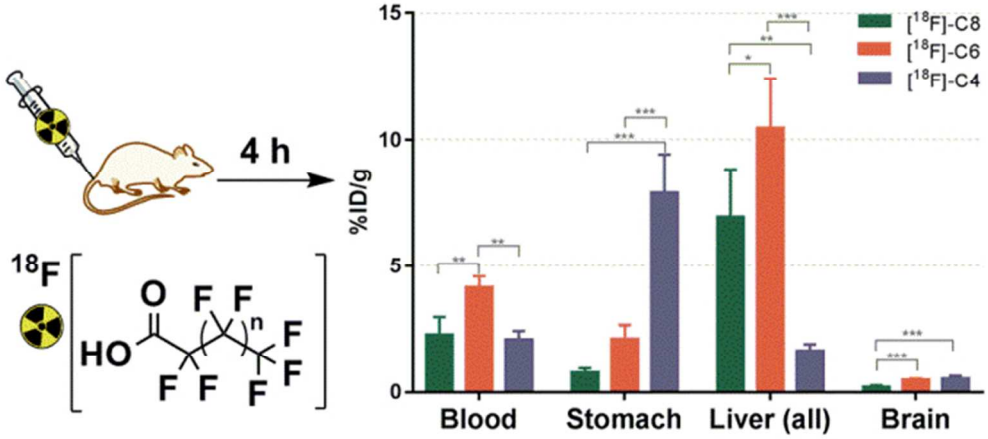
The authors would like to thank the UAB cyclotron facility isotope production team, including Harvey Doane, Jason Rider and Brian Brooks, for the production of fluorine-18.

REFERENCES.

1. Kudo, N.; Bandai, N.; Kawashima, Y., Determination of perfluorocarboxylic acids by gas-liquid chromatography in rat tissues. *Toxicol. Lett.,* **1998,** *99* (3), 183-190, doi: 10.1016/S0378-4274(98)00154-4.
2. Szostek, B.; Prickett, K. B., Determination of 8:2 fluorotelomer alcohol in animal plasma and tissues by gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2004,** *813* (1-2), 313-321, doi:10.1016/j.jchromb.2004.10.031.
3. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C., Biological monitoring of polyfluoroalkyl substances: A review. *Environ. Sci. Technol.,* **2006,** *40* (11), 3463-3473, doi: 10.1021/es052580b.
4. Perez, F.; Nadal, M.; Navarro-Ortega, A.; Fabrega, F.; Domingo, J. L.; Barcelo, D.; Farre, M., Accumulation of perfluoroalkyl substances in human tissues. *Environ. Int.,* **2013,** *59*, 354-362, doi:10.1016/j.envint.2013.06.004.
5. Olsen, G. W.; Burris, J. M.; Ehresman, D. J.; Froehlich, J. W.; Seacat, A. M.; Butenhoff, J. L.; Zobel, L. R., Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in

retired fluorochemical production workers. *Environ. Health Perspect.,* **2007,** *115* (9), 1298-1305, doi:10.1289/ehp.10009.
6. Taniyasu, S.; Kannan, K.; So, M. K.; Gulkowska, A.; Sinclair, E.; Okazawa, T.; Yamashita, N., Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *J. Chromatogr. A,* **2005,** *1093* (1-2), 89-97, doi:10.1016/j.chroma.2005.07.053.
7. Taniyasu, S.; Kannan, K.; Yeung, L. W.; Kwok, K. Y.; Lam, P. K.; Yamashita, N., Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2-C4) in precipitation by liquid chromatography-tandem mass spectrometry: comparison to patterns of long-chain perfluorinated acids (C5-C18). *Anal. Chim. Acta,* **2008,** *619* (2), 221-230, doi:10.1016/j.aca.2008.04.064.
8. Trojanowicz, M.; Koc, M., Recent developments in methods for analysis of perfluorinated persistent pollutants. *Mikrochim. Acta,* **2013,** *180*, 957-971, doi:10.1007/s00604-013-1046-z.
9. Miyake, Y.; Yamashita, N.; Rostkowski, P.; So, M. K.; Taniyasu, S.; Lam, P. K.; Kannan, K., Determination of trace levels of total fluorine in water using combustion ion chromatography for fluorine: a mass balance approach to determine individual perfluorinated chemicals in water. *J. Chromatogr. A,* **2007,** *1143* (1-2), 98-104, doi:10.1016/j.chroma.2006.12.071.
10. Sundstrom, M.; Bogdanska, J.; Pham, H. V.; Athanasios, V.; Nobel, S.; McAlees, A.; Eriksson, J.; DePierre, J. W.; Bergman, A., Radiosynthesis of perfluorooctanesulfonate (PFOS) and perfluorobutanesulfonate (PFBS), including solubility, partition and adhesion studies. *Chemosphere,* **2012,** *87* (8), 865-871, doi:10.1016/j.chemosphere.2012.01.027.
11. Vanden Heuvel, J. P.; Kuslikis, B. I.; Van Rafelghem, M. J.; Peterson, R. E., Tissue distribution, metabolism, and elimination of perfluorooctanoic acid in male and female rats. *J Biochem. Toxicol.,* **1991,** *6* (2), 83-92, doi:83-92, 10.1002/jbt.2570060202.
12. Katakura, M. K., N.; Tsuda, T.; Hibino, Y.; Mitsumoto, A.; Kawashima, Y., Rat organic anion transporter 3 and organic anion transporting polypeptide 1 mediate perfluorooctanoic acid transport. *J. Health Sci.,* **2007,** *53* (1), 77-83, doi: 10.1016/j.toxlet.2009.07.011.
13. Lupton, S. J.; Huwe, J. K.; Smith, D. J.; Dearfield, K. L.; Johnston, J. J., Absorption and excretion of 14C-perfluorooctanoic acid (PFOA) in Angus cattle (Bos taurus). *J. Agric. Food Chem.,* **2012,** *60* (4), 1128-1134, doi:10.1021/jf2042505.
14. Liu, J.; Mejia Avendano, S., Microbial degradation of polyfluoroalkyl chemicals in the environment: a review. *Environ. Int.,* **2013,** *61*, 98-114, doi:10.1016/j.envint.2013.08.022.
15. Hekster, F. M.; Laane, R. W.; de Voogt, P., Environmental and toxicity effects of perfluoroalkylated substances. *Rev. Environ. Contam. Toxicol.,* **2003,** *179*, 99-121, doi: 10.1007/0-387-21731-2_4.
16. Russell, M. H.; Nilsson, H.; Buck, R. C., Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey. *Chemosphere,* **2013,** *93* (10), 2419-2425, doi:10.1016/j.chemosphere.2013.08.060.

17. Polson, C.; Sarkar, P.; Incledon, B.; Raguvaran, V.; Grant, R., Optimization of protein precipitation based upon effectiveness of protein removal and ionization effect in liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.,* **2003,** *785* (2), 263-275, doi:10.1016/S1570-0232(02)00914-5.
18. Blau, M.; Nagler, W.; Bender, M. A., Fluorine-18: a new isotope for bone scanning. *J. Nucl. Med.,* **1962,** *3*, 332-334.
19. Blau, M.; Ganatra, R.; Bender, M. A., 18 F-fluoride for bone imaging. *Semin. Nucl. Med.,* **1972,** *2* (1), 31-37, doi:10.1016/S0001-2998(72)80005-9.
20. Czernin, J.; Satyamurthy, N.; Schiepers, C., Molecular mechanisms of bone 18F-NaF deposition. *J. Nucl. Med.,* **2010,** *51* (12), 1826-1829, doi:10.2967/jnumed.110.077933.
21. Giesy, J. P.; Kannan, K., Perfluorochemical surfactants in the environment. *Environ. Sci. Technol.,* **2002,** *36* (7), 146A-152A, doi: 10.1021/es022253t.
22. Lau, C.; Butenhoff, J. L.; Rogers, J. M., The developmental toxicity of perfluoroalkyl acids and their derivatives. *Toxicol. Appl. Pharmacol.,* **2004,** *198* (2), 231-241, doi:10.1016/j.taap.2003.11.031.



Radiosynthesis and biological distribution of [^{18}F]-labeled perfluorinated alkyl substances

Jennifer L. Burkemper, Tolulope A. Aweda, Adam J. Rosenberg, David M. Lunderberg, Graham F. Peaslee, Suzanne E. Lapi*

*Corresponding author

181x83mm (96 x 96 DPI)

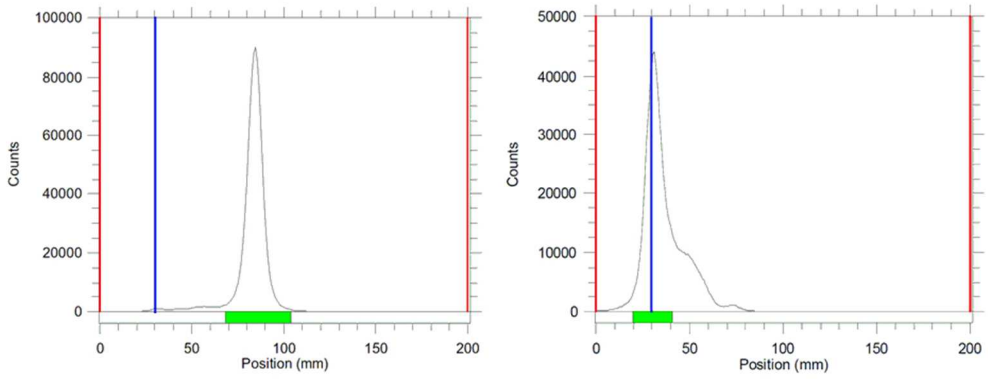


Figure 1. An example of iTLC results for (A) [^{18}F]PFHxA (Rf = 1) and (B) free [^{18}F]F^- (Rf = 0). The origin is marked with a blue line. Instant TLC strips were developed in 0.1% NH_4OH in methanol.

79x31mm (300 x 300 DPI)

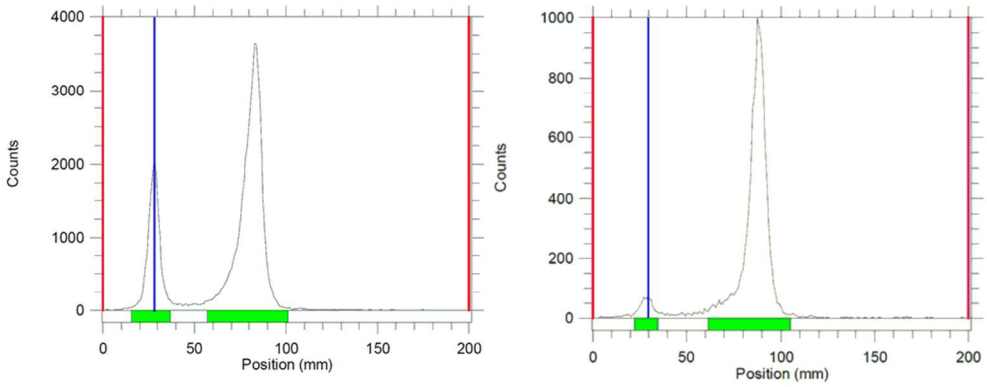


Figure 2. (A) Shows the iTLC result of the [^{18}F]PFHxA/rat serum mixture at 1 h after the end of synthesis. Rf of [^{18}F]PFHxA = 1, Rf of [^{18}F]PFHxA + serum = 0. (B) Shows the iTLC results of the acetonitrile supernatant of the same [^{18}F]PFHxA/serum mixture after the precipitation of the serum proteins. The origin is marked with a blue line.

88x35mm (300 x 300 DPI)

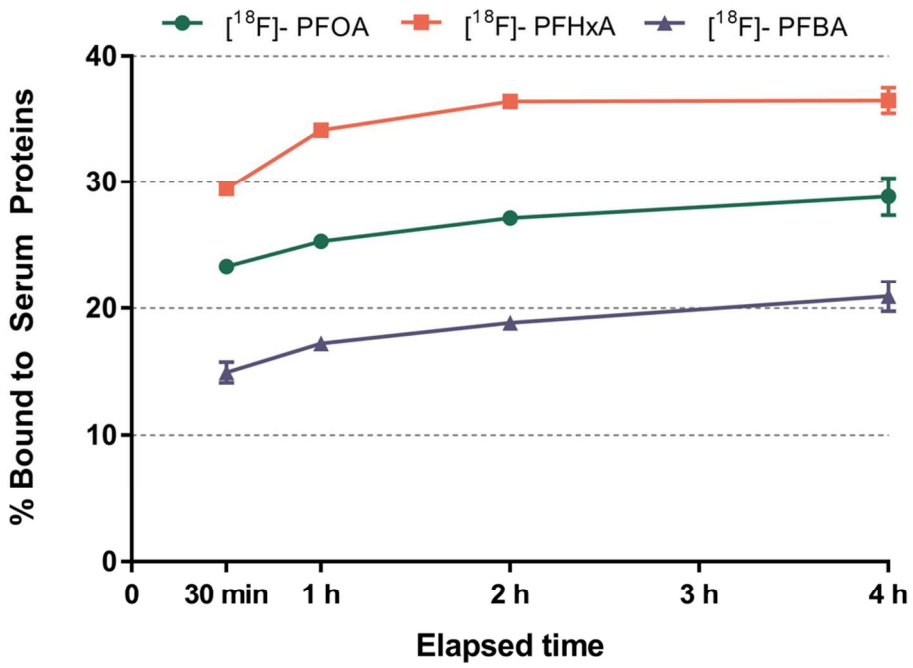


Figure 3. Results of the serum stability studies with [^{18}F]PFOA, [^{18}F]PFHxA and [^{18}F]PFBA in mouse serum. The data were calculated as the percent of total activity bound to the serum proteins.

95x67mm (300 x 300 DPI)

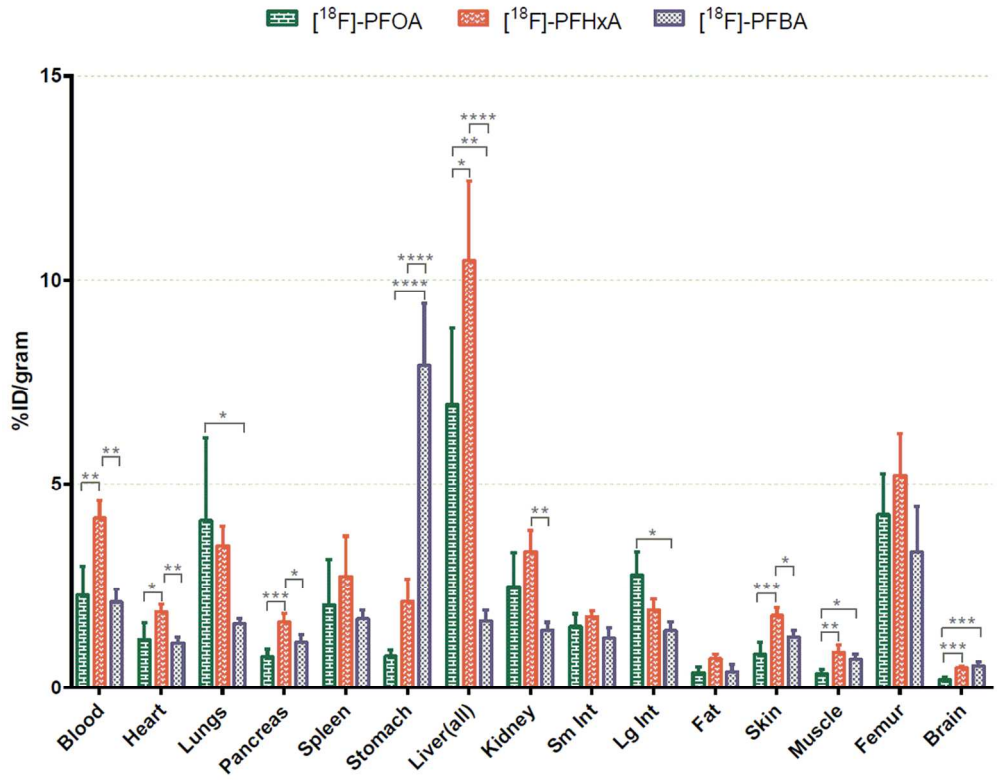


Figure 4. The complete biodistribution results of [^{18}F]PFOA, [^{18}F]PFHxA, and [^{18}F]PFBA in healthy mice, 4 hours after injection. The results are presented as the percent of injected dose per gram of tissue. Error bars are indicative of the standard deviation (n=4). Statistical analysis was performed to determine statistical differences (p values). Data are presented in table format in the supporting information, Table S1. * $p < 0.05$, ** $p < 0.008$, *** $p < 0.0006$, **** $p < 0.0001$.

135x106mm (300 x 300 DPI)