This article "New Concerns about PFAS in Food" has been reprinted with the kind permission of International Labmate Ltd – first printed in International Labmate, February 2021, Volume 46 Issue 1 – www.intlabmate.com.

Author – David C. Kennedy, Ph.D.

Per and Polyfluorinated Alkyl Substances (PFAS) are well-known environmental contaminants that have a newly recognized potential to taint certain food products through agricultural consumption via environmental transport from contaminated industrial sites [1]. The analysis of PFAS in food products requires more extensive analytical preparation techniques, compared to PFAS testing of simple matrices such as drinking water, in order to reduce the impact of sample matrix interferences on the subsequent instrumental analysis. An example is provided of a PFAS method applicable to milk, butter, cheese, and fish.

The PFAS in Food Prequel

Per and Polyfluorinated Alkyl Substances (PFAS) are an extensive family of synthetic, fluorochemicals with a unique set of physical and chemical properties. These properties have resulted in their widespread commercial use over the past 50 years in diverse applications ranging from firefighting foams to stain-resistant carpet to grease-proof pizza boxes. However, these same unique physical and chemical properties also have been found to bear serious environmental consequences: widespread dispersion ability, extreme environmental persistence, and a high degree of bioaccumulation [2]. Although PFAS do not exhibit acute toxic properties, researchers have found that PFAS can demonstrate a large number of subtle, chronic health effects, primarily affecting the endocrine and reproductive systems. Consequently, health experts have long been concerned that low-level, cumulative exposure to PFAS over an extended period of time could have serious health consequences

[3]. Therefore, chronic lifetime PFAS exposure pathways – such as through food or drinking water – are of particular concern to regulators and are receiving enhanced scrutiny.

Initial Concerns Regarding PFAS in Food

In the US, the initial US Food and Drug Administration (FDA) concern about PFAS centered on the contamination of food products through contact with PFAS containing food packaging (and to a lesser extent with food processing equipment). The classic examples are those PFAS coated pizza boxes, fast-food hamburger wrappers, and microwave popcorn bags that have done such a marvelous job of keeping grease off our clothes. That problem was summarily solved in late 2016 when FDA removed the approval for the use of PFAS in food packaging [4].

Likewise, the primary US Environmental Protection Agency (EPA) focus has been on drinking water as a primary source of lifetime PFAS exposure. EPA is continuing to conduct extensive nationwide testing for PFAS in drinking water under the Unregulated Contaminant Monitoring Rule (UCMR) program [5]. These efforts will very likely result in specific regulatory limits for the allowable concentration of certain PFAS in drinking water. Concurrently, other government agencies, such as the US Department of Defense (DOD) have been extensively studying the widespread environmental contamination of military facilities owing to the extensive historical use of PFAS firefighting foams, principally at airbases [6].

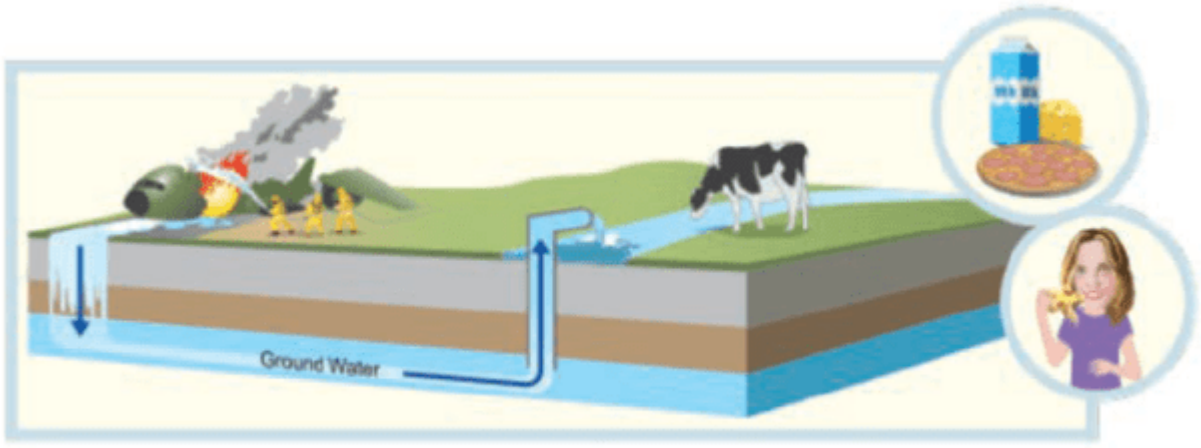

Figure 1. Pathway Model for Environmental Transmission of PFAS to Food and Consumer

This expanded concept of the PFAS problem is clearly a major step forward, but it has presented some analytical challenges. Much of the official PFAS methodology developed over the past decade has been focused on the analysis of drinking water and aimed at a very limited list of analytes. With little challenge from matrix interference, easily surmountable chromatography issues, and straightforward mass spectrometry, these official drinking-water-only methods proved to be inadequate when applied to the analysis of PFAS in soil, sediment, sludge, and wastewater. When applied to the analysis of foods with a myriad of complex matrices, they are quite ineffective, resulting in a surge in PFAS analytical method development centered on complex matrices, with food testing occupying a prominent position. The following section features one such application as an illustration of the approaches now being pursued in pursuit of the expanded PFAS challenge.

Analysis of PFAS in Food such as Dairy Products, Eggs,

and Fish by LC-MS/MS

Method Introduction – The following work was performed through a collaboration between Weck Laboratories, Inc, City of Industry, CA, USA, and Phenomenex, Inc, Torrance, CA, USA, for the development of new sample preparation and analysis procedures for determining low levels of PFAS in food products. This particular application was directed at achieving sub-ppb sensitivity for 23 PFAS analytes in dairy products (milk, butter, and cheese), eggs, and fish as representative of difficult to analyze fatty matrices. The following discussion is a synopsis of the full work [8].

Sample Preparation – One gram of homogenized sample was spiked with internal standards and surrogates and an analyte mix of 23 PFAS compounds (Table 1) at the 1ng/g level, followed by the addition of 10 mL acetonitrile and 10 ml water. Four replicates of each matrix (milk, eggs, butter, cheese, and fish) were prepared. The samples were processed by a modified QuEChERs procedure using a commercial kit (Phenomenex roQ Extraction Kit). An aliquot (500 uL) of the cleaned acetonitrile phase was transferred to an LC vial for analysis. Figure 2 displays an extraction blank and the five sample types following sample preparation.

Table 1. PFAS Analyte List .

Analytes:	1. PFBA	9. PFHpS	17. Et-FOSE
	2. PFPeA	10. PFOS	18. Et-FOSA
	3. PFBS	11. PFNA	19. PFDS
	4. PFHxA	12. FOSA	20. PFUdA
	5. PFHpA	13. Me-FOSE	21. PFDoA
	6. PFHxS	14. 8:2 FTS	22. PFTrDA
	7. 6:2 FTS	15. Me-FOSA	23. PFTeDA
	8. PFOA	16. PFDA	

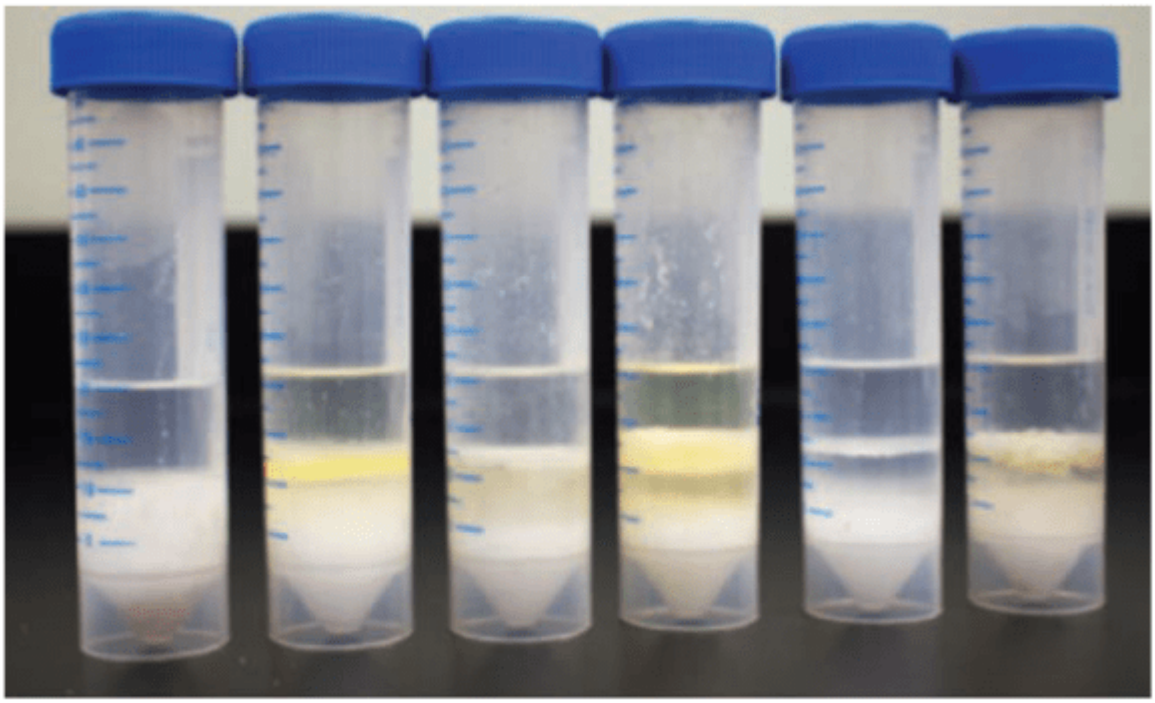

Figure 2. Samples after QuEChERs Cleanup: From left to Right: Blank, Butter, Cheese, Egg, Milk and Fish.

Optional Solid Phase Extraction – A dispersive SPE cleanup was used to achieve a 10-fold

lower level of quantitation. Four replicate samples of the egg matrix were spiked with the PFAS analyte mix at the 0.1ng/g level and processed by the QuEChERs procedure. Following extraction, 500uL of the acetonitrile phase was diluted with 15 mL of water and loaded onto a preconditioned, weak ion-exchange SPE tube (Phenomenex Strata-X-AW 200 mg). The analytes of interest were then eluted with 4 mL of 0.3% NH4OH-acetonitrile. The eluate was evaporated to dryness, reconstituted with 500uL of acetonitrile, and transferred to an LC autosampler vial for analysis. LC-MS/MS Analysis. The chromatography was performed on an Agilent 1290 UHPLC system. The LC column employed was a Phenomenex Luna Omega 1.6 um PS C18 operating at 40ºC with a flow rate of 0.55mL/min and an injection volume of 20 uL. The mass spectrometer used was an Agilent 6460 QQQ. Various LC-MS/MS conditions were explored and an ammonium acetate/acetonitrile gradient (Table 2) proved to be optimum, resulting in a run time of approximately 4 minutes.

Results and Discussion

System calibration showed a linear dynamic response from 0.05 ppb – 1000 ppb with a lower limit of quantization of 0.05 ppb as shown in Figure 3 and a calibration chromatogram at the 0.05 ppb level is shown in Figure 4. Recovery data for the five matrix types are summarised in Figures 5 – 9. Four replicates of each matrix were spiked at the 1 ng/g level and prepared for analysis as described above (but were not subjected to the solid phase extraction process). Figure 10 presents the recovery data for four replicates of the egg matrix spiked at 0.1 ng/g and prepared as described above, but with the addition of the solid phase extraction step to increase method sensitivity.

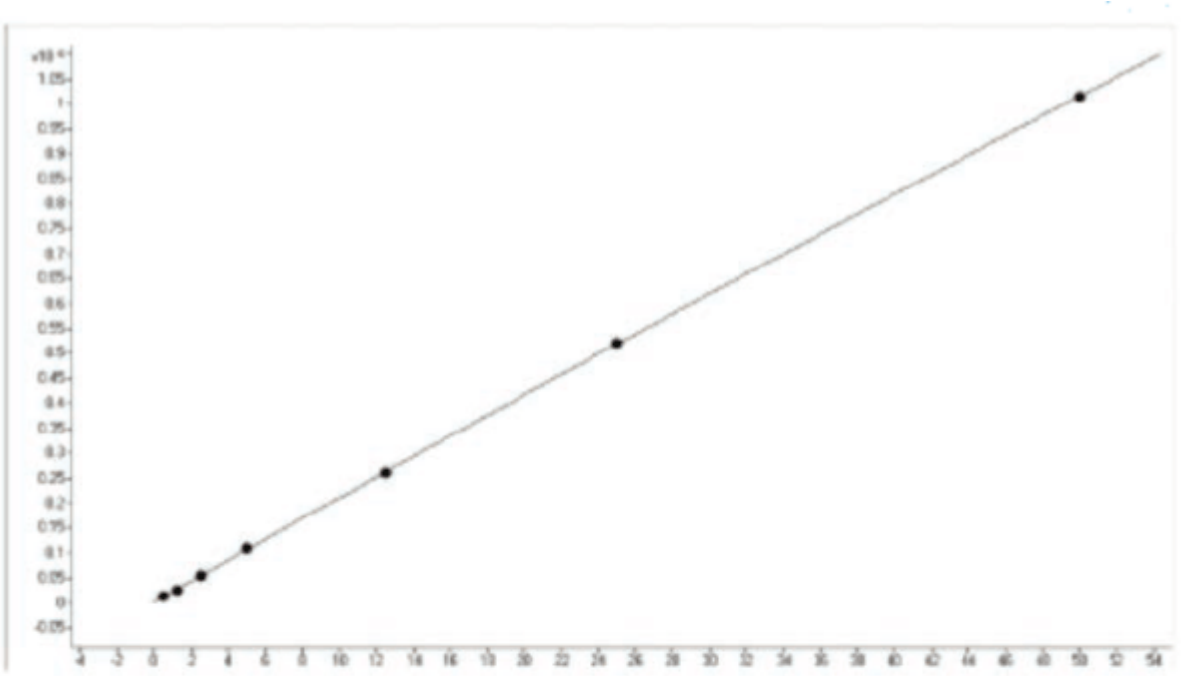

Figure 3. System Calibration Dynamic Range (0.05 – 1000 ppb).

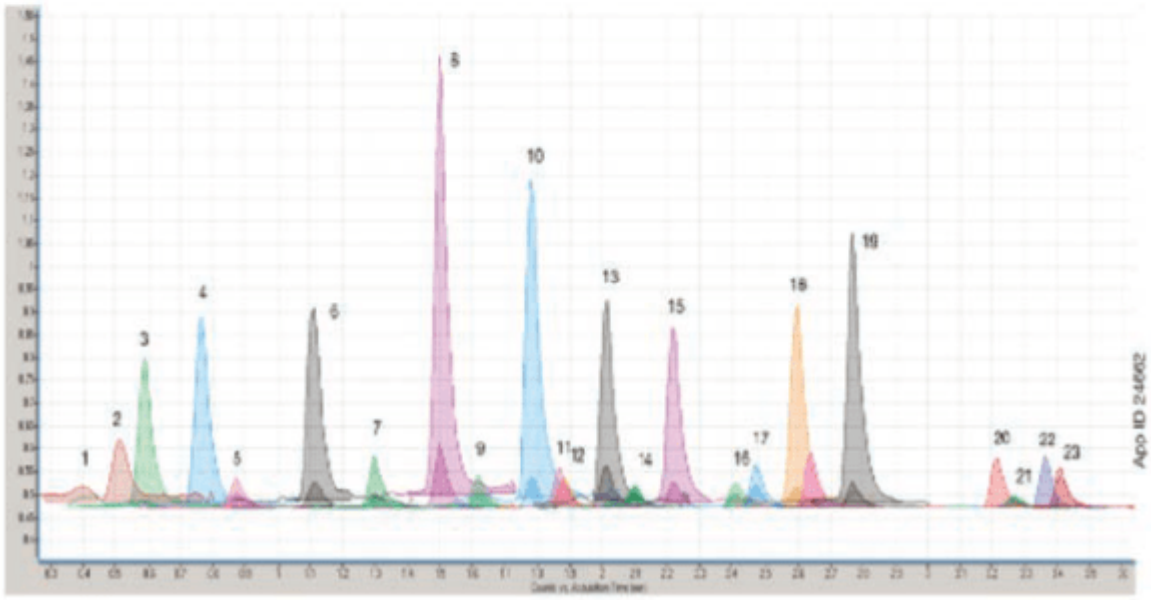

Figure 4. Chromatogram of 0.05 ppb Lower Limit of Quantization Standard.

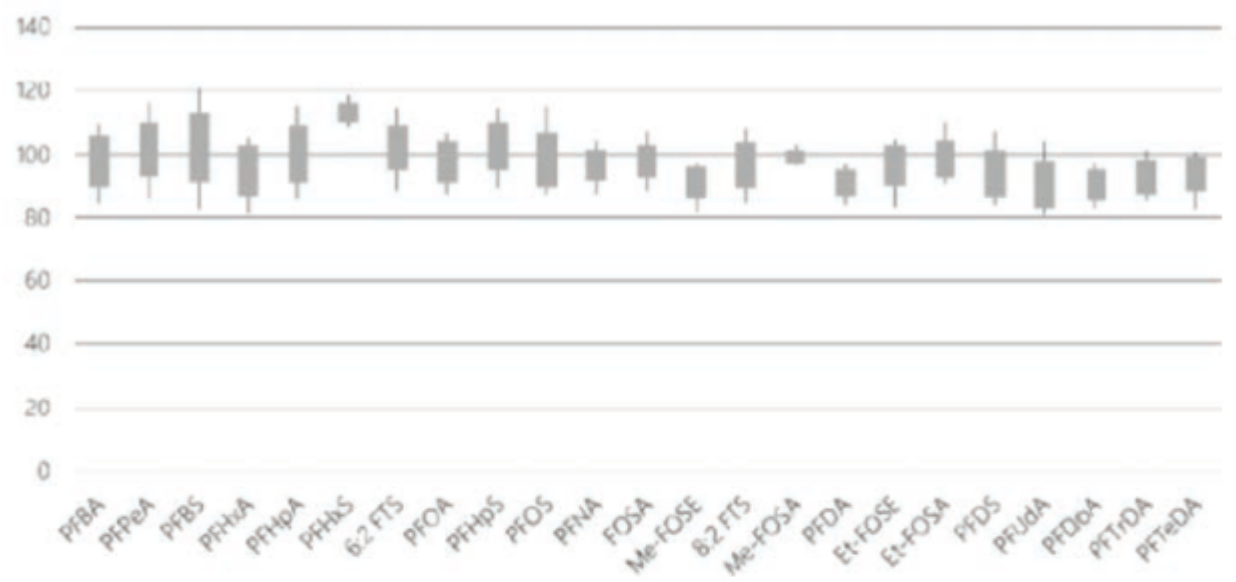

Figure 5. Milk Recoveries (QuEChERs: 1 ng/g, n=4).

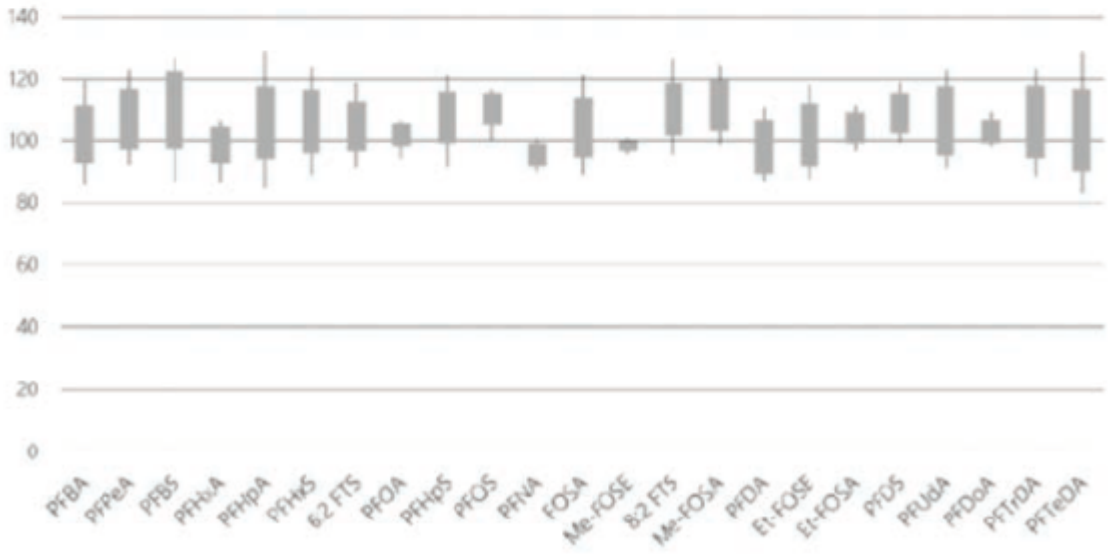

Figure 6. Butter Recoveries (QuEChERs: 1 ng/g, n=4).

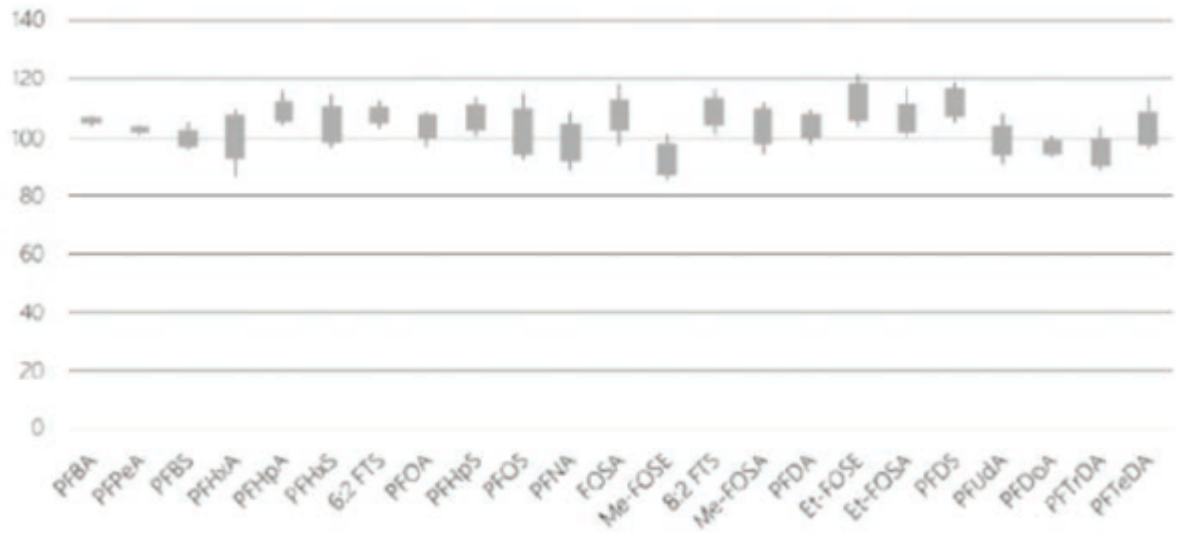

Figure 7. Tuna Recoveries (QuEChERs: 1 ng/g, n=4).

The recovery data show good recovery for all five matrices spiked at the 1ng/g level, with most analytes falling into the 80% – 120% recovery range. Precision is generally somewhat poorer for the higher fat dairy products than for the lower fat matrices. The recoveries on tuna fish are particularly good, considering the complexity of the matrix. In comparing the analyte recoveries from eggs at the 1 ng/g and 0.1ng/g levels (Figure 9 and Figure 10), both show comparable recoveries although, as expected, the higher spike level shows greater precision. Overall, the data suggest that the method has sufficient accuracy and precision to potentially be used to assess environmental PFAS contamination of food products.

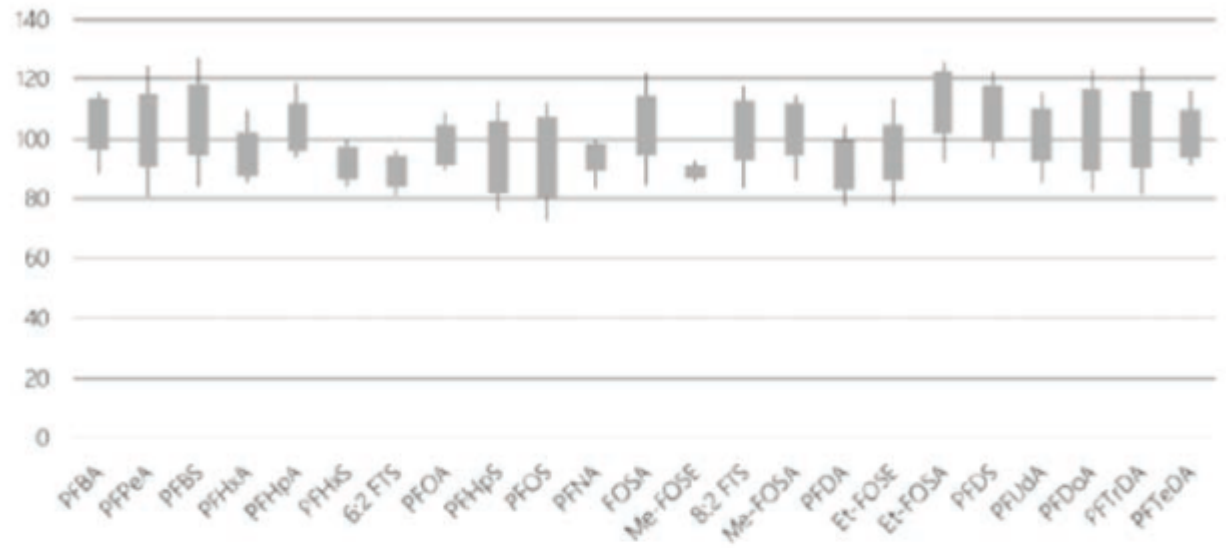

Figure 8. Cheese Recoveries (QuEChERs:1 ng/g, n=4).

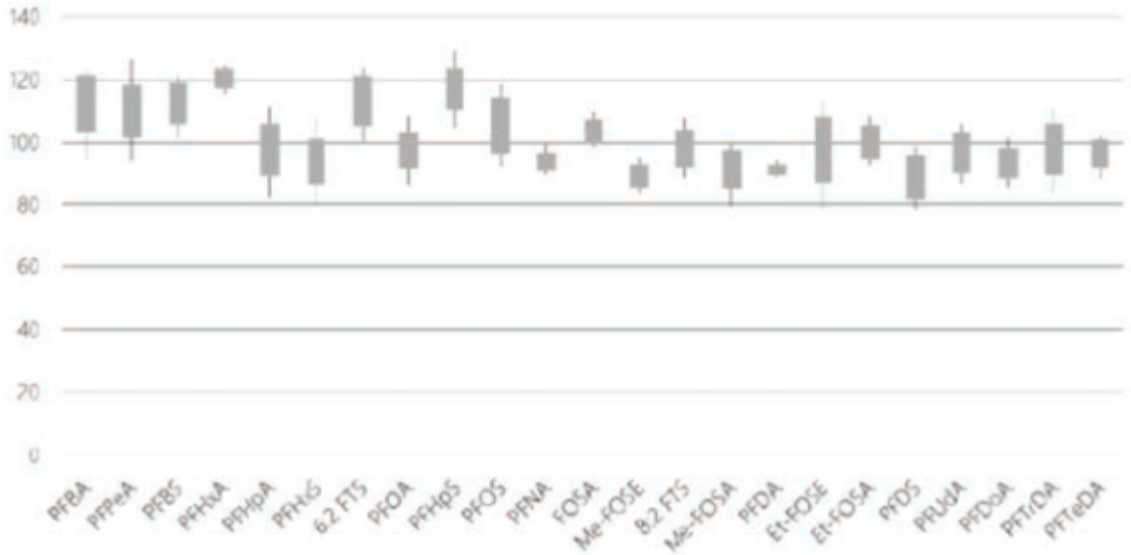

Figure 9. Egg Recoveries (QuEChERs: 1 ng/g, n=4).

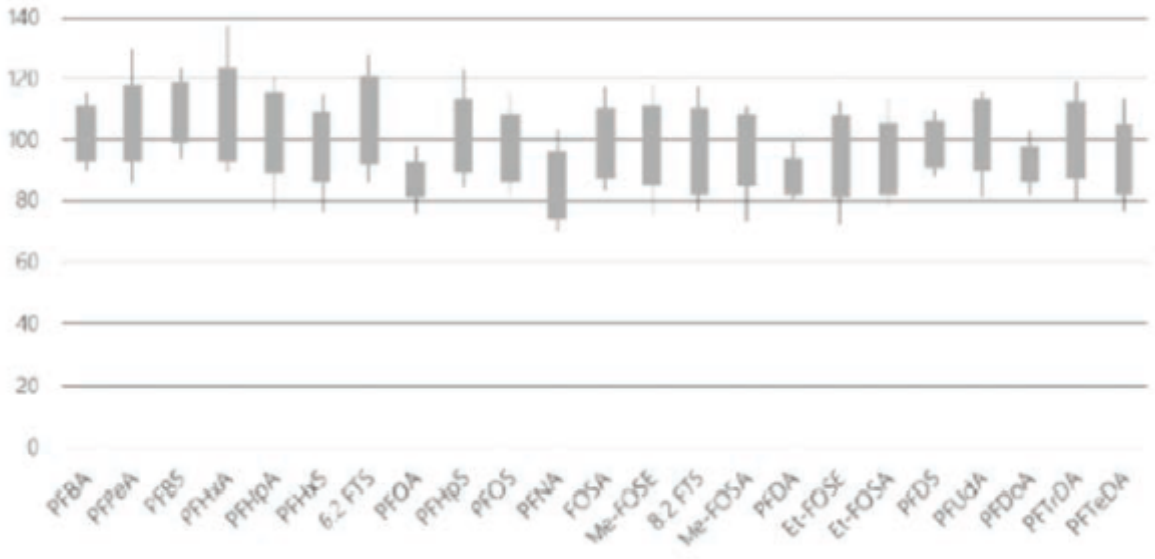

Figure 10. (Egg Recoveries (QuEChERs + SPE: 0.1 ng/g, n=4).

Clearly, this is preliminary data and further development and multi-laboratory validation would be required to demonstrate such a purpose. However, the data clearly show that current sample preparation techniques, coupled with the power of advanced chromatography and triple-quad mass spectrometry represent a suitable workflow.

The Sequel

The earlier discussion showed the use of current analytical technology to address the challenge of environmental PFAS contamination of the food supply. However, care should be taken since the experience with analytical chemistry teaches us that we will inevitably be facing further analytical challenges from the realm of the ‘unknown-unknowns’.

In PFAS analysis, we are currently discussing a target analyte list of 20, 30, or 40 compounds? However, the number of compounds in the PFAS universe has been estimated at 5000 and even as high as 8,000 which doesn’t include potential degradation products.

Toxicity is largely a function of the unique chemical and configurational state of a molecule that controls the biochemical interaction with the organism. So, there is much more analytical work to identify the most important PFAS compounds from a toxicity perspective.

Excellent work is being done with accurate mass and advanced data analysis to give us a broader understanding of the chemical complexity of the PFAS universe. However, given the complexity and extent of the problem of environmental PFAS contamination, it is clear that a lot of hard work has yet to be done.

Acknowledgments

The contribution of Dr. Agustin Pierri and his team at Weck Laboratories, City of Industry, California, USA is gratefully acknowledged.

References

1. *Analytical Results of Testing Food for PFAS from Environmental Contamination. (https://www.fda.gov/food//chemicals/analytical-results-testing-pfas-environmentalcontamination),*
   US Food and Drug Administration, October 20, 2020.
2. *Basic Information about PFAS (https://www.epa.gov/pfas/basic-information-pfas),*
   US Environmental Protection Agency, December 06, 2018.
3. *Toxicological Profile for Perfluoroalkyls (https://www.atsdr.cdc.gov/toxicprofiles/tp.asp?id=1117&tid=237), Agency for Toxic Substances and Disease Registry, Communicable Disease Center, September 30, 2020.*
4. *FDA Removes Approval for the Use of PFCs in Food Packaging Based on the*

Abandonment.
(https://www.fda.gov/food/cfsan-constituent-updates/fda-removesapproval-use-pfcs-food-packaging-based-abandonment), US Food and Drug Administration, November 21, 2016.

5. *Development of the Proposed Unregulated Contaminant Monitoring Rule for the Fifth Monitoring Cycle (UCMR 5): Public Meeting and Webinar (https://www.epa.gov/sites/production/files/2019-10/documents/ucmr5-stakeholdermeeting-190830.pdf), US Environmental Protection Agency, July 16, 2019*
6. *Installations Conducting Assessments for PFAS use or Potential Release. (https://media.defense.gov/2020/Mar/17/2002265608/-1/-1/1/MAP_OF_INSTALLATIONS_CONDUCTING_ASSESSMENTS_FOR_PFAS_USE_OR_POTENTIAL_RELEASE.PDF), US Department of Defense, September 30, 2019.*
7. *Per and Polyfluoroalkyl Substances (PFAS) (https://www.fda.gov/food/chemicals/and-polyfluoroalkyl-substances-pfas), US Food and Drug Administration, December 20,2019.*
8. *Per- and Polyfluorinated Alkyl Substances (PFAS) from Milk, Eggs, Butter, Cheese and Fish using QuEChERs, SPE and LC-MS/MS. Agustin Pierri Weck Laboratories) and*

Scott Krepich (Phenomenex, Inc,). Phenomenex Technical Note TN-0124 (https://phenomenex.blob.core.windows.net/documents/a9406d77-c88e-49cb-9347-81efd828fc25.pdf), Phenomenex, Inc., 2018.