Smart note | 000842

Environmental and food analysis

PFAS data processing: Forever chemicals do not need forever analysis with the right equipment and software

Challenges, from quantitation at low levels to identifying the growing number of new PFAS chemicals and their metabolites, give rise to the fear of missing the next variant of emerging concern.

It is important that your lab is armed with a range of PFAS testing capabilities focused on known targets, confident identification of unknowns, and analysis of emerging threats.

In this Smart Note, we address critical considerations to help ensure your success when meeting the evolving needs for PFAS data analysis.

Overview

Per- and polyfluoroalkyl substances (PFASs) were introduced in the 1940s and were rapidly incorporated into everyday products across multiple industries, from stain and water repellent materials to coatings, paints, and even foams used for fighting fires. Initially, these compounds were thought to be inert and were considered “wonder chemicals.” Their usage was widespread, and now it is estimated that there are well over 10,000 possible PFAS compounds.

The most well-known PFAS compounds, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been extensively studied for their chemical properties and toxicological effects. Both chemicals are persistent in the environment and accumulate in the human body over time. Human exposure to PFASs can be traced to multiple sources, including water, soil, the food chain, processing equipment, and packaging materials.

Numerous strategies can be deployed to provide confidence in your results and ensure consumer safety.

Why is there so much interest in PFASs and related compounds?

Human exposure to PFAS residues has been implicated in the incidence of cancer, obesity, endocrine system disruption, and other adverse health effects. Because PFASs bioaccumulate, are widespread and persistent pollutants (forever chemicals), and have multiple opportunities for human exposure, global regulatory bodies routinely investigate the prevalence of PFAS compounds in the environment.

Why are PFAS so hard to analyze?

Given the ubiquitous nature of PFASs and the large potential range of possible PFAS compounds, there are numerous analytical challenges: from quantitation at low levels to identifying the growing number of new PFAS chemicals (and their metabolites), giving rise to the fear of missing the next variant of emerging concern.

In the laboratory, many containers, sample preparation systems, and analytical systems can contribute towards background PFAS signals; therefore, robust experimental design and strategies to remove or account for background contamination/signals need to be considered to avoid skewing results. However, due to the number of exposure opportunities from processing through manufacturing to delivery, it is difficult to track compounds and understand how they react in unique situations, or if new/novel PFAS are introduced.

There are a range of flexible analytical strategies available to evaluate known PFAS compounds (e.g., liquid chromatography (LC)/gas chromatography (GC) and quadrupole-based mass spectrometry (MS)), as well as methods to capture information about potential unknowns without the need for standards (e.g., combustion ion chromatography (CIC) and LC or GC high-resolution MS).

How can I increase my data processing efficiency and ensure that my analysis has a rapid turnaround time?

Powerful, yet easily adoptable, software is key in ensuring increased productivity for your analytical laboratory, enabling you to address critical challenges today and tomorrow. Software requirements will vary based upon the technologies deployed and laboratory goals but can include compliance tools, networking or enterprise-cloud capabilities, instrument control across a broad range of instrumentation, data automation, intelligent data processing, and more.

Choosing the right software will ensure streamlined training, reduce errors, and minimize potential lab disruption, resulting in awareness of valuable information within your data.

How can I determine what and how many compounds are in potentially complex datasets, while ensuring confidence in these results?

For high-throughput targeted analysis of a predefined list of known PFAS compounds, a triple quadrupole mass spectrometer would be the most suitable option in combination with **Thermo Scientific™ TraceFinder™** software or **Thermo Scientific™ Chromeleon™** Chromatography Data System software. However, if in addition to targeted analysis, there is an interest in screening for and analyzing unknown compounds with the capabilities to retrospectively search for new compounds, a high-resolution accurate mass (HRAM) MS system is a more appropriate option.

The real challenge lies in detecting and identify emerging threats, and this is where **Thermo Scientific™ Compound Discoverer™** software in combination with the **Thermo Scientific™ mzCloud™** mass spectral fragmentation library expedites unknown compound identification, delivering accurate results with reduced turnaround time. Integrating high-quality accurate mass fragmentation information, accurate mass spectral fragmentation libraries and compound databases, and intelligent processing algorithms eliminates time-consuming manual steps when identifying unknowns.

From unknown to known – how can I streamline identification of potential new contaminants?

Acquiring high-resolution MS/MS or MS^n fragmentation data provides data-rich information to assist in unknown identification (Figure 1). **Thermo Scientific™ AcquireX™** intelligent data acquisition workflows can capture MS/MS or MS^n data for all compounds within a given sample, providing complete confidence that you always have mass spectral fragmentation information available, even after the sample is gone.

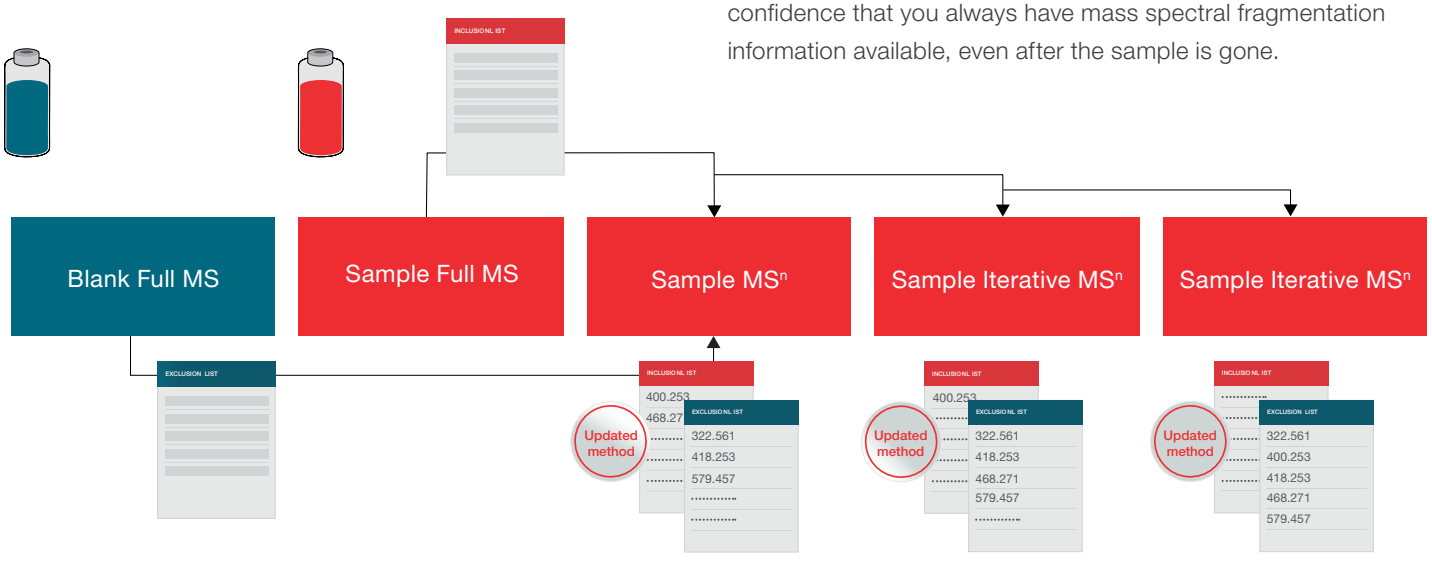

Figure 1. An illustration of the deep scan AcquireX workflow, where the analysis of fragmentation information for all relevant compounds can be fully automated to ensure complete coverage for any given sample where potential unknown PFAS compounds could be present

Analyzing PFAS compounds in environmental matrices, especially untargeted analysis, is really a challenge: these compounds often occur at very low levels in a complex background, so we need high sensitivity as well as rapid and high coverage MS/MS acquisition to get the information to be able to discern and annotate these compounds. Specifically, many of these compounds don't occur in chemical databases.

The combination of the ultra-high resolution and high mass accuracy, with AcquireX data-dependent analysis is a real game changer. We're able to push up to 100% MS/MS acquisition coverage; this really helps us to gain the information we need to be able to annotate those compounds using library matching as well as other annotation tools.

—Dr. Lee Ferguson, Associate Professor of Civil and Environmental Engineering, Duke University

AcquireX workflows and Compound Discoverer software enable you to identify more of your unknowns. Together, they automate the identification of unknowns more quickly through spectral library searching against the online mzCloud spectral library or offline **Thermo Scientific™ mzVault™ spectral libraries**, which can also be used to create your own proprietary spectral libraries to search against. As more PFAS standards become available, experts within Thermo Fisher Scientific curate extensive fragmentation data and continually add this information to the libraries, further increasing the effectiveness of any unknown identification analyses.

What if my unknown compound is not within a library?

As many PFAS standards are unavailable, direct matches with known compounds may not always be possible. However, the ability to search a wide range of online structural databases and combine these with the extensive MS^n fragmentation information contained within the mzCloud and mzVault mass spectral libraries on PFAS standards makes it possible to elucidate potential structures for true unknowns more easily.

As demonstrated in Figure 2, the **Thermo Scientific™ mzLogic™** data analysis algorithm compares mzCloud similarity matches against chemical structural database hits, looking for maximum common substructure from millions of fragmentation spectra. It then ranks database hits based on structure overlap, leveraging real fragmentation information to rank structural candidates for true unknowns.

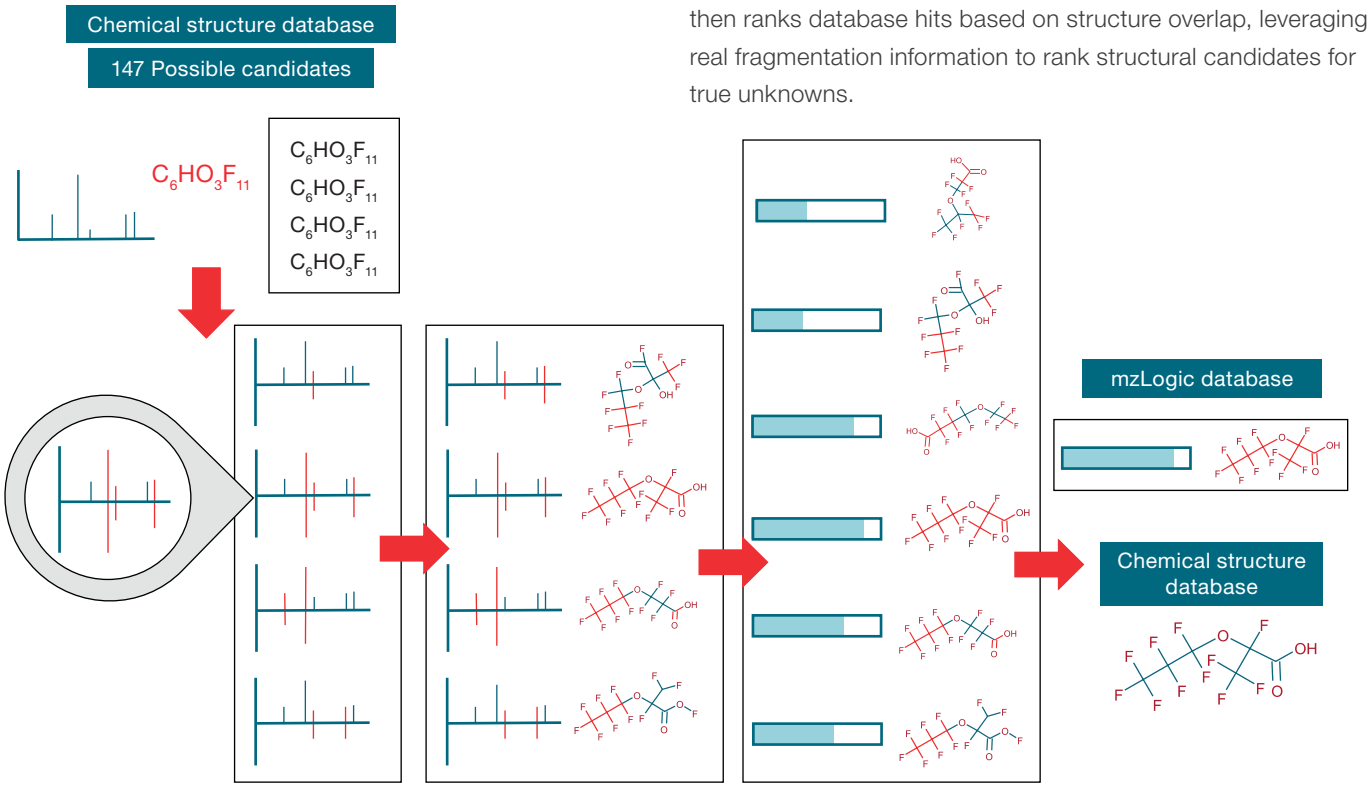

Figure 2. Unknown identification workflow example showing 147 possible candidates based upon the elemental composition. Using the extensive mass spectral fragmentation information within the mzCloud or mzVault mass spectral library in combination with compound structural databases, the level of confidence in any structural match can be determined through spectral fragmentation overlap to provide the most likely candidate for your unknowns

How can I transfer my compound identifications for high-throughput QQQ analysis?

There will be a continual need to quantify additional targets across a range of matrices for potential compliance monitoring as the scale of PFAS monitoring and detection continues to expand. To keep up with this ongoing evolution, the creation of effective selected reaction monitoring (SRM) transitions as part of method development for targeted methods can be simplified.

SRM information can be directly imported from the mzCloud mass spectral library into TraceFinder software for use with **Thermo Scientific™ TSQ™ Plus** mass spectrometers. Fragmentation breakdown curves enable exploration of optimal product ions for SRM transition determination for each targeted compound. Figure 3 illustrates the extensive collision energy information contained within the mzCloud library for each fragment, allowing the optimal SRMs and relevant collisions energies to be created and transferred for everyday analysis.

Although Thermo Scientific™ Orbitrap™-based HRAM mass spectrometers use different collision gases to acquire the high-resolution mzCloud spectral data, **it has been demonstrated** that the relative energetics for unimolecular fragmentation pathways will overlap, generating similar product ions and resulting in very good agreement of empirically determined collision energies with experimentally determined collision energies.

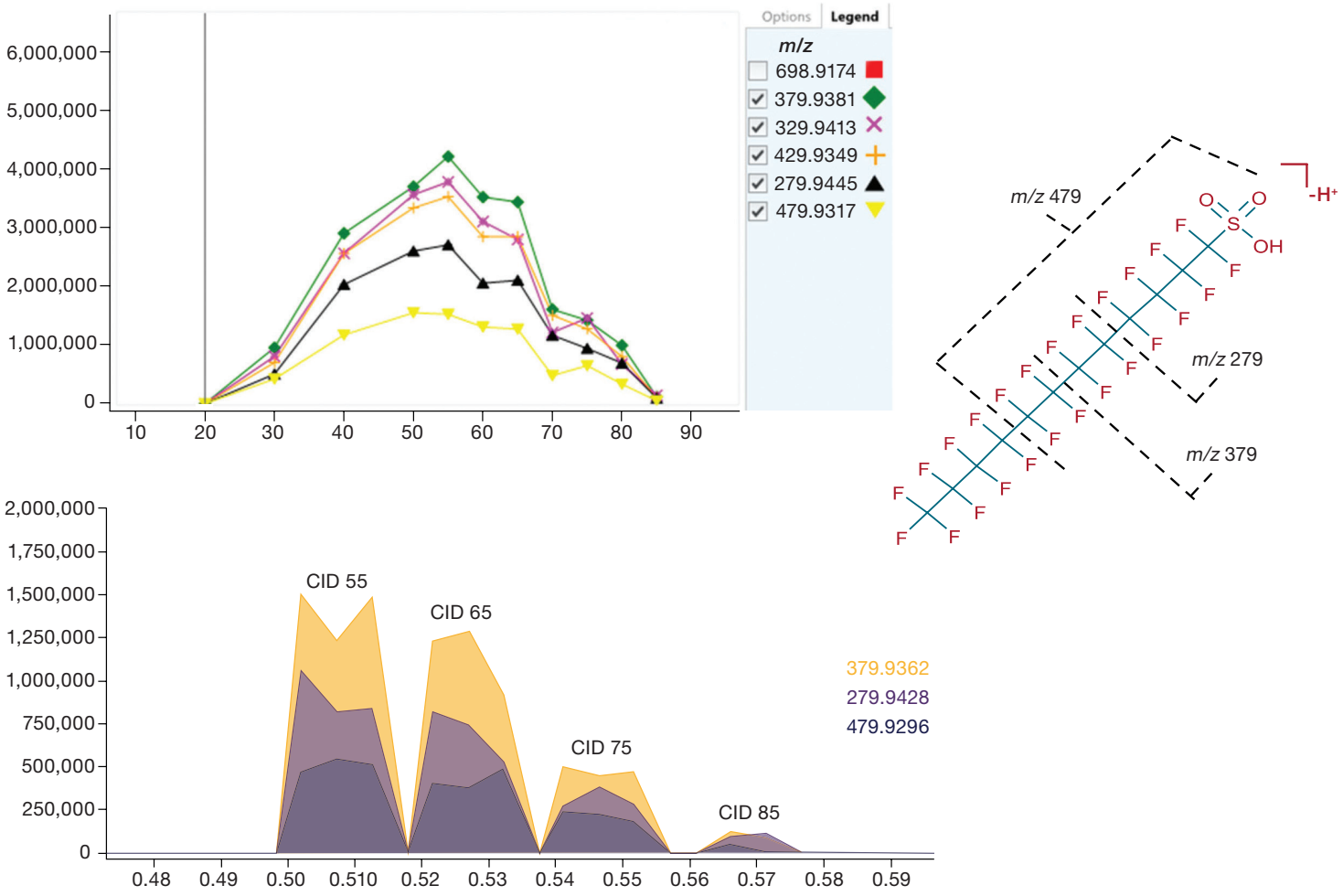

Figure 3. An example of the fragmentation breakdown curves for the compound perfluoro-1-dodecanesulfonate contained within the mzCloud mass spectral library (top left), with the fragmentation scheme for this structure (top right), and confirmation from triple quadrupole mass spectrometer analysis (bottom), demonstrating the applicability of using mzCloud HRAM fragmentation information to automate the creation of SRMs

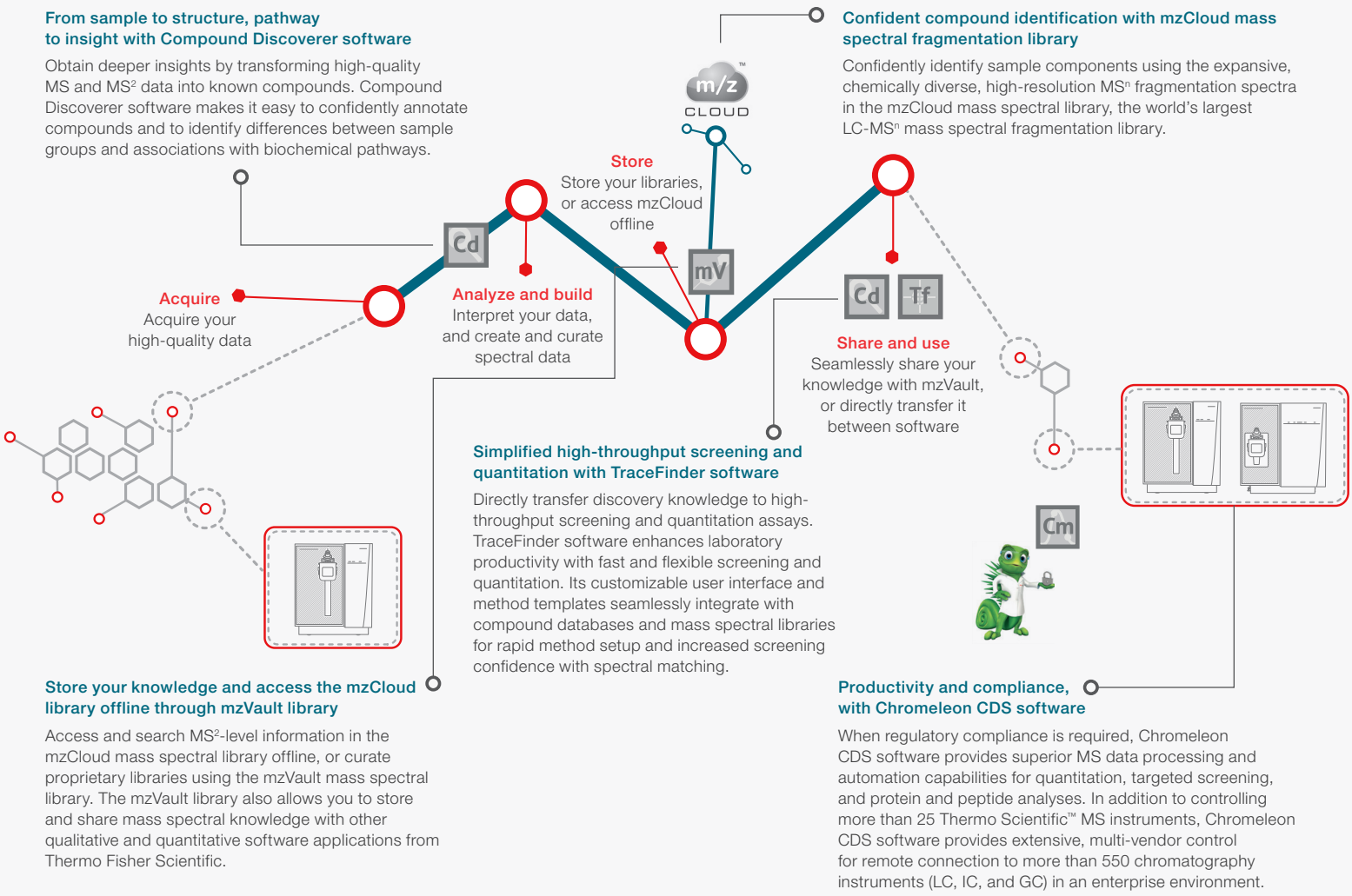

Figure 4. An overview of how the connected hardware and software ecosystem support your laboratory goals in ensuring these forever chemicals don't require a forever analysis

Where can I learn more about data processing options for PFAS analysis to streamline my workflows?

Here are helpful resources to get more information on products and services that support PFAS testing:

- **thermofisher.com/pfas-testing** provides information about a range of workflows for PFAS detection and monitoring, covering all aspects of sample preparation, separations, detection, and data processing.
- **thermofisher.com/compounddiscoverer** details how mzCloud and mzVault libraries can be used to aid the identification of unknown compounds, as well as a range of other powerful data analysis tools.
- **thermofisher.com/tracefinder** details how high-throughput screening can be simplified, with quick access to reports to ensure that insights from your data are rapidly delivered, allowing you to make business decisions faster.
- **thermofisher.com/chromeleon** provides more information about how enterprise-ready software combines with the necessary compliance tools and the ability to control instrumentation and process data from Thermo Fisher Scientific and a range of other vendors.

Learn more at **thermofisher.com/pfas-testing**