Non-Targeted Analysis and High Resolution Mass Spectrometry: The Basic and Beyond

Abstract #: 1029 | Session: E1. Advances in the Analysis of Non-Target Per- and Polyfluorinated Alkyl Substances (PFAS)

Monday, June 3, 2024

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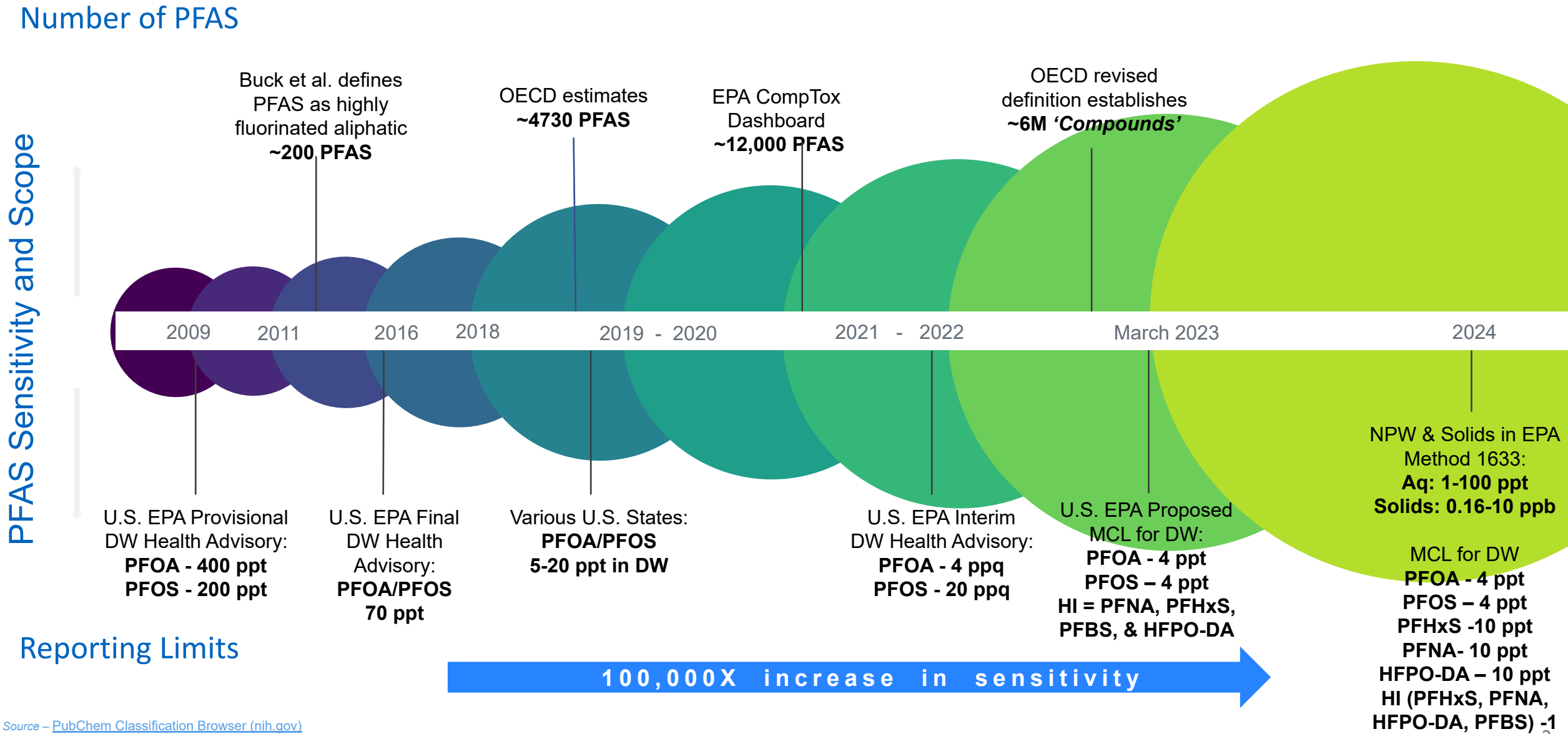
PFAS: Evolution of the Science



Source – PubChem Classification Browser (nih.gov)

Drinking Water

EPA Method 533
25 PFAS

EPA Method 537.1
18 PFAS

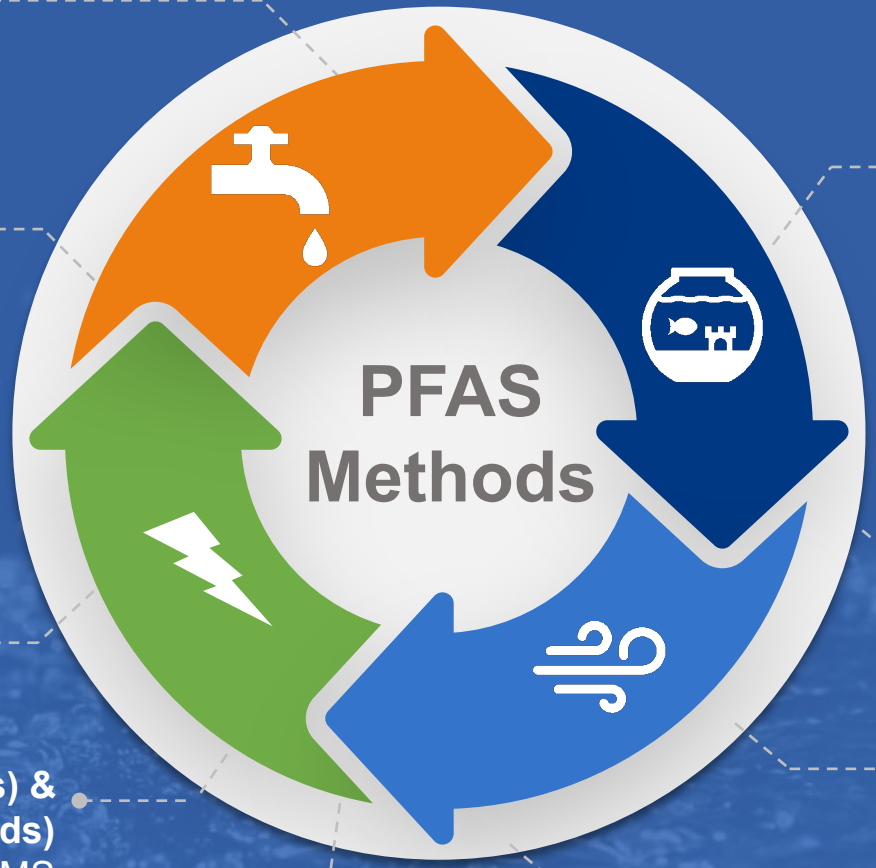
Screening

EPA Method 1621
Adsorbable Organic Fluorine (AOF) by CIC

- **TOP Assay**
- **EOF**
- **Branched/Linear**
- **Non-Targeted Analysis & Suspect Screening**

…us) & …olids)
…S/MS

…27 (Aqueous)
…by LC-MS/MS



Non-Potable Water, Solid, & Tissue

EPA Method 1633
40 PFAS

Air

OTM 45- Air Emissions
50 PFAS

OTM 50- Air Emissions
30 PFAS

Modified TO-13A/10A/17 – Ambient Air

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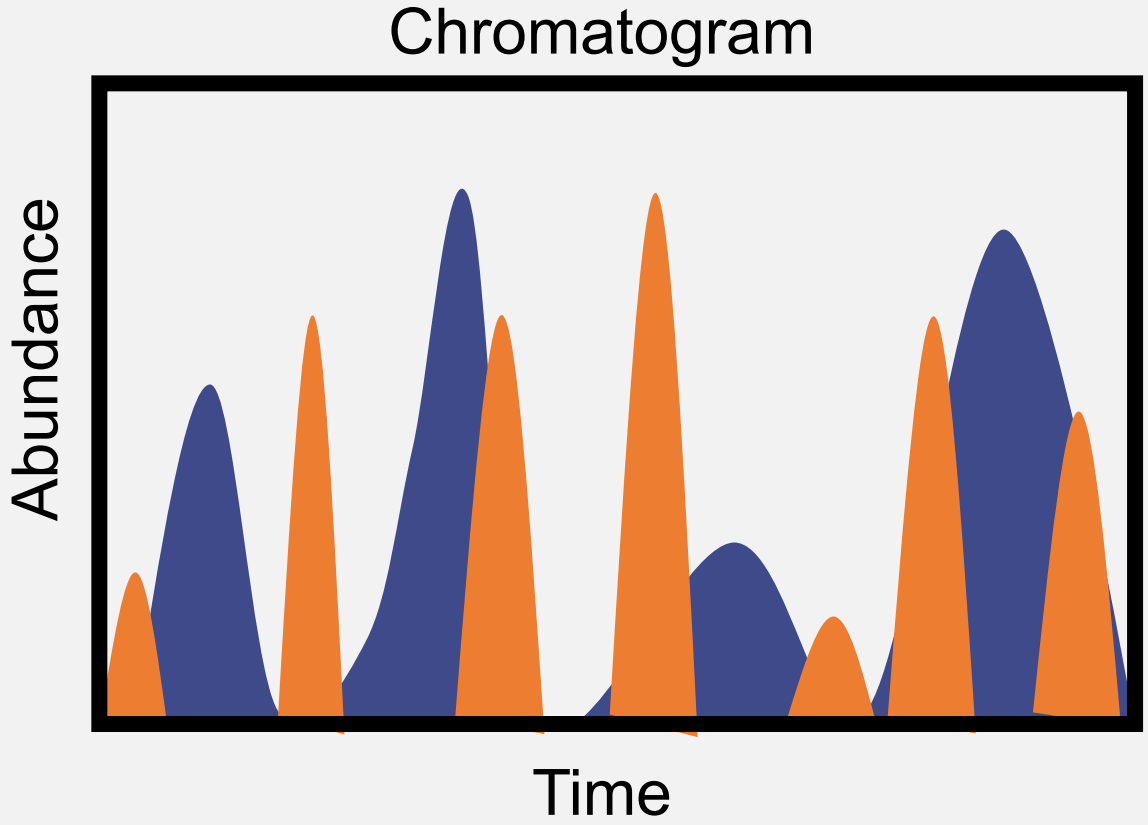
https://www.epa.gov/water-research/pfas-analytical-methods-development-and-sampling-research

Targeted Methods

How much Compound X do I have?

- Select compounds
- Specific matrix
- Analytical Standards
- Quantitative
- Closed Analysis



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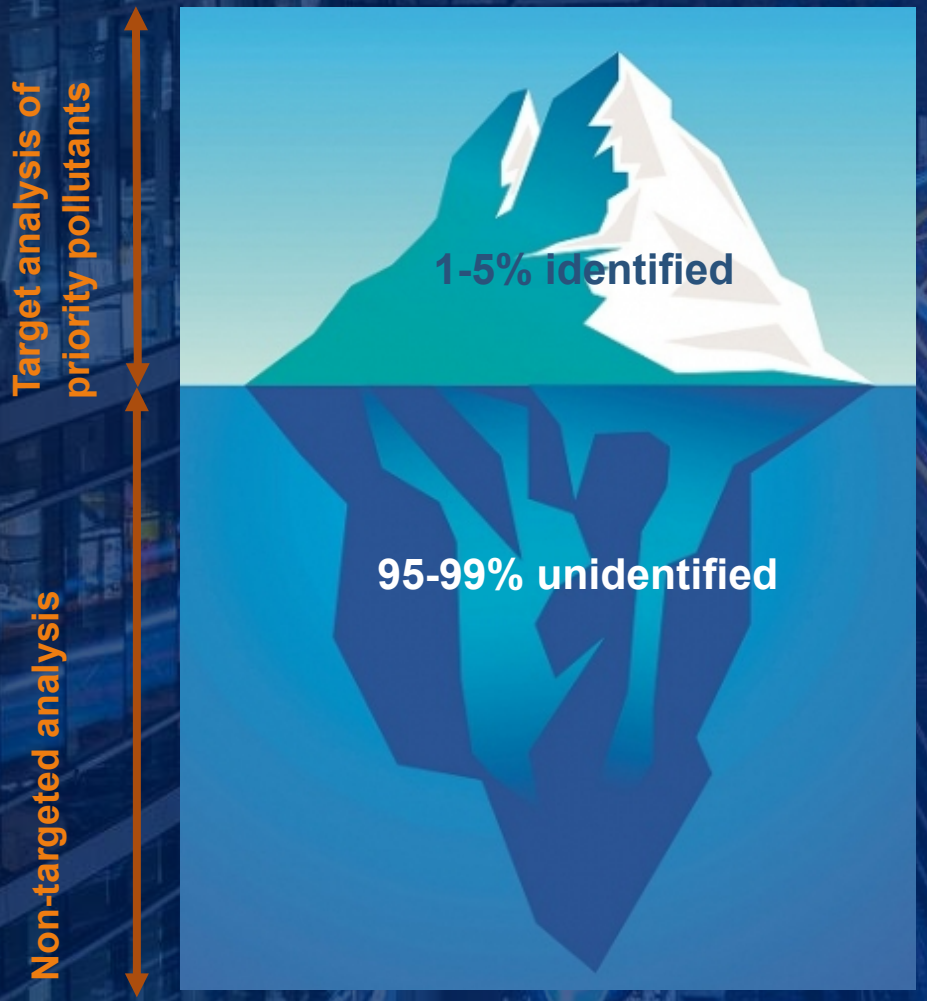
Why explore non-targeted analysis?



- TSCA inventory >86,000 chemicals in commerce (2019)
- Today there are >219 million entries in the CAS registry
- Current standard methods include a very limited number of chemicals

We will only find, what we are looking for!

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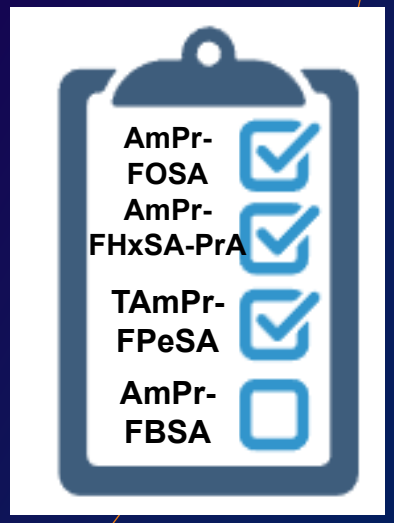
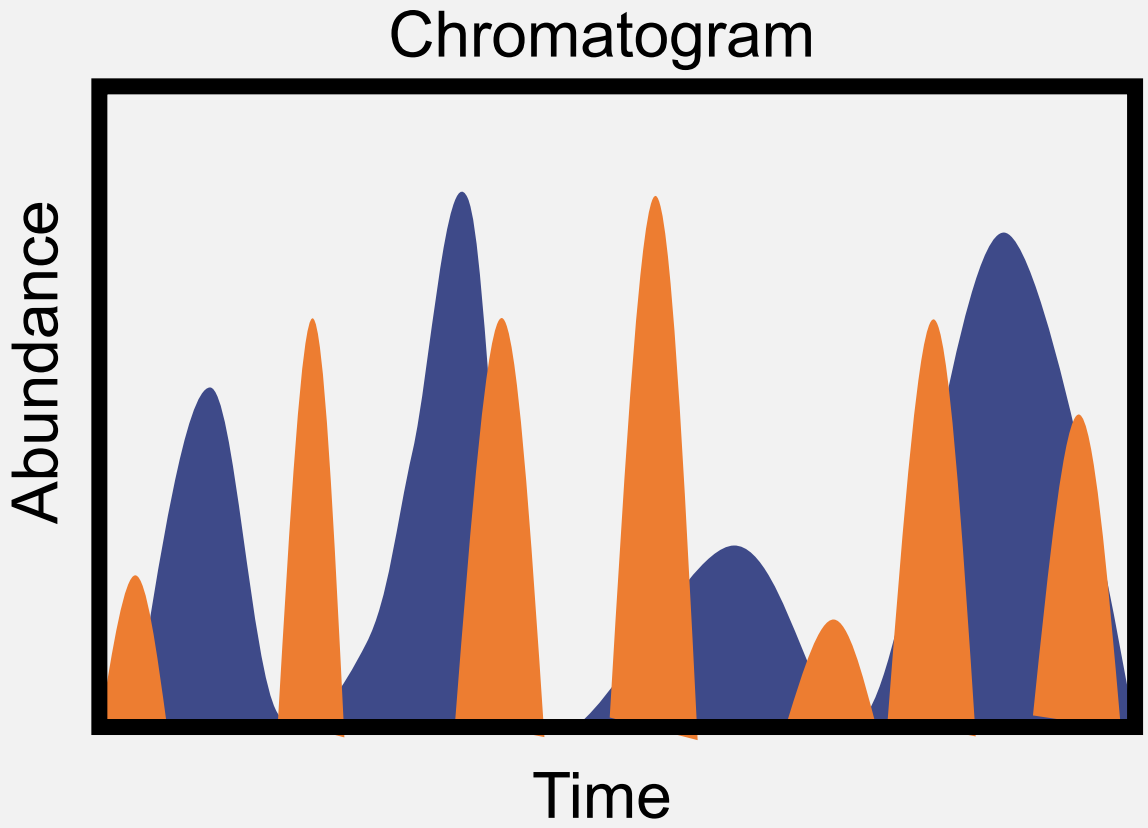
Non-Targeted & Suspect Screening

What is in my sample?

- High Resolution Mass Spectrometry
- User Defined Compound Lists
- No Standards required
- Semi-Quantitative
- Open Ended Analysis





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Wild West of Non-Targeted Analysis

Agenda:

- High Resolution Mass Spectrometry
- Reporting Details
- Applications

Image generated with AI

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Why do we need High Resolution Mass Spectrometry?

- **Mass Accuracy**
- **Resolution**

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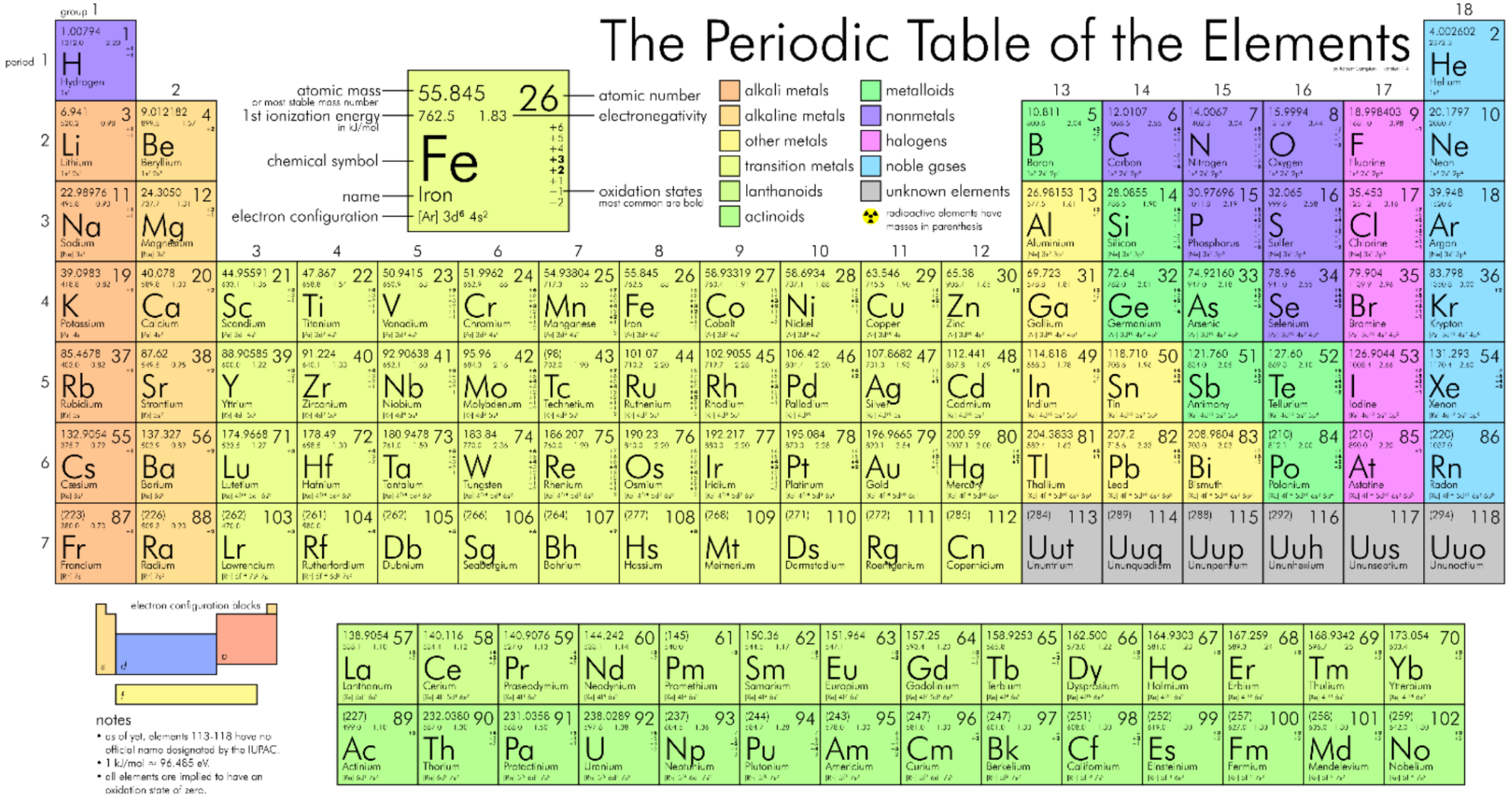
HRMS can reveal molecular formulas



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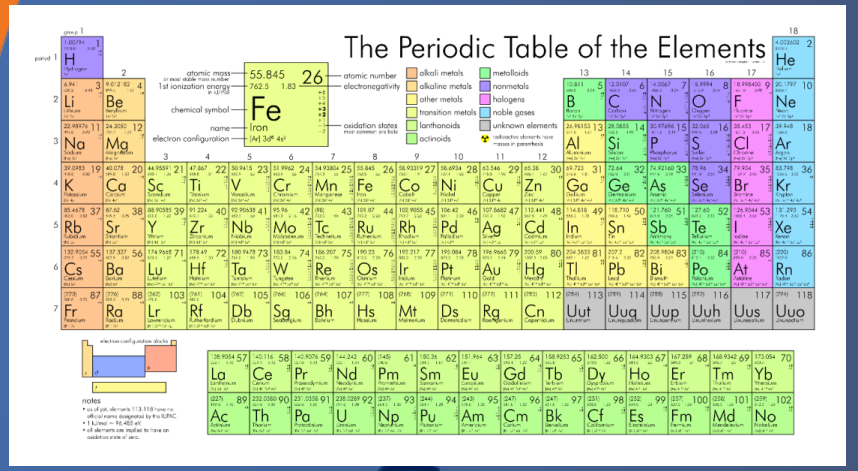
HRMS can reveal molecular formulas

	Exact Mass	Abundance	Exact Mass	Abundance
Carbon	12	98.91%	13.0034	1.09%
Hydrogen	1.0078	99.99	2.0141	0.01%
Fluorine	18.9984	100%	--	--
Oxygen	15.9949	99.76	17.9992	0.2%
Sulfur	31.9721	95.02	33.9679	4.22%



$$PFOS = C_8HF_{17}O_3S$$

- 1633 Ion: 499 *m/z*
- Exact Mass: *498.9302 m/z*

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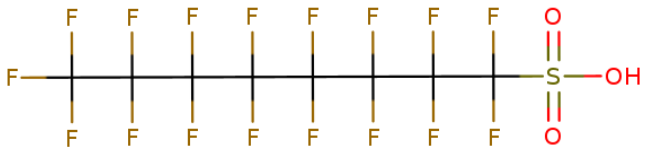
Mass Accuracy (ppm) is the agreement between the measured mass and the exact mass

$$Accuracy = \frac{Difference\ in\ masses}{Exact\ Mass}$$

Exact mass	Observed mass
498.9302	498.9308

$$Mass\ Accuracy = \frac{(498.9302 - 498.9308)}{498.9302}$$

$$Mass\ Accuracy = -1.2\ ppm$$



PFOS

General Rule:

$$Mass\ Accuracy \leq 5\ ppm$$

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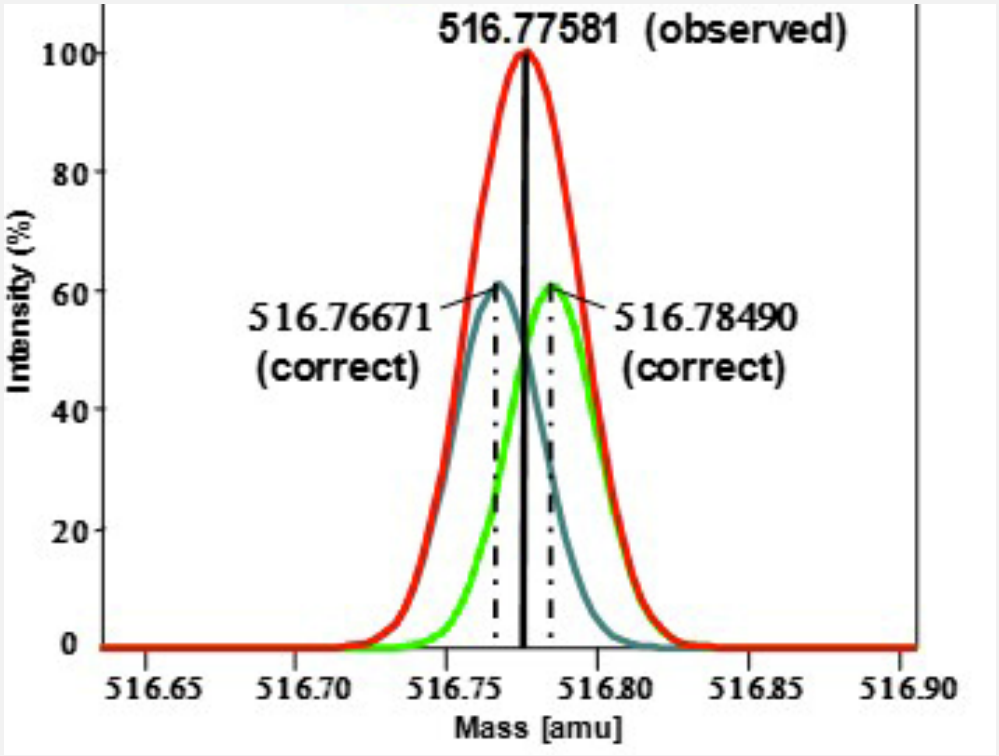
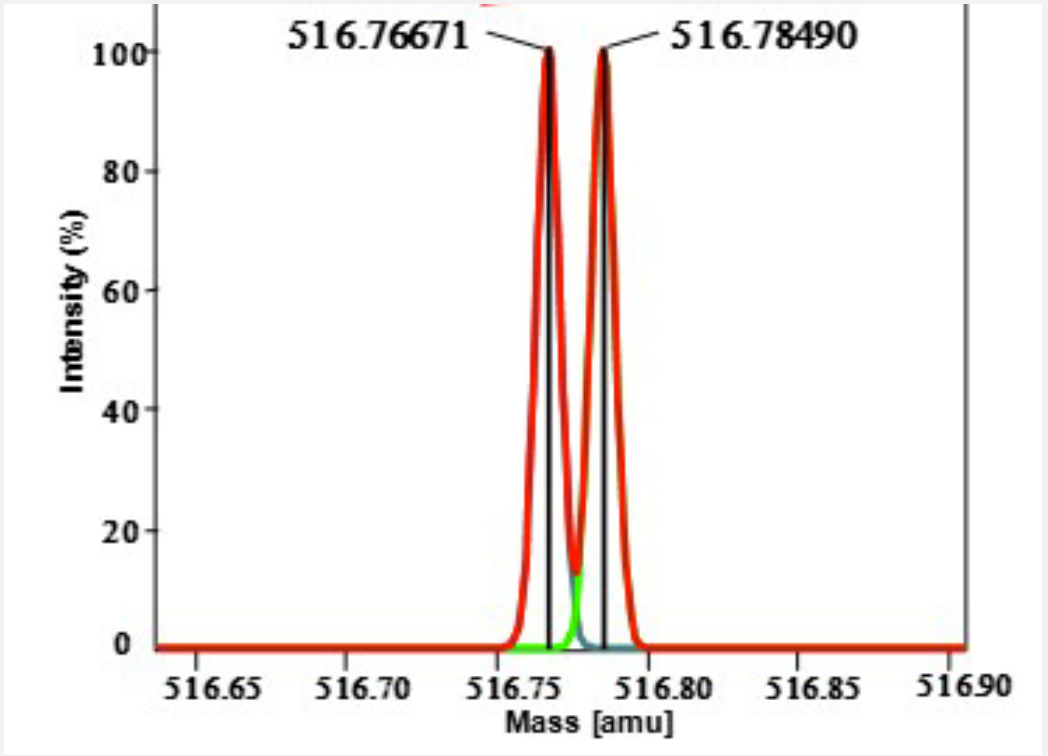
Resolution is the ability to distinguish 2 peaks with similar m/z

Low Resolution



High Resolution



- As **resolution** increases: Greater separation of peaks with similar m/z
- As **mass accuracy** increases: observed mass → exact mass
- Higher confidence in compound identification

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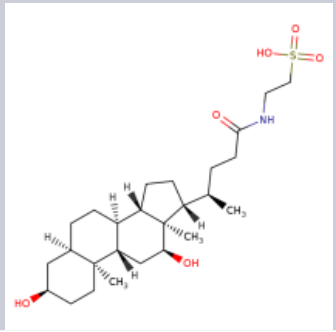
How much Resolution do we need?

PFOS vs. TDCA

	PFOS	TDCA (1633 Bile Acid)	Difference
Structure			
Molecular Formula	$C_8HF_{17}O_3S$	$C_{26}H_{45}NO_6S$	
Average Mass	499.1	498.7	0.4
Exact Mass (Monoisotopic Mass)	498.9302	498.2895	0.6407

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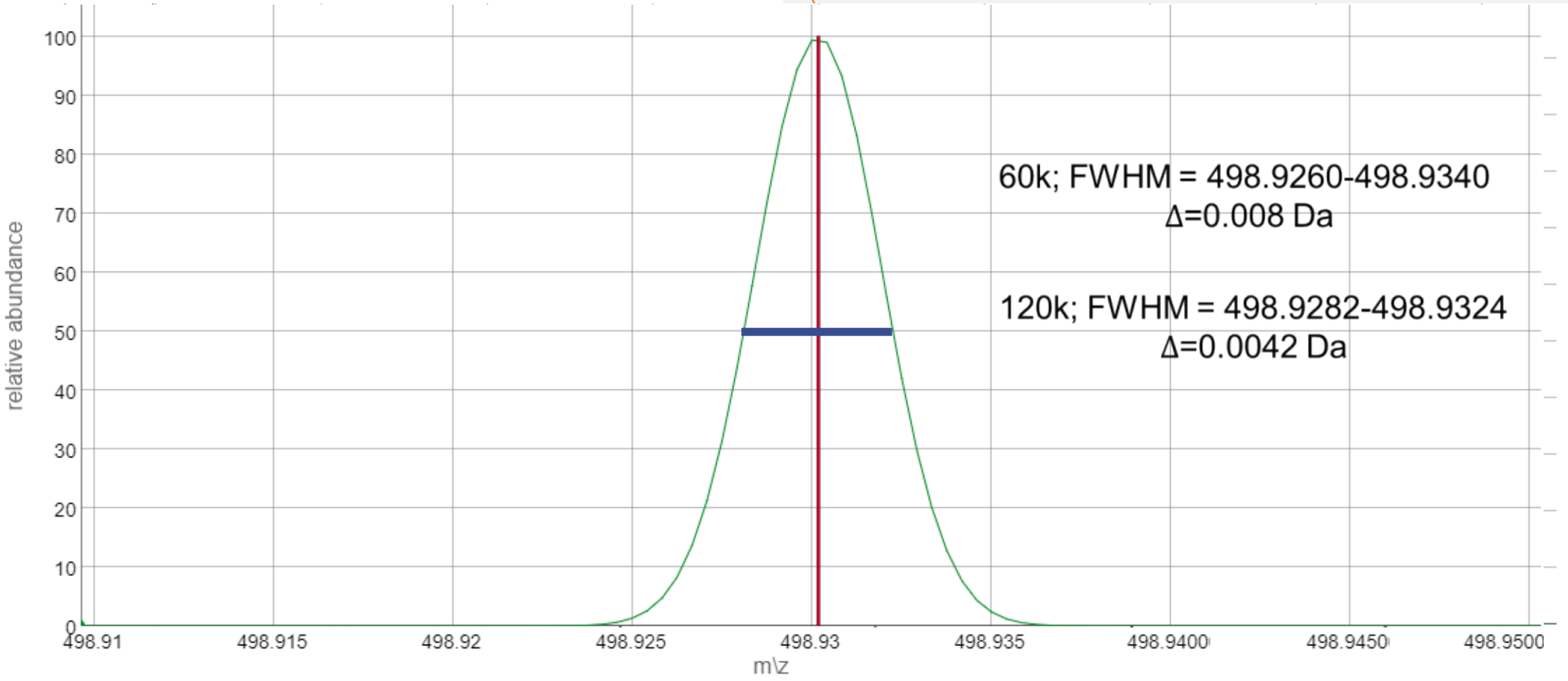
How much Resolution do we have? 60k (QToF) – 240k (Orbitrap)



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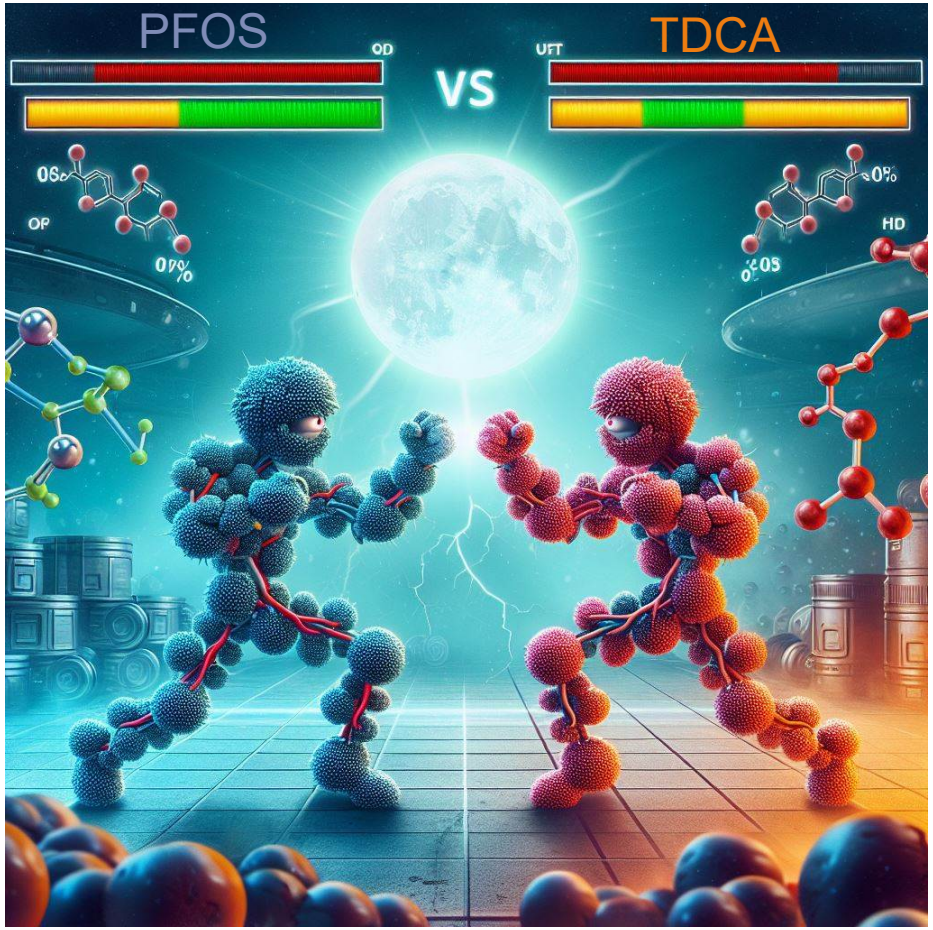
How much Resolution & Mass Accuracy do we need?

PFOS vs. TDCA



	PFOS	TDCA	Difference
Exact Mass	498.9302	498.2895	0.6407
Mass Error	1284.148 ppm		
Resolution	~700		

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Data Uses & Reporting Details

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Data Uses – Client Driven

Target Analysis "Known Knowns"	Suspect Screening "Known Unknowns"	Non-Targeted Screening "Unknown Unknowns"	Retrospective analysis
Know what to look for	Suspect list of compounds to look for	No compounds to look for	Reanalyze data using new knowledge to discover previous contamination
Analytical standards Quantitative	No standards Semi-Quantitative or Qualitative only	No standards	No standards
Is Compound X in my sample? How much?	Which compounds from the list are in the sample?	What is in my sample? How do these two samples compare statistically?	What

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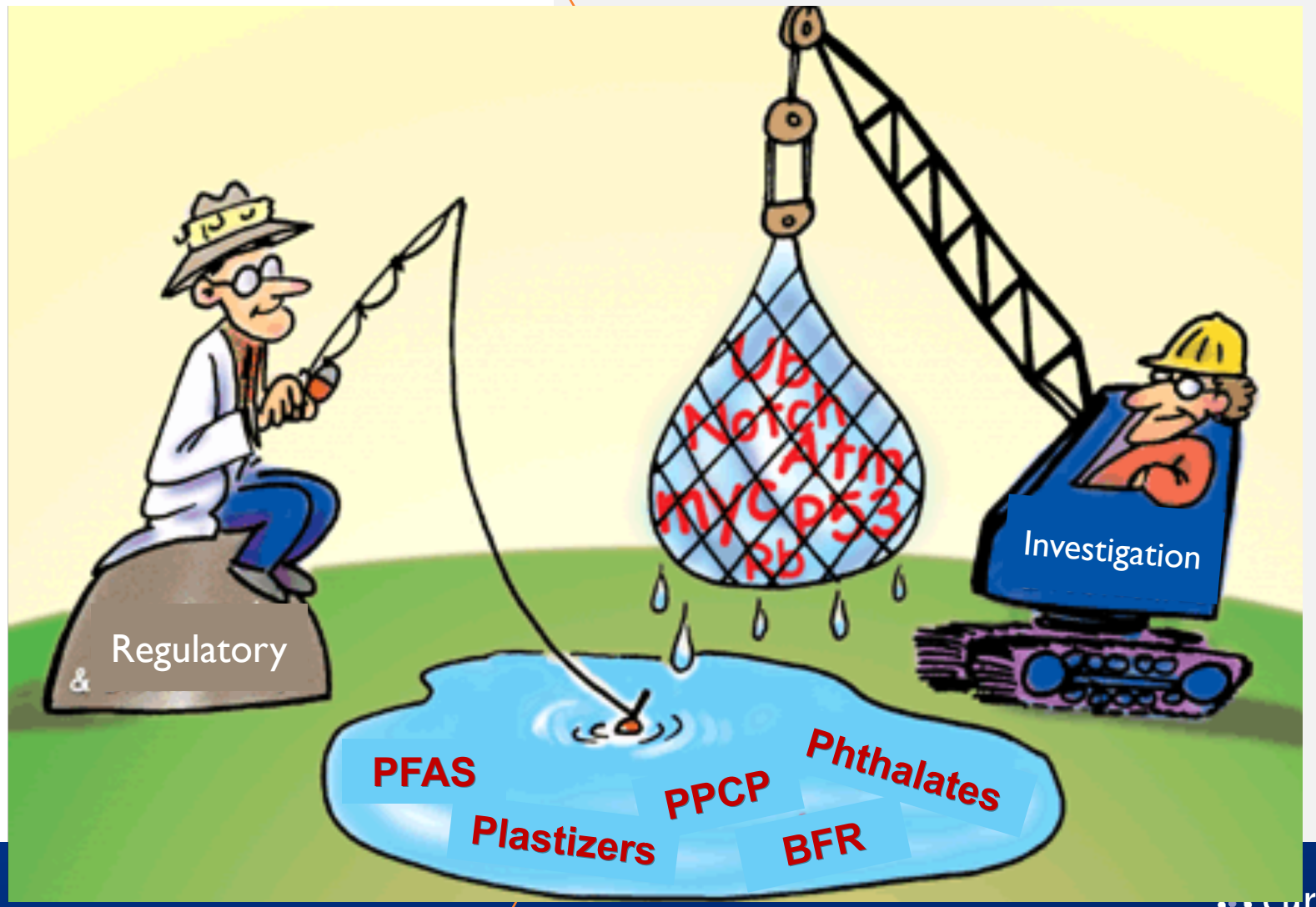
Non-Targeted Analysis & Suspect Screening



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Proteomics in Genomeland, DOI: (10.1126/science.291.5507.1221)

Data Quality Assessments

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Confidence	Schymanski Confidence Levels	Detail evidence of identification	Stds, Libraries, Lists
Quantitation	RLs are data dependent	Quantitative with standards	Semiquant for NTA
Transparence	BP4NTA Study Reporting Tool	Each report	Details

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Communicating Confidence – Schymanski Confidence Scale

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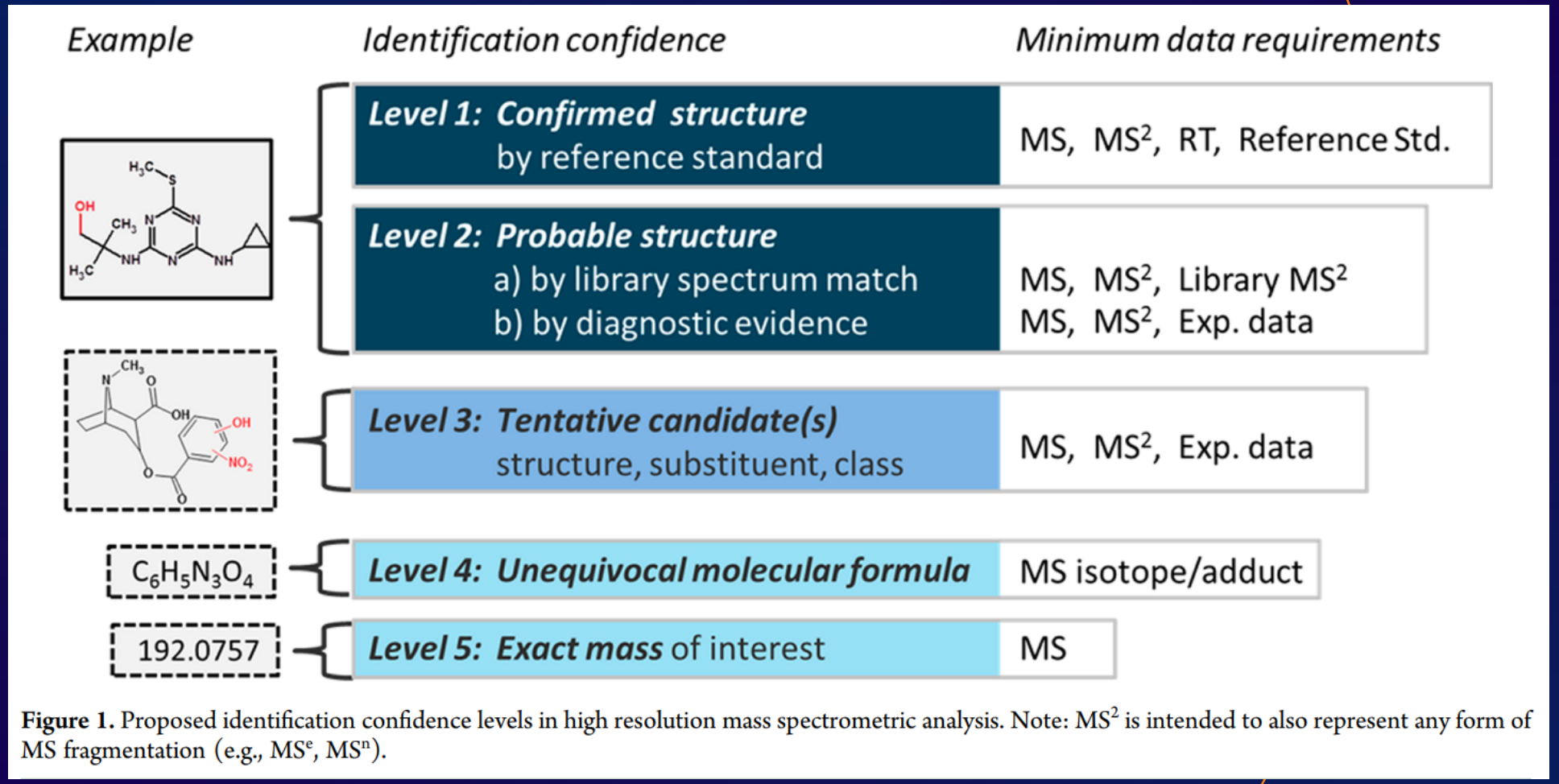

Figure 1. Proposed identification confidence levels in high resolution mass spectrometric analysis. Note: MS^2 is intended to also represent any form of MS fragmentation (e.g., MS^e, MS^n).

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Communicating Transparency
BP4NTA – Study Reporting Tool

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NTA Study Reporting Tool

Please read before using!

Purpose: This Tool was developed for use by NTA researchers and reviewers to assess the quality of NTA study reporting, and the resulting scores reflect solely whether the reporting is sufficiently complete and transparent (based on current, best available understanding of the environmental, food, and exposomics NTA communities). The Tool is not intended for evaluation of the quality of the study or resulting data.

We also encourage two supplementary uses of the Tool: 1) to guide study design - by considering what should be reported, a researcher is inherently encouraged to incorporate the necessary aspects into their study design, and 2) as a starting point for (or portal to) relevant reference content and resources, which are available via the BP4NTA website (www.nontargetedanalysis.org).

Notes & Guidance: The "Example Information to Report" column provides a brief list of representative items relevant to each sub-category - not all are required or necessary for every study. Researchers and reviewers should use their expertise and discretion to determine which points pertain to a given study, and whether additional details not explicitly listed are also critical to report. Additionally, certain sub-categories may not be relevant to a given study (hence the option to select "NA"), or may be less critical to the overall quality and completeness of reporting. To evaluate these aspects, we strongly encourage users to both consider the study type and objectives (e.g., method development, performance evaluation, field application), as well as conceptual linkages across subcategories (e.g., between Statistical Analysis and Statistical Outputs).

Please also note that the Sections (Methods and Results) are not intended to indicate the location in a manuscript where the information is reported - a user should consider the manuscript in its entirety (including any supporting documents and/or citations). We also encourage reviewers to include a rationale, so that authors/researchers may readily address concerns.

Scoring: *NA* = not applicable (gray); *3* (blue) is the highest score and *0* (red) is the lowest. See scoring system explanation provided below.

✔ **Toggle to show score colors vs. fillable fields**

Section	Category	Sub-Category	Example Information to Report	Score (drop-down menu) NA 0 1 2 3	Rationale for score
Methods	Study Design	Objectives & Scope	• Study goals and hypotheses • Scope of the study with respect to use of NTA / suspect screening • Expected chemical coverage of approach and potential limitations		
		Sample Information & Preparation	• Sample collection/replication, handling/storage, preparation, extraction, & clean-up methods (and related QA practices) • Intended use of samples (e.g., method development, compound identification, etc.) • Development and intended use of blanks		
		QC Spikes & Samples	• Development of QC spikes/samples (e.g., isotopically labeled standards/spikes, native standard spikes, matrix pools) • Intended use of QC spikes/samples (e.g., to monitor instrument performance, data normalization, etc.)		
	Data Acquisition	Analytical Sequence	• Sample randomization and use of replicate injections • Inclusion of blanks and QC samples in the acquisition sequence • Information about single vs. multiple analytical batches		
		Chromatography	• Instrument specifications • Method settings (e.g., column/guard, mobile phases, gradient, injection techniques)		
		Mass Spectrometry	• Instrument specifications • Instrument calibration and/or tuning procedures • Method settings (e.g., acquisition parameters, such as polarity, resolution, data-dependent vs. data-independent)		
		Data Processing	• File conversion information (e.g., to open-source format, centroiding) • Software program(s) used • Workflow steps (e.g., peak picking, RT calibration, alignment, gap filling) and settings		

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Non-Targeted Analysis (NTA) by LC-HRMS (in development)

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Target

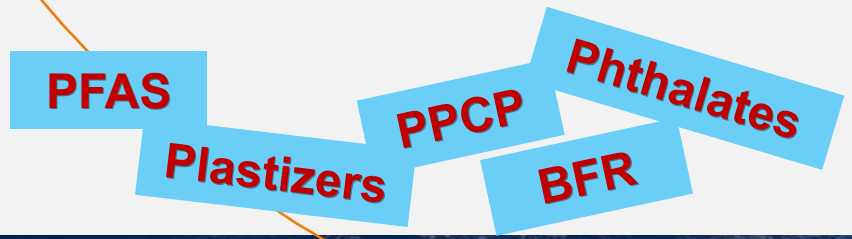
(Known-Knowns)

AND

Non-Targeted

(Known-Unknowns)

(Unknown-Unknowns)



- Applications:
  - Site investigation, research projects, suspected contamination, AFFF characterization, forensics, source tracking, non-regulatory work
- Results:
  - Client Driven: Qualitative or semi-quantitation results
- BP4NTA & ITRC CECs

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THANK YOU

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