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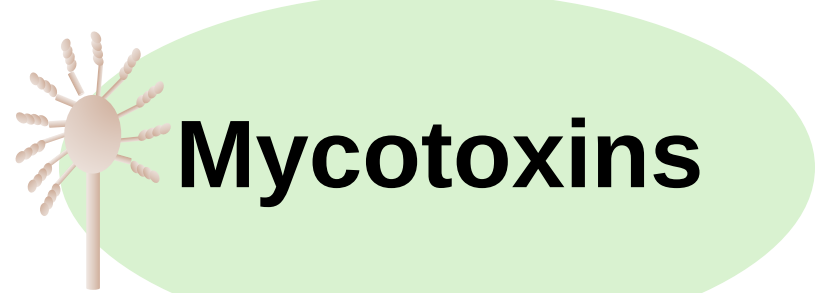
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Abstract

Monitoring contaminants, such as mycotoxin, veterinary drug, and pesticide residues is crucial for ensuring food safety and protecting public health. Several analytical challenges are associated with residue analysis, including the need to monitor many measurands and matrix combinations, analyze multiple analyte forms, metabolites, and degradation products, and detect residues at trace levels. There has been a trend of developing mass spectrometric-based, multi-class/residue methods to analyze samples to improve efficiency. Hundreds of analytes can be monitored using a single method for some multi-class/residue methods. Method validation of these “mega” methods can be challenging due to the large number of measurands and matrices. Matrix reference materials (RMs) are valuable when validating methods. However, developing RMs is challenging for national metrology institutes (NMIs) and RM producers. It is not feasible to produce an RM with hundreds of measurands, and hence, it is necessary to prioritize residues and matrices. The National Institute of Standards and Technology (NIST) has produced RMs to support residue testing in food. Standard Reference Material® (SRM) 1565 Mycotoxins in Corn was designed to contain various mycotoxin classes (by blending several sources of incurred samples together) to support the development and validation of mass spectrometry-based methods capable of detecting multiple mycotoxins simultaneously. Currently, another mycotoxin RM is being developed to support measurements in distiller's dried grains with solubles, which are used as animal feed. NIST is also examining the feasibility of producing small-batch research-grade test materials of food matrices with pesticide residues. One example is a prototype material consisting of ten pesticide residues in spinach, which was sent out in an interlaboratory study to determine community measurement capability and receive feedback on the material. Another small set of research-grade test materials was produced to compare two pesticide residues in incurred and spiked materials. These studies may help decide how to design fit-for-purpose RMs to support residue analysis. While some progress is being made in developing RMs to support multi-class/residue methods, more work is needed. To further help the residue testing community, it is essential to establish collaboration and communication between NMIs and RM producers, get feedback from stakeholders, and use other measurement services, such as interlaboratory studies, to help prioritize measurands and matrices.

Residue Analysis

Monitoring contaminants such as mycotoxin, veterinary drug, and pesticide residues is crucial for ensuring food safety and protecting human health



Mycotoxins

- Toxic metabolites produced by certain fungi
- Can proliferate on various commodities in the field or during storage
- Several hundred exist and include aflatoxins, deoxynivalenol, fumonisins, patulin, ochratoxin A, T-2 and HT-2 toxins, and zearalenone.

Veterinary Drugs

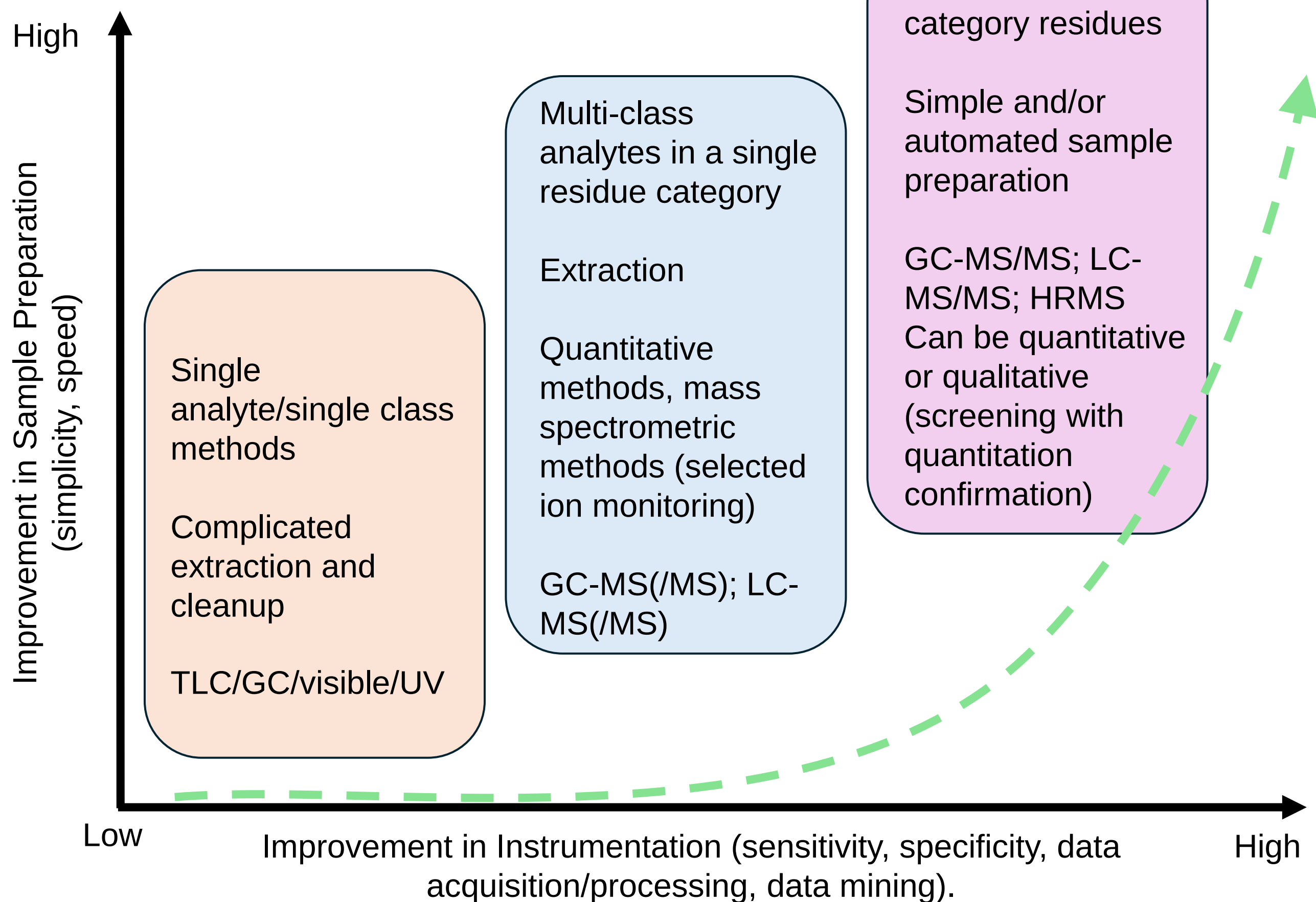
- Substances used to treat disease and/or enhance growth and production in food-producing animals
- Used as a prophylactic for disease prevention in food-producing animals
- Includes antimicrobials, corticosteroids, analgesics, antiparasitics, and hormones.



Pesticides

- Chemicals intended to prevent, destroy, or control pests
- Wide range of purposes, uses, and chemical structures and properties
- Includes organochlorines, organophosphorus, carbamates, triazines, etc.

The trend is to analyze multi-class/residues in a single method (mycotoxins + pesticide residues + veterinary drug residues + other contaminants)



Advantages of multi-class/residue methods [2]

- Allows for quantitation or screening of a large number of measurands in a single method
- Decreases the amount of sample preparation needed
- Decreases analysis times
- Offers the flexibility to add measurands as needed

Challenges of multi-class residue methods [2]

- Some measurands/classes may not be amenable to “generic” sample preparation and detection due to chemical properties (e.g., polar residues)
- Longer method development times
- Increased time for method validation and limited reference materials available for use as controls

Reference Materials

Validation/verification (matrix) RMs

- Typically, preparation of naturally occurring materials of a comparable matrix as unknown samples
 - Ideally at contaminant levels of interest
 - Processed for uniform composition
 - May be modified for improved stability
- Intended to be used to validate methods and check in-house reference materials

Reference Materials (RMs) [3]

- Homogenous
- Stable
- Well-characterized for one or more chemical or physical properties
- Fit-for-purpose

Certified Reference Materials (CRMs) [3]

- RM with documentation
- Issued by an authoritative body
- One or more specified property values with associated uncertainty and traceability

Challenges in producing matrix RMs to support multi-class/residue analysis

- Difficulty in finding sources of incurred materials
- Stability concerns
- Unmanageable number of measurands to include in a single RM

Standard Reference Material® (SRM)

- CRM issued by the National Institute of Standards and Technology (NIST)

How to Support Multi-Class/Residue Methods:

Prioritize RM Development

- Choose residues that are difficult to measure
- Choose residues and commodities with the highest violation rates and/or highest occurrence rates
- Choose residues that pose the most hazards to human health
- Choose residues that represent several different classes and groups
- Choose representative commodities from different food categories
- Feedback from stakeholders
- Using two or more above approaches

Identify Measurement Service Needs

- CRMs used for calibration
- Proficiency testing and interlaboratory studies
- MS databases
- Non-targeted analysis tools

Increase collaboration and communication among national metrology institutes and RM producers

NIST Recent Efforts to Support Residue Analysis

- Several mycotoxins are regulated in food and animal feed
- Advances in analytical protocols and instrumentation allow for multi-mycotoxin analysis
- RMs exist but multiple RMs are required for multi-mycotoxin methods
- RMs are also needed in a variety of matrices



<https://shop.nist.gov/>

SRM 1565 Mycotoxins in Corn		
Mycotoxin	Mass Fraction (ng/g)	
	SRM 1565 (Blank)	SRM 1565 (Incurred)
Aflatoxin B1	ND	7.5 ± 1.7
Aflatoxin B2	ND	1.43 ± 0.34
Aflatoxin G1	ND	0.98 ± 0.19
Aflatoxin G2	ND	0.87 ± 0.24
Total Aflatoxins	ND	10.2 ± 2.9
Deoxynivalenol	142 ± 36	466 ± 69
Fumonisin B1	10.4 ± 3.9	805 ± 190
Fumonisin B2	ND	217 ± 30
Fumonisin B3	ND	99.3 ± 8.4
Total Fumonisins	ND	1150 ± 170
Ochratoxin A	ND	9.4 ± 1.2
T-2 Toxin	ND	18.4 ± 4.2
Zearalenone	ND	62 ± 31

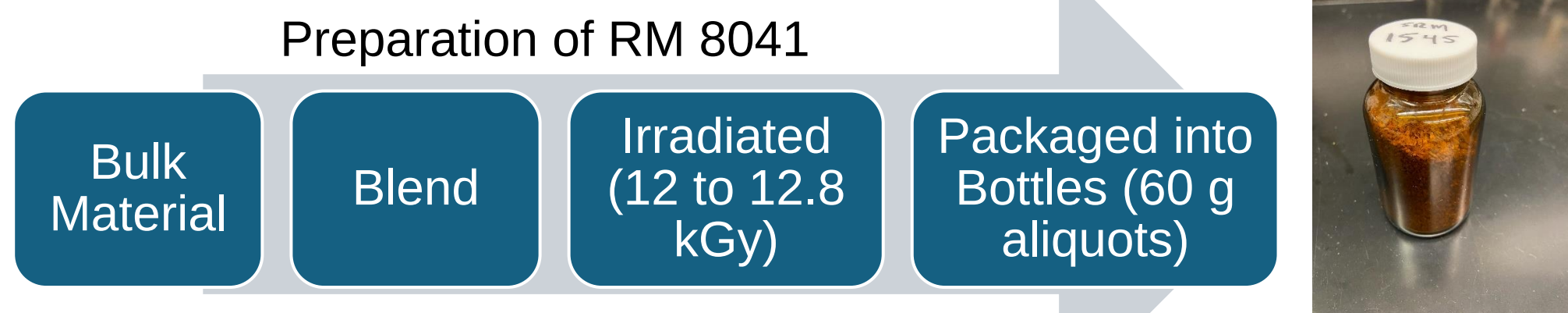
The bold values are certified values. All other values are non-certified. For more information on NIST certified and non-certified values, see [4].

RM 8041 Distillers Dried Grains with Solubles (DDGS) (under development)

- DDGS is a coproduct from the distillation process that is high in protein and used as animal feed
- Matrix can be difficult to analyze
- Will be characterized for fumonisins, deoxynivalenol, and zearalenone



70 kg of bulk material



Bottled material (1191 bottles)

Pesticide Residue Research Grade Test Materials

Preparation of small batch Research Grade Test Material (RGTM) to:

- Use as interlaboratory study materials
- Garner stakeholder feedback/interest
- Investigate feasibility and stability
- Compare variability of incurred and spiked materials

Spiked Pesticide Residues in Spinach

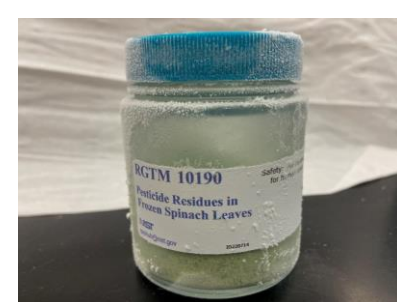
Pesticides Spiked into Spinach	
Acephate	Dimethomorph
Boscalid	Fluopicolide
Chlorantraniliprole	Flutriafol
Clothianidin	Mandipropamid
p,p'-DDE	Permethrin



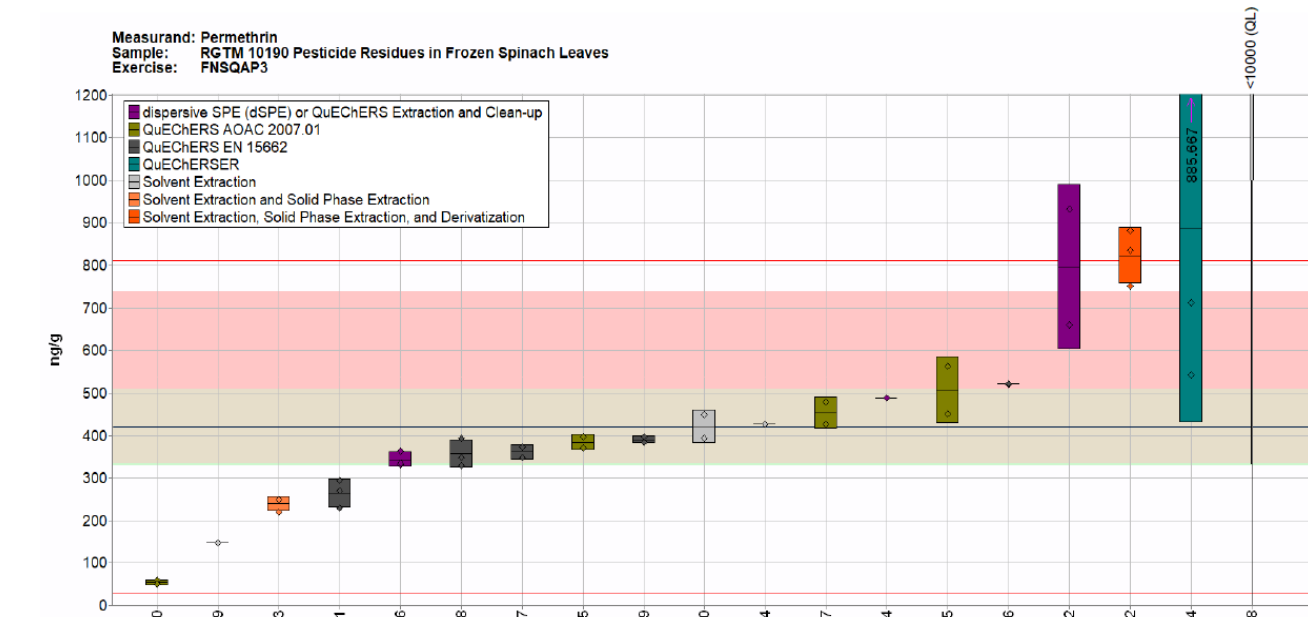
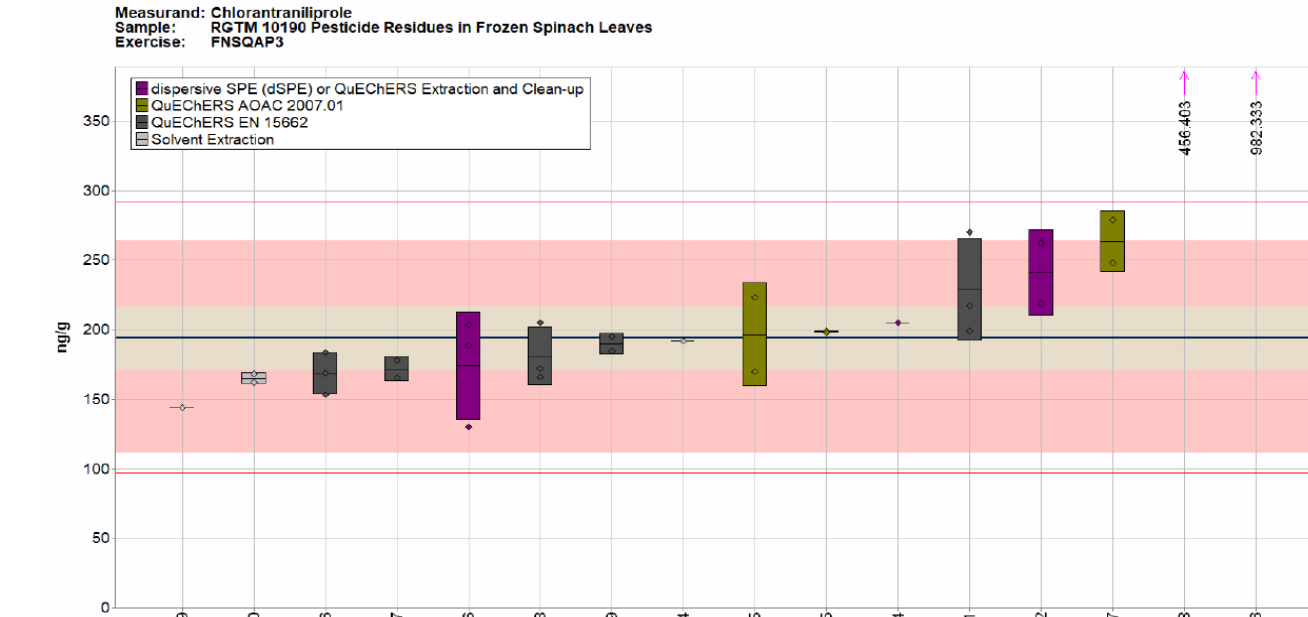
Organic spinach



Milled blank spinach with spiked solution of pesticides



Packaged in 20 g aliquots



Select results from an interlaboratory study. The solid black line represents the consensus mean. The beige shaded region represents the overlapping of the 95 % confidence interval for the consensus mean (green region) and the NIST range of tolerance (red region). For more details see [5].

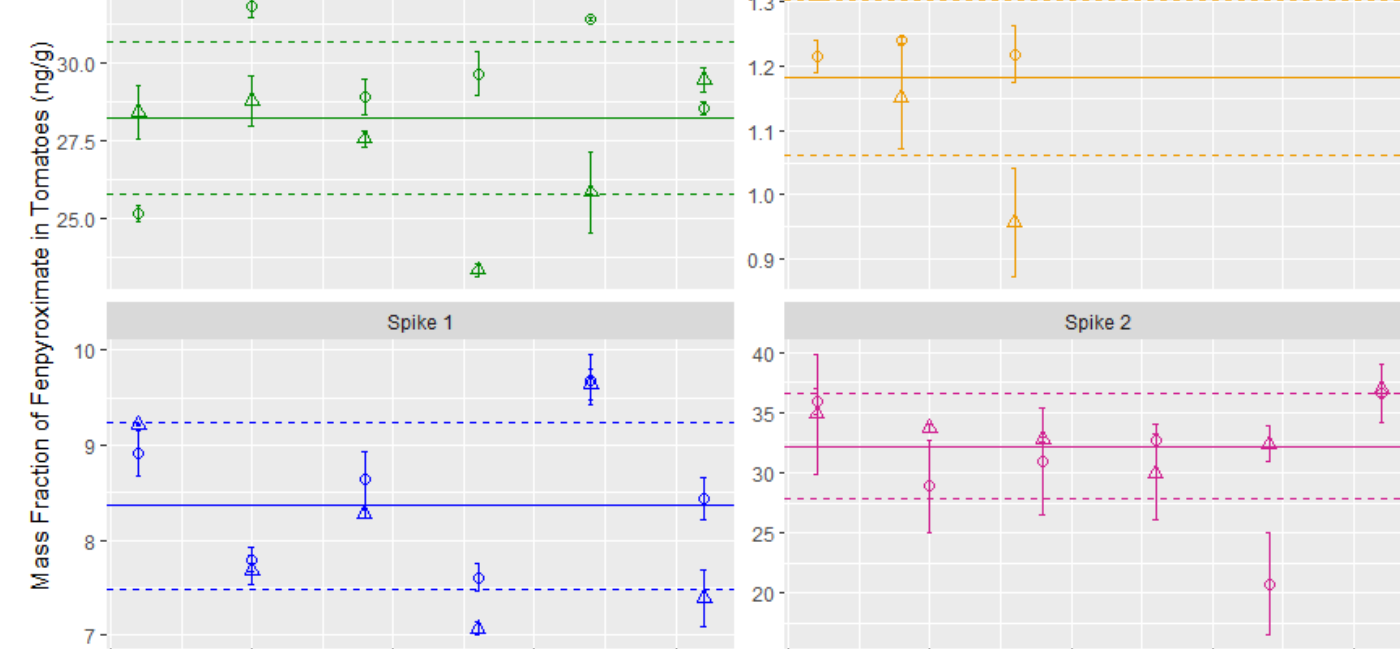
Comparison of Spiked and Incurred Tomatoes



Tomatoes



Spiked cryomilled tomatoes



Fenpyroximate Spike RSD (%)

Incurred	8.7 % - 10 %
Spiked	10.5 % - 13 %

Future work includes stability testing

Conclusions

Producing RMs to support residue analysis is challenging because of the large number of measurands and matrix combinations. RM producers need to prioritize measurands and matrices. Other measurement services should also be explored such as providing more proficiency testing and creating non-targeted analysis tools. NIST efforts in recent years in food safety include developing RMs with mycotoxins from multiple classes and matrices. Additionally, small batch research grade test materials (RGTM) have been produced to study the feasibility of preparing pesticide residues RMs in foods.

References

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