Welcome to the
AEIC Composition and Protein Working Group Meetings

2020 Fall AEIC CWG and PWG Meetings

October 13, 2020 – virtual

INDUSTRY STATEMENT FOR ANTITRUST COMPLIANCE

There shall be no discussion or activities for the purpose of arriving at any understanding or agreement regarding price, the terms or conditions of sale, distribution, volume of production, territories or customers. There shall be no discussion or activity for the purpose of preventing any person or persons from gaining access to any market or customer for goods or services, nor any agreement or understanding to refrain from purchasing or using any material, equipment, services or supplies. There shall be no discussion or activity that may be construed as forestalling or limiting research and development. We, of course, expect your consideration and full compliance with these guidelines, both while in attendance at this Industry meeting and at all times in your business.

Agenda

- Composition Working Group (CWG) meeting
- Protein Working Group (PWG) meeting

Protein Working Group Meeting

Kristen Kouba (Corteva)

Matt Cheever (BASF)

2020 Fall AEIC CWG and PWG Meetings

October 13, 2020 – virtual

PWG Mission Statement

The mission of the Protein Working Group is to leverage the collaborative expertise across the agricultural biotechnology and analytical industries to:

- Support the development and adoption of high quality and scientifically sound protein analytical methods, particularly for new techniques, applications or modernization of existing methods.
- Seek standardization of protein analytical methods, where beneficial, for increased efficiency and international acceptance.
- Produce and publish scientific literature and standard documentation to support protein analytical methodologies.

PWG Workstreams and Leads

Multiplex Method Validation

- Simone Cummings (Syngenta)
- Kristen Kouba (Corteva)

Mass Spectrometry for Protein Quantification

- Mark Bednarcik (Syngenta)
- Yanfei Wang (Bayer)

Allergen Analysis

- Tao Geng (Bayer)
- Norma Houston (Corteva)

Extraction Efficiency

- Ivan Birukou (Syngenta)

Intractable Proteins

- Rong Wang (Bayer)
- John Zhang (Corteva)

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PWG Meeting Agenda

- New topics
- Break?
- Allergen Analysis workstream update
- Mass Spectrometry for Protein Quantification workstream update
- Multiplex Method Validation workstream update
- Extraction Efficiency workstream update
- Intractable Proteins workstream update
- EFSA explanatory note – determining protein concentration in stocks of protein for quantitative standards
- Other topics

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New topics

Break

Allergen Analysis Workstream Update

Tao Geng (Bayer)

Norma Houston (Corteva)

October 13, 2020

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Mission Statement

The Allergen Workstream is committed to leveraging scientific expertise from across agricultural biotechnology and analytical industries to:

- Support the development, usage and acceptance of science-based methods for the quantification and characterization of potential allergens.
- Standardize methods and harmonize endpoints, where beneficial.
- Generate and publish scientific papers, when necessary, to support methods.

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Team Members

- Matt Cheever – BASF
- Tao Geng , Co-chair – Bayer
- Nancy Gillikin – BASF
- Norma Houston, Co-chair – Corteva
- Lucy Liu – Bayer
- Justin McDonald – Syngenta
- Hector Serrano – BASF
- Jeff Shippar – Covance
- Afua Tetteh – BASF
- Rong Wang – Bayer
- Yanfei Wang – Bayer

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Projects and activities

- Generate and publish a paper of MS approach to Evaluate *In Vitro* Digestion

- Standardize methods and harmonize endpoints for 10 soy endogenous allergens

A paper of MS approach to Evaluate *In Vitro* Digestion

- Titled “Can Mass Spectrometry Analysis of *In Vitro* Digestion Products Improve the Assessment of Allergenic Potential of a Newly Expressed Protein?”
- Key message “Therefore, the use of LC-MS/MS for a standard *in vitro* digestibility assessment provides no improvement in allergenicity prediction, and the value of LC-MS/MS analysis needs to be investigated further prior to adoption for allergenicity prediction of NEPs in GM crops.”
- Submitted to “Journal of Regulatory Science”

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Soy endogenous allergens

- EFSA requests 10 soy allergens to be assessed in an approach of the compositional analysis (EC, 2015 and Naegeli et al.,2017)
  - Tests of difference and equivalence
  - A database of natural variability
- A survey for allergen quantification method and endpoints
  - Companies are using LC-MS multiplexing for 10 allergens
  - Companies also published data of soybean allergens generated from multiple-year productions (Hill et al., 2017; McClain et al., 2018, Ahsan et al., 2018)

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Discussions on endogenous allergens

- Reporting harmonization
  - Reporting strategy the same as Composition?
- Database of natural variability
  - The similar database as CCDB?
- Dow Agroscience patents?

Welcome to the AEIC/PWG Mass Spectrometry for Protein Quantitation Working Group

Mark Bednarcik (Syngenta)

Yanfei Wang (Bayer)

October 13, 2020

Team Update

- **Workstream Leads**
  - Mark Bednarcik (Syngenta)
  - Yanfei Wang (Bayer)

- **Team Members**
  - Kristen Kouba (Corteva)
  - Simone Cummings (Syngenta)
  - ~~Valerie Messmer (Syngenta)~~
  - Jeff Shippar (Eurofins)
  - Lucy Liu (Bayer)
  - Matt Cheever (BASF)
  - Norma Houston (Corteva)
  - Tao Geng (Bayer)
  - Shawn Motyka (BASF)
  - Qiang Zhao (BASF)
  - Chris Ament (Eurofins)
  - Scott Young (Syngenta)

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Mission Statement

- The mission of the Protein Quantitation by Mass Spectrometry workstream is to leverage the agricultural biotech industry expertise to:
  - Obtain alignment within Ag Biotech on the space for mass spectrometry as a scientifically acceptable approach for transgenic protein quantitation in research and development and regulatory studies
  - Publish a scientific paper reviewing and summarizing evidence supporting both Immunoassay and LC/MS/MS as valid analytical techniques to assess protein concentrations in plant tissues

Workstream Progress

- Monthly/bi-monthly meetings to obtain alignment within Ag Biotech on the space for mass spectrometry as a scientifically acceptable approach for transgenic protein quantitation in research and development and regulatory studies
  - Draft v4 is in process and additional content is being added by all team members
    - Body of paper includes review of technologies as well as their strengths and weaknesses
  - Describe LC-MS/MS as an acceptable technology for measuring soybean allergens as well as plant incorporated proteins
    - Detection of intact MW is not needed and deleted per team agreement
  - Three immunoassay platforms will be highlighted (MSD, ELISA, and Luminex)
  - Paper will focus on quantitation not detection

Workstream Progress

- Publication will provide options based on situation; reiterate both options are scientifically sound and viable
- Discussion and conclusions sections need the most work
- All documents are saved at new Bayer sharepoint https://bayergroupus.sharepoint.com/sites/008221/AEIC/Shared%20Documents/Forms/AllItems.aspx?csf=1&RootFolder=%2Fsites%2F008221%2FAEIC%2FShared%20Documents%2FMass%20Spec%20Workstream&FolderCTID=0x012000AB460D0C32545B479B6F4C7350C391EE

Current Assignments

- **Introduction (Mark/Qiang)**
  - Rearranged to keep the flow and to have enough content for both immunoassay and mass spectrometry
- **LC/-MS/MS (Yanfei, Norma, Kristen, Jeff)**
  - Yanfei added an overall process and a flow diagram. **Norma** will make picture flow diagram and review the mass spec sections in detail
- **Immunoassay (Tao/Qiang/Kristen)**
  - Discussion regarding the strength and weakness in introduction as overall is needed (Tao/Qiang)
  - Each technique will discuss their own special strength and weakness.
  - Kristen will reach out to Luminex and MSD for use of figures

Current Assignments

- **Discussion (Simone, Jeff)**
  - Seeking additional volunteers for this section
  - Describe what situations or circumstances would lead to a preference for one technique over another, along with situations where both are equally appropriate
  - **All** to review the section for allergen quantitation made by Tao and decide on how to trim this section down

- **Conclusions (Yanfei/Mark/All)**
  - Multiple valid protein quantitative methods
  - Mass Spec techniques are relatively newer for quantifying proteins but well suited for many applications and better for some
  - Both Mass Spec and ELISA data (with a validated method) should be considered acceptable for regulatory submission
    - e.g. Trait protein expression studies

- **References (Yanfei/Tao)**
  - **Yanfei** will make Endnotes for some of the references and **Tao** will combine them all. Endnotes will be needed for the overall references

Next Steps and Discussion

- Next Steps
  - Mark and Yanfei will work with individual team members for each section
  - Paper will need to be reviewed by each company from a technical and legal point of view
  - Next meeting on Oct 28th
- Discussion
  - Ways of Working to improve progress and meeting efficiencies
  - Timelines for delivering final draft for company reviews

Validation of multiplex protein methods

Simone Cummings (Syngenta)

Kristen Kouba (Corteva)

October 13, 2020

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Team members

- Co-chairs:
  - Simone Cummings - Syngenta
  - Kristen Kouba – Corteva
- Matt Cheever - BASF
- Jeff Shippar – Eurofins (formerly Covance)
- Yongcheng Wang – Bayer
- Yanfei Wang - Bayer

Goals

- Produce scientific literature that:
  - Provides method validation guidance for protein-based technologies and platforms
  - Involves input and agreement from relevant parties
  - Outlines standardized validation parameters for multiplexed methods
  - Outlines standardized validation parameters for single-plex methods (this is an expansion of current literature)
  - Describes how to add a new trait to a validated multiplexed method
  - Is visible to others (targeted journals for maximum visibility)

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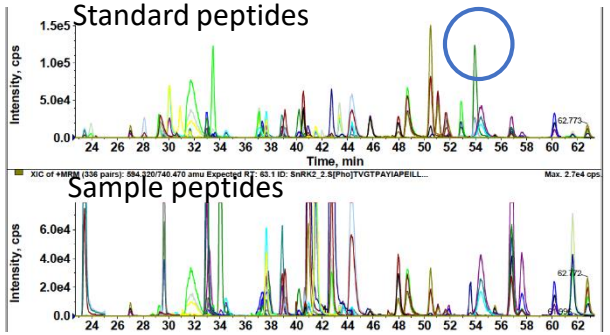
Technologies

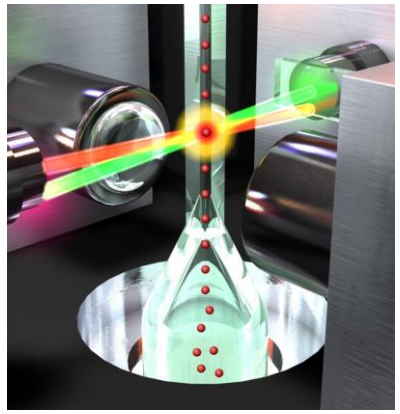
- Multiplexed
  - Mass Spec
  - Luminex
  - MSD
- Single-plexed
  - ELISA

Mass Spec

MRM Multiplexing Results



Luminex



MSD

Progress

- 5 meetings
- Paper outline completed
- We have volunteers working on all sections of the paper
- We have monthly discussions to align on acceptance criteria as well as validation guidance
  - We are finding common ground on all discussion points

Validation parameters

- **Completed**
  - Curve fit
  - Accuracy
  - Quantitative range
  - Repeatability precision
  - Intermediate precision
  - Extract stability (long and short term, freeze thaw)
  - Appropriate controls for all technological platforms
- **To be discussed**
  - Minimum required dilution
  - Limit of detection
  - Linearity (dilutional)
  - Extraction efficiency
  - Reference control stability
  - Specificity

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Timelines

- Finish validation parameter discussions - Spring 2021
- Draft paper - Fall 2021

Welcome to the
AEIC Extraction Efficiency Working Group

Ivan Birukou, Syngenta

October 13, 2020

EFSA explanatory note.
Protein extraction efficiency

- Extraction efficiency is defined as the amount of extracted protein relative to the total amount in the plant tissue, expressed as a percentage
- a significant amount of the NEP may remain in the insoluble fraction after extraction and should be quantified to estimate the extraction efficiency
- the NEP present in the insoluble fraction should be extracted under strong denaturing ('harsh') conditions and quantified by western blot analysis

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Workgroup discussion topics

- Define the workgroup goal: prepare a manuscript describing the harmonized approach to determine extraction efficiency
- Discuss the methodology currently applied to determine extraction efficiency, specifically the insoluble fraction
- Discuss the ways of harmonization of the extraction efficiency methodology and potential implications for previous, current and future registrations

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Current EE methodologies

Two main approaches

1. Soluble and insoluble fractions are analyzed using separate methods and then combined to determine the EE:
   a. Determination of the exhaustively extracted soluble fraction using quantitative method of choice (ELISA, MS-based, etc.). Mild extraction conditions.
   b. Determination of the insoluble protein fraction in the tissue pellets after exhaustive extraction of the soluble fraction by densitometric analysis of the Western blots. Harsh extraction conditions.
2. Determination of the EE by densitometric analysis of the Western blot.

   EE is calculated as a ratio of the 1st soluble extraction (mild extraction condition) to the total protein (includes insoluble fraction extracted using harsh conditions).

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Harmonization of EE methodology

- Both approaches include the insoluble fraction in the EE calculation and utilize harsh extraction conditions followed by Western blot to analyze the insoluble fraction – EFSA requirements are addressed
- By the time the EE WS was formed, WS participants were either in the process of conducting or submitting the studies to address EFSA requirements – harmonization by switching to a single methodology not feasible
- WS decided to prepare a manuscript describing all discussed methods and proving that all listed approaches are scientifically sound and address EFSA requirements.

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Intractable Proteins

Rong Wang, Bayer
John Zhang, Corteva

October 13, 2020

Intractable Protein Analysis

- The intractable protein analysis topic was brought up at 2019 AEIC Fall meeting. There was an interest in it.
- About intractable proteins
  - Definition of intractable proteins
    - Example classes discussed in 2014 ILSI Task Force and publication
  - Challenges presented by the intractable proteins
    - Production, extraction, isolation, characterization, etc.
  - Products containing intractable proteins
    - Examples of the de-regulated
    - Traits under development

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Perspective of the New Workstream

- **Benefit of having a workstream in AEIC**
  - Technical expertise in protein analysis
  - Complimentary to CLI protein characterization/expression expert team

- **Scope of this workstream**
  - Protein characterization methods
  - Focus on intractable protein first, expand when needed

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Kickoff of the New Workstream

- **Team name** (tentative):
  - Protein Characterization Workstream
- **Mission statement** (tentative):
  - The Workstream is committed to leveraging scientific expertise from across agricultural biotechnology and analytical industries to:
  - Support the development, usage and acceptance of science-based methods for the characterization of proteins, particularly those that are considered intractable
  - Standardize protein characterization methods and harmonize endpoints, where beneficial. Seek to address technical challenges, standardization of methods, and harmonization of endpoints associated with protein characterization, especially where beneficial for appropriate safety assessment and characterization of biotech crops
  - Generate and publish scientific papers, when necessary, to support methods
- **Call for participation**
- **Logistics**

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EFSA explanatory note (23July2018)

Determining protein concentration

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4.2.2 Additional considerations for the analytical method

4.2.2.1 Reference standards

Protein concentration determination using two independent methods is recommended for reference proteins (e.g. a non-colorimetric method such as amino acid analysis and a colorimetric method such as a Bradford assay, e.g. Noble and Bailey, 2009).

Other topics