

Welcome to the AEIC CHR/Intractable Protein Workstream

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Fall AEIC Protein Working Group Meeting

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Intractable Protein/Characterization Workstream

- Established in early 2021
- Meet monthly, currently 15 members
- Goal: review protein characterization, production and quantification methods and address technical challenges associated particularly with intractable proteins. Standardize methods and harmonize endpoints and publish scientific papers and communication documents when necessary
- **Current project:** manuscript for safety assessment of intractable proteins in GM crop



Intractable Protein/Characterization WS Progress



2021 Spring Meeting

-Roster

- 15 members
- 7 companies

-Logistics - monthly meeting

-Finalized mission statement

-Identified a project



2021 Fall Meeting

-Drafted a manuscript outline at high-level

-Worked on sub-bullet points by brainstorming details & scenarios, rationales on the content- production challenges & alternative solutions

-Completed outline in Oct meeting and start draft



2022 Spring Meeting

-Wrote in blocks, added content in each section (intro, production, CHR, Safety assess)

-Had rounds of additional re-work due to redundancy or incohesiveness

-Planned to complete the draft by June for companies' review



2022 Fall Meeting

- Refined the focus of the manuscript
- Modified sections
 - Emphasis on challenges and alternative approaches for production & characterization
- Made recommendations on safety assessment for intractable proteins
- Polishing the first full manuscript before submitting to company review

Navigation

Headings Pages Results

Safety assessment of intractable proteins expressed...

Abstract

1. Introduction

2. Protein Characterization and Production

2.1 Protein Characterization

2.2 Intractable protein isolation from plant source

2.3 Intractable protein isolation from heterologo...

3. Safety assessment of intractable proteins

3.1. Potential hazard identification

3.1.1 History of safe use

3.1.2 Mode of action (MOA) and specificity

3.1.3 Sequence analysis with bioinformatic too...

3.2. Hazard characterization or supplemental stu...

3.2.1. Toxicological assessment

3.2.2. Environmental risk assessment (ERA)

3.3. Exposure assessment or supplemental studies

3.3.1. Stability to food processing

3.3.2. Stability to digestion

4. Discussions

Reference

Safety assessment of intractable proteins expressed in genetically modified crops

Abstract

Both developers and global regulatory agencies have gained significant experience and knowledge in safety assessment of NEP ~30 years. Simplified safety assessments with protein safety studies should be considered by leveraging the extensive scientific data and knowledge. Grams to hundred grams quantities of purified active protein are typically involved for all assessments. Producing and characterizing proteins that support the core protein safety studies remains the biggest challenge for the proteins that are difficult to work with, here referring to “intractable” protein. This technical review paper leverages the growing experience and knowledge with proposals and examples of alternative methods for key characterization end points are discussed to offer options and solutions to support the production and use of surrogate proteins expressed with heterologous systems due to nature of the intractable proteins and technology limitations. The need for additional protein safety assessment should be performed on a case-by-case basis depending on the GM trait and intended use. Alternative approaches or methods of protein production for the intractable proteins are described and proposed.

1. Introduction

Intractable proteins: The current regulatory paradigm for assessing the safety of newly expressed proteins in genetically modified (GM) crops assumes that multiple-gram quantities of purified protein can be isolated in an active form for use in the characterization and safety studies typically outlined in regulatory guidance. Production of such protein preparations is most often facilitated through expression in a microbial system (heterologous overexpression system). However, it is sometimes infeasible to produce a protein preparation that will meet the current regulatory paradigm due to technical obstacles (CODEX + Ref). We relate proteins of this kind as intractable proteins. “Intractable proteins are defined here as those proteins with properties that make it extremely difficult or impossible with current methods to express in heterologous systems; isolate, purify, or concentrate; quantify (due to low levels); demonstrate biological activity; or prove equivalency with plant proteins.” (Bushey, 2014).

Intractable Protein WS

(i) Introduction

- **Intractable proteins and challenges:**
 - Expression in heterologous systems
 - Isolation or purification
 - Quantification (extremely low levels)
 - Demonstration of biological activity
 - Proving equivalency with plant proteins (Bushey *et al*, 2014)
- **Current regulatory paradigm for NEPs:**
 - Food, feed and environment safety assessment
 - Potential toxicity and allergenicity
 - Protein requirements:
 - **Multiple grams - purified, active**
 - Often produced with microbial expression systems
 - Equivalent to plant derived protein – biochemical and functional

Intractable Protein WS

(ii) Characterization and Production

- **Key message**

- Challenges in plant derived protein characterization
- Production of gram quantities of intractable proteins - not feasible

- **Key content**

- Overview of different production systems
- Demonstration of challenges and efforts through method development
- Alternative methods and examples
- Protein characterization & equivalence
 - **Core Analyses** – molecular weight, amino acid Sequence and function
 - **Alternatives** for intractable proteins:
 - Molecular data (gene, transcript)
 - Phenotype (efficacy)
 - Structure modeling

Intractable Protein WS

(iii) Safety Assessment

- **Key message:**
 - Weight of evidence based
 - Regulatory framework & methodologies - update needed
- **Key content :**
 - **Core studies** for hazard identification
 - History of safe use
 - Mode of action and specificity
 - Bioinformatic sequence analysis (toxins, allergens)

Intractable Protein WS

(iii) Safety Assessment

- **Supplementary studies - Toxicological assessment**
 - **Acute toxicity**
 - Routine study not scientifically justified
 - Only when a potential hazard identified
 - High limit dose not feasible (2 or 5 g/kg BW)
 - Alternative approaches for intractable proteins
 - Margin of exposure (e.g., 100X)
 - Enriched samples or whole food
 - **28-day repeated dose**
 - Scientifically unjustified
 - “protein toxins” typically act acutely

Intractable Protein WS

(iii) Safety Assessment

- **Supplementary studies (continued) :**
 - Stability to digestion, processing and exposure assessment
 - Environmental risk assessment
 - Hypothesis-driven, problem formulation
 - NTOs
 - Alternative approaches for intractable proteins
 - Partially purified/enriched protein samples, plant tissues
 - Studies in field or controlled environment

Intractable Protein WS

Next Step

- Additional review by the working group
- Seek feedback from immediate partners within each company
- Incorporate changes based on feedback
- Move to formal company review process

Thank you to WS members!

Name	Company
Scott Bloomer	AOCS
Rong Wang	Bayer
Yanfei Wang	Bayer
Tao Geng	Bayer
Matt Cheever	BASF
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Brandy Verhalen	Corteva
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Barry Schafer	Schafer Scientific
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