The Toxicity Assessment of Newly Expressed Proteins (NEPs): Intractable Protein Challenges & 28-day Study Considerations

Ad hoc **meeting EFSA – GMO applicants**

October 5, 2023

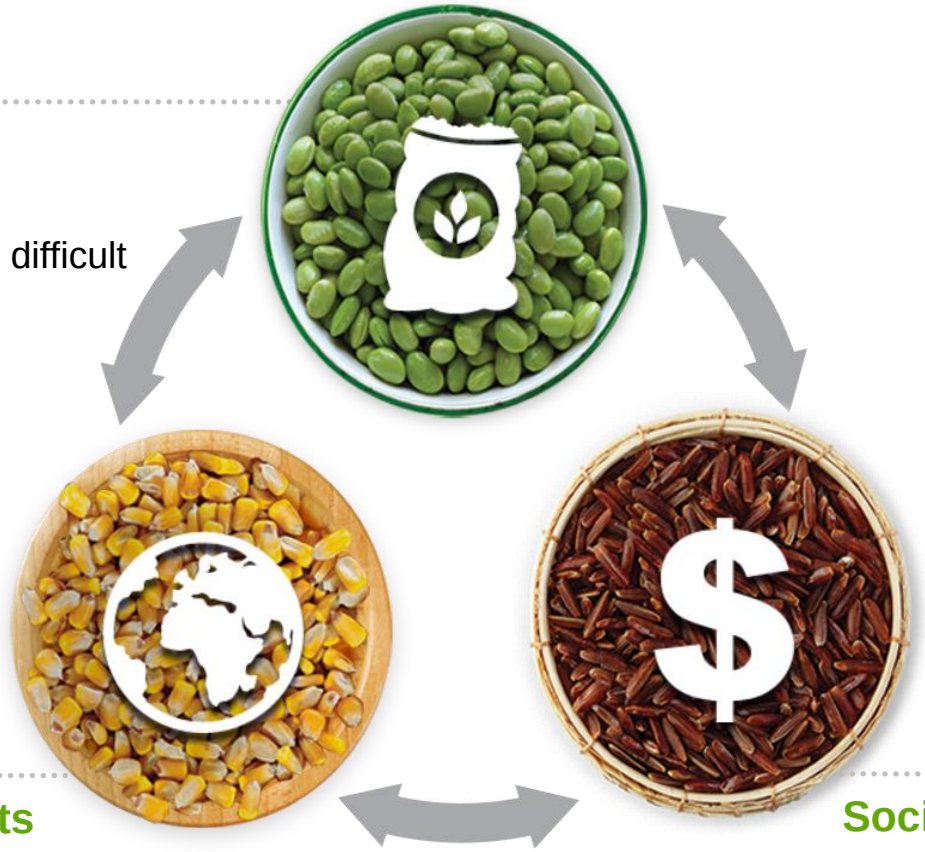
Agenda

- **Benefits of GM Crops**
- **Intractable Proteins**
  - Background
  - Challenges
  - Considerations
- **28-day Study Considerations**
  - 3R Legislation
  - Weight-of-evidence evaluation
  - Exposure based approach
- **New Perspective**
  - Based on 30 years of experience and data
  - Compatible with existing regulations
- **Conclusion**

Benefits of GM Crops*

Food security

- Increased yields
- Maintained yields during difficult growing seasons



Proven to be Safe

GM crops have been consumed by people and animals for 30 years, with zero confirmed health or safety issues.

Environmental benefits

- Reduced greenhouse gas emissions
- Improved soil health by tilling less
- Preserved natural resources

Socio-economic benefits

- Increased farmer income
- Created new jobs
- Improved quality of life

*Brookes, 2022

Late blight resistant GM potato: Example from a public sector project

- Potato is one of the most important crops globally for human consumption*
- Late blight causes 16% crop loss globally @ annual cost of €6.1 billion*
- Three stacked R-genes from wild potato relatives (Rpi-blb2, Rpi-vnt1, Rpi-mcq1)
- Confer resistance to *Phytophthora infestans*
- Prevent yield loss and greatly reduce fungicide use
- **R-genes/proteins are considered intractable due to very low expression levels***
- Developed by the International Potato Center (CIP)

Non-GM control

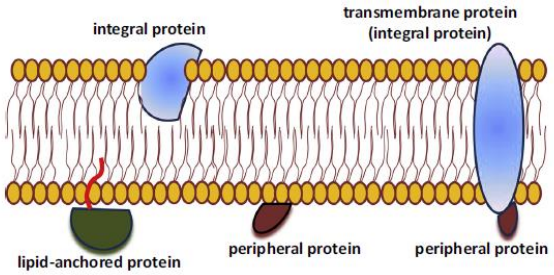
Late blight resistant GM potato

*Bubolz et al., 2022; Habig et al. 2018

Intractable Proteins

Background: Intractable Proteins

- Intractable proteins have properties that make it extremely difficult or impossible to:
  - Express in heterologous systems
  - Quantify (due to very low expression levels)
  - Isolate, concentrate, or purify from either heterologous expression systems or the GM plant
  - Demonstrate functionality of the isolated protein (e.g., the active form)
  - Demonstrate equivalency of the heterologously produced protein with the plant protein



Membrane proteins are an example of intractable proteins.
Bushey et al., 2014

Challenges: Intractable proteins cannot meet current data requirements

- Current data requirements for protein safety studies include:
  - Large amount of surrogate protein (~50g-100g)
    - Plant protein expression is low therefore, a surrogate protein is used in safety studies
  - Dosing concentration
  - Biologically functional form of protein

- Challenge to produce enough active and intact intractable proteins from heterologous expression systems (e.g., bacterial expression) or plant sources (e.g., seed or leaf)
  - Resulting in challenges to demonstrate equivalency to support the use of surrogate protein

Consideration: Weight of Evidence (WOE) approach is important for proteins, particularly intractable proteins

- The WOE approach for safety assessment should consider all the available information to support hazard identification and the evaluation of the NEP
- In the recent EFSA Network Meeting on Risk Assessment of GMO (June 2023), support for a WOE approach was given by some member countries.
- "The WOE approach is critical, as in a vast majority of cases no single assay or biochemical characteristic can identify a protein as a hazard. A stepwise approach is recommended to evaluate the safety of [proteins] taking the totality of information into account" (Roper et al., 2021).
  - Protein's source, function or activity
  - Evidence of Exposure or Consumption
  - Bioinformatics and/or higher order structure
  - History of Safe Use (HOSU)
  - Resistance to Digestion *In Vitro*
  - Stability to Processing Conditions
  - Expression Level and Dietary Intake

Due to the nature of intractable proteins, it is extremely difficult, or impossible, to generate the data required using the methods available for tractable proteins, and therefore a technically feasible and weight-of-evidence approach is needed.

Delaney et al, 2008; Hammond et al, 2013; Bushey et al, 2014; Brune et al, 2021; Roper et al, 2021; Anderson et al, 2019

Consideration: Exposure-based approach should be used for dose selection for intractable proteins

- No hazard to date has been identified for a NEP when a stepwise WOE approach has indicated no toxicological concern. The 28-day study has only been confirmatory.
  - "The need for a shift in experiments within the given weight-of-evidence approach seems worth discussing".*
- If a supplemental study, such as 28-day study is required, then an exposure-based approach should be used to meet the regulatory data requirement.
    - Unlike chemical exposure scenarios, testing NEPs in GM crop products at the limit dose of 1000 mg/kg/day results in doses orders of magnitude higher and in excess of realistic human and animal exposure.
- An exposure-based approach to dose selection should be adopted instead of requiring the limit dose.
    - Use GM protein concentration and human/animal dietary consumption levels to establish relevant doses to be tested

*EFSA. Network on Risk Assessment of GMO; Minutes of the 15Th meeting (June 2023).

Currently available *in silico* tools are fit-for-purpose
Regulatory-accepted *in vitro* assays are not available

In silico tools to identify hazard

- **Currently using bioinformatic framework to assess protein toxicity risk**
  - Current bioinformatics framework has built confidence in excluding mammalian toxic proteins from GM products
  - No protein is required therefore no concern for intractable protein

In vitro toxicity testing

- **Currently, *in vitro* assays to replace *in vivo* studies require further discussion**
  - *In vitro* toxicity testing for proteins continues to remain of considerable interest (Bushey et al., 2014, Roper et al., 2021)
  - Proof of concept research with human intestinal epithelial cell line monolayers is one example of an on going effort but **requires further development** and acceptance by Regulatory Authorities (**long-term solution**)
  - Regulatory-accepted *in vitro* assays to assess protein toxicity, including for intractable proteins are not available
  - Collaboration amongst industry, government, and academia is needed to advance *in vitro* toxicity testing

Next Opportunity: Intractable proteins play a role in delivering sustainability goals

"The potential benefits of intractable proteins include a broad range of valuable traits such as disease resistance, drought tolerance, nitrogen use efficiency, and enhanced nutrient value" (Bushey et al., 2014).

"Many newer GM crops will express different types of intractable proteins to withstand drought, to enhance utilization of nitrogen, or to alter their composition for nutritional purposes" (Delaney et al., 2017).

Summary for Intractable Proteins

Due to the nature of intractable proteins, CLE is requesting fit-for-purpose considerations when assessing these proteins, including acceptance of a technically feasible data set.

- There are several technical challenges related to intractable proteins that make it difficult to meet data requirements
- Recognizing these challenges, a way forward is needed
  - Leveraging a WOE approach is important for all proteins, particularly intractable proteins
  - An exposure-based approach should be used for dose selection for all proteins, not only intractable proteins
  - *In vitro* testing would require development, validation, and acceptance
    - Feasibility not yet demonstrated

28-day Study Considerations

28-day toxicity studies have not identified a hazard for NEPs, and do not align with the 3R principles or legislation

- **DIRECTIVE 2010/63/EU** (protection of animals used for scientific purposes) requires "an evaluation of the objectives of the project, the predicted scientific benefits or educational value"
- "Defaulting to *in vivo* toxicology studies, as is often required for regulatory approvals, does not reflect ethical use of animals in scientific research and testing as outlined by the 3R's of responsible animal use" (Roper *et al.*, 2021).

Eliminating the 28-day study requirement is consistent with the EU Commission's planned roadmap to further reduce animal testing with the aim to ultimately move to an animal-free regulatory system under chemicals legislation.

28-day toxicity studies are performed only for EU (EFSA)

- To date, CLI member companies have performed
  - Thirty-nine 28-day toxicity studies for EFSA*
  - Using a total number of over 4,600 animals
- None of these 28-day toxicity studies identified a hazard for the NEPs
- Some regulatory authorities do not require submission of any *in vivo* data to conduct their risk assessment

*submitted by three CLI companies

A 28-day toxicity study does not provide additional information to support the protein safety assessment

- Testing of NEPs (Regulation (EU) No 503/2013)
  - The 28-day toxicity study is triggered in the case where the history of safe use for consumption is not “duly documented”.
- To date, 28-day toxicity studies have not provided additional information on the protein safety assessment, but instead have only confirmed existing *in silico* and *in vitro* data.

Summary for the 28-day Study

- To date, 28-day toxicity studies have not provided additional value to the protein safety assessment
  - CLI member companies have performed thirty-nine 28-day toxicity studies for EFSA using over 4600 animals to meet the data requirements of the 28-day toxicity study
- Consideration of the entire WOE approach supports the safety assessment
  - Should not solely rely on HOSU or HOSC
  - If testing is needed, a weight of evidence approach provides relevant data to support the safety assessment of the protein
- **"If the NEP is related to a family of proteins that has a history of safe use based on bioinformatics and literature review, and is not homologous to known protein toxins, then any supplementary toxicology study is not necessary"** (Brune et al., 2021).

New Perspective

Years of experience and knowledge working with NEPs

- Over the past 30 years, significant experience and knowledge in the safety assessment of NEPs has been gained.
- The Codex (Codex 2003) framework has been successfully applied for over two decades in the evaluation of NEPs.
- It is noteworthy that such approaches can be equally applicable to intractable proteins as well as to tractable proteins currently subject to traditional safety assessment.

Interpretation of EU 503/2013

- HOSU "duly documented"
  - If YES, then no specific toxicity testing shall be required
    - In most instances, HOSU is "duly documented" with information from the literature or by demonstrating that a homologous protein found in food is consumed safely.
  - If NO, then specific toxicity testing shall be selected on a case-by-case basis, depending on what is known about the protein's source, function or activity, and HOSU
    - In most cases, a protein's source, function or activity can be addressed from information derived from the literature, homologs from another crop or organism, or another protein within the family using sequence and/or structural similarity.

Data requirements should be applied on a case-by-case basis

- The NEPs in the earliest GM crops were produced in heterologous expression systems, isolated in large quantities, and in their active form. These early regulatory packages shaped the current standard for assessing safety.
- Historically, all data has been provided and is now expected even though it goes beyond what is outlined in the EU 503/2013.
- Appreciate that EU 503/2013 is legally binding, but CLE believes it allows for a pragmatic and science-based application and case-by-case interpretations.

When is protein needed?

- Specific toxicity testing (a – e) shall be selected on a <u>case-by-case basis,</u> depending on what is known about the protein's source, function or activity, and HOSU
  - a) Protein characterization – most endpoints require protein
  - b) Bioinformatics – no protein required
  - c) Stability to processing – requires protein (and protein and animals for antibody production)
  - d) Resistance to digestion – requires protein (and protein and animals for antibody production)
  - e) 28-d repeated dose study – requires large quantities of protein and animals
- Most NEPs are expressed at very low concentrations
  - Consideration of a NEP's expression level and dietary exposure when assessing the need for specific toxicity testing should be taken into account – no protein required

Conclusion

- Due to the nature of intractable proteins, CLE is requesting fit-for-purpose considerations when assessing these proteins, including acceptance of a science-based, technically feasible limited data set.
- The case-by-case approach prescribed in Regulation (EU) No 503/2013 should be adopted rather than defaulting to the 28-day toxicity study.
- The 28-day studies with NEPs have only confirmed the results demonstrated by a WOE evaluation and have not provided additional value to the protein safety assessment.
- Collaboration amongst industry, government, and academia would be needed to advance *in vitro* oxicity testing for NEPs.

CLE looks forward to working together with EFSA to modernize the safety assessment of proteins, particularly for intractable proteins where current approaches are not technically feasible.

Working together will enable the availability of innovative and sustainable solutions to support the agricultural community.

Thank you

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