PROTEIN SAFETY OF PRESENT AND FUTURE GM PLANTS

Applicants meeting

October 2023

EUROPEAN FOOD SAFETY AUTHORITY

PURPOSE OF DISCUSSION

- Continue discussion on protein safety – difficult cases
- Share/discuss potential solutions to the protein safety overall
- Future development needs
- Protein safety to cover both humans (food) and animals (feed)

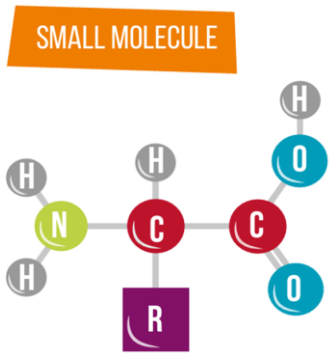
RISK ASSESSMENT PRINCIPLES – PROTEIN SAFETY

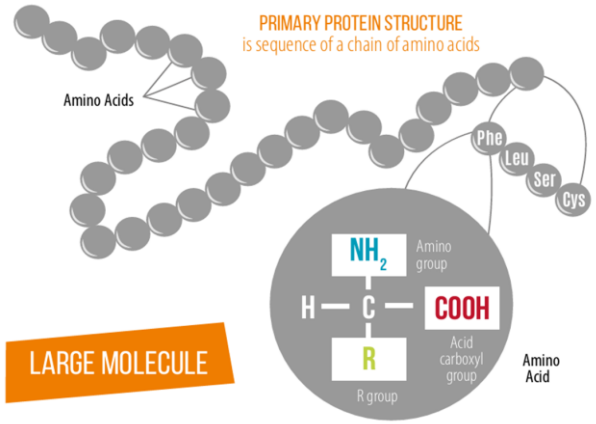
Foods derived from modern biotechnology

Second edition

2003-2009

Adapted from chemical risk assessment





Urgent need to improve and modernise protein safety assessments

But HOW?

EFSA GMO Network Meeting

Present and upcoming challenges

HoSU: Definition and criteria needed

3R Principle

Need for alternative methods

Complex cases, e.g. high number of proteins

SynBio vs. HoSU?

CHALLENGES

Revision of existing methods needed??

Proteins difficult to extract/purify, e.g.membrane-bound proteins

Adequate testing specificity

Proteins difficult to test

Need for a shift in experiments within a weight-of-evidence approach

Development of new methods: standardization and validation

Partial similarity to toxins/allergens

SCENARIOS AND PRESENT/FUTURE ISSUES

- GM plant with 1 protein easy to purify, e.g. mEPSPS protein
- GM plant with high number of proteins easy to purify
- GM plant with 1 protein difficult to purify
- GM plant with high number of proteins difficult to purify

Aspect reflected in Allergenicity and Synbio Opinions in 2022

But how to deal with this in a credible&practical manner?

DEVELOPMENT NEEDS

Weight of evidence approach Protein safety

- History of safety use
- Protein characterisation
- Mode of action
- Stability
- Source organism
- Phylogeny
- Structural/Functional similarity to known proteins
- Similarity to known toxins/allergens
- Fate in the gastrointestinal tract
- Interaction between proteins
- Others

Toxicological assessment

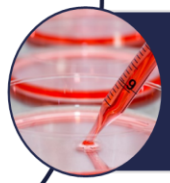
Tiered approach using *in vivo* studies only if concerns identified

Allergenicity assessment

Ranking of allergens[2] and post-market monitoring

New Approach Methodologies (NAMs) [3]

In silico tools: information on the derived structure of the novel protein



In vitro testing: stability tests could better inform about the fate of the novel proteins during processing, storage and after digestion in the gastrointestinal tract

EUROTOX 2023 – Toxicology letters – https://toxlet-384-s1.elsevierdigitaledition.com/
[2] EFSA GMO Panel, 2022. Scientific Opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. EFSA Journal 2022;20(1):7044
[3] Cattaneo et al., 2023. Implementing New Approach Methodologies (NAMs) in food safety assessments: Strategic objectives and actions taken by the European Food Safety Authority. Trends in Food Science & Technology, 133:277-290

HISTORY OF SAFE USE

- Intuitively easy for extreme cases but difficult to implement
- Definition and criteria needed as not yet one internationally recognised
- Fundamental concept in GMOs – comparative assessment
- Concepts to be considered:
  - HOSU for plant/varieties
  - HOSU for proteins
- SO on Criteria for NGTs published in 2022
  - Function/Structure – today mainly considered primary sequence but….interesting developments in protein 3D modelling

IN SILICO ANALYSIS

- Current bioinformatics only based on primary sequence
- Extrapolations for the assessment of proteins, e.g. highly similar proteins
- Some new proposals might need consensus with international community (e.g. 35% cut-off in allergenicity)
- Developmental projects
  - Allergenicity: ranking/targeted databases and new approaches
  - Toxicity: new *in silico* tools

IN VITRO ANALYSIS

- Protein characterisation and equivalence
- Substrate specificity – criteria on selection
- Studies on protein stability, but mainly pepsin test (past assessments, EFSA guidance 2017, procurement 2019 and EFSA opinions in 2021-2022)
- Gastrointestinal digestion tests (including human/animals)
- Future interest to expand *in vitro* testing, when needed

IN VIVO + OTHER

- Toxicity studies (e.g. 28-day and others) – reference to OECD but 3Rs

- Other additional aspects:

  Exposure (expression levels)

  Post-market monitoring

QUESTIONS

- How to define a new logic flow for the stepwise, case-by-case, weight of evidence approach in the protein safety assessment?
- Does each protein have to be tested individually or are there also experiments/strategies that can be used to test proteins in combination?
- How to effectively assess additive, synergistic or antagonistic effects of a combination of multiple proteins?
- Which components/tests of the current weight-of-evidence approach could be replaced by new/revised components/tests? What could the new components/tests look like?

Also discussed at EFSA GMO Network Meeting

QUESTIONS

- What criteria can be used for History of Safe Use?
- What is considered an acceptable degree of similarity in primary aa sequence (probably not possible to set a threshold)?
- How to consider 2D and/or 3D structure of proteins?
- How to use info on function and stability of proteins?
- What *in vitro* test can be considered in the weight of evidence approach?
- Are animal studies needed? If so, when? Can they be replaced/reduced?
- In which cases would animal testing be essential?

Also discussed at EFSA GMO Network Meeting

FUTURE DEVELOPMENTS

Scientific Opinion on protein safety

End 2024 / Beginning of 2025

Thank you very much!!!!

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