# PROTEIN SAFETY OF PRESENT AND FUTURE GM PLANTS

Applicants meeting

April 2024

EUROPEAN FOOD SAFETY AUTHORITY

# PROTEIN SAFETY – RISK ASSESSMENT REQUIREMENTS AT PRESENT

**Protein safety = protein toxicity and allergenicity**

**<u>Codex 2003/2009 defined the principles for the assessment</u>**

- Main information considered:
  1. Knowledge on the source/protein – HoSU
  2. Bioinformatics analysis
  3. *In vitro* studies
  4. *In vivo* studies

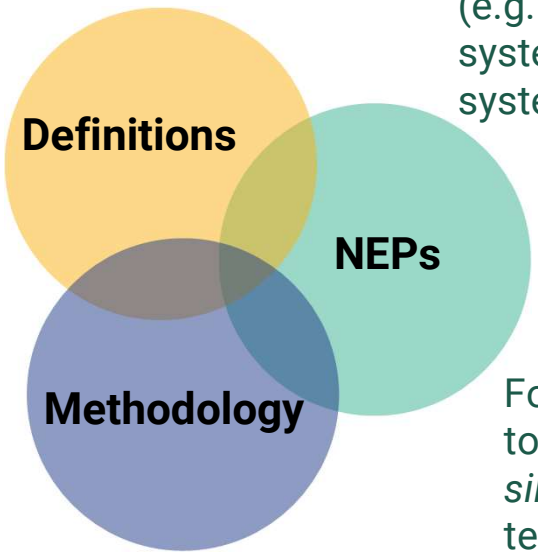

2003-2009

# FUTURE ADDITIONAL NEEDS – FROM EXPERIENCE GAINED

Similarity

HoSU

Thresholds




For proteins difficult to extract/purify (e.g. membrane-bound): purification systems or alternative expression systems

For high number of proteins: agreement on testing the mixture or testing each individually

For proteins with partial similarities to toxins/allergens: more targeted *in silico* tool or hypothesis driven *in vitro* tests

Standardisation and validation of new methods

Experimental shift within a weight-of-evidence approach

Need for complementary/alternative methods

# 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**

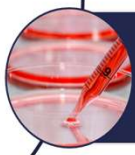
Tiered approach using *in vivo* studies only if concerns identified

**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

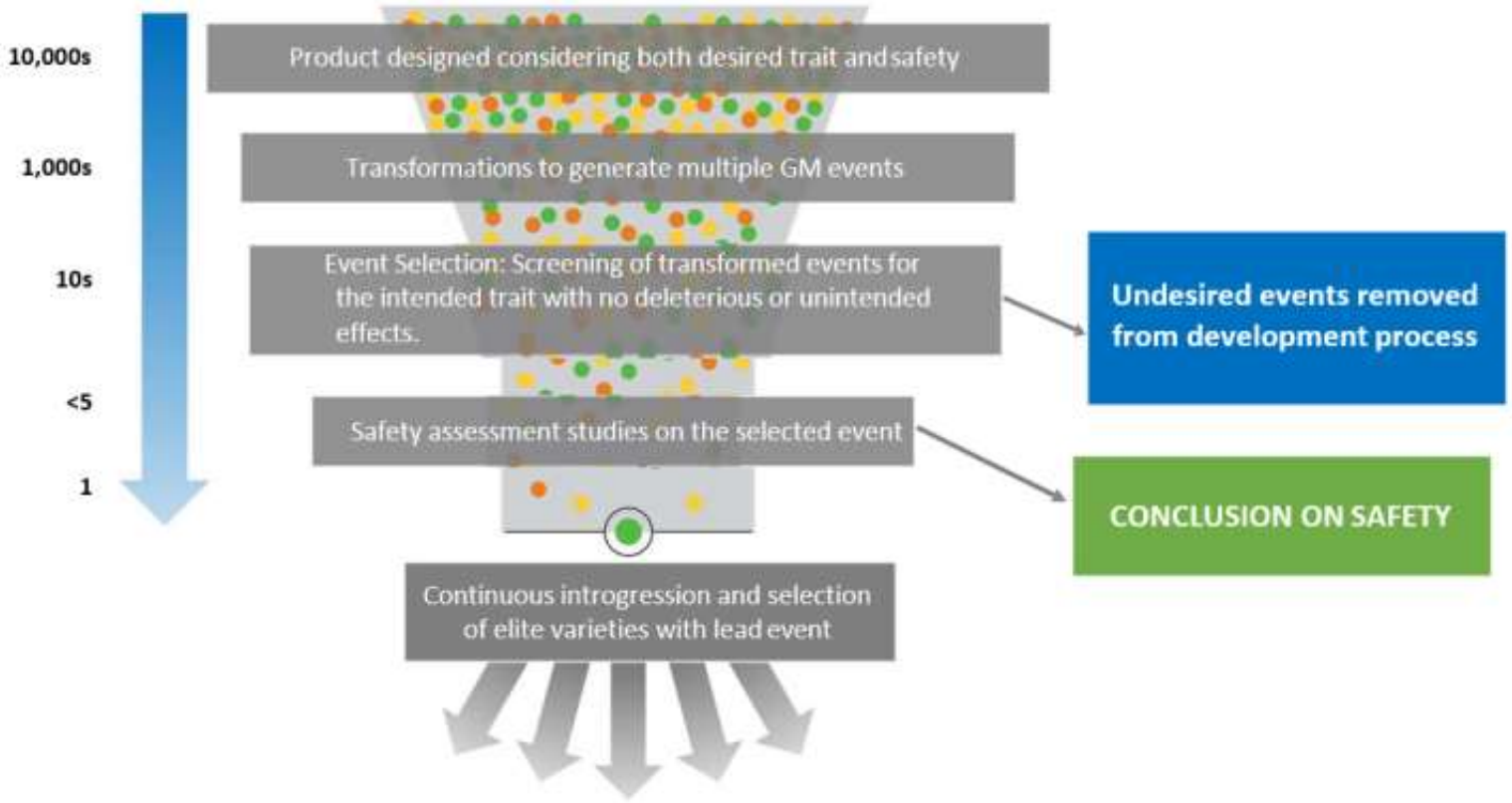
[3] Cattaneo et al., 2023. Implementing New Approach Methodologies (NAMs) in food safety assessments: Strategic objectives and actions taken by the European Food Safety Authority. Trends in Food Science & Technology, 133:277-290

# EFSA GMO PANEL MANDATE

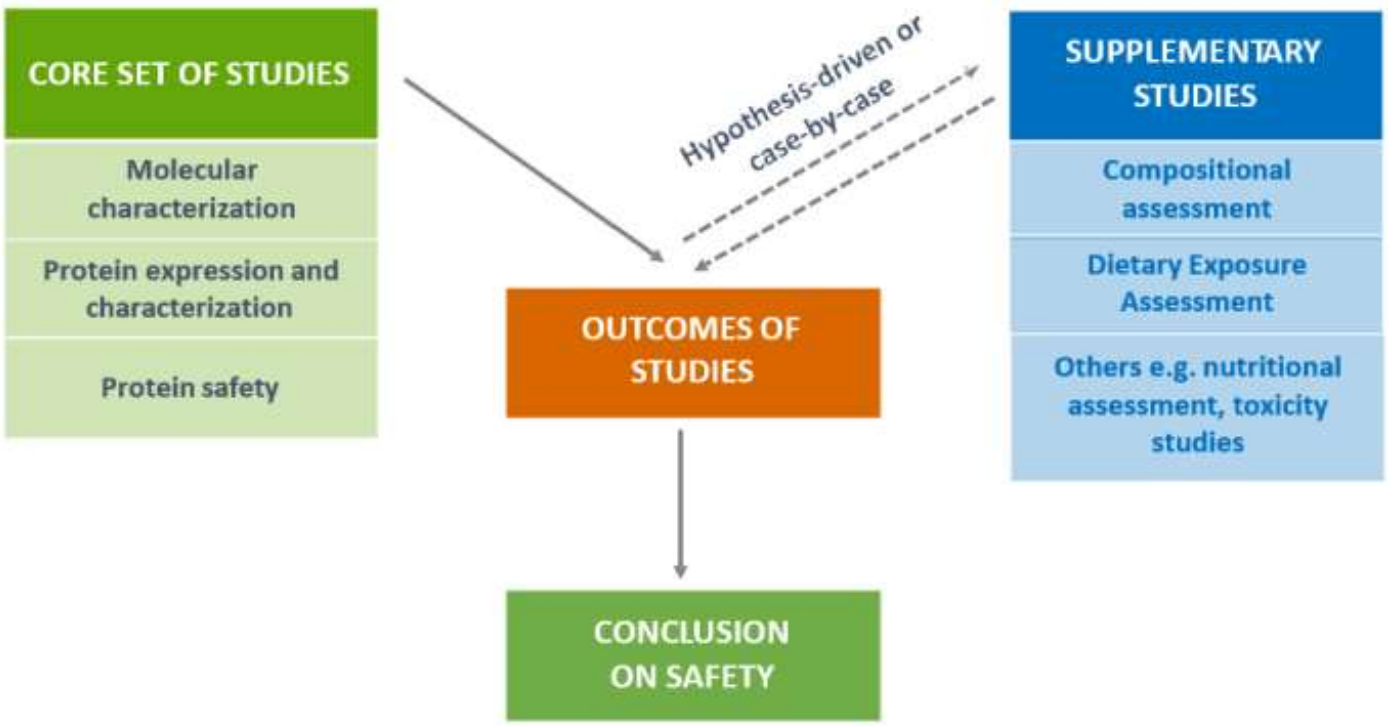
Scientific Opinion reflecting on current practice, challenges and future opportunities of protein safety in GMOs

1. Lessons learned from experiences in the assessment of newly expressed proteins in the last 20 years, including more recent complex cases
2. Building on the experience and issues identified, develop a critical appraisal of new methodologies available with the potential to be used as complementary/ alternative testing strategies to current methodologies described in legal frameworks
3. Road map for future implementation of such complementary/alternative methods in risk assessment strategies
4. Recommendations for further research to address methodological development needs

# WATERS ET AL 2021



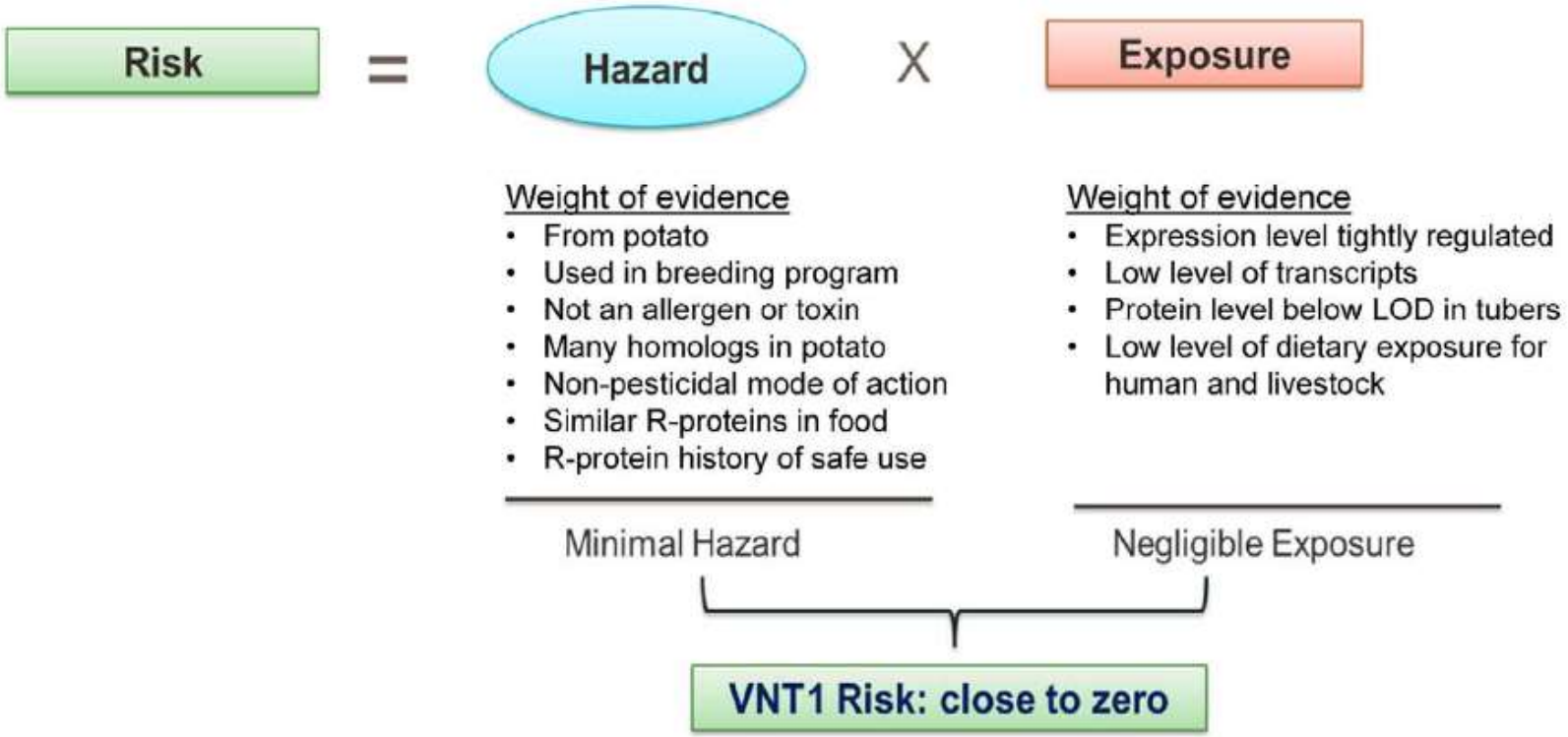
# WATERS ET AL 2021 AS WELL AS BRUNE ET AL 2021



# ROPER ET AL 2021

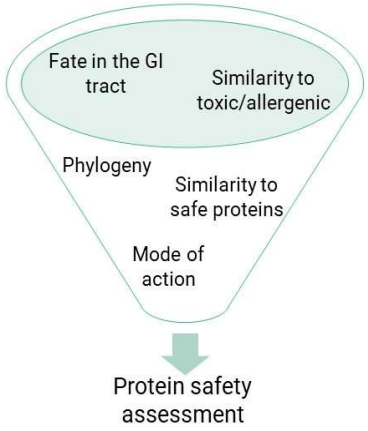
- A stepwise approach is recommended to evaluate the safety of NEPs taking the totality of information into account
- Core studies
  - HoSU of the NEP – demonstration of prior human/animal consumption or closely related proteins
  - No need for any specific toxicity or allergenicity testing in cases where both the plant and proteins expressed in the GM plant have a history of safe consumption by humans and animals – reference to EFSA guidance 2011
  - HoSU structural and/or functional similarity and exposure to other endogenous proteins
  - **The appropriate methods for establishing this similarity need to be determined on a case-by-case basis**
  - Bioinformatics results should be regarded as guiding rather than predictive
  - **Intestinal epithelial cell line monolayers from rodents and humans have been investigated** to evaluate the effects of known hazardous proteins, including ricin and PHA-E

# HABIG ET AL 2018



# STEPWISE APPROACH IN THE PROTEIN SAFETY ASSESSMENT

- Protein vs simple chemical safety assessments
- Comparative approach as baseline–HoSU, familiarity, knowledge on proteins
- What is considered safe?
- What is considered a hazard in protein safety?
- Structural/functional similarity; but how similar is similar?
- How can evidence of consumption of a protein or source be established?
- Is there a need or possible to have additional thresholds/cut-off values (e.g. bioinformatics)?
- Is *in vitro* testing ready to be used when needed?
- How can exposure be considered in protein safety – WoE?

# PROTEIN SAFETY ASSESSMENTS

Thank you very much!!!!

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# BRUNE ET AL 2021

- Protein safety - CORE STUDIES
  - Toxicological assessment: As a result of the acidic conditions and digestive enzymes of the gastrointestinal tract, dietary proteins are typically rapidly degraded into small peptides and individual amino acids before absorption and metabolic use by the body
    - HoSU of the NEP
    - HoSU of the source organism
    - Bioinformatics for sequence comparison
    - Mode of action and functional specificity: If the mode of action and functional specificity of the NEP are well understood and have been shown to have low relevance to humans or animals, this provides confidence that it is unlikely to cause harm when consumed
  - Allergenicity
    - A stepwise approach is recommended where hazard identification is first performed for all NEPs. If a hazard is identified, exposure characterization should be done (supplementary info)
    - HoSU of the NEP and familiarity with the source organism
    - Aminoacid sequence similarity and bioinformatics: sequence level, structural relatedness, structural considerations

# BRUNE ET AL 2021

- Protein safety – SUPPLEMENTARY STUDIES
  - Protein abundance in food and feed
  - Processing
  - Resistance to digestion
  - Tox studies with animals
  - Compositional analysis
  - Dietary exposure assessment

  - Case-by-case studies
    - Post translational modifications – if identified, further studies needed as it can change physicochemical charac.
    - Mode of action
    - Substrate specificity