PROTEIN SAFETY ASSESSMENT MANDATE

UPDATE

Advisory Forum
March 2025
NIF Unit - CSO

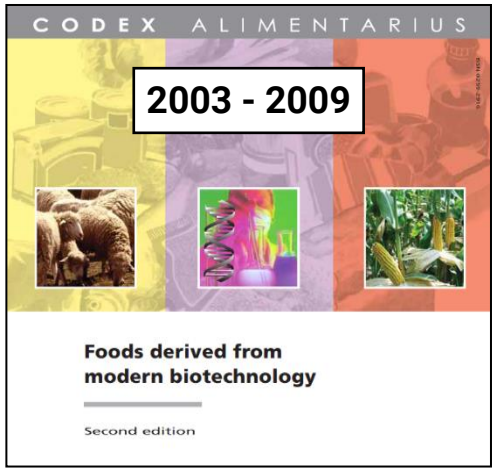
EUROPEAN FOOD SAFETY AUTHORITY

PROTEIN SAFETY – NEWLY EXPRESSED PROTEINS (NEPS)

Protein safety = protein toxicity and allergenicity

Codex 2003-2009 principles for risk assessment

1. Knowledge on the source/protein – History of safe use (HoSU)
2. Bioinformatics analyses
3. *In vitro* studies
4. *In vivo* studies

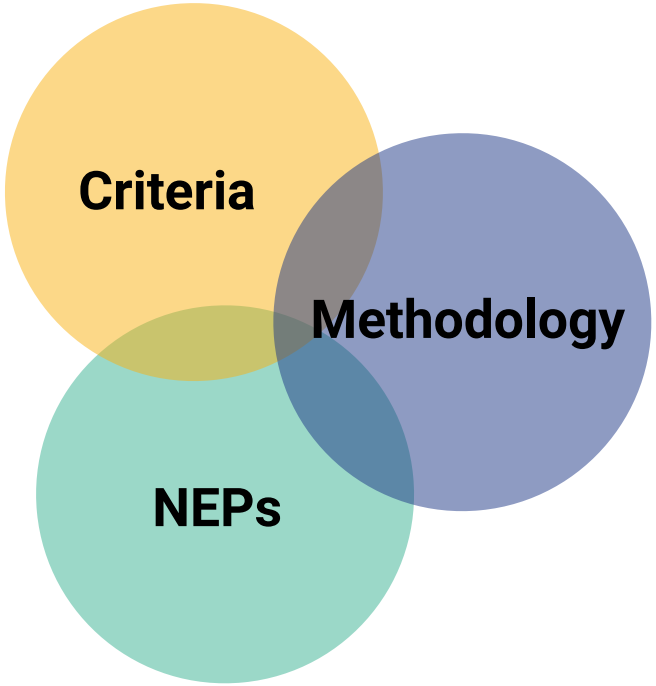


PROTEIN SAFETY – CHALLENGES

Proteins difficult to extract/purify

High number of proteins

Proteins with partial similarities to toxins/allergens



Need for complementary/ alternative methods

PROTEIN SAFETY – EFSA PREPARATORY WORK

- **Allergenicity assessment:**
    - Developing novel approaches to **increase reliability of predictions** (Published)
    - Development of an **adverse outcome pathway**, celiac disease (Published) and EFSA/OECD
    - Peptide binding **prediction HLA-DQ2/HLA-DQ8** (Published)
- **Toxicity assessment:** exploring *in silico* (Published) and *in vitro* tools (Published)
- **Effects of processing:** relevance on protein safety (Published)
- **Methodology for Open Reading Frames:** GMO applications (Published)
- **Consultation:** Stakeholders, Member States, Scientific community

PROTEIN SAFETY OPINION

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

1. **Lessons learnt**
2. **Complementary/ alternative testing strategies**
3. **Roadmap for future implementation**
4. **Recommendations for further development and research**

GAPS AND DEVELOPMENT NEEDS

- **HoSU, familiarity and related terms**
- ***In silico* tools**
- ***In vitro* tools**
- ***In vivo testing***
- ***De novo sensitisation***
- **Post Market Monitoring**

EFSA welcomes feedback on the necessary developmental and research activities and seeks input on how these should be prioritised

CURRENT STATUS AND NEXT STEPS

Survey

Finalised on 18th Feb 2024

Public consultation

Q1-2025

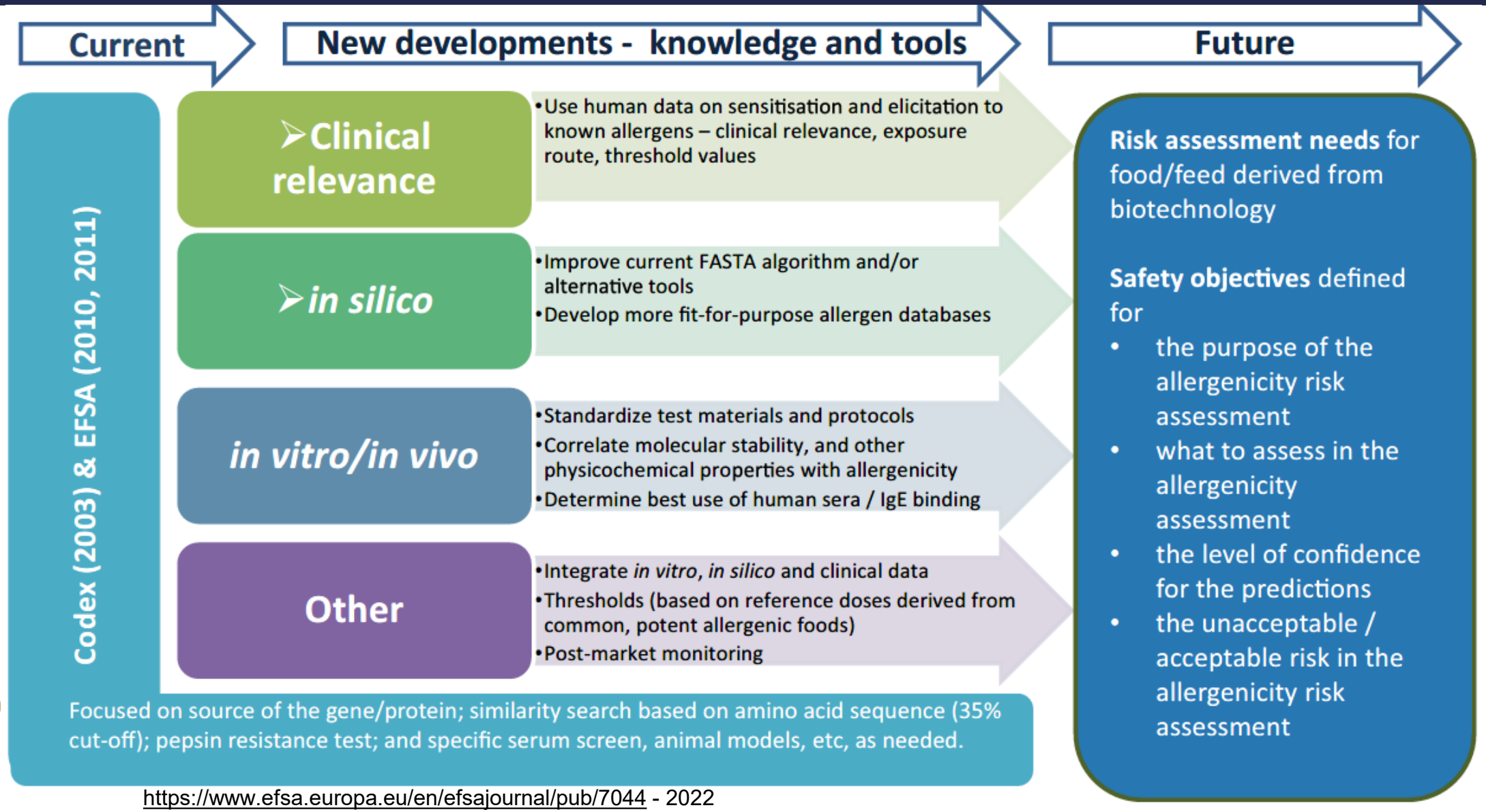
Adoption of the Scientific Opinion

Mid-2025

Open till March 12th

https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk000003SPsz/pc1278

ALLERGENICITY – ROADMAP TO IMPROVED 'WEIGHT OF EVIDENCE' IN RA



https://www.efsa.europa.eu/en/efsajournal/pub/7044 - 2022

ALLERGENICITY PREDICTION

- **Bioinformatics tool for cross-reactivity assessment** supported by a **clinically-relevant allergen database**
- **Novel, clinically relevant allergies** (primary sensitisation) – ***In silico*** assessment of the antigenic potential of insect proteins by modelling their **binding capacity to antigen-presenting cells** (HLA)
- Scenarios -**when and how- *in vitro* testing** (and others when required) is needed to confirm the allergenic potential of the predictions

ACKNOWLEDGMENTS

GMO Panel: Josep Casacuberta, Francisco Barro, Albert Braeuning, Pilar Cubas, Ruud de Maagd, Michelle M. Epstein, Thomas Frenzel, Jean-Luc Gallois, Frits Koning, Antoine Messéan, F. Javier Moreno, Fabien Nogué, Giovanni Savoini, Alan H. Schulman, Christoph Tebbe, Eve Veromann

NIF Unit: Ana Afonso, Reinhilde Schoonjans, Michele Ardizzone, Tilemachos Goumperis, Aleksandra Lewandowsky, Tommaso Raffaello, Kostas Paraskevopoulos, Nikol Papadopoulou, Antonio Fernandez

CSO: Estefanía Noriega Fernández, Carlos Das Neves

Engagement Team: Margherita Guidi/Matthew Ramon

Thank you very much!

Questions?

Feedback?

STAY CONNECTED

SUBSCRIBE TO
efsa.europa.eu/en/news/newsletters
efsa.europa.eu/en/rss
Careers.efsa.europa.eu – job alerts

LISTEN TO OUR PODCAST
Science on the Menu –Spotify, Apple Podcast and YouTube

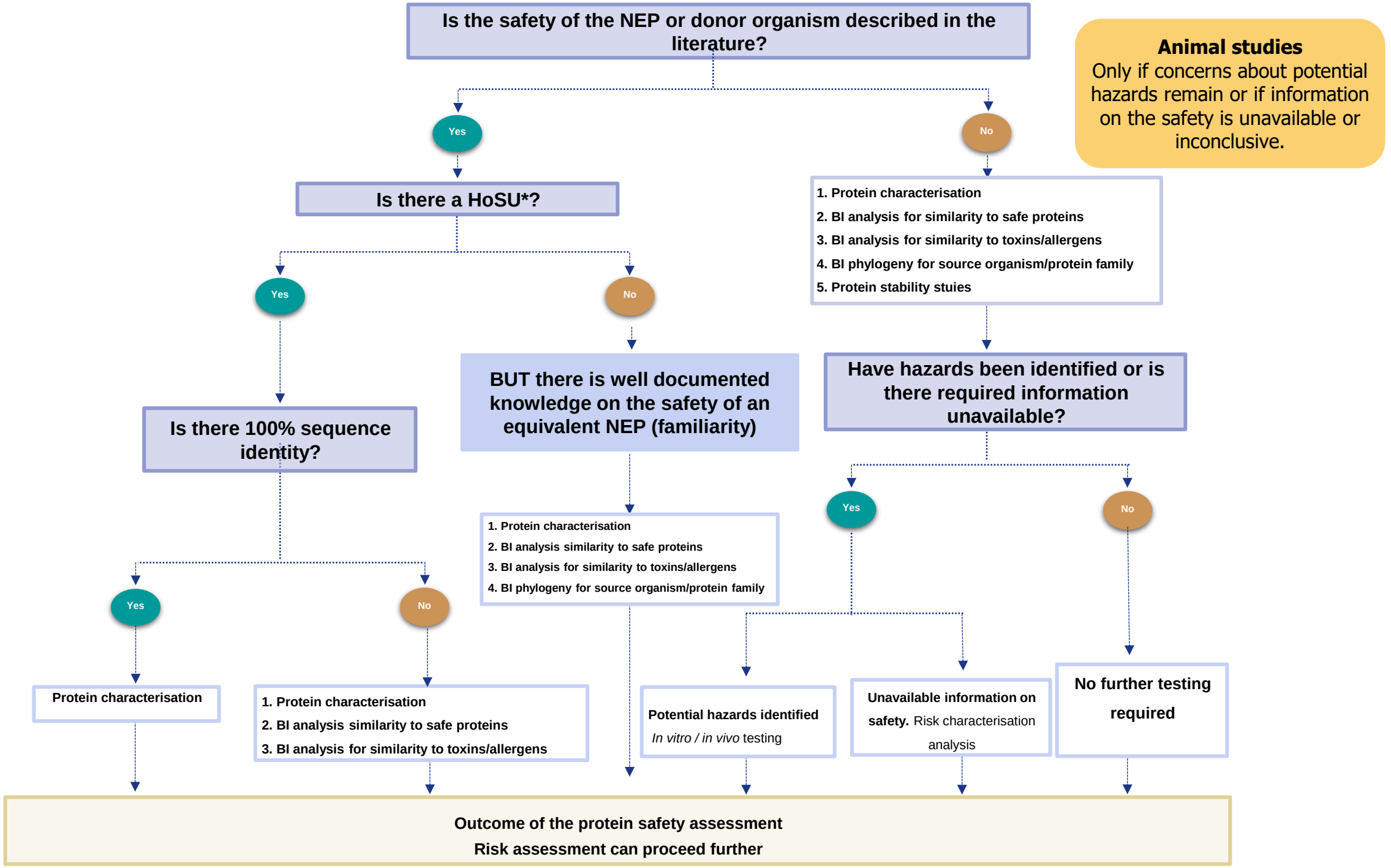
FOLLOW US ON TWITTER
@efsa_eu @methods_efsa
@plants_efsa @animals_efsa

FOLLOW US ON LINKEDIN
Linkedin.com/company/efsa

FOLLOW US ON INSTAGRAM
@one_healthenv_eu

CONTACT US
efsa.europa.eu/en/contact/askefsa

efsa
EUROPEAN FOOD SAFETY AUTHORITY


Is the safety of the NEP or donor organism described in the literature?
Yes
No
Animal studies
Only if concerns about potential hazards remain or if information on the safety is unavailable or inconclusive.
Is there a HoSU*?
1. Protein characterisation
2. BI analysis for similarity to safe proteins
3. BI analysis for similarity to toxins/allergens
4. BI phylogeny for source organism/protein family
5. Protein stability stuies
Yes
No
Is there 100% sequence identity?
BUT there is well documented knowledge on the safety of an equivalent NEP (familiarity)
Have hazards been identified or is there required information unavailable?
1. Protein characterisation
2. BI analysis similarity to safe proteins
3. BI analysis for similarity to toxins/allergens
4. BI phylogeny for source organism/protein family
Yes
No
Yes
No
Protein characterisation
1. Protein characterisation
2. BI analysis similarity to safe proteins
3. BI analysis for similarity to toxins/allergens
Potential hazards identified
In vitro / in vivo testing
Unavailable information on safety. Risk characterisation analysis
No further testing required
Outcome of the protein safety assessment
Risk assessment can proceed further

PHASE 1

- Building a fit-for-purpose risk assessment database of proteins involved in IgE-mediated allergic reactions
- Ranking of proteins with different allergenic potential according to their clinical relevance (based on quality criteria; e.g., method of detection and diagnosis, prevalence, and severity of symptoms)

Novel protein

Development of a fit-for-purpose risk assessment bioinformatic tool specific to the database developed and capable of allocating novel food proteins in any of these quality levels according to their amino acid sequence and physicochemical properties, secondary structure properties, 3D conformation, HLA alleles, etc.

Increasing risk of IgE allergenicity

No risk	Low	Medium	High
(e.g., lack of any evidence of allergenicity)	(e.g., evidence of *in vitro* specific IgE binding but missing biological activity)	(e.g., evidence of basophil activation, histamine release, or skin test reactivity)	(e.g., evidence of challenge test reactivity using subjects allergic to the source)

PHASE 2

In vitro tests (e.g., specific human sera screening studies and/or digestion)	Skin prick and/or cell activation tests	Oral challenge

Fernandez et al. 2021. Trends in Biotechnology, Volume 39, Issue 1, 4 - 8

Trends in Biotechnology

https://www.efsa.europa.eu/en/supporting/pub/en-8840 - 2024

UNIVERSITY OF SURREY

EuroFIR
European Food Information Resource