PROTEIN SAFETY MANDATE AFTER PUBLIC CONSULTATION

EFSA NIF Unit

June 2025

EUROPEAN FOOD SAFETY AUTHORITY

AGENDA

Objective: ensuring a full understanding of the comments submitted prior final adoption by June/July-2025. This meeting is not intended as a forum for submitting new or additional comments, nor for providing any prior indication of the GMO Panel's final conclusions

Time	No.	Item
14:00	1	Welcome
14:05	2	Summary of the comments provided – Participants to present followed by exchange of views
15:30	3	Terms of Reference 4, Gaps and development needs identified in the context of comments received at Public consultation – EFSA/Participants exchange of views
16:30	*End of the meeting*	

Details of the section	Number of comments
Abstract	3
1 Introduction	4
2 Data and Methodologies	1
2.2 Consultations	1
3 Assessment	2
3.1 ToR1: Lessons learned from EFSA experience in the assessment of NEPs in the last 20 years, including more recent complex cases	10
3.2 ToR2: Building on experience above and issues identified, a critical appraisal of new methodologies available with the potential to be used as...	11
3.3 ToR3: Road map for future implementation of such complementary/alternative methods in risk assessment strategies	10
3.4 ToR4: Recommendations for further research or for addressing methodological development needs	9
4 Conclusions	7
5 Documentation as provided to EFSA	1
6 References	1
7 Annexes	1
8 Abbreviations	2

PROTEIN SAFETY ASSESSMENT MANDATE

Summary of the comments provided – all to present

Stakeholder	Category[a]	Country
ANSES (French Agency for Food, Environmental and Occupational Health & Safety)	Not indicated	France
Atova Regulatory Consulting SL	Not indicated	Spain
BASF Belgium Coordination Center Comm.V.	Not indicated	Belgium
Bayer Agriculture BV	Not indicated	Belgium
BFR - Bundesinstitut für Risikobewertung	Not indicated	Germany
Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	Not indicated	Germany
Corteva Agriscience Belgium B.V.	Not indicated	Belgium
CropLife Europe	Not indicated	Belgium
Hjelle Consulting Group	Consultant	Belgium
Undisclosed	Submission on Personal Capacity	Belgium
Sciensano	Not indicated	Belgium
Testbiotech e.V. - Institute for Independent Impact Assessment of Biotechnology	Not indicated	Germany
University Medical Center Utrecht	Academia/Research Institute	Netherlands

PUBLIC CONSULTATION

Main comments:

1) Overall agreement on the spirit of the document and the need to modernise approaches/methods

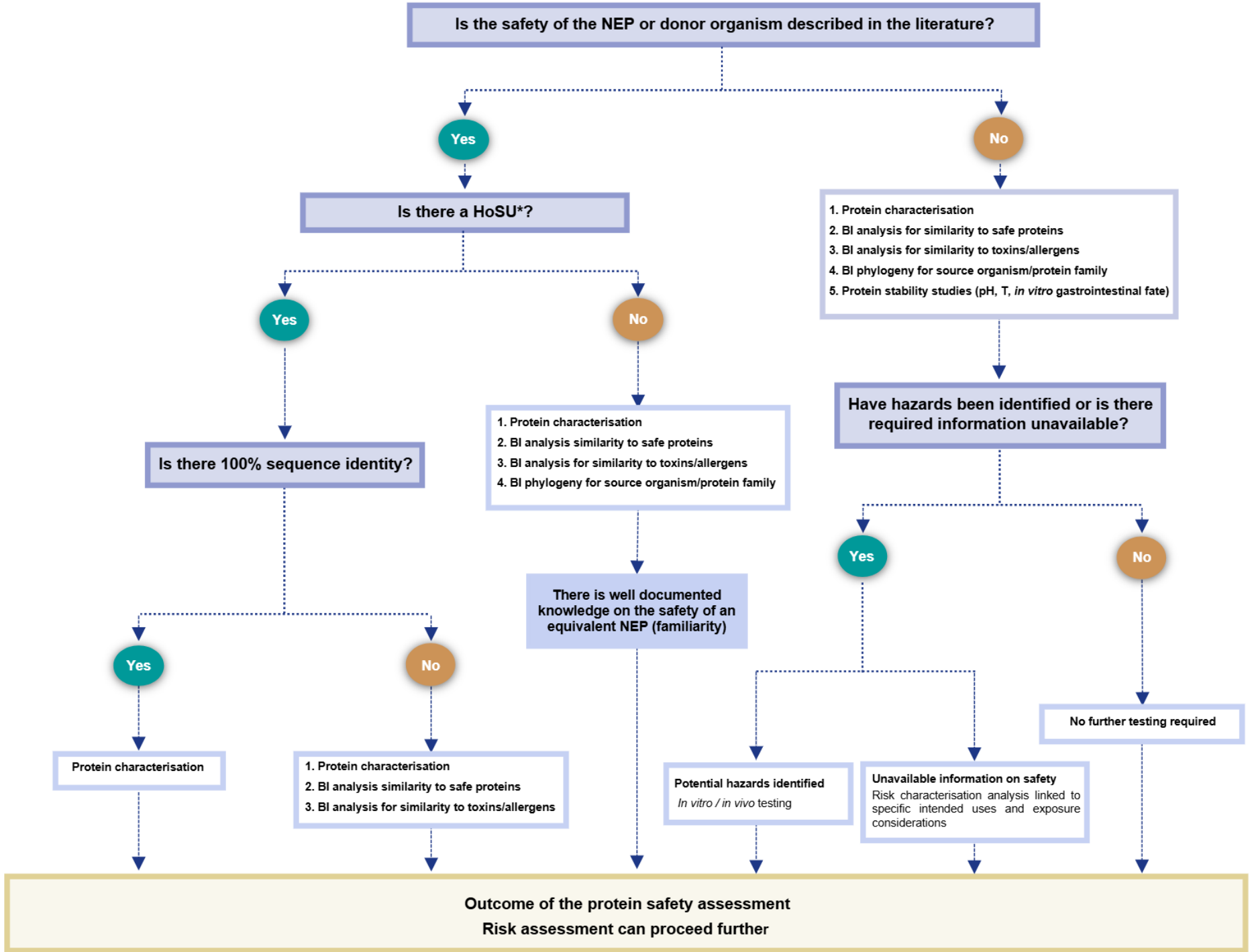
2) Need to work further on priorities

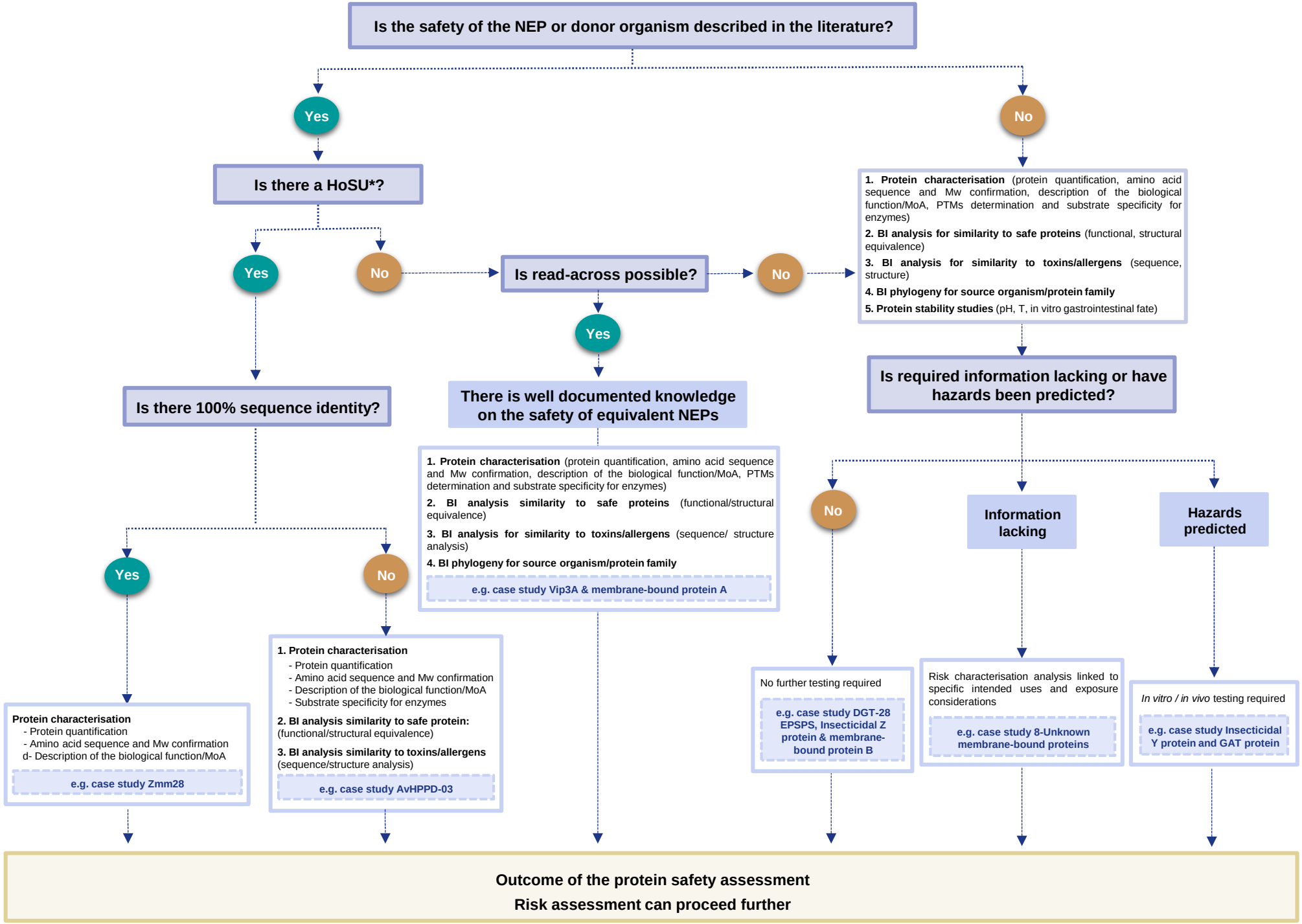
   - History of safe use (HoSU), read-across, phylogeny, etc
   - Fit-for-purpose databases (including 'known safe' proteins)
   - Regulatory acceptance/validation (e.g. new *in silico* & *in vitro* studies) in risk assessment

3) Few modifications to Figure 3

4) Additional improvements of ToR4 – mainly on development needs

5) Proceed towards finalisation for adoption in June/July GMO Panel meeting


Is the safety of the NEP or donor organism described in the literature?
Yes
No
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 studies (pH, T, in vitro gastrointestinal fate)
Yes
No
Is there 100% sequence identity?
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
Have hazards been identified or is there required information unavailable?
Yes
No
Yes
No
There is well documented knowledge on the safety of an equivalent NEP (familiarity)
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 linked to specific intended uses and exposure considerations
No further testing required
Outcome of the protein safety assessment
Risk assessment can proceed further


Is the safety of the NEP or donor organism described in the literature?
Yes
No
Is there a HoSU*?
Yes
No
Is read-across possible?
No
1. Protein characterisation (protein quantification, amino acid sequence and Mw confirmation, description of the biological function/MoA, PTMs determination and substrate specificity for enzymes)
2. BI analysis for similarity to safe proteins (functional, structural equivalence)
3. BI analysis for similarity to toxins/allergens (sequence, structure)
4. BI phylogeny for source organism/protein family
5. Protein stability studies (pH, T, in vitro gastrointestinal fate)
Yes
Is there 100% sequence identity?
There is well documented knowledge on the safety of equivalent NEPs
Is required information lacking or have hazards been predicted?
1. Protein characterisation (protein quantification, amino acid sequence and Mw confirmation, description of the biological function/MoA, PTMs determination and substrate specificity for enzymes)
2. BI analysis similarity to safe proteins (functional/structural equivalence)
3. BI analysis for similarity to toxins/allergens (sequence/ structure analysis)
4. BI phylogeny for source organism/protein family
e.g. case study Vip3A & membrane-bound protein A
No
Information lacking
Hazards predicted
Yes
No
Protein characterisation
- Protein quantification
- Amino acid sequence and Mw confirmation
d- Description of the biological function/MoA
e.g. case study Zmm28
1. Protein characterisation
- Protein quantification
- Amino acid sequence and Mw confirmation
- Description of the biological function/MoA
- Substrate specificity for enzymes
2. BI analysis similarity to safe protein: (functional/structural equivalence)
3. BI analysis similarity to toxins/allergens (sequence/structure analysis)
e.g. case study AvHPPD-03
No further testing required
e.g. case study DGT-28 EPSPS, Insecticidal Z protein & membrane-bound protein B
Risk characterisation analysis linked to specific intended uses and exposure considerations
e.g. case study 8-Unknown membrane-bound proteins
In vitro / in vivo testing required
e.g. case study Insecticidal Y protein and GAT protein
Outcome of the protein safety assessment
Risk assessment can proceed further

GAPS and/or UNCERTAINTIES in the PROTEIN SAFETY ASSESSMENT	DEVELOPMENTAL and RESEARCH ACTIVITIES NEEDED
1. SHORT-TERM core priorities: HoSU / read-across	
HoSU / Read-across lacks criteria and definitions	Develop consensus definitions, meaningful criteria, and databases of safe-use proteins
2. SHORT- to MID-TERM core priorities: *in silico*	
Outdated *in silico* methods & databases	Modernise tools, create fit-for-purpose databases, validate criteria for structural/functional similarity
***In silico* methods not validated / insufficient alone**	Integrate *in silico* with experimental data; establish robust validation/regulatory acceptance pathways
3. MID- to LONG-TERM core priorities: *in vitro*	
Lack of standardised *in vitro* GI digestibility tests	Update methodologies (e.g., INFOGEST), define interpretation criteria, demonstrate relevance in case studies
Limited validated in vitro NAMs	Validate more in vitro NAMs; integration into the weight-of-evidence

Additional priorities

***In vivo* testing not protein-specific / overuse of 28-day test**	Develop targeted, protein-focused *in vivo* protocols meeting 3Rs principles/Hypothesis driven
Exposure role not clearly defined in current frameworks	Define exposure assessment strategy; research dose-response; establish thresholds for toxicology/allergenicity
PMM lacks guidance on design/use	Design PMM systems to confirm exposure and reduce pre-market uncertainty; integrate with other systems
De novo allergen sensitization, mechanisms unclear	Conduct basic research into immunological mechanisms underlying sensitization
Unknown effects of food processing on proteins	Research processing effects on toxicity, allergenicity, digestibility, nutrition
Omics tools are hard to interpret in risk context	Explore how to incorporate and interpret - Omics data in compositional/protein safety assessment
Complex cases challenge current methods	Develop alternative risk assessment strategies tailored for membrane-bound, designed proteins, multi-NEP GMOs

PROTEIN SAFETY ASSESSMENT MANDATE

Thank you very much!!!!

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