Draft scientific opinion on current practice, challenges, and future opportunities in the safety assessment of newly expressed proteins in GM plants

KEY COMMENTS SUBMITTED BY CROPLIFE EUROPE

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General comments

- We are pleased that EFSA aims to utilize the significant experience and knowledge gained over the decades in the assessment of newly expressed proteins (NEPs) to improve future GM assessments.
- CropLife Europe recognises the opportunity for a revision of the best practices for protein safety assessment, however it is important to highlight that **no safety concerns have been detected in the 30 years of GMOs risk assessments** by multiple regulatory agencies, including EFSA.
- The weight-of-evidence approach, combined with a stepwise methodology, provides a robust framework for evaluating protein safety.
  - The WoE allows for a comprehensive assessment that considers all available data, including existing literature, *in silico* predictions, and *in vitro* studies.
  - By integrating diverse lines of evidence, researchers can draw informed conclusions about the potential allergenicity or toxicity of NEPs using case-by-case approach.
- We agree that new tools/methods (e.g., in silico/in vitro) can add value to the safety assessment of (NEPs); however, the use of these **new tools or methods should be hypothesis-based** and used only **on a case-by-case basis** and should not become additional *de facto* requirements.
  - Only if core studies indicate additional tools or methods are needed to assess an identified hazard of a NEP should these new tools or methods be leveraged as supplementary data.

3.2 ToR2: Critical appraisal of new technologies

- It is important that **any proposed additional assay is validated and fit for purpose** for protein assessment, and that it does not generate redundant information.
  - Instead of proposing a completely new sequential approach using several in vitro assays, it is more appropriate to investigate how to future in vitro assay(s) would fit within the current framework to evaluate protein safety.
- We do not agree with the statement that protein aggregation is relevant for the assessment of NEPs.
  - There is **low relevance of protein aggregation** in protein safety assessment in the context of GM crops.
  - Aggregated proteins lose their functionality and cannot be used for testing, making them less relevant for evaluating safety.
- We question the added value for the risk assessment "*to carry out a comprehensive peptide mapping of digesta and identify stable digestion fragments*".
  - The presence of peptides is a natural result of digestion, and LC-MS analysis has not indicated any correlation to any immunological outcomes (Mackie et al., 2019).
- We question the usefulness of INFOGEST 2.0 as an *in vitro* digestion model for the safety assessment of NEPs at this point in time as it does not fully replicate true physiological digestion.
  - While INFOGEST represents an important step forward in in vitro digestion modeling, this model simplifies the complex dynamics of digestion, lacks biological context and has predictive limitations.
  - Its limitations highlight the need for caution in relying solely on its findings for safety assessments and underscore the importance of integrating core studies using weight-of-evidence approach.

3.2 ToR2: Critical appraisal of new technologies

Regarding the proposal for a combined animal study for toxicity and allergenicity assessment

- Toxicity is dependent on protein function, which is lacking in the case of allergens.
- Toxins act indiscriminately, whereas allergens are restricted to genetically predisposed individuals.
- If an *in vivo* study is needed on a case-by-case basis to test for a protein toxin, then *in vivo* toxicity studies are available. However, currently no definitive *in vivo* test for novel allergens exists.
- Designing another study specifically for EFSA is not aligned with the need to reduce the use of animal studies and the principles of the 3Rs.
- The elimination of the 28-d repeated dose study, which is only performed for EFSA, would be in the spirit of a globally harmonized consensus approach.
    - Should an animal study be needed to address a specific hypothesis, then an exposure-based approach to dosing should be considered using a refined human and animal dietary animal exposure assessment.
    - Unlike chemical exposure scenarios, testing NEP at the limit dose results in doses orders of magnitude higher and in excess of realistic human and animal exposure.

3.2 ToR2: Critical appraisal of new technologies

Regarding exposure assessment

- Exposure assessment provides critical information on the likelihood and magnitude of human and animal exposure to NEPs.
- We agree with the conclusion that exposure should be more effectively integrated into protein safety assessments.
  - Most NEPs are expressed at very low concentrations.
  - NEPs are susceptible to heat, pH extremes, and processing conditions typically resulting in loss of biological activity and function during processing. As most GM derived products are highly processed, the NEP is denatured before being consumed (Waiblinger et al., 2023).
    - Exposure to an active, intact protein is negligible.
  - Since Risk = Hazard x Exposure. The absence of an identified hazard or the lack of exposure would imply that there is no risk.
- The expression level and dietary consumption of the NEP should be considered when determining the need for allergenicity and toxicity testing.
  - If exposure to the NEP is negligible, then there is no need for a hazard assessment.

3.4 ToR4: Recommendations for further research

In our opinion, a new “protein toxin database” is not needed since high quality public databases addressing toxin activity are already available and the use of a protein toxin database intentionally restricts the type of information that can be retrieved on the NEP (Bauman et al, 2022).

- Existing databases (including those with protein or allergen sequences) are fit-for-purpose. They are routinely updated and improved as we gain more insights.

The use of omics-based methodologies are not considered useful in food/feed risk assessment

- The omics methodologies for regulatory purposes have limitations, such as data complexity, lack of standardization, and interpretation of large data sets (Sauer et al., 2017, Harrill, 2021).

“Currently, there is no detailed guidance addressing the specific methodologies for substrate specificity testing in the GMO risk assessment”.

- Each enzyme or protein is unique in its catalyst function, necessitating tailored methodologies for substrate specificity testing.
- A one-size-fits-all guideline is inadequate for this purpose; instead, a scientific approach that evaluates each case individually is essential.

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