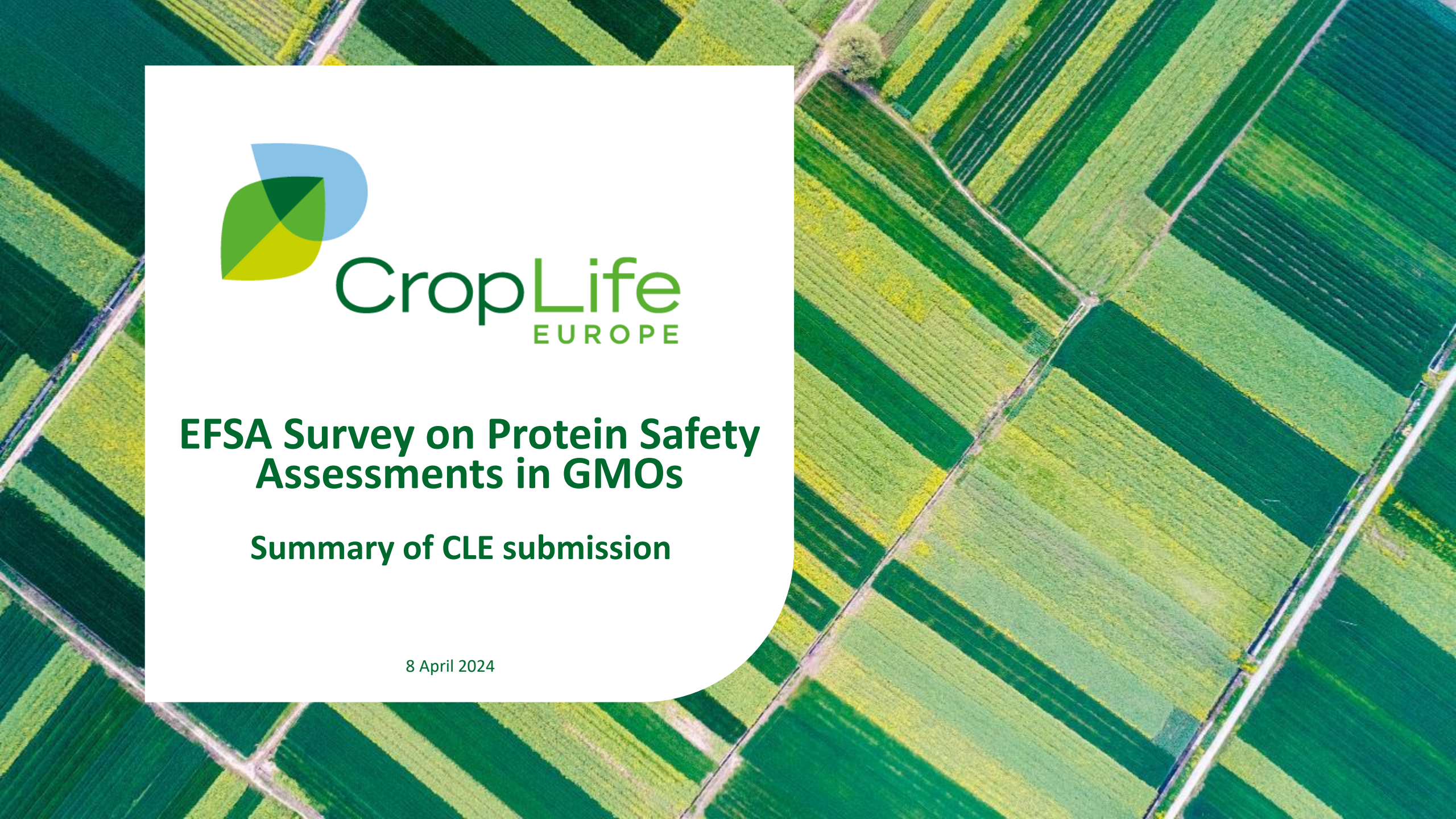

CropLife EUROPE

EFSA Survey on Protein Safety Assessments in GMOs

Summary of CLE submission

8 April 2024

Q1. Which is the common strategy for the assessment of the new expressed proteins in GM food and feed?

A weight-of-evidence (WOE), tiered-testing strategy.

The WOE approach for safety assessment should consider all the available information to support hazard identification and the evaluation of the newly expressed protein (NEP).

If no hazard is identified using these *in silico* and *in vitro* studies, then additional hazard characterization studies (e.g., acute oral toxicity, 28-d toxicity, hypothesis-based studies) are not needed to conclude on the safety of the protein.

Q1.1 In how many cases/GMO applications have the current methods identified issues/safety concerns for human/animal health?

GM crops are safe by design and have been reviewed by numerous independent regulatory agencies globally for the past 30 years. Thousands of risk assessments have unanimously found in each case that the GM crop was as safe as its conventional counterpart.

EFSA has adopted more than 100 scientific opinions related to GM plant applications, comprising more than 40 NEPs. Issues/safety concerns for human/animal health have not been identified.

The development of any GM crop variety undergoes stringent extensive screening process to eliminate undesired and unintended characteristics, which includes lack of homology to known protein toxins and allergens.

Q1.2 Do risk assessment authorities request animal studies in addition to their basic/core requirements?

28-day toxicity studies are performed only for EU (EFSA).

Acute toxicology studies are still required by some regulatory authorities.

Some regulatory authorities do not require submission of any *in vivo* data to conduct their risk assessment.

Q1.3 Which studies/methodologies have contributed most/least to identifying potentially adverse effects?

Most

A WOE approach is critical, should take the totality of information into account, which should include bioinformatics and/or higher order structure for toxicity and allergenicity screening, mode of action/functional specificity, protein characterization and expression, history of safe use (HOSU) of the NEP, and HOSU of the source organism.

Q1.3 Which studies/methodologies have contributed most/least to identifying potentially adverse effects?

Least

The use of *in vivo* studies as default for toxicological evaluation of NEP is not hypothesis-driven nor supported by the current WOE.

The 28-d toxicity study is irrelevant to risk assessment, and unjustifiable based on scientific or animal welfare considerations when testing at the limit dose, which is many thousands to hundreds of thousands of times more than realistic human and animal exposure. If the need for a toxicity study is determined, an exposure-based approach to dose selection should be considered.

Q2. Complex cases requiring a different approach; criteria for HOSU; testing proteins individually or in combination

Current approaches do not work for certain NEPs (e.g., intractable proteins).

- It is noteworthy that any new approach can be equally applicable to intractable proteins as well as to tractable proteins currently subject to traditional safety assessment.

A HOSU of a NEP can be established through safe consumption of closely related proteins by humans and/or animals.

- Evidence of structural and/or functional similarity and exposure to other endogenous proteins is necessary to demonstrate a HOSU.
- Absence of a clear HOSU does not automatically indicate a hazard, only that some further evidence and analysis is needed for the safety assessment.
- The safe consumption of the source organism for the gene encoding the NEP provides additional evidence of its safety.
  - Not every protein from an allergenic or toxic organism is an allergen or toxin.

In general, testing of NEPs individually is scientifically appropriate, but there may be instances where testing in combination is scientifically justified based on known interactions or relationships.

- The decision to test proteins individually or in combination should be based on scientific knowledge and technical feasibility, and applicants should provide a rationale for their approach.

Q3. Complementary/alternative methodologies for protein safety assessment, considering hazard identification and hazard characterisation

There is no need for new data sources or databases for protein safety assessment as existing databases are regularly updated with new information.

New methodologies need to be fit for purpose before they can be validated.

In vitro toxicity testing of proteins requires further development, validation, and acceptance by regulatory authorities (long-term (many years) alternative).

In vitro toxicity testing may not be feasible for all proteins.

Collaboration among industry, government, and academia is needed to advance *in vitro* toxicity testing.

New methodologies require investment, training, time, and effort. They should be open for use by both private and public sectors.

Q4. What stepwise approach following a WOE should be used for the safety assessment of NEPs in GMOs?

A WOE, stepwise, and science-based approach that uses a set of core studies should be the first step to conduct the assessments of the NEPs.

Core Studies

- Bioinformatics and/or higher order structure for toxicity and allergenicity screening
  - Bioinformatics and *in silico* toxicity and allergenicity screening continue to be important for hazard identification.
- Mode of Action/Functional Specificity
- Protein Characterization and Expression
- HOSU of the NEP
  - Evidence of consumption of the NEP or a closely related protein determined either by primary amino acid sequence similarity or structural/functional similarity.
  - Restricted use in computationally designed gene of interest
- HOSU of the Source Organism
  - Evidence of consumption or exposure
  - If the source organism is not allergenic or toxic, then proteins from that organism will not be allergenic or toxic. However, not every protein from an allergenic or toxic organism is an allergen or toxin.
  - Restricted use. This may not be relevant in the following context: a computationally designed gene of interest with multiple source organisms, or unknown origin of a sequence from a mixed environmental population.

If the NEP is related to a family of proteins that has a HOSU based on bioinformatics and literature review, and is not homologous to known protein toxins, then any supplementary toxicology study is not necessary.

Q4. What stepwise approach following a WOE should be used for the safety assessment of NEPs in GMOs?

Depending on the introduced trait, intended use, and data obtained from the core studies, supplementary studies (hypothesis-driven, case-by-case) may be considered to further evaluate the safety of NEPs in GM food and feed.

Supplementary Studies

- Additional *in silico* tools and/or prediction models
- Other new methodologies – As more complex traits become desirable, more challenging proteins will be needed and thus, the flexibility for use of other new fit-for-purpose methodologies will continue to evolve. Applicants should provide rationale for approach taken.
- Risk = Hazard x Exposure. Both hazard and exposure are needed to have a risk. For most NEP, the level of expression is very low to negligible.
  - Exposure Assessment (human dietary exposure assessment, animal exposure assessment)
  - *In vitro* testing evaluations (e.g., digestibility, heat stability)

Only when the information from the core and supplementary studies is clearly not sufficient to conclude on risk may additional hazard characterization studies (e.g., acute oral toxicity, 28-d toxicity, hypothesis-based studies) be considered. If performed, these studies should follow an exposure-based approach regarding the dose tested.

Q5. How these new methodologies can be introduced as complementary/alternative testing strategies in the overall WOE approach for protein safety?

Depending on the new methodologies, they may be introduced as supplementary studies when the core studies are insufficient to confirm negligible hazard in NEP.

Q6. How should the outcome of these new methodologies be interpreted to inform the overall WOE approach for protein safety?

Depending on the new methodologies, they may be introduced as supplementary studies when the core studies are insufficient to confirm negligible hazard in NEP.

Q7. What are the main gaps and/or uncertainties in the protein safety assessment that would need to be addressed in the future?

Intractable proteins cannot meet current data requirements for protein safety studies because it is difficult, if not impossible, to produce enough active and intact protein from heterologous expression systems or plant sources.

A computationally designed protein such as a protein with multiple source organisms, or unknown origin of a sequence from a mixed environmental population.

Q8. What developmental and research activities are needed to address the above gaps? And how would this change if tests in live animals were not performed or minimized?

Any new methodologies for protein safety assessment would require further development, validation, and acceptance by global regulatory authorities.

In vivo toxicity studies have not provided additional information on protein safety assessment, but instead have only confirmed *in silico* and *in vitro* data.

Defaulting to *in vivo* toxicology studies for regulatory approvals is not ethically aligned with the responsible use of animals in scientific research and testing.

Minimizing or eliminating animal testing aligns with the Conventions on the protection of animals and the 3R legislation at the EU level.

Eliminating *in vivo* toxicity studies from protein safety assessments in GMOs is consistent with the EU Commission's roadmap to reduce animal testing and move towards an animal-free regulatory system under chemicals legislation.

Q9. What factors could be considered for building trust and confidence in these new methodologies?

Peer-reviewed publications.

GM crops are safe by design and have been reviewed by numerous independent regulatory agencies globally for the past 30 years.

The environmental, socioeconomic, and food security benefits of GM crops are well documented and should be considered in the context of the world's population growth and climate change.

Q10. Please use the field below if you have additional comments not considered in the previous questions (e.g. adjuvanticity)

Most NEPs are expressed at very low concentrations

- The expression level (protein concentration in the plant) and dietary consumption (human and animal) of the NEP should be taken into account when determining the need for toxicity testing.
- If exposure to the NEP is negligible, then there is no need for toxicity testing.
- Highly refined products such as oil and sugar do not contain detectable levels of protein.

Proteins are an essential part of the diet of humans and other mammals. The mammalian digestive system degrades dietary proteins into its building blocks for incorporation into new proteins. NEPs consumed via the dietary route will be degraded in the same manner as any other dietary protein. For this reason, proteins are different from chemicals, which display ADME (absorption, distribution, metabolism and excretion) properties. Thus, proteins should not be treated like chemicals in a risk assessment evaluation.

In summary

A WOE, step-wise, science-based approach should be adopted with core and supplemental studies.

With the rich experience gained over the years, it has become evident that animal studies do not provide additional insights to safety and hence the need for animal studies should be reconsidered (aligning with 3R initiatives).

Effective partnership will enable us to tackle future challenges/gaps with difficult proteins (e.g., intractable proteins, computationally design proteins).