GMO RISK ASSESSMENT OUTSOURCING AND PARTNERSHIP

Ana Afonso

Nutrition & Food innovation Unit

EUROPEAN FOOD SAFETY AUTHORITY

GMO RISK ASSESSMENT OUTSOURCING PREPARATORY WORK

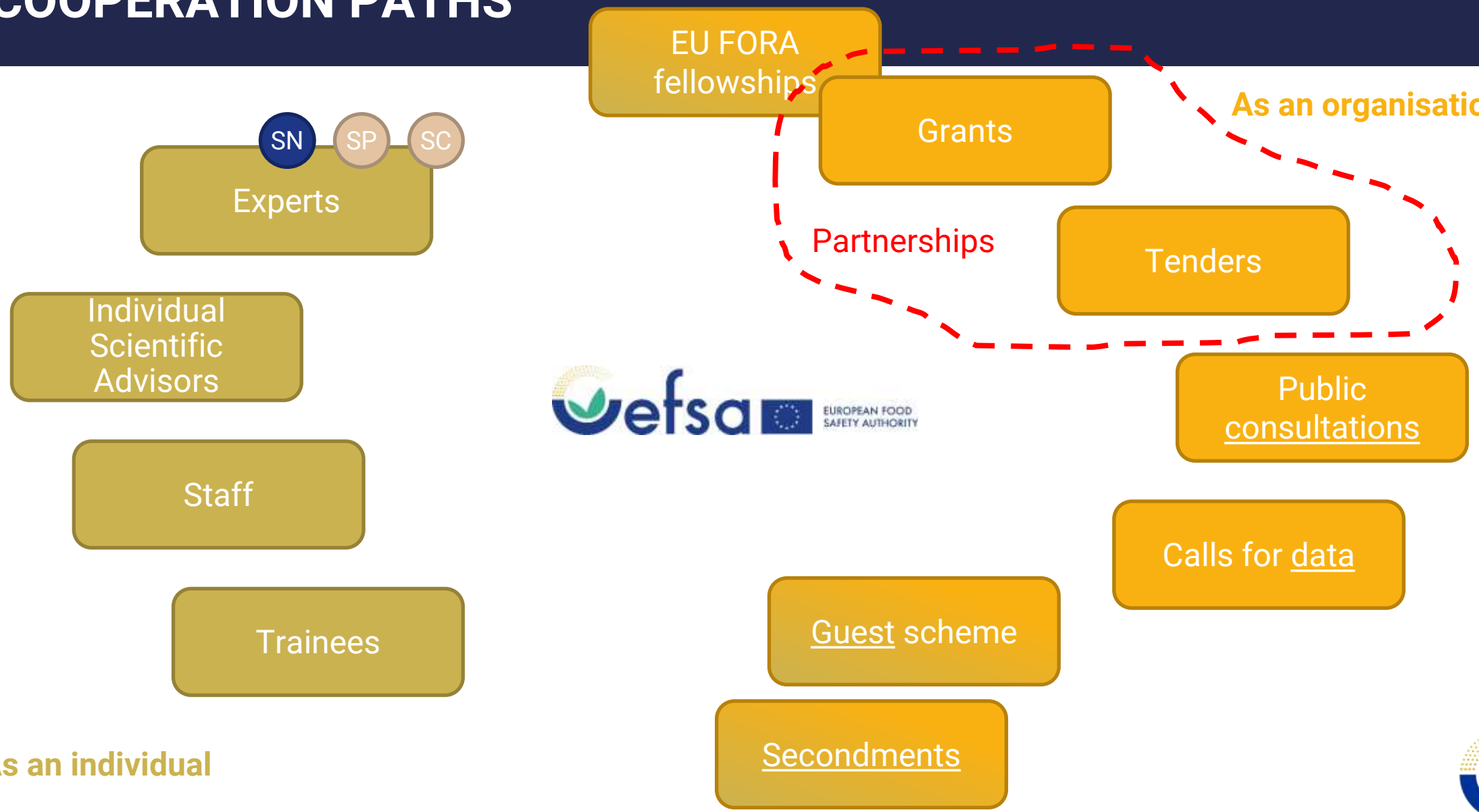
EOI/EFSA/SCIENCE/2020/01 - CT 02 NIF - SCIENTIFIC AND TECHNICAL SUPPORT IN THE AREAS OF SAFETY STUDIES ON FOOD/FEED (GENETICALLY MODIFIED ORGANISMS AREA GMO	Yearly contract 20/07/2022 to 19/07/2023
OC/EFSA/GMO/2020/01 - DNA SEQUENCING QUALITY CHECK OF GMO APPLICATIONS	Contract ends 20/12/2024
OC/EFSA/GMO/2021/06 PREPARATORY SUPPORT ON BIOINFORMATICS FOR THE EVALUATION OF THE RISK ASSESSMENT OF GMO DOSSIERS	Contract ends 29/06/2026
Statistical quality assessment	EFSA FWC
Literature review quality assessment	EFSA FWC

TRANSPARENCY REGULATION

(18) It is essential to ensure the efficient operation of the Authority and to improve the sustainability of its expertise. It is therefore necessary to strengthen the support provided by the Authority and the Member States to the work of the Scientific Committee and the Scientific Panels. In particular, the Authority should organise the preparatory work supporting the tasks of the Scientific Panels, including by requesting the Authority's staff or national scientific organisations networking with the Authority to draft preparatory scientific opinions to be peer-reviewed and adopted by those Scientific Panels. That should be without prejudice to the independence of the Authority's scientific assessments.

5e. The Authority shall support the tasks of the Scientific Committee and Scientific Panels by organising their work, in particular the preparatory work to be undertaken by the Authority's staff or by designated national scientific organisations referred to in Article 36, including by organising the possibility for preparing scientific opinions to be peer-reviewed by the Scientific Panels before they adopt them.

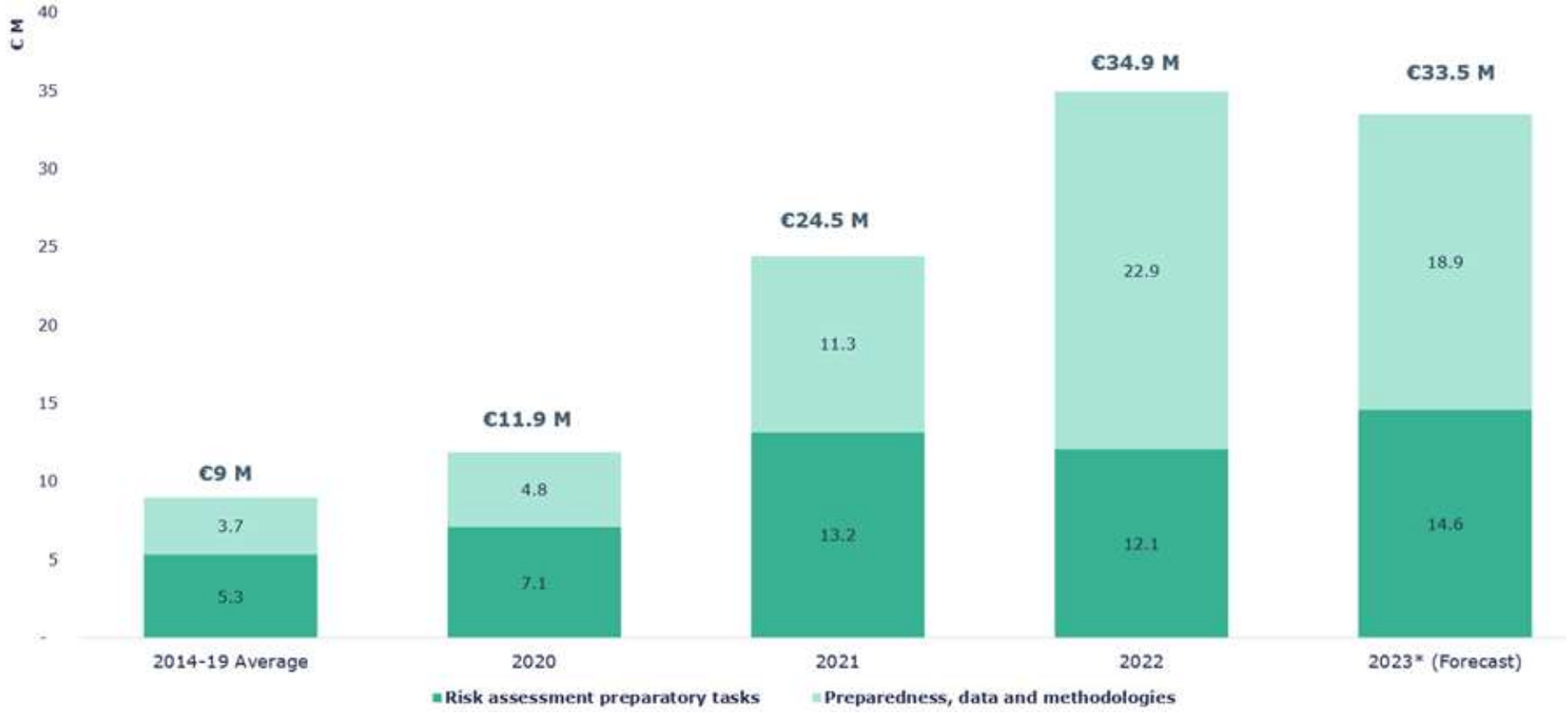
COOPERATION PATHS



AN INCREASING ROLE FOR PARTNERING WITH MS ORGANISATIONS

A Partnership is ‘a long-term, trust-based cooperation, built on common values and goals, with attractive win-win elements, primarily between EFSA and MS competent organisations, EU Agencies, EC Services, where risks and benefits are shared and that generate, among others, tangible outputs.’ (85th AF meeting, October 2022)

GRANTS AND PROCUREMENT, 2014-2023



ARTICLE 36 /COMPETENT ORGANIZATIONS

- are active in fields within EFSA's mission;
- fulfil a set of eligibility criteria, ensuring for example independence and scientific expertise; and
- are designated by a Member State for inclusion in the List of Competent Organisations.

- Eligibility criteria - Regulation 2230/2004
- Art.36 List – the latest updated version

151 organizations with GMO competencies

PARTNERSHIP OPPORTUNITIES IN THE GMO DOMAIN

- Are there activities of EFSA in the evaluation of GMO dossiers where MS competent bodies can support?
- Are there needs that the Network has identified that can be covered by MS competent bodies?
- Are there capacities in MS competent bodies that can be leveraged via partnership projects?

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EUROPEAN FOOD SAFETY AUTHORITY

PROTEIN SAFETY OF PRESENT AND FUTURE GM PLANTS

INTRODUCTION

Antonio Fernandez & Tommaso Raffaello

Network meeting

June 2023

EUROPEAN FOOD SAFETY AUTHORITY

PURPOSE OF DISCUSSION

- Frame a common understanding of the topic
- Share/discuss potential solutions to the issues – starting point
- Plan for today:
    - EFSA introducing the topic
    - The Netherlands aspects on *in silico* and *in vitro*
    - Germany other areas and questions for discussion

BACKGROUND

Protein safety = protein allergenicity and toxicity

Codex 2003-2009 defined the principles for the assessment

- Regulation 503/EFSA GMO Panel 2011 borrowed such principles
- Main information considered:
  - 1.- Knowledge on the source/protein – HoSU
  - 2.- Bioinformatics analysis
  - 3.- *In vitro* studies
  - 4.- *In vivo* studies

REGULATION 503 – PRESENT REQUIREMENTS

Toxicity assessment of NEPs:

- Case by case approach. Depending on knowledge on protein's source, function or activity and history of human or animal consumption
- If history of safe consumption is duly documented, specific toxicity studies not needed
- Where specific testing is required, the applicant shall provide:
  - Molecular and biochemical characterisation of the NEP
  - Bioinformatics searching for homology to proteins known to cause adverse effects
  - Stability of the protein, e.g. influences of temperature, pH
  - Degradation of the NEP to proteolytic enzymes (pepsin test)
  - 28 day toxicity study, depending on the outcome additional targeted investigation may be needed

Allergenicity assessment of NEPs:

- Case by case approach. The approach shall include:
  - Bioinformatics searching for homology with know allergens
  - Specific serum screening, cases where there is a sequence homology or structure similarity and where the source of the gene is considered allergenic
  - Pepsin resistance and *in vitro* digestibility tests
  - Additional studies, if needed

SCENARIOS AND PRESENT/FUTURE ISSUES

- GM plant with 1 protein easy to extract/purify (native, semi native, chimeric, etc), e.g. mEPSPS protein
- GM plant with high number of proteins easy to extract/purify – issue related to number of testing and animals required
- GM plant with 1 protein difficult to extract/purify – e.g. AP75&AP87
- GM plant with high number of proteins difficult to extract/purify – issues…

Aspect reflected in Allergenicity and Synbio Opinions from 2022

But how to deal with this in a credible manner?

1. HISTORY OF SAFE USE

- Intuitively easy for extreme cases but difficult to implement
- Definition and criteria needed as not yet one internationally recognised
- Fundamental concept in GMOs – comparative assessment
- Concepts to be considered:
  - HoSU for plant/varieties
  - HoSU for proteins
- SO on Criteria for NGTs published in 2022
  - Function/Structure – today mainly considered primary sequence but….see next point on *in silico* analysis

2. *IN SILICO* ANALYSIS

- Current bioinformatics only based on primary sequence
- Developmental projects
  - Allergenicity: ranking/targeted databases and new approaches
  - Toxicity: new *in silico* tools
- New scenarios, e.g. hits with toxins/allergens, assessment for celiac disease
- Extrapolations for the assessment of proteins, e.g. highly similar proteins
- Any new proposals might need consensus with international community (e.g. 35% cut-off in allergenicity)

3. *IN VITRO* ANALYSIS

- Protein characterisation and equivalence
- Substrate specificity – criteria on selection
- Studies on protein stability, but mainly pepsin test (past assessments, EFSA guidance 2017, procurement 2019 and EFSA opinions in 2021-2022)
- Applicants now tend to use gastrointestinal digestion
- Future interest to develop other *in vitro* testing, when needed

4. *IN VIVO* + OTHER

- 28 day studies (and others) – reference to OECD

- Other additional aspects:

  Exposure (expression levels)

  Post-market monitoring

QUESTIONS IDENTIFIED:

- How to define a new logic flow for the stepwise, case-by-case, weight of evidence approach in the protein safety assessment?
- What criteria can be used for HoSU?
- What is considered an acceptable degree of similarity in primary amino acid sequence (is it possible to set a threshold)? How to consider 2D and/or 3D structure?
- How to use info on function and stability of proteins for protein safety assessments?
- How to use info on the fate of a protein in the GI tract for protein safety assessments?
- What other *in vitro* test can be considered in the weight of evidence approach?
- Are animal studies needed? If so, when? Can they be replaced/reduced?

Way forward?

Thank you very much!!!!

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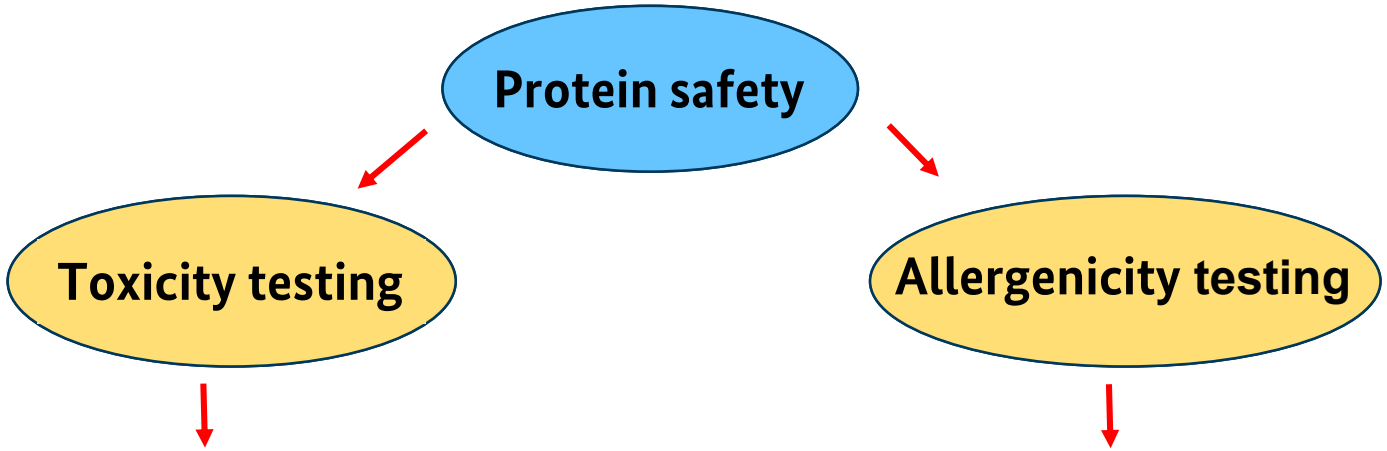
Federal Office of Consumer Protection and Food Safety

Protein safety of present and future GM plants

Upcoming challenges and (possible) questions to discuss

EFSA GMO NETWORK MEETING – June 2023

Assessment of protein safety



<u>Principles:</u>

1) HoSU (→ source)

2) Bioinformatics analysis

3) *In vitro* studies

4) *In vivo* studies (→ on a case-by-case basis)

Present and upcoming challenges

HoSU: Definition and criteria needed

3R Principle

Avoiding animal testing and need for alternative methods

Complex cases, e.g. high number of proteins

SynBio vs. HoSU?

CHALLENGES

Revision of existing methods needed??

Proteins difficult to extract/purify, e.g.menbrane-bound proteins

Adequate testing specificity

Proteins difficult to test

Need for a shift in experiments within a weight-of-evidence approach

Development of new methods: standardization and validation

Partial similarity to toxins/allergens

HoSU – Definition, concept(s) and criteria

Focus: History of safe consumption for human beings and/or animals

Problem: There is no internationally recognized general definition

⟹ Different concepts and criteria to be considered and combined

⟹ Keep in mind cases where HoSU cannot be easily demonstrated (e.g. SynBio-sources or SynBio-proteins)

Concepts:

- HoSU of plants/varieties
- HoSU of proteins within a crop/plant/microorganism/etc.

HoSU – Definition, concept(s) and criteria

Criteria:

- Assessment of proteins according to their

  a) FUNCTION

  b) STRUCTURE

  Questions:

  ➡ How to use information on function for protein safety assessments?

  ➡ What is considered an acceptable degree of similarity in primary amino acid sequence (is it possible to set a threshold?)?

  ➡ How to consider 2D and/or 3D structure?

- What other criteria can be used?

Risk assessment of complex/complicated cases - e.g. high number of newly expressed proteins

(Possible) questions

- To what extent does the previous test strategy make any sense here or is it even feasible in terms of capacity?
- Does each protein have to be tested individually or are there also experiments/strategies that can be used to test proteins in combination?
- Which components/tests of the previous weight-of-evidence approach could be replaced by new/revised components/tests? What could the new components/tests look like?
- How to effectively assess additive, synergistic or antagonistic effects of a combination of multiple proteins?
- Are there cases where animal testing would be essential?

3R Principle (refine, reduce, replace) - risk assessment while avoiding animal testing

(Possible) questions

Toxicity testing

- Do we even need animal experiments for the safety assessment of newly expressed proteins?
- In which cases are animal experiments indispensable from today's perspective?
  → Keeping in mind the weight-of-evidence approach!
- What could an alternative for the 28-day toxicity study look like?
- Who is responsible for the development of new methods?
- How much time must be scheduled for the standardization and validation of new methods?

Allergenicity testing

- Do animal experiments or their (new) development make sense here at all?

Let’s start to support EFSA defining a new logic flow

for a stepwise, case-by-case, weight of evidence

approach in the protein safety assessment

Share and discuss open questions, opinions, proposals

and potential solutions to the mentioned issues

Thank you for your attention!

Contact:
Dr. Andrea Scheepers

Federal Office of Consumer Protection and Food Safety

Email: andrea.scheepers@bvl.bund.de

Recent insights/initiatives from NL on in-vitro testing of protein safety

Gijs Kleter (Netherlands)

Comparability of in-vitro to in-vivo digestion

INFOGEST Static Model (COST Action)

- Developed and validated for digestion of in humans
  - Constant pH and ratio of meal to digestive materials
  - Not just for proteins but also for micronutrients, fats, etc.
  - Method 2.0, *e.g.*, gastric lipase
- Extended to livestock animals:
  - Pigs: model for human ileal digestibility
  - Validation with in-vivo data from pig
  - High correlation with amino acid digestibility
    - Variety of proteins tested: whey, maize zein, bean proteins (black bean, pigeon pea), collagen

Reference: Sousa et al., 2023, https://doi.org/10.1016/j.foodchem.2022.134720

INFOGEST model

1. • Amylase (simulated *salivary* fluid), pH7
2. • Pepsin (simulated *gastric* juice), pH3
3. • Pancreatin & bile (simulated intestinal juice), pH7
4. • NaOH, protease inhibitor AEBSF (stop reaction) pH7
5. • MeOH precipitation: soluble fraction, pellet

Impact of intestinal transport and digestion on allergenicity of peanut allergens

In-vitro model (TNO)

- Peanut proteins Ara h 1, Ara h2, Ara h 3, and Ara h 6
- Processing of samples
  - Digestion with pepsin (pH 2.5, 5 min.)
  - Intestinal transport in-vitro (InTESTine)
- Testing:
  - Protein characterization
  - Indirect basophil activation assay
  - Human mast cell activation assay
- Ara h 1 and 3: unstable towards pepsin, digested *ànd* transported forms activate basophils
- Ara h 2 and 6: not affected by pepsin, activate basophils after transport

- Reference: Smits et al., 2021, https://doi.org/10.1002/mnfr.202000712

GMM NGT MANDATE

Dafni Maria Kagkli

efsa
EUROPEAN FOOD SAFETY AUTHORITY

TASKS OF THE MANDATE

Terms of reference

The European Commission requests EFSA to produce an opinion (in accordance with Article 29 of Regulation (EC) No 178/2002) on new developments in biotechnology applied to microorganisms.
For the purpose of this opinion, and in accordance with the conclusion of the Commission's study on NGTs, EFSA is requested to perform the following two-step work on i) microorganisms and products of **category 4** to be released into the environment or placed on the market as or in food and feed ; ii) products of **category 3** to be placed on the market as or in food and feed.

Two tasks:

I. **Horizon scanning** on microorganisms and their products obtained by new developments in biotechnology, and based on it,

II. **EFSA opinion** on potential novel hazards/risks from new developments in biotechnology applied to microorganisms and adequacy of the current EFSA risk assessment guidance

HORIZON SCANNING

I. **Horizon scanning on microorganisms and their products obtained by new development in biotechnology**

- The expected outcomes of this activity are:

1. **Identify microorganisms and their products** obtained by new development in biotechnology described since 2001 including their traits and uses.
2. **List the techniques and modifications used**, including explanation of relevant terminology.
3. **Identify microorganisms and their products developed since 2001 subject to authorisation** procedures by international authorities as well as the available risk assessment, should they already exist.
4. **Information** on risk assessment approaches taken by risk assessors and **potential available guidances for the risk assessment**.

INTERMEDIATE RESULTS – LITERATURE SEARCH

SOURCE	Number of hits
MEDLINE	15,268
EMBASE	17,751
Web of Science	3,093
Total	34,316
Duplicates	16,855
Total unique	**17,461**

- Based on these findings the contractor has so far identified **15 cases of interest** which are currently under evaluation by the WG for the selection of case studies
- Task to be finalised in the coming days

EFSA OPINION

- A dedicated WG was set up at the end of 2022. Minutes are published here

The WG is requested to address the following ToR:

- **Identify novel potential hazards and risks** that new developments in biotechnology applied to microorganisms could pose for humans, animals and the environment.

- **Determine** whether the **existing guidelines for risk assessment of GMM are applicable**, fully or partially, and sufficient to risk assess new developments in biotechnology applied to microorganisms.

- In case existing guidelines for risk assessment are considered not applicable, partially applicable or not sufficient, to **identify on which aspects existing guidelines should be updated, adapted or complemented**

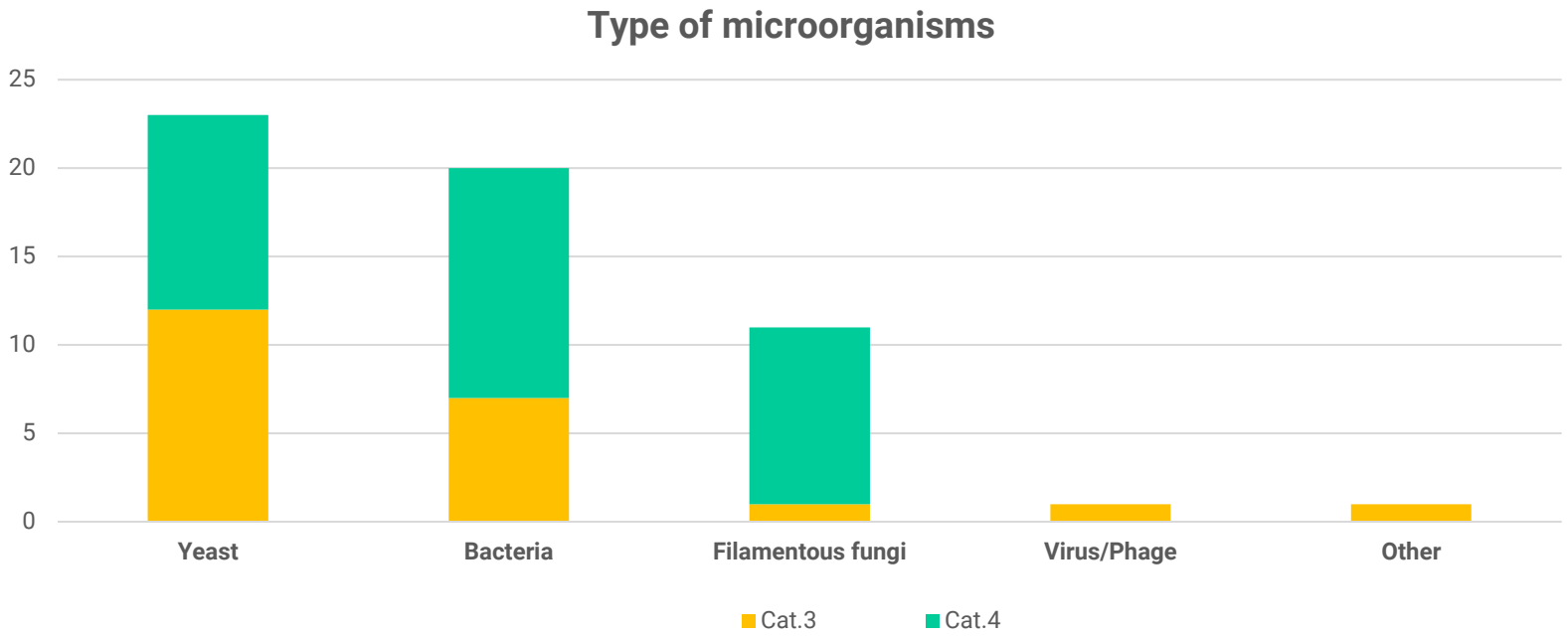
To do so, the WG defined a protocol and strategy on how to address the ToRs of the mandate in accordance with EFSA 2020

TOR2: EFSA SURVEY ON NEW DEVELOPMENTS IN BIOTECHNOLOGIES APPLIED TO MICROORGANISMS

EFSA will take into consideration the **horizon scanning** and the **survey results** launched on the EFSA website https://www.efsa.europa.eu/en/call/survey-new-biotechnologies-microorganisms

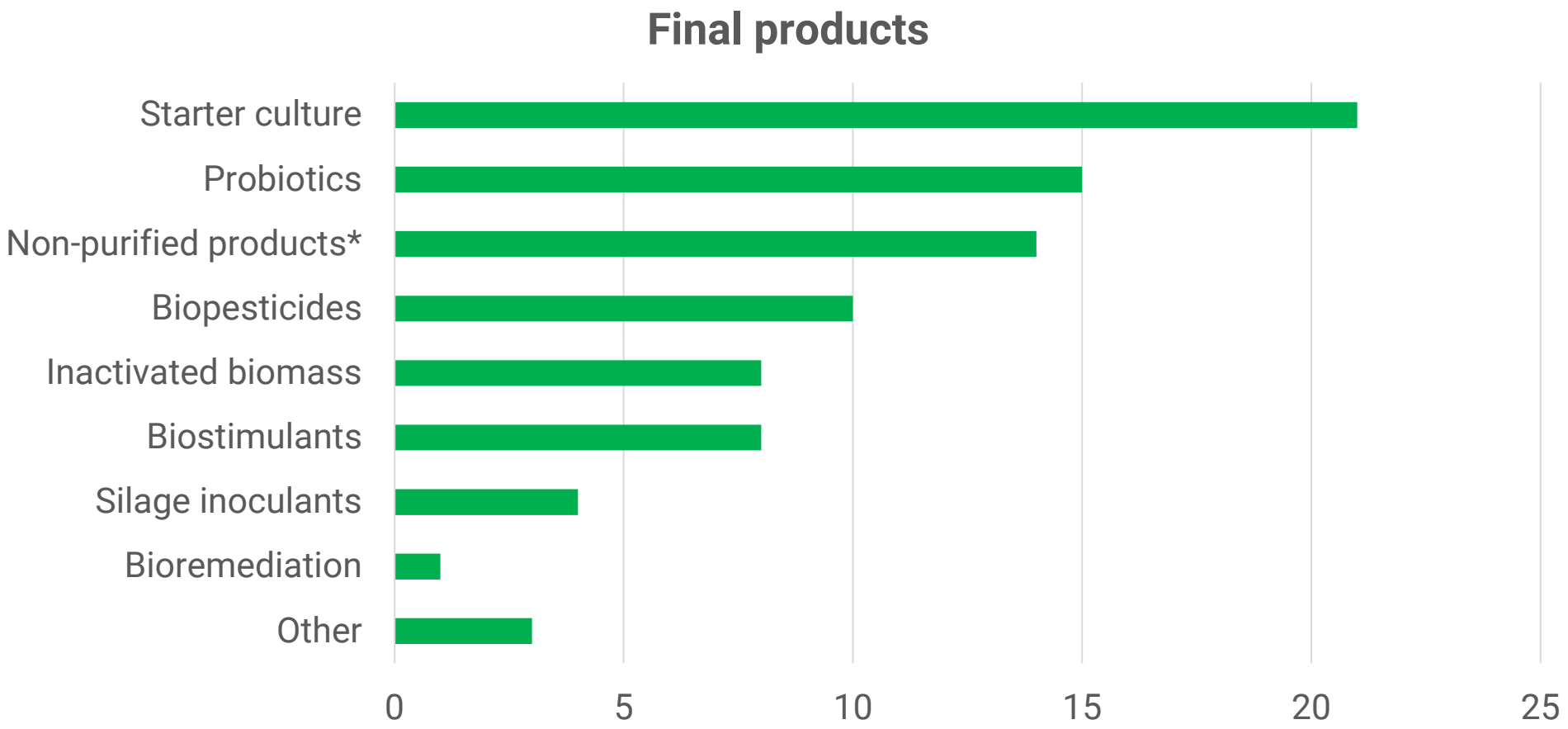
- 56 responses received from EU and non-EU countries
  - Academia 28.6%
  - Industry 64.3%
  - Others 7.1%

INSIGHTS FROM THE SURVEY



- 39.3 % Category 3
- 60.7 % Category 4
- 54% mentioned use of CRISPR
- 75% mentioned a combination of EGTs with NGTs

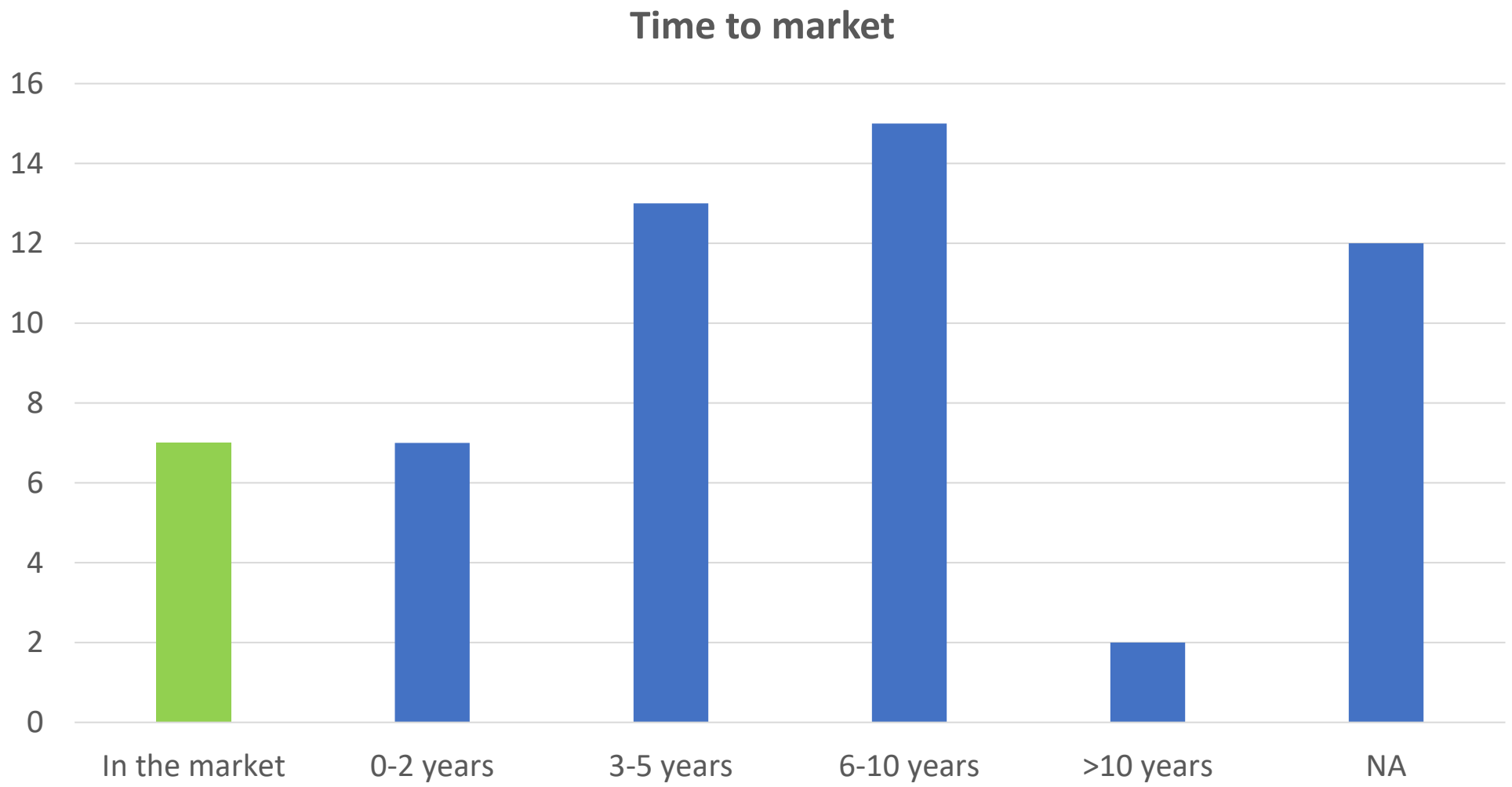
INSIGHTS FROM THE SURVEY



* Non-purified products include enzymes, amino acids, proteins, lipids, flavours and specialty chemicals.

Note: some respondents indicate more than one use (thus the total is >56)

EFSA SURVEY RESULTS



EFSA SURVEY RESULTS

- **7** NGT GMM products already on the market:

- 6 Cat.3 and 1 Cat.4
- Yeasts, bacteria and phages
- Authorised in US, Canada, India, Brazil and other South American countries
- The uses include: starter cultures, inactivated biomasses (e.g. for feed uses) and production of enzymes, flavours, colours, amino acids

EFSA SURVEY RESULTS: COMMENTS FROM THE PARTICIPANTS

The participants report:

- Worldwide quick spread of NGTs for various scopes
- Precision and accuracy of the NGTs can have safety advantages (off-targets reduction, targeted elimination of genes of concern, etc.)
- NGTs could substantially contribute to sustainability goals (see Green Deal and Farm to Fork)

- The EU legislative burden is discouraging NGT development in the EU
- The current requirements for GMO labelling would cause consumer rejection
- Several products (e.g. biostimulants, feed additives, etc.) are assessed under specific regulatory frameworks. No need for additional burden for safety
- Difficult legal enforcement: Impossible to prove that certain modifications are the result of NGTs

- Request for a future product-centric (rather than process-oriented) regulatory framework for food/feed produced via NGTs, where requirements are proportionate to safety risk: similar requirements for products with similar risk profiles.
- Overall: Time to market will depend on future legislative proposals for NGT GMMs

REQUEST FOR YOUR INPUT

So far, it seems that “new biotechnologies” in GMMs only include CRISPR/CAS techniques

- Do you have any examples of specific GMM cases developed with CRISPR/CAS currently under assessment by you (category 3 and 4)? If so, how is the assessment done? For instance, in the case of field trials
- Do you/would you demand WGS after the CRISPR-Cas mutation and comparison with the parental organism?
- Shall QPS be extended to the GMMs developed through NGTs?
- Which guidances would you feel shall be applied for the risk assessment of these organisms? Do you have any suggestions for updates of specific sections?
- If the same GMM can be obtained through different technologies how would the assessment be done?

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Vitenskapskomiteen for mat og miljø

Norwegian Scientific Committee for Food and Environment

New developments in biotechnology applied to microorganisms (Agenda No.6, Day1.)

Ville Erling Sipinen

EFSA 15th GMO Network meeting

8-9 June 2023

VKM Report 2021: 18

Genome editing in food and feed production – implications for risk assessment

Opinion of the Steering Committee of the Norwegian Scientific Committee for Food and Environment

European Commission

Statement by the Group of Chief Scientific Advisors

A Scientific Perspective on the Regulatory Status of Products Derived from Gene Editing and the Implications for the GMO Directive

On 25 July 2018, the Court of Justice of the European Union ('the Court') decided that organisms obtained by the new techniques of directed mutagenesis are genetically modified organisms (GMOs), within the meaning of the Directive 2001/18/EC on the release of genetically modified organisms into the environment ('GMO Directive')[1,2], and that they are subject to the obligations laid down by the GMO Directive.

New techniques of directed mutagenesis include gene editing such as CRISPR/Cas9 methodologies. The legal status of the products of such techniques was uncertain, because it was unclear whether they fell within the scope of the GMO Directive.

Biotechnology' (SAM, 2017a), we have examined the GMO Directive taking into account current knowledge and scientific evidence.

1. The Ruling of the Court of Justice

On request by the French *Conseil d'État*, the Court was asked to determine whether organisms obtained by mutagenesis[4] should be considered GMOs and which of those organisms are exempt according to the provisions of the GMO Directive. In particular, the Court was asked to determine whether organisms obtained by new directed mutagenesis techniques are exempt from the obligations imposed by the GMO Directive, as are

VKM

Terms of Reference (ToR)

1. **Describe the various methods that constitute the genome editing technologies.** Different methods and their technologies, including the variation within these and the genomic alterations they result in, should be described.

2. **Describe the use of genome-editing technologies today, including future perspectives.** The main applications of new genome editing technologies within plant breeding, animal breeding (including farmed fish) and microorganisms should be described, and examples relevant for Norway should be highlighted.

3. **Discuss implications for risk assessment regarding genome-edited organisms.** Potential challenges for risk assessment of genome-edited organisms (and products thereof) with the EFSA guidance for genetically modified organisms should be investigated and described.

4. **Discuss possible implications for biodiversity in Norway.** Potential effects stemming from the spread and establishment following the use or production of genome-edited organisms should be discussed.

VKM

Use of genome-editing technologies in microorganisms (ToR 2)

Rarely relevant as a single defined food or feed source

- important in food and feed processing, e.g., as additives

Bacteria and yeasts

- Baked goods/pastries, dairy products, fermented beverages and meats
- Pharmaceutical compounds, in cosmetics, additives in food and feed products

Genome editing tools, esp. CRISPR

- many different species of bacteria and yeasts
- more rapid development of probiotics and starter strains/cultures for food and feed

Synthetic biology

- *Saccharomyces cerevisiae* lacks the metabolic potential present in many other yeast species
- CRISPR allows use of non-conventional species as synthetic biology platforms
- Unexplored metabolic potential among prokaryotes

Potential application of genome-edited microorganisms (short term)

(modified after Donohoue et al., 2018).

Species	Purpose of study/industrial relevance	Modifications/type of alteration	References
Fungi			
S. cerevisiae	Common production strains	- Donor-mediated gene disruption - Multiplexed donor-mediated gene disruption - Multiplexed recombination - CRISPRa, CRIISPRi *	(David and Siewers, 2015; Jakociunas et al., 2015) (Bao et al., 2015; Biot-Pelletier and Martin, 2016; DiCarlo et al., 2013; Gilbert et al., 2013; Ronda et al., 2015; Ryan et al., 2014; Smith et al., 2016; Zalatan et al., 2015)
Aspergillus oryzae	Industrial-scale production of recombinant proteins and enzymes	Mutagenesis: multiple gene deletions/integrations	(Katayama et al., 2019)
Aspergilus niger	Constitutive production of pectinase. Pectinase produced by *A. niger* are used in food industry. Pectin as polysaccharides in plants is the substrate for *A. niger*.	Mutation in Gaar, the regulator of D-galacturonic acid-responsive genes	(Alazi et al., 2019)

*CRISPRa; CRISPR activation, CRIISPRi; CRISPR interference.

Species	Purpose of study/industrial relevance	Modifications/type of alteration	References
Bacteria			
L. reuteri	Probiotic strain and producing of biotherapeutics	Recombination: oligonucleotide-mediated deletion	(Oh and van Pijkeren, 2014)
L. casei	Biotechnological production of acetoin (flavouring compound naturally occurs in wine, honey, milk, coffee, etc.	Using single plasmid genome (system), 4 different genes (*hicD3, pflB, ldh*, and *phdC*), responsible for acetoin biosynthesis	(Xin et al., 2018)
Lactococcus lactis	Nisin-controlled inducible expression. High nisin concentration important for inhibiting pathogenic bacteria in fermented food	Single plasmid system; simultaneous inducible co-expression of multiple recombinant genes	(Berlec et al., 2018)
Streptococcus thermophilus	Probiotic and industrial fermentation strains provided resistance against phages attacks	Engineered immunity because of acquired spacers in phage resistance mutants	(Barrangou et al., 2007)

VKM

The products of gene technology used on microorganisms intended for food and feed are categorised into four categories by EFSA

1. Chemically defined purified compounds and their mixtures in which both genetically modified microorganisms and newly introduced genes have been removed (e.g., amino acids, vitamins)

2. Complex products in which both genetically modified microorganisms and newly introduced genes are no longer present (e.g., cell extracts, most enzyme preparations)

3. Products derived from genetically modified microorganisms in which genetically modified microorganisms capable of multiplication or of transferring genes are not present, but in which newly introduced genes are still present (e.g., heat-inactivated starter cultures)

4. Products consisting of or containing genetically modified microorganisms capable of multiplication or of transferring genes (e.g., live starter cultures for fermented foods and feed)

Key areas of information required for the risk assessment

- Information relating to the characteristics of the recipient or (when appropriate) parental organism, including the origin of the inserted sequences [donor organism(s)], the genetic modification, and comparison of the genetically modified microorganisms with an appropriate comparator
- Information relating to the product, including information relating to the production process, the product preparation process, description of the product and considerations of the genetically modified product for human health
- Exposure assessment/characterisation related to food and feed consumption
- Potential environmental impacts of genetically modified microorganisms and their products
- Post-market environmental monitoring, including considerations of case-specific monitoring and general surveillance

VKM

VKM conclusion on the applicability of the EFSA guidance on risk assessment of GMM for genome-edited microorganisms

"…Due to the heterogenous uses of microorganisms/products their regulatory landscape can be considered complex, falling under both a directive, different EU regulations and various guidance documents developed by several of the EFSA panels. The product categorisation presented in the guidance allows for differentiation in the amount of data needed for the assessment. In contrast to animals and plants, the core concept of qualified presumption of safety (QPS) provides a clear baseline for the comparative approach. This combined with a case-by-case approach provides both structure and flexibility to the risk assessment process. The same flexibility is offered to genome-edited organisms within this regulatory framework."

VKM

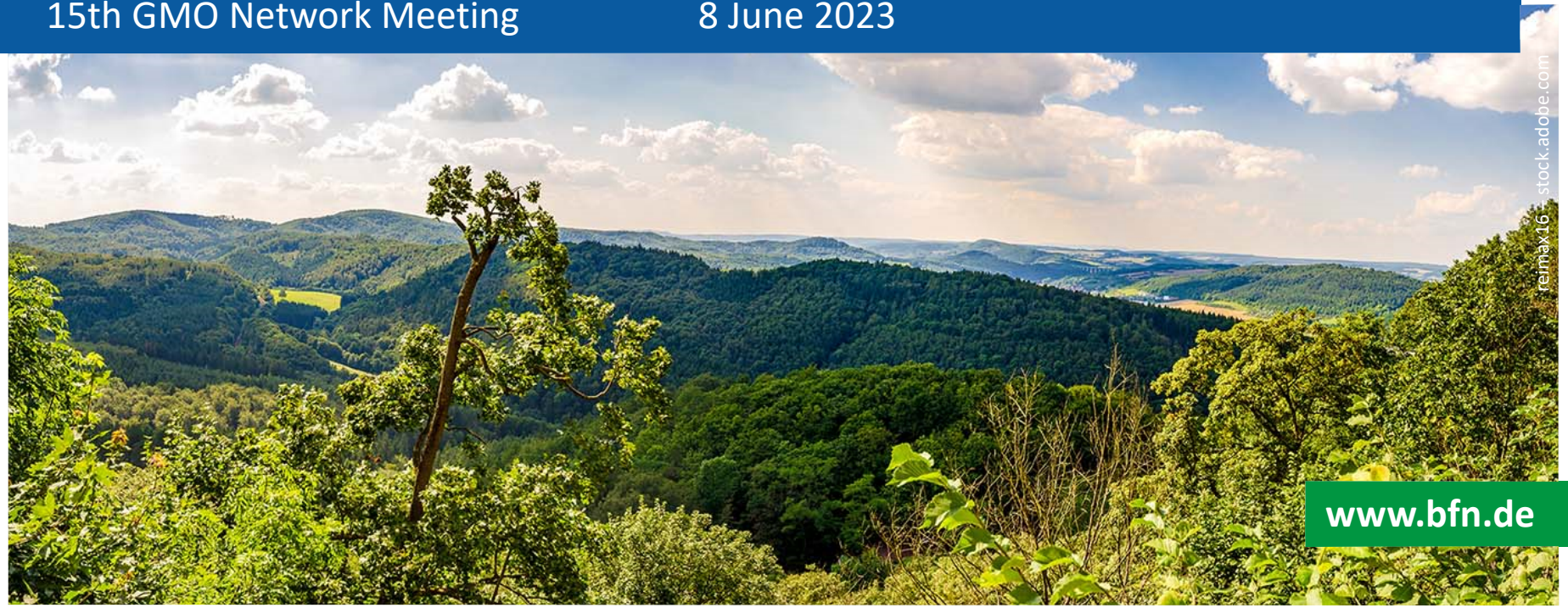
Browse vkm.no and our social media channels

Federal Agency for Nature Conservation

Horizon scan and environmental risk assessment of GM virus applications

Wolfram Reichenbecher

15th GMO Network Meeting 8 June 2023



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The Project

- Runs from Q4/2021 – Q4/2023
- Environment Agency Austria
- Commissioned by BfN (F. Waßmann)
- Assist horizon scanning exercises at CBD and OECD level
- Support implementation and enforcement of current GMO regulation
- Support further policy development

Steps involved

- Horizon Scan on scientific and on grey literature
- Focused on a broad range of GM virus applications with possible need for ERA
- Four case studies selected for further investigation
- Expert consultations on
  (1) Horizon Scan and ERA of GM virus applications
  (2) Sustainability of GM virus applications
  (3) Recommendations for ERA

Challenges with horizon scan and selection of case studies

Are applications really based on GM viruses?

- Live attenuated/„modified“ viruses, WT strains with specific character

Assessment of status of development

- Different criteria for different types of applications

Selection of case study examples

- Extent of environmental exposure

Available info and guidance on RA and sustainability assessment

Relevant publications describing

- GM virus applications intended for release into environment
- GM virus applications as tools for genetic modification of plants and animals

Not considered publications

- GM virus applications intended in contained systems or for medical purposes

Case study categories and examples

– For different fields of GM virus applications –

1) **GM Citrus Tristeza Virus:** *in field application*
   - GM CTV-SoD in orange plantations to control HLB disease
   - Agriculture

2) **Virus-based vector tools:** *contained use/ in field application*
   - Viruses used as vector tools for VAGE or VIGS
   - Tobacco rattle virus, Baculoviruses, BYSMV and others
   - Tools for R&D in laboratory and proposals for in-field use (VAGE, VIGS, HEGAAs)
   - R&D; Agriculture

3) **GM vector vaccines for lifestock/wildlife:** *non-transmissible or transmissible*
   - GM vaccine against facial tumours in Tasmanian devils or myxomatosis in rabbits
   - R&D; Veterinary Health; Nature Conservation

4) **GM bacteriophages:** *contained use/in field application*
   - Xylencer, i.e. proposal for biocontrol of plant pathogen *Xylella fastidiosa*
   - Food Safety; Agriculture

Some ERA considerations for the application of GM CTVs

Available ERA information

- USDA-APHIS (2020): Pest Risk Assessment/Environmental Impact Assessment
- EFSA (2020) Evaluation of existing guidance and EFSA (2017) CTV pest risk assessment
- *Lack of guidance regarding 'specific areas of risk'* (EFSA, 2020)

Possible environmental effects

- Used CTV strain of low infectivity and pathogenicity: Similar in Europe with other environments and other endemic CTV strains? Changes to infectivity and pathogenicity?
- Used CTV strain not meant to spread via insect vectors or be present in harvested fruit: Similar in Europe with other environments, other (wild) host plants and vector insects?
- Resistance development in target organism to defensin (-> application sustainable?)
- Potential effects on NTOs – uncertainty regarding SoD effects on European insect species

Uncertainties

- Data availability; are conclusions of USDA-APHIS assessments applicable?

Challenges for risk assessment of GM virus applications

Scope and diversity of GM virus applications

- Technical tools for GM modification (GM virus vectors)
- What are they for: agriculture, food safety, nature conservation?

GM virus applications are developed for different intended environmental exposure

- Contained use vs. intentional spread/release with possible complex environment interactions

GM virus applications are developed for different purposes

- Different protection goals need to be considered

Existing guidance not sufficient

- Cf. conclusions by More et al. (2020) with regard to MC and ERA of GM CTV-SoD

Uncertainty with intended environmental spread

- Substantial with complex interactions (CTV, HEGAAs, transmissible vaccines and others)

Challenges for sustainability analysis of GM virus applications

No well-developed framework for sustainability analysis of GMOs

- Current initiatives address GM plants only
- Concepts need to be developed for GM virus applications

Current EC initiatives for a sustainability analysis relevant

- Implementation of sustainability analysis
- Involvement of authorities and stakeholders
- Relation between risk assessment & sustainability analysis

GM virus applications developed for different application types

- Different issues regarding sustainability need to be addressed

GM virus applications developed for different scopes of environ. exposure

- Contained use & limited spread vs. applications for intentional spread

Outlook

Third expert consultation on recommendations for ERA

Final report

The issue of GM viruses should be considered essentially from the perspective of nature conservation. This is because of the risk assessment and for conceptual reasons.

Publications

Genetic engineering, nature conservation and biological diversity: Boundaries of design. Viewpoint. Bonn. DOI:10.19217/pos222en. https://www.bfn.de/en/publications/position-paper/genetic-engineering-nature-conservation-and-biological-diversity

Synthetic Biology – Scan the horizon for impacts on biodiversity. https://attachments.cbd.int/567962e74dc1af45194e3f51e4acc1ae/SyntheticBiology.pdf

Verma et al. (2022): The effect of mating complexity on gene drive dynamics. The American Naturalist, Artikel 722157. https://doi.org/10.1086/722157

Ad hoc-Stellungnahme zum Urteil des Europäischen Gerichtshofs in der Rechtssache C-688/21 https://www.bfn.de/sites/default/files/2023-03/adhoc_Urteil_C_688_21_0.pdf

Eckerstorfer et al. (2023) Recommendations for the Assessment of Potential Environmental Effects of Genome-Editing Applications in Plants in the EU. Plants, 12(9), 1764. https://doi.org/10.3390/plants12091764

Thank you for your attention!

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New developments in biotechnology applied to animals

Michele Ardizzone
EFSA, NIF Unit

EUROPEAN FOOD SAFETY AUTHORITY

EC mandate M-2018-0205
new developments in biotechnology applied to animals

EUROPEAN COMMISSION
DIRECTORATE-GENERAL FOR HEALTH AND FOOD SAFETY

Food Safety, Sustainability and Innovation
Biotechnology

"EC *Mandate*" for an EFSA opinion on new developments in biotechnology applied to animals (including synthetic biology and new genomic techniques)

- … *not* a new mandate
- … *but* the continuation of the mandate M-2018-0205
- … *with* adjusted Terms of Reference

EC mandate M-2018-0205 background

Jun 2018	Request for an EFSA opinion on GMOs *(microorganisms, plants and animals)* developed through synthetic biology and their implications for risk assessment methodologies, covering aspects related to food & feed and environment
Oct 2018 **Step 1**	Agreement to complete two opinions on the evaluation of existing guidelines for their adequacy for the: • microbial characterisation and ERA of microorganisms obtained through SynBio (EFSA SC 2020) • molecular characterisation and ERA of GM plants obtained through SynBio (EFSA GMO Panel 2021)
Oct 2020 **Step 2**	Agreement to complete two opinions on the evaluation of existing guidelines for their adequacy for the: • food and feed risk assessment of GM microorganisms obtained through SynBio (EFSA SC 2022) • food and feed risk assessment of GM plants obtained through SynBio (EFSA GMO Panel 2022)
Dec 2022 **Step 3**	Agreement to complete an opinion on new developments in biotechnology applied to animals • including synthetic biology and new genomic techniques (NGTs)

EC mandate M-2018-0205 aim and timelines

EC has given a mandate to EFSA to deliver two outputs:

1. **Knowledge gathering report** on known cases of animals and their food and feed products obtained by new developments in biotechnology
2. **Scientific opinion** on potential novel hazards/risks from new developments in biotechnology applied to current and near market animals and adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety & welfare, and environmental impact

1. Knowledge gathering on known cases of animals and their food and feed products obtained by NGT

Knowledge gathering on known cases of animals and their food and feed products obtained by NGT #1

NGTs in animals are mainly used for:

Focus of the mandate

Food purposes: farmed animals
- ❑ cattle (hornless cattle; heat-resistant cattle)
- ❑ pigs (porcine reproductive and respiratory syndrome-resistant pigs)
- ❑ fish (yield-enhanced/fast-growing tilapia)
- ❑ birds (gene-edited hens to end cull of male chicks)

Environmental control: NGT-based gene drive applications
- ❑ insects, especially mosquitos and some invasive species

Research on human diseases e.g.:
- ❑ mice: disease models for a gene therapy (cancer and genetic diseases)
- ❑ pigs: donor of organs to be transplanted into human patients (no transplant rejection)
- ❑ rats and monkeys still at the early R&D (model human diseases)

Knowledge gathering on known cases of animals and their food and feed products obtained by NGT #2

Gene-edited cattle produce no horns

New technique could eliminate the need for painful dehorning

CRISPR cattle cleared for the first time by FDA

The animals carry a gene that helps them beat the heat.

AquaBounty gets Argentina go-ahead for edited tilapia

Transgenic salmon producer AquaBounty and its majority owner, Intrexon, today announced that their jointly developed gene-edited line of tilapia, FLT 01, has been exempted from GM regulation in Argentina.

Agreement targets disease-resistant gene-edited pigs

Researchers and commercial partners to continue collaboration on developing pigs resistant to Porcine Reproductive and Respiratory Syndrome.

Photo credits: Shutterstock

Knowledge gathering on known cases of animals and their food and feed products obtained by NGT #3

January 2023 → **April 2023** → **July 2023**

Procurement for the knowledge gathering report

Aimed to support the activity of the NGT animal WG by:

- ❑ identifying animals and their products obtained by NGTs, described since 2001, including their traits and uses
- ❑ listing the techniques and modifications used, including explanation of relevant terminology
- ❑ identifying NGT animals and their products developed since 2001 that are subject to authorisation procedures by non-EU national and international authorities, and the corresponding available risk assessments (e.g. opinions, guidances, authorisations) that exist
- ❑ collecting per case the information relevant for the risk assessment of NGTs applied to farmed animals and their agri/food/feed products

University of California
Survey

New Genomic Techniques (NGTs) Animals and their Agri/food/feed products

The overall objective of this survey is to identify near-market (i.e. under research/development) farmed NGT animals and their agri/food/feed products globally for a report being prepared for the European Food Safety Authority (EFSA).

New Genomic Techniques (NGTs) are defined as a particular method or methodology "capable of altering the genetic material of an organism" - and "New" is further defined as having been developed after April 2001.

Definitions for "agri/food/feed products":

- Food/feed products are obtained by NGT animals that are farmed for food/feed uses, e.g.:
  - meat or milk from mammals (e.g. ruminants, pigs, horses)
  - meat or eggs from birds (e.g. broilers, laying hens, turkeys, quails and other fowls)
  - meat or eggs fishes (e.g. see/fresh water aquaculture)
  - meal or honey from insects (e.g. black soldier fly, honey bee)
- Agri products are obtained by NGT animals that are farmed but not for food/feed uses, e.g.:
  - fur animal (e.g. mink)
  - ornamental fishes
  - sericulture (e.g. silk worms)
  - insect frass

Please fill out the survey for each NGT animal/trait combination

https://surveys.ucanr.edu/survey.cfm?surveynumber=40529

2. Scientific opinion on

a) Potential novel hazards/risks from new developments in biotechnology applied to current and near market animals

b) Adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety & welfare, and environmental impact

a) Potential novel hazards/risks from new developments in biotechnology applied to current and near market animals

Definitions for “stages of NGTs applications”:

- Commercial stage: NGTs applications in farmed animals for agri, food and feed uses, currently marketed in at least one country worldwide.
- Pre-commercial stage: NGTs applications in farmed animals for agri, food and feed uses, ready to be commercialised in at least one country worldwide but not yet on the market (products that are undergoing authorisation procedures in at least one country).
- Research & Development stage: NGTs applications in farmed animals for agri, food and feed uses, at proof of concept stage (e.g. testing gene targets for trait enhancement of commercial interest).

b) Adequacy of current EFSA RA guidance, covering all aspects of MC, FF safety & welfare, and environmental impact

European Food Safety Authority EFSA Journal 2012;10(1):2501

SCIENTIFIC OPINION

Guidance on the risk assessment of food and feed from genetically modified animals and on animal health and welfare aspects[1]

EFSA Panels on Genetically Modified Organisms (GMO) and Animal Health and Welfare (AHAW)[2, 3]

European Food Safety Authority EFSA Journal 2013;11(5):3200

SCIENTIFIC OPINION

Guidance on the environmental risk assessment of genetically modified animals[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

Expertise identified for the NGT animal WG

BACK UP SLIDES

EC mandate M-2018-0205
terms of reference #1 (from the mandate)

1) Knowledge gathering report on known cases of animals and their food and feed products obtained by new developments in biotechnology

- identify animals and their products obtained by new development in biotechnology described since 2001 including their traits and uses
- list the techniques and modifications used, including explanation of relevant terminology
- identify animals and their products developed since 2001 that are subject to authorisation procedures by international authorities, and the corresponding available risk assessments (e.g. opinions, guidances, authorizations) that exist
- collect per case the data and information relevant for risk assessment, and structure it according to the EFSA guidances

EC mandate M-2018-0205
terms of reference #2 (from the mandate)

2) Scientific opinion on potential novel hazards/risks from new developments in biotechnology applied to current and near market animals and adequacy of the current EFSA risk assessment guidance, covering all aspects of molecular characterisation, food feed safety & welfare, and environmental impact. The expected outcome of this activity will be an opinion which:

- identifies, where possible, novel potential hazards and risks which new developments in biotechnology applied to current or near market animals could pose for humans, animals and the environment compared to conventional breeding or established techniques of genetic modification.
- determines whether the existing guidelines for risk assessment of genetically modified animals are applicable, fully or partially, adequate and sufficient to risk assess new developments in biotechnology applied to animals.
- in case existing guidelines for risk assessment are considered not applicable, partially applicable, not adequate or not sufficient, identifies on which specific areas and aspects existing guidelines should be updated, adapted or complemented.

Thank you!

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EUROPEAN FOOD SAFETY AUTHORITY

Vitenskapskomiteen for mat og miljø

Norwegian Scientific Committee for Food and Environment

New developments in biotechnology applied to animals (Agenda No.7, Day2.)

Ville Erling Sipinen

EFSA 15th GMO Network meeting

8-9 June 2023

Use of genome-editing technologies in animal breeding (ToR 2)

- Generating production animals from elite individuals has limitations in traditional breeding
  - Identification of high merit individuals, generation times, maintenance of genetic diversity, conversion of genetic variation into genetic gain
- Broad range of potential applications for genome-editing tools
  - disease resistance, growth, fertility, animal welfare

VKM

Examples of studies documenting genome editing in Atlantic salmon.

Purpose of the study	Target gene	Trait associated with the gene	Genome-editing technique	Type of genetic alternation	Delivery system	Reference
Production of germ-cell free salmon	Dead end (*dnd*)	Fertility	CRISPR/Cas9	Indels at the *dnd* gene	RNA-injection into fertilised eggs	(Wargelius et al., 2016)
Increase of endogenous synthesis of omega-3 polyunsaturated fatty acids (PUFA)	Elongation of very long chain fatty acid protein 2 (e*lovl2*)	Elongation of omega-3 PUFAs	CRISPR/Cas9	Indels in the coding region of *elovl2*	RNA-injection into fertilised eggs	(Datsomor et al., 2019b)

Examples of studies documenting genome editing in cattle.

Purpose of the study	Target gene	Trait associated with the gene	Genome-editing technique	Type of genetic alternation	Delivery system	Reference
Production of hornless dairy cattle	*P_C POLLED*	Hornlessness	TALENs	Homozygous and heterozygous introgression of *P_C POLLED*	Somatic cell nuclear transfer	(Carlson et al., 2016)
Increased muscle growth	Myostatin (*mstn*) gene	Regulation of muscle mass	ZFN	5-bp indels at the *mstn* gene	Somatic cell nuclear transfer	(Luo et al., 2014)
Knockout of prion protein (PRNP)	Bovine *prnp* gene, encoding the PrP^C glycoprotein	Bovine spongiform encephalopathy caused by accumulation of the misfolded isomer PrP^{BSE} in the brain	CRISPR/Cas9	Indels and large deletions at the *prnp* gene in bovine cell lines and embryos	Somatic cell nuclear transfer and zygote editing	(Bevacqua et al., 2016)
Knockout of bovine beta-lactoglobulin (BLG)	*blg* gene	Hypoallergenic milk lacking the whey protein BLG	ZFN	17- and 16-bp indels leading to frameshift mutations	Somatic cell nuclear transfer	(Sun et al., 2018b)

Examples of studies documenting genome editing in domestic pig.

Purpose of the study	Target gene	Trait associated with the gene	Genome editing	Type of mutation	Method used	Reference
Resistance to Porcine reproductive and respiratory syndrome (PRRS) virus	*cd163*	A lectin, present on the surface of macrophages, involved in the entry process of PRRS virus	CRISPR/Cas9	Deletion of exon 7 at scavenger receptor cysteine-rich (SRCR) domain 5	Zygote editing	(Burkard et al., 2017)
Cold resistance	Uncoupling protein 1 (*ucp1*)	Heat generation and regulation of energy homeostasis	CRISPR/Cas9	Insertion of exons 3-5	Knock-in of mouse adiponectin-*ucp1* combined with somatic cell nuclear transfer	(Zheng et al., 2017a)
Removal of boar meat taint	Kisspeptin receptor 1 (*kiss1r*)	Trigger of puberty and regulation of Gonadotropin-Releasing Hormone	TALEN	Indels ranging between 1-3 bp	Somatic cell nuclear transfer	(Sonstegard et al., 2016)

Five cases were used to discuss the applicability of the EFSA guidance (ToR 3 and 4)

Genome-edited animals

Case 1 represents two examples of genome-edited farmed Atlantic salmon (*Salmo salar* L.) developed with theCRISPR/Cas9 technique and categorised as SDN1 (Datsomor et al., 2019a; Datsomor et al., 2019b). In both cases, genes encoding enzymes involved in the production of polyunsaturated fatty acids (PUFA) were edited, resulting in altered fatty acid composition.

Case 2 is a genome-edited farmed Atlantic salmon developed with the CRISPR/Cas9 technique and categorised as SDN1 (Wargelius et al., 2016). Introduced edits in the dead end (dnd) gene leading to knockout of this gene resulted in a sterile fish without germ cells. The dnd gene is a factor required for germ cell survival in vertebrates.

Case 3 is a genome-edited channel catfish (*Ictalurus punctatus)* developed with CRISPR/Cas9 technique and categorised as SDN1 (Khalil et al., 2017). Knockout of the MSTN gene encoding the protein myostatin which normally suppresses muscle growth, resulting in enhanced growth of the fish.

Case 4* is a genome-edited cattle (*Bos taurus*) developed with the TALEN technique and categorised as a SDN3 (Carlson et al., 2016). Insert of a 212 bp duplication (homology-directed) into bovine embryo fibroblasts leads to alteration of the gene responsible for development of horns. The altered gene resembles a gene variant found naturally in cattle of Celtic origin (Polled Celtic, PC POLLED) that does not produce horns.

**This example also illustrates the occurrence of unintended effects of the engineering approach. Independent analyses of sequencing data made available by the developers revealed that vector sequences remained in the final cow genome. FDA discovered a stretch of bacterial plasmid DNA including several genes conferring antibiotic resistance. The unintended integration of the DNA fragment is likely to have occurred during the genome-editing process (Norris et al., 2020).*

Case 5 is a genome-edited pig (*Sus scrofa domesticus*) developed with the CRIPSR/Cas9 technique and categorised as SDN1 (Burkard et al., 2017; Burkard et al., 2018). Resistance towards porcine reproductive and respiratory syndrome (PRRS) was achieved by a deletion in the CD163 gene. The virus causing the disease enters immune cells via the CD163-receptor to establish an infection. Animals carrying the modified CD163 receptors seem to be fully resistant to PRRS virus infection.

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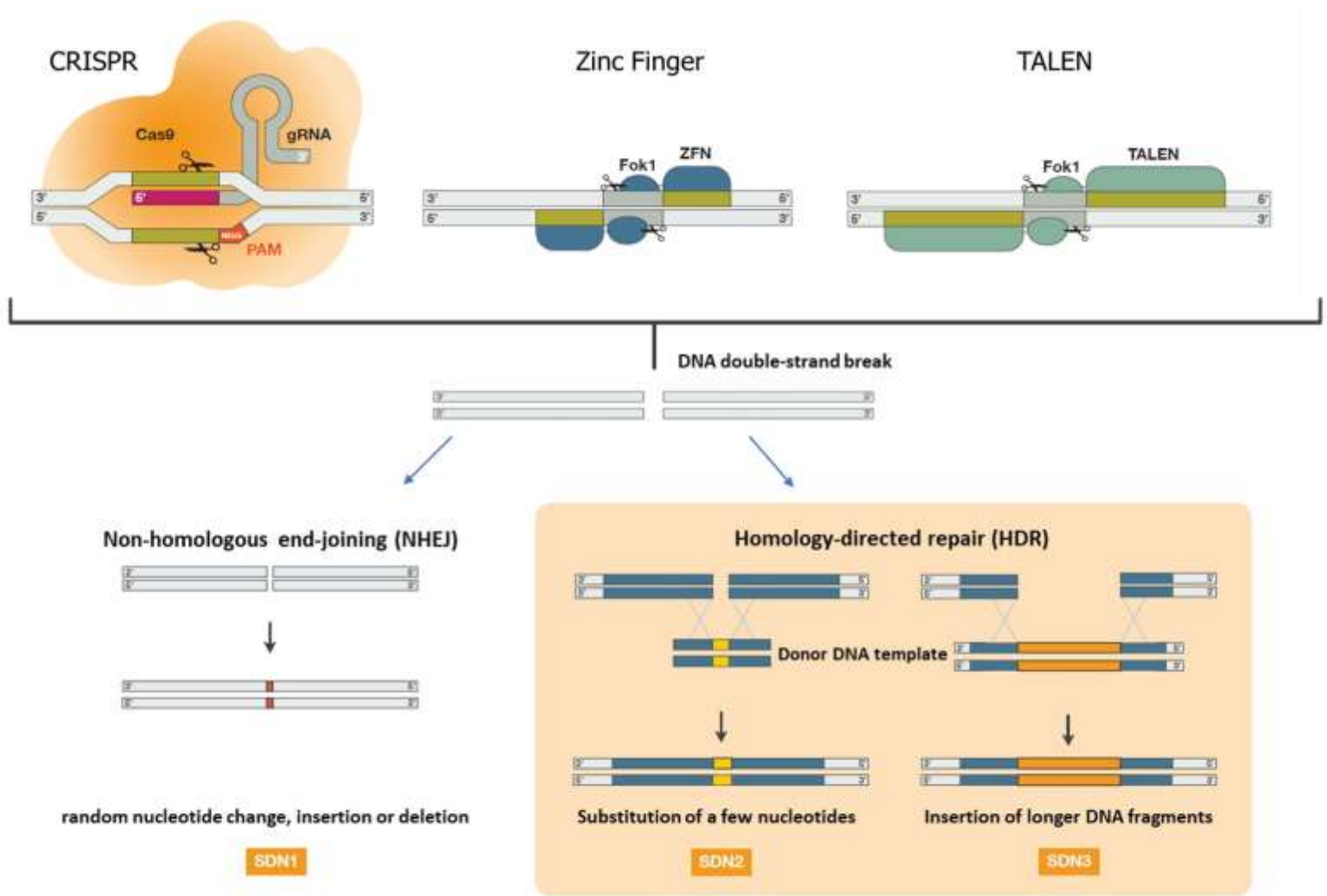
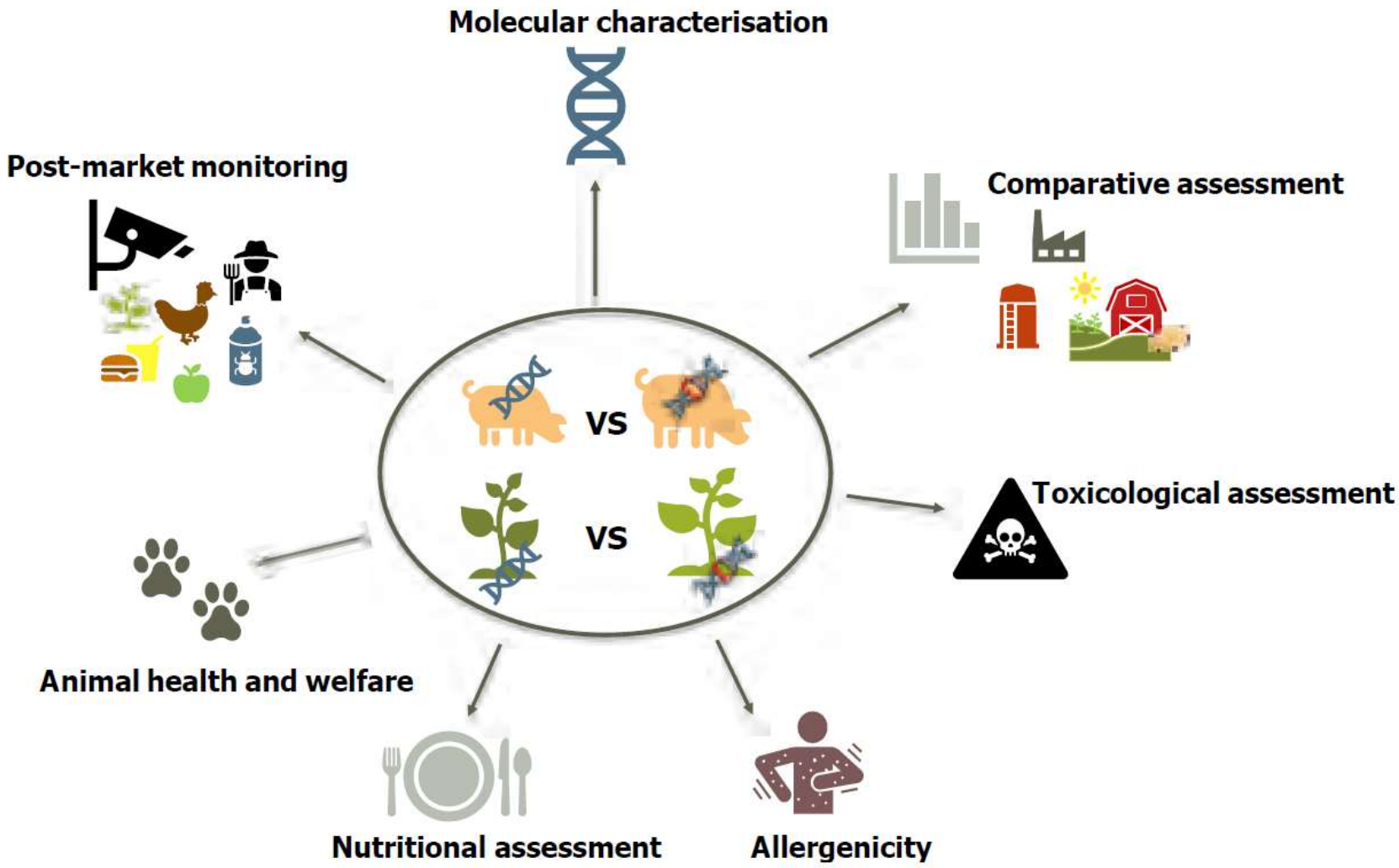
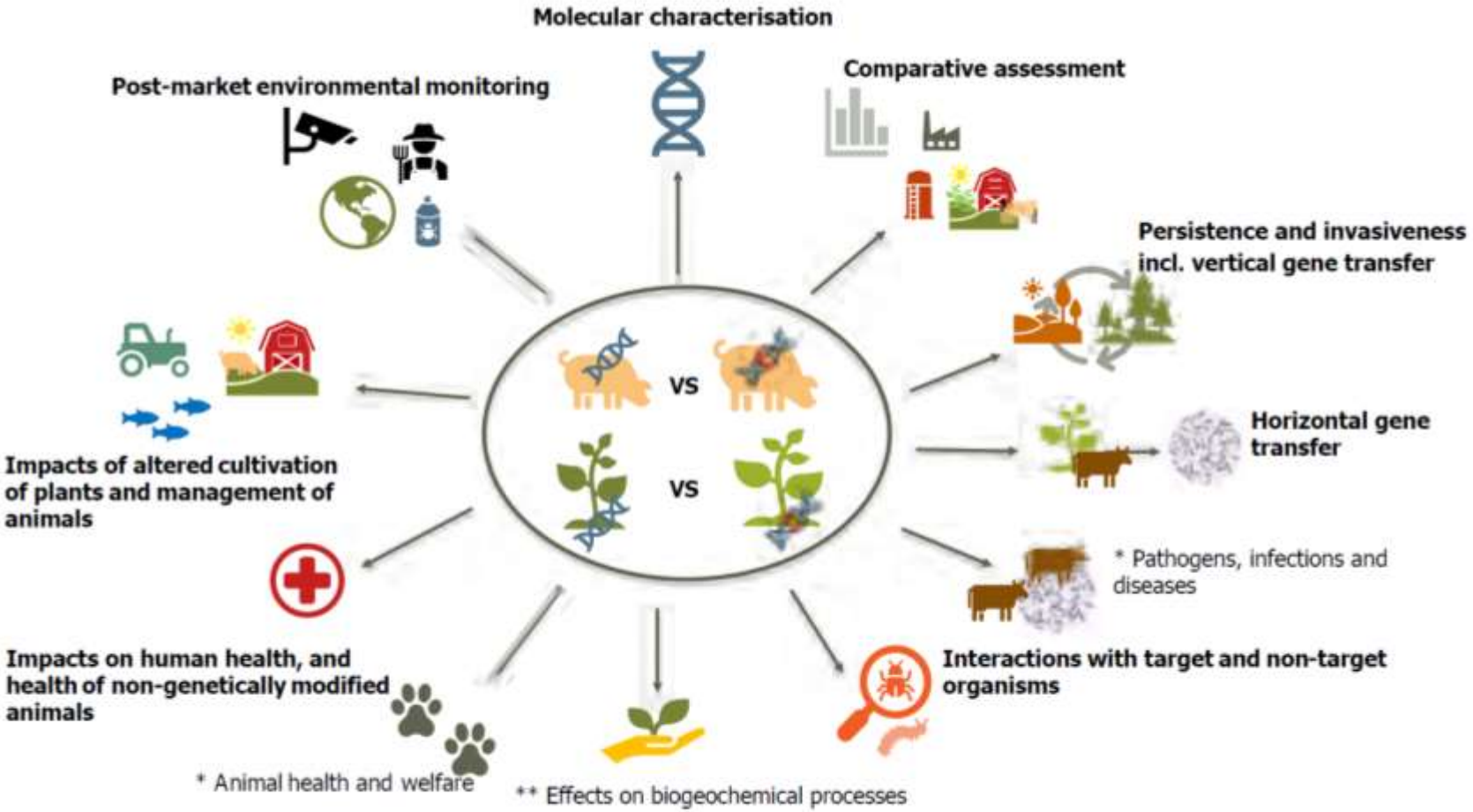

CRISPR
Cas9
gRNA
PAM
Zinc Finger
Fok1
ZFN
TALEN
Fok1
TALEN
DNA double-strand break
Non-homologous end-joining (NHEJ)
random nucleotide change, insertion or deletion
SDN1
Homology-directed repair (HDR)
Donor DNA template
Substitution of a few nucleotides
SDN2
Insertion of longer DNA fragments
SDN3

Food and Feed



ERA



* Risk assessment of genetically modified animals, ** Risk assessment of genetically modified plants.

...and specific areas of ERA

Fish

- Persistence and invasiveness of genetically modified fish and vertical gene transfer to wild and feral relatives
- Horizontal gene transfer
- Impacts of genetically modified fish on biotic components and processes in the ecosystem
- Fish pathogens, infections and diseases
- Interactions of genetically modified fish with the abiotic environment
- Environmental impacts of the specific techniques used for the management of genetically modified fish
- Impacts of genetically modified fish on human health

Mammals and birds

- Persistence and invasiveness of genetically modified mammals and birds and vertical gene transfer to wild and feral relatives
- Horizontal gene transfer
- Pathogens, infections and diseases
- Interactions of genetically modified mammals and birds with target organisms (TO)
- Interactions of genetically modified mammals and birds with non-target organisms (NTOs)
- Interactions of genetically modified mammals and birds with the abiotic environment
- Environmental impacts of the specific techniques used for the management of genetically modified mammals and birds
- Impacts of genetically modified mammals and birds on non-genetically modified animal's health and welfare
- Impacts of genetically modified mammals and birds on human health

VKM

VKM conclusions

- *The inherent flexibility of the EFSA guidance makes it suitable to cover health and environmental risk assessments of a wide range of organisms with various traits and intended uses. Combined with the embedded case-by-case approach including the initial hazard identification step, that determines the type and extent of information needed for the assessment, the guidance is applicable to genome-edited organisms. VKM's evaluation has not identified new hazards specific to genome-edited organisms that fall outside the areas of concern established in the guidance.*

- *The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with novel traits (i.e. the phenotype of the organism) may be fully applied to all categories of genome-edited organisms. The guidance on environmental risk assessment is largely concerned with novel traits and assessment of potential effects on biodiversity (e.g. in Norway) stemming from the spread and establishment of genome-edited organisms is fully applicable.*

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Cont…

- *The evaluation of the guidance demonstrates that the parts of the health and environmental risk assessment concerned with the genetic modification (i.e. the genotype of the organism) may be fully applied to genome-edited organisms with inserted genes or long fragments of DNA, i.e. edits categorised as Site-Directed Nuclease type 3 (SDN3).*

  However, these parts are not fully applicable for genome-edited organisms with minor insertions, deletions or single mutations, i.e. edits categorised as Site-Directed Nuclease type 1-2 (SDN1-2), edits obtained by oligonucleotide directed mutagenesis (ODM) or base editing (BE).

In summary, VKM finds that the EFSA guidance on risk assessment of genetically modified organisms provides a functional framework for risk assessment of genome-edited organisms. However, inclusion of specific considerations in the guidance regarding different properties of genome-edited organisms would be beneficial to ensure a common understanding between product developers and risk assessors regarding the type and extent of data needed to perform a risk assessment.

VKM

Dynamic nature of EFSA guidance

"Collectively, the guidance supplemented with technical notes covers new technological developments such as the potential use of omics and next generation sequencing technologies, as well as new genome-editing approaches"

"VKM emphasises that the overall relevance and suitability of the guidance is based on its dynamic nature. An assessment of the suitability of guidance should therefore not be limited to a narrow interpretation of the suitability of single documents."

VKM

Ongoing application in Norway

VKM

Browse vkm.no and our social media channels

AP162

JUNE 2023

R. Schoonjans

efsa
EUROPEAN FOOD SAFETY AUTHORITY

Updates on key issues in the evaluation

Clock stopped by EURL and EFSA

Additional information received

PRODUCT(S) HISTORY

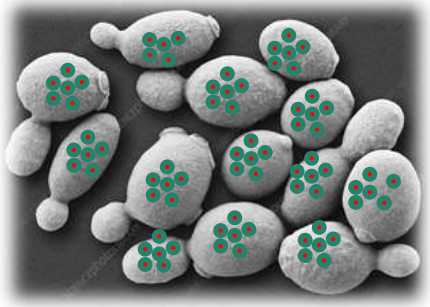
Soy → LBG2 gene

GM Pichia → Production organisms containing LBG2 gene

LegH prep → •Liquid solution containing rDNA

Meat Analogue → 0,8 % containing LegH prep

GM



To be marketed in Europe

SCOPE IN THE DOSSIER AND IN REG. 1829/2003

Soy → LBG2 gene

GM Pichia → Production organisms containing LBG2 gene

LegH prep → •Liquid solution containing rDNA

Meat Analogue → 0,8 % containing LegH prep

1. This Section shall apply to:

(a) GMOs for food use;

(b) food containing or consisting of GMOs;

(c) food produced from or containing ingredients produced from GMOs.

4. The authorisation referred to in paragraph 2 may cover:

(a) a GMO and foods containing or consisting of that GMO as well as foods produced from or containing ingredients produced from that GMO; or

(b) food produced from a GMO as well as foods produced from or containing that food;

(c) an ingredient produced from a GMO as well as food containing that ingredient.

GMM Guidance 2011

Scope Applicant: This application is submitted to gain **authorisation for the use of** soy leghemoglobin (the liquid preparation is referred to as "**LegH Prep**") produced from genetically modified *Pichia pastoris* (*P. pastoris*) **as a flavouring** ("meaty taste") in meat analogue products that will be marketed in the European Union (EU).

ADDITIONAL FOOD ADDITIVE DOSSIER

- Preamble 13 of Reg. 1829/2003: Flavourings falling within the scope of Council Directive 88/388/EEC of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (3) which contain, consist of or are produced from GMOs should also fall **within the scope of this Regulation for the safety assessment of the genetic modification**.
- Preamble 12 of Reg.1829/2003: "…..on to this authorisation procedure, food additives containing, consisting of or produced from **GMOs should fall also within the scope of this Regulation for the safety assessment of the genetic modification**, while the final authorisation should be granted under the procedure referred to in Directive 89/107/EEC.

OUTLINE

MC: Details of the genetic modification and of **the NEP**

ERA: Lysed cells with presence of rDNA in the meat analogue: ERA (HGT) assessment

FF: The impact of the genetic modification on the ingredient (= the LegH preparate)

- For **Toxicity**
- For **Allergenicity**
- For **Composition** and **Nutrition**

MC

- Genetic modifications mapped and described
- DNA sequence delivered according to the WGS note for the final production strain 541
- ADR3 + 5: Absence of Viable cells

MC

- Bioinformatics on the NEP
- Protein characterisation taking into account case specific elements
- Mass spec data analysis ongoing to demonstrate equivalence of proteins
- Deposit number of the strain
- Pending

ERA

- HGT
- No ADR
- Conclusions ready

MC&FF: STRAINS USED IN THE DOSSIER

- Table listing all tests and the corresponding strains/batches that were used
- Total of 4 different strains were used in the safety dossier, the final production strain being 541
- Equivalence questions for proteins pending

FF - TOX IN VIVO (UPDATES END MAY)

- **90-day done with final strain 541** is the main driver for the assessment, performed with the highest doses of the protein
- Cooperation with FIP/FAF ongoing – agreement on the conclusions for endpoints
- All other tox studies 28 day and 14 day: short narratives are foreseen with the relevant RA conclusions.
- Pending

EXPOSURE

- **Dietary exposure** only for humans; no animal exposure
- Not linked to the genetic modification + linked to the meat analogue comprising the food additive >> referring to the remit of the FAF Panel
- Pending

FF - ALLERGENICITY (UPDATES END MAY)

- SoyLegH protein
  - no hits
  - In vitro degradation study details
- Soy origin LegH and meat analogue– labelling recommendation “The imported and consumed meat analogues will be labelled “containing soy””
- Yeast background
  - hits
  - Independent from the impact of the genetic modification
  - Pending

FF - COMPOSITION AND NUTRITION

- Minerals
- Anti-foam
- Microfiltration
- Pending

LITERATURE SEARCH

Pending collaboration with FAF WG: Literature search following practices under the Guidance for submission for food additive evaluations ((see below)), which describes the requirements for literature searches in Appendix B - Data Requirements For The Evaluation Of Food Additive Applications (# B.2).

5.3. Review of published literature

Applicants should review the published literature for relevant references. This should be based on the principles underpinning systematic reviews. The methods used to identify relevant data and other information, including the scope and criteria of literature searches, should be described.

ENVISAGED PLAN FOR CO-PUBLISHING AND CROSS REFERRING

- FAF Opinion can refer to GMO opinion for
  - MC of the strain and the NEP
  - Allergenicity
  - Nutrition (general)
  - HGT

- GMO Opinion can refer to FAF opinion for
  - Toxicity *in vitro* (incl. ADME, Pepsine degradation)
  - Toxicity *in vitro* (incl. 14d, 28d and 90d studies)
  - Human Dietary exposure
  - Tbd Nutrition - Fe upper levels ?
  - Tbd Composition – FA specifications?

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CRISPR/Cas9 Specificity in Tomato

Ellen Slaman and Ruud de Maagd, EFSA GMO MS Network, June 9th, 2023
(presented by Gijs Kleter, NL delegation)

Objectives:

- Investigate the specificity and side-effects of CRISPR-mediated mutagenesis in plants
- Increase knowledge base for risk assessors and regulatory officials

Research Programme
Biotechnology and Safety

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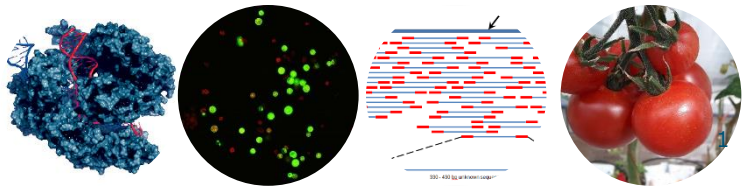
UNIVERSITY & RESEARCH

100years 1918 — 2018

NWO

Ministerie van Infrastructuur en Waterstaat



Conclusions

- With the proper experimental design (requires a reference genome), CRISPR/Cas-mediated genome editing in tomato is very specific
- In general, *and if deemed necessary*, amplicon sequencing of predicted off-target sites can rule out off-target mutations
- GUIDE-seq works as an extra, unbiased method but did not yield more, unexpected mutations in our hands (sensitivity could be increased)
- Whole Genome (Re)sequencing even with a proper reference genome does not likely produce more useful information

Publication (recent)

https://www.frontiersin.org/articles/10.3389/fgeed.2023.1196763/full

frontiers | Frontiers in Genome Editing

TYPE Original Research
PUBLISHED 06 June 2023
DOI 10.3389/fgeed.2023.1196763

OPEN ACCESS

EDITED BY
Matthew R. Willmann,
Pairwise, United States

REVIEWED BY
Changtian Pan,
Zhejiang University, China
Ning Zhang,
Cornell University, United States

High-throughput sgRNA testing reveals rules for Cas9 specificity and DNA repair in tomato cells

Ellen Slaman[1,2], Michiel Lammers[2], Gerco C. Angenent[1,2] and Ruud A. de Maagd[2]*

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Assessing safety-by-design in novel plant breeding techniques by comparing native gene based modification with classical breeding

Jack Vossen, Jasper Engel (creators)

Presented by Gijs Kleter (Netherlands), EFSA GMO MS network, June 9th, 2023

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100years
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Introduction to the project

- Native genes from crop wild relatives
    - Example: late blight resistance genes in potato
- Deployment of native genes in crops
    - Classical breeding (slow, linkage drag)
    - Cisgenesis (fast, precise non-native genomic location)
    - Knock-on of inactive alleles (fast, precise, native location)
- Safety by design principles
    - Native genes have a history-of-safe-use
    - CRISPR-Cas application without genomic integration
    - Equivalence tests through comparative Metabolomics

Red font: Project objectives

Publication (recent)

https://link.springer.com/article/10.1007/s11248-023-00344-y

Transgenic Res
https://doi.org/10.1007/s11248-023-00344-y

REVIEW

Check for updates

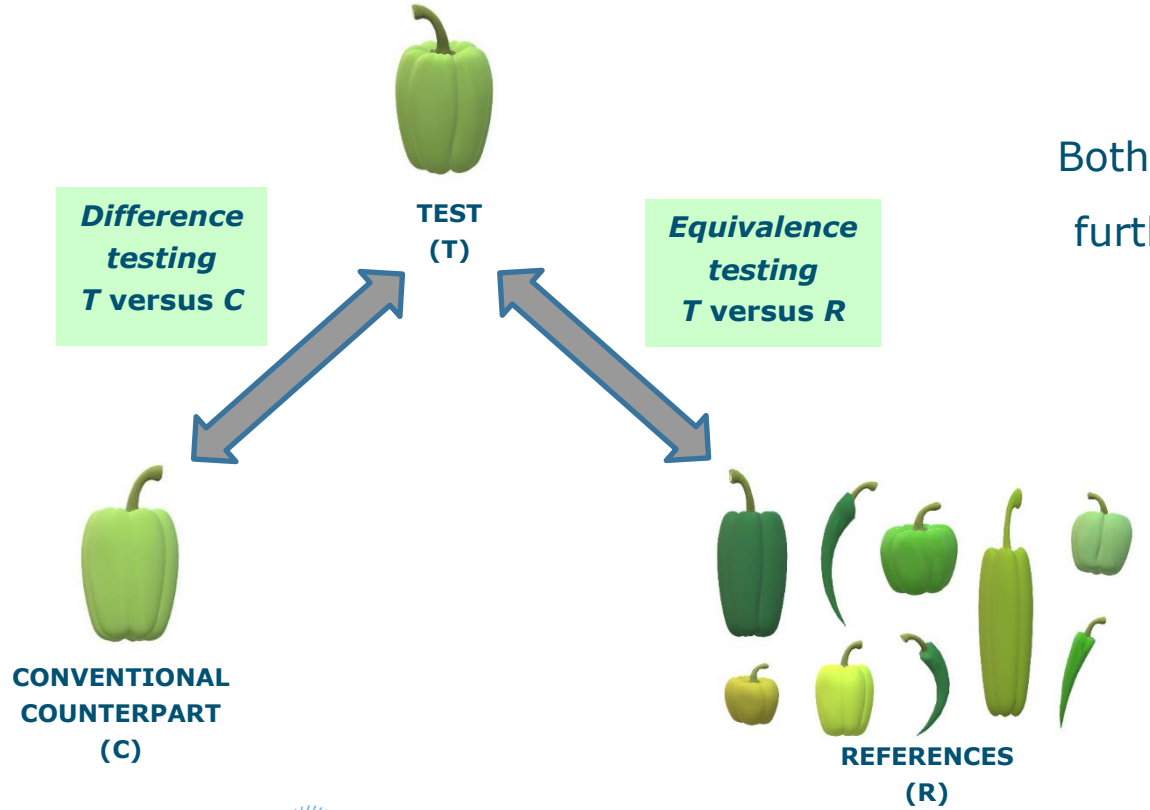
Comparative safety assessment of genetically modified crops: focus on equivalence with reference varieties could contribute to more efficient and effective field trials

Gijs A. Kleter · Hilko van der Voet · Jasper Engel · Jan-Pieter van der Berg

Received: 24 September 2022 / Accepted: 28 March 2023

Abstract The initial compositional analysis of plants plays an important role within the internationally harmonized comparative safety assessment (value for cultivation and use) or other, independent variety trials.

The current EU approach

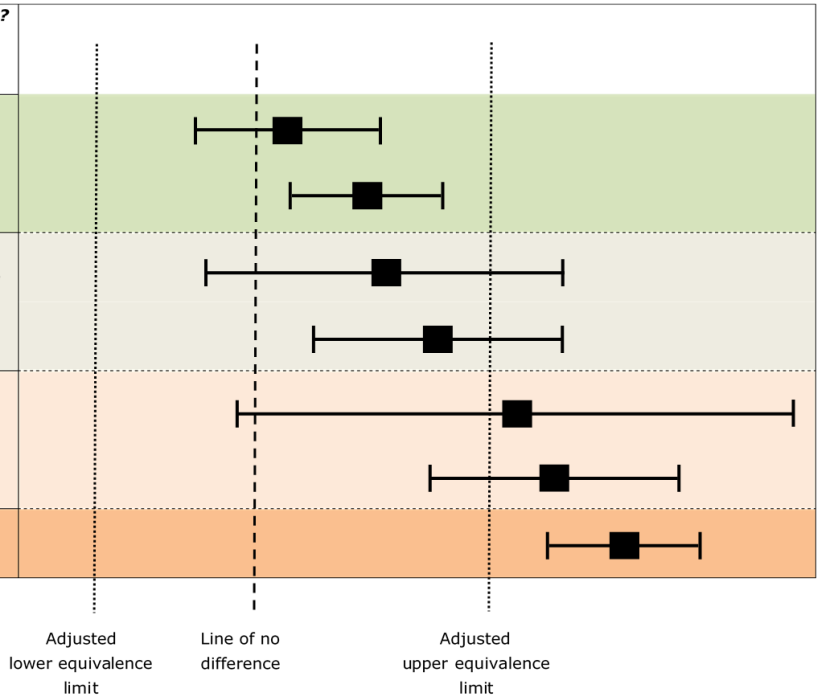


Both tests significant
→
further assessment

EFSA: recommended approach

Outcome scenarios

Outcome type	*Statistically significant different?*	*Equivalence category*	*Equivalent?*
1	No	I	Yes
2	Yes		
3	No	II	More likely than not
4	Yes		
5	No	III	Less likely than not
6	Yes		
7	Yes	IV	No



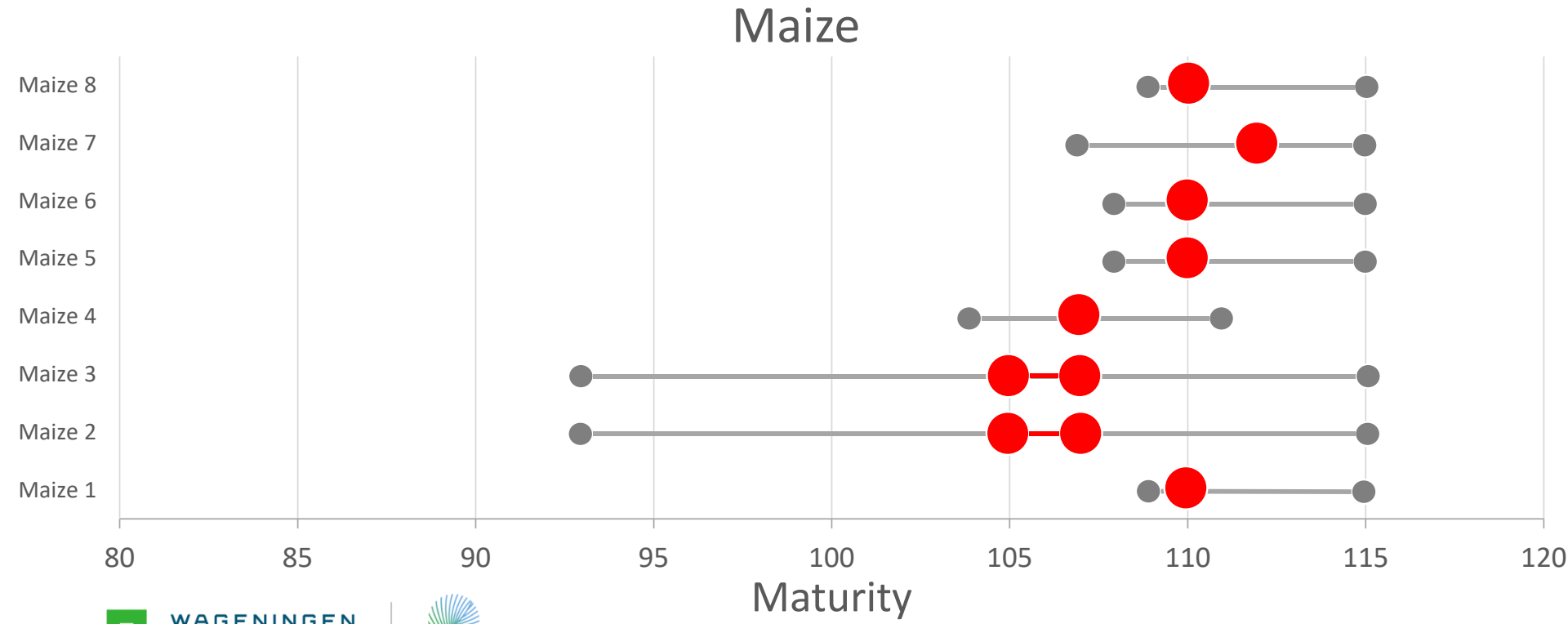
Putting the equivalence test in a wider context

- 2011: guidelines for statistical tests published by EFSA
- What are the experiences gained from a decade of use?
  - Screening of EFSA GMO panel opinions from the last 5 years (2017 – 2021)
  - Evaluation of the role of R and C in regulatory practice
- Possibilities for increasing the efficiency and effectiveness of the approach

Screening of 33 EFSA GMO panel opinions

Event	*Number of reference varieties*	*Number of endpoints (total/tested)**	*Statistical findings*			*Reference*
			1. Statistically significant differences	*2 Not-shown equivalences (cat. III/IV)*	*Both 1 and 2*	
Cotton						
Single events						
GHB811	7	73/56	29	2	1	EFSA, 2021a
GHB614 × T304-40 × GHB119	6	73/53	68	8	6	EFSA, 2018b
Maize						
Single events						
4114	19	84/71	68	0	0	EFSA, 2018c
DAS-40278-9	6	82/59	80	24	20	EFSA, 2016a
…						
Oilseed rape						
Single events						
73496	6	131/103	109	11	7	EFSA, 2021e
…						
Soybean						
Single events						
GMB151	9	112/89	67	6	2	EFSA, 2021f

Maturity classes of test & conventional counterpart varieties (●), and references (●)



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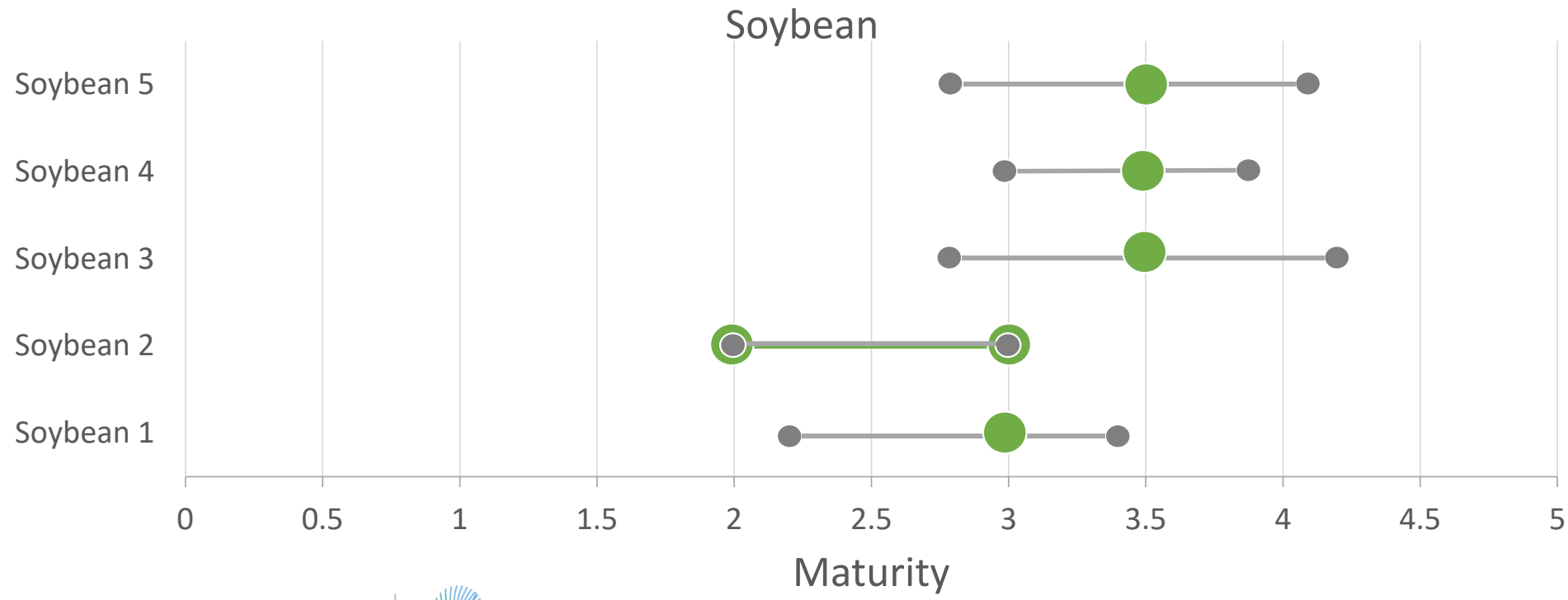
Maturity classes of test & conventional counterpart varieties (●), and references (●)



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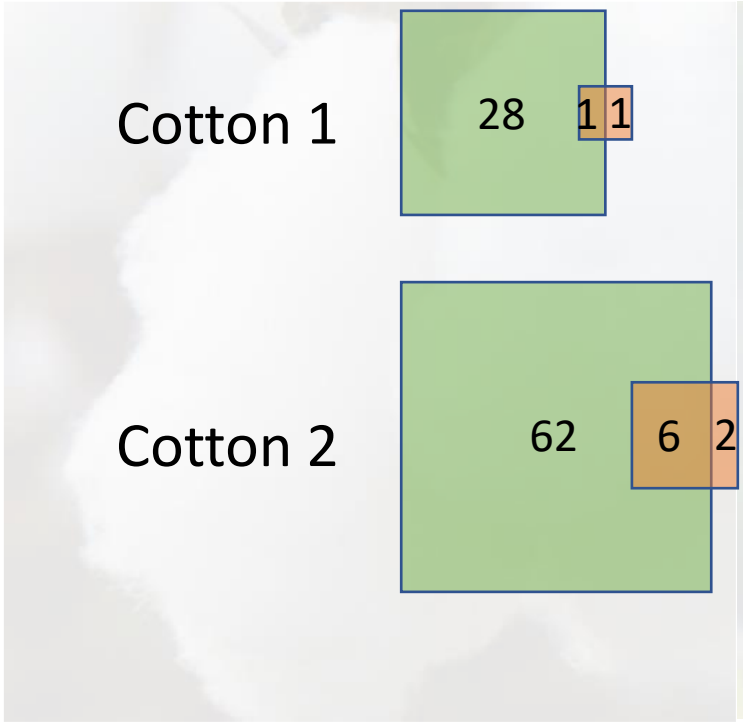
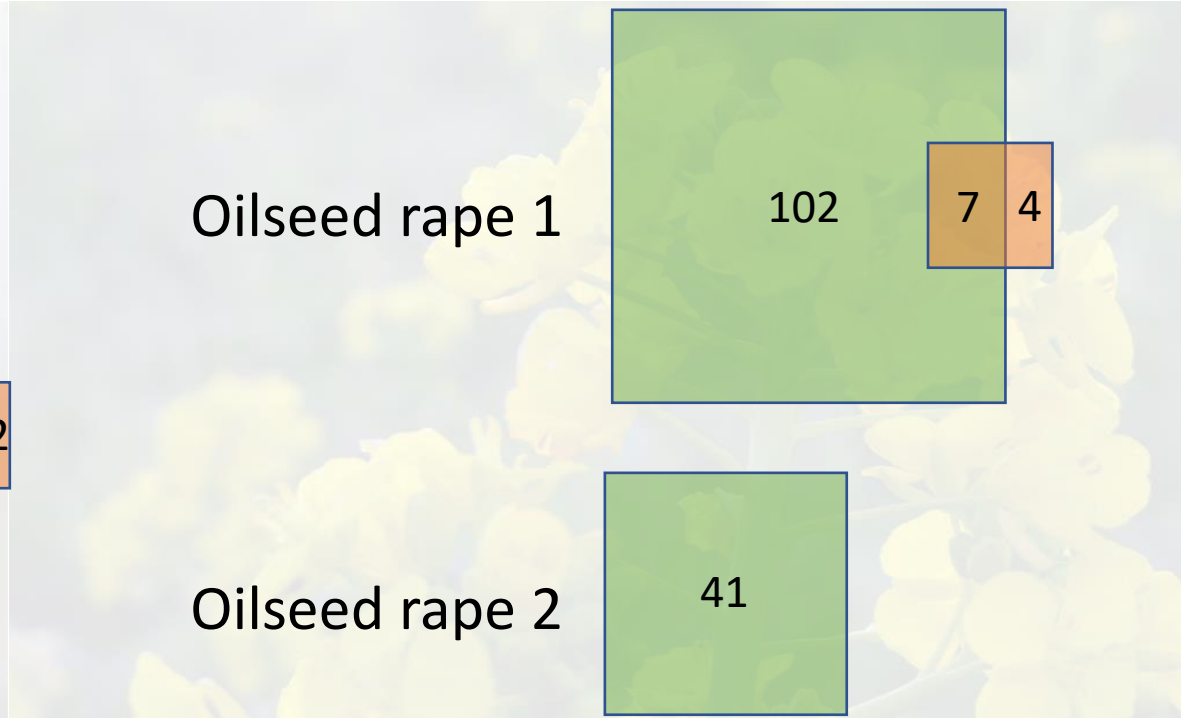
Statistically significant differences ( ), parameters not showing equivalence ( ) and their overlap





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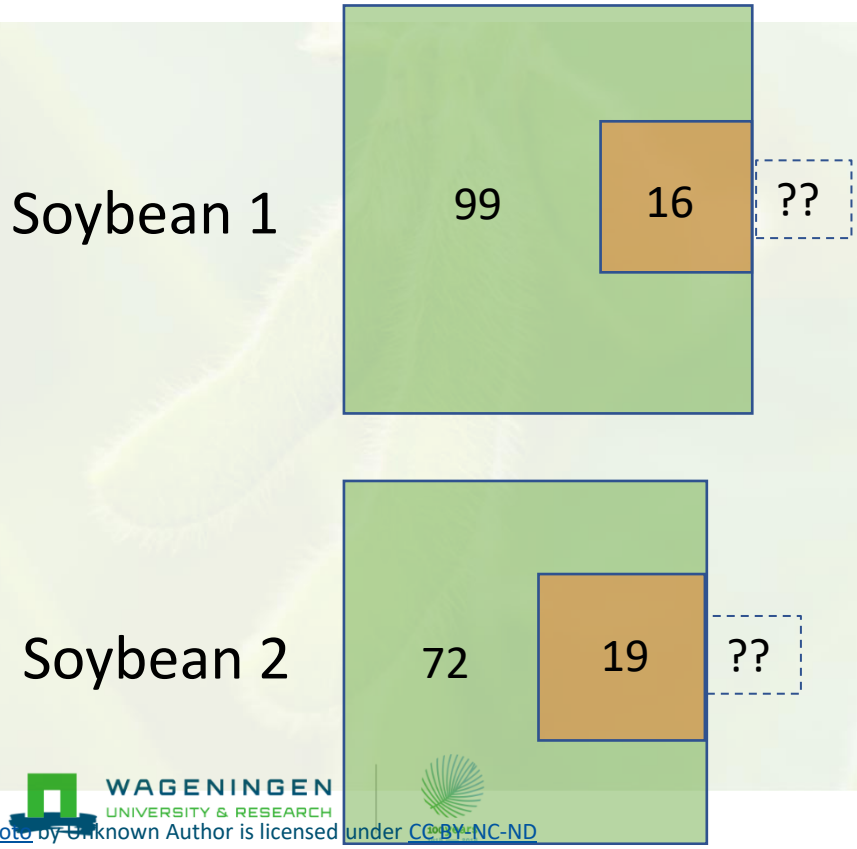
Statistically significant differences ( ), parameters not showing equivalence ( ) and their overlap



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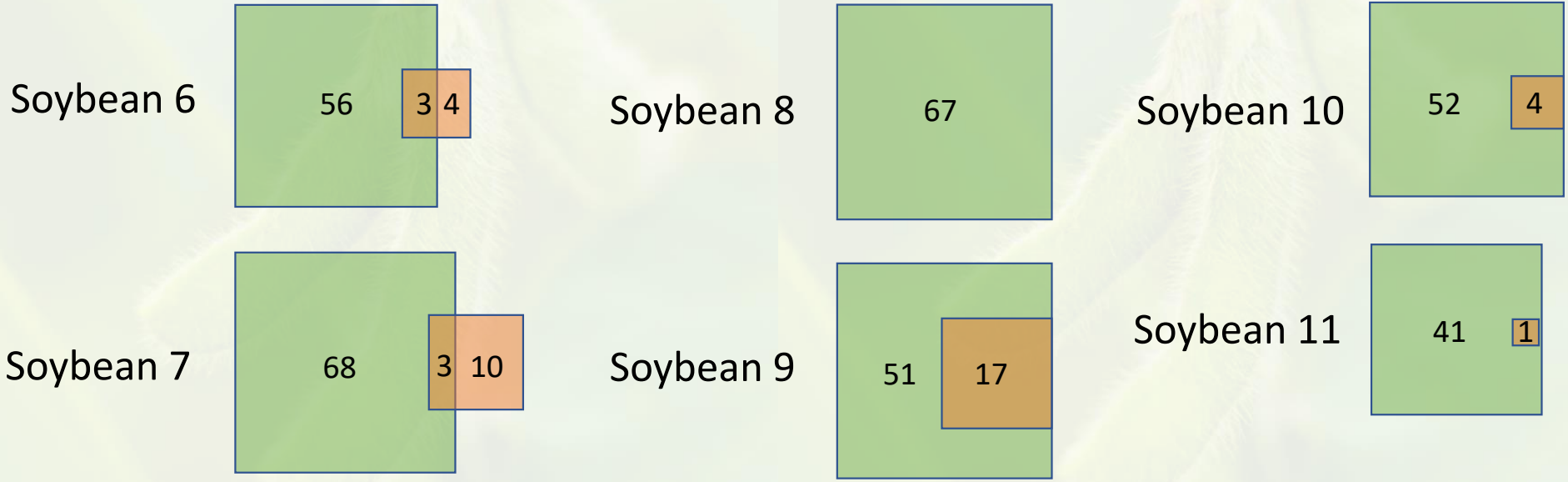

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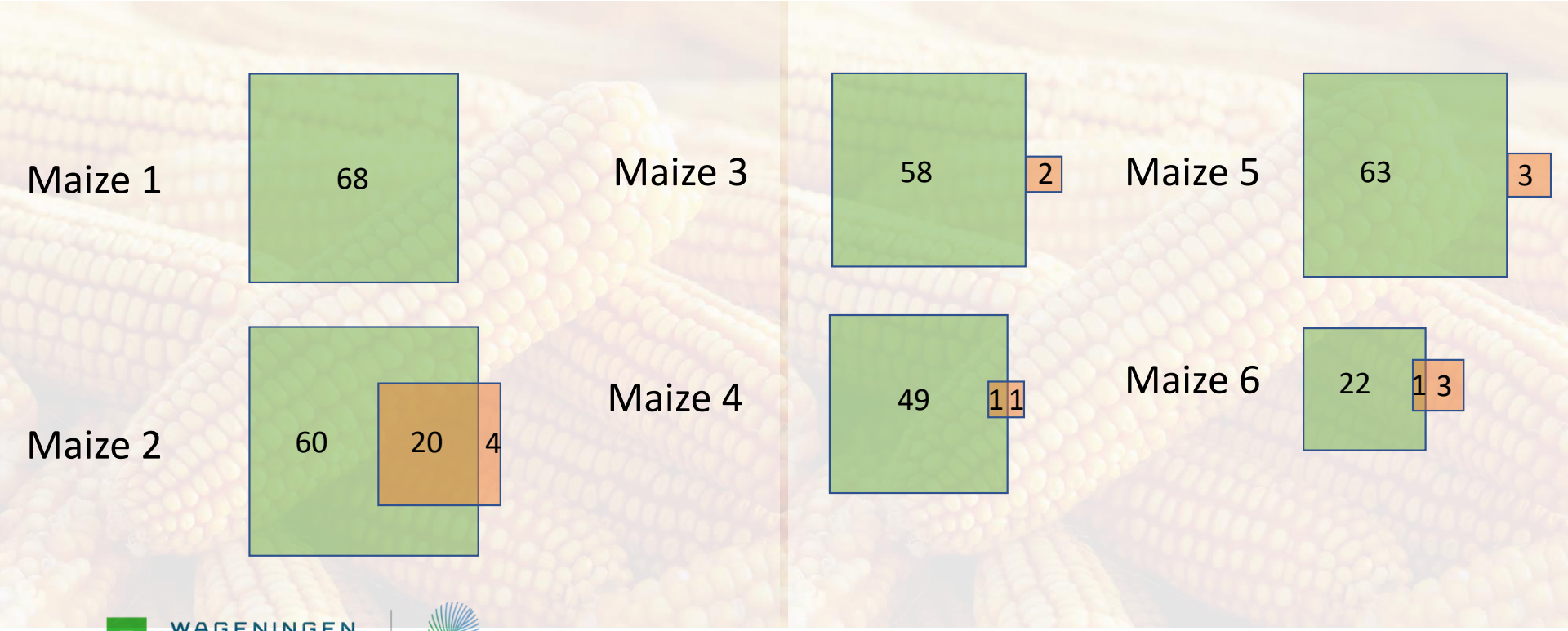

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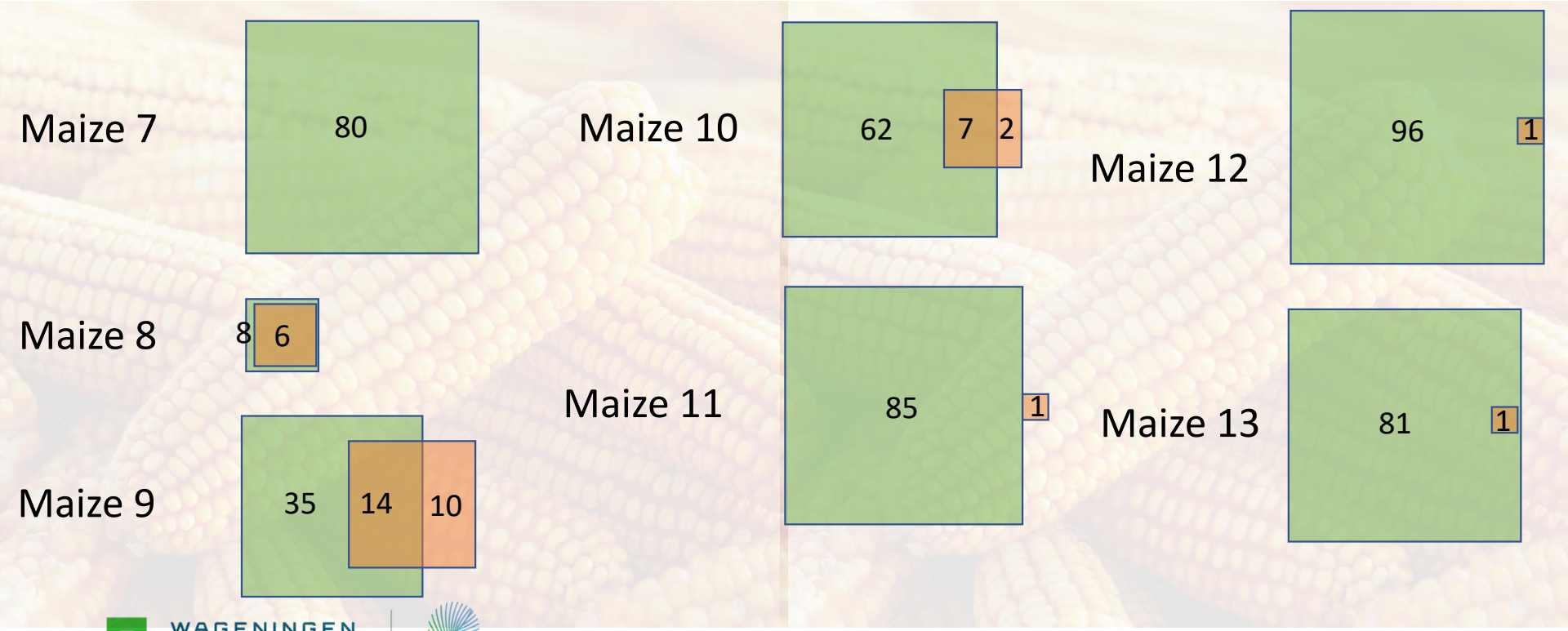

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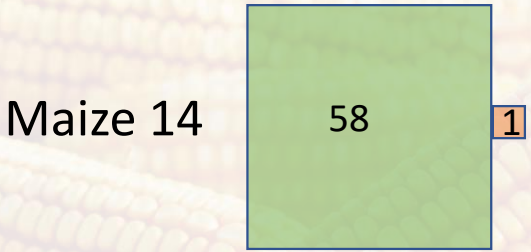

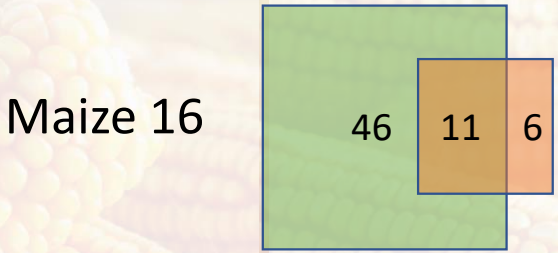

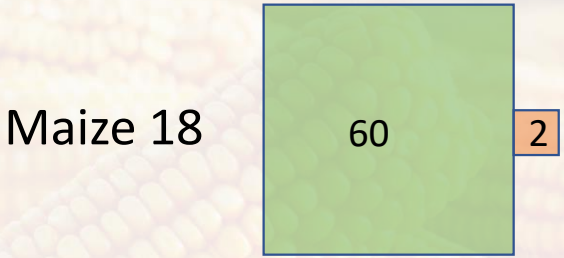

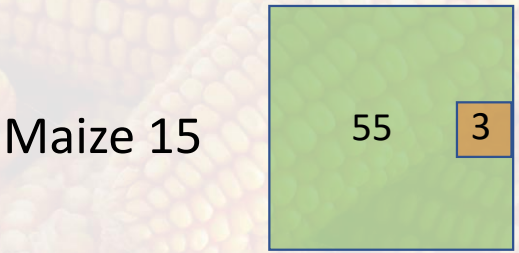

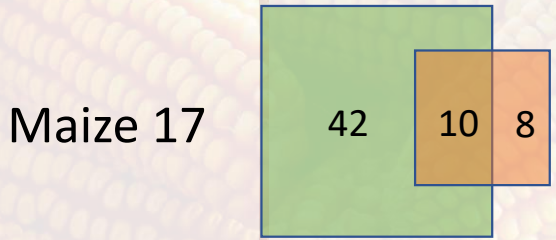

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Screening of 33 EFSA GMO panel opinions

Observations:

- Appropriateness of comparators
  - Controls usually registered variety (indicative of history of use)
  - References within same maturity groups
- Large number of significant T-C differences
- Small number of T-R not-shown equivalences
- Apparently, many parameters that differ from the control fall within background spanned by R's
- Might equivalence testing *per se* already be sufficient?

Proposal for comparative experiments:

- Focus on test-versus-references comparisons
- Inclusion in variety registration trials, i.e. VCU trials
- This includes the use of data from reference varieties, obtained in the trial or (possibly) historical data can be used

08 June 2023

EFSA Scientific Colloquium 27 “Cell Culture-derived Foods & Food Ingredients”

Dafni Maria Kagkli & Antonio Fernández Dumont

EUROPEAN FOOD SAFETY AUTHORITY

EFSA SCIENTIFIC COLLOQUIUM 27 (EFSA SC27)

EFSA's Scientific Colloquium 27 "Cell culture-derived foods and food ingredients"

11 May 2023, 09.00 - 12 May 2023, 12.30 (CEST)

Brussels, Belgium and online

Share:

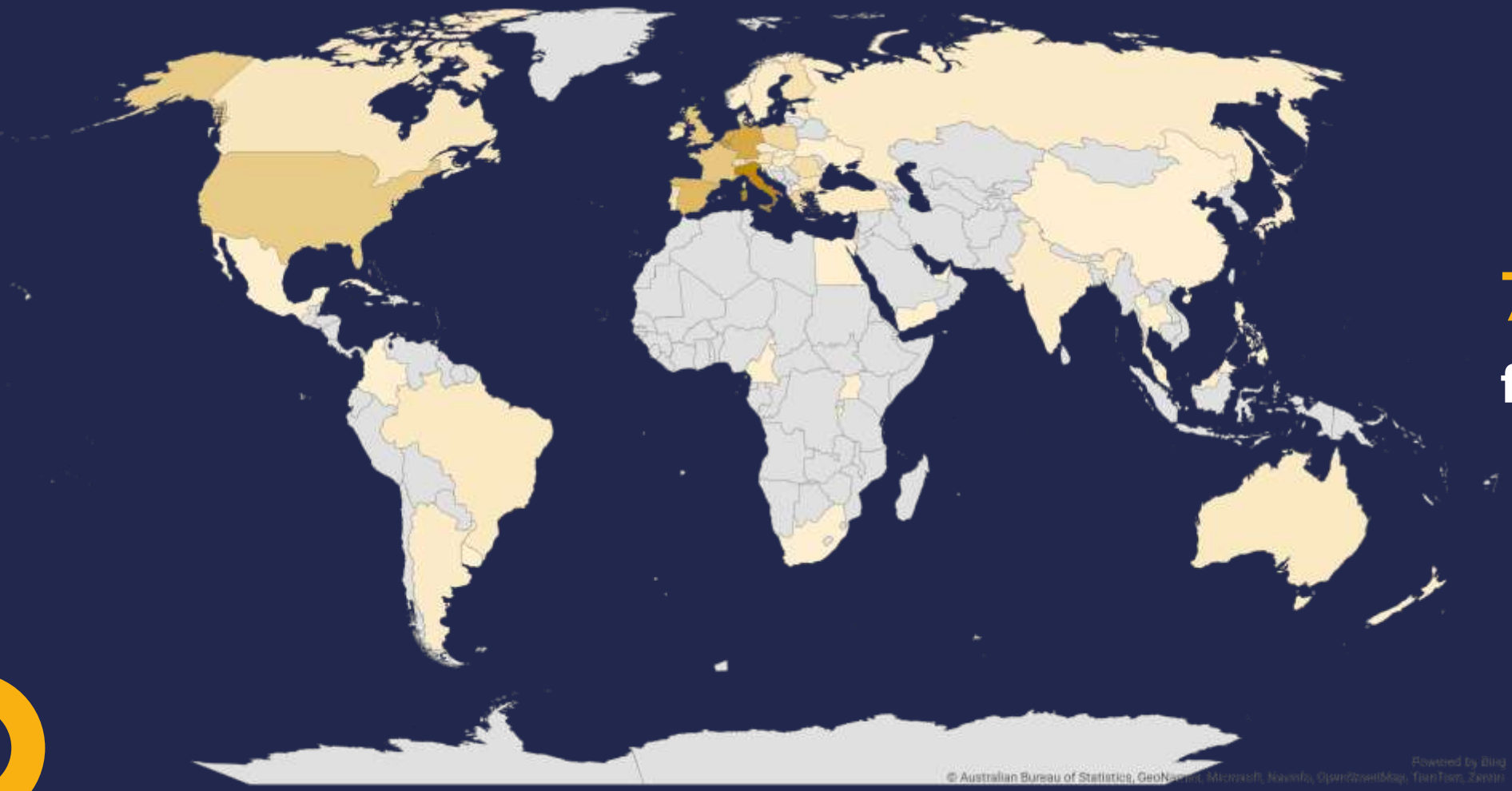
CELL CULTURE-DERIVED FOODS

PRECISION FERMENTATION

- Sectors in the agri-food system
- State of the art of relevant concepts, technologies, and derived products
- Emerging safety and methodological aspects and their impact on EFSA's risk assessment approaches

Programme, abstracts, bios, presentations, recordings & final report (link)

EFSA'S SCIENTIFIC COLLOQUIUM 27
"CELL CULTURE-DERIVED FOODS AND FOOD INGREDIENTS"



720 registrants
from **62** countries

SCIENTIFIC COLLOQUIUM 27
"CELL CULTURE-DERIVED FOODS AND FOOD INGREDIENTS"

720 registrants from:

SCIENTIFIC COLLOQUIUM 27
"CELL CULTURE-DERIVED FOODS AND FOOD INGREDIENTS"

What did registrants expect from the Colloquium?

Break-out sessions 3 and 4 on Precision Fermentation

EFSA SC27 PF BO – BACKGROUND & OBJECTIVES

PRECISION FERMENTATION (PF)

In **absence of a regulatory definition** and for the purpose of the EFSA's SC27, PF refers to the use of **engineered microbial cell factories** in the production of **food ingredients**

- **Pre-market authorisation** under different regulatory frameworks (e.g., novel foods, food additives and flavourings, GMOS, etc.)
- Several **sectoral & cross-cutting EFSA guidance** documents (scientific requirements for the **risk assessment**)
- Product categories **1-2** (free from DNA & viable cells) and cat **3** (contain DNA but not viable cells)
  (EFSA GMO Panel, 2011; EFSA Scientific Committee, 2022)

EFSA SC27 PF BO – BACKGROUND & OBJECTIVES

Emerging safety and methodological aspects associated to PF (e.g., **knowledge & methodology gaps, development needs**) and their impact on **EFSA's risk assessment** approaches were discussed with relevant experts and stakeholders

BO3

New developments on engineered microbial cell factories: considerations for their safety assessment

BO4

Development needs for the safety assessment of food ingredients derived from precision fermentation (PF)

EFSA SC27 PF BO3 – STAKEHOLDERS' FEEDABCK

- No disruption in current approaches for risk assessment – **Safety-by-design** approach
  - **Phenotypic** data in addition to genomic data
  - Sufficient knowledge about the **metabolism** of the host strain to allow predictions of possible adverse effects (e.g., computational models) and optimise production
  - **Strain stability** only relevant for category 4 products
  - **Horizontal gene transfer** relevant if genes of concern are present. Safety of newly introduced sequences (e.g., allergenicity) to be assessed (GM strain traceability)

- **QPS** concept could be extended to GMMs generated by **NGTs** (strain *vs.* species)
  - Hazards (GMMs) are **independent** of the genetic modification technique
  - **Off-target** effects (point mutations introduced by NGTs *vs.* naturally occurring) could be assessed through, e.g., toxigenicity/pathogenicity testing

Limitations of **OMICS** (other than genomics) for routine use in risk assessment. Future developments in **automation** are expected to reduce costs and allow standardisation

EFSA SC27 PF BO4 – STAKEHOLDERS' FEEDABCK

- Comparative approach could be followed when native counterparts exist
- A consensus is needed between risk assessors & managers on the **acceptable level of identity** (how similar must the native & recombinant products be?)
- **Post-translational modifications** (product integrity and/or protein function)

- **Harmonisation** of methodology to assess the fate in the GI tract (i.e., digestibility, bioavailability, ADME)
- Classical tox studies might not be needed for the risk assessment of macro-nutrients (e.g., proteins) – **NAMs** to be integrated RA
- **Allergenicity** WoE approach – Sufficient for products similar to native substances

New-to-nature products

- Concerns for allergenicity – Lessons can be drawn from ICH guidelines for biotechnology products (pharmaceuticals)
- Imbalanced nutrition, e.g., by altering bioavailability

OVERALL CONCLUSIONS

EFSA received input on the set objectives of the colloquium:

- sectors in the agri-food system relevant to cell-culture derived food
- state of the art of relevant concepts, technologies, and derived products
- emerging safety and methodological aspects and their impact on EFSA's risk assessment approaches

EFSA is currently "digesting"/considering the input received in order to be prepared and have fit-for-purpose risk assessment methodologies

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Development of risk assessment methodology for RNAi-based GM plants

15th GMO Network

9/06/2023

EUROPEAN FOOD SAFETY AUTHORITY

PROJECT AIMS

EFSA's objective: in order to update EFSA's guidance documents, the scientific literature is regularly reviewed and the RA methodologies are updated to follow the progress in knowledge in the field

Literature searches to determine the need for update of EFSA's strategy for the RA of RNAi-based GM plants

Bioinformatic tool delivered by EFSA to the applicant to harmonise plant RNAi off-target searches (depending on the applicants' interest)

PROJECT TIMELINE

Project start: December 2022

Literature search start: January 2023

Milestone review: Q3 2023

Project end: Q3 2024

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