14 March 2019

# GMO Risk Assessment Future perspectives

**Anna Lanzoni**

Senior Scientific Officer, GMO Unit

Trusted science for safe food

European Food Safety Authority

# ROADMAP

- GMO Risk Assessment in EU – the frame
- Future perspectives

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# GMO IN EUROPE – THE FRAME

**An organism is "genetically modified" if its genetic material has been changed in a way that does not occur under natural conditions through cross-breeding or natural recombination.**

EU Directive 2001/18/EC (Art. 2)

**In the EU, products that are, contain, or are produced from Genetically Modified Organisms (GMOs) must have an authorisation prior to entering the market**

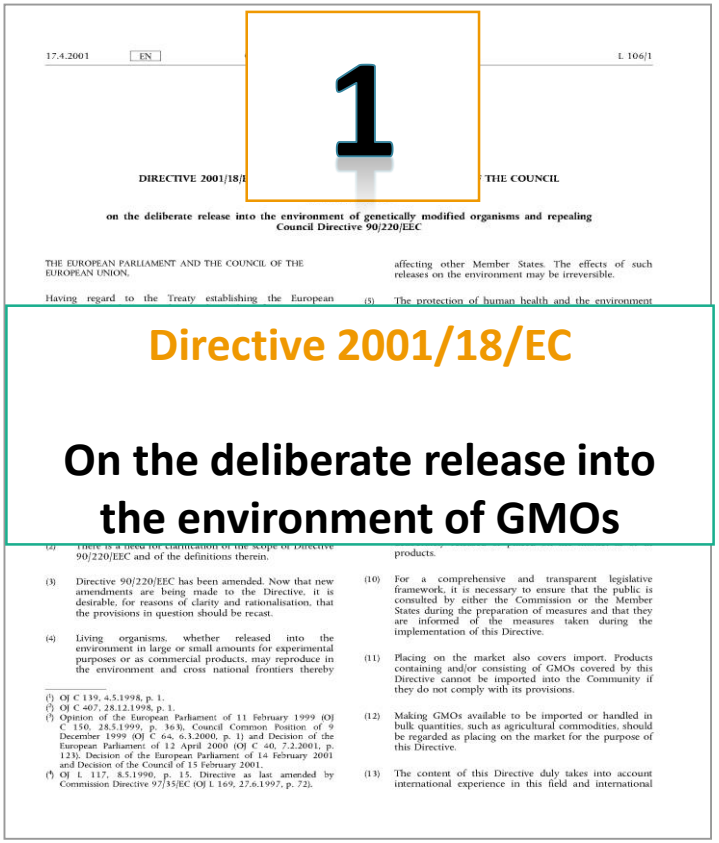
# THE REMIT OF EFSA

- EFSA is responsible to perform a **risk assessment of GMOs** with regard to **human and animal health** and **the environment**

- **What EFSA cannot do**
  - **Give authorisations** (for products such as GMOs, feed additives, food additives, pesticides etc)
  - **Be responsible for food safety legislation** (sampling, labelling or other risk management issues)
  - **Take charge of food safety/quality controls**

# EU LEGAL FRAMEWORK FOR GMO RA

EFSA's role is to carry out scientific Risk Assessment on GMOs under two regulatory frameworks:



17.4.2001 EN L 106/1

1

DIRECTIVE 2001/18/[illegible] THE COUNCIL

on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European

affecting other Member States. The effects of such releases on the environment may be irreversible.

(5) The protection of human health and the environment

90/220/EEC and of the definitions therein.

(3) Directive 90/220/EEC has been amended. Now that new amendments are being made to the Directive, it is desirable, for reasons of clarity and rationalisation, that the provisions in question should be recast.

(4) Living organisms, whether released into the environment in large or small amounts for experimental purposes or as commercial products, may reproduce in the environment and cross national frontiers thereby

products.

(10) For a comprehensive and transparent legislative framework, it is necessary to ensure that the public is consulted by either the Commission or the Member States during the preparation of measures and that they are informed of the measures taken during the implementation of this Directive.

(11) Placing on the market also covers import. Products containing and/or consisting of GMOs covered by this Directive cannot be imported into the Community if they do not comply with its provisions.

(12) Making GMOs available to be imported or handled in bulk quantities, such as agricultural commodities, should be regarded as placing on the market for the purpose of this Directive.

(13) The content of this Directive duly takes into account international experience in this field and international

(1) OJ C 139, 4.5.1998, p. 1.
(2) OJ C 407, 28.12.1998, p. 1.
(3) Opinion of the European Parliament of 11 February 1999 (OJ C 150, 28.5.1999, p. 363), Council Common Position of 9 December 1999 (OJ C 64, 6.3.2000, p. 1) and Decision of the European Parliament of 12 April 2000 (OJ C 40, 7.2.2001, p. 123). Decision of the European Parliament of 14 February 2001 and Decision of the Council of 15 February 2001.
(4) OJ L 117, 8.5.1990, p. 15. Directive as last amended by Commission Directive 97/35/EC (OJ L 169, 27.6.1997, p. 72).

**Directive 2001/18/EC**

**On the deliberate release into the environment of GMOs**

18.10.2003 EN L 268/1

2

REGULATION (EC) No 182[illegible] AND OF THE COUNCIL

on genetically modified food and feed

(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Com-

(4) Differences between national laws, regulations and administrative provisions concerning the assessment and authorisation of genetically modified food and feed may hinder their free movement, creating conditions of unequal and unfair competition.

feed is an essential aspect of the internal market and contributes significantly to the health and well-being of citizens, and to their social and economic interests.

(2) A high level of protection of human life and health should be ensured in the pursuit of Community policies.

(3) In order to protect human and animal health, food and feed consisting of, containing or produced from genetically modified organisms (hereinafter referred to as genetically modified food and feed) should undergo a safety assessment through a Community procedure before being placed on the market within the Community.

(7) Feed consisting of or containing genetically modified organisms (GMOs) has so far been authorised, subject to the authorisation procedure provided by Council Directive 90/220/EEC of 23 April 1990 (7) and Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms (8); no authorisation procedure exists for feed produced from GMOs; a single, efficient and transparent Community authorisation procedure for feed consisting of, containing or produced from GMOs should be established.

(8) The provisions of this Regulation should also apply to feed intended for animals which are not destined for food production.

(1) OJ C 304 E, 30.10.2001, p. 221.
(2) OJ C 221, 17.9.2002, p. 114.
(3) OJ C 278, 14.11.2002, p. 31.
(4) Opinion of the European Parliament of 3 July 2002 (not yet published in the Official Journal), Council Common Position of 17 March 2003 (OJ C 113 E, 13.5.2003, p. 31), Decision of the European Parliament of 2 July 2003 (not yet published in the Official Journal) and Council Decision of 22 July 2003.

(7) OJ L 43, 14.2.1997, p. 1.
(8) OJ L 117, 8.5.1990, p. 15. Directive repealed by Directive 2001/18/EC.
(9) OJ L 106, 17.4.2001, p. 1. Directive as last amended by Council Decision 2002/811/EC (OJ L 280, 18.10.2002, p. 27).

**Regulation (EC) No 1829/2003**

**On GM food and feed including derived products**

Scope: Food/Feed

Deliberate release: Import and processing, Cultivation

# IMPLEMENTING REGULATION

## Regulation (EU) No 503/2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003

- Mandatory from 8 December 2013
- Defined the scientific information to be provided in applications for GM food and feed under Regulation (EC) No 1829/2003
- Reflects the EFSA GD to a large extent with additional mandatory elements
  - **90 day feeding study** with whole food/feed for all single events
  - **re-sequencing of DNA inserts** and their flanking regions in **GM stacks** & comparison with the sequence of the respective single events
  - **quantitative measurement of allergens** in the frame of compositional analysis as referred to in relevant OECD documents

# GUIDANCE DOCUMENTS

- **Environmental Risk Assessment (ERA) of GM Plants (2010)**
- **Guidance for risk assessment of food and feed from GM plants (2011)**
- **Guidance for Post-Market Environmental Monitoring (PMEM) (2011)**
- **Guidance on the agronomic and phenotypic characterisation of GM plants (2015)**
- **Guidance on Allergenicity Assessment of GM plants (2017)**

**All guidance documents available at**

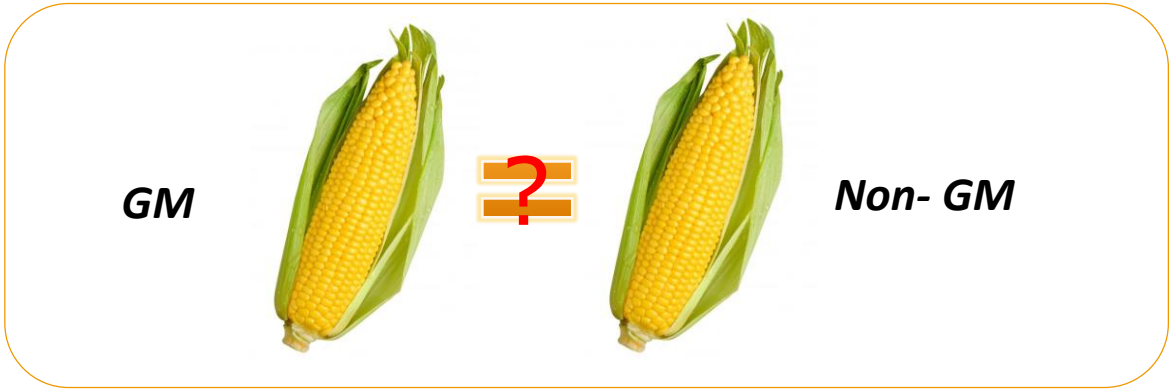
http://www.efsa.europa.eu/en/gmo/gmoguidance.htm

# GMO RISK ASSESSMENT – THE PRINCIPLE

## COMPARATIVE APPROACH

CODEX ALIMENTARIUS

FAO/WHO Food Standards - Normes Alimentaires FAO/OMS - Normas Alimentarias FAO/OMS




**Focus on Intended and Unintended effects:**

*New or altered hazards*

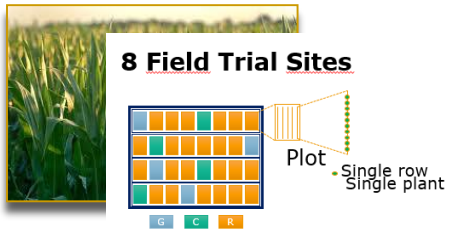
*Changes in key nutrients*

# GMO RISK ASSESSMENT – THE TOOLS

## Molecular Characterisation

- Genetic modification
- Characteristics of the GM plant

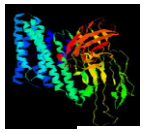
## Comparative analysis




- Agronomic-phenotypic characteristics
- Compositional analysis (OECD list)

## Food and Feed safety:

**new proteins, new compounds, altered levels of constituents**



- Toxicological assessment
- Allergenicity assessment
- Nutritional assessment

## Exposure assessment

## Environmental risk assessment

# ROADMAP

- GMO Risk Assessment in EU – the frame
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  - Omics
  - Re-thinking and streamlining the safety assessment of new proteins

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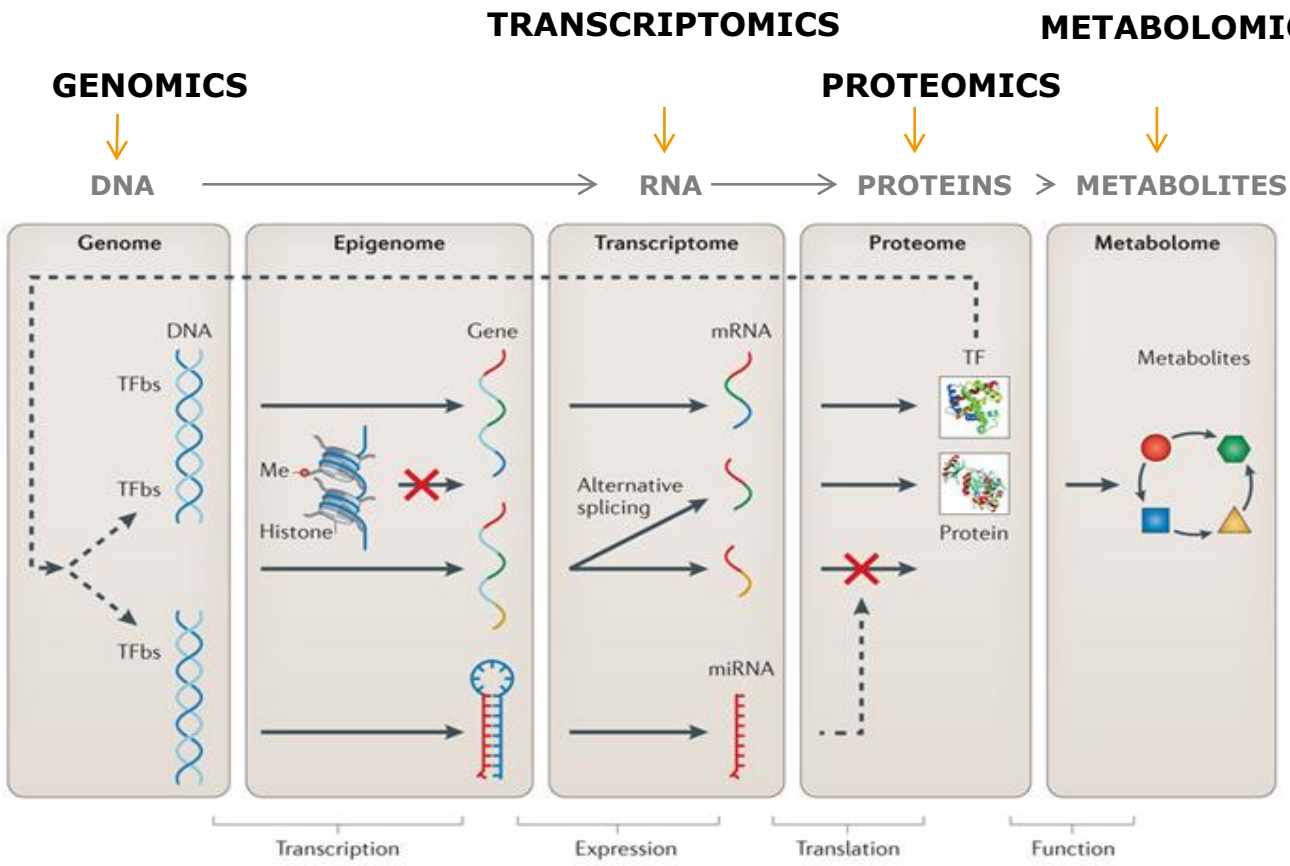
# OMICS




- **Information on functional status of an organism**
- **Information on impact of external factors on an organism**

Nature Reviews | Genetics

Adapted from Ritchie et al., 2014, Nature review Genetics, 16, 85-97

# OMICS IN RISK ASSESSMENT

- **OMICS technologies in research**
  - used for more than a decade to study basic biological problems
  - vast amounts of analytical data are being collected and shared
- **OMICS technologies in support of risk assessment**
  - is still in an initial phase
  - OMICS datasets are starting to be used in some risk assessment areas;
    - WGS data in analysis of food borne diseases
    - WGS data in dossiers for genetically modified plants

Technical Note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants (EFSA Journal 2018;16(7):534)

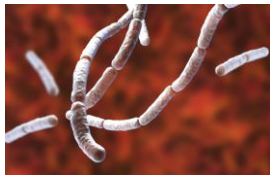
Guidance on the characterisation of microorganisms used as feed additives or as production organisms (EFSA Journal 2018;16(3):5206)

# EFSA activities in relation to OMICS

- In April 2018, EFSA held its 24th colloquium focusing on omics and aiming to
  - Explore the potential use of OMICS datasets to support the scientific safety evaluation
  - Advance further on concrete paths of implementation to support risk assessors in the process of incorporating OMICS tools into the risk assessment of food and feed products

- The programme
  - 4 Plenary talks introducing the topics addressed in the different discussion groups
  - 4 Discussion groups addressing challenges for the implementation of OMICs in the risk assessment
  - Feed back from the discussion group to all participants in a final plenary session

# Discussion groups



- **DG1**: Genomics for identification and characterisation of microbial strains in food and feed products

- **DG2**: The use of Metabolomics in the comparative risk assessment of GM plants

- **DG3**: The use of OMICS in human risk assessment of chemicals

- **DG4**: The use of OMICS in environmental risk assessment

# DG2: Metabolomics in risk assessment of GM plants

- **Setting:**
- Comparative approach - GM plants are compared to their non-GM counterpart and non-GM commercial varieties by analysing a set of compositional endpoints
  - internationally agreed standard set of key compounds is analysed
  - each compound compared individually (GM vs non-GM plants
- **Issue discussed:**
- Can metabolomics add to or substitute the current approach?
  - Basis for discussion: approach developed in research projects (group E. Kok and collaborators, RIKILT Institute, Netherlands)
    - uses omics data to generate „general profiles" of the plants
    - The profiles of commercial varieties are used to establish a safe „one class" against which the GM plant profile is tested

# DG2: Metabolomics in risk assessment of GM plants

- **Outcome**: Metabolomics could be used to either fully substitute or to complement the existing approach on a case-by-case basis
- **Advantages:**
  - More compounds can be analysed, increased level of information
  - **focus on pathways** rather than individual endpoints (holistic picture of the metabolism)
  - in the **"profile – one class" approach**, there is **no endpoint-by-endpoint** comparison but a holistic comparison of the GM plant profile against a safe class of commercial varieties
  - Could be cost-efficient, if the approach is globally accepted and depending on exact conditions
- **Development needs:**
  - Standardisation of experimental protocols and data analysis (statistics)
  - Global regulatory harmonisation and frame for interpretation in RA

# More details

Published November 2018 !

EFSA (European Food Safety Authority) and Aguilera J, Aguilera-Gomez M, Barrucci F, Cocconcelli PS, Davies H, Denslow N, Dorne JL, Grohmann L, Herman L, Hogstrand C, Kass GEN, Kille P, Kleter G, Nogué F, Plant NJ, Ramon M, Schoonjans R, Waigmann E and Wright MC 2018.
**EFSA Scientific Colloquium 24 – 'omics in risk assessment: state of the art and next steps.**
EFSA supporting publication 2018:EN-1512. 30 pp.
doi:10.2903/sp.efsa.2018.EN-1512

# ROADMAP

- GMO Risk Assessment in EU – the frame
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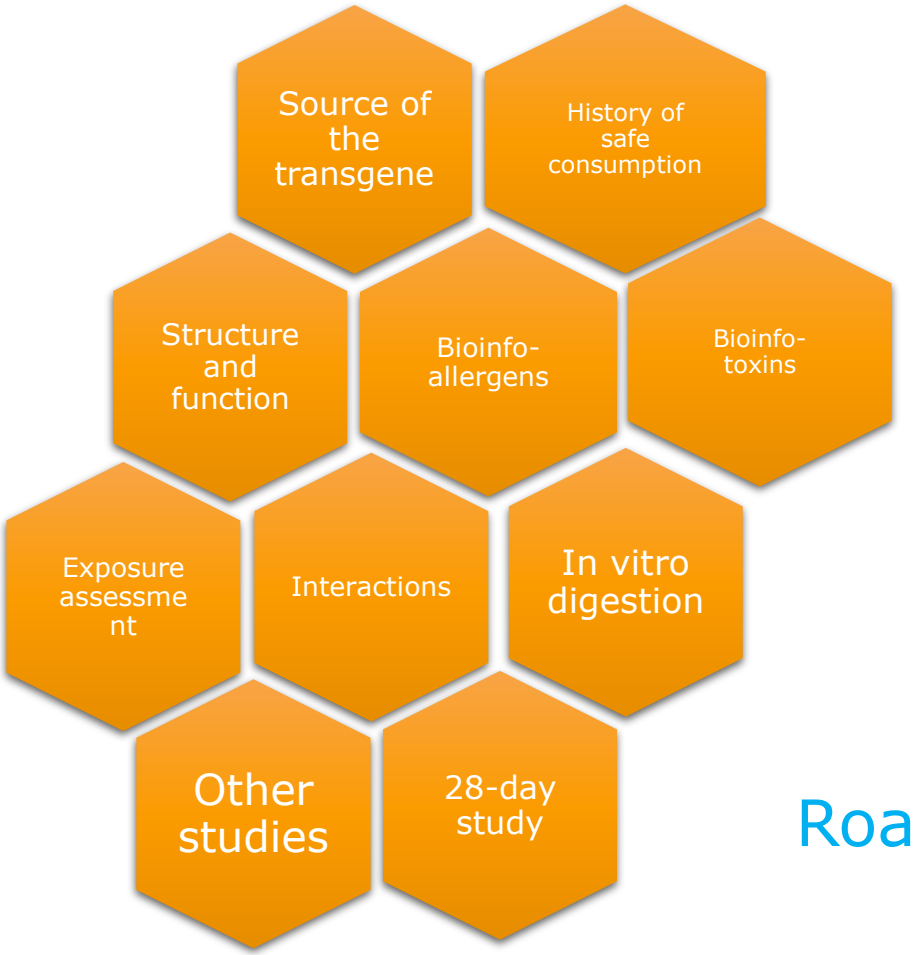
  - Omics
  - Re-thinking and streamlining the safety assessment of new proteins

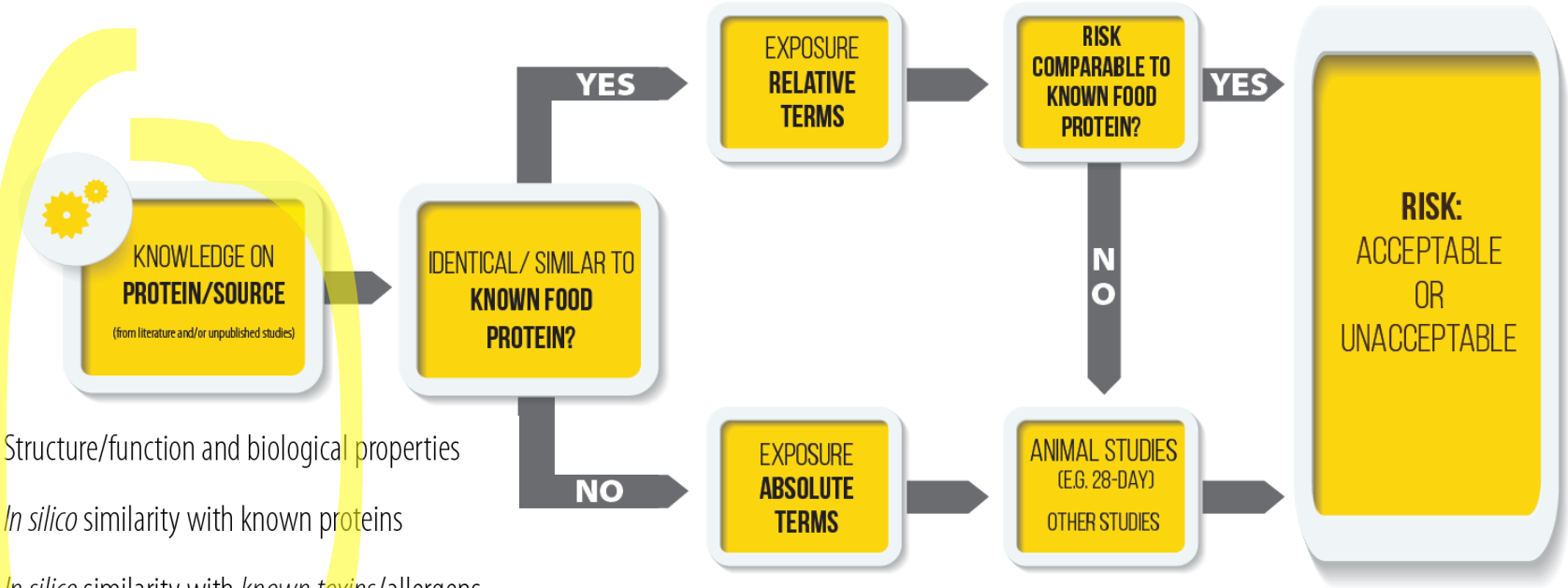
# SAFETY ASSESSMENT OF NEW PROTEINS IN GMO

Weight of evidence




Roadmap needed!

# Re-thinking protein safety assessment




- Structure/function and biological properties
- *In silico* similarity with known proteins
- *In silico* similarity with *known toxins*/allergens
- *In vitro* degradation studies
- Physicochemical characteristics
- Other studies

Fernandez Dumont A, Paoletti C, Gomez Ruiz J, Ardizzone M and Lanzoni A. 2018. The safety assessment of proteins in food: where do we stand? Toxicology Letters, 295 (Supplement 1): S69–S266. https://doi.org/10.1016/j.toxlet.2018.06.722

# Future developments needed

- Improved in silico analysis
- In vitro digestibility studies
- Targeted in vitro studies
- Animal models
- Framing risk characterisation
- Etc…

- Integrated strategies

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- Improved in silico analysis
- In vitro digestibility studies
- Targeted in vitro studies
- Animal models
- Framing risk characterisation
- Etc…

Toxicity

- Integrated strategies

# Future developments needed

- **Improved in silico analysis**
- **In vitro digestibility studies**
- Targeted in vitro studies
- Animal models
- Framing risk characterisation
- Etc…

- **Integrated strategies**

Immunogenicity/Allergy:
Celiac Disease

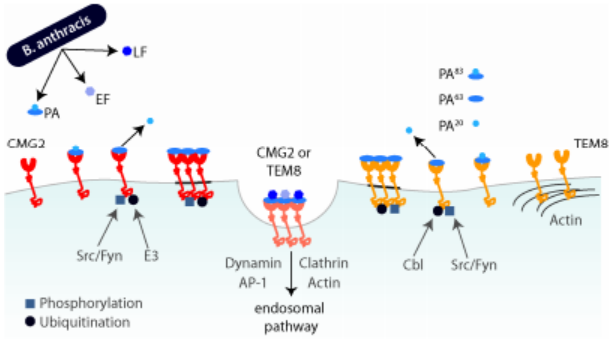
# IN SILICO TOOLS - TOXICITY

## The present

- The current *in silico* investigations on proteins in GMO dossiers are based on comparisons of the protein sequence to company internal databases

## Limitations

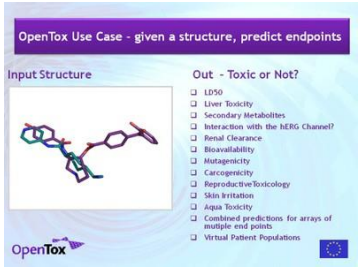
- Structural similarity cannot be used as sole criteria for defining a protein as a toxin:
  - 3D structures are relevant
  - complex formation
  - specific protein binding sites
- Comprehensiveness of databases as regards experimentally validated toxins




Friebe,S et al., 2016 Toxins, 8, 69

Is it a better approach possible?
Are there protein predictive toxicity tools available?

# IN SILICO TOOLS - TOXICITY




Differently from the small molecules area

no tools to predict protein toxicity are available:

- lack of comprehensive, public curated databases of toxic proteins
- 3D structures to be considered
- incomplete knowledge on the determinants of pathogenetic effects

The future:

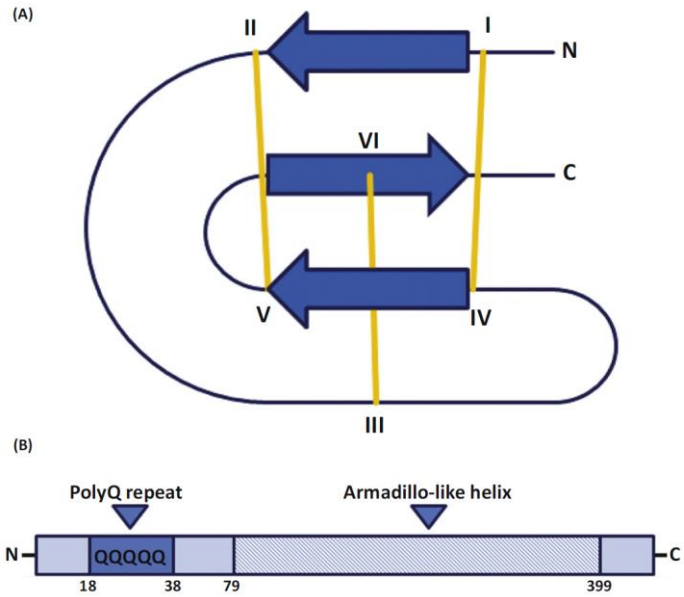
Extensive information is now available in public databases and can serve as the basis for establishing in silico tools to determine the potential risk of toxicity

- aa sequences
- 3D structures
- biochemical and biological functions of proteins

# EXAMPLES

Motif analysis

homologous proteins with similar function share similar motifs (critical region relevant for function and folding), even the overall identity is 20-30%.




Franceschi et al, 2017
Trends in Biotechnology, 2017, 35, 483

Structural Representation of the (a) Inhibitor Cystine Knot (ICK) and (b) Polyglutamine (polyQ) Motifs.

# NP/EFSA/GMO/2018/01

**Literature search – Exploring in silico protein toxicity prediction methods to support the food and feed risk assessment**

1. identifying, list and cluster proteins known to be associated with adverse effects in humans and animals
2. to identify molecular signatures (e.g. motifs, domains) of these "toxic" proteins and the pathogenesis leading to adverse effects in humans and animals.
3. to identify available databases and evaluate their relevance with respect to the scope.

Deadline Jan 2020

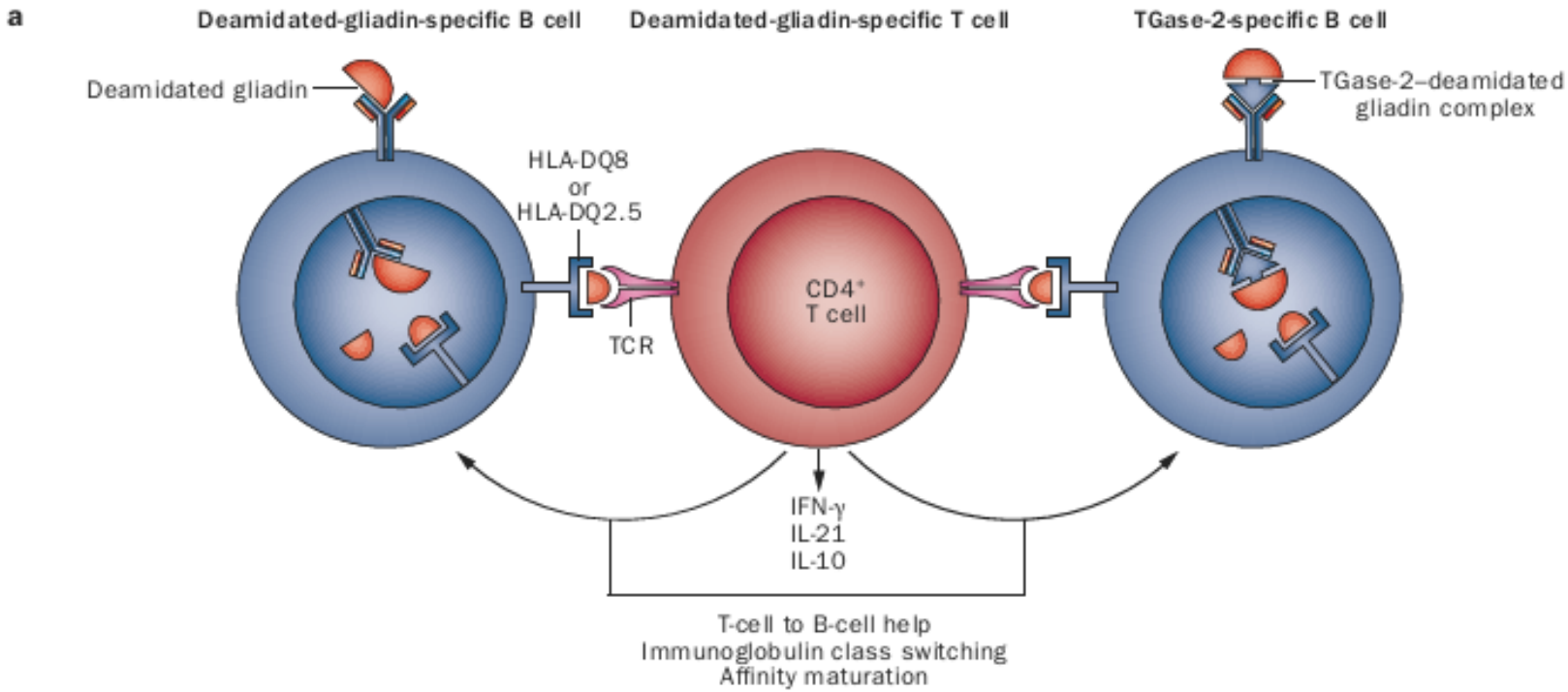
## Coeliac disease: the integrated EFSA strategy

Celiac disease (CD): non-IgE-mediated adverse immune reaction to foods

- caused by an uncontrolled intestinal immune response to gluten proteins in wheat (Triticum spp), gluten-like hordeins in barley (Hordeum vulgare) and secalins in rye (Secale cereal).
- Oat (Avena sativa) is generally considered safe for patients although exceptions were reported
- The only available treatment is a lifelong gluten-free diet implying the exclusion of all food products that contain wheat, barley and rye or gluten and gluten-like proteins from these cereals.
- CD affects approximately 1% of the world population

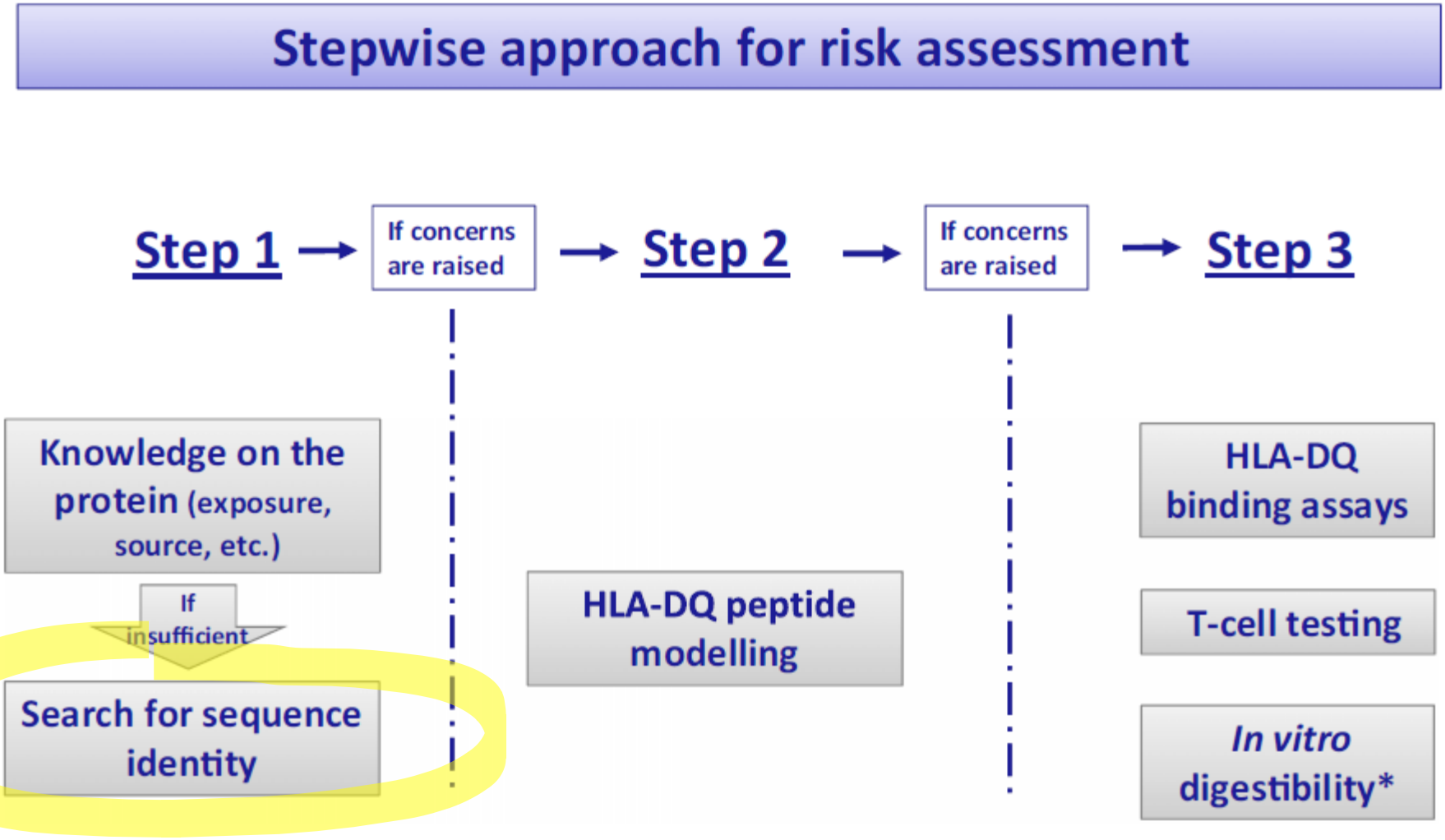
EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017.
Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

# CD – INSIDE THE MECHANISM




Koning et al 2015. Nature Reviews Rheumatology, 11, 450-461.

# CD – the EFSA strategy

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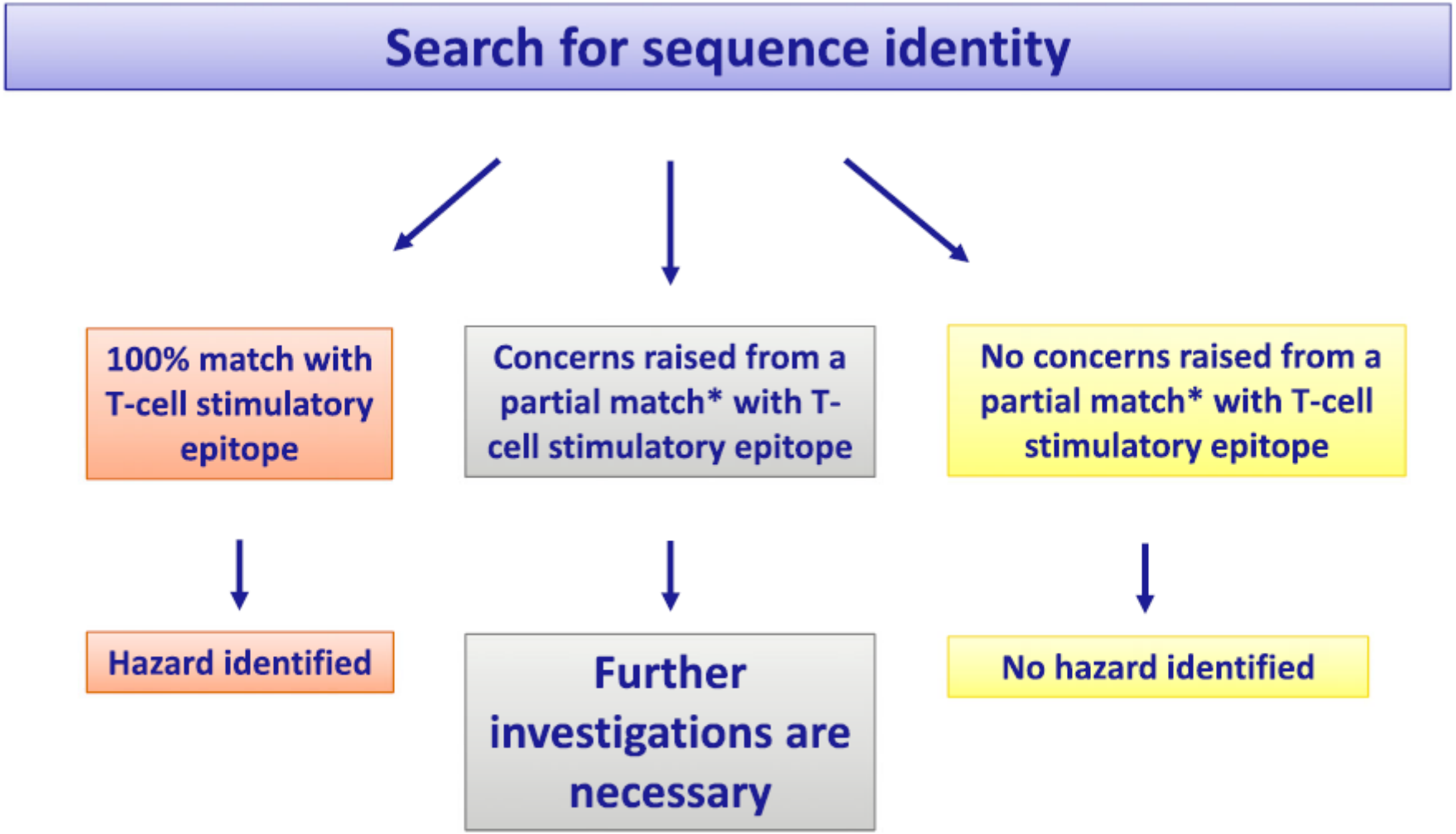



*A partial match with a known T-cell-stimulatory peptide raises concern because of the position and nature of the identical amino acids.

# Celiac disease – DQ2 T-cell epitopes

**DQ2 restricted epitopes**

Sollid et al., 2012. Immunogenetics, 64, 455-460

| Epitope | Motif | Reference |
|---|---|---|
| DQ2.5-glia-α1a | P F P Q P **Q L P Y** | Arentz-Hansen et al. (2000) |
| DQ2.5-glia-α1b | P Y P Q P **Q L P Y** | Arentz-Hansen et al. (2002) |
| DQ2.5-glia-α2 | P Q P **Q L P Y** P Q | Arentz-Hansen et al. (2000) |
| DQ2.5-glia-α3 | **E L P Y** | Vader et al. (2002b) |
| DQ2.5-glia-γ1 | **Q Q F** | Sjöström et al. (1998) |
| DQ2.5-glia-γ2 | **F** | Qiao et al. (2005), Vader et al. (2002b) |
| DQ2.5-glia-γ3 | **F A** | Arentz-Hansen et al. (2002) |
| DQ2.5-glia-γ4a | **S V** | Arentz-Hansen et al. (2002) |
| DQ2.5-glia-γ4b | **E Q** | Qiao et al. (2005) |
| DQ2.5-glia-γ4c | | Arentz-Hansen et al. (2002) |
| DQ2.5-glia-γ4d | | Qiao (unpublished) |
| DQ2.5-glia-γ5 | | Arentz-Hansen et al. (2002) |
| DQ2.5-glia-ω1 | | Tye-Din et al. (2010) |
| DQ2.5-glia-ω2 | | Tye-Din et al. (2010) |
| DQ2.2-glut-L1 | | Vader et al. (2002b) |
| DQ2.5-glut-L2 | | Stepniak et al. (2005), Vader et al. (2002b) |
| DQ2.5-hor-1 | | ye-Din et al. (2010), Vader et al. (2003) |
| DQ2.5-hor-2 | | ader et al. (2003) |
| DQ2.5-sec-1 | P F P Q P **Q Q P F** | Tye-Din et al. (2010), Vader et al. (2003) |
| DQ2.5-sec-2 | P Q P **Q Q P F** P Q | Vader et al. (2003) |
| DQ2.5-ave-1 | P Y P E Q **Q E P F** | Arentz-Hansen et al. (2004), Vader et al. (2003) |
| DQ2.5-ave-1b | P Y P E Q **Q Q P F** | Arentz-Hansen et al. (2004), Vader et al. (2003) |

**Q/E-X1-P-X2**

# Celiac disease – DQ8 T-cell epitopes

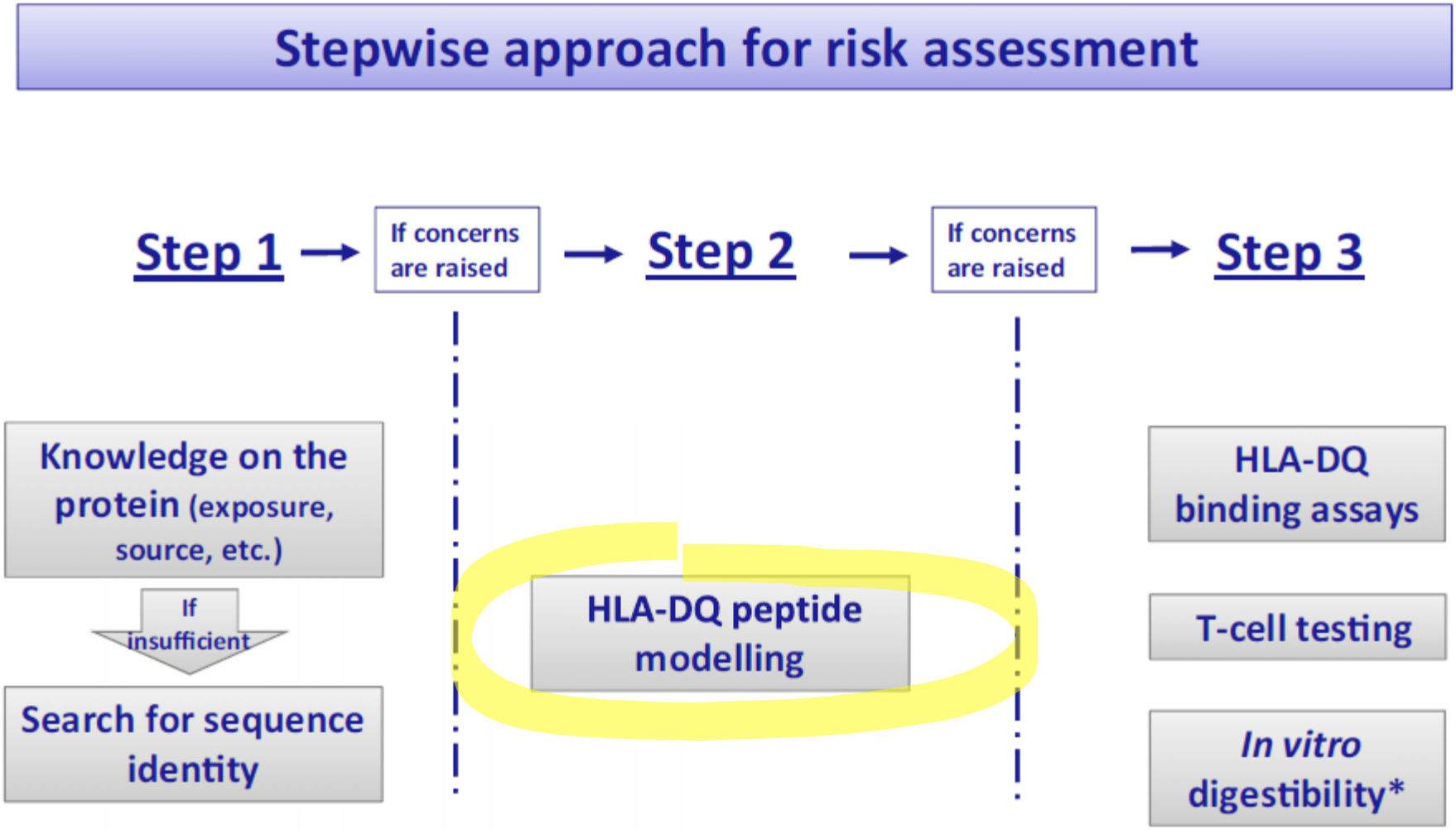
Sollid et al., 2012. Immunogenetics, 64, 455-460

**DQ8 restricted epitopes**

| Epitope | Motif | Reference |
|---|---|---|
| DQ8-glia-α1 | **Q** G S F Q P S Q **Q** | van de Wal et al. (1998b) |
| DQ8-glia-γ1a | **Q** Q P Q Q P F P **Q** | Tollefsen et al. (2006) |
| DQ8-glia-γ1b | **Q** Q P Q Q P Y P **Q** | Tollefsen et al. (2006) |
| DQ8-glut-H1 | **Q** G Y Y P T S P **Q** | van de Wal et al. (1999) |

Partial matches without the Q/E-X1-P-X2 to be investigated

# CD – the EFSA strategy

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# OC/EFSA/GMO/2019/01

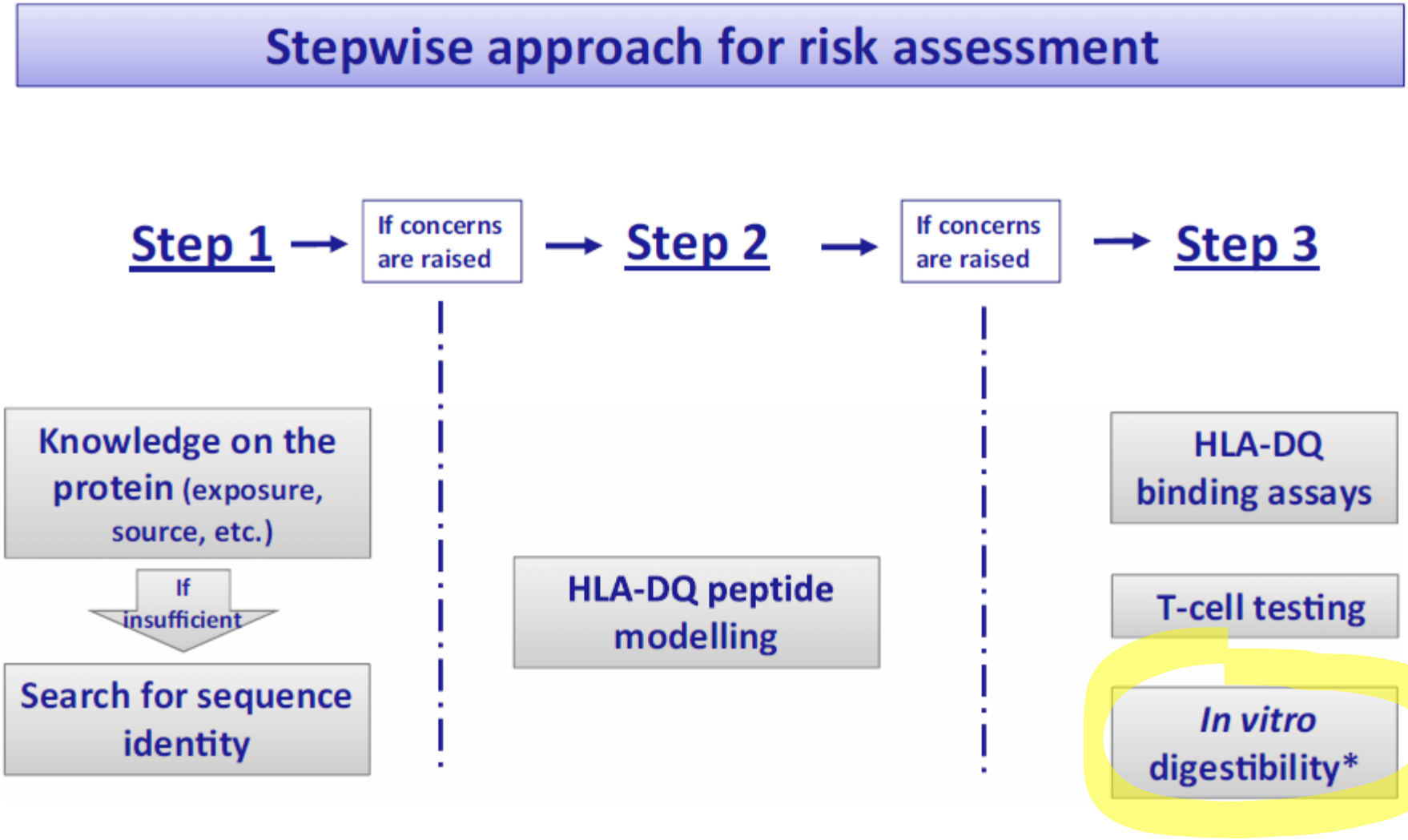
**HLA-DQ peptide modelling software – Developing a software tool for peptide modelling regarding its capacity to bind to HLA-DQ molecules and to activate T-cells**

1. Collecting info on tools available for protein modelling related to celiac disease
2. Developing a software tool for predicting the capacity of a protein to cause celiac disease based on peptide binding to specific molecules and activation of immune cells
3. Testing and adjusting the software tool

https://etendering.ted.europa.eu//cft/cft-display.html?cftId=4505

Deadline for submission 8/4/2019 at 14:30(CET)

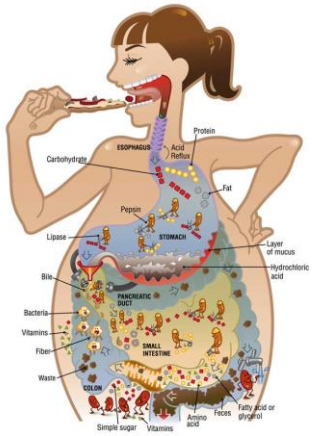
# CD – the EFSA strategy




EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, Jones H, Manachini B, Messean A, Nielsen EE, Nogue F, Robaglia C, Rostoks N, Sweet J, Tebbe C, Visioli F, Wal J-M, Eigenmann P, Epstein M, Hoffmann-Sommergruber K, Koning F, Lovik M, Mills C, Moreno FJ, van Loveren H, Selb R and Fernandez Dumont A, 2017. Guidance on allergenicity assessment of genetically modified plants. EFSA Journal 2017;15(5):4862, 49 pp. https://doi.org/10.2903/j.efsa.2017.4862

# IN VITRO TOOL - PROTEIN DIGESTION

- There is evidence that gastrointestinal digestion can affect the immunogenicity of dietary proteins related to both IgE and non-IgE-mediated adverse reactions to foods.
- **In vitro protein digestion** can be used as an additional piece of information in the weight-of-evidence approach followed for the allergenicity assessment of newly expressed proteins, because no single test is fully predictive. (Codex Alimentarius, 2003, 2009; EFSA GMO Panel, 2011)



# OC/EFSA/GMO/2017/01

## In vitro protein digestibility– defining a fit for purpose protocol for in vitro protein digestion

- In 2017 the EFSA GMO Panel proposed a **refined in vitro digestion test** extending the conditions currently used in the classical pepsin resistance test
  - Gastric digestion phase (pH, pepsin)
  - Intestinal digestion phase
- More informative read-outs of the test define the extent to which either the intact protein or resistant fragments remain after in vitro digestion
- **Interim phase (~ 2 years duration):** the laboratories involved, working with EFSA, will further detail and apply the refined digestion test methodology
- **Deadline: 2019**

# CONCLUSIONS

- The GMO risk assessment investigates intended and unintended effects related to the introduced genetic modification and verifies whether the GM is as safe and nutritionally equivalent to conventional, consumed crops.
- Scientific and technological advances in molecular biology, in silico and in vitro science could provide valuable information and could be used to integrate the current approaches and tools in GMO risk assessment.
- Areas under exploration by EFSA include the use of **omics** data in the context of compositional analysis of GM plants and the **re-thinking and streamlining of the risk assessment of proteins** newly expressed in GM plants, taking onboard tools such as **in silico investigations and in vitro protocols**.
- EFSA is engaged in activities investigating these aspects
- Work in progress!

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# BACKUP SLIDES

# GMO RISK ASSESSMENT – THE TOOLS

## Molecular Characterisation

- Description of methods used for the genetic modification
- Source and characterisation of nucleic acid used for transformation
- Nature and source of vector(s) used
- Description of the traits introduced or modified
- Information on the sequences actually inserted/deleted (sequence of the insert(s) + flanking regions; copy number of insert)
- Information on the expression of the inserted/modified sequences (typically protein expression levels)
- Bioinformatic analysis to
  - identify ORFs
  - Identify homology to toxins and allergens
  - Support problem formulation for HGT
- Genetic and phenotypic stability

# GMO RISK ASSESSMENT – THE TOOLS

## Comparative analysis – Agropheno and Composition

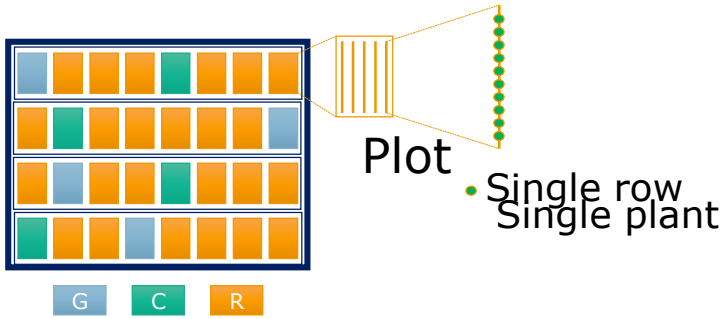
**Test of Difference**

to verify if the GMO is different from the non-GM comparator (identification of possible hazard)

**Test of Equivalence**

to verify if the GMO is equivalent to non-GM (commercial) reference varieties (natural variation)

**8 Field Trial Sites**



# COMPARATIVE ANALYSIS

## Comparative analysis –Endpoints

- **Seeds**
  - Seedling
  - Seed purity
  - Seed germination and health
- **Seedling**
  - Initial stand count
  - Emerged plants
  - Herbicide injuries

**Reproductive phase**

- Flowering
- Lodging
- Pod shattering / dropped ear
- Plant height
- Days to maturity/harvest
- Final stand count
- Seeds/plant
- #Seeds/pod soybean
- Yield
- Seed weight
- Seed moisture

**Composition (OECD)**

- Proximates
- Key macro- and micro-nutrients
- Anti nutritional compounds
- Natural toxins

Specific analysis on a case-by-case basis

# GMO RISK ASSESSMENT – THE TOOLS

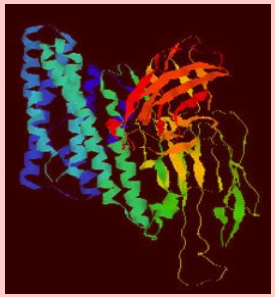
## Food and feed safety - Toxicology and allergenicity

**newly expressed proteins**

- Protein characterisation
- Source of the transgene
- History of safe use
- Bioinformatics
- Interactions (stacked events)

*On a case by case basis :*

- *28-day toxicity study (OECD TG 407)*
- *Specific human sera*
- *Animal models/cell based assays*



**other components**

- ad hoc studies, if needed

**whole food/feed**

- 90-day study in rodents (single events)
- Common allergenic food (e.g. soybean): endogenous allergens to be analysed, human sera, mass spectrometry, animal antibodies

## Food and feed safety - Nutrition

Based on the outcome of compositional analysis; humans and animals

# GMO RISK ASSESSMENT - ERA

## Strategies for ERA of GM plants

Issues to be addressed

1. Persistence and invasiveness
2. Horizontal gene transfer
3. Target organisms (TO)
4. Non-target organisms (NTO)
5. Farming practices
6. Biogeochemical processes
7. Human and animal health
8. PMEM