GMO Risk Assessment
Future perspectives

Anna Lanzoni
Senior Scientific Officer, GMO Unit
Roadmap

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

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

1. **Directive 2001/18/EC**
   - **On the deliberate release into the environment of GMOs**
   - **Scope:** Food/Feed
   - **Deliberate release:** Import and processing, Cultivation
   - **Regulation (EC) No 1829/2003**
   - **On GM food and feed including derived products**
   - **Scope:** Food/Feed
   - **Food consisting of or containing genetically modified organisms (GMOs) has not been authorised, subject to the authorization procedure provided by Council Directive 90/220/EEC on the deliberate release into the environment of GMOs**

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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)

All guidance documents available at
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
- Future perspectives
  - Omics
  - Re-thinking and streamlining the safety assessment of new proteins
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OMICS

DNA → RNA → PROTEINS → METABOLITES

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

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)
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.
Published November 2018!


ROADMAP

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

- Source of the transgene
- History of safe consumption
- Structure and function
- Bioinfo-allergens
- Bioinfo-toxins
- Exposure assessment
- Interactions
- In vitro digestion
- Other studies
- 28-day study

Roadmap needed!
SAFETY ASSESSMENT OF NEW PROTEINS IN GMO

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

Future developments needed

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

- Integrated strategies
RE-THINKING PROTEIN SAFETY ASSESSMENT

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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- Etc...

- Integrated strategies

Immuno-genicity/Allergy: Celiac Disease
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?
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
Motif analysis homologous proteins with similar function share similar motifs (critical region relevant for function and folding), even the overall identity is 20-30%.

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

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

Search for sequence identity

- 100% match with T-cell stimulatory epitope
  - Hazard identified

- Concerns raised from a partial match* with T-cell stimulatory epitope
  - Further investigations are necessary

- No concerns raised from a partial match* with T-cell stimulatory epitope
  - No hazard identified

*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

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<tr>
<th>Epitope</th>
<th>Motif</th>
<th>Reference</th>
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<tr>
<td>DQ2.5-glia-α1a</td>
<td>PFPQPQLPY</td>
<td>Arentz-Hansen et al. (2000)</td>
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<td>Vader et al. (2002b)</td>
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<td>Sjöström et al. (1998)</td>
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<td>Qiao et al. (2005), Vader et al. (2002b)</td>
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<td>DQ2.5-glia-γ4c</td>
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<td>Arentz-Hansen et al. (2002)</td>
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<td>Arentz-Hansen et al. (2004), Vader et al. (2003)</td>
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## Celiac disease – DQ8 T-cell epitopes

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

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<td>van de Wal et al. (1998b)</td>
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<td>DQ8-glia-γ1b</td>
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<td>Tollefsen et al. (2006)</td>
</tr>
<tr>
<td>DQ8-glut-H1</td>
<td>QGYYPSTS</td>
<td>van de Wal et al. (1999)</td>
</tr>
</tbody>
</table>

Partial matches without the Q/E-X1-P-X2 to be investigated
CD – the EFSA strategy

IN SILICO TOOLS - MODELLING/CELIAC DISEASE

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

Deadline for submission 8/4/2019 at 14:30(CET)
**Stepwise approach for risk assessment**

1. **If concerns are raised**
   - **Knowledge on the protein (exposure, source, etc.)**
     - If insufficient
     - **Search for sequence identity**

2. **If concerns are raised**
   - **HLA-DQ peptide modelling**

3. **If concerns are raised**
   - **HLA-DQ binding assays**
   - **T-cell testing**
   - **In vitro digestibility**

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)
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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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

8 Field Trial Sites

- **Plot**: Single row, Single plant
- **Plot layout**: Grid of plots with different colors (G, C, R)
## 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