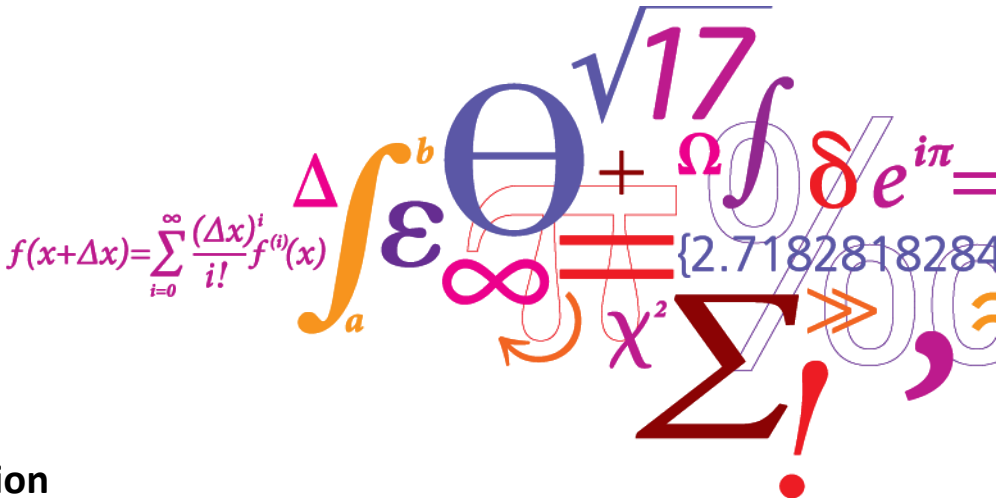
Molecular assessment of GMOs



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Molecular characterization

Objective : to assess the level of documentation necessary for the evaluation of the insertion and expression of the new gene products expressed by the GMO.

(an overview of the different inserted genes and gene products)

Generation	Period	New Traits	Principal Benefits
I	1995-2005	Herbicide, pest and virus resistance	Increased productivity, reduced use og agro-chemicals, and health benefits owing to reduced pesticide use and seed and other edible plant products with fewer or no toxins
II	2005-2015	Herbicide, pest and pathogen resistance; drought, salt, heavy metal and temperature tolerance; improved flavours and fragrances; elimination of allergens; vaccines, human therapeutic proteins, pharmaceuticals; phytoremediation; Male sterility, fertility restoration control system displaying glufosinate herbicide resistance; elevated lysine; seeds for fuel ethanol production	The highest direct benefit to consumers
III	2015	Onward	Altering plant architecture, manipulation of flowering time; manipulation of fruit/seed quality, size and number; improved photosynthetic efficiency; improved nutrient assimilation; exploiting and manipulating heterosis.

GM crops with altered composition
(Processing, Nutrient Deficiencies, Health)

- **Soybean** – Enriched omega-3 fatty acids
- **Soybean** - Increased oleic acid/decreased linoleic acid
- **Potato** - High amylopectin content
- **'Golden' rice** - Containing β-carotene
- **Rice** - Fortified with iron, lower allergen content
- **Tomato** - β-carotene/lycopene/anthocyanin/enriched
- **Lupin** - Higher methionine levels
- **Maize** - Higher levels of lysine, detoxification of mycotoxins
- **Sweet potato** - Enhanced β-carotene, higher protein content
- **Cassava** - Detoxification of cyanogens
- **Kidney beans** - Lower levels of lectins
- **Alfalfa** - Transgenic phytase, P-availability
- **Rape seed** - Vitamin E enriched

Molecular characterization

Each of the following 4 elements is considered for all applications during the risk assessment process

Molecular characterization of the GM product, taking into the account the characteristics of the donor and recipient organism.

The compositional, nutritional and agronomic characteristics of the GM product

The potential toxicity and allergenicity of the GM product.

The potential environmental impact following deliberate release of the GMO.

Molecular characterization

Guidance for risk assessment of food and feed from GM plants. EFSA 2011;9(5):2150 (is laying ground for the level of documentation)

3.1.2 Molecular characterization:

- Should provide data on the structure and expression of the insert(s) and on the stability of the intended trait(s).
- Should be specifically indicated whether the molecular characterization of the genetic modification(s) raises safety concerns with regard to the interruption of endogenous genes.
- Should specifically aim to identify whether the g. modification(s) raises any issues regarding the potential for producing new toxins or allergens.
- Should address potential unintended changes, which shall be also addressed in the relevant complementary parts of the risk assessment.

Molecular characterization

More specifically – applicants have to provide:

- A clear description of the insert, including all information necessary to interpret molecular data: primer binding sites, restriction sites, probe location
- Sub-cellular location of inserts
- History of safe use (if any) for the sequences intended to be inserted
- Southern blotting should cover the insert flanking regions.
- The requirement for the description of the helper plasmid (if used).
- All sequences between stop codons, not limiting the length of the sequences should be considered when searching for new ORFs.

ORF – is defined as any nucleotide sequence that consists of a string of codons that is uniterrupted by the presence of a stop codon in the same reading frame.

Molecular characterization

- **Bioinformatic searches** should be conducted on the possible new ORFs not just at the insert-genomic DNA junctions, but also at the junctions sites arising due to internal rearrangements of the insert(s).

- **Expression analysis** of potential new ORFs identified at the junction sites created as a result of the genetic modification shall be provided only in cases when complementary information(e.g. potential for transcription/ translation and similarity to known toxins/allergens) indicates a potential safety issue

Molecular characterization

contribution of bioinformatics to hazard identification

- **New proteins encoded by the transgenes: similarity with allergens, with toxins?**
  Bioinformatics analysis of the newly expressed proteins: similarity search with known allergens and toxins in public databases.

- **Unforseen peptides encoded by new ORFs created by the genetic modifications?**
  Bioinformatic search for similarity with known allergens and toxins in public databases.

- **Disruption of endogenous genes at the insertion sites?**
  Bioinformatic analysis of the insertion locus and serarch for known endogenous genes.

- **Bioinformatic analysis of similarities with microbial genomes (likelihood of homologous recombinations). Recombinogenic sequences on the T-DNA promoting horizontal gene transfers?**

Molecular characterization

- **Protein expression** data is only acceptable from field trials. The same material should be used as for compositional analysis.
- **Developmental protein expression** levels are not required in all cases (f. ex. Food –feed import and processing).
- **On case-by –case basis** data may be required on potential reduction of protein levels other than those intended. (RNA techniques).
- RNA levels might be required on a case by case basis
- Multiple generation is defined **as five** to demonstrate trait stability.

Molecular characterization

Stacked events:

- The risk assessment of GM plants containing stacked events requires the risk assessment of the GM plants containing these events independently (i.e. GM plants containing single events).

- For GM plants containing a combination of transformation events (stacked events) the primary concern for risk assessment is to establish that the combination of events is stable and that there are no interactions between the stacked events , that may raise safety concerns compared to single events

Examples from the audience of possible interactions on the level of genes and proteins

EFSA guidance EFSA Journal 2011; 9(5):2150

Molecular characterization

Stacked events

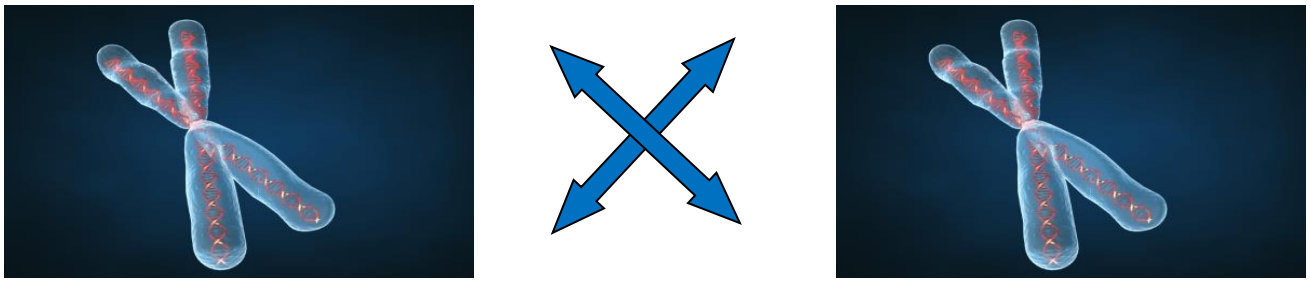
- The risk assessment of GM plants containing stacked events focuses on issues related to:
- Stability of the inserts.
- Expression of the introduced genes and their products and potential synergistic or antagonistic effects resulting from the combination of the events.

Depending on the outcome of this analysis further toxicological and nutritional information may be required.

Molecular characterization

GENE interaction - expression of one gene depends on expression (presence or absence) of another gene

- In genetics, **epistasis** is the phenomenon where the effects of one gene are modified by one or several other genes, (which are modifier genes).
- **Pleiotropy :** Expression of one gene has multiple phenotypic effects



Molecular characterization

MON 810 as example :

- Evaluation of donor and the insert: (sequence actually inserted included sufficient cry1Ab coding region encoding insecticidal Cry1AB protein). Size and copy numbers determined by Southern blotting./ satisfactory provided by the applicant/
- History of safe use of gene product, possible relationship of the gene product with known toxins, antinutrient and allergens. /satisfactory provided by the applicant/
- Description of the methods used for genetic modification.
- Information of expression of the insert: expression levels of Cr1Ab were measured in samples of various maize tissues, kernels, from field trials (f.ex.one site over 3 seasons)
- Inheritance and stability of the inserted DNA. (Southern blotting of good quality). The applicant has provided data from 5 generation of vegetative cycles.
- **EFSA guidance EFSA Journal 2011; 9(5):2150**

Molecular characterization

Questions from the EU Commission to EFSA

- Is there: Need for new guidance or update of the existing EFSA guidance on risk assessment of products obtained by new breeding techniques?

- Risks that these new techniques could pose, irrespective of whether or not they fall under the GMO legislation.

- Compare plants obtained by the new techniques with plants obtained by conventional plant breeding or with genetic modification (transgenesis).

Molecular characterization

New Techniques..

1. CISGENESIS/INTRAGENESIS.
2. **Z**INC **F**INGER **N**UCLEASE TECHNOLOGY (ZFN-1, ZFN-2 and ZFN-3) and CRISPR-Cas9
3. OLIGONUCLEOTIDE DIRECTED MUTAGENESIS (ODM).
4. RNA-DEPENDENT DNA METHYLATION (RdDM).
5. GRAFTING ON GM ROOTSTOCK.
6. AGRO-INFILTRATION.
7. REVERSE BREEDING.
8. SYNTHETIC BIOLOGY.

Molecular characterization

- **Transgenesis: Covered by EFSA guidelines.**

- **Cisgenesis:**

  Genetic modification of an organism with a gene from a crossable organism (same species or closely related)

  Cisgenic plants do not contain any parts of transgenes or foreign sequences (question to the audience- do we then need risk assessment?)

- **Intragenesis:**

  Similar to cisgenesis, but new combinations of DNA-elements

  from the same species are possible.

  Novel hazards ? (question to the audience).

  Similar methods are used as for transgenesis (Agrobaterium,

  electroporation, bolistics).

Molecular characterization

Conclusions concerning cisgenesis and intragenesis.

- EFSA Guidance Documents for Food/Feed Risk Assessment and for the Environmental Risk Assessment developed for transgenic (GM) plants apply also to Cisgenic/Intragenic Plants.
- **Data requirements can be reduced** where there is familiarity with the donor and/or recipient plants
- Less data on toxicity and consumption of newly expressed proteins
- (history of safe use)
- Less data for the environmental risk assessment

EFSA Opinion Cisgenesis/Intragenesis, EFSA Journal 2012;10(2):2561

Conclusions:

1. Molecular characterization (MC) contributes to hazard and risk identification, but must be completed by biological evidence.
2. Both intended and unintended effects are assessed.
3. Beyond the basic requirements of MC , case by case assessment may request further, hypothesis driven analyses.
4. New molecular techniques are emerging for the characterization of GMPs.
5. New breeding techniques are emerging for the genetic modification of plants, challenging current risk assessment of plants.

Molecular characterization

Conclusions

- EFSA Guidance Documents for Food/Feed Risk Assessment and for the Environmental Risk Assessment developed for transgenic plants are applicable for SDN-3 (site directed nuclease-3) plants.

- The targeted insertion of DNA by SDN-3 techniques can diminish hazards associated with the disruption of genes and regulatory elements.

- Flexibility in data requirements may be considered.

Molecular characterization

- **Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function**

- Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate genome editing by creating a double-stranded break in DNA at a user-specified location.

- A double-stranded break is important for site-specific mutagenesis in that it stimulates the cell's natural DNA-repair processes, namely homologous recombination and Non-Homologous End Joining (NHEJ).

Hazards from random integration are decreased compared with transgenesis

Molecular characterization

Zink finger technology mode of action