



EFSA Guidance for the Safety Assessment of GM Food and Feed in the European Union

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**Analisi del rischio di alimenti e mangimi
geneticamente modificati**

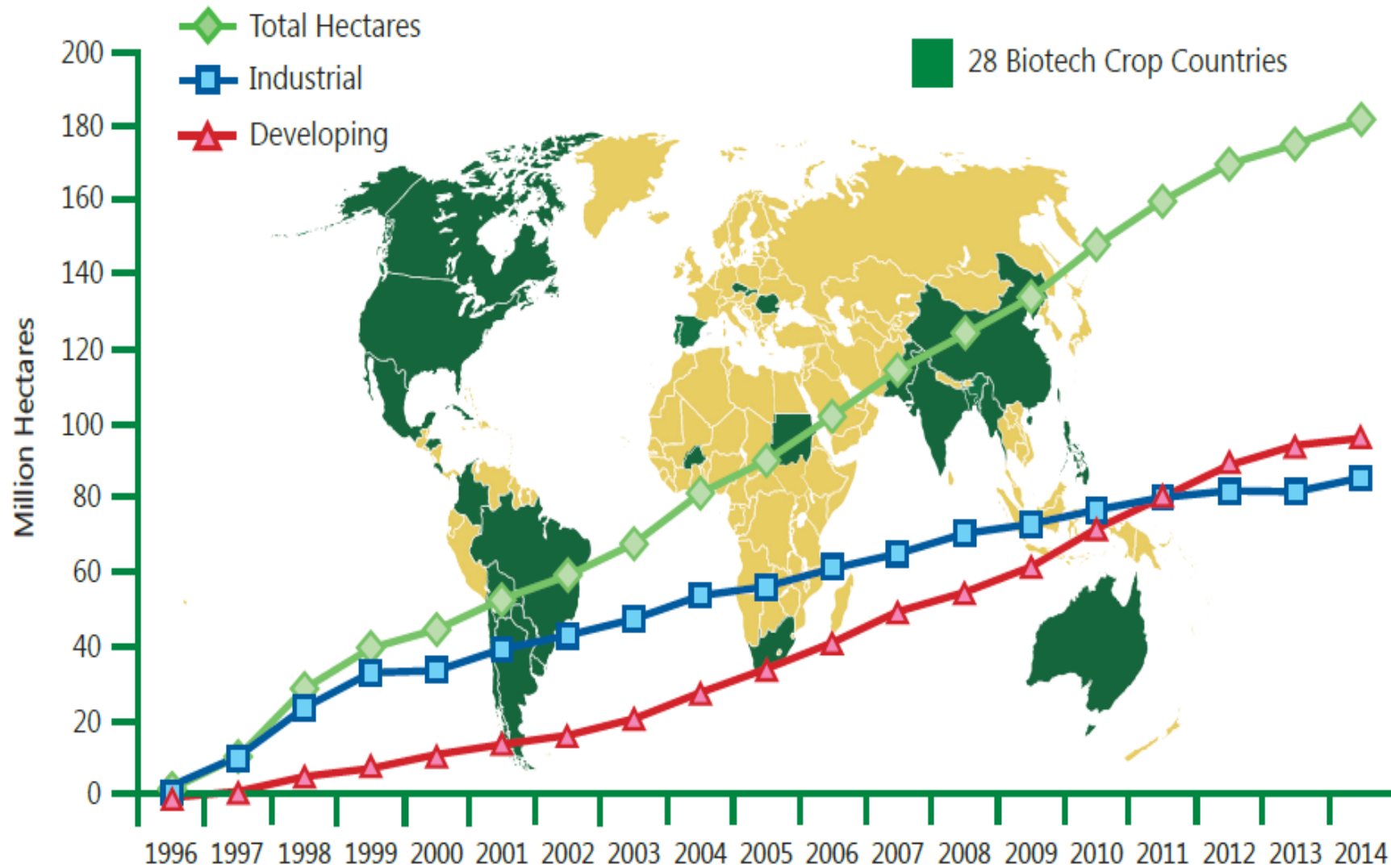
25 e 26 febbraio 2015, Istituto Superiore di Sanità, Roma

- Safety and Nutritional Assessment Strategy for GM Food/Feed
 - *EFSA Guidance Document (2011)*
 - *New Commission Implementing Regulation (EU) No 503/2013*

EU Member States

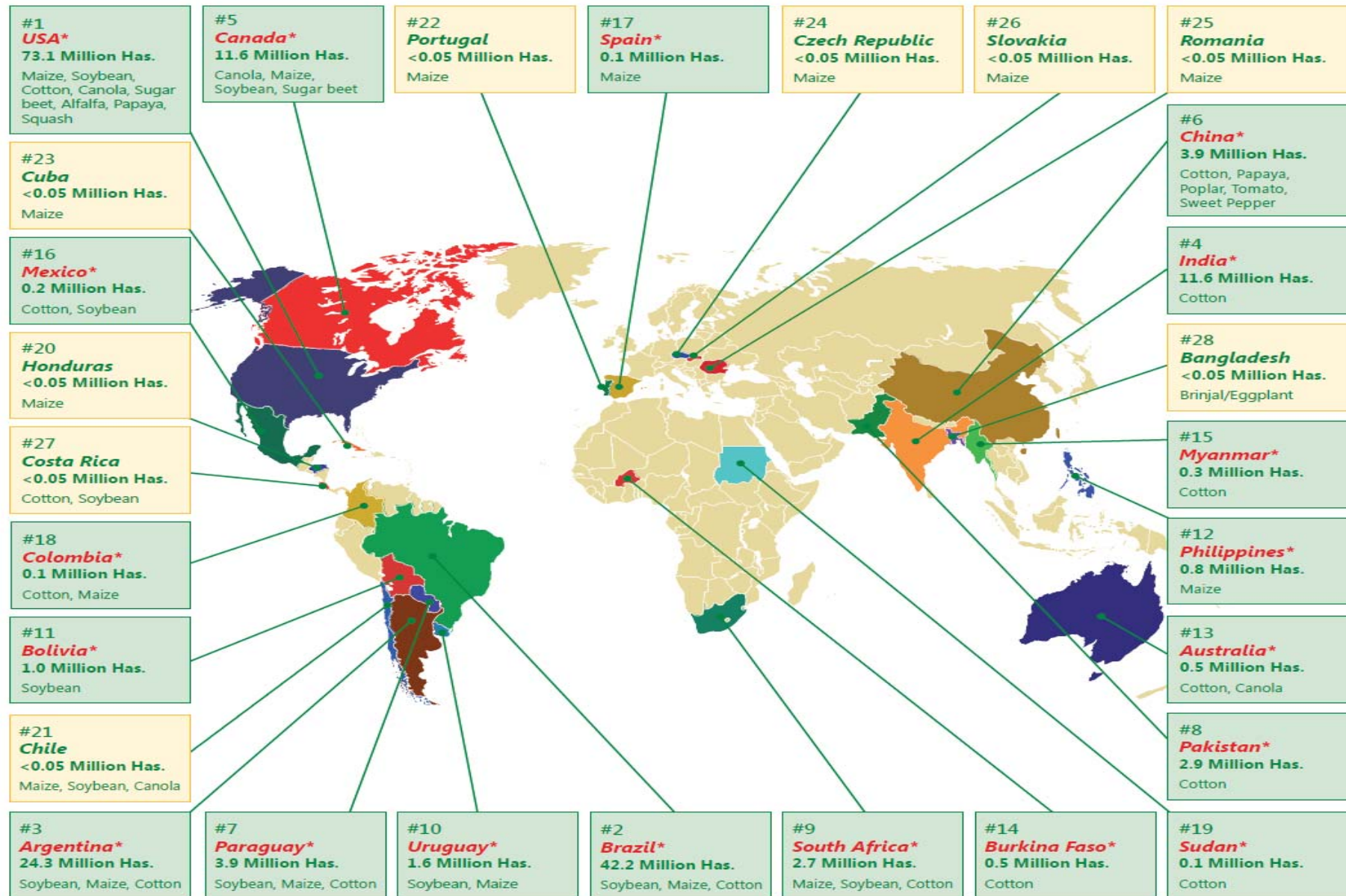


GLOBAL AREA OF BIOTECH CROPS Million Hectares (1996-2014)



Clive James, 2014. International Service for the Acquisition of Agri-biotech Applications, Isaaa

Biotech Crop Countries and Mega-Countries*, 2014



■ *19 biotech mega-countries growing 50,000 hectares, or more, of biotech crops.

Source: Clive James, 2014. [Isaaa](#)

First Generation of GM Plants with Agronomical (Input) Traits

- Improved disease resistance (viruses, fungi)
 - Improved pest resistance (lepidoptera, beetles)
 - Tolerance for herbicides (glyphosate, glufosinate)
 - Combined herbicide tolerance/ pest resistance (stacks)
-
- Main Commercial Crops:
Soybean, Maize, Rapeseed, Cotton



Further Developments of Input Traits to Combat Abiotic Stress Conditions



Soil acidity
Metal tolerance
Drought tolerance

Second Generation of GM Crops with Output Traits

Output traits improve the nutritional content (health benefits) or processing characteristics

'Golden' rice
Rice

containing β -carotene
fortified with iron

Tomato

lower allergen content

Lupin

β -carotene / lycopene enriched

Maize

higher methionin levels

higher levels of lysin

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

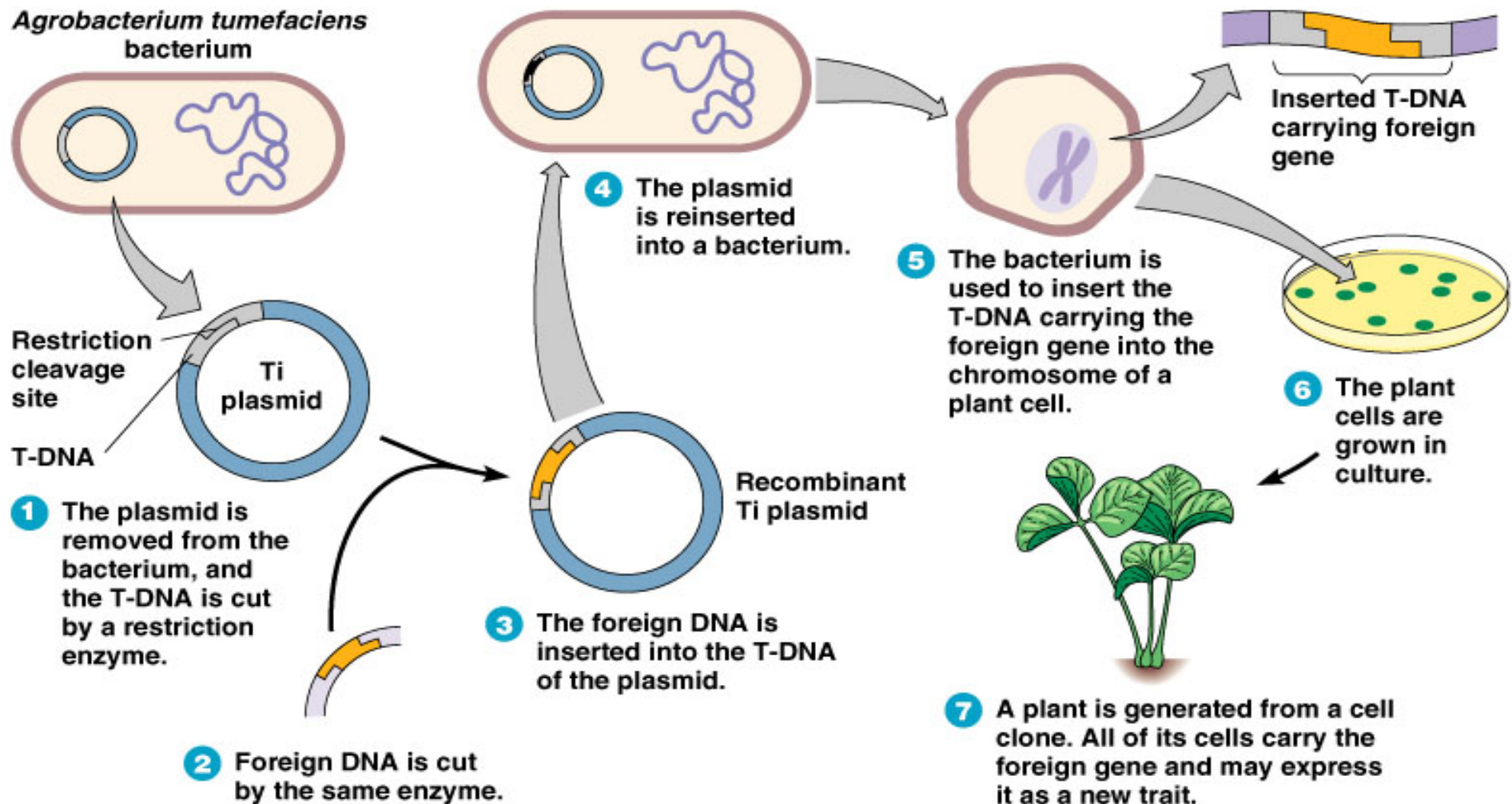
Canola

vitamin E enriched

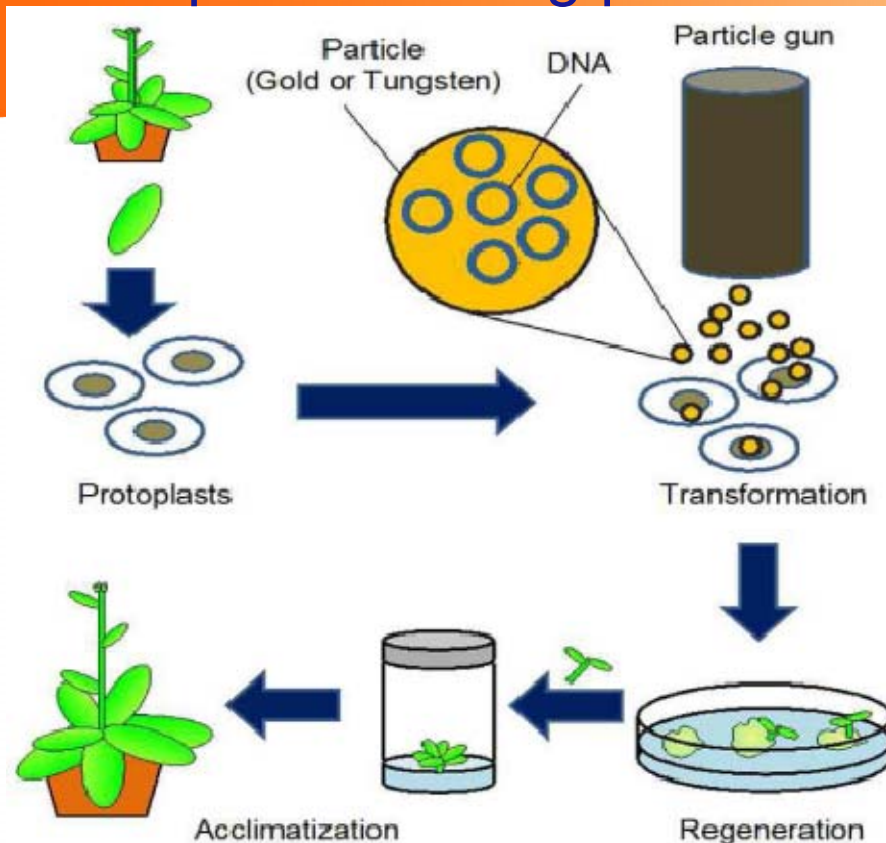


Generation of GM plants using *Agrobacterium tumefaciens*

Herrera-Estrella, Luis; Depicker, Ann; Van Montagu, Marc; Schell, Jeff (1983), *Nature* **303** (5914): 209–13.



Plant transformation process using particle bombardment:



- (1) Isolate protoplasts from leaf tissue
- (2) Inject DNA-coated particles into protoplasts using particle gun
- (3) Regenerate into whole plants.
- (4) Acclimate the transgenic plants in a greenhouse

Singh A, Kumar V, Poonam, Gupta HR (2014) Genetically Modified Food:
A Review on Mechanism of Production and Labeling Concern. Adv Plants Agric Res 1(4): 00020.

European Food Safety Authority



EFSA

- Structure:
 - Executive director
 - Management Board
 - Advisory forum
 - Scientific committee and panels
 - 9 Scientific Panels
- Independent, e.g. from European Commission (EC)
- Advice to EC and Member States
- Risk Assessment, not Risk Management



Parma

<http://www.efsa.europa.eu>

EFSA Scientific Committee and Panels

Horizontal scientific issues

- Scientific committee (SC)

Risk Assessment of Regulated Products

- Feed additives (FEEDAP)
- Nutrition (NDA)
- Food ingredients and packaging (ANS and CEF)
- **Genetically modified organisms (GMO)**
- Pesticide (PPR)

Risk Assessment and scientific assistance

- Animal health and welfare (AHAW)
- Biological hazards (BIOHAZ)
- Contaminants (CONTAM)
- Plant health (PLH)



- Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms
- Regulation (EC) No 1829/2003 on GM food and feed
- Commission Implementing Regulation (EU) No 503/2013 for authorization of GM food/feed

Genetically modified organism (GMO) means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.

Source: Article 2 of European Union Directive 2001/18/EC



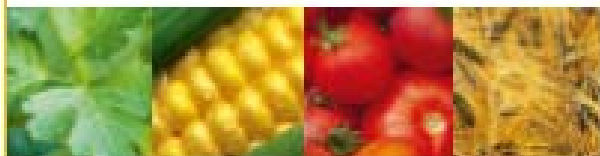
Regulation (EC) 1829/2003 (1)

- Risk assessment under responsibility of EFSA
- Covers both GM food and feed, no single (separate) authorisation
- Not products from animals fed GM feed
- Post-market monitoring may be required for GM foods and feed where appropriate
- Methods for sampling, identification and detection of GM food and feed should be provided by the applicant
- Methods should be validated by the Community Reference Laboratory

Regulation (EC) 1829/2003 (2)

- Clear labelling irrespective presence of DNA or protein
- No labelling in case of adventitious or technically unavoidable presence of minute traces of GMOs:
 - 0,9% for GM material authorised in the EU
 - 0,5% for GM material not authorised in the EU, but favourably evaluated (transitional measure)
- Unintended presence of GMOs in other products should be avoided and guidelines for co-existence of GM, conventional and organic crops should be developed

EFSA Guidance for GM Plants and derived Food and Feed



GUIDANCE DOCUMENT
OF THE SCIENTIFIC PANEL
ON GENETICALLY MODIFIED
ORGANISMS FOR THE RISK
ASSESSMENT OF GENETICALLY
MODIFIED PLANTS AND
DERIVED FOOD AND FEED

Adopted on 24 September 2004
First, edited version of 8 November 2004

March 2005

- Adopted on 24 September 2004,
- Updated in December 2005 (PMEM)
- Complemented in
 - December 2006 (Renewals)
 - March 2007 (Stacked events)
- Updated
 - May 2008, for Public Consultation,
 - May 2011 final version



Commission Implementing Regulation (EU) No 503/2013

REGULATIONS

COMMISSION IMPLEMENTING REGULATION (EU) No 503/2013

of 3 April 2013

on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006

(Text with EEA relevance)



8.6.2013 Official Journal of the
European Union L 157/1



GMO Risk Assessment

Role of EFSA



To introduce a GM product on the EU market:
an **Authorization** is required

For an authorization the **Applicant** needs to prepare a **Dossier** with required data and a scientific evaluation of any possible risk for humans, animals and the environment

EFSA prepares a **Scientific Opinion** based on dossier and other information

The Comparative Risk Assessment Approach

Regulation EC 1829/2003

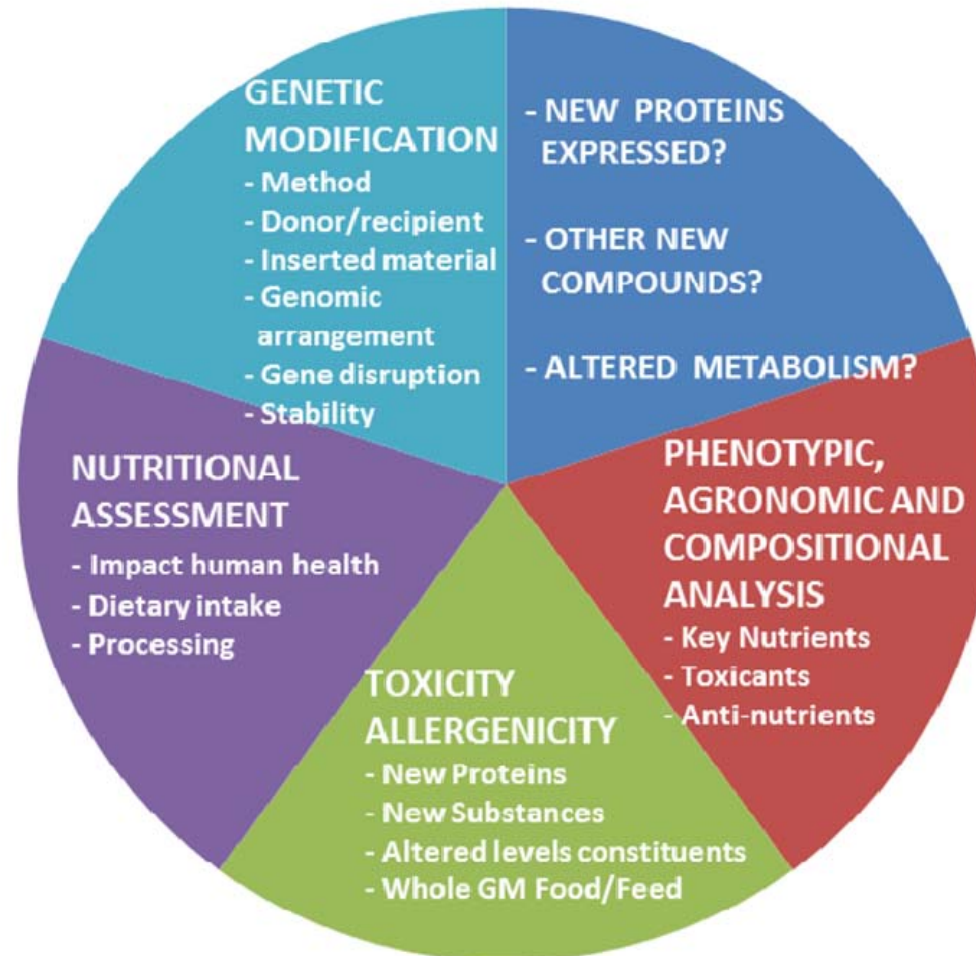
- Compare the agronomic, phenotypic and compositional characteristics of the GMO and derived products with those of the non-GM conventional counterpart
- Conventional counterpart:
 - Similar food/feed produced without the help of genetic modification and for which there is a well established **history of safe use**

Two –Step procedure for the Safety Assessment of GM Crop derived Food/Feed



- Find the *Differences* between the GM and its conventional counterpart
– (Difference and Equivalence Testing)
- Assess the identified *Differences* with respect to environmental, food/feed safety and nutritional impact

Key Elements Safety Assessment GM Food



Compositional Parameters

- **Fat, fatty acid profile**
- **Protein, amino acid profile**
- **Micronutrients**
- **Anti-nutrients**
- **Crude fibre**
- **Ash**
- **Moisture content**
- **Crop specific toxicants**



–OECD Consensus Documents

To identify Differences between the GM and non-GM plants: Perform Field Trials



Two types of tests

1. Test of Difference:

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

2. Test of Equivalence:

To verify whether the identified difference(s) 'fall' within natural variation ranges of reference (commercial) varieties

Experimental design for field trials –(ii) between sites



There may be different commercial varieties at each site

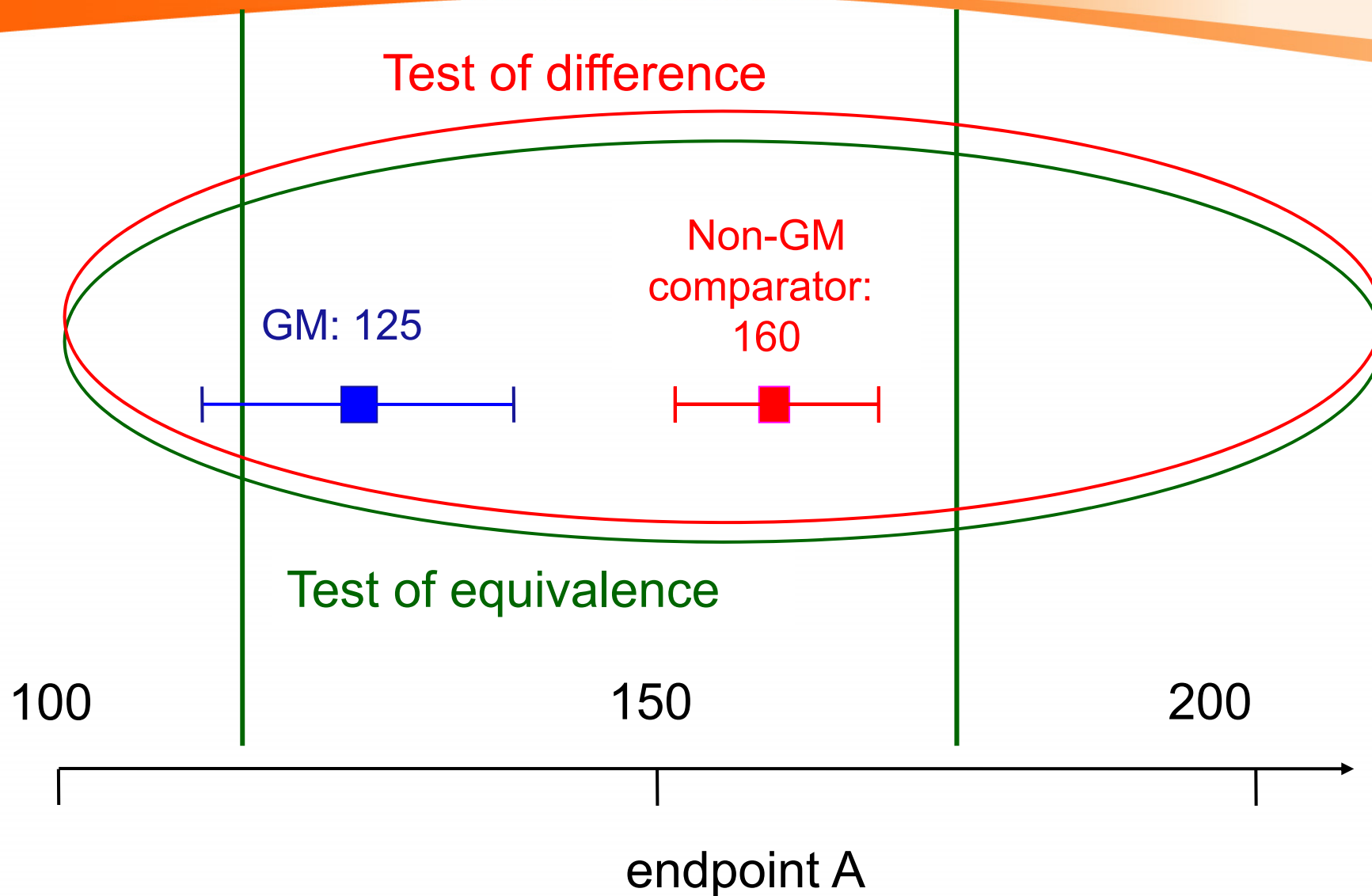
The same GM, and non-GM comparator at each site

At least 6 commercial varieties over all the sites

There must be at least 8 sites, over one or more years

GM	C	CV1	CV2	CV3	CV4
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The Difference and Equivalence Tests

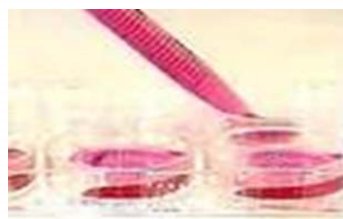


Toxicological Assessment: Safe Level of Consumption

- Newly expressed proteins
- Possible new constituents
- Possible alterations in content of natural food and feed constituents
- Animal feeding trials with whole GM food/feed
only if there are Indications for adverse effects

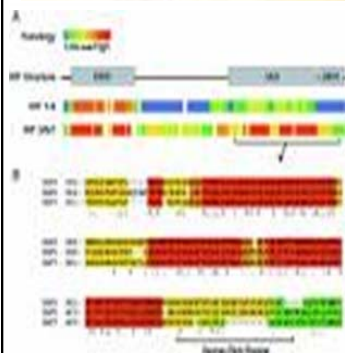
Toxicological Assessment of Newly Expressed Proteins

- Protein's source, function/activity and history of use and human/animal consumption



- Protein characteristics:

- Stability, digestibility, amino acid sequence, molecular weight, post-translational modifications, function, enzyme activity, interactions



- Sequence homology to proteins known to be toxic



- Repeated dose toxicity studies unless information exists on the safety of the protein

Toxicological tests for Chemicals which may be applied for testing of GMO food/feed

- *In vivo* tests in laboratory animals (OECD test guidelines, European Commission Directives)
 - *Single dose toxicity testing*
 - *Repeated-dose toxicity testing including 28/90-day oral toxicity, chronic toxicity, carcinogenicity*
 - *Reproductive and developmental toxicity testing*
 - *Immunotoxicity testing*
- Specific tests
 - *In vitro* tests
 - *In silico* search for sequence homology
 - *In vitro* stability tests of proteins under gastro-intestinal conditions
 - *Genotoxicity tests*
 - *Immunochemical cross-reactivity tests*
 - *Profiling technologies*
- **Studies under (GLP) described in Council Directive 2004/10/EC**

Random integration of transgenes

- ⇒ **insertional mutagenesis**
- ⇒ **disruption of endogenous gene functions**
 - **gene activation / inactivation**
 - **production of new proteins**
- ⇒ **changes in**
 - **enzymes**
 - **metabolites**
 - **phenotype**

Examples Unintended Effects in Conventional Breeding

Potato glycoalkaloids

- Pest resistance: glycoalkaloids up
- Cases of human poisoning



Celery

- Furanocoumarins
- Insect / Fusarium resistance
- Contact dermatitis in field workers



Compositional Analysis to Detect (Un)intended Effects

Compositional analysis



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graph TD; A((Compositional analysis)) --> B[Targeted analysis of single compounds]; A --> C[Non-targeted Profiling Methods];
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Targeted analysis of single compounds

- ✓ (Broad) spectrum of physiological pathways
- ✓ (Biased) selection of compounds

Non-targeted Profiling Methods

- Transcriptomics
- Proteomics
- Metabolomics

- ✓ Assessment of a broad spectrum of compounds
- ✓ Interconnective pathways
- ✓ Increased probability to detect unintended effects

Allergenicity

- Allergy is an immune-mediated reaction, involving IgE antibodies
- Affects individuals with a genetic predisposition (atopic individuals)
- More than 170 foods cause food allergies
- Most common foods “The Big Eight”:
 - cow's milk
 - eggs
 - fish
 - crustaceans
 - peanuts
 - soybeans
 - tree nuts
 - wheat

Assessment of the Allergenic Potential of GM Foods

Source of Gene Allergenic?

Sequence Homology with
Known Allergens

Specific Serum Screening
(persons with a known allergy to
the protein source)

Targeted Serum Screening
(persons with a known allergy to
related foods)

Pepsin Resistance

Animal Models

Weight of Evidence Approach

No single test to predict allergenic responses in humans

Assessment Allergenicity of whole GM Food

- Potential change in allergenicity of the food?
 - Human serum screening, proteomics, animal models
- Not one test is conclusive
- Weight-of-Evidence Approach (WHO/FAO)

Animal Testing of Whole GM Food/Feed Needed?

EFSA Guidance 2011: Only whole food/feed testing in case of indications for unintended effects

- In case relevant differences have been identified by molecular, compositional, phenotypic/agronomic analyses, which need further characterization
- In case of indications or remaining uncertainties for the potential occurrence of unintended effects
- Stacks with interacting proteins

Commission New Implementing Regulation 2013:
Compulsary testing of all GMO single events and stacks from retransformation

Risk Assessment Strategy for GM Food/Feed



European Food Safety Authority

Phase 1

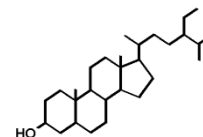
Tracing of differences between the GM food and its Conventional Counterpart (Comparative Analysis)



Introduced genes



(New) proteins (New) metabolites and toxins



Toxicological / Nutritional Assessment



Gene transfer



**Degradation
Toxicity
Allergenicity**



Toxicity



Whole foods

Phase 2

Phase 3

Exposure Assessment



- Role of the product in the diet
- Intake of the product by the consumer



Phase 4

Risk Characterization of GM food/Feed

Conclusions

- The risk assessment strategy for GM foods/feed as developed by EFSA is robust and comprehensive
- The EFSA risk assessment strategy is in line with guidelines developed at the international level (OECD,FAO/WHO)
- The risk assessment of the GM plant and derived products is carried out in *a comparative way*: non-GM products with a well known history of safe use serve as a baseline for the assessment
- The risk assessment is focused on:
 - (i) the genetic modification,
 - (ii) the expression of new proteins and other compounds,
 - (iii) identification of potential unintended compositional alterations,
 - (iv) the impact of observed alterations on human and animal health

Acknowledgements

- Dr. Claudia Paoletti (Deputy Head of the EFSA GMO Unit)