Ad hoc meeting with Industry _23 June 2021

Allergenicity assessment

GMO Unit

Trusted science for safe food

efsa
European Food Safety Authority

Allergenicity assessment

GUIDELINES FOR PREDICTION

Codex Alimentarius 2003-2009

EFSA GMO Panel - 2010
- 2011
- 2017

CEP Panel - 2009

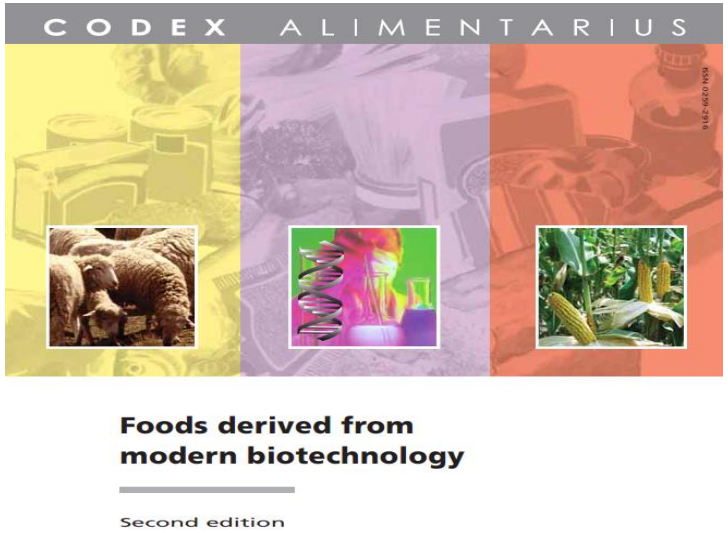
FEEDAP Panel - 2008
- 2017

NDA Panel - 2016

2003-2009

WoE approach

Allergenicity assessment – Weight of evidence



The information in the WoE includes:

- Source of the protein (IgE and non-IgE)
- Amino acid sequence comparison
- *In vitro* degradation studies (mainly pepsin test)
- Specific serum screening
- Cell based / *in vivo* assays

On a case-by-case basis

Protein safety – Allergenicity

Complex questions raised decades ago:

What makes a protein an allergen?

How much is too much?

- Whole foods as well as individual proteins to be risk assessed
- The risk assessment (RA) should take into account:
  - not only cross-reactivity of a reaction
  - but also *de novo* sensitisation
- Different sources of information taken into account in the RA
- No validated method available ➡ Weight-of-evidence (WoE)

Future innovative foods

European Food Safety Authority

RESEARCH & INNOVATION

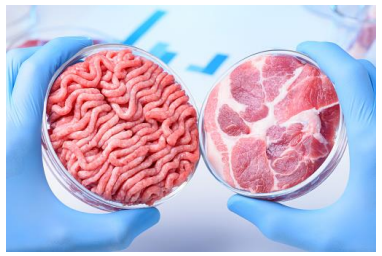
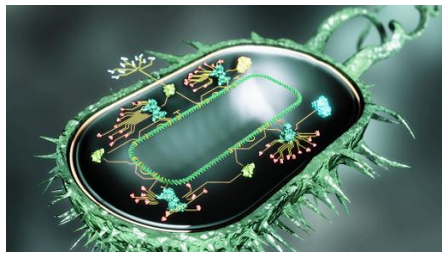
European Commission

Novel food sources: Food 2030 - Innovative EU research ensures food system is future-ready

https://ec.europa.eu/knowledge4policy/publication/food-2030-innovative-eu-research-ensures-food-system-future-ready_en

Nutrition for more sustainable and healthy diets; Climate resilience and environmental sustainability; Circularity and resource efficiency; and finally, Innovation and the empowerment of communities



Protein safety – Allergenicity

Complex questions still remains:

What makes a protein an allergen?

How much is too much?

- An urgent need to improve and better modernise the allergenicity assessment prediction

- EU projects in this direction: e.g. https://imparas.eu/

- EFSA activities with the focus on allergenicity assessment: Stakeholders involvement

EU projects

Verhoeckx *et al. Clin Transl Allergy* (2020) 10:13
https://doi.org/10.1186/s13601-020-00318-x

Clinical and Translational Allergy

REVIEW Open Access

COST Action 'ImpARAS': what have we learnt to improve food allergy risk assessment. A summary of a 4 year networking consortium

Kitty Verhoeckx[1*], Katrine Lindholm Bøgh[2], Anne Constable[3], Michelle M. Epstein[4], Karin Hoffmann Sommergruber[5], Thomas Holzhauser[6], Geert Houben[1], Annette Kuehn[7], Erwin Roggen[8], Liam O'Mahony[9], Ben Remington[1] and René Crevel[10]

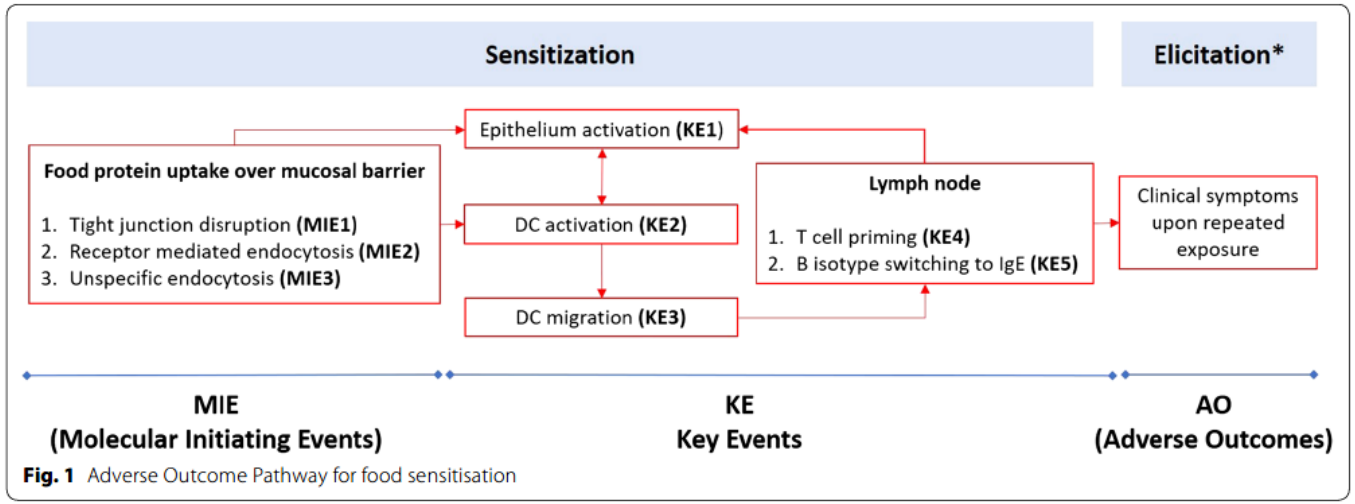
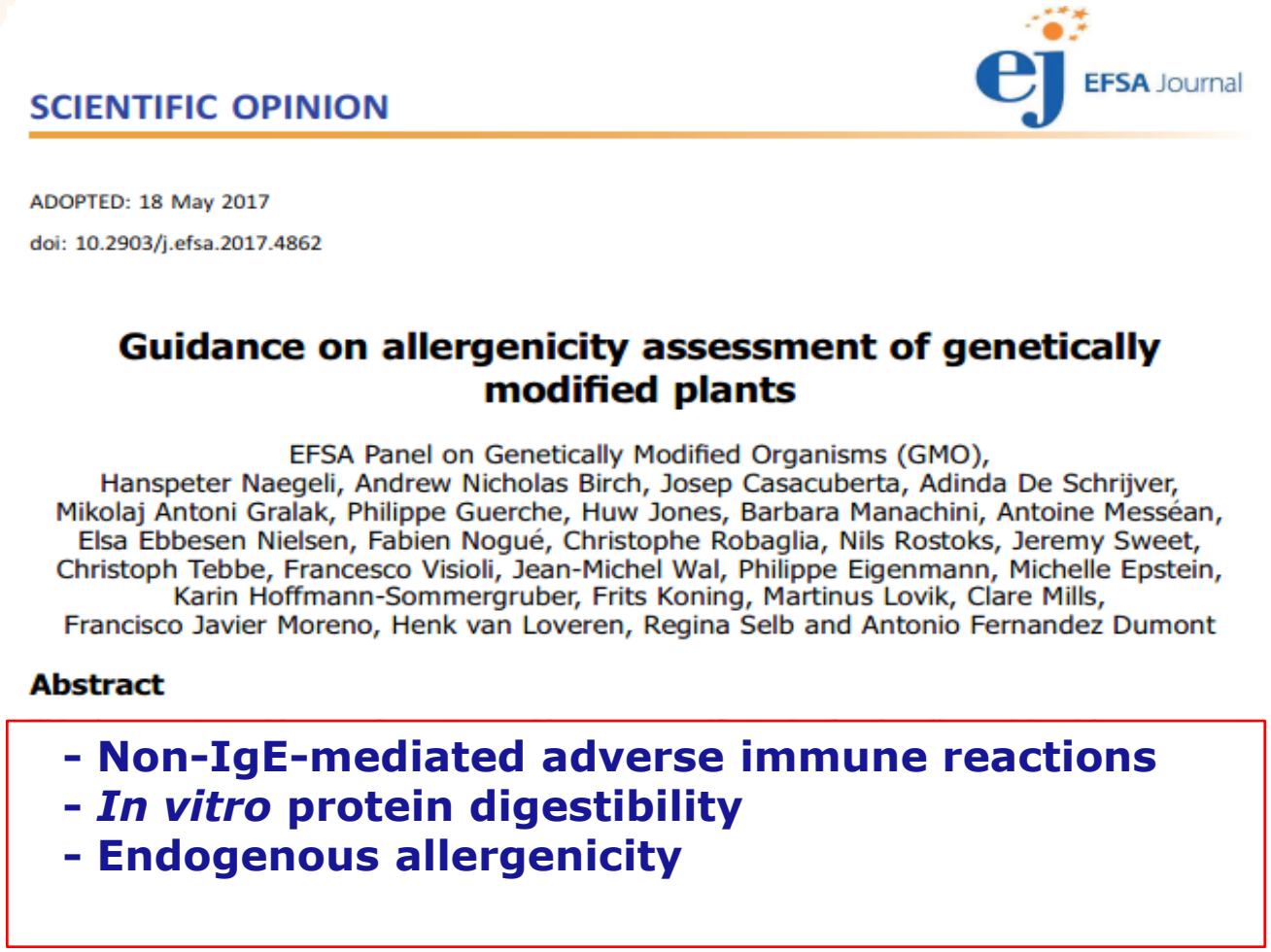


Fig. 1 Adverse Outcome Pathway for food sensitisation

- Introduction of new foods should not add to the burden of food allergy
- Need of a reliable, harmonized, evidence-based and validated allergenicity RA strategy
- To make use of novel methods and knowledge
- To focus on future research

EFSA activities



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 18 May 2017

doi: 10.2903/j.efsa.2017.4862

Guidance on allergenicity assessment of genetically modified plants

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Philippe Eigenmann, Michelle Epstein, Karin Hoffmann-Sommergruber, Frits Koning, Martinus Lovik, Clare Mills, Francisco Javier Moreno, Henk van Loveren, Regina Selb and Antonio Fernandez Dumont

Abstract

- Non-IgE-mediated adverse immune reactions
- *In vitro* protein digestibility
- Endogenous allergenicity

Keywords: guidance, allergenicity assessment, newly expressed proteins, endogenous allergenicity, GMO

EFSA activities focused on:

- A statement on the usefulness of the pepsin resistance test
- A workshop organised 15-16 June 2021
- A scientific opinion on future development needs

Looking closer at the future needs

- **More robust bioinformatic approaches**:
  - Current methodologies date back to 2001
  - Databases development
  - EFSA procurement on peptide modeling ongoing
- **More refined *in vitro* protein digestion protocols**
  - Classical pepsin resistance test in place but questioned
  - EFSA procurement on *in vitro* digestion finalised but more work needed
- **More predictive cell-based approaches/ HLA-phenotyping**

EFSA procurement on *in vitro* digestion

Report on EFSA project OC/EFSA/GMO/2017/01

"*In vitro* protein digestibility" (Allergestion)

Alan Mackie[1], Didier Dupont[2], Amelia Torcello-Gómez[1], Julien Jardin[2], Amélie Deglaire[2]

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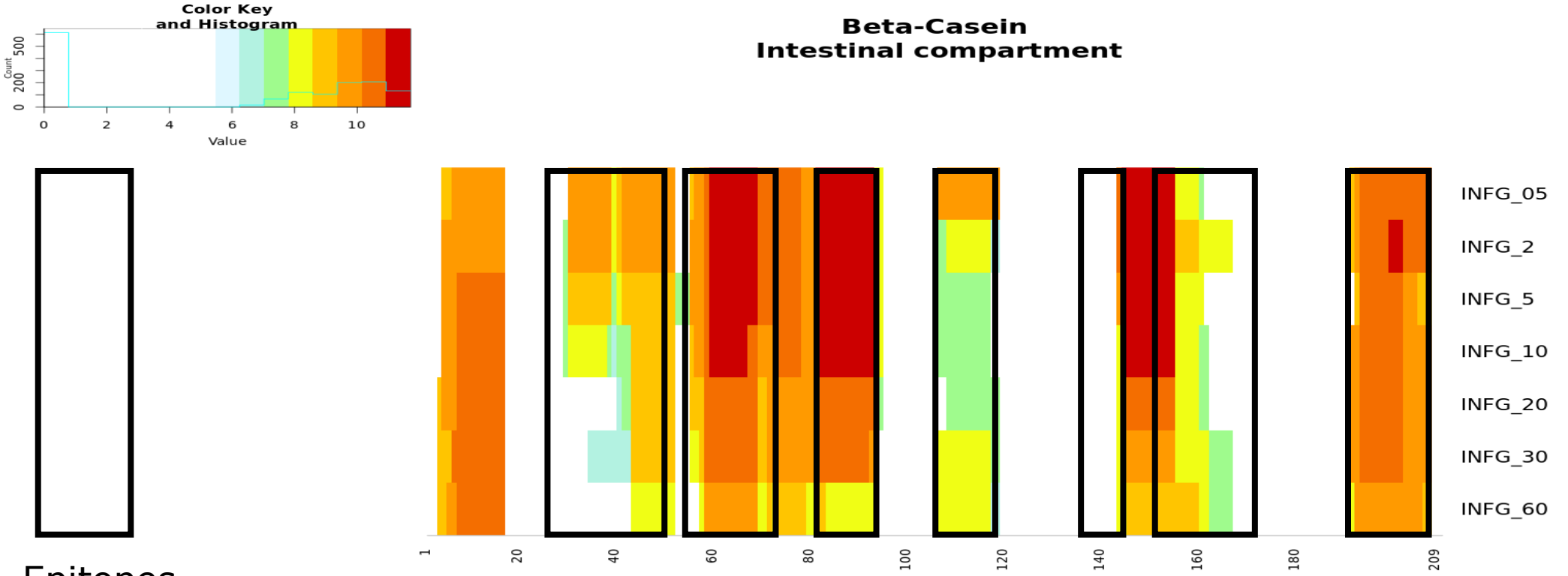
https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2019.EN-1765

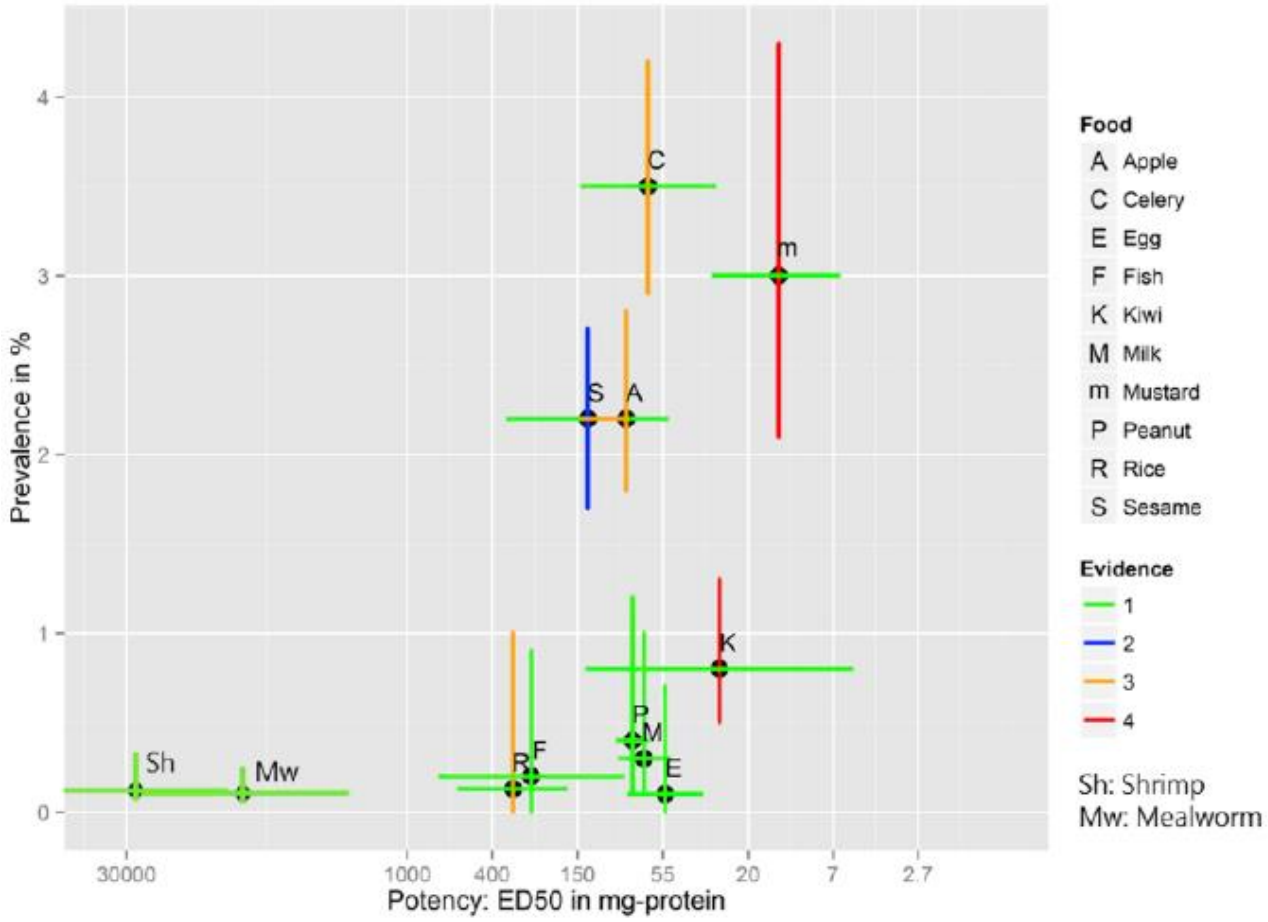


Epitopes

The primary amino acid sequence of BCAS evaluated with IgE-binding epitopes identified in the study

But with other control proteins not so inspiring results

Example: Scaling allergy risks of foods relatively



Houben et al 2019. Food and chemical toxicology, 127, pp 61-69

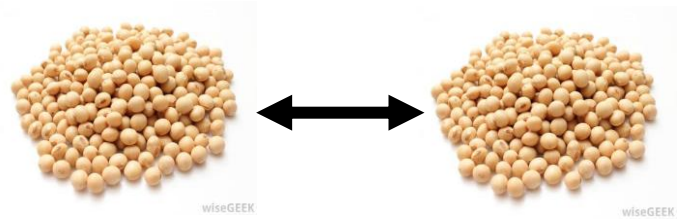
Strategy for ranking the allergic potential of known proteins as a way forward

(FAO/WHO, 2001; EFSA GMO Panel, 2017; Remington et al 2018; Verhoeckx et al 2020; Fernandez et al 2020)

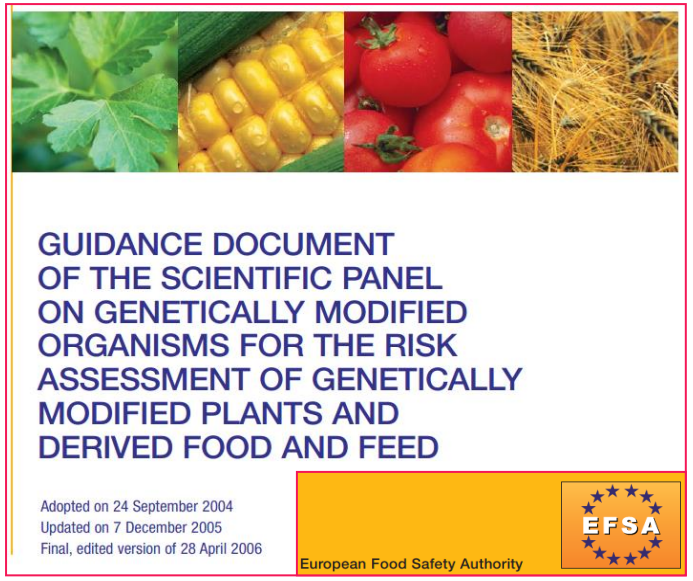
Whole foods → **Individual allergens?**

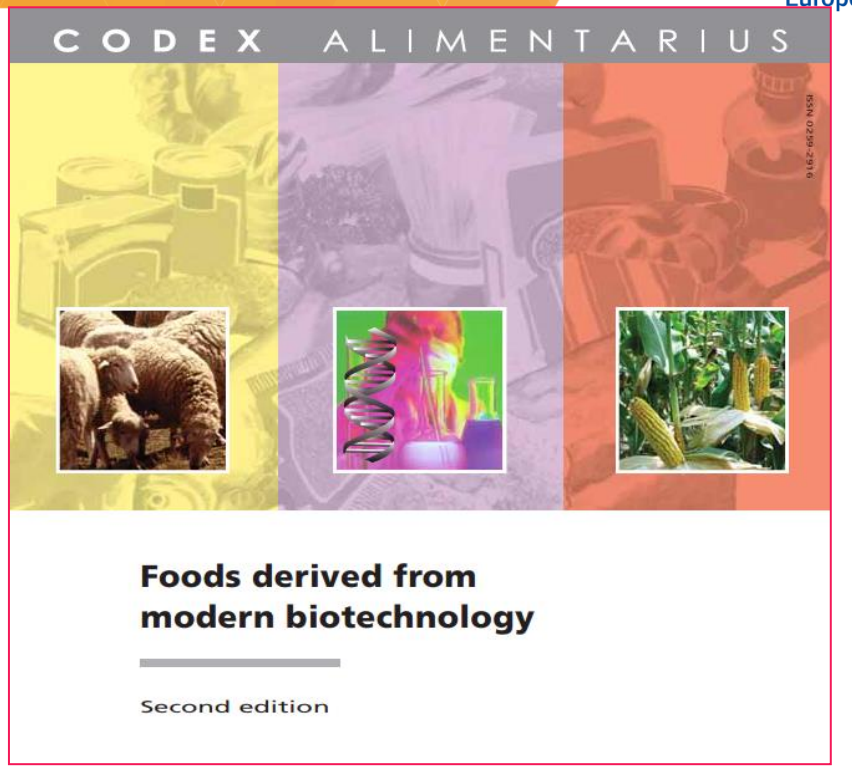
Endogenous allergenicity

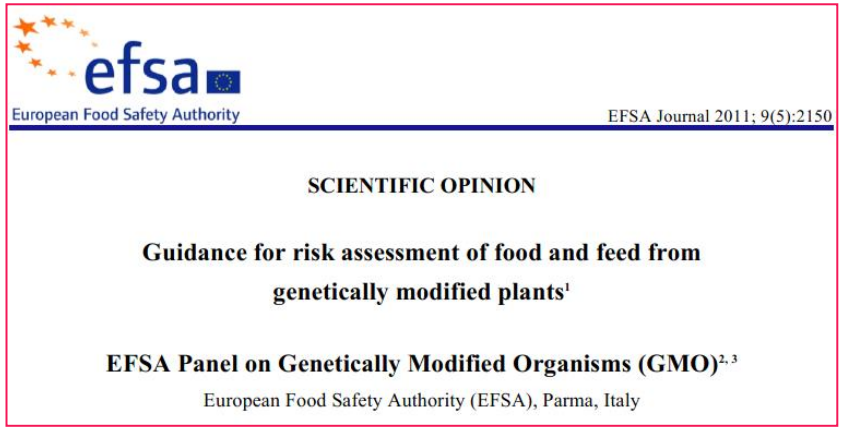
Comparative approach



“non-GM” “GM”







Relevant plants for analysis

- **Analysis performed on a case-by-case basis**
- **For «common allergenic foods» (EU Reg. 2003, 2011)**
- **To date: soybean is the main crop analysed**
- **Other GM plants than soybean: whenever considered necessary**

Risk managers | **Risk assessors** | **Health professionals** | **Stakeholders**

Relevant allergens for quantification

EU Regulation 503/2013

OECD consensus soy 2012
Soy allergen list:
'potential soybean allergens'

Table 20. Potential soybean allergens

IgE-binding proteins	Allergen nomenclature	Molecular weight (kDa)	Family
Hydrophobic proteins	Gly m 1[1]	7.0-7.5	Lipid transfer proteins
Beta-conglycinin	Gly m 2[1]	8.9	Storage proteins
Profilin	Gly m 3[1]	14	Profilin
SAM22	Gly m 4[1]	18.0	Pathogenesis related protein PR-10
P34	Gly m Bd 30 K	34	Protease
Unknown Asn-linked glycoprotein	Gly m Bd 28 K	28	Unknown
β-Conglycinin (vicilin, 7S globulin)	Gly m 5[1]	140–170	Storage proteins (with subunits)
Glycinin (legumin, 11S globulin)	Gly m 6[1]	320–360	Storage proteins (with subunits)
2S albumin	Not assigned	12	Prolamin
Lectin	Not assigned	120	Lectins
Lipoxygenase	Not assigned	102	Enzyme
Kunitz trypsin inhibitor	Not assigned	21	Protease inhibitor
Unknown	Not assigned	39	Unknown
Unknown	Not assigned	50	Homology to chlorophyll A-B binding proteins
P52-53	Not assigned	52–53	Unknown

Source: adapted from L. Ricciardi and Bonsi, (2007); updated with information from WHO/IUIS (2011)

[1] WHO/IUIS (2011) Allergen nomenclature recognized by NIAID and IUIS

Evidence check

Evaluation of literature for all single allergens

and

Comparison and complementation with databases (EFSA, 2010)

and/or

Systematic Reviews

EFSA Guidance 2017

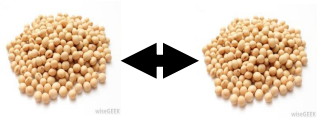
Clinical relevance shown

Relevance for GMO risk assessment

Methodology

- **Quantitative ELISA**
- **Quantitative mass spectrometry**

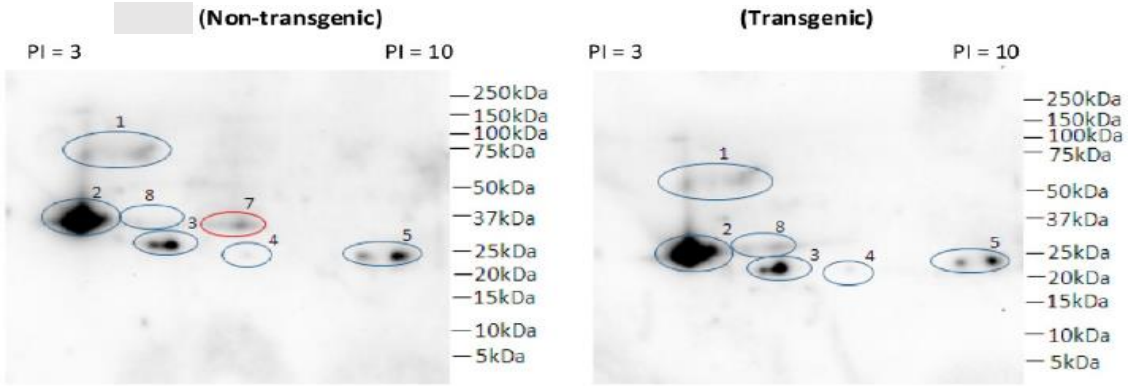
comparative approach



"non-GM" "GM"

Future development of an allergen database (natural variability)

Historically: **human sera (IgE-binding)**

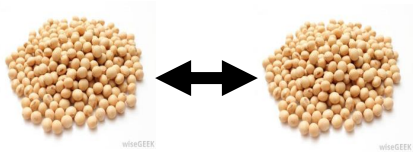


Goodman et al. 2013, J. Agric. Food Chem. 2013, 61, 8317-8332

Data interpretation

- **Natural variability of allergens**

comparative approach



"non-GM" "GM"

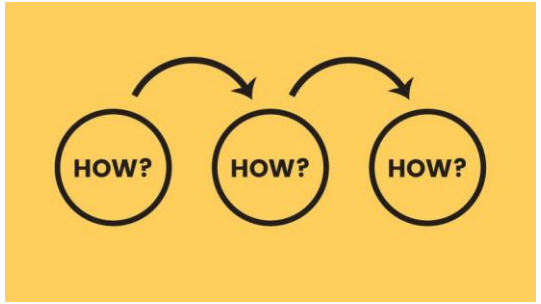
- **On case-by-case basis**
  - Magnitude and number of changes
  - Clinical relevance of the allergen(s) involved
  - Exposure considerations
  - Clinical evaluation

Few overall remarks

Scientific developments → Translated into → Risk assessment requirements

A reliable, harmonised, and evidence-based allergenicity RA strategy
(e.g., Codex, EFSA, Cost actions, others)

Consensus permeable to evolution



- **Session on clinical relevance and risk assessment**

How to better use available human data on allergic reactions to known allergens?

- **Session on *in silico* approaches**

What is the usefulness of current FASTA algorithms and of alternative approaches?

How can the current allergen database be improved?

- **Session on *in vitro/in vivo* approaches**

What are the most relevant test materials to predict allergenicity?

When and how should human sera or alternatives be used to address challenges?

- **Session on outstanding questions**

What are the most effective methods to predict allergenicity? How should they be integrated?

How best to validate *in silico* and *in vitro* methods (e.g., animal models)?

How can PMM tools be developed and how to determine acceptable levels and/or thresholds of protein?

Thank you very much

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