Endogenous Allergenicity – EFSA view

Meeting with Stakeholders: EFSA Allergenicity Guidance, Nov 23rd 2016

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Endogenous Allergenicity Assessment

- **EFSA GDs: when the plant receiving a transgene is considered to be allergenic (e.g. soybean), the allergen repertoire should be examined**

- **In line with internationally agreed standards (e.g. Codex Alimentarius 2003-2009)**

comparative approach

“non-GM” **“GM”**

Endogenous Allergenicity Assessment

Implementing Regulation 503/2013

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)

compositional analysis of already identified allergens (IR 503/2013 refers to OECD consensus documents)

→ New EFSA guidance to help implementing these requirements

Endogenous Allergenicity Assessment

- Relevant plants for the analysis
- Relevant allergens to be quantified
- Methodology
- Data interpretation

Relevant plants for the analysis

- IR 503/2013: performed on a case-by-case basis
- To date: for foods recognised as "common allergenic foods"
  - → European Regulation for labelling purposes (EC, 2003, 2011)
- Until now, EFSA has not received applications involving recognised allergenic food other than soybean
- Other GM plants than soybean: whenever considered necessary

Relevant allergens to be quantified

Fig. 1: Current requirements

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

Table 20. Potential soybean allergens

IgE-binding protein	Allergen nomenclature	Molecular weight (kDa)	Family
Hydrophobic protein	Gly m 1[1]	7.0-7.5	Lipid transfer protein
Defensin	Gly m 2[1]	8.0	Storage protein
Profilin	Gly m 3[1]	14	Profilin
SAM22	Gly m 4[1]	16.6	Pathogenesis related protein PR-10
P34	Gly m Bd 30 K	34	Protease
Unknown Asn-linked glycoprotein	Gly m Bd 28 K	26	Unknown
β-Conglycinin (vicilin, 7S globulin)	Gly m 5[1]	140–170	Storage protein (with subunits)
Glycinin (legumin, 11S globulin)	Gly m 6[1]	320–360	Storage protein (with subunits)
2S albumin	Not assigned	12	Prolamin
Lectin	Not assigned	120	Lectin
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 protein
P22-25	Not assigned	22–25	Unknown

Source: adapted from L'Hocine and Boye, (2007); updated with information from WHO/IUIS (2011)

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

Evidence check

Evaluation of literature for all single allergens

and

Comparison and complementation with databases (EFSA, 2010)

and/or

Systematic Reviews

Clinical relevance shown

Relevance for GMO risk assessment

Relevant allergens to be quantified

EFSA example: Identification of potential soybean allergens

Fig. 1: Current requirements

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

Evidence check

Evaluation of literature for all single allergens
and
Comparison and complementation with databases (EFSA, 2010)
and/or
Systematic Reviews

↓

Clinical relevance shown

↓

Relevance for GMO risk assessment

Table C1 – Potential soybean allergens

19 proteins/ 69 papers identified from the scientific literature:

- **Protein data**
  - Available information on the protein in question
- **Data on allergic individuals**
  - Number of individuals tested, origin, age group
- **Clinical relevance**
  - Allergic reactivity of the tested allergic individuals with soybean (DBPCFC, cutaneous reactivity)
  - Methodology to confirm reactivity with the individual potential soybean allergens
  - Evidence for clinical reactivity to soybean in connection to reactions to the individual potential allergens in question

Endogenous Allergenicity Assessment

Proteins to be considered – EFSA example

- Challenging to connect clinical reactivity to whole soy to a single protein in question
- WHO/IUIS defines criteria for a protein to be included as an allergen
  - → Primary requirements
  - → Peer-review of available scientific data

Proteins to be considered – EFSA example

- **Proteins peer reviewed and included in WHO/IUIS**
  - **Gly m 1, Gly m 2, Gly m 3, Gly m 4, Gly m 5, Gly m 6, Gly m 7, Gly m 8**
  - **Currently no sequence available: Gly m 2**
- **Fulfill primary requirements of WHO/IUIS**
  - **Gly m Bd 28K, Gly m Bd 30K, lectin, lipoxygenase, KTI, Gly m 50 KD**
  - **No considerable peer-literature available or sequence currently not available: lectin, lipoxygenase, Gly m 50 KD**
- **Do not fulfill the primary requirements**
  - **Gly m 39KD, P22-25, Gly m CPI, Gly m EAP, "unknown" possible allergen**

Endogenous Allergenicity Assessment

Proteins to be considered – EFSA example

10 allergens currently identified to be considered further

Clinical Relevance

-severity of reactions

-prevalence of reactions

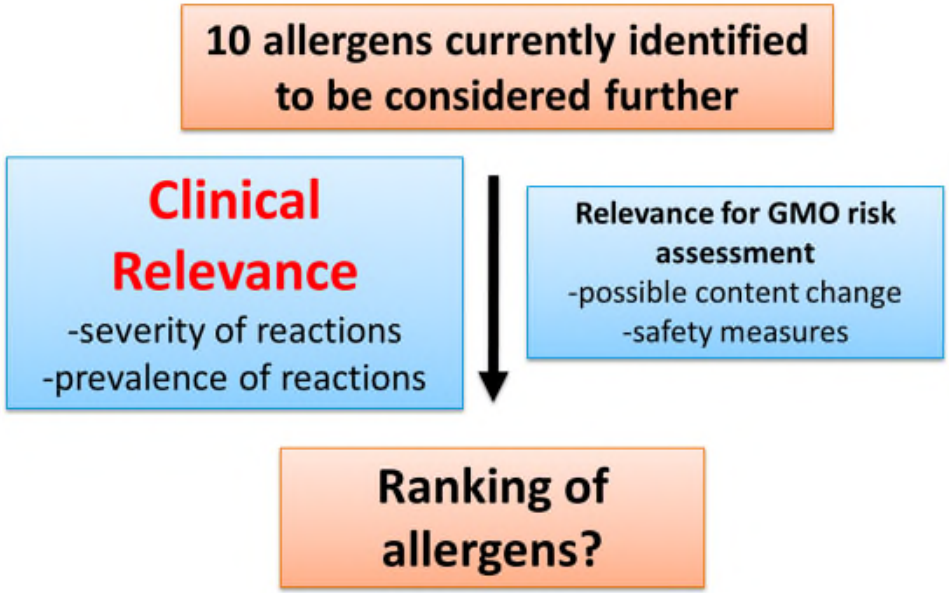
Relevance for GMO risk assessment

-possible content change

-safety measures

Ranking of allergens?

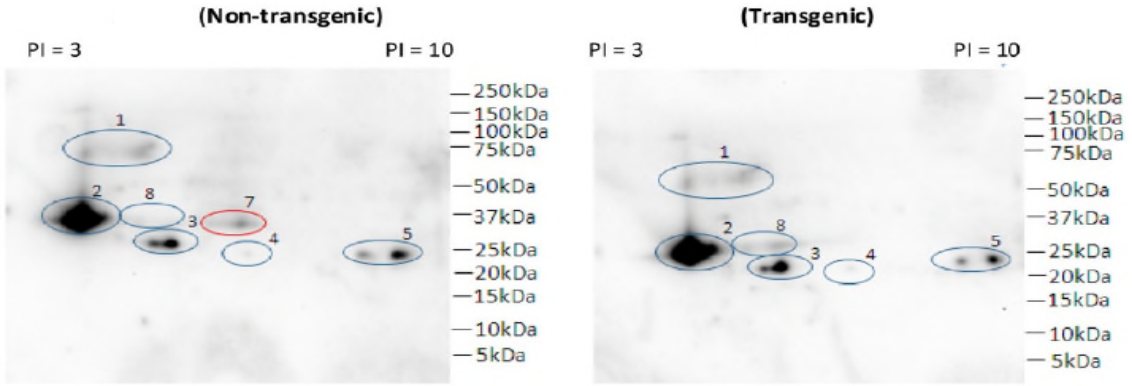
Proteins to be considered – EFSA example



→ This strategy could be applied to other plants than soybean, whenever considered necessary

EFSA guidance: Methodology (I)

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



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

- EFSA GD 2011: **recommended new approaches for quantification of known allergens (e.g. MS)**
- New EFSA draft Guidance: **Absolute quantification is mandatory according to IR 503/2013**

Methodology – EFSA experience from dossiers

Patients' IgE: comparison of binding patterns
and/or
2D GE/spot quantification: single proteins
and/or
Absolute protein quantification

↓ **IR503/2013**

Absolute protein quantification

EFSA guidance: Methodology (II)

Quantitative ELISA

Quantitative mass spectrometry

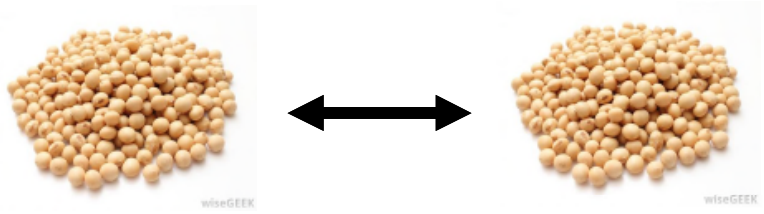
Advantages, limitations of each method

Further development and harmonisation of methods

Data interpretation

- Consider the natural variability of allergens
- How to identify a significantly enhanced allergen content
- Which enhanced content is a possible safety concern?

comparative approach



“non-GM” “GM”

Consequences of a statistically significant enhanced allergen content

- Considerations based on the allergen in question - clinical relevance evaluated with the help of clinicians
  - Potency of the allergen etc. (→ranking)
  - Magnitude and number of the change
- Exposure considerations
- Clinical evaluation (in case of need)
  - DBPCFC in comparison with an appropriate comparator
  - Dose-distribution curves obtained by DBPCFC to single allergens

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Thank you for your attention!

Technical meeting with stakeholders on supplementary guidance for allergenicity assessment of GM plants

Parma, 23 November 2016