Overall Safety Assessment of GM Crops- Allergenicity

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Biotechnology is an Extension of Traditional Plant Breeding

**TRADITIONAL PLANT BREEDING**

Desired Gene

Donor Plant

X

Commercial Plant Variety

New Plant Variety

Many genes are transferred

**PLANT BIOTECHNOLOGY**

Desired Gene

Donor

+ Desired Gene

Commercial Plant Variety

Improved Commercial Plant Variety

A single gene is transferred
Safety for humans, animals and the environment

- Safety assessment for plant biotech products is mandatory worldwide
- Considers human + animal health as well as environmental safety
- Approval only if authorities conclude:
  - Genetically optimized plant is as safe as a conventionally bred plant
  - Safety assessment starts early in the development process
Food Safety is a Global Concern:

Consumers need assurance that food will not cause harm and covers contamination by chemical and biological agents and concerns about inherent food nature.

Key Global Concerns (http://www.who.int/features/factfiles/food_safety/en/) include:

- Spread of microbiological hazards
  - 1,500,000 deaths/yr

- Chemical Food Contaminants
  - >300,000 affected by melamine and urea in milk in 2008

- Genetic Modification
  - 0 deaths or illnesses resulting from GM foods
Safety Assessment is a multi-pronged undertaking

- Gene(s)
- Protein(s)
- Crop Characteristics
- Food/Feed Composition
- Environmental Safety
Regulatory Studies are grouped in four categories:

- Molecular characterization
- Protein Characterization/Food/feed safety
- Agronomic and Compositional Equivalence
- Environmental safety
Protein characterization, Food / Feed safety

- Gram quantity protein production and purification
- Establishing protein level in plant tissues
- Protein characterization and equivalence
- **Allergenicity assessment**
- Toxicity assessment
Why proteins do not typically represent a hazard

- Proteins are relatively large and labile.

- Proteins are an essential part of the diet (avg. consumption 100 g/day).

- Digestive systems have evolved to convert the protein to its building blocks for incorporation. (very efficient only 6 – 12 g protein lost/day)

- The human body synthesizes approx. 300 g protein/day.
Protein Hazards

- Pathogenic bacterial toxins - botulinum, diptherium, active <100 mg/kg body weight.
- Plant toxins - ricin (0.5 mg MLD)
- Animal toxins - prions
- Allergens – Ara H2, b-lactoglobulin, glutens
- Antinutrients - trypsin inhibitors, some lectins
Categories of Potential Health Risks of Agricultural Biotechnology Relative to Allergenicity

• Transfer an existing allergen or cross-reactive protein into another crop.

• Creation of food allergens *de novo* 
  (*i.e.*, potential to become a new allergen.)

• Alteration or quantitative increase of endogenous (existing) allergens 
  (*i.e.*, increasing the hazard of currently allergenic foods)
Safety Assessment Approach - IgE-Mediated Allergy

• Avoid transfer of known allergens
  • Assume genes from allergenic sources encode an allergen until proven otherwise

• All introduced proteins evaluated

• Endpoints
  • gene source, sequence homology with known allergens, immunologic analyses and physicochemical properties

• ‘Weight-of-the-evidence’ provides reasonable assurance that foods will not become more allergenic
CODEX Guidelines (2003; 2009)

- CODEX recommended allergy assessment includes:
  - Source of the introduced protein
  - Similarity of the introduced protein to known allergens
  - Susceptibility to enzymatic digestion (pepsin)

Currently, no single test can predict food allergy for humans
CODEX Guidelines (2003; 2009)

- If introduced protein from a non-allergenic source
  ✓ Assess amino acid sequence similarity to known allergens
  ✓ Assess pepsin resistance

- If introduced protein from an allergenic source
  ✓ Assess amino acid sequence similarity
  ✓ Assess in vitro pepsin resistance
  ✓ Assess specific IgE binding
  ✓ Assess skin prick testing
CODEX Guidelines (2003; 2009)

• CODEX recommended allergy assessment

• Other considerations
  ✓ Exposure level of the introduced protein
  ✓ As science and technology evolves other methods may be considered
    ➢ T-cell epitopes and structural motifs associated with allergens (glutens)
    ➢ Animal models
Non-IgE Mediated Immune Reactions to Foods

Limited information in CODEX regarding the evaluation for the potential of non-IgE mediated reactions:

- “The transfer of genes from organisms known to elicit gluten-sensitive enteropathy in sensitive individuals should be avoided unless it is documented that the transferred gene does not code for an allergen or for a protein involved in gluten-sensitive enteropathy.”

- “This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies.”
Safety Assessment Screening: Focus on Celiac Disease

- Gene Source: avoid genes from wheat, barley, rye and oats

- In silico exact peptide mapping of novel protein to those peptides identified in the peer reviewed literature as inducing celiac disease

- Evaluation occurs early in product development- (i.e., before constructs are made).
EFSA Draft Guidance on Allergenicity Assessment of GM Plants

Non-IgE-Mediated Immune Adverse Reactions to Foods

Stepwise Approach for Risk Assessment

**in silico**
- identity search (epitope/motif)
- HLA-DQ peptide modelling

**in vitro**
- in vitro digestibility
- HLA-DQ binding assays
- T cell testing
Identity Searches (epitope/motif)

100% match with T stimulatory epitope

Hazard identified

“potentially relevant”* Partial match with T Stimulatory epitope or Q/E-X1-P-X2 motif

Further investigations are necessary

no “potentially relevant”* match with with T stimulatory epitope or Q/E-X1-P-X2 motif

No hazard identified

*Match with a known T cell-stimulatory peptide which raises concern due to the position and nature of the identical amino acids.
Questions/Comments for Discussion

• Stepwise approach for risk assessment versus a weight-of-the-evidence approach is suggested in the draft guidance.

• If the source of the gene is not from a gluten-containing cereal or taxonomically related species and has no homology to a gluten protein, is there a need for further assessment?

• If the source of the gene is from a gluten-containing cereal and/or has homology to a gluten protein, suggest to search for exact match with T-cell epitopes.

• Is there any data to otherwise suggest that only very specific peptides from glutens are responsible for eliciting celiac disease (i.e., mismatched peptides concept)?

• What is the threshold for similarity? How similar should a peptide be for cross-reactivity to occur? How many amino acid mismatches are allowed before a peptide sequence is indicated to be a potential T-cell epitope (biologically significant)?

• What is the relevance of the Q/E-X1-P-X2 motif search? Preliminary data suggest that it is relatively common in proteins from organisms not associated with celiac disease (high level of false positives).

• If there are no stable fragments following pepsin digestion, are additional in vitro studies still needed with positive bioinformatic findings?