

Protein Allergy Assessment

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Food Safety in Context

- Millions of people are sickened each year due to food borne diseases; many thousands die, even in “developed” countries
- Biotech crops are some of the most tested foods; tested to a much greater extent than foods produced by other breeding methods

Food Safety

Food Safety definition

'...a reasonable certainty that no harm will result from intended uses under the anticipated conditions of consumption.'

OECD, 1993

- Traditional food bears a presumption of safety based on 'a history of safe use'

Constable et al. 2007

- While 'zero-risk' is unattainable, food should be 'safe and wholesome'
- Whole GM food is assessed in comparison with traditional food to be 'as safe & nutritious as..'

Food/Feed Safety Assessment



RELATIVE SAFETY

Historically we learned to eat “safely” through experience:

- ❑ Wheat must be avoided by those with celiac disease
- ❑ Legumes (beans/peas) must be cooked to inactivate lectins and trypsin inhibitors
- ❑ Allergic individuals must avoid specific foods

GM-Crops must be “as safe as” Non-GM

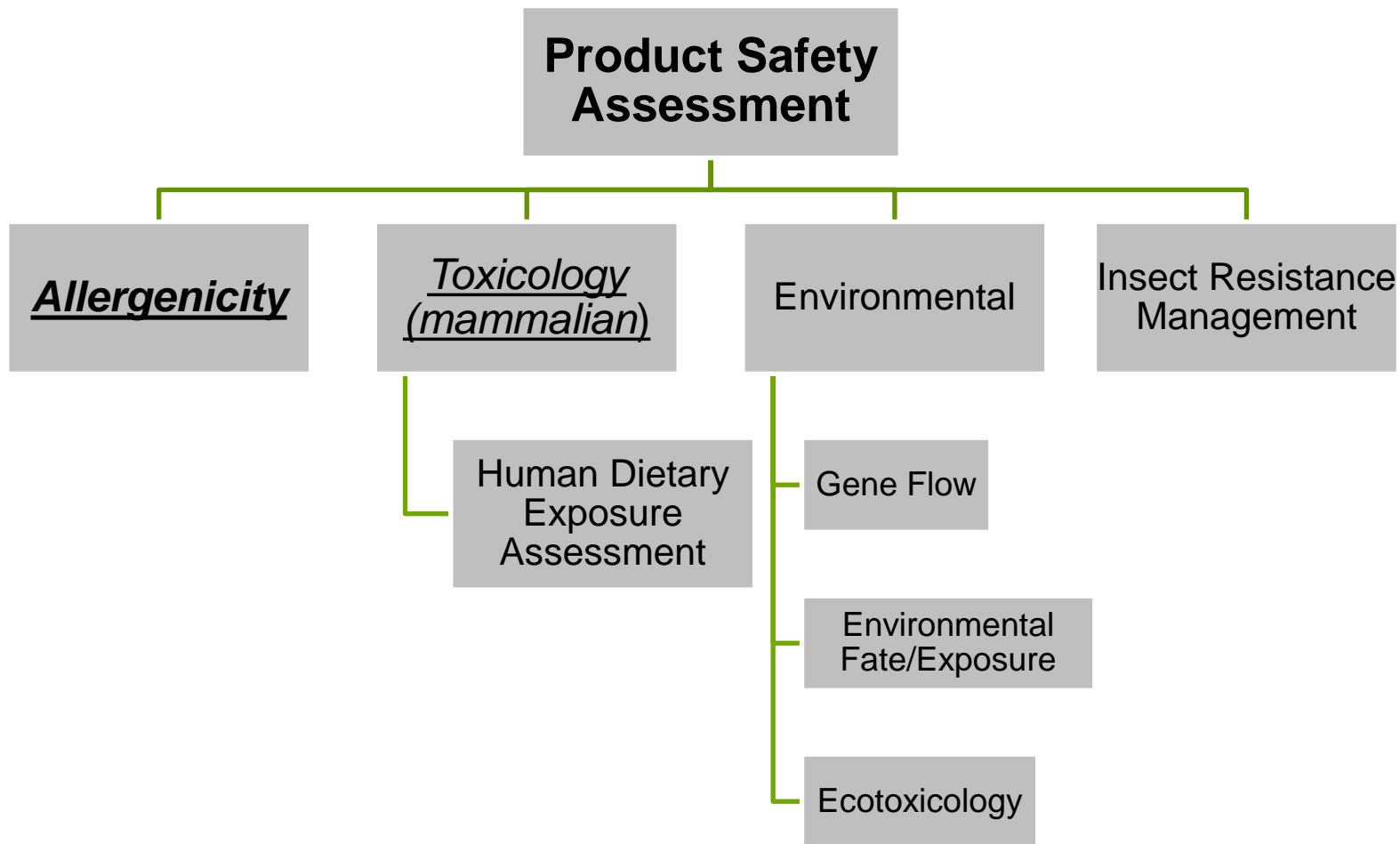
GM-Crops must be “as safe as” Non-GM

But, you can NOT guarantee absolute safety!

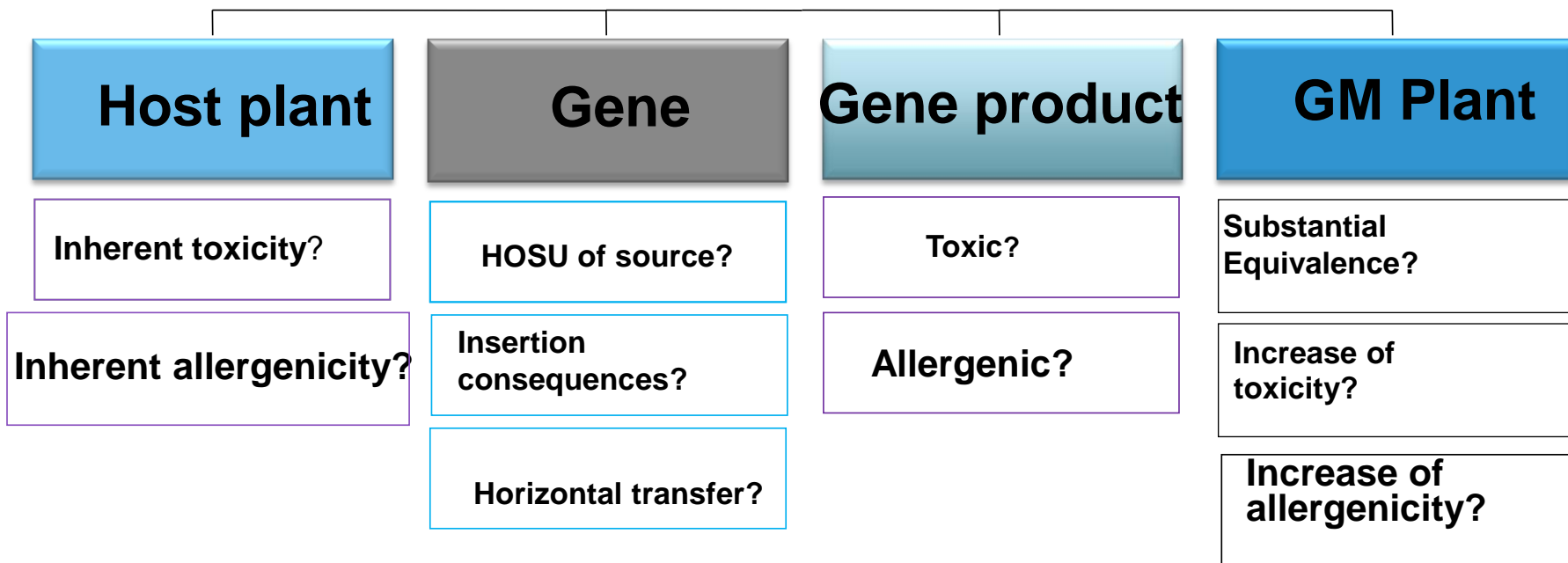
SAFETY FOCUS IS ON THE NEW GENE-PROTEIN

How do we know GM products are safe?

- **There is a comprehensive safety assessment program!**



Holistic Approach



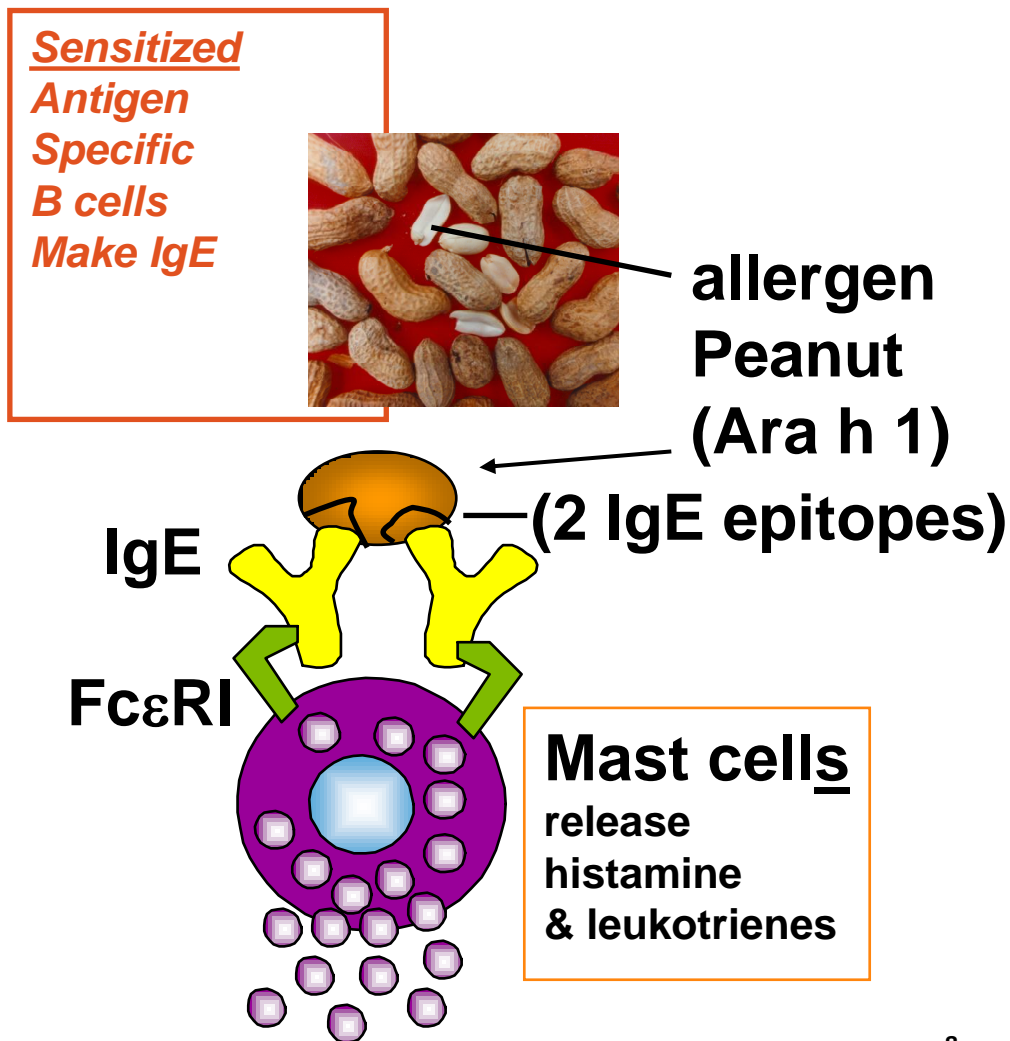
Comparison of the GM crop to a conventional equivalent with a History of Safe Use (HOSU) guides the safety assessment

Protein Allergy Assessment

Protein-specific IgE is the key mediator in Food Allergy

IgE Mediated Symptoms
10 to 20 minutes after eating:

- hives
- angioedema
- asthma
- diarrhea/vomiting
- atopic dermatitis
- anaphylaxis



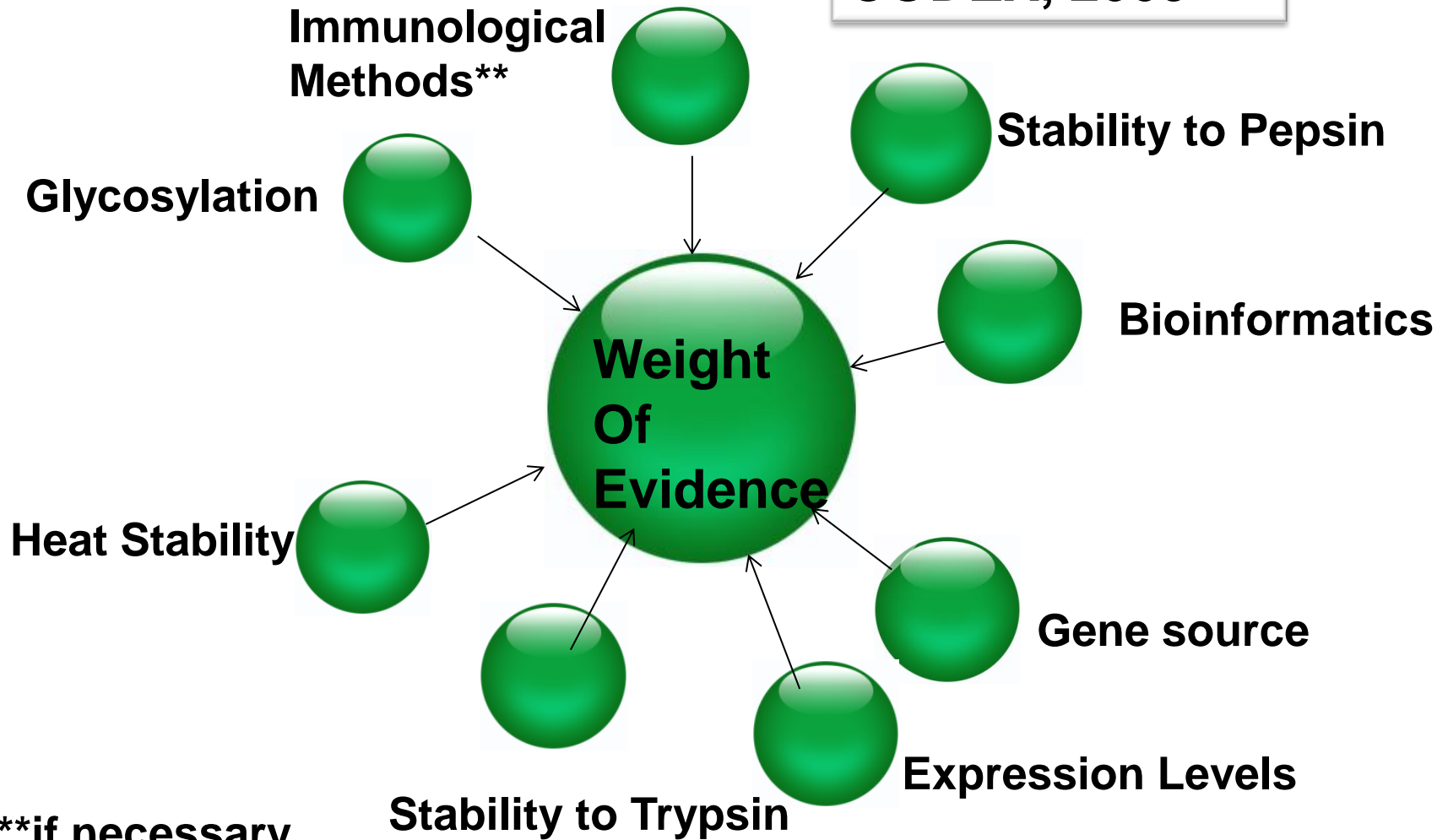
What Are The Protein Allergenicity Concerns with Ag Biotechnology?

Categories of Potential Health Risks Relative to Protein Allergenicity (in order of risk)

1. Transfer an existing allergen or cross-reactive protein into another crop.
2. Creation of food allergens de novo (i.e., potential to become a new allergen)
3. Alteration or quantitative increase of endogenous (existing) allergens (i.e., increasing the hazard of currently allergenic foods)

Weight-of-the-Evidence

CODEX, 2009



**if necessary

Categories of Potential Health Risks

Risk:

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

Alteration or quantitative increase of endogenous (existing) allergens

Creation of food allergens *de novo*

Endpoints to reduce risk per CODEX (2009):

Bioinformatics/Immunological methods

Analytical methods

Physical properties of protein (e.g., stability in SGF; heat)

How does bioinformatics help?

Allows one *primary* question to be asked:
Is the protein an existing allergen?

Allows one *secondary* question to be asked:
Is the protein likely to cross-react with an
existing allergen?

Bioinformatics is not intended to answer
whether a protein will “become” an allergen

Search Strategy

- **Allergen Search**
 - **Compare amino acid sequence of query protein to database containing sequences of food, dermal and respiratory allergens.**

- **Industry sponsored, peer-reviewed allergen database at Univ. Nebraska**
 - Peer-reviewed by clinical and research allergists from around the world: Japan, Europe, and U.S.
 - Well-defined criteria; posted on database website.
 - Inclusion of protein allergens (food, dermal, respiratory) based on available data in the public literature.
 - Updated once a year (Version 13)
 - Available free to the general public
 - www.allergenonline.org

Allergen Search Strategy

- Compare amino acid sequence of query protein to database containing sequences of food, dermal and respiratory allergens.
- Evaluate sequence for amino acid identity using local alignment programs, such as BLAST (or FASTA)
 - > 35% identity over an 80 or greater amino acid window

and potential (theoretical) IgE epitope matches.

- ~~≥ 8 contiguous identical amino acids~~ (EFSA 2011; Ladics et al., 2011, Reg. Toxicol. Pharmacol., 60:46-53).

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Specific IgE Sera Screening

- For proteins originating from an allergenic source, or having significant homology with a known allergen, specific serum screening is conducted.
- An issue of critical importance to sera screening is the availability of well characterized, quality human sera from a sufficient number of patients. **HIPAA; patient disclosure; IRB; utilizing serum from private citizens to support a private enterprise.**
- Potential false positives/equivocal results

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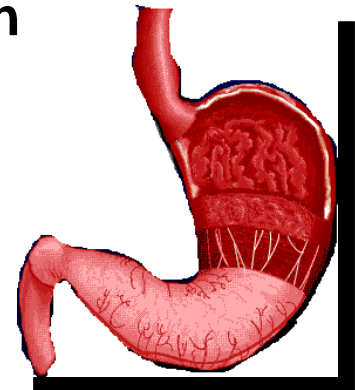
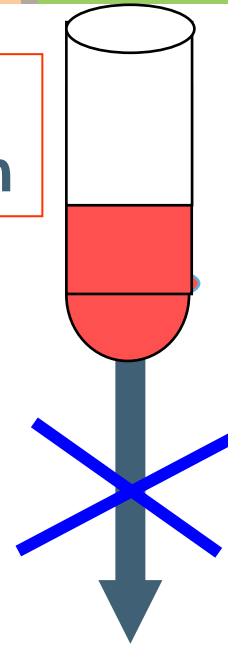
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Stability to Pepsin In Vitro

- Protein resistance to pepsin evaluated in simulated gastric fluid (pH 1.2) containing 0.3% (w/v) pepsin.
- Digestions performed for time intervals 0, 15 and 30 seconds, 1, 2, 5, 10, 15, 20, 30, and 60 minutes at 37°C.
- Samples (each protein at each time point) then analyzed by SDS polyacrylamide gel electrophoresis and/or Western blot analysis.
- A standardized protocol for evaluating the *in vitro* pepsin resistance of proteins was established (Thomas *et al.*, Regulatory Toxicology Pharmacology, 39:87-98, 2004).

pH 1.2
Pepsin



Provides a loose correlation for major food allergens (stable).

This test is not meant to “mimic” real digestion

- **Active research area; no consensus**
- **Definite need for further evaluation**
 - **selectivity**
 - **sensitivity**
 - **testing with a range of proteins**
- **None (rodent or non-rodent) validated or widely accepted**

Ladics et al., (2010). Reg. Toxicol. Pharmacol., 56:212-224

No scientific evidence that a biotech protein or a GM crop increased allergenic risk to the susceptible public

Goodman, R.E., et al., (2008). Nat. Biotechnol., 26(1):73-81.

**Goodman, R.E. and Tetteh, A.O. (2011). Curr. Allergy Asthma Rep.,
DOI 10.1007/S11882-011-0195-6**