Current Allergy Assessment Process – 1996-2007

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Prevalence of Food Allergy

Prevalence of IgE antibody-mediated food allergies among the general population-

1-2% of adults

4-6% of children

6-7 million (U.S)

Public Perception: 30%

Common Allergenic Foods

Eight foods or food groups account for over 90% of food allergies

(peanuts, soybeans, cow's milk, hen's egg, fish, crustacean, wheat, and tree nuts)

Prevalence to allergy varies geographically

- Buckwheat and rice allergy: Asia
- Fish allergy: Scandinavia
- Walnut/pecan: U.S.
- Hazelnut: Europe
- Fruit allergy: Spain

"Emerging" : avocado/kiwi; sesame seeds; spices

What Are The Protein Allergenicity Concerns with Biotechnology?

Categories of Potential Health Risks Relative to Allergenicity

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

2. Alteration or quantitative increase of endogenous (existing) allergens

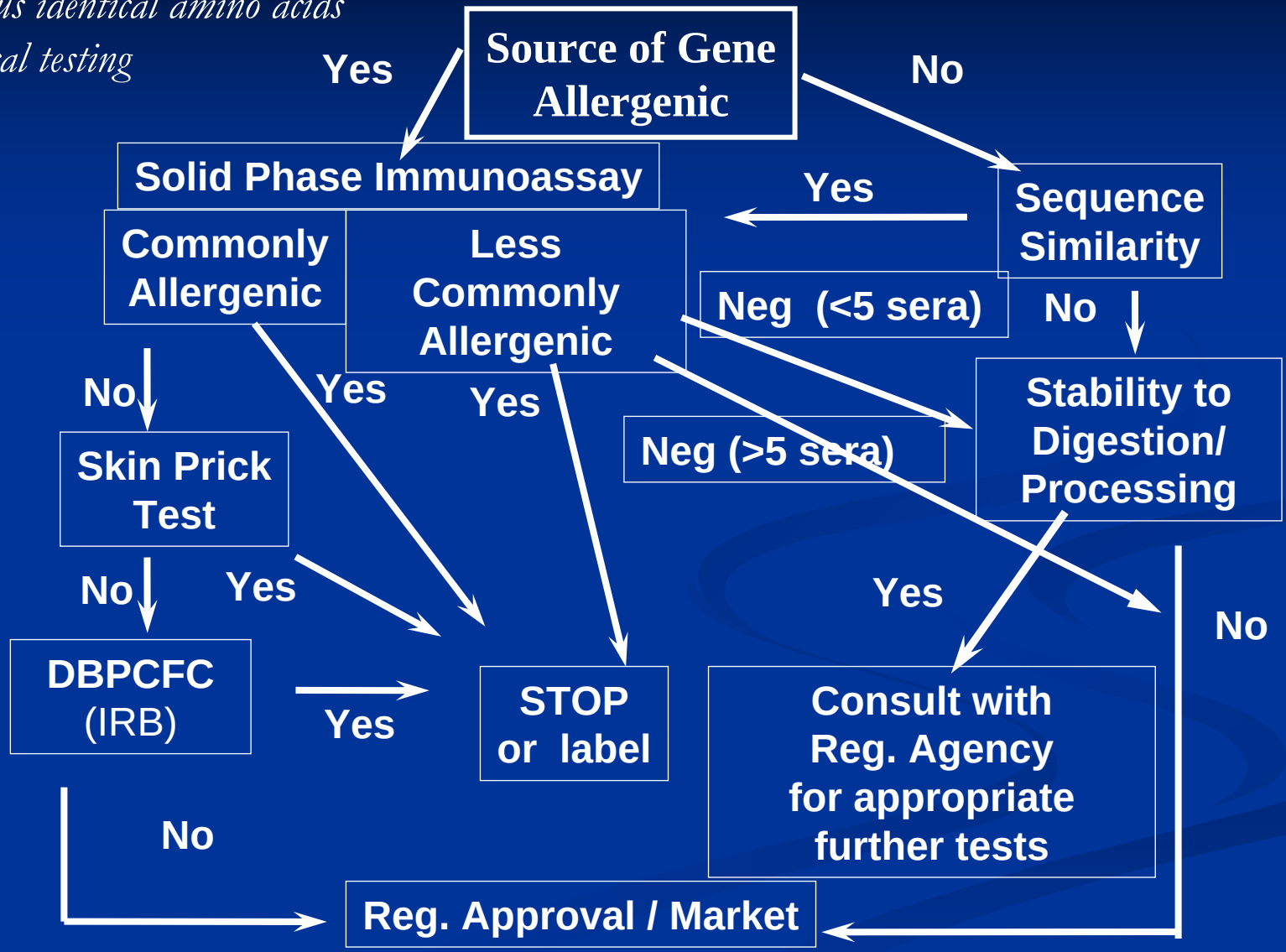
   (*i.e.*, increasing the hazard of currently allergenic foods)

3. Creation of food allergens *de novo*

   (*i.e.*, potential to become a new allergen.)

1996 IFBC/ILSI Decision-Tree

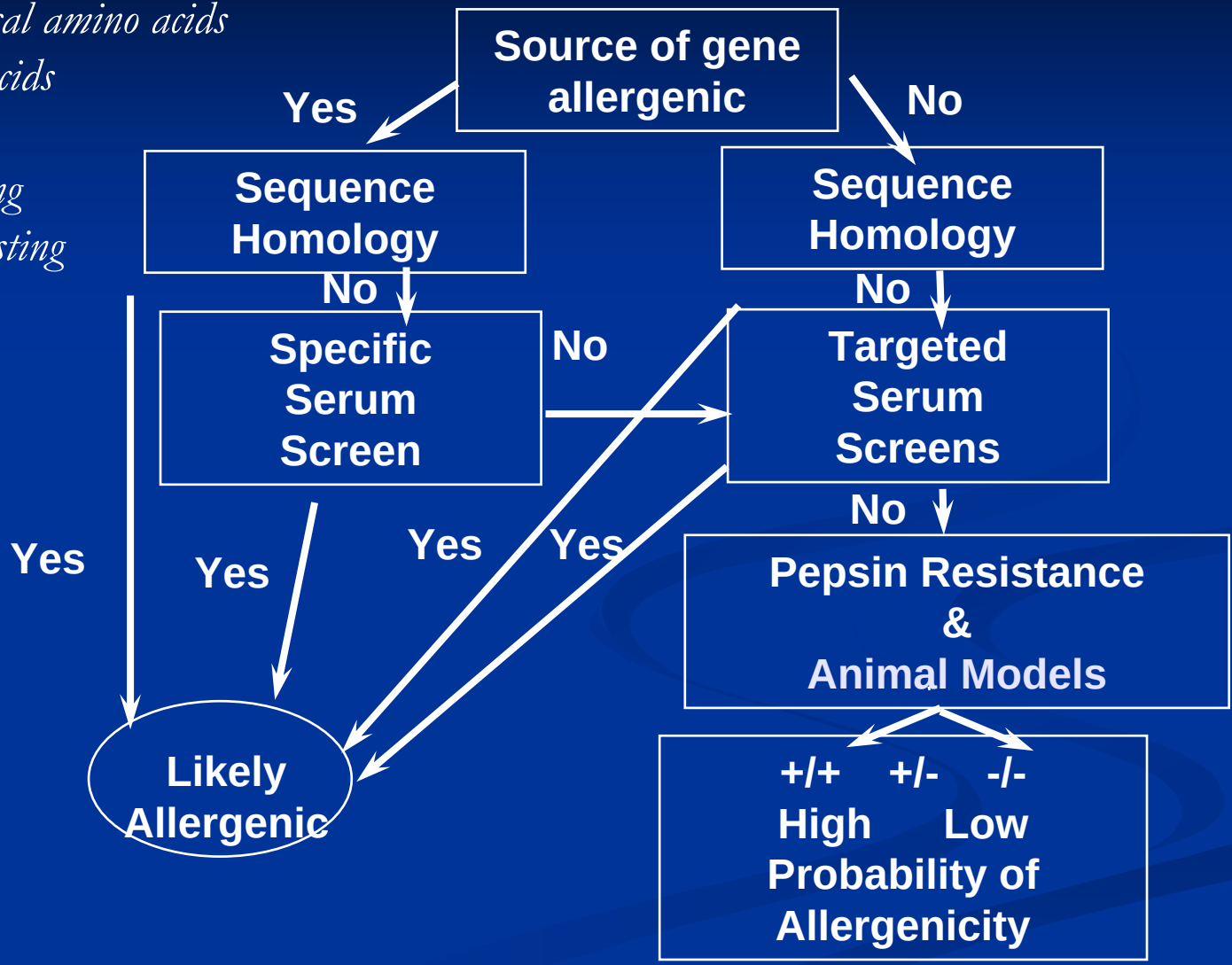
- ≥ *8 contiguous identical amino acids*
- *In vivo clinical testing*



Metcalfe et al. (1996) Crit. Rev. Food Sci. Nutr. 36:165-186

FAO/WHO 2001

- *≥ 6 contiguous identical amino acids*
- *> 35%/80 amino acids*
- *Animal models*
- *Targeted sera screening*
- *No in vivo clinical testing*



FAO/WHO 2001 Inspired Activities

6 contiguous amino acids

- Hileman et al., 2002, Int. Arch. Allergy Immunol. 128:280-291.
  - Used 6 aa and 8 aa search of corn proteins to determine % of total that matched allergens
    - 82% of corn proteins "matched" allergens on 6 aa search
- Stadler and Stadler, 2003, FASEB J. 17:1141-1143
  - 67% of Swiss-Prot sequences are "allergens" by 6 aa criteria

The use of a 6 aa sliding window search yields an unacceptably high number of false positives and does not provide any useful information in terms of regulatory decisions.

FAO/WHO 2001 Inspired Activities

- Silvanovich et al., 2006, Toxicol. Sci. 90:252-258.
  - Examined "The Value of Short Amino Acid Sequence Matches for Prediction of Protein Allergenicity"
  - Conducted a series of analyses and calculated match probabilities between a peptide sequence derived from a query protein and a sequence from a protein allergen.
  - Identification of short amino acid sequence matches (e.g., 6) using a sliding window is a product of random chance.
- Thomas et al., 2006, Mol. Nutr. Food Res., 50 (7):591-670.
  - Universal agreement - 6 contiguous identical amino acid searches were declared to lack utility in predicting protein cross-reactivity; some debate on utility of sliding window search in general

The use of a 6 aa sliding window search yields an unacceptably high number of false positives and does not provide any useful information in terms of regulatory decisions.

FAO/WHO 2001 Inspired Activities

> 35% similarity over 80 amino acid window

- Greater than 35% identity over 80 amino acids is a conservative estimate of the potential for cross-reactivity (Thomas et al., 2006, Mol. Nutr. Food Res., 50 (7):591-670).

- Conventional FASTA analysis (overall sequence alignments) produced fewer false positive findings and equivalent false negative rates; generally more significant *E* scores; a more relevant identity to the query protein; and better reflected functional similarity compared to the 80 amino acid search (Ladics et al., 2007, Mol. Nutr. Food Res. 51:985-998).

FAO/WHO 2001 Inspired Activities

Animal models for predicting protein allergenicity

- Active area of research (rodents, dogs, pigs)
- Definite need for further evaluation
  - assay selectivity
  - assay sensitivity
  - broad testing with a range of proteins
- Presently, no animal models (rodent or non-rodent) have been validated or are widely accepted.

  Thomas, et al., 2005 meeting poster, AAAAI. Multi-lab study with various mouse strains and several purified allergenic and non-allergenic proteins. Responses of allergenic proteins were similar or less than those of the putative non-allergenic proteins.

Codex Allergenicity Annex, July 2003

- *AdHoc* Intergovernmental Task Force on Foods Derived from Biotechnology

- Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants, including Annex: Assessment of Possible Allergenicity.

CODEX Guidance (2003)

- CODEX recommended allergy assessment includes:
  - Source of the introduced protein
  - Similarity of the introduced protein to known allergens
  - Susceptibility to enzymatic digestion and/or heat stability

Currently, no single test can predict food allergy for humans (weight-of-the-evidence approach)

CODEX Guidance (2003)

- CODEX recommended allergy assessment
  - ***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 to known allergens
    - assess in vitro pepsin resistance
    - assess specific IgE binding
    - assess skin prick testing on appropriate individuals

Short contiguous amino acid matches- window size should be scientifically justified

- 'scientifically defensible window size'

CODEX Guidance (2003)

- CODEX recommended allergy assessment
  - ***Other considerations***
    - Exposure level of the introduced protein
    - As science and technology evolves other methods may be considered
      - targeted sera screens
      - animal models
      - examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens

Weight-of-the-Evidence Approach

Weight-of-the-Evidence Approach

- Source of gene(s) / Crop
- Structural features of protein
  - amino acid sequence comparisons
- Biochemical / biophysical characteristics
  - pepsin resistance
  - post-translational effects
- Abundance in crop / food

Consistencies Across Recommendations

- Avoid introduction of known allergens
- Protein from allergenic source or has significant amino acid sequence identity, conduct specific IgE binding studies
- In vitro pepsin resistance
  - Standardized protocol for the *in vitro* pepsin resistance of proteins (Thomas *et al.*, 2004, Regul. Toxicol. Pharmacol., 39:87-98).

Differences Across Recommendations

- Decision tree vs. weight-of-the-evidence
- 6 vs. 8 or greater contiguous identical amino acids
- In vivo clinical testing
- Inclusion of non-validated methods
  - Animal models
  - Targeted sera screening
    - The value of targeted sera screening, as recommended by FAO/WHO 2001, has not been fully characterized or validated (Thomas et al., 2007, Toxicol. Sci. 97:27-31).

Protein Allergenicity Safety Assessment

- *Current State* - multiple documents/differing recommendations have resulted in confusion and arbitrary inclusion of tests. Decisions based on non-validated (e.g. animal models) or refuted (i.e., 6 amino acid matches) tests.
  - not consistent across geographies
- *FAO/WHO 2001*- continual impact

Desired "*Future*" State

- Harmonization of testing requirements across geographies (Codex?)
- Inclusion of endpoints based on 'sound science' (peer reviewed published data)
- Use of only 'validated' endpoints for safety assessment purposes