



Health and Environmental Sciences Institute (HESI)

## **HESI Protein Allergens, Toxins and Bioinformatics (PATB) Committee:**

**Overview of the Contributions and Research to Bioinformatics Analysis of the Potential Allergenicity of Novel Proteins**

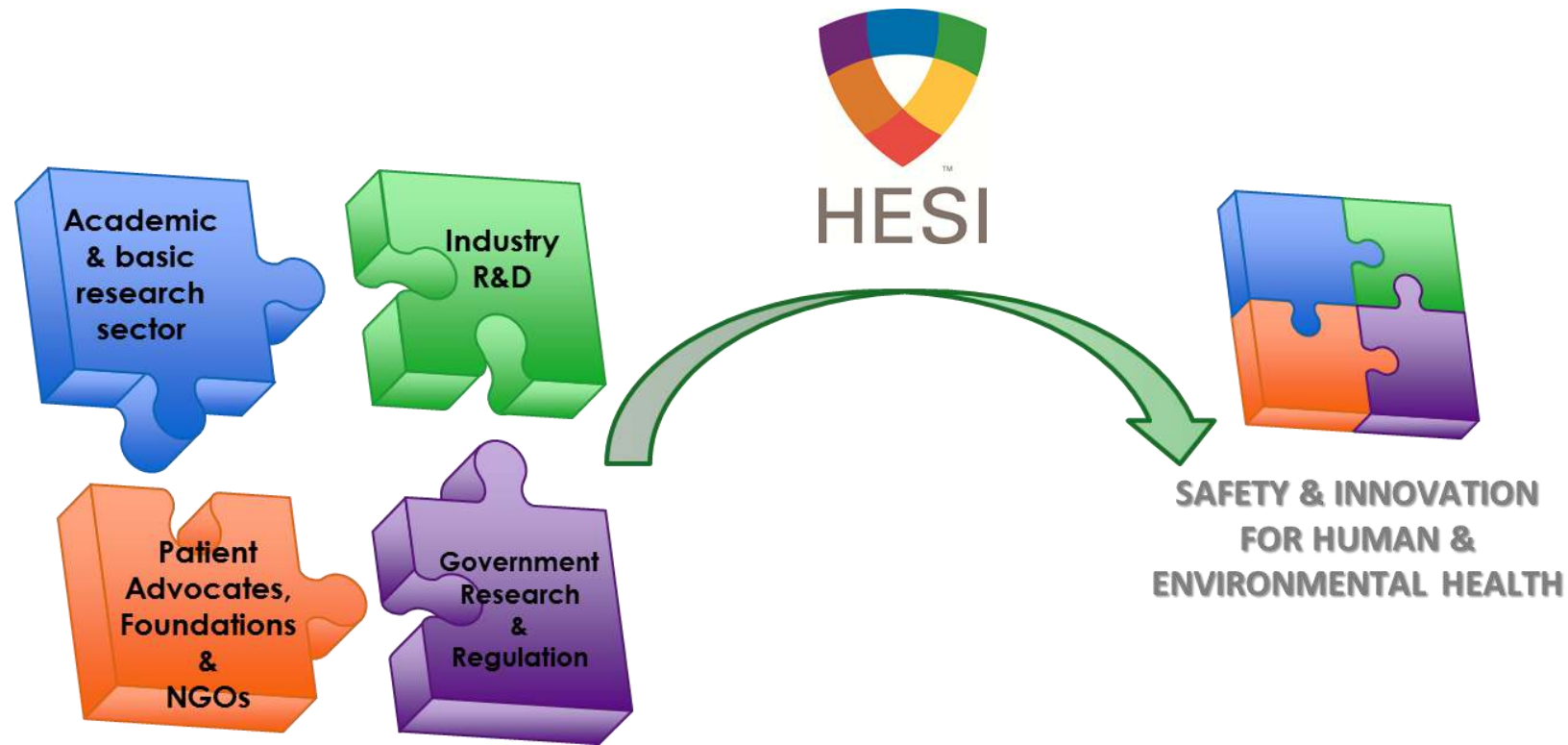
Gregory S. Ladics  
DuPont Industrial Biosciences  
Wilmington, DE  
HESI PATB Co-Chair

# Outline

- HESI & PATB Committee Background
- How did we get here?- brief history of bioinformatics and predicting potential allergenicity
- PATB activities pertaining to Bioinformatics
- Back to the future?



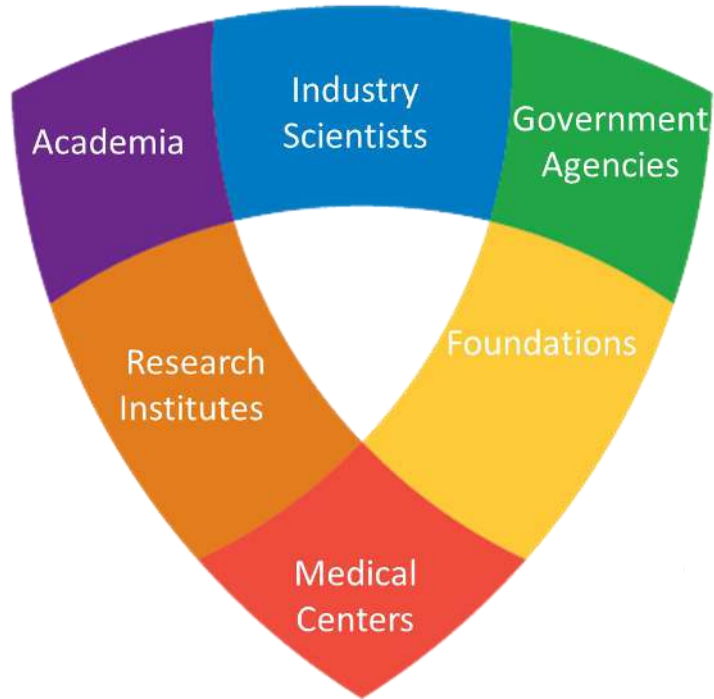
# The HESI Model: Bridging Research to Application, for almost 30 years



**HESI** is an independent non-profit dedicated to bringing together cross-sector scientists from around the world, to solve the most pressing risks and safety challenges facing humans and the environment today.



# **HESI's Mission:** *Create science-based solutions for a sustainable, healthier world*



**Connecting the best expertise to define needs and solutions.**



**Convene**



**Translate**

**Driving programs that move knowledge into application.**

**Implementing science for a safer, more sustainable world.**



**Protect**



# PATB Leadership and Staffing

## PATB Committee Co-Chairs:

- ❖ Dr. Gregory Ladics (DuPont)
- ❖ Dr. Scott McClain (Syngenta USA)
- ❖ Prof. Ronald van Ree (Academic Medical Center, University of Amsterdam)

**Staff:** Lucilia Mouriès; Lauren Peel (HESI)



# Public and Private Sector Participation

## Public Sector Participants:

- ❖ Academic Medical Center, University of Amsterdam, Netherlands
- ❖ Copenhagen University Hospital at Gentofte, Denmark
- ❖ Guangzhou Medical University (China)
- ❖ US Environmental Protection Agency
- ❖ US Food and Drug Administration

## Private Sector Participants:

- ❖ BASF Plant Science
- ❖ Bayer
- ❖ DowDuPont
- ❖ Syngenta USA



# PATB Mission

*To advance the scientific understanding of the relevant parameters defining allergenic proteins & protein toxins, as well as to encourage the development of reliable and accurate methodologies for characterizing the allergenic potential of novel proteins.*



# Committee Objectives

- ❖ Promote understanding of what makes a protein allergenic;
- ❖ Promote understanding of what makes a protein a toxin;
- ❖ Establish processes useful in a weight-of-evidence approach to evaluate the potential allergenicity of novel proteins;
- ❖ Develop scientific uniformity for these evaluations; and
- ❖ Communicate scientific findings to the academic, industry, and regulatory communities.





# Strategy to Fulfill Mission

- ❖ Focused workshops/symposia with experts from government, academia, and industry
- ❖ Harmonize the development of common approaches for *in vitro* and *in silico* assessments
- ❖ Peer-reviewed publications
- ❖ Outreach activities to update the state-of-the-art in allergy science and the role played by new information in regulatory safety assessment of food and feeds.



# PATB Areas of Interest

❖ **Biochemical Parameters** associated with allergenic proteins

❖ **Sequence Identity/**  
**Bioinformatic evaluations**

❖ ***In vitro* Models** for predicting potential allergenicity of novel proteins

❖ **Detection Methods** to support endogenous allergen assessments



# What Are The Potential Protein Allergenicity Concerns with Agriculture Biotechnology?



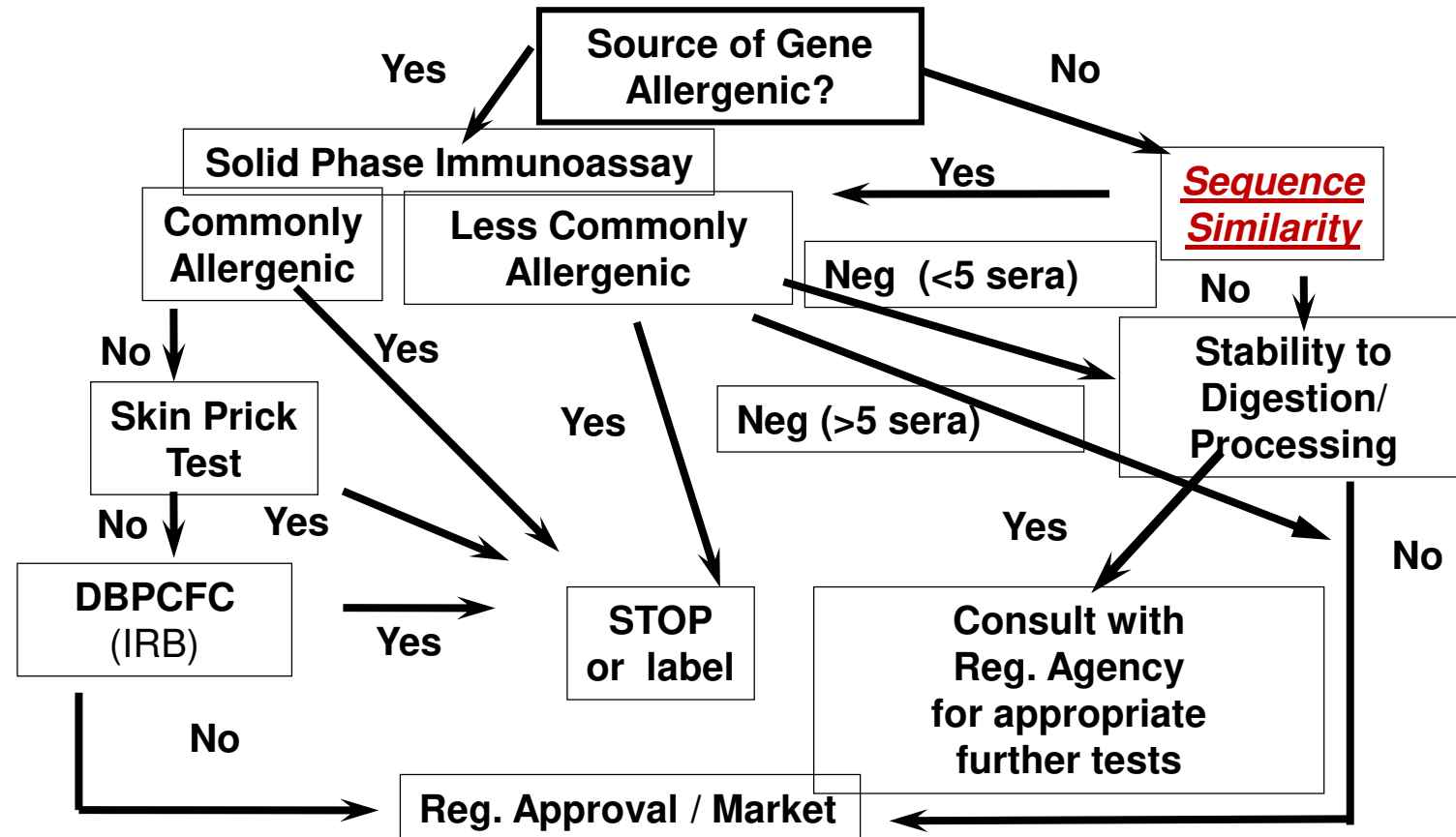
# Categories of Potential Health Risks Relative to Allergenicity

- ***Transfer an existing allergen or cross-reactive protein into another crop.***
- Creation of food allergens *de novo*
- Alteration or quantitative increase of endogenous (existing) allergens (*i.e.*, increasing the hazard of currently allergenic foods)



# In the Beginning... 1996 IFBiC/ILSI Decision-Tree

- $\geq 8$  contiguous identical amino acids



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



## ≥ 8 contiguous identical amino acid comparison

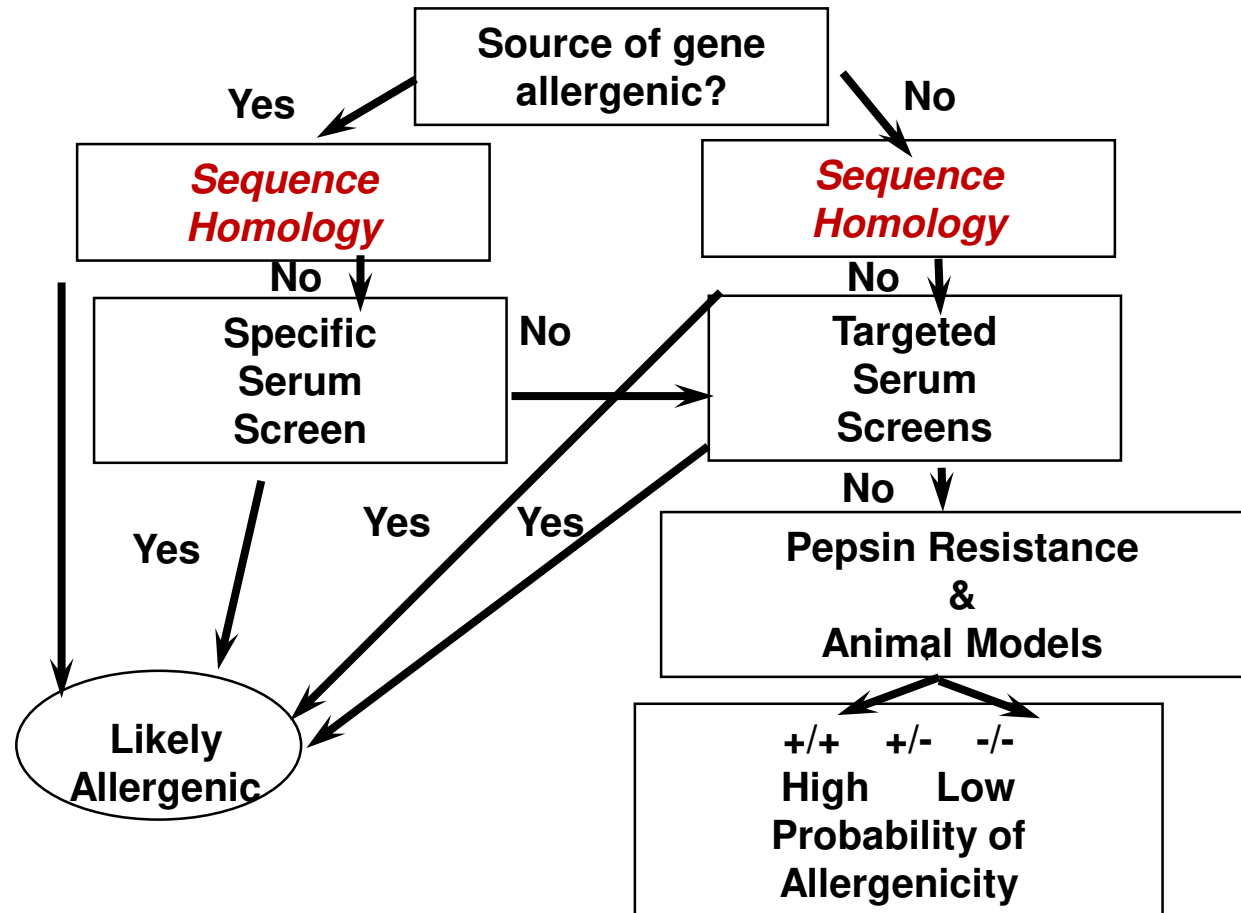
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- Original concept was suggested at 1994 workshop held by the US FDA, EPA and USDA
  - Immunologists knew that epitopes come in small sizes
  - Also knew that most IgE epitopes were “not described”
- Metcalfe et al, 1996 recommend a comparison of each theoretical 8 amino acid of a protein to all known allergens



# FAO/WHO 2001

- $\geq 6$  contiguous identical amino acids
- $> 35\%/80$  amino acid windows (FASTA or BLAST)



# Codex Guidance (2003, 2009)

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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





# PATB has been active in Bioinformatics to predict potential protein allergenicity

- I. **International Workshop on Bioinformatics, February 2005**  
(Mallorca, Spain)
- II. **International workshop October 2007:** Current and future methods for evaluating the allergenic potential of proteins, (Nice, France).
- III. **October 2008 Workshop:** Safety assessment of biotechnology products for potential risk of food allergy: implications of new research, (Washington DC, USA)



# I. February 2005 Bioinformatics workshop

- To review the state-of-the-science for conducting a bioinformatics evaluation in the context of a comprehensive allergenicity assessment for novel proteins.
- To obtain consensus on the value and role of bioinformatics in evaluating novel proteins.



# 1. February 2005 Bioinformatics workshop

1. Agreement that matches of 35% identity over 80 or more amino acids were useful for identifying proteins that could potentially cross-react.
2. The 6-mer amino acid homology is not meaningful in a safety assessment.
3. Participants recognized that bioinformatics methods were evolving, and that new methods, once they are tested and validated, may lead to improved methods for identifying potentially allergenic characteristics of novel proteins.

*Thomas, K. et al., (2005). In Silico Methods for Evaluating Human Allergenicity to Novel Proteins: International Bioinformatics Workshop Meeting Report, 23–24 February 2005, TOXICOLOGICAL SCIENCES 88(2):307–310*



## II. International workshop October 2007: Current and future methods for evaluating the allergenic potential of proteins

- Addressed issues related to current bioinformatics methods and proposed several technical improvements to this process.
- Discussed new approaches based on protein conformational structure that may be useful for refining the WOE approach in the future, when proven predictive.
- The positive predictive value of the 35% identity over an 80 aa 'sliding window' algorithm was questioned (Ladics et al., 2007). Data indicated that a conventional FASTA analysis across the whole protein sequence produced fewer false positives and equivalent false negative rates to the 'sliding window' FASTA search.
- An *E* score threshold was discussed. A FASTA *E* score cutoff of  $4.7 \times 10^{-7}$  was proposed. The cutoff was 100% effective at identifying known allergens, but was sufficiently conservative as to have a 95% false positive rate.



## II. International workshop October 2007: Current and future methods for evaluating the allergenic potential of proteins

- The workshop consensus was that the bioinformatics techniques based on linear sequence comparisons could be improved by using more advanced tools, such as  $E$  score thresholds.
- To increase the power of the bioinformatics analyses, the determination of the degree of similarity between proteins and known allergens by using a structural database and appropriate comparison scripts may prove useful in the future.

*Thomas, K., et al. (2008). Current and future methods for evaluating the allergenic potential of proteins: International workshop report 23-25 October 2007. Food Chem. Toxicol., 46:3219-3225.*



### III. October 2008 Workshop: Safety assessment of biotechnology products for potential risk of food allergy: implications of new research

- Data presented on a structural database of allergenic proteins (SDAPs) that is integrated with a variety of computational tools

**Tools Available for Assessing Potential Allergenicity Based on Structural Analyses**

Structural characterization	Source <sup>a</sup>	Comments
35% shared identity over any 80 amino acids	<a href="http://www.ebi.ac.uk/Tools/webservices/services/fasta">http://www.ebi.ac.uk/Tools/webservices/services/fasta</a>	Current approach; highly conservative
Protein families	<a href="http://pfam.sanger.ac.uk/">http://pfam.sanger.ac.uk/</a>	Allergens limited to a small subset of families <sup>b</sup>
PD	Ivanciuc <i>et al.</i> (2009b)	Based on physicochemical properties <sup>b</sup>
3D structure	Oezguen <i>et al.</i> (2008)	Conformation of epitope is important; 433 reliable 3D models <sup>b</sup>

*Selgrade, M.K., Bowman, C.C., Ladics, G.S., Privalle, L., and Laessig, S.A. (2009). Safety assessment of biotechnology products for potential risk of food allergy: implications of new research. Toxicol. Sci., 110(1):31-39.*



## *Back to the Future.....2001*

The criteria for identifying a protein as a potential allergen *has not changed* although more information about allergens has accumulated, numerous peer-reviewed publications have become available, and search algorithms have become more sophisticated.





# *Thank you!*

To Learn more:

- **PATB events and publications:**  
<http://hesiglobal.org/protein-allergens-toxins-and-bioinformatics-committee-patb/>
- **PATB 2017-2018 Fact Sheet:** *in your meeting folder!*



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## *Interested in PATB's work?*

The PATB is seeking **new public and private sector participants** with relevant technical expertise. The program also seeks **creative funding partners** and encourages inquiries by those with interest in developing innovative public resources.

Please contact Lucilia Mouriès ([lmouries@hesiglobal.org](mailto:lmouries@hesiglobal.org)) for more information.

