



## ILSI Health and Environmental Sciences Institute PROTEIN ALLERGENICITY TECHNICAL COMMITTEE

### MISSION

The mission of the HESI Protein Allergenicity Technical Committee (PATC) is to advance the scientific understanding of the relevant parameters defining allergenic proteins, as well as encourage the development of reliable and accurate methodologies for characterizing the allergenic potential of novel proteins.

### OBJECTIVES

- Promote understanding of what makes a protein allergenic.
- Establish processes useful in a weight-of-evidence approach to the evaluation of novel proteins expressed in biotech products.
- Develop scientific uniformity for these evaluations.
- Communicate findings to the academic, industry, and regulatory communities.

### STRATEGY

- Convene focused workshops and symposia with experts from government, academia, and industry.
- Support and direct basic research to evaluate utility of *in vivo* methods.
- Harmonize the development of common approaches for *in vitro* assessments.
- Report research and perspectives in peer-reviewed, scientific publications.
- Conduct outreach activities to update and communicate the state-of-the-art in allergy science and the role played by new information in regulatory safety assessment of food and feeds.

### SCOPE

The PATC's well-established reputation for unbiased scientific consensus-building has provided an excellent forum for government, academic, and industry scientists to work collaboratively to improve the science associated with conducting comprehensive allergenicity evaluations of novel proteins. PATC participants are experts in the fields of biochemistry, allergy, and toxicology and have an extensive professional network that helps support its workshops, basic research, and outreach on a global scale. This PATC represents the only HESI committee that is devoted exclusively to science issues associated with agricultural biotechnology and food safety.

An important component of the safety assessment of biotechnology products is making a determination of the allergenic potential of newly expressed proteins. The PATC engages in activities to advance the science related to predicting the risk of human allergy from exposure to novel proteins and genetically modified organisms (GMOs). These activities have largely focused on the various components of a weight-of-evidence approach for evaluating the

allergenicity of novel proteins, as described in the FAO/WHO Codex Alimentarius 2003 and 2009 guidelines for Novel Food and Feed Safety.

Research and workshop activities are focused on areas where scientific understanding of protein allergenicity assessment has been or remains ambiguous or unavailable. Projects, education, and outreach are accomplished through international workshops, symposia and roundtable discussions with recognized experts. These activities result in publications in the scientific, peer-reviewed literature. The goal is to better understand the basic science and/or technological needs for developing improved allergen assessment methodologies, and to register safe products through global regulatory product development and safety review processes. To collaboratively assess the current state of the science, identify gaps, and assist in the development of a plan to improve allergenicity assessments, the PATC engages experts from the public sector on the committee and maintains ongoing partnerships with international experts.

Since its formation in 1997, the PATC has addressed the following specific areas:

- Biochemical parameters associated with allergenic proteins
- Sequence homology / bioinformatics evaluations
- Animal models for predicting human food allergy
- Sera bank development
- Detection methods to support endogenous allergen assessments
- Development of a common *in vitro* digestive stability (SGF) protocol
- Impact of food processing on allergenicity
- Sensitizing properties of proteins

## **PARTICIPATION**

**Co-Chair:** Dr. Gregory Ladics (DuPont Pioneer)

**Co-Chair:** Dr. Scott McClain (Syngenta USA)

**Co-Chair:** Prof. Ronald van Ree (Academic Medical Center, University of Amsterdam)

**Staff:** Nancy G. Doerr, MS (HESI)  
Brianna A. Farr (HESI)

### **Public Sector:**

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

### **Private Sector:**

- BASF Plant Science
- Bayer SAS / Bayer CropScience
- DuPont Pioneer
- Monsanto Company
- Dow AgroSciences
- Syngenta Crop Protection, LLC

## RECENT AND ONGOING FOCUS

- Increasing global regulatory requests for highly technical evaluations of endogenous soybean allergens.
  - Open collaboration among industry members.
  - Two workshops bringing together technical experts who perform 2-D gels, serology, and other proteomic approaches.
  - Basic research into the technical capabilities of quantitatively determining soybean allergen content.
  - Extensive discussions with EU regulators.
  - Multiple publications in support of quantitative mass spectrometry methods for soybean allergens.
- Novel protein digestibility
  - Pepsin enzyme digestibility remains a cornerstone of novel protein safety assessments.
  - 2004 PATC initiative: Evaluated a standardized in vitro protocol to support the Simulated Gastric Fluid (SGF) methodology. Successfully completed a general protocol built on the ring-trial concept. 2004 publication.
  - 2013-2014 PATC initiative: Due to impending changes in European Commission regulatory guidance, the PATC is sponsoring an intra- and inter-laboratory evaluation of a more physiologically-based SGF assay.
- 2D-DIGE phase 2 validation: analysis of rice proteins with different cultivars
  - Inter-laboratory validation of 2D-DIGE method (two-dimensional difference in gel electrophoresis) to quantify rice allergens in four different non-transgenic rice cultivar seeds.
  - Five laboratories (two in Japan and three in the US) quantified rice allergens using 2D-DIGE analysis to determine the reproducibility of the method when performed using a common protocol.
  - In parallel, two different laboratories quantified the same rice allergens using coomassie-blue stained 2D-gel electrophoresis.
  - Final analysis of results is underway, and a manuscript will be prepared for publication.
- Protein toxins
  - Investigate approaches for identifying protein toxins.
  - Focus on bioinformatics approaches to characterize existing protein toxins.
  - Discuss modes of action and likely exposure scenarios for known protein toxins.
  - Identify the appropriate search tools (BLAST, FASTA, etc.) and homology criteria (e.g., E-score, % identity, 3-D structural information, clustering and classification of protein families and superfamilies) for comparison purposes.
  - Suggest specific guidelines to identify new protein toxins.

## PUBLICATIONS

Doerr N, Ladics G, McClain S, Herouet-Guicheney C, Poulsen L, Privalle L, Stagg N. 2010. Evaluating biological variation in non-transgenic crops: executive summary from the ILSI Health and Environmental Sciences Institute workshop, November 16-17, 2009, Paris, France. *Regul Toxicol Pharmacol* 58, S2-S7.

Houston NL, Lee DG, Stevenson SE, Ladics GS, Bannon GA, McClain S, Privalle L, Stagg N, Herouet-Guicheney C, MacIntosh SC, Thelen JJ. 2011. Quantitation of soybean allergens using tandem mass spectrometry. *J Proteome Res* 10, 763-773. [research supported by the HESI PATC]

Ladics GS, Fry J, Goodman R, Herouet-Guicheney C, Hoffmann-Sommergruber K, Madsen CB, Penninks A, Pomés A, Roggen EL, Smit J, Wal J-M. 2014. Allergic sensitization: screening methods. *Clin Transl Allergy* 4, 13.

Lee D-G, Houston NL, Stevenson SE, Ladics GS, McClain S, Privalle L, Thelen JJ. 2010. Mass spectrometry analysis of soybean seed proteins: optimization of gel-free quantitative workflow. *Anal Methods* 2, 1577-1583. [research supported by the HESI PATC]

McClain S, Bowman C, Fernández-Rivas M, Ladics GS, van Ree R. 2014. Allergic sensitization: food- and protein-related factors. *Clin Transl Allergy* 4, 11.

McClain S, Jones W, He X, Ladics G, Bartholomaeus A, Raybould A, Lutter P, Xu H, Wang X, Jia X, Chen J. 2014. Agricultural biotechnology safety assessment. *Chi J Prev Med. Submitted.*

Poulsen LK, Ladics GS, McClain S, Doerrner NG, van Ree R. 2014. Sensitizing properties of proteins: executive summary. *Clin Transl Allergy* 4, 10.

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu TJ, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herouet C, Holsapple M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, Van Ree R, Woolhiser M, Zawodny J. 2004. A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. *Regul Toxicol Pharmacol* 39, 87-98.

Thomas K, Bannon G, Hefle S, Herouet C, Holsapple M, Ladics G, MacIntosh S, Privalle L. 2005a. In silico methods for evaluating human allergenicity to novel proteins: International Bioinformatics Workshop meeting report, February 23–24, 2005, *Toxicol Sci* 82, 307-310.

Thomas K, Bannon G, Herouet-Guicheney C, Ladics G, Lee L, Lee S, Privalle L, Ballmer-Weber B, Vieths S. 2007a. The utility of an international sera bank for use in evaluating the potential human allergenicity of novel proteins: workshop report. *Toxicol Sci* 97, 27-31.

Thomas K, Herouet C, Bannon GA, Ladics GS, MacIntosh S, Privalle L, Woolhiser M. 2005b. Evaluation of mouse models for assessing the allergenic potential of proteins. *Toxicologist* 84 (S-1), 1307. (Abstract)

Thomas K, Herouet-Guicheney C, Ladics G, Bannon G, Cockburn A, Crevel R, Fitzpatrick J, Mills C, Privalle L, Vieths S. 2007b. Evaluating the effects of food processing on the potential human allergenicity of novel proteins: international workshop report. *Food Chem Toxicol* 45, 1116-1122.

Thomas K, Herouet-Guicheney C, Ladics G, McClain S, MacIntosh S, Privalle L, Woolhiser M. 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.

Thomas K, MacIntosh S, Bannon G, Herouet-Guicheney C, Holsapple M, Ladics G, McClain S, Vieths S, Woolhiser M, Privalle L. 2009. Scientific advancement of novel protein allergenicity evaluation: an overview of work from the HESI Protein Allergenicity Technical Committee (2000-2008). *Food Chem Toxicol* 47, 1041-1050.

van Ree R, Hummelshøj L, Plantinga M, Poulsen LK, Swindle E. 2014. Allergic sensitization: host-immune factors. *Clin Transl Allergy* 4, 12.

van Ree R, Poulsen LK, Wong GWK, Ballmer-Weber BK, Gao Z-S, Jia X, Chen J. 2014. Food allergy: definitions, prevalence, diagnosis and therapy. *Chi J Prev Med. Submitted.*

## CONFERENCES, SYMPOSIA AND JOINT WORKSHOPS

Events sponsored or co-sponsored by the PATC between 2001 and 2014:

- August 2014 Food Allergy and Safety Assessment Workshop, with the Kenya National Biosafety Authority, Kenya, Africa
- August 2014 GM Food / Feed Safety Assessment: Training Workshop for Regulators, with the Kenya National Biosafety Authority, Kenya, Africa
- January 2014 Meeting on the Genetic Basis of Unintended Effects in Modified Plants with the Canadian Food Inspection Agency, the ILSI International Food Biotechnology Committee (IFBiC), the ILSI Research Foundation, and CropLife International, Ottawa, Ontario, Canada  
<http://www.hesiglobal.org/i4a/pages/index.cfm?pageID=3654>
- September 2013 Scientific Workshop on Biotech Safety Assessment with ILSI India, the ILSI International Food Biotechnology Committee (IFBiC), and the Ministry of Science and Technology of the Government of India, New Delhi, India
- September 2013 Food Allergy Session at the IUNS 20th International Congress of Nutrition (ICN), with ILSI Europe, ILSI North America, Granada, Spain
- June 2013 International Meeting on Comparative Approaches to Safety Assessment of GM Plant Materials, with ILSI Argentina, the ILSI International Food Biotechnology Committee (IFBiC), and SENASA, Buenos Aires, Argentina
- May 2013 joint NAFTA Biotechnology Update Symposium with the ILSI International Food Biotechnology Committee (IFBiC), Arlington, VA  
<http://www.hesiglobal.org/i4a/pages/index.cfm?pageID=3619>
- April 2013 joint Food Allergy and Safety Assessment Workshop with the ILSI Focal Point in China, the ILSI International Food Biotechnology Committee (IFBiC), the China National Centre for Food Safety Risk Assessment, and the China Key Laboratory on Food Safety Risk Assessment, Beijing, China  
<http://www.hesiglobal.org/i4a/pages/index.cfm?pageID=3618>
- November 2012 joint Workshop on Food Safety Evaluation & Environmental Risk Assessment of GM Plants with ILSI Brasil, the ILSI Center for Environmental Risk Assessment, and the ILSI International Food Biotechnology Committee (IFBiC), Brasilia, Brazil
- November 2012 International Seminar on Protein Allergenicity hosted by ILSI Argentina, Buenos Aires, Argentina
- September 2012 joint poster at 12th International Symposium on Biosafety of Genetically Modified Organisms (ISBGMO12), with the ILSI International Food Biotechnology Committee (IFBiC), the ILSI Research Foundation, and the ILSI Center for Environmental Risk Assessment, St. Louis, MO
- June 2012 posters at European Academy of Allergy and Clinical Immunology (EAACI) Congress, Geneva, Switzerland (2D-DIGE phase 2 validation; absolute quantitation of seed allergens from three varieties of soy from eight geographical locations)
- April 2012 Symposium on Sensitizing Properties of Proteins, Prague, Czech Republic  
<http://www.hesiglobal.org/i4a/pages/index.cfm?pageid=3595>
- November 2011 joint Workshop on Safety Assessment of Novel Proteins and GM Crops with the ILSI Focal Point in China, the Chinese Centre for Disease Control and Prevention, and the ILSI International Food Biotechnology Committee (IFBiC), Beijing, China

- May 2011 joint Biotechnology Workshop 2011 with the ILSI International Food Biotechnology Committee (IFBiC) for the OECD Working Group on the Harmonization of Regulatory Oversight in Biotechnology (WGHROB) and the OECD Task Force on the Safety of Novel Foods and Feeds (TFSNFF), Paris, France
- May 2011 joint Biotechnology Update Workshop with the ILSI International Food Biotechnology Committee (IFBiC) for the Canadian Food Inspection Agency (CFIA), Ottawa, Canada
- October 2010 joint symposium with ILSI Europe, EuroPrevall, UK Food Standards Agency, and FAARP on Frontiers in Food Allergen Risk Assessment, Nice, France.
- September 2010 joint NAFTA Biotechnology Update Symposium with the ILSI International Food Biotechnology Committee (IFBiC), Washington, DC.
- November 2009 workshop on Evaluating Biological Variation in Non-transgenic Crops, Paris, France.
- October 2008 host of symposium on Efforts to Improve Techniques for Identifying and Evaluating Food Allergens, as part of the 45<sup>th</sup> Eurotox Annual Meeting, Rhodes, Greece.
- September 2008 joint symposium with ILSI SEA and the Thai National Science and Technology Development Agency (NSTDA), Bangkok, Thailand.
- September 2008 joint symposium with ILSI SEA on Biotechnology and Nutritionally Enhanced Food and Crops, Cebu, Philippines.
- April 2008 joint meeting with IFBiC, ILSI Research Foundation on ILSI Activities Related to Biotechnology, Washington, DC.
- February 2008 joint workshop with ILSI Japan and Japanese regulators on Sequence Homology and Bioinformatic Assessments, Tokyo, Japan.
- February 2008 joint workshop with the Biotechnology Coalition of the Philippines, ILSI Southeast Asia (SEA), Department of Agriculture (Philippines), and the International Service for the Acquisition of AgriBiotech Applications on Novel Protein Safety Evaluation, Manila, Philippines.
- October 2007 PATC workshop on New Methods for Allergenicity Assessments, Nice, France.
- November 2006 seminar with ILSI Argentina and Food and Feed Safety Authority of Argentina (SENASA), International Course on Food Risk Analysis, Buenos Aires, Argentina.
- November 2006 joint workshop with ILSI Brazil on Conducting a Comprehensive Allergenicity Evaluation of Novel Proteins, Sao Paula, Brazil.
- June 2006 International Effects of Food Processing on Allergenicity Workshop, hosted in partnership with ILSI Europe, ILSI International Food Biotechnology Committee (IFBiC), and ILSI Research Foundation, Estoril, Portugal.
- April 2006 International Sera Bank Development Workshop, Seoul, Korea.
- March 2005 poster at the American Academy of Asthma, Allergy, and Immunology (AAAI) meeting, San Antonio, TX.
- March 2005 poster at the Society of Toxicology (SOT) Annual Meeting, New Orleans, LA.

- February 2005 International Sequence Homology / Bioinformatics Workshop, Mallorca, Spain.
- July 2004 Symposium at International Congress of Toxicology Meeting (ICTX), Tampere, Finland.
- September 2003 poster at the Eurotox Annual Meeting, Florence, Italy.
- September 2003 joint workshop with ILSI Japan to present committee activities to scientists at National Institute of Health Sciences, Tokyo, Japan.
- March 2002, Committee-led ILSI delegation at CODEX Ad Hoc Task Force on Food Derived from Biotechnology Meeting, Yokohama, Japan.
- September 2001, Committee-led ILSI delegation at CODEX Ad Hoc Open-Ended Working Group on Allergenicity Meeting, Vancouver, Canada.

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