



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.

SCOPE

The PATC's well-established reputation for non-biased 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. This committee represents the only HESI group that is devoted exclusively to science issues associated with agricultural biotechnology.

An important component of the safety assessment of biotechnology products is to make a determination of the allergenic potential of newly expressed proteins. Consequently, the PATC engaged in a number of 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 have been focused on areas where the scientific understanding required for progress in assessing protein allergenicity was either ambiguous or unavailable. Our program areas are accomplished through international workshops, symposia and roundtable discussions with recognized experts. These activities result in publications in the scientific, peer-reviewed literature. We typically orient workshops and symposia around the goal of better understanding the basic science and/or technological needs for developing improved allergen assessment methodologies, with the ultimate goal of registering safe products through the global regulatory product development and safety review processes. We rely on a growing number of public sector PATC participants, as well as targeted collaboration with international experts, to assess the current status of the science, identify gaps, and assist in the development of a plan to improve allergenicity assessments.

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

- Development of a common in vitro digestive stability (SGF) protocol
- Assessment of molecular characteristics of food allergens
- Sequence homology evaluation / bioinformatics assessment
- Sera bank development, including coordination of regional clinical managers
- Development of animal models
- Impact of food processing on allergenicity
- Workshop on the newest methods in characterizing allergens
- Basic research to develop new soybean protein characterization methods using mass spectrometry
- Workshop on multiple “-omics” that represent cutting-edge plant profiling technologies
- Symposium on 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. Doerrer, MS (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
- DuPont Pioneer
- Monsanto Company
- Dow AgroSciences
- Syngenta USA

CONFERENCES, SYMPOSIA AND JOINT WORKSHOPS

The PATC engages scientists internationally to advance the science for novel protein allergenicity evaluations through workshops, seminars, symposia, and lectures. The following workshops and presentations have been conducted between 2001 and 2013 (beginning with the most recent):

- May 2013 joint NAFTA Biotechnology Update Symposium with the ILSI International Food Biotechnology Committee (IFBiC), Arlington, VA.
- 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

- 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
- 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 45th 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.

PUBLICATIONS

- *In preparation: Four manuscripts from the April 2012 PATC Symposium on Sensitizing Properties of Proteins, Prague, Czech Republic, for submission to Clinical and Experimental Allergy in summer 2013.*
- Doerrer, N., Ladics, G., McClain, S., Herouet-Guichenev, 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, N.L., Lee, D.G., Stevenson, S.E., Ladics, G.S., Bannon, G.A., McClain, S., Privalle, L., Stagg, N., Herouet-Guichenev, C., MacIntosh, S.C., Thelen, J.J. (2011). Quantitation of soybean allergens using tandem mass spectrometry. *J Proteome Res* 10, 763-773. [research supported by the HESI PATC]
- Dong-Gi, L., Houston, N.L., Stevenson, S.E., Ladics, G.S., McClain, S., Privalle, L., Thelen, J.J. (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]
- Thomas, K., MacIntosh, S., Bannon, G., Herouet-Guichenev, C., Holsapple, M., Ladics, G., McClain, S., Vieths, S., Woolhiser, M., and 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.
- Thomas, K., Herouet-Guichenev, C., Ladics, G., McClain, S., MacIntosh, S., Privalle, L., and 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., Bannon, G., Herouet-Guichenev, C., Ladics, G., Lee, L., Lee, S., Privalle, L., Ballmer-Weber, B., and 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(1), 27-31.
- Thomas, K., Herouet-Guichenev, C., Ladics, G., Bannon, G., Cockburn, A., Crevel, R., Fitzpatrick, J., Mills, C., Privalle, L., and 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., Bannon, G., Hefle, S., Herouet, C., Holsapple, M., Ladics, G., MacIntosh, S., and 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(2), 307-310.
- Thomas, K., Herouet, C., Bannon, G.A., Ladics, G.S., MacIntosh, S., Privalle, L., and Woolhiser, M. (2005b). Evaluation of mouse models for assessing the allergenic potential of proteins. *Toxicologist* 84 (S-1), 1307. (Abstract)
- Thomas, K., Aalbers, M., Bannon, G.A., Bartels, M., Dearman, R.J., Esdaile, D.J., Fu, T.J., Glatt, C.M., Hadfield, N., Hatzos, C., Hefle, S.L., Heylings, J.R., Goodman, R.E., Henry, B., Herouet, C., Holsapple, M., Ladics, G.S., Landry, T.D., MacIntosh, S.C., Rice, E.A., Privalle,

L.S., Steiner, H.Y., Teshima, R., van Ree, R., Woolhiser, M., and 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, 87-88.

RECENTLY COMPLETED AND ONGOING PATC-SPONSORED RESEARCH

- Proteomics method development: A quantitative approach to measuring the content of specific allergens in soybean (Dr. Jay Thelen, University of Missouri)

Soybean (*Glycine max*) seeds contain some proteins that are allergenic to humans and animals. However, the concentration of these allergens and their expression variability among germplasms is presently unknown. To address this problem, ten protein allergens from mature seed of 20 non-genetically-modified commercial soybean varieties were quantified using mass spectrometry-based approaches for relative and absolute quantitation. The results show that while the total quantity of allergens measured among the 20 soy varieties was mostly similar, individual levels of allergens were variable among germplasms. Two publications were developed on behalf of the PATC from this research by University of Missouri investigators (Lee et al., 2010; and Houston et al., 2011.)

- Absolute quantitation of seed allergens from three varieties of soy from nine geographical locations (Dr. Jay Thelen, University of Missouri)

Soybean (*Glycine max*) is an important food stock, but is considered an allergenic food with at least 16 well characterized allergens. Soybean is incorporated into commonly consumed foods, and therefore, the endogenous allergens pose a potential concern for individuals sensitive to one or more of these proteins. The protein profile of soybean can be affected by several factors including genetic, environmental, and biological. To investigate how soybean allergen content may be affected by germplasm variation and/or environment, nine soy allergens were quantified from three commercial soybean varieties grown in nine locations in three states within a single climate zone in North America; Iowa, Illinois, and Indiana, USA. Quantification was achieved using liquid chromatography-multiple reaction monitoring tandem mass spectrometry (LC-MRM) with AQUA peptide standards specific to the nine target allergens. Quantitation of allergen levels indicated that both genetics and location affected specific allergen content. Seven of the nine allergens were significantly influenced by genetics; with the exceptions of Glycinin G4 and KTI 3. The allergens P34, Gly m Bd 28k, Glycinin G3 and KTI 1 showed statistically significant impact from location as well, but at a lower threshold of significance compared with genetics (cultivar/variety). The University of Missouri investigators are preparing a manuscript for publication on behalf of the PATC.

- Comparison of 2D assay with AQUA MS approach (Donald Danforth Plant Science Center, St. Louis, MO)

This research was implemented to measure soybean allergen protein levels from one variety grown in multiple locations, using both 2D gel electrophoresis coupled with protein quantitation using tandem mass spectrometry. The purpose of this work was to address the technical limitations of electrophoresis and to compare the levels of inherent technical variability with the less established mass spectrometry methodology using side-by-side data comparisons. The European Union was fundamentally interested in endogenous allergen studies for biotech crops and promoted electrophoretic methods because they were the technology available at the time.

- 2D-DIGE phase 2 validation: Analysis of rice proteins with different cultivars (Dr. Reiko Teshima, Japan National Institute of Health Sciences)

Understanding the natural variability of the plant proteome is crucial for the interpretation of biological and safety-relevant differences between transgenic and non-transgenic parental lines. The PATC undertook an inter-laboratory validation of 2D-DIGE analysis (two-dimensional difference gel electrophoresis) of three known rice allergens in four different non-transgenic rice cultivar seeds. Five laboratories, two in Japan and three in the US, quantified the 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. The study demonstrated that some rice allergens could be efficiently quantified using the 2D-DIGE method; however, the extraction and detection method for basic proteins needs to be optimized in order to obtain more consistent data among different laboratories. Final analysis of results is underway, and a manuscript will be prepared by Dr. Teshima for publication.

- Intestinal stem cells to assess safety and efficacy of various compounds (Dr. Raymond Pieters, Utrecht University of Applied Sciences, Utrecht, The Netherlands)

Currently, there are no available endpoints to predict whether a protein will become an allergen *de novo*. Although some animal models have been evaluated and used for this purpose, none of them demonstrate the required level of specificity and reproducibility to predict whether a protein has allergenic potential. In this multi-phase project, a mouse intestinal stem cell-line is used that contains epithelial subsets known to play an essential role in food allergy and to secrete various cytokines in response to external stimuli. The PATC has provided funding for Phase I of the research, which explores whether there are unique cytokine fingerprints for allergens vs. non-allergens. Phase I of the research should be completed by the end of 2013, after which Utrecht University investigators will prepare a manuscript for publication on behalf of the PATC.

- New digestibility model(s) for investigating allergenicity of proteins. (Academic Medical Center / University of Amsterdam, The Netherlands; Bayer SAS, Sophia Antipolis, France)

The current pepsin resistance test (i.e., the human simulated gastric fluid [SGF] test) does not correlate well with allergenicity nor does it reflect physiological conditions of digestion. Recently, the European Food Safety Authority (EFSA) recommended that other *in vitro* digestibility tests on newly expressed proteins be performed under more realistic physiological conditions. To address this need, the PATC will identify and test a new digestibility model that is based on a stomach simulated digestion (modified SGF) combined with a duodenal simulated digestion (modified SIF). In a phased approach, the PATC will sponsor the following research: 1) Set up the model and define experimental conditions; 2) evaluate correlation with allergenicity by testing pairs of allergenic and non/low-allergenic proteins using the conditions determined during Phase 1; 3) evaluate robustness and reproducibility by inter-laboratory comparison; and 4) investigate the food matrix effect. Phases 1 and 2 are currently underway, and will be completed in the spring of 2014. One or more manuscripts for publication will be developed.

HESI PATC Contact:

Nancy G. Doerrer, MS
HESI Associate Director
202-659-3306, x116; ndoerrer@hesiglobal.org