HESI PROTEIN ALLERGENICITY TECHNICAL COMMITTEE (PATC)

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HESI PATC Co-Chair

ILSI Health and
Environmental Sciences
Institute

Leadership and Staffing

Co-Chairs:

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

Staff: Nancy G. Doerrer, MS (HESI)

Public and Private Sector Participation

Public 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

Sponsors:

- BASF Plant Science
- Bayer SAS
- DuPont Pioneer
- Monsanto Corporation
- Dow AgroSciences
- Syngenta USA

PATC Mission

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

HESI PATC is Qualified to Address Allergy Safety Issues

- **The PATC's reputation for unbiased, scientific consensus-building provides an excellent forum for government, academic, and industry scientists to work collaboratively to move the science in this area forward.**
- **This is the only HESI committee devoted exclusively to science issues associated with agricultural biotechnology – a technology that impacts 14 million farmers in 25 countries and millions of consumers (Crop Life, 2010).**
- **PATC members are experts in the fields of biochemistry, allergy, and toxicology, and have an extensive professional network that helps support the various workshops, basic research, and outreach on a global scale.**
- **For the last ~2 years, we have had consistent participation by two primary academic advisors (1 is a co-chair) and 2-3 government representatives (US EPA and US FDA). The PATC has also built an extensive network of academic experts in various fields that provides a basis for ongoing symposia concepts.**

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 biotechnology products;
- Develop scientific uniformity for these evaluations; and
- Communicate scientific findings to the academic, industry, and regulatory communities.

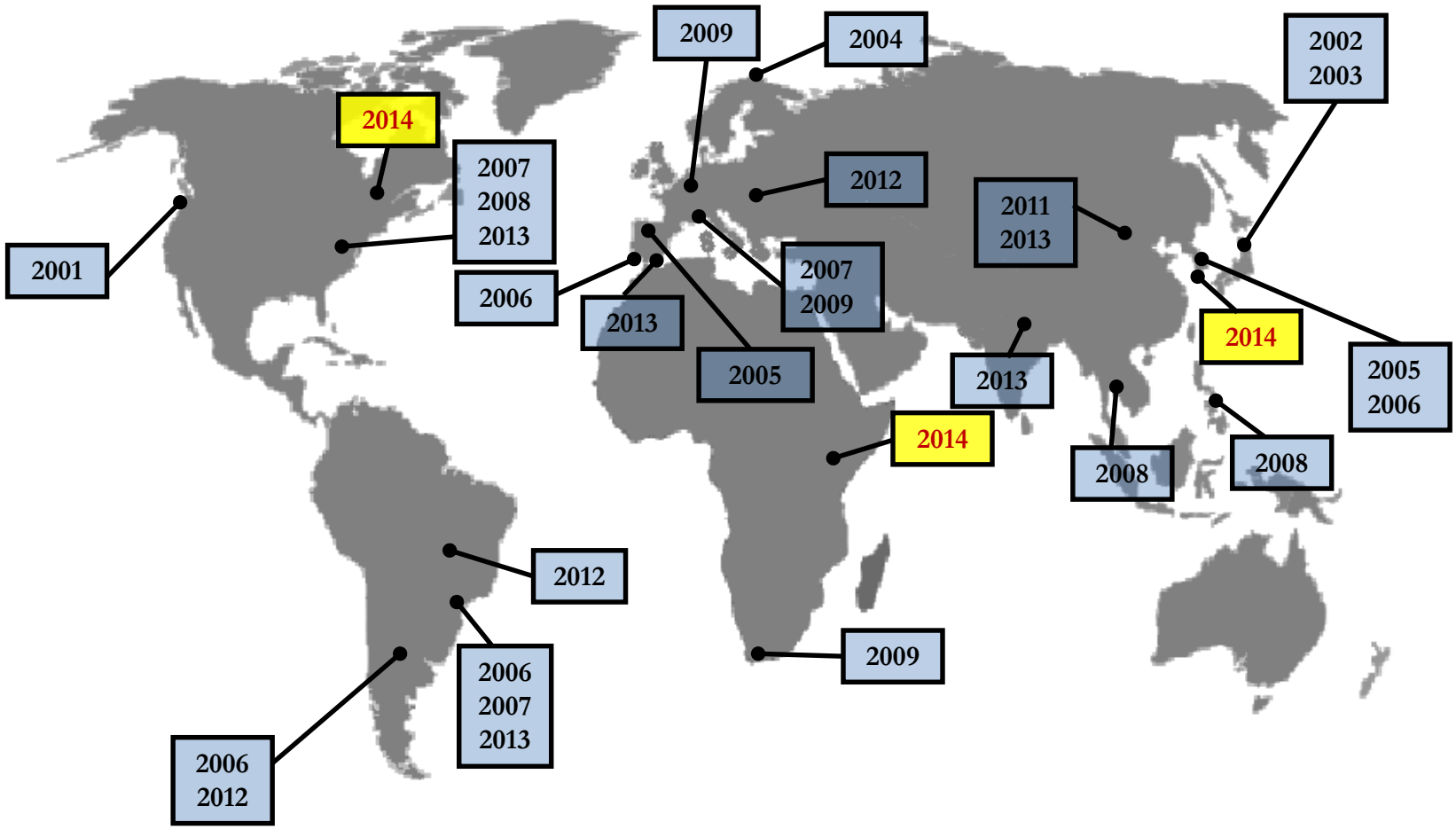
Strategy to Fulfill Mission

- **Focused workshops/symposia with experts from government, academia, and industry**
- **Support and direct basic research to evaluate utility of *in vivo* methods**
- **Harmonize the development of common approaches for *in vitro* 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**

PATC Areas of Interest

- **Biochemical Parameters** associated with allergenic proteins
- **Sequence Homology / Bioinformatics** evaluations
- **Animal Models** for predicting human food allergy
- **Protein Toxins**
- **Detection Methods** to support endogenous allergen assessments

Global PATC Impact



Collaboration with International Agencies and ILSI Branches: *Examples*

- **Joint Workshop, Ottawa, Canada, January 2014**
  - Canadian Food Inspection Agency (CFIA)
  - IFBiC

- **Joint Workshop, Beijing, China, April 2013**

At the request of Chinese regulators, the PATC supported and coordinated a broadly-scoped meeting on food allergy and food and feed safety assessment of biotech (GM) crops. (Follow-up to a November 2011 allergy symposia in Beijing.)

  - China National Centre for Food Safety Risk Assessment
  - China Key Laboratory on Food Safety Risk Assessment, Ministry of Health
  - ILSI Focal Point In China
  - ILSI International Food Biotechnology Committee (IFBiC)

Collaboration with International Agencies and ILSI Branches: *Examples, cont'd.*

- **Joint Workshop, Brasilia, Brazil, November 2012**
  - ILSI Brasil
  - ILSI Center for Environmental Risk Assessment (CERA)
  - ILSI International Food Biotechnology Committee (IFBiC)

- **Joint Workshop, Beijing, China, November, 2011**
  - Institute of Nutrition and Food Safety, Chinese CDC
  - ILSI Focal Point in China
  - IFBiC

- **Joint Workshop, Nice, France, Oct 2010**
  - UK Food Standards Agency
  - EuroPrevall
  - ILSI Europe
  - Food Allergy Research and Resource Program (FARRP), University of Nebraska

Completed Scientific Objectives: *Examples*

Symposium on Genetic Basis of Unintended Effects in Modified Plants (January 2014)

- **Symposium Objective**: The objective of this meeting was to explore current knowledge and data gaps on unintended effects and discuss how this information can inform and improve risk assessments. The meeting featured presentations on the molecular basis for unintended changes, a hypothesis-driven look at unintended effects in conventional and GM crops, and the consequences of unintended effects from a safety assessment perspective.

- **Deliverables**: Publication on meeting proceedings which will be submitted to *Transgenic Research* in 2014.

Completed Scientific Objectives: *Examples, cont'd.*

Symposium on Sensitizing Properties of Proteins, Prague, Czech Republic (April 2012)

- **Symposium Mission**: Significantly less attention has been given to assessing whether a new protein may become a food allergen independent of previous sensitizations. The purpose of the symposium was to summarize current knowledge about the sensitizing properties of proteins and to explore the applicability of emerging experimental techniques.
  - Global experts from various disciplines and fields provided overviews of the present knowledge of the mechanisms by which proteins in foods may cause sensitization. Speakers also provided a broad overview of experimental models of protein sensitizing potential spanning from in silico procedures to in vivo techniques.
- **Deliverables**: Four summary papers published in *Clinical and Translational allergy* in 2014.

Completed Scientific Objectives, *Examples, cont'd.*

- **Proteomics Workshop (2009)**
  - **Natural variability in non-genetically engineered crops. Special issue publication of twelve papers in *Regul Toxicol Pharmacol* (2010).**
- **New Methods Workshop (2007)**
  - **State of allergy science and new methods. Special issue publication of eleven papers in *Regul Toxicol Pharmaco*l (2009).**
- **Food Processing Workshop (2006)**
  - **Processing effects on allergens and methods to determine allergen content in foods. Special issue publication of six papers in *Mol Nutr Food Sc*i (2009).**
- **Sera Bank Workshop (2006)**
  - **Evaluation of a coordinated effort in serum collection and application of in vitro studies. Special issue publication of seven papers in *Food Chem Toxicol* (2008).**
- **Bioinformatics Workshops (2005)**
  - **Outreach to support science-based approaches in allergy safety. *Toxicol Sci* (2005).**
- **Animal Model Development (2005)**
  - **Multi-laboratory study with standardized proteins. *Regul Toxicol Pharmacol* (2010).**

Recent Biotech Issues Engaged by PATC

- **Increases in global regulatory requests for highly technical evaluations of endogenous soybean allergens.**
  - Addressed through open collaboration among industry members.
  - Supported 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.

Recent Biotech Issues Engaged by PATC, *Continued*

NOVEL PROTEIN DIGESTIBILITY (SGF)

- **Ongoing PATC challenge**. Pepsin enzyme digestibility remains a cornerstone of novel protein safety assessments. This represents a unique continuation of a 2004 PATC initiative with more recent changes in regulatory guidance.
- **Original Goal**: Evaluate a standardized in vitro protocol to support the Simulated Gastric Fluid methodology. In 2004, the PATC completed a successful general protocol built on the ring-trial concept. Publication in *Regul Toxicol Pharmacol* (2004).
- **2014 Initiative**. Due to impending changes in European Commission regulatory guidance, the PATC is addressing forthcoming requests to industry for improved methods. The PATC is directly supporting research into the technical considerations for a new method.

Research and Activities for 2014

- **Intra- and Inter-laboratory evaluation of a more physiologically-based SGF assay**
- **2D-DIGE phase 2 validation with rice (Dr. Reiko Teshima, Japan National Institute of Health Sciences)**
- **Protein toxin working group**

Ongoing and New Research for 2014, *cont'd.*

- **Intra- and Inter-laboratory evaluation of a more physiologically based SGF assay (i.e., digestibility).**
  - **Purpose:** Address requests by the EU guidance to provide a more physiologically relevant pepsin digestion assay to assess novel protein digestion potential.
  - **Plan:** A protocol will be assessed whereby pairs of proteins that are either known allergens or known non-allergens will be evaluated under a number of parameters (pH, time of exposure, etc.).
  - **Status:** Proteins have been identified and initial development of protocol completed. A ring-trial design will be initiated.

Ongoing and New Research for 2014, *cont'd.*

- **2D-DIGE phase 2 validation with rice (Dr. Reiko Teshima, Japan National Institute of Health Sciences)**
  - **Purpose:** Ring-trial assessment of the 2D-DIGE method (two-dimensional difference in gel electrophoresis) to quantify rice allergens several rice varieties.
  - **Plan:** Five independent academic laboratories have tested three known rice allergen families in four different rice cultivars using a common protocol and the same starting biological material.
  - **Status:** 2/3 rice allergen families could be efficiently quantified using 2D-DIGE method. The data also indicate that the extraction and detection method for basic proteins (third allergen family) needs to be optimized in order to obtain more consistent data among different laboratories.

Ongoing and New Research for 2014, *cont'd.*

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.
- Potentially develop a protein toxin database.

PATC Publications (2014)

Four publications from the April 2012 PATC Symposium on Sensitizing Properties of Proteins.

- McClain, S., Bowman, C., Fernández-Rivas, M., Ladics, G.S., and van Ree, R. (2014). Allergic sensitization: Food-and-Protein-Related Factors. *Clinical and Translational Allergy* 4:11.

- Ladics, G.S., Fry, J., Goodman. R., Herouet-Guicheney, C., Hoffmann-Sommergruber, K., Madsen, C.B., Penninks, A., Pomés, A., Roggen, E.L., Smit, J. and, Wal, J.M. (2014). Allergic sensitization: Screening Methods. *Clinical and Translational Allergy* 4:13

- Poulsen, L.K., Ladics, G.S., McClain, S., Doerrer, N.G., and van Ree, R. (2014). Sensitization properties of proteins: Executive Summary. *Clinical and Translational Allergy* 4:10

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

PATC Publications

Houston, N.L., Lee, D.G., Stevenson, S.E., Ladics, G.S., Bannon, G.A., McClain, S., Privalle, L., Stagg, N., Herouet-Guicheney, 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]

Doerrer, 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.

Lee, D.-G., 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-Guicheney, 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-Guicheney, 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.

PATC Publications, cont'd.

Thomas, K., Bannon, G., Herouet-Guicheney, 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-Guicheney, 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.