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Identifying food proteins with allergenic potential: Evolution of approaches to safety assessment and research to provide additional tools

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ABSTRACT

A safety assessment process exists for genetically engineered crops that includes the evaluation of the expressed protein for allergenic potential. The objectives of this evaluation are twofold: (1) to protect allergic consumers from exposure to known allergenic or cross-reactive proteins, and (2) protect the general population from risks associated with the introduction of genes encoding proteins that are likely to become food allergens. The first systematic approach to address these concerns was formulated by Metcalfe et al. [Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., and Fuchs, R.L. 1996. Assessment of the allergenic potential of foods from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. 36(5), 165–186.] and subsequently Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) [FAO/WHO, 2001. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. January 22–25, 2001. Rome, Italy]. More recently, Codex [Codex Alimentarius Commission, 2003. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June–5 July, 2003. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity. pp. 47–60], noting that no single factor is recognized as an identifier for protein allergenicity, suggested a weight of evidence approach be conducted that takes into account a variety of factors and approaches for an overall assessment of allergenic potential. These various recommendations are based on what is known about allergens, including the history of exposure and safety of the gene(s) source; amino acid sequence identity to human allergens; stability to pepsin digestion *in vitro*; protein abundance in the crop and processing effects; and when appropriate, specific IgE binding studies or skin-prick testing. Similarities and differences between these various suggested recommendations, as well as data gaps, are discussed. The US Environmental Protection Agency (EPA)'s Office of Research and Development (ORD) has initiated a targeted research effort to address data gaps and improve the various recommended methods/endpoints for assessing the allergenic risks associated with plant incorporated pesticides (PIPs) through both intramural and extramural (grant supported) research. The areas of primary focus for EPA include: (1) development and evaluation of animal models; (2) targeted or specific serological assays; and (3) structure–activity relationships. Details on the current as well as proposed EPA funded research are discussed. More recently US EPA has partnered with the National Institute of Allergy and Infectious Disease (NIAID), National Institutes of Health to support research in areas of mutual interest with respect to food allergy.

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1. Introduction

The incorporation of new proteins into food crops using genetic engineering to promote resistance to pests and other stressors, improve nutrition, or otherwise modify the phenotype is an important, relatively new technology that has many advantages over more conventional approaches. Genetically engineered foods, however, have raised a number of concerns (see e.g., US GAO, 2002), including the possibility that introduction of a novel protein into the food supply could result in the unintentional introduction of a new or cross-reactive food allergen and could pose a risk to susceptible individuals. Importantly, conventional breeding tactics, such as chemical and radiation mutation, can also alter existing proteins. Over the last 10 years, approaches to identifying potential food allergens for purposes of safety assessment of genetically engineered crops have been developed and modified, and research

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is underway to better understand what characteristics make a protein an allergen and provide additional predictive tools.

A food allergy is a reaction of the immune system to an otherwise harmless protein in food. Typically, such food allergic reactions are mediated by IgE and occur in atopic individuals who are genetically predisposed to allergy and who have been previously sensitized to the allergen (Sicherer, 2000). Upon initial exposure of an offending food (i.e., sensitization phase), antigen specific immunoglobulin E (IgE) is produced and binds to the surface of mast cells and basophils. Subsequent exposure of sensitized subjects to the offending food antigen produces an allergic response (i.e., the elicitation phase) by cross-linking antigen specific IgE on the surface of mast cells and basophils causing the release of various chemical mediators. These mediators in turn produce the clinical signs typically associated with food allergy (e.g., rhinitis, itching, hives, or gastrointestinal symptoms and occasionally bronchoconstriction and anaphylaxis). It should be noted that despite the large extent of food antigen exposure, only a small percentage of individuals (even among atopics) experience adverse immunologic reactions to food. The normal immune response to dietary proteins is associated with the induction of oral tolerance, a state of active inhibition of immune responses to an antigen by means of prior exposure to that antigen via the oral route. The mechanisms responsible for the development of oral tolerance are still the subject of research but involve the presentation of antigen via dendritic cells to T lymphocytes and the development of various types of regulatory T cells (Chehade and Mayer, 2005).

The incidence of food allergy ranges from 1% to 2% in adults and 6% to 8% in children (US GAO, 2002; Ladics et al., 2003). The most serious manifestation, severe anaphylaxis, occurs in approximately 3 individuals per 100,000/year (Burks and Sampson, 1997). Relatively few foods are responsible for the vast majority of significant food-induced allergic reactions. In general, the most common causal foods in children are cow's milk, egg, peanut, wheat, soy, tree nuts, fish, and shellfish. In adults the most common allergies are to shellfish, peanut, tree nuts, fish, and more recently sesame seeds. Reactions to fruits and vegetables are also common but usually not severe (Sicherer and Sampson, 2006). The prevalence of food allergy to specific foods can also vary geographically (e.g., increased buckwheat allergy in Asia; celery allergy in Europe) and is believed to be increasing worldwide (Metcalfe et al., 1996; Sampson, 1997). In the United States, this translates into approximately 6–7 million individuals with a clinically documented food allergy. Importantly, food, whether developed by conventional means or through biotechnology, is a potential source of allergens.

Before marketing genetically modified crops, such products are required to undergo an evaluation of the potential allergenic activity of the protein(s) that are produced from the introduced genes. The objectives of this evaluation are twofold: (1) protect allergic consumers from exposure to known allergenic or cross-reactive proteins that may trigger an adverse reaction in those already allergic to such proteins, and (2) protect atopic individuals from risks of allergic sensitization associated with the introduction of genes encoding proteins that are likely to become food allergens. Over the last decade, there have been three key documents published that have provided recommendations for evaluating the potential allergenicity of transgenic proteins. The first systematic approach to address the potential allergenic concerns with genetically modified crops was published by the International Food Biotechnology Council (IFBC), in collaboration with the Allergy and Immunology Institute (AII) of the International Life Sciences Institute (ILSI) in 1996 (Fig. 1; Metcalfe et al., 1996). The IFBC/ILSI report suggested the use of a decision tree approach and introduced the use of bioinformatics (i.e., $\geqslant$8 contiguous identical amino acids to identify 'theoretical' IgE epitopes) and pepsin resistance (Herman et al., 2006) for the assessment of potential allergenicity. Central to the IFBC/ILSI assessment was a consideration of the source of the transgene (i.e., allergenic or non-allergenic source). If the protein was derived from an allergenic source, an IgE binding study using sera from well-characterized patients allergic to the source was suggested, followed if necessary by additional clinical studies (e.g., skin-prick testing, food challenge studies). If from a non-allergenic source, an 8 or greater contiguous identical amino acid search and a pepsin resistance study were suggested. If a significant bioinformatics match occurred, an IgE binding study and additional clinical studies were recommended.

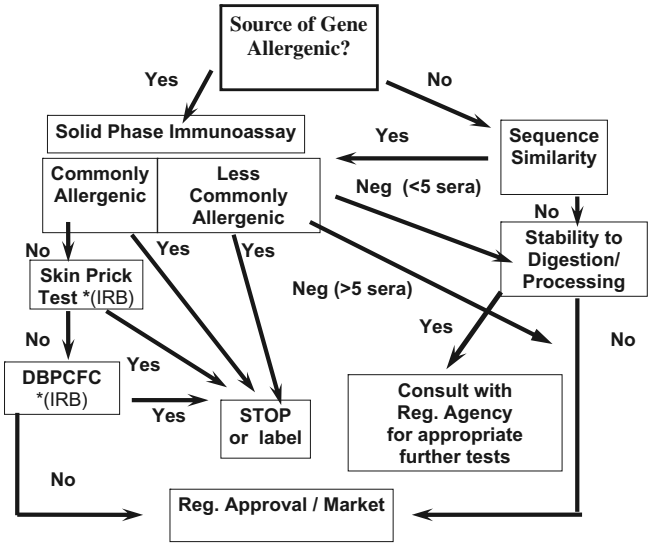

Fig. 1. 1996 International Food Biotechnology Council/the Allergy and Immunology Institute of the International Life Sciences Institute (IFBC/ILSI) Decision Tree (Metcalfe et al., 1996). Double blind placebo control food challenge (DBPCFC) studies; *institutional review board (IRB) approval required.

In 2001, the Joint Food and Agriculture Organization/World Health Organization of the United Nations (FAO/WHO) Consultation on Foods Derived from Biotechnology developed a new decision tree approach and included a number of additional recommendations (Fig. 2; FAO/WHO, 2001). Similar to the IFBC/ILSI approach, the FAO/WHO approach initially considered the source of the transgene (allergenic or non-allergenic) and subsequent bioinformatics and pepsin resistance analysis. Specific IgE binding studies using well-characterized sera from individuals allergic to

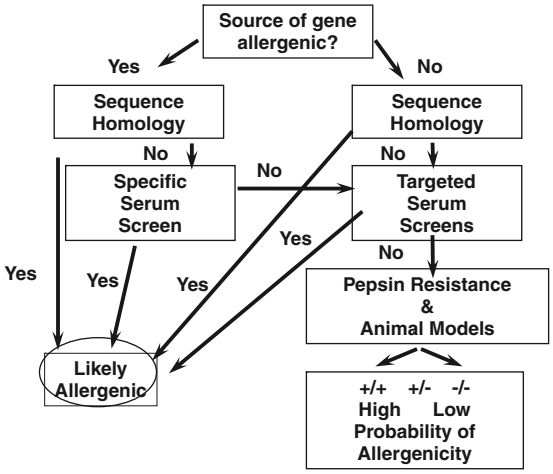

Fig. 2. The Joint Food and Agriculture Organization/World Health Organization of the United Nations (FAO/WHO) 2001 Decision Tree.

the identified allergenic source were also recommended. In contrast, the FAO/WHO eliminated human testing and modified the bioinformatics parameters (i.e., decreased the threshold from $\geqslant 8$ to $\geqslant 6$ contiguous identical amino acids and introduced a new parameter of >35% amino acid identity over an 80 amino acid window to identify potential cross-reactive allergenic proteins using FASTA or other equivalent programs). FASTA (Pearson and Lipman, 1988) is a program used for amino acid sequence (or nucleotide) comparisons and database searches. Subsequent studies, however, have indicated that the use of a 6 contiguous identical amino acid match occurs too commonly across proteins resulting in an unacceptably high number of false positive findings and should not be utilized (Hileman et al., 2002; Stadler and Stadler, 2003; Ladics et al., 2006; Silvanovich et al., 2006). Further, Ladics et al. (2007) reported that a conventional FASTA analysis (overall sequence alignments) produced fewer false positive findings (i.e., fewer non-allergenic proteins identified as allergens) and equivalent false negative rates (i.e., allergenic proteins identified as non-allergens) compared to the 80 amino acid window search suggested by FAO/WHO (2001). In addition, Ladics et al. (2007) observed generally more significant expectation (*E*) scores, which reflect the potential random occurrence of aligned sequences and can be used to evaluate the significance of an observed alignment; a more relevant identity to the query protein; and a better reflection of functional similarity with a conventional FASTA analysis. The FAO/WHO also included a number of new recommendations. These included: (1) targeted serum screening (assessment of IgE binding in sera from subjects with allergic responses to broadly related categories of foods) of proteins from non-allergenic sources; (2) targeted serum screening of proteins with no amino acid sequence homology to known allergens; and (3) use of animal models. To date, these additional recommendations have not been validated for their specificity, sensitivity, or reproducibility (Ladics et al., 2003; McClain and Bannon, 2006; Thomas et al., 2007).

As noted above, the approach by which allergy assessment was initially recommended to be conducted involved the use of a stepwise, decision tree (Metcalfe et al., 1996; FAO/WHO, 2001). Currently, however, no single factor has been recognized as predicting food allergy in humans. Therefore, a "weight of evidence" approach, which accounts for a variety of factors and experimental approaches for an overall assessment of the allergenic potential of the new protein, has more recently been recommended by the Codex Alimentarius Commission (Table 1; Codex, 2003). These assessments are currently based on what is known about food allergens such as history of exposure and safety of the gene product(s) (i.e., whether the source of the gene for the new protein is known to induce allergy), protein sequence (*in silico* amino acid sequence homology comparisons to known human allergens), physicochemical properties (e.g., stability to pepsin digestion *in vitro*; glycosylation status; heat stability), and protein abundance in the crop. When appropriate (i.e., an amino acid sequence match is observed to a known allergen or the transgenic protein is derived from a known allergenic source), specific IgE binding studies using well-characterized serum from individuals allergic to the identified source or skin-prick testing with relevant allergic subjects are also conducted. The Codex Commission also recognized that certain methods previously recommended (e.g., animal models; targeted serum screening) were not validated, but may prove useful in the future in assessing the allergenicity potential of transgenic proteins "as scientific knowledge and technology evolves" (Codex, 2003).

Table 1
Codex Guidance (2003).

Codex recommended allergy assessment
If introduced protein from a non-allergenic source
Assess amino acid sequence similarity to known allergens*
Assess *in vitro* 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
Other considerations as scientific knowledge and technology evolves
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

* Short contiguous amino acid matches-window size should be scientifically justified (i.e., a 'scientifically defensible window size').

Over the last 12 years, there have been multiple documents that have provided differing recommendations for assessing the potential protein allergenicity of transgenic proteins (Metcalfe et al., 1996; FAO/WHO, 2001; Codex, 2003). These differing recommendations have often resulted in confusion and arbitrary inclusion of tests across different geographies and subsequent safety assessment evaluations based on non-validated (e.g., animal models) or refuted (i.e., 6 contiguous amino acid matches) tests. Ideally, in the future, there needs to be better harmonization of testing requirements for assessing potential protein allergenicity across geographies and inclusion of only those endpoints that have been 'validated' based on 'sound science' (peer reviewed published data).

To address data gaps associated with methods for assessing the potential protein allergenicity of transgenic proteins, The US Environmental Protection Agency's (EPA) Office of Research and Development (ORD) in 2002, initiated a targeted research effort to enhance methods for assessing the risks associated with plant incorporated pesticides (PIPs). Currently, the EPA regulates biotechnology products that are pesticides (either produced by plants or by microorganisms) and non-pesticidal substances such as industrial enzymes, biosensors, and bioremediation agents produced using microorganisms. This initiative included both intramural and extramural (grant supported) research and addressed both ecological concerns and the potential risk to humans related to allergenicity. Described here are the research efforts associated with the latter.

In 2001, a workshop co-sponsored by the National Institute of Environmental Health Sciences (NIEHS), EPA/ORD, and the Food and Drug Administration (FDA) was held to assess the state of science related to food allergy and protein allergenicity and identify research needed to improve hazard identification in this area (Bannon et al., 2003; Bernstein et al., 2003; Germolec et al., 2003; Kimber et al., 2003; Metcalfe 2003; Selgrade et al., 2003). A number of research needs were identified: (1) Develop, evaluate, and validate animal models; (2) human studies [relationships between antigen specific IgE and overt disease, susceptibility, incidence, non-IgE allergy, and systematic recording of adverse events]; (3) better tools [human sera banks, improved skin tests, and to identify, purify, and bank proteins, both positive, and negative controls, for allergy testing]. ORD chose to focus on two areas: sequence homology approaches and development of animal models.

These research areas were selected to address a number of issues associated with identifying food allergens. Implicit in the concept of hazard identification is the assumption that there is something about the protein that makes it an allergen. However, there are currently no known structural motifs that flag a protein as an allergen, and it is unclear how structural attributes might confer allergenicity. For food allergens, characteristics such as digestibility are likely important, and the matrix in which the protein is presented may play a role. It is also assumed that allergenicity is related to the manner in which the protein interacts with the immune system. IgE is the most widely used biomarker of

allergy. In the case of food allergy, IgE production implies a deficiency in oral tolerance, also an important consideration. Hence, the interactions with the immune system likely include dendritic cells and the balance between T helper (Th)1, Th2, and T regulatory (Treg) lymphocytes. Finally, it is recognized that genetic predisposition plays a critical role in susceptibility to food allergy, although exactly what genes are involved is unclear. Like asthma, food allergy susceptibility probably involves a number of genes. Developing a laboratory rodent model of food allergy entails a number of considerations including selection of the most appropriate route of exposure, sensitization regimen, species and strain of rodent, and endpoints or biomarkers of effect. An ideal model should mimic human disease as nearly as possible. However, oral tolerance presents a major obstacle. Use of alternate routes of exposure, adjuvants, and genetic manipulation to weaken oral tolerance are among the approaches currently being used by EPA-supported investigations to develop animal models.

In 2004, EPA/ORD initiated an intramural research program to develop a mouse model of food allergy. The results of this effort are described by Bowman and Selgrade (2008a,b,c). In 2006, EPA/ORD released its first request for (grant) applications (RFA). The request listed hazard identification as the primary interest including development and evaluation of animal models, targeted or specific serological assays, and structure–activity relationships. Basic issues underlying sensitization to food allergens were listed as a secondary interest, including genetic, developmental, and other determinants of susceptibility; mechanisms underlying food allergy including the development of oral tolerance; and influences of route, duration and timing of dietary exposure on development of allergic sensitization (Fig. 3). Four grants were awarded: (1) further development of a structural database of allergenic proteins described by Braun et al. (2008); (2) delineation of appropriate specific and targeted IgE serum testing to assess potential allergenicity described by Goodman et al. (2008); (3) safety assessment of dietary proteins for allergenicity using an adjuvant free mouse model, and (4) an improved animal model for assessment of allergenic potential of foods through selective deletion of T cells and global gene expression analysis. The two grants funded to develop rodent models proposed different approaches to overcoming oral tolerance. The adjuvant free model utilizes a transdermal sensitization protocol with subsequent oral challenge (Birmingham et al., 2007). Eleven known allergenic food extracts (peanut, tree nut, egg, fish, wheat, soybean, shellfish, celery root, mustard seed, sesame seed, and milk) will be used to test the positive predictability

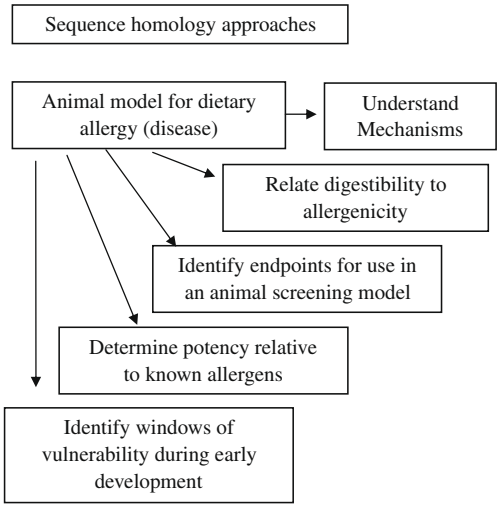

Fig. 3. US Environmental Protection Agency (EPA) Office of Research and Development (ORD) Research Strategy.

Table 2
Joint NIAID/EPA 2007 solicitation.

Research of interest primarily to EPA
Methods for assessing the risks associated with introduction of novel proteins into the food supply (including animal models, *in vitro* assays, and computational methods)
Biomarkers of exposure or effects of novel proteins in the diet of existing human or animal populations
Research of interest primarily to NIAID
Pathogenesis of food allergy, including mechanisms of allergen processing and presentation to specific T cells, T cell activation, and role of the innate immune system
Pathogenesis of severe food allergy, including role of mediators, and of pathophysiologic responses in airways or other relevant organs
Relationship between IgE-mediated food allergy and co-morbid conditions, such as atopic dermatitis, asthma, and eosinophilic gastroenteritis
Genetic components of human food allergy and of severe food allergy
Research of interest to both NIAID and EPA
Biomarkers of human food allergy and of the severity of food allergic reactions
New food allergens, including non-protein allergens and post-translationally modified protein allergens
Immune mechanisms that underlie the loss of oral tolerance and result in the development of food allergy
Food allergen epitopes and their relationship to food allergy
Genetic and environmental (e.g., microbial flora) factors, and their interactions, that modulate sensitization versus tolerance to food

of this model, and negative predictability will be assessed using 11 non-allergenic food extracts (spinach, pinto bean, vanilla, blueberry, kidney bean, mung bean, pigeon pea, amaranth, quinoa, rape seed, and sorghum). Endpoints assessed in this model include allergic antibodies, as well as a clinical score for anaphylaxis. The T cell deletion model proposed to develop a food allergy sensitive mouse by deleting T cells known to be important in the development of oral tolerance. Both projects have been underway for approximately two years. More recently EPA has released a second RFA jointly with the National Institute of Allergy and Infectious Disease (NIAID). The purpose of the current solicitation is to encourage high impact, innovative, exploratory/developmental investigations to determine mechanisms of and risk factors associated with IgE-mediated food allergy and related co-morbid conditions, focusing on ex vivo studies with human specimens and studies with current or new animal models of food allergy. A number of specific research interests are mentioned in the RFA (Table 2).

2. Summary

A number of differing recommendations for assessing the potential allergenicity of transgenic proteins have been suggested (Metcalfe et al., 1996; FAO/WHO, 2001; Codex, 2003). Some have included non-validated (e.g., animal models, targeted serum screening) endpoints (FAO/WHO, 2001). To help address data gaps associated with methods for assessing the potential protein allergenicity of transgenic proteins, the US EPA/ORD initiated a targeted research effort. EPA/ORD's research effort, although small, represents a sizeable increase in the number of investigators pursuing methods to improve hazard identification with respect to the potential risk of allergenicity associated with plant incorporated pesticides and other genetically engineered organisms. Progress is being made on several fronts in an effort to develop endpoints that have been 'validated' based on 'sound science' and subsequently resolve some of the issues that hamper risk assessment in this area.

3. Disclaimer

This paper has been reviewed by the National Health and Environmental Effects Research Laboratory, US Environmental Protec-

tion Agency and approved for publication. Approval does not signify that the contents necessarily reflects the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Bannon, G., Fu, T.J., Kimber, I., Hinton, D.M., 2003. Protein digestibility and relevance to allergenicity. Environ. Health Perspect. 111, 1122–1124.

Bernstein, J.A., Bernstein, I.L., Bucchini, L., Goldman, L.R., Hamilton, R.G., Lehrer, S., Rubin, C., Sampson, H.A., 2003. Clinical and laboratory investigation of allergy to genetically modified foods. Environ. Health Perspect. 111, 1114–1121.

Bowman, C.C., Selgrade, M.J.K., 2008a. Differences in allergenic potential of food extracts following oral exposure in mice reflect differences in digestibility: Potential approaches to safety assessment. Toxicol. Sci. 102 (1), 100–109.

Bowman, C.C., Selgrade, M.J.K. 2008. Failure to induce oral tolerance in mice is predictive of dietary allergenic potency among foods with sensitizing capacity. Toxicol. Sci. Adv. Access doi:10.1093/toxsci/kfn200.

Bowman, C.C., Selgrade, M.J.K. 2008. Utility of rodent models for evaluating protein allergenicity. Same series of papers.

Birmingham, N.P., Parvataneni, S., Hassan, H.M., Harkema, J., Samineni, S., Navuluri, L., Kelly, C.J., Gangur, V., 2007. An adjuvant-free mouse model of tree nut allergy using hazelnut as a model tree nut. Int. Arch. Allergy Immunol. 144, 203–210.

Braun, W., Schein, C.H., Barcia, T, Oezguen, N., Negi, S., Ivanciuc, O. 2008. Structural analysis of linear and conformational epitopes of allergens. Same series of papers.

Burks, A.W., Sampson, H.A., 1997. Anaphylaxis and food allergy. In: Metcalfe, D.D., Sampson, H.A., Simon, R.A. (Eds.), Food Allergy: Adverse Reactions to Foods and Food Additives. Blackwell Science, Cambridge, MA, pp. 25–245.

Chehade, M., Mayer, L., 2005. Oral tolerance and its relation to food hypersensitivities. J. Allergy Clin. Immunol. 115, 3–12.

Codex Alimentarius Commission, 2003. Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June–5 July, 2003. Appendix III, Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants, and Appendix IV, Annex on the assessment of possible allergenicity. pp. 47–60.

FAO/WHO, 2001. Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology. January 22–25, 2001. Rome, Italy.

Germolec, D.R., Kimber, I., Goldman, L., Selgrade, M., 2003. Key issues for the assessment of the allergenic potential of genetically modified foods: breakout group reports. Environ. Health Perspect. 111, 1131–1139.

Goodman, R.E., Vieths, S., Sampson, H.A., Hill, D., Ebisawa, M., Taylor, S.L., van Ree, R., 2008. Allergenicity assessment of genetically modified crops – what makes sense? Nature Biotechnol. 26, 73–81.

Herman, R.A., Storer, N.P., Gao, Y., 2006. Digestion assays in allergenicity assessment of transgenic proteins. Environ. Health Perspect. 114, 1154–1157.

Hileman, R.E., Silvanovich, A., Goodman, R.E., Rice, E.A., Holleschak, G., Astwood, J.D., Hefle, S.L., 2002. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. Int. Arch. Allergy Immunol. 128, 280–291.

Kimber, I., Dearman, R.J., Penninks, A.H., Knippels, L.M., Buchanan, R.B., Hammerberg, B., Jackson, H.A., Helm, R.M., 2003. Assessment of protein allergenicity on the basis of immune reactivity: animal models. Environ. Health Perspect. 111, 1125–1130.

Ladics, G.S., Holsapple, M.P., Astwood, J.D., Kimber, I., Knippels, L.M., Helm, R.M., Dong, W., 2003. Workshop overview: approaches to the assessment of the allergenic potential of food from genetically modified crops. Toxicol. Sci. 73, 8–16.

Ladics, G.S., Bardina, L., Cressman, R.F., Mattsson, J.L., Sampson, H.A., 2006. Lack of cross-reactivity between the Bacillus thuringiensis derived protein Cry1F in maize grain and dust mite Der p 7 protein with human sera positive for Der p 7-IgE. Regul. Toxicol. Pharmacol. 44 (2), 136–143.

Ladics, G.S., Bannon, G.A., Silvanovich, A., Cressman, R.F., 2007. Comparison of conventional FASTA identity searches with the 80 amino acid sliding window FASTA search for the elucidation of potential identities to known allergens. Mol. Nutr. Food Res. 51 (8), 985–998.

McClain, S., Bannon, G.A., 2006. Animal models of food allergy: opportunities and barriers. Curr. Allergy Asthma Rep. 6, 141–144.

Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson, H.A., Taylor, S.L., Fuchs, R.L., 1996. Assessment of the allergenic potential of foods from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. 36 (S), 165–186.

Metcalfe, D.D., 2003. Introduction: what are the issues in addressing the allergenic potential of genetically modified foods? Environ. Health Perspect. 111, 1110–1113.

Pearson, W.R., Lipman, D.J., 1988. Improved tools for biological comparison. Proc. Natl. Acad. Sci. USA 85, 2440–2448.

Sampson, H.A., 1997. Immediate reactions to foods in infants and children. In: Metcalfe, D.D., Sampson, H.A., Simon, R.A. (Eds.), Food Allergy: Adverse Reactions to Foods and Food Additives. Blackwell Science, Cambridge, MA, pp. 169–182.

Selgrade, M.K., Kimber, I., Goldman, L., Germolec, D.R., 2003. Assessment of allergenic potential of genetically modified foods: an agenda for future research. Environ. Health Perspect. 111, 1140–1141.

Sicherer, S.H., 2000. Determinants of systemic manifestations of food allergy. J. Allergy Clin. Immunol. 106, 251–257.

Sicherer, S.H., Sampson, H.A., 2006. Food allergy. J. Allergy Clin. Immunol. 117 (2 Suppl Mini-Primer), S470–S475.

Silvanovich, A., Nemeth, M.A., Song, P., Herman, R., Tagliani, L., Bannon, G.A., 2006. The value of short amino acid sequence matches for prediction of protein allergenicity. Toxicol. Sci. 90 (1), 252–258.

Stadler, M.B., Stadler, B.M., 2003. Allergenicity prediction by protein sequence. FASEB J. 17, 1141–1143.

Thomas, K., Bannon, G., Herouet-Guicheney, C., Ladics, G., Lee, L., Lee, S., Privalle, L., Ballmer-Weber, B., Vieths, S., 2007. The Utility of an international sera bank for use in evaluating the potential human allergenicity of novel proteins. Toxicol. Sci. 97 (1), 27–31.

United States General Accounting Office (GAO), 2002. Genetically modified foods: Experts view regimen of safety tests as adequate, but FDA's evaluation process could be enhanced, GAO-02–566. Washington, DC. Available from: <http://www.gao.gov/new.items/d02566.pdf>.