Key Issues for the Assessment of the Allergenic Potential of Genetically Modified Foods: Breakout Group Reports

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On the final afternoon of the workshop "Assessment of the Allergenic Potential of Genetically Modified Foods," held 10–12 December 2001 in Chapel Hill, North Carolina, USA, speakers and participants met in breakout groups to discuss specific questions in the areas of use of human clinical data, animal models to assess food allergy, biomarkers of exposure and effect, sensitive populations, dose–response assessment, and postmarket surveillance. Each group addressed general questions regarding allergenicity of genetically modified foods and specific questions for each subject area. This article is a brief summary of the discussions of each of the six breakout groups regarding our current state of knowledge and what information is needed to advance the field. ***Key words:*** **animal models, food allergy, hazard identification, IgE, immunoassay, postmarket surveillance, safety assessment, sensitization, skin prick test, threshold.** ***Environ Health Perspect*** **111:1131–1139 (2003). doi:10.1289/ehp.5814 available via** ***http://dx.doi.org/*** **[Online 19 December 2002]**

On the final afternoon of the workshop "Assessment of the Allergenic Potential of Genetically Modified Foods," held 10–12 December 2001, in Chapel Hill, North Carolina, speakers and participants met in breakout groups of 8–12 individuals to discuss key issues in the following areas: use of human clinical data, animal models to assess food allergy, biomarkers of exposure and effect, sensitive populations, dose–response assessment, and postmarket surveillance. Each group was asked to address general questions regarding what can be done to assess the potential allergenicity of genetically modified (GM) foods and what is needed to improve this process, as well as questions specific to each particular topic. In many instances, the discussion topics overlapped such that a number of topics were addressed by multiple groups, and in some cases their conclusions differed. Following is a brief summary of the discussions of each of the six breakout groups regarding our current state of knowledge and what information is needed to advance the field. Each breakout group contained individuals with a wide variety of expertise so that the subject material could be covered fully. The text below is an effort to capture the expertise and opinions of diverse participants and as detailed in the text below, in some instances consensus was not achieved.

Use of Human Clinical Data

How important are the following end points in hazard identification and dose response: immune indicators of sensitization (IgE, skin test positivity), clinical symptoms from skin, gut, respiratory tract after provocation (DBPCFC), and anaphylaxis? A clinical syndrome suggestive of an IgE-mediated reaction (flushing, urticaria, angioedema, wheeze, stridor, abdominal pain, vomiting, or cardiovascular collapse) after the ingestion of an allergenic food can be confirmed with a skin prick test (SPT) or serum-specific IgE. However, in the absence of a clinical history suggestive of allergy, IgE detection, whether SPT or specific IgE, serves as a good indicator of sensitization but not necessarily of disease. Conversely, in the clinical setting, the absence of detectable IgE may be useful at excluding IgE-mediated food allergy. However, this depends somewhat on the specific antigen and techniques used. It is possible to obtain positive SPT results in individuals who test negative for serum IgE, as antigen-specific IgE may be predominantly cell bound when present at low levels. Interpretation of the usefulness of SPT or food-specific IgE rests with the comparison of SPT/specific IgE results with the outcome of double-blind, placebo-controlled food challenge (DBPCFC), which is currently the "gold standard" for determining food allergy. Approximately 50% of positive SPTs correlate with confirmed DBPCFCs, suggesting that sensitization to food allergens occurs in the absence of clinical symptoms (Bock et al. 1977, 1978; Eigenmann and Sampson 1998). The magnitude of the SPT or specific IgE measurement is useful in predicting the likelihood of clinical allergy (as confirmed by DBPCFC) but is not useful in predicting severity (Eigenmann and Sampson 1998; Sampson 2001).

The DBPCFC is an excellent method for confirming suspected allergy. In a controlled setting with experienced clinicians, the DBPCFC can be safely performed (Bock et al. 1978, 1988; Watson 1995; Williams and Bock 1999). The rapidity of IgE-mediated reactions (> 90% within 1 hr) allows the DBPCFC to reproduce objectively IgE-mediated symptoms resulting from the food administered.

Theoretically, any food containing a protein could elicit an allergic reaction; however, eight common foods are responsible for > 90% of food allergies. The remaining 10% of food allergies result from over 150 different proteins (Hefle et al. 1996). Data are becoming available on threshold doses required to provoke an allergic reaction in previously sensitized individuals. Recently, the results of 10 independently conducted clinical challenge studies have been reported (Taylor et al. 2002). In these 10 well-defined clinical studies involving peanut, milk, egg, fish, and mustard allergens, 0.25 mg peanut protein (equivalent to 1 mg whole peanut) was the lowest provoking dose and was therefore considered to be the lowest observable adverse effect level (LOAEL) for elicitation. Of the 10 studies, one reported four subjects, from a study cohort of 74, who developed an allergic response to this LOAEL. In the other nine studies, no other individuals with this degree of sensitivity were identified. It should also be noted, however, that the responses in these four individuals were mild and reversed spontaneously (Taylor et al. 2002).

What end points are appropriate for the premarket assessment of previously sensitized individuals to source proteins versus the postmarket assessment of potentially sensitized individuals to source proteins as well as the potential sensitization and allergenicity of novel proteins? In premarket assessment of novel proteins for hazard identification, the group fealt tht there was no role for SPT or measurement of specific IgE, as sensitization would not have occurred. However, when the protein in question originates from a known

This article is part of the mini-monograph "Assessment of the Allergenic Potential of Genetically Modified Foods."

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The authors declare they have no conflict of interest.

Received 31 May 2002; accepted 9 December 2002.

allergic source or a potentially allergic source, clinical testing would be valuable. Clinical testing could include specific serum testing from well-defined allergic individuals and/or SPT. SPT may only be necessary in situations where human serum IgE is detected to the GM protein and the product is to be further developed. It is expected, however, that in most circumstances where specific serum screening is positive, there would not be any further development of the product.

In a postmarket assessment, if IgE is detected either serologically or by skin testing, the relationship between the detected IgE and clinical symptoms may be confirmed using DBPCFC.

What constitutes "harm" (a term used in standards for food safety evaluation by both the U.S. Food and Drug Administration and U.S. Environmental Protection Agency)? Is sensitization harm? What about clinical symptoms? What evidence links antigen-specific IgE with these responses? Using an accepted legal definition of harm as "reasonable certainty of no harm," several factors must be considered. These include at-risk populations, potency and exposure to the allergen, and background prevalence of food allergy.

Development of clinical symptoms unequivocally constitutes harm, but development of sensitization does not. Not all individuals who have IgE against a specific protein will develop clinical symptoms (Sarlo and Kirchner 2002). However, IgE-mediated allergic symptoms and signs will not occur in the absence of sensitization; therefore, sensitization leads to the potential for harm. In other words, if the sensitization can be detected and if the exposure is removed prior to the occurrence of clinical symptoms and signs, harm may not result. There is evidence to suggest that sensitization to respiratory allergens in the occupational setting may occur at lower levels of exposure than doses capable of eliciting systemic hypersensitivity, and that reduction of exposure can also remove the chances for harm (Schweigert et al. 2000). However, respiratory tract sensitization may not predict the risk of allergic sensitization from oral ingestion in the case of foods. For example, baker's asthma is a common occupational disease, yet very few bakers have allergic responses after eating wheat bread, even though many have wheat protein–specific IgE (Smith et al. 2000). Additionally, it has been observed that some IgE antibodies are not clinically relevant and will not result in clinical sequelae (Aalberse et al. 2001).

As discussed above, the positive predictive value of antigen-specific IgE, whether SPT or serum measurements, varies with the food in question and the disease prevalence. In general, the positive predictive value of an SPT ranges from 25 to 75%.

What kinds of tests can be done in the clinical setting, and should they be conducted prior to approval or in the postmarket evaluation? If the gene is derived from a source known to be allergenic, *in vitro* studies such as immunoblots should precede clinical studies. If IgE binding by the protein in question is detected, further development should only proceed with caution. Clinical studies, including specific serum screening, and/or SPTs should be performed prior to marketing. Specific serum screening should be performed with serum obtained from well-defined clinical populations with allergies to the source protein. The feasibility of this step depends on the availability of banks that contain sera from well-defined, clinically allergic individuals. SPTs can also be performed to correlate with the outcome of the specific screening, but this may not be necessary unless serum data banks are inadequate or unavailable.

If the gene is derived from a source not known to be allergenic, no clinical tests are likely to be relevant in an unexposed population. However, as recommended by the Food and Agriculture Organization/World Health Organization Expert Consultation (FAO/WHO 2001), targeted serum screening for cross-reactivity with sera from patients allergic to proteins broadly related to the source gene could be conducted. If cross-reactivity is found, there may be a role for skin testing of individuals allergic to the related source protein with the novel protein. In this scenario, it may also be important to use appropriate nonclinical tests, such as animal models with appropriate positive and negative controls, in conjunction with human clinical trials. The ethical and technical constraints of conducting human trials, and the necessity of doing so, is a subject that requires considerable attention. However, these types of studies may be necessary for the validation of animal models as predictors of human allergic disease.

Postmarket assessment of novel foods. Clinical testing with specific IgE should be developed and employed. In addition, SPT with purified protein would be useful to detect IgE *in vivo*. Positive tests should be followed by DBPCFC, under appropriate clinical observation, to correlate demonstrable IgE with clinical symptoms. The availability of purified protein may limit the development of specific IgE tests.

What evidence, if any, is there for cross-reactivity between different food allergens and between respiratory allergens and food allergens? Within food families, there is considerable *in vitro* cross-reactivity. However, this frequently does not correlate with clinical cross-reactivity. Bock and Atkins (1990) described the results of 480 food challenges where < 1% of the children demonstrated clinical allergy to more than one member of a food family. Similarly, Bernhisel-Broadbent and Sampson (1989) demonstrated that in 41 children with two or more positive SPTs to legumes, only two of these children had positive challenges to more than one legume. Similar data exist for cereal grains, nuts, and fish (Bernhisel-Broadbent et al. 1992; Bock and Atkins 1989; Jones et al. 1995). Although *in vitro* cross-reactivity greatly exceeds clinical cross-reactivity, there is significant variability between patients and their reactivity to members within food families (Bernhisel-Broadbent et al. 1989).

Cross-reactivity can also be demonstrated between foods and aeroallergens, where pollens may cross-react with fruits, vegetables, or nuts. Examples include birch pollen that cross-reacts with apples, pears, and cherries, and ragweed pollen that cross-reacts with gourds.

The potential utility of targeted serum screening is well described in the FAO/WHO Expert Consultation Report (FAO/WHO 2001), but widespread use would require standardized panels of food and inhalant IgE obtained from individuals with well-characterized allergies. Although targeted serum screening may be an appropriate approach to the detection of potential allergens, many of the tools needed to routinely conduct this type of testing are not widely available (e.g., pooled banks of sera from allergic individuals), and both development and validation of the associated tests would be necessary prior to their use for risk assessment. Some IgE epitopes may not be clinically relevant, and such information should be considered when serum screening is used.

Animal Models to Assess Food Allergy

What attributes should an animal model have to be a reasonable method for safety evaluation of allergic potential? This group defined allergy (as opposed to immunogenicity) as the adverse health effects that may result from the stimulation of a specific immune response. For the purposes of this discussion the focus was on IgE-mediated responses induced against dietary proteins and resulting in food allergy and excluded such reactions as celiac disease. However, it is important to note that not all IgE responses are harmful (e.g., protection against roundworm infection). Models should be able to distinguish between immunogenicity (IgG, IgM, IgA, and cellular immune responses) and allergenicity (IgE). The participants felt that to meet this criterion an animal model would likely be genetically predisposed to have an atopic or T-helper cell type 2 (Th2) phenotype involving a skewed response toward the production of IgE antibodies and Th2 cytokines [interleukin (IL)-4, IL-5, IL-6, IL-10, and IL-13].

Models for safety assessment should show antigen (usually protein)-specific IgE antibodies. There was some discussion regarding pure carbohydrates as allergens, recognizing that many allergens are glycosylated proteins. It was also noted that lipids or oils in highly allergenic foods such as peanuts and tree nuts might influence immune responses. For all participants in this group, the ability to demonstrate antigen-specific IgE antibodies mediated by mast cells/basophils was the critical factor in the utility of any model. Additional markers such as cytokine or chemokine profiles could be used in conjunction with IgE.

Animal models should focus on hazard identification concerning potential allergenicity of proteins. As responses measured within the models may be used to assess the potency of specific allergens, validation of the models might include the determination of potency of known allergens. A second focus of animal models could then be the assessment of potency of novel allergens, which may be of use in risk assessment. Although dog and swine models are useful for studying mechanisms of food allergy, as they have clinical manifestations similar to those seen in humans, they may be less practical than the use of rodent models to study hazard identification.

Similarly, while adjuvants may be used to enhance allergic responses for the study of mechanisms of food allergy and pathology, the use of adjuvants in models meant for hazard identification should be considered with caution. It is possible that the use of adjuvants could confer on nonallergic proteins the ability to cause sensitization, thus creating false positives. However, few data are available to substantiate this hypothesis.

Finally, there must be a great deal of confidence on the level of false negatives in any model, as false negatives might lead to an inappropriate conclusion that a novel food is safe.

What characteristics must the administered allergen have to represent accurately the risk of effects in humans? Safety evaluation of foods derived from GM crops is necessary to ensure that the novel food is as safe as the conventional food. Given this goal, the protein being tested should be identical, at least as is practically possible, to the protein expressed in the plant. For testing purposes, the novel protein is usually expressed in a vector such as *Escherichia coli* or yeast. It must be determined in advance if molecular differences exist between proteins expressed in the new crop or end product and those expressed in the vector. An additional consideration is whether the introduced gene results in the expression or upregulation or alteration of endogenous genes (thus potentially coding for allergenic proteins) or results in changes in posttranscriptional processing of endogenous proteins with the potential of creating new allergens. Finally, the matrix within which the protein will appear needs to be considered. Is it appropriate to test purified proteins, or should they be evaluated along with other food components as they are seen in the digestive tract?

What are appropriate positive and negative controls? Positive and negative allergens should be used to evaluate and validate any model. There was some controversy among the participants regarding the use of only pure proteins or even pure epitopes, such as arachis hypogaea 2 (Ara h2) from peanut, as the sole positive versus the use of crude protein extracts. Such extracts would more closely mimic human exposure by preserving glycosylation and/or binding to natural adjuvants found in the crop of interest. Positive controls should be antigens positive in humans, such as peanut Ara h2 and peanut lectin, brazil nut 2S-albumin, ovomucoid, ovalbumin, and β-lactoglobulin. Positive allergens should induce at least a moderate response and dose–response relationships should be demonstrable. Negative controls should be proteins that the human population is widely exposed to but seldom lead to allergenic responses such as rubisco or corn phosphoenolpyruvate–carboxylase. Ideally, both positive and negative controls should be easily stored, relatively stable, and reasonably affordable.

What questions can be potentially addressed using animal models? In addition to questions regarding the sensitizing potential and relative potency of individual allergens, animal models may be used to complement or provide corroborative information for other testing methods. For example, there is considerable debate about how much sequence homology is required with a known allergen in order for a novel protein to be allergenic and about the relevance of stability in pepsin as a marker for allergenicity. Animal models could be used to examine the properties of foods that have some sequence homology or pepsin stability to determine if the novel protein is an allergen, and if so, what its relative potency would be. *In vivo* models could also be used to determine dose–response relationships and whether there are thresholds below which allergenic proteins have no clinical effects. In the detergent industry, exposure levels have been reduced and managed so that between 0 and 3% of the workforce become sensitized in a given year (Sarlo and Kirchner 2002; Schweigert et al. 2000). Under these conditions of exposure, clinical disease has been virtually eliminated, indicating that, at least for aeroallergens, there is a threshold for sensitization that may be different than that for elicitation. Determination of acceptable limits for both sensitization and elicitation of clinical symptoms may have important implications for risk evaluation and management.

Animal models can also be useful tools to determine basic information on the mechanisms of the allergic response. The specific properties that make a protein an allergen and how tolerance is induced are of critical importance in understanding allergic responses. *In vivo* models may elucidate how specific proteins could be made to induce tolerance rather than sensitization. In addition, these types of models could provide data on cross-reactivity between specific allergens. External factors such as environmental tobacco smoke, particulate materials, and infectious agents may act as adjuvants to enhance sensitization. These types of effects are best examined using *in vivo* models.

How can data generated in animal studies be used in the safety evaluation process? Results of appropriate animal studies pertaining to hazard identification and potency evaluation can make a significant contribution to the safety evaluation process. These types of models can be used to screen proteins in conjunction with information from stability and *in vitro* studies.

What are the limitations in current animal models, and how can we improve them? Validation studies are needed to assess whether animal models can accurately predict allergenicity in humans. Although allergic responses in animals and humans share many common mechanisms, there are clearly some cross-species differences such as in reaginic antibodies and complement components that induce anaphylaxis. Allergy or atopy in both humans and animals depends on genetic factors that differ between individuals and between species. Introduction of novel genes may alter the metabolism or expression of endogenous proteins that may differ across species. Because of genetic differences across species, the ability to sensitize or alter endogenous protein expression may not readily be captured in some models. In addition to species differences, there are questions regarding differences in responses to specific allergens between rodent strains.

It is unlikely that a single animal model will be sufficient to address all the issues concerning prediction of allergenicity to humans. Rather, some species (such as rodents) that are less expensive and technically challenging may provide information on hazard identification and statistical validity of nonallergenicity. Other species, for example, dogs and swine, that more closely follow the clinical symptoms in the atopic human population may provide important mechanistic information. Although reagents to evaluate allergic responses, such as cytokines, chemokines, and monoclonal antibodies to cell surface markers, are readily available for rodents, they are only now being developed for dogs and swine. It is clear that we must evaluate the currently available animal models, validate their utility to predict human responses, and decide how best to integrate one, some, or

none of them into a coherent strategy for safety assessment.

Biomarkers of Exposure and Effect

The appropriateness of measuring antigen-specific IgE and other potential biomarkers of allergy and exposure was considered for both premarket and postmarket evaluations of the safety of GM foods. Factors were identified that were deemed important to the development and validation of the recommended analytical procedures.

What biomarkers are available that can be applied to safety evaluation and risk assessment of novel foods? Is IgE sufficient? When a gene is transferred into a food crop, using the techniques of biotechnology, the newly expressed protein should be evaluated regarding its potential allergenicity (Metcalfe et al. 1996). If the gene donor organism is known to be allergenic, or if the amino acid sequence of the introduced protein is similar to a known allergen, the potential allergenicity is most appropriately evaluated by testing for antigen-specific reactions in those individuals with documented allergies to the donor or to the homologous allergen. Definitive proof that a protein is a food allergen would be the observation of a clinical reaction within a short time after ingestion of the food, which is most appropriately performed in a DBPCFC (Sampson 1997). Because of practical and safety considerations, a biomarker of potential effect, such as measurement of antigen-specific serum IgE, or positive skin test results may be more appropriate in the premarket phase of testing. However, these methods typically have modest to significant levels of false-positive and false-negative results, which may be minimized by proper assay design and reagent selection (Bindslev-Jensen and Poulsen 1997). A variety of antigen-specific IgE immunoassays have been used for preliminary diagnosis of food allergy. The same type of tests could also be used to monitor potential allergic reactions once the product is in the marketplace.

The potential utility of other biomarkers was discussed, including Th2-cell cytokine expression or proliferation, serum basophil or mast cell protease levels, and serum histamine levels. There is little evidence to support the use of these as biomarkers of allergic responses to dietary proteins. Measurements of other antibody isotypes including IgG4, IgA, or IgM were similarly dismissed as not being specific markers of allergic reactions.

In addition to the need for an antigen-specific IgE assay, the utility of measuring antigen-specific IgG was considered. Opinions were mixed regarding the usefulness of the expected data except in the case of negative IgE test results. In that case a positive IgG response demonstrates that individuals were exposed to the introduced protein and that this protein was immunogenic. If they do not have protein-specific IgE or IgG, it is difficult to ascertain whether they were exposed or are just immunologically unresponsive to the protein. Measurement of IgG levels in IgE-positive individuals is not likely to provide additional information on exposure or responsiveness.

What parameters are important in assays used to measure biomarkers of exposure and effect? **Protein standards.** For all assays discussed, the protein used as the test reagent and/or standard must be appropriate and representative of the material that will be in the food supply. Further, because the introduced protein in the currently approved GM products are expressed at low levels (< 1 ppm to ~ 400 ppm fresh weight), considerable time and effort are required to produce gram quantities of purified protein needed for use in assays and as a standard for the immunoassays. Because the development of GM plants suitable for commercial production is complex and time consuming, a recombinant protein produced from bacteria, yeast, plant, or animal cell culture system will typically be used. However, both the recombinant protein produced in the plant and in the heterologous organism will need to be characterized to demonstrate equivalence.

Because the epitopes recognized by some antibodies are formed by noncontiguous amino acids in close proximity because of the secondary structural folding of the antigen, optimal binding may require native conformation of the protein used in the immunoassays. However, a number of the most important allergenic epitopes found in food allergens involve only the primary structure and may require a denatured form for optimal detection. There is no single correct answer for all proteins regarding the question of which form is the correct one to use in evaluating IgE binding. Further, it is technically impossible to evaluate the absolute secondary, tertiary, and quaternary structure of all proteins in complex mixtures found in foods. However, as most proteins purified from either microbial sources or plants will be present as a population of native and denatured forms, antibodies from exposed individuals or immunized animals are expected to bind to a substantial fraction of either form.

With regard to the traceability of the introduced protein in foods, some proteins are enzymatically cleaved in the plant or during processing. Detection of fragments of the protein in processed foods may therefore not be possible unless immunoassays are developed with antibodies that recognize different segments of the protein. It is therefore recommended that the epitopes recognized by the antibodies used in these assays be mapped in order for the limitations of the assay to be understood. Traceability is important for exposure assessment in the case of a protein determined to be allergenic or if certain types of postmarket surveillance were performed.

Immunoassays. Direct enzyme-linked immunosorbent assay (ELISA), ELISA inhibition, direct radioallergosorbent test (RAST), RAST inhibition, Western blot or Western inhibition, or similar assay can be used to measure antigen-specific serum IgE. ELISA and RAST assays provide quantitative data of antibody binding to the solid-phase antigen. These assays have a qualitative measurement of the antibody–epitope interaction as well when dose responses and the slope of the dose–response curves are compared, although the inhibition assays are most appropriate for that evaluation. Western blot assays are rarely used for quantitative measurement but are quite useful to determine antibody specificity and, if appropriate, to evaluate the ability of the antibody to recognize fragments of the protein. General considerations for each assay were discussed in varying detail, with most emphasis placed on direct ELISA assays.

Appropriate positive and negative controls must be chosen to verify the specificity of binding and detection as well as day-to-day assay reliability. Licensed diagnostic allergy tests are required to have appropriate positive controls (NCCLS 1997). However, a direct positive control for antigen-specific human IgE is only available if there are individuals identified who have the specific allergy and who have significantly high titers of antigen-specific IgE in their sera. That is not likely to be the case for novel proteins, as they are intentionally chosen to avoid known allergens. Therefore alternative indirect controls must be used. Animal sera may be used to test for the presence of the antigen in the assay. Further, a different allergen-specific IgE assay and sera may be used to test the specificity and sensitivity of detection reagents to allow evaluation of the test articles and reagents. Antigen-specific human IgG may be assayed to evaluate whether an individual has been exposed to the antigen and whether the test protein is appropriately immunogenic. If there are no positive human sera, animal sera from specifically sensitized animals may be used to evaluate the protein antigen and test system.

Selection of subjects for human studies. Premarket testing may be used to determine whether allergic subjects who have been previously exposed to a protein have developed IgE that binds to the novel protein. Subjects will normally be consenting adults who have been identified through clinical allergy practices or by specific searches or surveys. These individuals are evaluated by careful interviews and screening of serum reactivity. The history of exposure of the serum donors must be clearly defined and documented. Individuals who have been occupationally exposed to the novel

protein may be appropriate subjects for premarket testing if exposure is clearly defined and documented. Even if specific IgE were detected in serum, definitive proof of allergy to the novel protein can be achieved only if the subjects also have clear clinical histories consistent with the specific allergy and if controlled challenge tests are positive. However, allergic responses after occupational exposure, which is typically through inhalation, may not be predictive of responses that may result from oral exposure to a novel protein. For example, baker's asthma is a common occupational disease, yet very few bakers have allergic responses after eating wheat bread, even though many bakers have wheat protein–specific IgE. Identifying a sufficient number of appropriate subjects may be another hurdle when designing human studies. Aside from the major allergens, the prevalence of allergy to any one complex allergen may be significantly less than 1 in 10,000 randomly chosen individuals. Reactions to a single protein may be one-third that number. In practice, it is very difficult to identify 10 soybean-allergic subjects to provide sera to perform simple screening assays, even though allergy to soy is thought to be common (Goodman RE. Personal communication).

Postmarket testing could be performed if there are reports of allergic reactions to specific foods produced from the commodity containing the GM product. Alternatively, new patients with allergies to appropriate foods may be asked to participate in a screen to specifically evaluate IgE binding to the introduced protein. As indicated above, it is unlikely that screening of a randomly chosen population of consumers would be productive in identifying allergies to a specific protein. In postmarket testing, antigen-specific IgG may be measured to evaluate exposure of the subjects and immunogenicity of the protein but should not be considered as a marker of potential allergenicity, as antigen-specific IgG is not considered sufficiently robust or predictive for the diagnosis of allergies. Further, the only two plausible mechanisms of action by which IgG binding would cause immediate allergic disease are through either complement activation, which may enhance the IgE effect in mast cells, or receptor-mediated IgG activation of mast cells, for which the evidence in human allergic reactions is quite weak, although some studies demonstrate activity in rodents (Siraganian 1997). Although there have been occasional demonstrations of antigen-specific IgGs that may enhance IgE binding and allergic responses (Denepoux et al. 2000), other clear studies provide evidence that IgG antibodies are important in blocking IgE reactions after allergen-specific immunotherapy (van Neerven et al. 1999). Therefore, measurement of antigen-specific IgG has not been found to correlate with clinical allergy.

Testing of sera from human subjects should always be done with careful attention to ethical standards. These include obtaining informed consent from subjects and the right of the subject to access the results from their own sera.

How should biomarkers of exposure and effect be validated? Validation must include tests to verify the specificity of binding, and measurement of the dose–response characteristics of the antibodies and protein similar to what would be required for a clinical diagnostic assay (NCCLS 1997). Assay performance should be verified in an independent laboratory. Positive cutoff values must be established that would indicate probable clinical significance, not just a given statistical level above background. Significance scores for commercially available *in vitro* allergy tests such as the Unicap or CapRAST system (Pharmacia Diagnostics, Uppsala, Sweden) (Sampson 2001) may serve as a model for these tests. Although levels should not be set to the level found to be 95% predictive of clinical symptoms, the cutoff should be high enough to minimize false-positive reports. There are many examples in the literature of positive IgE binding to proteins that do not seem to cause clinical reactions (e.g., Fujita et al. 2001). Sera from many individuals without clinically identifiable allergic reactions to foods will contain some low-level binding to common food proteins. The IgE in that case is typically much less abundant than in those with clinical symptoms, or may have significantly lower affinity for binding. Some cross-reactivity is due to the binding of carbohydrate-specific IgE that is of questionable clinical significance (Aalberse et al. 2001). A positive *in vitro* IgE reaction is not necessarily proof that a protein would cause clinical allergies but would indicate the need to test by clinical methods for definitive proof.

Sensitive Populations

What do we currently know about sensitive populations; what populations do we think are most at risk? Why? Children are more susceptible to food allergies than adults and therefore are most in need of protection from food allergies (reviewed in Sampson 1997). In particular, because allergies have a genetic component, children of atopic parents might be at even higher risk. The age of the child also probably plays a role, with younger children and infants being more susceptible. The higher susceptibility of children in general, and younger children specifically, may be due to the immature immune system not being able to develop tolerance, to higher gut permeability in the infant, and/or to higher dietary exposure, for example, from milk- and soy-based formulas (Sampson 1997). It has been suggested that the type and patterns of exposure to specific allergens may be an important factor in the increased prevalence of food allergy in infants (Zeiger 2000). In addition, children who have preexisting food allergies are more likely to develop allergic reactions to other foods introduced into their diets. Finally, the issue of exposures to unexpected sources of allergenic proteins, such as exposure via milk from soy-fed cattle ("food chain proteins"), needs to be considered.

Atopic adolescents and adults represent other potentially sensitive groups. As there is evidence that people with atopic dermatitis are more likely to develop allergies from exposure by other routes (e.g., aeroallergens; Burks et al. 1988), and allergic dermatitis is easily observed, it is possible that individuals with atopic dermatitis might serve as sentinels for food allergies to novel food products.

Workers who process GM food represent a fourth potentially sensitive population. Data support the idea that exposure to aerosols of food products could lead to food allergy (Leser et al. 2001; Roberts et al. 2002). Sensitization might occur via dermal, respiratory, or gastrointestinal exposure. This population, having a known exposure, might also be useful from a research standpoint.

Is* in utero *exposure or exposure via breast milk something we need to be concerned about? *In utero* exposure and exposure via breast milk are important considerations. There is suggestive evidence that children can become sensitized via breast milk to food allergens consumed by the mother (Frank et al. 1999; Hourihane et al. 1996; Vadas et al. 2001). Studies have found food antigens present in uterine fluid. Likewise, specific IgE has also been found in cord blood. It is less clear if there are windows of vulnerability to sensitization during *in utero* development.

Is there any evidence for infections or concurrent exposures to agents with adjuvant effects influencing the development of food allergies? Although the role of infections in development of food allergy is an important issue, definitive data on this question are lacking.

There are data suggesting that cryptochrome-1 Ac toxin from *Bacillus thuringiensis* is a highly potent systemic and mucosal adjuvant (Vazquez et al. 1999) and that phylogenetically distant Cry toxins may bind to the same receptors (Shinkawa et al. 1999). However, the question of whether GM foods or existing food proteins could have an adjuvant effect, thereby increasing the allergenicity of other foods, is still controversial.

Are the strategies currently proposed for safety evaluation (risk assessment) adequate to protect sensitive populations? The group agreed that no realistic strategy has been proposed to protect the general population, including sensitive populations. Overall, the procedures represented within the individual steps in the decision tree need to be better validated. The use of human serum for screening needs validation. Although targeted serum screening tests

may be valuable, efforts need to be made to identify appropriate individuals to provide serum. In addition, the use of postmarket surveillance challenge testing (e.g., DBPCFC) could be a valuable and safe part of the evaluation process with proper study design. The use of SPTs and DBPCFCs for evaluating novel food allergens was a controversial topic. However, the group agreed that the use of postmarket surveillance skin testing (for detection of sensitization) and challenge testing (e.g., DBPCFC for establishing reactivity) presents minimal safety concerns if done properly, and therefore the benefits outweigh the risks.

The value of animal testing in evaluating safety is also an important consideration. However, currently it is difficult to extrapolate animal results to human, and more research is needed to overcome this obstacle.

The group agreed that developing standards for safety that protect the public, are acceptable to the public, and are not too restrictive is an important but difficult goal, and that it will be impossible to achieve zero risk. The concept of substantial equivalence has been applied to other areas of risk assessment, and the group agreed that it could be applied to the area of food allergy. Novel proteins could be evaluated against a panel of known food allergens of varying potency as a framework.

Models of Dose Response

Evidence for thresholds for sensitization and/or allergic reactions were presented and discussed. The need for dose–response information in the regulatory decision-making process was considered for evaluating the safety of GM crops. Additionally, the current tools available for developing dose–response information as well as factors such as immunologic tolerance and sensitization doses (e.g., number, dose, route, etc.) were discussed.

What is the evidence that there are thresholds for sensitization and/or allergic reactions? Data from food-challenge studies in humans were provided for a number of the known allergenic foods (e.g., peanut, shrimp, milk, egg, soybean, tree nuts) that demonstrated the existence of a threshold level for eliciting an allergic reaction to either the food or the purified allergenic food protein (Table 1). For example, Hourihane et al. (1997) and Wensing et al. (2001) both reported a threshold of 100 μg total peanut protein or 6 μg of the peanut allergen Ara h2, and a no-observable effect level (NOEL) of between 30 and 50 μg peanut protein or 2–3 μg of Ara h2 in patients with peanut allergy, using DBPCFCs. From this study, the authors concluded that the threshold dose of peanut protein needed to elicit a response in a group of individuals allergic to peanuts varies. In patients with egg allergy, Moneret-Vautrin et al. (1998) reported a threshold of < 100,000 μg egg and 54,000 μg ovalbumin and a NOEL of 5,000–10,000 μg egg or 5,400 μg ovalbumin, whereas Sicherer et al. (2000) reported a threshold of 100,000 μg egg and 54,000 μg ovalbumin and a NOEL of 100,000 μg of egg (i.e., the first dose tested). These data are important for establishing the relative risk associated with a potential food allergen. In contrast to the elicitation phase of allergic reactions, only limited data are available in humans regarding food allergens and thresholds for sensitization. Furthermore, there may be populations or subpopulations of individuals (e.g., based on age, ethnicity) that are more sensitive to the induction or elicitation phase of protein exposure. In addition, limited data are available from industrial exposures to proteins (i.e., the detergent industry) regarding thresholds for sensitization and/or elicitation for aeroallergens that may be relevant (Schweigert et al. 2000). Thresholds for sensitization have also been reported in various animal models for both dermal (Basketter et al. 1997, 1999; Kimber et al. 1999) and respiratory (Hillebrand et al. 1987; Karol 1983) sensitizers.

Is there a need for dose–response information, or is hazard identification alone adequate to warrant regulatory action? The consensus was that dose–response information was needed, particularly for setting relative risk. However, current regulatory action regarding the potential allergenicity of foods derived from GM plants is driven predominately by hazard identification. For example, the food allergy decision tree developed by FAO/WHO (1996, 2001) involves determining the source of the gene(s) (is the source of the gene allergenic?), evaluating the physicochemical characteristics of the protein (amino acid sequence homology to known allergens; pepsin resistance) (Taylor and Lehrer 1996), conducting specific and targeted serum screening, and using validated animal models. If one or more of the latter evaluations is positive, the protein is labeled as likely allergenic and removed from further consideration. Therefore, the relative risk associated with such a protein is usually not considered.

What is the role of immunologic tolerance in establishing dose–response information? Currently, little is known regarding the mechanisms of immunologic tolerance. The group concurred that the dose–response curve begins once tolerance is broken or fails to be established. Furthermore, because tolerance to a particular protein can be induced by both low and high doses, it was agreed that a wide range of doses should be used to maximize the chance of falling within the responsive range of the dose–response curve.

What tools are available to develop dose–response information? The current tools available to develop dose–response information include animal models and human food-challenge studies (Table 1). For both technical and ethical reasons, it would be very difficult to conduct human food-challenge or sensitization studies with novel proteins. Therefore, it was the opinion of the group that a validated animal model(s) that assesses the allergenic potential of proteins currently represents our best tool for establishing dose-response information. As with humans, the genetic predisposition of a particular animal strain may influence the degree of allergenicity observed with a particular protein. Thus, it is important to ensure that haplotypic differences do not result in misleading results in animal models. In initial validation studies multiple strains of the same species could be evaluated to determine those strains most sensitive and specific in discriminating between both known allergenic and nonallergenic proteins.

Is one large sensitization dose the same as many small sensitizing doses? The group agreed that one large sensitization dose is not the same as many small sensitizing doses. Among the factors that need to be considered are the age at which the sensitization dose(s) occurs, the time between sensitizing doses, and the route(s) of administration of the sensitizing dose. For example, Woolhiser et al. (2000), using natural rubber latex proteins, reported differences in latex-specific IgE immunoblot profiles and pulmonary function between four different sensitization routes. These authors concluded that the sensitization route of exposure might partially determine the primary allergen(s) and the clinical symptoms of the allergic response.

Postmarket Surveillance

What tools and strategies are currently available for postmarket surveillance, and what can we learn from them? The feasibility of a range of tools and strategies that could be applied to postmarket surveillance of foods produced through biotechnology and their role in providing information for safety assessment were considered. Such tools and strategies included the collection and analysis of case reports of adverse reactions; conduct of classical epidemiology studies such as cohort, case–control, ecologic, and cross-sectional studies (telephone surveys, food frequency questionnaires); and clinical trials (DBPCFCs). The identification of highly exposed populations in the context of specific foods (e.g., occupational groups, populations with limited variety in the diet such as children, infants, ethnic populations) was considered helpful for the conduct of epidemiologic studies. Marketbasket surveys were discussed as a means of assessing exposure to specific types of food and would reflect regional food preferences. It was suggested that modern technologies such as grocery-scanning devices could assist in data collection. However, exposure to GM foods would be

difficult to establish in the absence of specific labeling.

The most useful tool was considered to be the development of an adverse event reporting system that would allow the systematic collection of case-report data on adverse reactions to foods. This offered the possibility of identifying sensitized individuals. In the United States, such data are currently collected through state health departments, emergency rooms, and self-reporting. The existing infrastructure limits the capability for collecting such information. In addition, a reliable method for detection of recombinant proteins in food products is essential to establish that exposure to a protein that is the suspected cause of an adverse reaction did in fact occur. It is important to note that this kind of system is most useful in identifying individuals sensitized to specific food items rather than for assessing the potential for GM food–induced allergy.

For data on adverse food reactions collected from postmarket surveillance to be meaningful, they must be compared against baseline data collected prior to the introduction of the food under surveillance. It was suggested that such data could be collected from pilot surveillance projects to reflect regional food preferences across the country.

DBPCFCs were considered essential as a postmarket tool for the validation of causation between a specific food ingredient and an adverse reaction. Such studies were not considered useful screening tools to establish the potential of adverse food responses related to a particular food. The limitations and issues surrounding the use of such methods were discussed. These include difficulties associated with proving the negative (how many people would need to be tested) and ethical issues (e.g., the potential for induction of sensitivity

Table 1. Thresholds for eliciting allergic responses to food proteins.

Food	Allergen	Allergen serving	Threshold (µg)	Symptoms	NOEL (µg)
Peanut	Ara h2	6% of total protein[a]	100 peanut protein or 6 Ara h2 (Wensing et al. 2001)	Itching of throat, tongue, and/or lips (Wensing et al. 2001)	30 peanut protein or 1.81 Ara h1 (Wensing et al. 2001)
			100 peanut protein or 6 Ara h2 (Hourihane et al. 1997)	Oropharyngeal itching (Hourihane et al. 1997)	50 peanut protein or 3 Ara h2 (Hourihane et al. 1997)
Shrimp	Tropomyosin	No data on level of shrimp tropomyosin in shrimp equivalents	0.02 g shrimp equivalents per gram ice cream (Daul et al. 1988)[b]	No data	1 shrimp equivalent (Daul et al. 1988)[b]
Milk	β-Lactoglobulin	9% of total protein[a]	100,000 milk or 400,000 β-lactoglobulin (Sicherer et al. 2000)	Skin, gastrointestinal, and/or respiratory reactions	100,000 was the first dose (Sicherer et al. 2000)
			0.5 β-lactoglobulin (breast milk–exposed infants[c]) (FAO/WHO 2001)	"Adverse reactions"	No data
			43 β-lactoglobulin (breast milk–exposed infants[c]) (Host et al. 1988)	Skin, gastrointestinal, and/or respiratory reactions	No data
Egg	Ovalbumin	54% of total protein[a]	< 100,000 egg or 54,000 ovalbumin (Moneret-Vautrin et al. 1998)	Atopic dermatitis, angioedema, asthma, anaphylactic shock, digestive symptoms	5,000 or 10,000 egg or 5,400 ovalbumin (Moneret-Vautrin et al.1998)
			100,000 egg or 54,000 ovalbumin (Sicherer et al. 2000)	Skin, gastrointestinal, and/or respiratory reactions	100,000 was first dose of egg (Sicherer et al. 2000)
			3,330 SDWE or SDWE 1800 ovalbumin (15.1% SDWE) (Christie et al. 2001)	No data	300 SDWE or 180 ovalbumin (Christie et al. 2001)
			500 egg protein or 270 ovalbumin (breast milk–exposed infants[c]) (FAO/WHO 2001)	"Adverse reactions"	500 was the first dose (FAO/WHO 2001)
Wheat	Sucrose synthetase	< 1% of total protein[d]	< 500,000 wheat or 5,000 allergen (Sicherer et al. 2000)	Skin, gastrointestinal, and/or respiratory reactions	500,000 was the first dose (Sicherer et al. 2000)
Tree nuts (hazelnut)	Cor a1	No data on level of Cor a1 allergen in hazelnut meal	1,000 hazelnut meal (Wensing et al., 2002)	No data	1,000 hazelnut meal was the first dose (Wensing et al. in press)
Soybean	β-Conglycinin	18.5% of total protein[d]	< 500,000 soy (Sicherer et al. 2000)	Skin, gastrointestinal, and/or respiratory reactions	500,000 was the first dose (Sicherer et al. 2000)
Fish	Gad c1	0.1% of total protein[d]	6,000 (codfish or 6 Gad c1 (Hansen and Bindslev-Jensen 1992)	Irritation, itching, swelling of oropharynx, gastrointestinal symptoms, or cutaneous reactions	No data
			1,000,000 fish or 1,000 Gad c1 (Helbling et al. 1999)	Emesis, oropharyngeal symptoms, urticaria, or respiratory reactions	1,000,000 was the first dose (Helbling et al. 1999)
			< 500,000 fish or 500 Gad c1 (Sicherer et al. 2000)	Skin, gastrointestinal, and/or respiratory reactions	500,000 was the first dose (Sicherer et al. 2000)

SDWE, spray-dried whole egg.
[a]Data from Metcalfe et al. 1996. [b]Dose levels were 1, 4, or 16 g shrimp equivalents. NOEL was 4 g shrimp equivalents/207 g ice cream. No effects were elicited in this cohort with 1 g shrimp equivalents/207 g ice cream (Daul et al. 1988). [c]Results from infants exposed to these allergens in mother's breast milk. These studies are not applicable to nonnursing children or to adults. [d]Soybean allergen expressed in soy milk.

in previously unexposed individuals or induction of life-threatening allergic response in the process of screening).

When should postmarket surveillance be done? The issue of whether the conduct of postmarket surveillance should be routine for foods produced through biotechnology or should be triggered by a specific event in relation to a particular food (as in the case of StarLink corn) was discussed by the breakout group. Participants with concerns about the uncertainties inherent in the current procedure for allergenicity assessment of recombinant proteins favored routine monitoring. In contrast, a number of participants were confident that the present assessment process was adequate to identify novel proteins with allergenic potential and supported the use of postmarket surveillance only when triggered by a specific event.

What tools are needed to improve our ability to do postmarket surveillance and interpret results? The most useful tool was considered to be the collection of case reports of adverse responses to food. Modification of existing adverse events reporting systems was suggested for this purpose. Among the features of such a system that needed to be determined were *a*) establishing who would be responsible for notifying, *b*) who should be notified, and *c*) who would be responsible for following up the case reports to collect necessary information. In terms of the current infrastructure available for the collection of these kinds of data, two scenarios were envisioned: improvements to the current system to capture data that would assist assessment of foods produced through biotechnology, or developing an entirely new system for collection and analysis of data. Creation of a nonprofit industry association group such as the Drug Safety Alliance that would be provided with access to the desired information was suggested.

Several members of the group felt that exposure assessment would be assisted by labeling foods produced through new technologies, but this clearly was an area in which consensus was not reached. Difficulties were acknowledged in using such labeling in products containing commodity crops in which mixing of harvested crops from different suppliers is a typical practice. Foods in which identity preservation is part of the marketing strategy, as in cases of nutritional modification or allergen reduction of products, were considered to be more amenable to labeling.

The lessons of previous experience, (e.g., from events where products not accepted for human consumption were found in consumer products) were considered useful in terms of guiding development of postmarket strategies. Such strategies would benefit from the adoption of a multidisciplinary approach.

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Overall Summary

Each breakout group addressed specific issues in the areas of use of human clinical data, animal models to assess food allergy, biomarkers of exposure and effect, sensitive populations, dose–response assessment, and postmarket surveillance. These groups discussed the adequacy of the current tools available for assessment of the allergenic potential of novel foods and described how these tools need to be improved for better safety assessment. In several instances the groups reached different conclusions or could not agree on the utility of specific tools, such as the use of human sera for hazard identification or labeling of foods containing GM crops. Each group was asked to identify specific areas in which data gaps should be the focus of future efforts. These research needs are discussed in detail in the article by Selgrade et al. (2003).

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