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Digestive Stability in the Context of Assessing the Potential Allergenicity of Food Proteins

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INTRODUCTION

Assessment of the potential allergenicity of proteins introduced into food crops through biotechnology is required by international regulatory agencies that govern the release and production of genetically modified plants. Currently, one aspect of this assessment includes analysis of the protein in a simulated gastric fluid (SGF) assay that tests the digestibility of the target protein to pepsin. The logic behind this test was that proteins that are nutritionally desirable tend to be rapidly digested and have greater bioavailability of amino acids than stable proteins. In addition, proteins that are highly digestible would be expected to have less opportunity to exert adverse health effects when consumed. The assay was not meant to predict the fate of the protein of interest under in vivo conditions, but rather to evaluate the susceptibility of the protein to digestion under fixed conditions in vivo. The purpose is to provide information that, in conjunction with other evidence, would be useful in predicting whether a dietary protein may become a food allergen. Therefore, the relationship of the resistance to digestion by pepsin and the likelihood that a dietary protein is an allergen was identified as a means of aiding the assessment of proteins added to commodity crops through biotechnology. In this article, we discuss the predictive value of this assay and the practical and theoretical aspects of allergen resistance to pepsin digestion in the context of food safety.

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IgE-mediated food allergic reactions usually are elicited by a relatively small group of foods including peanuts, milk, soy, crustacea, fish, egg, tree nuts, and wheat. Prospective studies have indicated that about 5% of children less than 4 years of age experience IgE-mediated food allergic reactions, with about 1.5% of young children reacting to cow's milk, about 1.3% to hen's egg, and 0.5% to peanuts (1). Children with atopic disorders, especially atopic dermatitis, are more often affected by food allergies. About 35% of children with moderate to severe atopic dermatitis have skin symptoms provoked by food hypersensitivity (2). Given the estimated frequency of allergy to a variety of foods, it is likely that about 2% of the adult population, or about 5.5 million Americans are affected by food allergies (1).

MECHANISM OF IgE-MEDIATED FOOD ALLERGIC REACTIONS

The clinical symptoms manifested by food allergic reactions are primarily due to the release of histamine and a large variety of other bioactive compounds from mast cells and basophils. These cells contain numerous secretory granules in which these substances are stored at extremely high concentrations. Activation of the mast cell results in the fusion of these granules with the cell surface membrane, leading to the exocytosis of the granule contents and the induction of allergic symptoms. The mast cell plasma membrane contains receptors for the Fragment of crystallation portion of IgE. This receptor binds IgE that recognizes the allergen. Mast cell activation is accomplished through the binding of an allergen simultaneously to more than one molecule of IgE. This cross-linking of at least two surface-bound IgE molecules brings the receptor proteins into close association with one another in the plane of the mast cell membrane. Kinases associated with these receptors become activated as a result of this proximity, initiating the second messenger cascade that results in mast cell degranulation.

COMPLETE AND INCOMPLETE ALLERGENS

Food allergens have been classified into two broad groups based on their ability to only elicit clinical symptoms in previously sensitized individuals or their ability to sensitize and elicit reactions in susceptible individuals (3). The first category, known as incomplete food allergens, comprises those proteins that can only elicit clinical symptoms due to their homology with another allergen. Incomplete allergens typically are not the primary sensitizing protein, but share significant sequence homology with that protein to elicit clinical symptoms. An allergic disorder known as oral allergy syndrome (OAS) (4) can be caused by cross-reactivity of allergens in pollens (sensitizing allergen) and vegetable foods (eliciting allergen) (5). Whereas this is the typical relationship that exists between the sensitizing and eliciting

allergen in OAS, anecdotal evidence indicates that some of these vegetable allergens in rare instances also may sensitize susceptible individuals through the orophayrngeal or respiratory route. Their inability to sensitize a susceptible individual via the gastric route is believed to be due to their sensitivity to the proteases found in the gastrointestinal tract (pepsin, trypsin, chymotrypsin). Examples of incomplete food allergens are typified by the fruit allergens that share sequence homology with latex allergens (6). The physicochemical characteristics potentially contributing to allergenicity of incomplete food allergens and complete aeroallergens such as pollens of grass, weed, or tree species may be different from complete food allergens. Researchers suggest that for aeroallergens, sensitizing exposures may be influenced by topological properties of pollen allergens and their ability to present in the upper airway (7).

The second category, known as complete food allergens, comprises those proteins that can both sensitize and elicit clinical symptoms. Complete food allergens have several biochemical characteristics in common including their abundance in the food and the ability to promote IgE production and elicit IgE-mediated clinical symptoms (3). Another significant characteristic is that complete food allergens are stable to the proteolytic and acidic conditions of the digestive tract. Protein stability is believed to impart on the allergen an increased probability of reaching the intestinal mucosa intact where absorption of significant quantities may lead to sensitization. In addition, this characteristic has an impact on the exposure rate of this protein to susceptible populations, an important variable in any risk assessment paradigm. Examples of complete food allergens are typified by the major allergens found in the commonly allergenic foods. Characteristics of the major allergens found in the commonly allergenic foods include abundance and stability. Although these are characteristics of most major food allergens, there are exceptions. One notable exception is patatin (Sol t 1), the major allergen of potato (8), that is unstable in the pepsin digestion assay but is also the most abundant protein in potatoes. Although it is the major allergen of potato, it is not as common or as potent an allergen as some of the complete allergens referred to previously. Furthermore, all stable proteins are not food allergens. Table 1 provides a partial listing of complete and incomplete allergens and their characteristics.

SAFETY ASSESSMENT OF FOODS DERIVED FROM GENETICALLY MODIFIED CROPS

Canola, corn, cotton, potatoes, soybeans, sugarcane, tomatoes, and other crops have been genetically modified (GM) by the introduction of specific genes encoding proteins that provide protection from insect pests or tolerance to herbicides. These GM crops are extremely useful for reducing the need for insecticides and allowing reduced tillage farming, and all of them have been

TABLE 1 Characteristics of Allergens in a Partial List of Complete and Incomplete Food Allergens

Allergen	Abundant[a]	Release time[b]	Sensitizing protein	Eliciting protein	Stable[c]
Complete[d] Food Allergens					
Peanut					
Ara h 1	yes	N/A	Ara h 1	Ara h 1	yes
Ara h 2	yes	N/A	Ara h 2	Ara h 2	yes
Milk					
β-lacto-globulin	yes	N/A	β-lacto-globulin	β-lacto-globulin	yes
Casein	yes	N/A	Casein	Casein	yes
Egg					
Ovomucoid	yes	N/A	Ovomucoid	Ovomucoid	yes
Ovalbumin	yes	N/A	Ovalbumin	Ovalbumin	yes
Soybean					
β-conglycinin (α/β)	yes	N/A	β-conglycinin (α/β)	β-conglycinin (α/β)	yes
Gly m 1	yes	N/A	Gly m 1	Gly m 1	yes
Incomplete[d] Food Allergens					
Apple					
Mal d 1	no[e]	N/A	Bet-v-1	Mal d 1	no
Pear					
Pyr c 1	no	N/A	Bet v 1	Pyr c 1	no
Pyr c 5	no	N/A	Bet v 6	Pyr c 5	no
Celery					
Api g 1	no	N/A	Bet v 1	Api g 1	no

Complete[d] Aeroallergens					
Birch					
Bet v 1	yes	yes	Bet v 1	Bet v 1	N/D
Grass					
Phl p 1	yes	yes	Phl p 1	Phl p 1	N/D
Lol p 1	yes	yes	Lol p 1	Lol p 1	N/D
Ragweed					
Amb a 5	yes	yes	Amb a 5	Amb a 5	N/D

[a]Abundant refers to the amount of the protein relevant to other proteins found in the matrix. A food protein was classified as abundant if it represented >1% of the total protein in a food (9).

[b]Release times refer to the kinetics of diffusion and correlates with sensitization potential (10).

[c]Stable refers to whether the allergen has been reported to be stable in the pepsin digestion assay (11, 12).

[d]Incomplete/complete refers to whether the allergen would be considered a complete or incomplete allergen as defined by Aalberse (3).

[e]May be considered abundant in certain parts of the apple such as the skin.

[N/A = not applicable; ND = not determined.]

approved for commercial production in at least one country (13). The approval process that allows each of these products to be used in commercial production involves safety assessments designed to protect the health of the human and/or animal consumer and the environment. The safety assessment of GM crops is quite extensive, usually involving comparative studies of the modified crop with current commercial varieties with regard to composition, nutrition, and environmental impact. An essential part of the safety assessment is the evaluation of the potential allergenicity of any newly introduced protein. The allergy assessment testing strategy, as originally proposed by the U.S. Food and Drug Administration (FDA) and further modified by FAO/WHO scientific panels (14–16), proposes that all proteins introduced into crops be assessed for their similarity to structural and biochemical characteristics of known allergens. Interestingly, the first GM crop considered by the U.S. FDA and commercialized in 1994, Flavr Savr™ tomatoes, did not produce a newly introduced protein but instead used antisense RNA technology to affect the delayed ripening trait (17).

Models of digestion are commonly used to assess the stability of dietary proteins (11, 18, 19). A digestion model using simulated gastric fluid (SGF) was adapted to evaluate the allergenic potential of dietary proteins (11). In this model stability to digestion by pepsin has been used as criterion for distinguishing food allergens from safe, nonallergenic dietary proteins. Although these digestibility models are representative of human digestion, they are not designed to predict the half-life of a protein in vivo. In this article, we review the currently available data supporting the utility of the pepsin digestion assay in predicting whether a protein introduced into a crop has the potential to be an allergen.

PEPSIN DIGESTIBILITY ASSAY

Practical Considerations

The pepsin digestibility assay was conceived as a means to determine the relative stability of a protein to the extremes of pH and pepsin protease encountered in the mammalian stomach and was originally developed and utilized as a method to assess amino acid bioavailability (20–22). The logic behind this test was that proteins that are nutritionally desirable tend to be rapidly digested and have greater bioavailability of amino acids than stable proteins. In addition, proteins that are highly digestible would be expected to have less opportunity to exert adverse health effects when consumed. This logic appears to have been confirmed, at least for milk and wheat allergy. Buchanan and colleagues have shown that when stability of the major allergens from these foods is disrupted by reduction of disulfide bonds, the allergens were strikingly sensitive to pepsin digestion and lost their allergenicity as determined by their ability to provoke skin test and

gastrointestinal symptoms in previously sensitized dogs (23, 24). Standardization of the assay conditions (i.e., pepsin concentration, pH, temperature, etc.) has been described in the *U.S. Pharmacopia* (25) and is sometimes referred to as SGF.

The assay was not meant to mimic precisely the fate of proteins in in vivo conditions, but rather to evaluate the susceptibility of the protein to digestion under fixed conditions in vitro. The purpose is to provide information that, in conjunction with other evidence, would be useful in predicting whether a dietary protein may become a food allergen. Therefore, the relationship of the resistance to digestion by pepsin and the likelihood that a dietary protein is an allergen was identified and subsequently recommended by the U.S. FDA, Environmental Protection Agency (EPA), and U.S. Department of Agriculture (USDA) in 1994 as a means of aiding the assessment of proteins added to commodity crops through biotechnology (14).

The digestive stability of the major allergens found in the most common allergenic foods was the first to be studied. The stability of some of the major allergens of peanut, soybean, egg, and milk relative to the stability of common nonallergenic food proteins were determined in the standard pepsin digestion assay (11). Under the conditions described for SGF in this study, all food allergens were more resistant to pepsin hydrolysis than were common plant proteins. For example, the Ara h 2 allergen of peanut was stable for at least 60 min in the pepsin digestion assay, while other nonallergen plant proteins such as rubisco (spinach leaf) or acid phosphatase (potato) were digested in less than 15 sec. However, not all allergens from the most common allergenic foods were stable in the pepsin digestion assay for 60 min. Stability of the whole protein or fragments from the allergens tested ranged from 8 min to 60 min, whereas all the nonallergen plant proteins tested did not survive in the pepsin digestion assay for more than 15 sec.

Since this initial report, numerous studies have repeated the pepsin digestion assay on these major food allergens (12). In general, the original findings that these allergens were stable to pepsin digestion relative to nonallergenic proteins were confirmed, but the length of time that either the whole protein or fragments of the allergen were stable did not always agree. The most likely explanation for this quantitative difference is due to subtle changes in the pepsin digestibility assay or in the method by which the proteins of interest were detected. For example, changes to enzyme concentration, pH, protein purity, and method of detection could have large effects on the interpretation of any in vitro assay. Table 2 summarizes many of the variables associated with the pepsin digestibility assay and the effect they may have on target protein stability.

Figure 1 illustrates results from a pepsin digestibility assay performed in which two different enzyme to target protein ratios were used and all other assay conditions were held constant. This figure shows the importance of laboratories using a standard set of conditions for all proteins tested in the pepsin digestibility assay. For this reason, the International Life Sciences

TABLE 2 Variables Affecting the Pepsin Digestion Assay

Variable	Range[a]	Comment
pH	1.2–3.5	Optimum pepsin activity at pH 1.5–3.5.
Enzyme concentration (enzyme/protein)[b]	1/1000–2200/1	Increase/decrease will affect protein half-life.
Target protein concentration	1–5 mg/ml	Increase/decrease will affect protein half-life.
Detection method	Immunoblot/stain	Increase/decrease in sensitivity will affect apparent protein half-life.

[a]Range refers to the range of values reportedly used in pepsin digestion assays from a variety of publications and laboratories.

[b]Enzyme concentrations have been reported as units of enzyme added and milligrams of enzyme added depending on the laboratory performing the assay.

Institute (ILSI) has proposed a standardization process for the assay that attempts to assess these variables so that results from different laboratories can be directly compared. Federal, academic, and industry laboratories from Europe, North America, and Japan will participate in this test in which pH (1.2/2.0), pepsin concentration, allergen purity, and method of detection have all been standardized (26).

Allergens from less commonly allergenic foods also have been studied using the pepsin digestion assay. For example, allergens from fruit and vegetable foods have been tested for their ability to survive pepsin digestion. Allergens from these sources are typically classified as incomplete (cross-reactive) allergens because they share significant structural homology with another allergen. Good examples of this are the allergens of fruit that share significant sequence homology with aeroallergens. In these cases, the individual is sensitized to an allergen via the respiratory route and then exhibits clinical symptoms after ingestion of a food that contains a protein of sufficient sequence homology to the sensitizing allergen. For example, the Bet v 1 homologous allergens of apple (Mal d 1), pear (Pyr c 1), apricot (Pru ar 1), and cherry (Pru av 1) are, in general, labile to enzymic digestion (12). The pepsin sensitive cross-reactive proteins typically cause localized symptoms of the oropharynx including tingling or swelling of the lips, tongue, or glottis and are less likely to cause systemic or gastrointestinal symptoms. The lack of stability of this class of food allergens in the pepsin digestion assay suggests that pepsin stability may not be a useful predictor of sensitization. More likely, the characteristics of this class of allergen simply reflect the discrete sensitization and elicitation processes unique to patients exhibiting oral allergy syndrome.

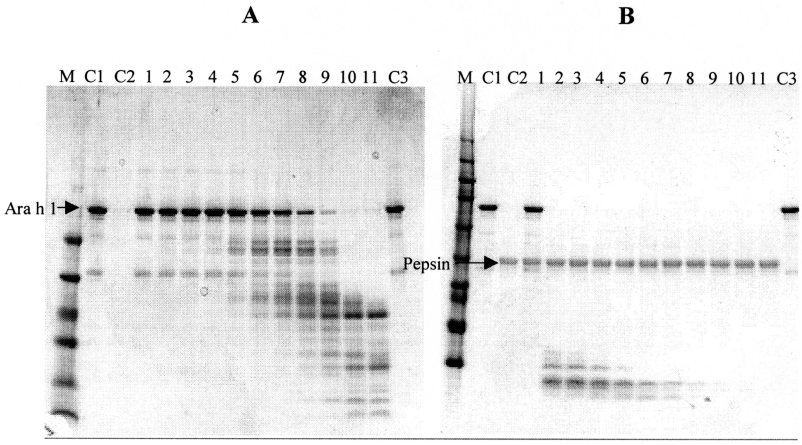

FIGURE 1. Pepsin digestion assay shows effects of different pepsin/target protein (Ara h 1) ratios. SDS-PAGE gel electrophoresis of different time points of a pepsin digestion assay using an enzyme/target protein ratio of either 1unit pepsin/1000 μg Ara h 1 (A) or 9 units pepsin/μg Ara h 1 (B). Lanes are M-molecular weight markers; C1-SGF buffer and target protein; C2-SGF buffer and pepsin; C3-SGF buffer and target protein; lanes 1–3 correspond to 0, 15, 30-sec time points; and lanes 4–11 to 1, 2, 4, 8, 15, 30, 60, and 120 min, respectively, after initiation of the experiment. Ara h 1 was the target protein in this assay and the arrow points to the full-length protein. A protein band corresponding to pepsin (arrow) can only be seen in (l B) because it is not in high enough concentrations in (A).

To assess the positive and negative predictive values (PPV and NPV, respectively) (27) for the pepsin digestion assay in identifying potential food allergens, known food allergens and known nonallergens were compared with respect to their stability to pepsin. To perform this analysis, four assumptions had to be made.

1. A food allergen was defined as any protein that bound IgE from a patient diagnosed as suffering from immediate hypersensitivity reactions on ingestion of that food.
2. Only complete food allergens were included in this analysis.
3. Pepsin stability had to be assessed under the conditions described by Astwood et al (11).
4. A positive for pepsin stability was defined as any protein (allergen or nonallergen) that survived pepsin digestion, either intact or fragments, for greater than 8 min.

With these assumptions, 20 food allergens and 10 nonallergens were identified (11, 28, 29) and analyzed (Table 3). As described by Forthofer and

TABLE 3 Summary of Positive and Negative Predictive Values for Proteins Tested in Pepsin Digestion Assay

	SGF Assay		
Allergen	Positive	Negative	Total
Yes	18[a]	2[b]	20
No	2[c]	8[d]	10
Total	20	10	30

Positive predictive value $[a/(a+c)] = 0.90$; negative predictive value $[d/(b+d)] = 0.80$.

[a]True positives are proteins classified as allergens and stable in the SGF assay.

[b]False-negatives are proteins classified as allergens and unstable in the SGF assay (i.e., potato allergen Sol t 1, milk allergen α-lactalbumin).

[c]False-positives are proteins classified as nonallergens that are stable in the SGF assay (i.e., beef tropomyosin, wheat γ-thionin).

[d]True negatives are proteins classified as nonallergens and are unstable in the SGF assay.

Lee (27), the PPV for the evaluation was 0.95 and the NPV was 0.80. This analysis indicates that the pepsin digestion assay is a good positive and negative predictor of the potential of a protein to be an allergen.

However, the results should be interpreted with some caution as food allergens associated with OAS were not included in this analysis and only 30 proteins were tested in this manner. Allergens associated with OAS may be detected by the bioinformatics search performed on all proteins introduced into commodity crops (see the previous article by Goodman et al. in this issue for a description of this portion of the safety assessment). In any event, assay standardization and the study of many proteins (allergens and nonallergens) inform the allergy assessment strategy with respect to the robustness and predictive power of this physicochemical property of proteins.

Theoretical Considerations

As described, the pepsin digestion assay can be a reasonable contributor to an overall allergy assessment of specific proteins. However, even more enlightening information may be obtained if the underlying structural basis for an allergen's ability to resist pepsin digestion was known. With this in mind, the sequence specificity of the pepsin substrate and the minimum peptide size required for eliciting the clinical symptoms of allergy are discussed.

Pepsin is an aspartic endopeptidase obtained from the gastric mucosa of vertebrates. However, all mammalian pepsins have similar specificities. Pepsin preferentially cleaves the peptide bond between any large hydrophobic residue (L, F, W, or Y) and most other hydrophobic or neutral residues except P (30). To cleave the peptide bond between two hydrophobic residues, the active site groove of pepsin binds to a segment of the protein containing the sessile peptide bond and 4 amino acids on either side of the cleavage site. A number of studies have evaluated the efficiency of pepsin cleavage and the effect of various amino acids around the sessile peptide bond. To facilitate discussion, the positions have been assigned identification labels such that the amino acid (aa) residues located on the amino-terminal side of the sessile bond are labeled P_1, P_2, P_3, or P_4, and on the carboxyl-side labeled $P1'$, P_2', etc. The bond between P_1 and P_1' is the sessile bond. The most efficiently cleaved peptides have aromatic or hydrophobic residues at both the P_1 and P_1' positions. The rate of pepsin cleavage is slowed if a proline is at amino acid position P_2' or if arginines are in the P_2, P_3, or P_4 positions (31, 32).

The resistance of a protein to pepsin digestion raises the possibility that it will be taken up by antigen processing cells at the mucosal surface of the small intestine and could sensitize susceptible individuals who have consumed the protein leading to the production of antigen-specific IgE. In addition, a pepsin resistant peptide might provoke an IgE-mediated allergic response in those who are already sensitized. IgE plays a pivotal role during the induction of an allergic response by triggering effecter cells such as the tissue mast cells (and possibly blood basophils) to release histamine, leukotrienes, and inflammatory proteases. This trigger occurs when two or more IgE molecules are bound to a single peptide fragment while the antibody is bound to the high-affinity IgE receptors (FcεRI) on these effecter cells. Studies of rat basophilic leukemia (RBL) cells indicate that it probably requires the cross-linking of well over 1,000 of the 200,000 or so FcεRI receptors on a single cell to cause degranulation of that cell (33). IgE antibody cross-linking occurs through binding multivalent antigens by IgE molecules bound to the surface of mast cells. While various IgE-antigen binding arrangements are possible, only certain ones lead to the productive signaling and degranulation of the mast cells (34, 35). The binding is only effective if it is maintained long enough (by a high-affinity interaction) and if the spatial relationship and rigidity of the antigen are sufficient to cross-link and induce intracellular signaling.

A series of studies was undertaken by Kane et al. using haptens with linkers of various sizes to determine the effective spacing for degranulation and to study intracellular signaling. Results demonstrated that oligomerization of the FcεRI-IgE-antigen molecules was more effective at inducing degranulation. Further, minimum spatial distances were identified using the artificial hapten-spacer constructs indicating that whereas tight IgE binding can occur with bivalent haptens spanning 30 Å (angstroms) the RBL cells were not induced to degranulate. Bivalent haptens of ~50 Å were required to

obtain modest degranulation while similar haptens spaced between 80 and 240 Å apart seemed to provide optimum degranulation (36, 37). These results may provide guidance on the sizes of peptides that might be required to cause an allergic reaction upon challenge.

To evaluate the minimum peptide size that might effectively cross-link receptors on mast cells, the maximum overall spacing (length) may be calculated but various assumptions must be made regarding epitope size and peptide conformation. The first assumption regards the size of a typical IgE-binding epitope observed in a food allergen. Most food allergen IgE-binding epitopes are range in size from 6–15 amino acids in length (38). Therefore, the absolute minimum size of a peptide would have to be 12–30 amino acids long and contain 2 IgE-binding epitopes. However, this does not take into account the data of Kane et al. that show the IgE-binding epitopes must be at least 80–240 angstroms apart to provide optimum degranulation (36, 37). Assuming the 2 IgE-binding epitopes are separated by the minimum length of 80 angstrom and that the diameter size for an amino acid such as alanine is 5 angstroms, the minimum size for a peptide that would be expected to elicit the clinical symptoms of an allergic reaction would be 29 amino acids long or a peptide of about 3190 daltons (29 aa $\times$ 110 avg aa molecular weight).

These calculations do not take into account the secondary structure of the peptide. For example, the peptide could be in an alpha-helical arrangement, a beta-pleated sheet, or a random coil dependent on its amino acid sequence. Dependent on the secondary structure of the peptide, mast cell degranulation would only be possible if each end of the fragment represents a strong IgE-binding epitope and if the peptide is in a beta-strand conformation. Based on this rationale, it appears improbable that the presence of a protease resistant fragment of <3 kDa in the in vitro pepsin digestion assay would have the ability to degranulate mast cells and therefore would not likely pose a risk to consumers.

CONCLUSIONS

The allergy assessment testing strategy, as presently formulated, is a tiered, hazard identification approach that utilizes currently available scientific data regarding allergens and the allergic response. The available practical and theoretical rationale for the use of the in vitro pepsin digestion assay as one of several parameters in the testing strategy appears to be supported (39). The pepsin digestion assay seems to have a relatively high positive predictive value for detecting potential complete food allergens. Potential incomplete food allergens would most likely not be detected by this assay but would be identified on the basis of another arm of the safety assessment testing strategy, the bioinformatic search. Therefore, it is extremely important to emphasize that all aspects of the current safety assessment testing strategy need to be considered when assessing a novel

protein, not just the results from a single arm of this strategy. Whereas a hazard assessment approach generally has worked to ensure the safety of the current wave of pest-resistant and herbicide-tolerant crops, it may not be adequate to use in the assessment of the next wave of nutritionally enhanced GM foods.

It is evident that the testing strategy will need to be integrated into a risk assessment mode where risk is defined as a function of the level of the hazard and the level of exposure to the hazard. This strategy consists of four steps: hazard assessment, dose-response evaluation, exposure assessment, and risk characterization (40). To apply risk assessment principles to allergenicity of proteins and GM crops new scientific data must be collected for each step in this process. In fact, this has already begun. For example, the issue of dose-response evaluation is beginning to be addressed by a variety of investigators exploring threshold doses for different foods in clinically allergic patients (41). Exposure assessment consists of three parts: the abundance of the protein in the food, the stability of the protein in the gastrointestinal tract, and the amount of the GM crop consumed in the diet. Efforts to collect data on all three components of this important step in the risk assessment paradigm have already begun.

In summary, protein stability as assessed by the pepsin digestion assay has been a good indicator of potential allergic sensitization when considered with other allergy hazard assessment methods. It will continue to provide important data relevant to the exposure assessment step in a risk assessment strategy for allergenicity of GM crops. However, we emphasize that safety assessment of any novel food currently is based on the concept of substantial equivalence with available food commodities. Additional tests performed on novel proteins introduced into these foods, such as bioinformatic searches as described in another article of this volume and the pepsin digestion assay described here, provide yet an additional layer of protection to the consuming public.

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