



Short communication

Allergenic sensitization versus elicitation risk criteria for novel food proteins

Rod A. Herman^{a,*}, Gregory S. Ladics^b^a Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268, United States^b DuPont Haskell Global Centers for Health and Environmental Sciences, 1090 Elkton Road, Newark, DE 19711, United States

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ABSTRACT

The value of criteria used in the weight-of-evidence assessment of allergenic risk of genetically modified (GM) crops has been debated. This debate may originate, in part, from not specifying if the criteria are intended to contribute to the assessment of sensitization risk or elicitation risk. Here, this distinction is explicitly discussed in the context of exposure and hazard. GM crops with structural relationships with known allergens or sourced from an organism known to cause allergy (hazard) are screened for IgE-antibody reactivity using serum from sensitized individuals. If IgE reactivity is observed, the GM crop is not developed. While digestive and heat stability impact exposure and thus the elicitation risk to sensitized individuals, these attributes are not interpretable relative to sensitization risk. For novel food proteins with no identified hazard, heat stability cannot be validly assessed because relevant IgE antibodies are not available. Likewise, the uncertain and sometime non-monotonic dose relationship between oral exposure to allergens and sensitization makes digestive stability a poor predictor of sensitization risk. It is hoped that by explicitly distinguishing between sensitization risk and elicitation risk, some of the debate surrounding the weight-of evidence criteria for predicting the allergenic risk of GM crops can be resolved.

1. Introduction

The value of several of the weight-of-evidence criteria used to predict the allergenic risk of novel food proteins has been debated (Astwood et al., 1996; Fernandez et al., 2013; Goodman et al., 2008; Herman et al., 2009, 2015, 2006; Ladics et al., 2006, 2007; Privalle et al., 2011; Schnell and Herman, 2009; Silvanovich et al., 2005). Allergenic risk criteria were developed to assess the safety of novel proteins produced in genetically modified (GM) crops and include: amino acid sequence similarity with known allergenic proteins, concentration in food, heat and digestive stability, and the allergenic status and history of safe use (HOSU) for the proteins and the source organisms from which they were isolated (Ladics, 2008; Metcalfe et al., 1996). Some of the debate surrounding the use of specific risk criteria is a consequence of not clearly specifying whether the potential risk is predicted for sensitization to the novel food protein or elicitation of an allergic response in persons with a pre-existing allergy to the protein or cross-reactive allergens. Here we attempt to discuss each of the risk criteria in relation to sensitization and elicitation potential. The objective is to clarify the value of each criterion in assessing the allergenic risk of novel food proteins produced in GM crops.

2. Risk

A classical paradigm in toxicology is that risk is the product of exposure and hazard. For the criteria used to assess the allergenic risk of novel food proteins, concentration in food, heat stability, and digestive stability are exposure criteria, while structural similarity with known allergens and history of safe use are hazard criteria. The relationship between exposure level and elicitation of allergic symptoms has been established for previously sensitized individuals (to the sensitizer and cross-reactive proteins) with several proteins (Taylor et al., 2009). However, this relationship may not apply to the sensitization process. The lack of dose-response knowledge in food allergy sensitization makes it difficult to interpret results in a risk assessment context. Since the relationship between dose and sensitization (the shape of the dose-response curve) is unknown, it is not possible to include this information to assess sensitization risk. It is currently not possible to include dose-response relationships in the interpretation of sensitization studies, and it is, therefore, not possible to characterize the sensitization risk relative to dose. Further, while zero exposure prevents sensitization (including no *in utero* exposure), oral exposure, especially early in life, may be required to induce the tolerance needed to prevent sensitization (Du Toit et al., 2016; Sicherer et al., 2017; Turcanu et al., 2017).

Reliable tests are not available to definitively predict the potential

* Corresponding author.

E-mail address: raheman@dow.com (R.A. Herman).

of novel food proteins to sensitize atopic individuals. Such potential depends on intrinsic characteristics of the protein (e.g. structure, function, and physiochemical properties), but also on complex interactions between the genetic background and physiology of the consumers and the exposure environment (McClain et al., 2014). Extrinsic factors such as the composition of the food matrix, food processing, the dose, and route, and frequency of exposure, are of major importance in modulating the potential of a protein to induce sensitization versus tolerance. Also, relevant pathways of toxicological concern have not yet been accurately described for sensitization and allergy triggered by food proteins (van Bilsen et al., 2017). In addition, it appears that dermal and/or inhalation exposure may play a key role in sensitization to some food allergens such that the classical “exposure x hazard = risk” paradigm may not be applicable to sensitization based on oral exposure (Turcanu et al., 2017). In fact, there is growing evidence that sensitization through the skin and lungs, as in the gut, is the result of an inflammation reaction that is driven by both epithelial cells and dendritic cells (Wills-Karp et al., 2010). Thus, the sensitization properties of proteins result from intrinsic, structural, and physiochemical properties of the protein that interact with the host genetics and physiology and with environmental conditions. For these reasons, the criteria used to predict sensitization and elicitation risks likely differ (Ladics et al., 2014; Remington et al., 2018).

3. Elicitation

From a practical standpoint, GM crops expressing proteins with a realistic allergenic hazard will not be developed. If a protein is isolated from a source known to cause allergy, or if bioinformatic analysis indicates potential cross reactivity with a known allergen, then screening serum from sensitized individuals for IgE reactivity to the novel food protein is a robust way to determine elicitation hazard (Nordlee et al., 1996). Likewise, low concentration of an allergen in food and digestive instability would be predicted to reduce the severity of symptoms in sensitized individuals. Testing the IgE reactivity to the allergen in sensitized individuals before and after heating the protein would also help predict the elicitation severity after cooking or processing of the crop. While these exposure criteria are relevant to risk assessment, it is highly unlikely that a GM food crop expressing a protein with IgE reactivity in sensitized individuals will ever be developed for public consumption.

4. Sensitization

The more challenging allergenic risk assessment for a novel protein expressed in a GM crop is for sensitization potential. If bioinformatic analyses indicate that there is a cross-reactive risk, or if the protein was sourced from an organism known to cause allergy, then serum screening should determine if an elicitation hazard exists, and if so, development of that GM crop will stop (Nordlee et al., 1996). As noted earlier, risk requires both hazard and exposure. Therefore, the components of the weight of evidence approach employed to evaluate exposure and hazard will be discussed in the context of protein sensitization.

Exposure - Heat Stability: Only proteins not known to cause allergy and with low cross-reactive risk are developed for use in GM crops. As a consequence, sensitized individuals are not available. This means that relevant IgE antibodies are also not available to determine if allergenic epitopes that might be present within the novel food protein are destroyed by heating. In the absence of this resource, some regulatory agencies have used loss of functional activity and/or IgG reactivity after heating as a surrogate for IgE binding. However, these endpoints have not generally been shown to correlate with loss of IgE binding or sensitizing potential (Nordlee et al., 1996; Privalle et al., 2011). IgE reactivity and elicitation of allergenic symptoms associated with oral exposure are known to both decrease and increase after heating

depending on the allergen and the nature of the heat treatment (Breiteneder and Mills, 2005a). Denaturation and/or fragmentation of the protein may destroy or reveal hidden allergenic epitopes. Thus, this criterion is generally not useful for predicting the sensitization risk for a novel food protein.

Exposure – Digestive Stability: While initial results indicated a good correlation between stability in simulated gastric fluid and the allergenic status of proteins (Astwood et al., 1996), subsequent studies with an expanded array of proteins have largely failed to confirm this relationship (Bøgh and Madsen, 2016; Fu et al., 2002; Herman et al., 2007). This poor correlation may stem from non-monotonic dose-responses with respect to sensitization potential. Unlike elicitation responses, oral exposure to food allergens is required to induce the tolerance response that prevents food allergy. In addition, for food allergens where sensitization may occur dermally and/or by inhalation (Turcanu et al., 2017), degradation in the digestion system may be of low relevance to the sensitizing exposure. Therefore, this criterion has questionable value in predicting the sensitization risk for a novel food protein.

Exposure – Concentration in Food: Most sensitizing food allergens are present at high concentration (> 1%) in food (Breiteneder and Mills, 2005b). This seeming contradiction with the hypothesized non-monotonic oral dose response for sensitization by food allergens may stem from the dermal and inhalation routes of exposure postulated for the sensitization process. High doses by the dermal and inhalation routes do not cause oral tolerance (Turcanu et al., 2017). Thus, there may be a balance between the sensitizing potential of high exposure via the dermal and/or inhalation routes of exposure and the oral tolerance response induced by exposure in the intestines. However, based on the presence of most food allergens at a high concentration in food, a low concentration of a novel protein in a GM crop is a reduced risk factor for sensitization.

Hazard – Bioinformatics: While high structural similarity with known allergens might indicate potential cross reactivity (elicitation risk) and/or sensitization risk, more distant protein relationships might also increase the risk for *de novo* sensitization. Most allergens belong to just a few protein families, even though most members of these same protein families are not known to cause allergy (Breiteneder and Mills, 2005b; Hoffmann-Sommergruber and Mills, 2009; Radauer et al., 2008). Thus, proteins in families without known allergens have a reduced risk for sensitization.

Hazard – History of Safe Use (HOSU): If exposure to the proteins expressed in GM crops (or to the organism from which it was derived) has a history of use in food without allergy, then the risk of sensitization from the proteins expressed in GM crops is greatly reduced. Evaluation of HOSU involves assessing past exposure to the protein of interest, including the concentrations in food and the environment, the timing and frequency of exposure, and potential exposure routes.

5. Endogenous food allergens

The European Food Safety Authority (EFSA) uniquely requires the levels of endogenous allergens be measured in GM crop-composition studies to inform the risk assessment (Fernandez et al., 2013; Herman and Ladics, 2011). The interplay between higher exposure potentially reducing sensitization but increasing the severity of elicitation in sensitized individuals makes predicting the overall epidemiology of exposure to food with higher endogenous allergen content difficult. This is exemplified by the recent change in the pediatric recommendation from withholding allergenic foods to providing allergenic foods to young children (Sicherer et al., 2017). The new recommendations were made after studies showed that exposure to allergenic foods at a young age reduced sensitization rates (Du Toit et al., 2015). This is great example of the value of evidence-based medicine compared with expert opinion (Herman and Raybould, 2014; Sackett and Rosenberg, 1995).

Table 1
Criteria for predicting sensitization and elicitation risk for novel food proteins.

Sensitization			Elicitation (after previous sensitization)
Hazard	Bioinformatics	Belonging to a family known to contain allergens increases risk.	Homology with known allergens proteins increases risk (triggers serum screening).
Hazard	Source organism	History of exposure without allergy reduces risk.	Being from an allergenic source triggers serum screening.
Exposure	Digestive stability	Complex/uncertain relationship between oral exposure/dose and sensitization vs. tolerance makes results uninterpretable.	Rapid digestion decreases severity of reaction.
Exposure	Concentration in food	Higher concentration is common for sensitizers.	Higher concentration increases exposure and severity of reaction.
Exposure	Heat stability	Not useful due to lack of relevant IgE antibodies.	Reduction in relevant IgE antibody binding after heating reduces severity of reaction.

6. Discussion

The debate concerning the usefulness of criteria supporting the weight of evidence for assessing the allergenic potential of novel food proteins expressed in GM crops may stem, in part, from a lack of clarity on whether one is addressing sensitization or elicitation risk, and lack of consideration of potential non-monotonic oral dose responses relative to sensitization. The latter may be reflective of dermal and/or inhalation exposure routes for sensitization to food allergens and the need for oral exposure to induce tolerance. It is hoped that this brief discussion brings forward concepts that will aid in selecting and interpreting the criteria used to build the weight of evidence for assessing the allergenic risk for novel food proteins and GM crops. Specifically, this approach highlights that for the typical case involving GM proteins with no identified allergenic hazard, exposure assessment is not required to conclude negligible risk, and thus, heat and digestive stability assessments are largely irrelevant to the allergy risk assessment for such proteins (Table 1).

Conflicts of interest

The authors are both employed by companies that develop and market transgenic seed.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.yrtph.2018.02.016>.

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