Allergic potential of novel foods

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Concerns have been expressed that the introduction of novel foods into the diet might lead to the development of new food allergies in consumers. Novel foods can be conveniently divided into GM and non-GM categories. Decision-tree approaches (e.g. International Life Sciences Institute-International Food Biotechnology Council and WHO/FAO) to assess the allergenic potential of GM foods were developed following the discovery, during product development, of the allergenic potential of GM soyabean expressing a gene encoding a storage protein from Brazil nut (*Bertolletia excelsa*). Within these decision trees considerations include: the source of the transgene; amino acid homology with known allergens; cross-reactivity with IgE from food-allergic individuals; resistance to proteolysis; prediction using animal models of food allergy. Such decision trees are under constant review as new knowledge and improved models emerge, but they provide a useful framework for the assessment of the allergenic potential of GM foods. For novel non-GM foods the assessment of allergenic potential is more subjective; some foods or food ingredients will need no assessment other than a robust protein assay to demonstrate the absence of protein. Where protein is present in the novel non-GM food, hazard and risk assessments need to be made in terms of the quantity of protein that might be consumed, the identity of individual protein components and their relationships to known food allergens. Where necessary, this assessment would extend to serum screening for potential cross-reactivities, skin-prick tests in previously-sensitised individuals and double-blind placebo-controlled food challenges.

Allergenicity: Novel foods: GM foods

Novel foods and food ingredients are defined as those that have not been used for human consumption within the EU before 15 May 1997. Detailed rules for the authorisation of such novel foods and food ingredients are laid out in Regulation (EC) 258/97 of 27 January 1997 (European Commission, 1997). Such legislation was necessary because of the increasing use of food technologies, including biotechnology, leading to the development of new food products that included GM crops. At the same time, increasing globalisation of the food supply was leading to the prospect of European consumers being exposed to an increasing variety of new foods from outside the EU. The novel food regulations attempted to provide a framework for the authorisation of all such foods, both GM and non-GM. To a large extent, consumer resistance to novel foods within the EU has been focused on GM foods. The reasons for this focus are multifactorial, but may include a concern about the regulation of biotechnology, ethical debates about gene manipulation and suspicion about the motives of multinational corporations. By contrast, novel non-GM foods meet less consumer resistance; indeed, the groups who are highly critical of GM technologies are often supportive of non-GM novel foods. This disparity may be because some non-GM novel foods represent the output of small local producers and some of the products are accompanied by shrouded suggestions of health and nutritional benefits.

The present review focuses on the allergenic potential of novel foods, and the reality is that it does not matter whether the novel food is from a GM source or non-GM source; both have the potential to add to the allergenic burden of the diet of the European consumer. However, because of the political sensitivities of the GM debates, frameworks for the assessment of the allergenic potential of GM foods have been extensively developed and are more structured than those for non-GM foods, for which the assessment will necessarily be more subjective.

Food allergies are of increasing concern to the European consumer, most of whom know of, or are acquainted with, an individual with food allergy who has to practise strict
dietary control to manage their condition. About 1–2% of 
adults suffer from food allergies and the prevalence may be 
as high as 5% in children (Bock & Atkins, 1990; Young 
et al. 1994). Symptoms can vary from the mild discomfort of 
oral allergy syndrome through to the potentially-fatal systemic anaphylaxis. Whilst the majority of food allergies are 
caused by a small number of foods, particularly pean-
uts (Arachis hypogaea), soybean, tree nuts (which include 
cashew (Anacardium occidentale L.), almond (Amygdalus 
communis L.), hazelnut (Corylus avellana), pecan (Carya 
illinoensis (Wangen.) K. Koch), walnut (Juglans regia), 
Brazil nut (Bertolletia excelsa), pistachio nut (Pistacia 
vera) and macadamia nut and Queensland nut (Macadamia 
temifolia)), milk, eggs, cereals, fish and shellfish, there are 
occasional case reports of allergies being triggered by a 
wide variety of foods (Young et al. 1994; Food and Agri-
culture Organization, 1995). In some cases the specific 
proteins within the foods responsible for triggering the 
allergic reaction are known, and those proteins have been 
studied in detail to characterise the allergenic epitope, the 
precise region of the protein that interacts with the host 
immune system. In other cases the nature of the allergenic 
protein is not yet known. Databases of allergenic foods, of 
allergenic food proteins and of allergenic epitopes are 
being constructed (e.g. InformAll database on allergenic 
foods; Informall EU Project, 2005) but they are as yet far 
from complete. It is fair to say that whilst the ability to 
manage existing food allergy has improved greatly, the 
ability to predict new food allergies is less well developed, 
largely because there is so much of the fine detail of the 
allergic immune response that is yet to be understood. 
Against this background of imperfect knowledge, judge-
ments have to be made on whether or not a novel food, be 
it GM or non-GM, is likely to be an allergenic hazard for 
consumers. The following sections will outline the types of 
approaches that can be taken.

Allergenic potential of novel GM foods

The much-reported incident of the Brazil nut allergen in a 
transgenic soyabean occurred in the mid-1990s (Nordlee 
et al. 1996). Essentially, an attempt was made to develop a 
GM soyabean for use as cattle feed. Soyabean is relatively 
low in the essential amino acid methionine, and animal 
feed normally has to be supplemented with S-containing 
amino acids. In this particular case a gene encoding one of 
the storage proteins from Brazil nut (known to contain high 
levels of methionine) was inserted into the soyabean. 
Development of the product was stopped when it was 
shown that the GM soyabean cross-reacted with serum 
from individuals previously sensitised to Brazil nut. In 
effect, the researchers had managed to transfer the gene for 
what turned out to be one of the major allergens of Brazil 
ut into the soyabean. This incident highlighted one of the 
potential pitfalls of GM technology in terms of transfer of 
allergenic potential and led to intense discussion of the 
development of regulatory frameworks; conversely, it also 
provided some reassurance there were certain tests avail-
able that could, and indeed did, prevent such a product 
being commercialised.

Soon afterwards, the first decision tree for the assess-
ment of the allergenic potential of biotechnology-derived 
foods was published under the auspices of the International Life Sciences Institute-International Food Biotechnology 
Council (Metcalfe et al. 1996). Within this decision tree 
the first question is whether the source of the transferred 
gene is known to be allergenic. If so, then a series of 
in vitro and in vivo immunoassays are prescribed, ending 
in a double-blind placebo-controlled food challenge, the 
so-called ‘gold standard’ of food allergy research. In prac-
tice, a product that follows this route through the decision 
tree and is positive in all tests would be highly unlikely to 
make it into the market place. In the case of a product for 
which the source of the transferred gene is not known to be 
allergenic, two hurdles have to be overcome. First, the 
transferred-gene product should have no sequence similar-
ity with known allergenic proteins and second, the 
transferred-gene product should not be resistant to proteo-
lysis using simulated gastric fluids. Failure at these hurdles 
would lead to more clinical tests and possibly labelling, or 
to consultation with the appropriate regulatory agency.

Whilst this decision tree represents a useful framework 
within which to assess allergenic potential, it is by no means perfect. In particular, there has been much debate 
about the homology searching and the usefulness of prote-
lysis tests, a debate that is still ongoing. In addition to 
overall homology searching, there are programs that 
search the databases of allergenic proteins for homology 
with sequences of contiguous amino acids present in the 
transferred gene. The minimum size of this amino acid 
sequence is the topic of debate. Based largely on a con-
sensus view that the minimum size of a linear epitope in an 
immunogenic protein would be about eight amino acids, 
the original homology searches were established using an 
eight amino acid match (International Life Sciences Insti-
tute Health and Environmental Sciences Institute, 2001; 
Hileman et al. 2002). Opponents of this strategy insist that a 
six amino acid match (as proposed by Food and Agri-
culture Organization/World Health Organization, 2001) is 
more appropriate and would avoid any false negatives. In 
practice, a six amino acid match tends to throw up large 
numbers of false positives that can confound interpretation. 
Which is the correct strategy? The answer is that the 
homology searching approach is far from perfect. In some 
cases an epitope is likely to be contained within more than 
eight amino acids, whereas there are examples of other 
epitopes that are shorter. However, it is also likely that not 
all amino acids within that linear sequence contribute to 
the epitope, i.e. the binding to molecules within the host 
immune system. In theory, therefore, there could be a 
variety of conservative substitutions within that linear 
sequence that would still retain the epitope structure. 
Clearly, there is scope for further research and develop-
ment of more predictive search programs.

The second problem occurs with the tests for resistance 
to proteolysis using simulated gastric fluids (Bannon et al. 
2003). A large number of proteins have now been tested 
using such models and, although resistance to proteolysis 
is a characteristic of some allergenic proteins, there are 
also many proteins that are known allergens that are 
susceptible to proteolysis. Thus, it is not necessarily safe to
conclude that a protein that degrades rapidly in simulated gastric fluid is not an allergen. An additional issue would be the physiological relevance of the test. In reality, protein does not enter the acid environment of the stomach as a pure test solution, but rather as part of a complex food matrix. Within a bolus of food passing through the stomach, it is unlikely that all protein is exposed to the extremes of acid pH, and some protein is likely to survive intact into the lower intestine.

The second incident to make headlines in relation to the allergenic potential of GM foods was the StarLink™ maize event (see Environmental Protection Agency, 2001). StarLink™ maize was genetically engineered to express Cry9C, a Bacillus thuringiensis-derived toxin conferring resistance against the corn borer (Ostrinia nubilalis). During the development of the product certain characteristics were observed in relation to the Cry9C protein, including its resistance to proteolysis in gastric-simulated fluid and the fact that IgE antibodies to Cry9C could be raised within an animal model of food allergy, which led to suspicions that it might be a potential allergen. Although these observations were not conclusive, they led the US Environmental Protection Agency (1998) to approve the use of Cry9C protein in StarLink™ maize for animal feed only, and not for human food use. However, it was almost inevitable that cross-contamination would occur, and in September 2000 it was announced that StarLink™ products had been found in tortilla chips that had been sold for human consumption (see Environmental Protection Agency, 2000). A hugely expensive recall soon followed, with ongoing threats of legal action. Although it is unlikely that any individual actually suffered an allergic reaction to Cry9C, since the quantities involved were small and the window of opportunity for both sensitisation and elicitation phases of the allergic reaction was restricted, the incident serves as a reminder of just what can go wrong. The integrity of the separate channels of supply for animal feed and food for human consumption should never be assumed, and the allergenic potential of GM products needs to be fully evaluated, even when they are intended for animal feed only. A Joint FAO/WHO Expert Consultation (Food and Agriculture Organization/World Health Organization, 2001) has proposed a revised decision tree for the assessment of the allergenic potential of GM novel foods. The pivotal question is still whether or not the source of the transferred gene is a known allergen, but in both cases a sequence homology search must be conducted. Positive sequence homology or positive results in a specific serum screen leads to definition of the transgene as a likely allergen. Where sequence homology is negative and a targeted serum screen is also negative, then a combination of pepsin-resistance tests and animal modelling is used to define a high, intermediate or low probability of allergenicity. This decision tree is an improvement on the previous version and in a sense reflects the uncertainty and imperfect knowledge, in that there is no outcome of the decision tree that can lead to categorisation as a ‘non-allergen’, only a low probability of being an allergen. The decision tree relies heavily on the use of either specific or targeted serum screens, depending on whether or not the transferred gene is from a known allergenic source. In the case of targeted serum screens, it is recommended that at least twenty-five individual serum samples are used. This sample size may be the ideal, but in reality it is often very difficult to source such numbers of well-validated (from patients who have undergone a double-blind placebo-controlled food challenge) sera for some of the rarer food or aero-allergens. The decision tree continues with the use of the pepsin-resistance tests, but performance in such a test has to be considered in association with performance in an animal model. This latter area of animal modelling is an area in which much research effort is needed in order to define a well-validated system.

Allergic potential of novel non-GM foods

Assessment of the allergic potential of novel non-GM foods presents some interesting challenges. In contrast to GM food crops there may be instances for novel non-GM foods in which there is no protein present and therefore no allergenic hazard. This situation would certainly be true for refined oils and carbohydrates. The issue is therefore to demonstrate convincingly the absence of contaminating protein, which may be done using a conventional protein assay (e.g. the Lowry method or the Bradford assay), or by visualisation of protein-banding patterns following SDS–PAGE. Alternatively, for novel food products that should be N-free, e.g. oils, total N estimation in the sample would give a theoretical maximum protein content. Where contaminating protein is present, it may be necessary to perform a qualitative analysis, depending on the source of that protein. Very low levels of protein (sub-microgram) are unlikely to present any allergenic hazard, although it is difficult to generalise because it is becoming clear that different food proteins may have different thresholds for elicitation of the allergic reaction (Bindslev-Jensen et al. 2002).

Where protein is clearly present at substantial levels in the novel non-GM food, either as a major contaminant or because the novel food is a whole food, then a formal assessment of allergenic potential needs to be performed. This assessment process will have some similarities to the decision tree for novel GM foods, in that the source of the protein needs to be defined. The outcome will then determine whether a specific serum screen or a targeted serum screen is appropriate. Homology searching is less appropriate for novel non-GM foods because there is no specific transgene to sequence. Where the protein is related to a major food allergen, then a specific serum screen to identify potential IgE-binding capacity is appropriate. Positive findings within such a screen would indicate the necessity for skin-prick tests or a double-blind placebo-controlled food challenge in a clinical environment. Ultimately, precautionary labelling may be the only way to manage any perceived risk.

Where the protein in the novel non-GM food is unrelated to any major food allergen or comes from an exotic source for which there is little information, e.g. imported fruits, then an investigation into the phylogenetic relationships of the food source with other known foods should be conducted. It is possible that the food may share partial
protein identity with other family members; for example, there are certain proteins that are considered pan-allergens (e.g. profilin is a pan-allergen that is recognised by IgE from about 20% of the patients with allergies to birch pollen and plant food; Scheurer et al. 2001), occurring in a wide variety of species with some notable conservation of homology that underlines their important role within the plant cell biology. The conclusions of such an exercise would lead to the design of a targetted serum screen, in which sera from individuals previously sensitised against botanically-related foods would be screened for potential cross-reactivity. Again, skin-prick tests and a double-blind placebo controlled food challenge are warranted if the outcome of a targetted serum screen is positive.

**Conclusion**

Novel GM foods currently offer a variety of agro-economic benefits and will probably in the future offer nutritional benefits to the consumer. Similarly, novel non-GM foods offer the consumer increased choice with the possibility of nutritional benefits. However, with a marked percentage of the population suffering from food allergies, care must be taken that new technologies and new choices do not equate to new risks of allergenicity. The decision-tree approaches outlined in the present review are very helpful in predicting allergenic potential, although they are not perfect, reflecting the imperfect state of current knowledge. The majority of these approaches for allergenicity assessment have been included in the recently-issued European Food Safety Authority (2004) guidance document for risk assessment of GM plants and derived food and feed, although it is likely that these approaches will evolve as the state of the knowledge on the development of the allergic response evolves. Rigorous application of these approaches, coupled with some judicious decision-making on a case-by-case basis, will help to ensure that it is convincingly demonstrated that a GM or novel food strategy does not add to the allergenic burden of the human diet.

**References**


