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GM organisms and the EU regulatory environment: allergenicity as a risk component

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The European Food Safety Authority, following a request from the European Commission, has published a guidance document for the risk assessment of GM plants and derived food and feed to assist in the implementation of provisions of Regulation (EC) 1829/2003 of the European Parliament and Council on GM food and feed. This regulation has applied since 18 April 2004. In principle, hazard identification and characterisation of GM crops is conducted in four steps: characterisation of the parent crop and any hazards associated with it; characterisation of the transformation process and of the inserted recombinant DNA, including an assessment of the possible production of new fusion proteins or allergens; assessment of the introduced proteins (toxicity, allergenicity) and metabolites; identification of any other targetted and unexpected alterations in the GM crop, including changes in the plant metabolism resulting in compositional changes and assessment of their toxicological, allergenic or nutritional impact. In relation to allergenicity specifically, it is clear that this property of a given protein is not intrinsic and fully predictable but is a biological activity requiring an interaction with individuals with a predisposed genetic background. Allergenicity, therefore, depends on the genetic diversity and variability in atopic human subjects. Given this lack of complete predictability it is necessary to obtain, from several steps in the risk-assessment process, a cumulative body of evidence that minimises any uncertainty about the protein(s) in question.

GM organism: Risk assessment: Allergenicity

Genetic engineering was first applied in the 1970s and, unlike other genetic-improvement methods, the application of this technology is strictly regulated. GM organisms (GMO) can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination. As an application of modern biotechnology, this technique allows selected individual genes to be transferred from one organism into another, and also between non-related species.

A GMO or a food product derived from a GMO can only be put on the market in the EU after it has been authorised on the basis of a detailed procedure. This procedure is based on a scientific assessment of the risks to health and the environment. EU legislation on GMO has been in place since the early 1990s, with the objective of protecting health and the environment and ensuring the free movement of safe GM products in the EU. In relation to crop plants, the primary relevant legislation concerning safety are: (a) Directive 2001/18/EC (European Commission, 2001), which covers issues related to the deliberate release of GMO into the environment; (b) Regulation (EC) No 1829/2003 (European Commission, 2003*a*), which applies to applications for the placing on the market of GMO for food and feed use, and also covers food and feed containing GMO, consisting of such organisms or produced from GMO. The regulation stipulates that the products must not: have adverse effects on human health, animal health or the environment; mislead the consumer or user; differ from the food or feed they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for human subjects (and for animals in the case of GM feed). In the case of GM food and feed they must not harm or mislead the consumer by impairing the distinctive features of the animal products.

GMO and food products derived from GMO that are placed on the market must also satisfy labelling and traceability conditions. These conditions are laid down in Regulation (EC) No 1829/2003 (European Commission, 2003*a*) and in Regulation (EC) No 1830/2003 (European

Abbreviations: EFSA, European Food Safety Authority; GMO, GM organism.
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Commission, 2003*b*), which concerns the traceability and labelling of GM organisms and the traceability of food and feed products produced from GM organisms and amend Directive 2001/18/EC (European Commission, 2001).

The authorisation procedure under Regulation (EC) No 1829/2003

This authorisation, valid throughout the EU, is granted subject to a single risk-assessment process under the responsibility of the European Food Safety Authority (EFSA) and a single risk-management process involving the European Commission and the member states through a regulatory committee procedure. Notably, it is up to the Commission to adopt the final decision and grant or reject the authorisation if the Standing Committee on the Food Chain and Animal Health (composed of representatives of the member states), and the Council of Ministers have not managed to adopt the decision by qualified majority within the time limit in question. Hence, the adoption of the final decision by the Commission constitutes the democratic exercise of a responsibility that was vested in it by the Council and the European Parliament, which directly represents the European citizens.

Applications are submitted first to the competent authority of the member state in which the product is first to be marketed. The application must clearly define the scope of the application, indicate which parts are confidential and must include a monitoring plan, a labelling proposal and a detection method. The national authority must acknowledge receipt in writing within 14 d and inform the EFSA. The application and any supplementary information supplied by the applicant must be made available to the EFSA, which is responsible for the scientific risk assessment, covering both the environmental risk and the human and animal health safety assessment. Its opinion will be made available to the public and the public will have the opportunity to make comments.

In general, a time limit of 6 months for the EFSA opinion is applied. This time limit can be extended if the EFSA has to request further information from the applicant. A guidance document for the risk assessment of GM plants and derived food and feed has been adopted (European Food Safety Authority, 2004).

Within 3 months of receiving the opinion of the EFSA, the Commission will draft a proposal for granting or refusing authorisation. The Commission may diverge from the EFSA's opinion, but it must then justify its position. The Commission's proposal will be approved through a qualified majority by the member states within the Standing Committee on the Food Chain and Animal Health. If the Committee gives a favourable opinion, the Commission adopts the decision; if not, or in the event of rejection of the Commission's proposal by a qualified majority of the Committee, the draft decision is submitted to the Council of Ministers for adoption or rejection by a qualified majority. If the Council does not act within 3 months or does not obtain a qualified majority for the adoption or rejection of the Commission's proposal, the Commission will adopt the decision.

National safeguard measures

Member states can invoke the so-called 'safeguard clause' if they have justifiable reasons to consider that a GMO, which has received written consent for placing on the market, constitutes a risk to human health or the environment. It may provisionally restrict or prohibit the use and/or sale of that product on its territory.

The risk-assessment process

The first international and national provisions for the safety assessment and regulation of GMO, including GM crops and derived foods were drawn up by scientific experts in the mid-1980s (Organisation for Economic Co-operation and Development, 1986; US Office Science and Technology Policy, 1986). This process was undertaken nearly a decade before the first regulatory approval of a GM crop in 1995. Since then, the global area of commercial cultivation of such crops has risen to 81×10^9 ha in 2004, representing a 20% increase from 2003 (James, 2004). Within the EU the recently-established EFSA has an important role to play in risk assessment in several areas. Key to its operational success are the risk assessments carried out by panels comprising independent scientific experts from throughout the EU. The panels include those on:

food additives, flavourings, processing aids, materials in contact with food;
additives and products in animal feed;
plant health, plant protection products;
GMO;
dietetic products, nutrition and allergies;
biological hazards;
contaminants in the food chain;
animal health and welfare.

Sequential steps in risk assessment involve:

hazard identification: characteristics that may cause adverse effects;
hazard characterisation: potential consequences for man and the environment;
exposure assessment: likelihood of occurrence or exposure;
total risk characterisation: evaluation of risk(s) posed by each identified characteristic.

In relation to GM crops a major underlying assumption is that traditionally-cultivated crops have gained a history of generally-accepted safe use. These crops can therefore serve as a baseline for the environmental and food or feed safety assessment of GM crops. This approach brings in the concepts of familiarity and substantial equivalence or comparative safety assessment. The concept of familiarity is based on the fact that most genetically-engineered organisms are developed from organisms such as crop plants that have a biology that is well understood. It is not a risk or safety assessment in itself, but familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of similar crops (including GM crops) into the environment and food chain. Familiarity comes from the knowledge and experience

available for conducting a risk or safety analysis before the scale-up of any new plant line or crop cultivar in a particular environment. The concept of substantial equivalence is based on the idea that an existing organism used as food or feed with a history of safe use can serve as a comparator when assessing the safety of the GM food or feed (Organisation for Economic Co-operation and Development 1993*a*,*b*; World Health Organization/Food and Agriculture Organization, 2000). Application of this concept may result in the identification of similarities and potential differences between the GM crop, food or feed and the non-GM counterpart. The outcome of this comparative approach will further structure the safety assessment procedure, which may include additional toxicological and nutritional testing. Application of the substantial equivalence concept is a starting point for the safety assessment. It provides assurance that the GM food or feed may be as safe as the traditional counterpart, or that no comparison can be made because of the lack of an appropriate comparator. Analysis of substantial equivalence involves not only a comparison of the chemical composition between the new and the traditional food or feed, but also of the molecular, agronomical and morphological characteristics of the organism in question. Such comparisons should be made with GM and non-GM counterparts grown under the same regimes and environments. When the extent of equivalence is established as substantial, a greater emphasis is placed on the newly-introduced trait(s). Where substantial equivalence does not occur, this factor does not necessarily identify a hazard. Where a trait or traits are introduced with the intention of modifying composition markedly and where the extent of equivalence cannot be considered substantial, then the safety assessment of characteristics other than those derived from the introduced trait(s) becomes of greater importance.

The risk assessment of GM plants and products takes into account:

- the characteristics of the donor and recipient organisms;
- the genes inserted and expressed;
- the potential consequences of the genetic modification;
- the potential environmental impact following a deliberate release;
- the potential toxicity and allergenicity of gene products and metabolites;
- the compositional, nutritional, safety and agronomic characteristics;
- the influence of food processing on the properties of the food or feed;
- the potential for changes in dietary intake;
- the potential for long-term nutritional impact.

Specific analyses carried out on the GM plant will confirm the intended effects, i.e. those effects that are targeted to occur from the introduction of the gene(s) in question and fulfill the original objectives of the genetic-transformation process. However, the analyses may also uncover unintended effects, i.e. consistent differences between the GM plant and its appropriate control lines, which go beyond the primary expected effect(s) of introducing the target gene(s). These effects may or may not be explicable in terms of the knowledge of biochemical regulation and may trigger the need for specific and additional risk assessment requirements. Unintended effects that might impact on human health would not be limited to GM approaches to plant breeding and are documented to occur when conventional approaches are used (Cellini *et al*. 2004).

Risk assessment: allergenicity

Allergy is an adverse reaction (in this context to foods) that, by definition, is immune-mediated and particularly involves IgE antibodies. Allergenicity is not an intrinsic fully-predictable characteristic property of a given protein but is a biological property requiring an interaction with individuals with a predisposed genetic background. Given this lack of complete predictability it is necessary to obtain, from several steps in the risk-assessment process, a cumulative body of evidence that minimises any uncertainty in relation to the protein in question. There is clearly a need to ensure that the products of novel genes introduced into GM crops are not allergenic and that the process of transformation does not cause unwanted changes in the characteristics and/or levels of expression of endogenous allergenic proteins. Assessment of allergenicity of foods derived from GM crops therefore requires that the following questions are addressed:

- is the recombinant protein derived from an allergenic source or a known allergen;
- is the recombinant protein able to induce *de novo* sensitisation;
- is the recombinant protein cross-reactive with IgE antibodies raised by known allergens, and therefore potentially capable of eliciting allergic reactions in already-sensitised subjects;
- has transformation itself in some way altered the allergenic properties of a food derived from a GM crop (such as, for instance, a change in the level of allergens endogenous to the host plant).

Importance of molecular characterisation of the transformation event

A requirement of the risk-assessment process is that the gene insertion event is well characterised in the host plant. The characterisation procedure includes analysis of the sequence of the inserted genes and associated DNA, which will identify: (a) the extent to which the inserted sequence differs from that in the plasmid used for transformation, which may detect substantial DNA re-arrangements and duplications or deletions; (b) identification of the junction sequences between the inserted DNA and the host plant DNA, which may detect the interruption of host plant open reading frames that might influence the expression of genes not intended to be changed. Alternatively, such ‘junction sequence’ analysis may also indicate the formation of new open reading frames that might give rise to novel fusion proteins that could have biological effects (i.e. toxic or allergenic potential). The obtention of insert and flanking region sequence information is accompanied by bioinformatics analysis, comparing DNA sequences with those

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known to encode for established toxins and allergens. Where there is cause for concern additional data will be required. In-depth molecular analysis of the insertion event is therefore an important starting point for several reasons.

Bioinformatic sequence-similarity searches utilise various publicly-available databases (King *et al*. 1994) to compare the amino acid sequence of the introduced protein with those of known allergens, identifying contiguous identical amino acids that may represent linear allergenic epitopes. The size of the contiguous identical amino acids searched for should be based on a scientifically-justified rationale in order to minimise the potential for false negative or false positive results (Codex Alimentarius Commission, 2003). There is currently some debate on whether the identity of six or eight contiguous amino acids between the novel gene product and a known allergen should signal a potential concern. Available data suggest that classifying proteins that have a linear sequence homology with a known allergen of six contiguous amino acids as potential allergens, as proposed by the Joint FAO/WHO Expert Consultation (Food and Agriculture Organization/World Health Organization, 2001), would result in a great number of false positive predictions. A contiguous eight amino acid search is probably more effective in order to detect common epitopes with known allergens (International Life Sciences Institute Health and Environmental Sciences Institute, 2001; Hileman *et al*. 2002). Amino acid sequence searches cannot, however, identify discontinuous or conformational allergenic epitopes that depend on the tertiary structure of the protein. It is clear that currently the approach is partly empirical and there is a need for a more detailed understanding of the extent of structural similarity that signals a likely hazard. Progress is being made in the design and application of novel bioinformatic and biocomputational approaches to align sequence data and protein folding with allergenic potential, and there is a need to exploit these opportunities fully.

Allergenicity of the newly-expressed protein

An integrated stepwise case-by-case approach is supported to assess possible allergenicity of newly-expressed proteins (Fig. 1 shows an example of such an approach). In every case a search for sequence homologies and/or structural similarities between the expressed protein and known allergens should be made as the first step. Identification of potential linear IgE-binding epitopes should be conducted by a search for homologous peptidic fragments in the amino acid sequence of the protein. The size of the contiguous identical or chemically-similar amino acid search should be based on a scientifically-justified rationale in order to minimise the potential for false negative or false positive results.

If the source of the introduced gene is considered allergenic, but no sequence homology to a known allergen is demonstrated, specific serum screening of the expressed protein is then expected using appropriate sera from patients allergic to the source material and relevant validated immunochemical tests. If the source is not known

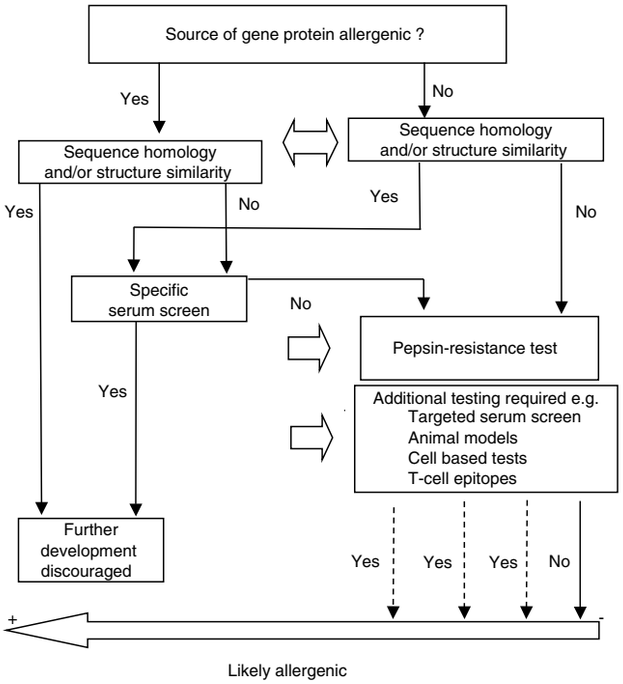

Fig. 1. Flow chart for assessment of allergenicity of newly-expressed protein in GM organisms. (Reproduced courtesy of JM Wal.)

to be allergenic but sequence homology to a known allergen is demonstrated, the specific serum screening should be conducted with sera from patients sensitised to this allergen. If the source of the gene or protein is not known to be allergenic and no sequence homology to a known allergen is demonstrated, or if the result of the specific serum screening of a newly-expressed protein from a source known to be allergenic is negative or equivocal, additional tests should be performed. These tests include pepsin resistance tests or targeted serum screening.

Stability to digestion by proteolytic enzymes has long been considered a characteristic of allergenic proteins. Although it has now been established that no absolute correlation exists, resistance of proteins to pepsin digestion is still proposed as an additional criterion to be considered in an overall risk assessment. In the case of resistance of a protein to degradation in the presence of pepsin under appropriate conditions, further analysis should be conducted to determine the likelihood of the newly-expressed protein being allergenic. It is also useful to compare intact, pepsin-digested and heat-denatured proteins for IgE binding. Targeted serum screening, as proposed in the Joint FAO/WHO Expert Consultation (Food and Agriculture Organization/World Health Organization, 2001), aims to assess the capacity of the newly-expressed protein to bind to IgE in sera of individuals with clinically-validated allergic responses to categories of foods broadly related to the gene source. If no relevant serum is available the expressed protein should be analysed for evidence of cross-reactivity and/or sensitising potential using other tests such as appropriate animal models or search for T-cell epitopes, structural motifs, etc. Complementary data on the biological origin and function and structural features of the

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newly-expressed protein may also be provided in order to increase the body of facts to support a conclusion.

Assessing allergenicity of the whole GM plant or crop

If the host plant of the introduced gene is known to be allergenic, any potential change in the allergenicity of the whole GM food or feed should be tested by comparison of the allergen repertoire with that of the conventional non-GM variety. These approaches should be applied on a case-by-case basis, depending on the available information on the allergenic potential of the source and/or the host. Data on the prevalence of occupational allergy in workers or in farmers who have marked exposure to the GM plant and crops or to the airborne allergens they may contain will provide useful information for the risk-assessment process.

'Model' approaches

Current uses of animal models to assess allergenicity are based on assessment of induced antibody responses and/or the frequency of responders in the test groups. Alternative or supplementary end points based on a more detailed understanding of the immuno-biological basis for sensitisation and an appreciation of why proteins differ in their sensitising potential should hopefully be possible. It will also be important to consider the sensitising potential when proteins are present in a complex food matrix and the ways in which a protein allergen is encountered. There is a definite need for more research on the immuno-biology of allergy to identify molecular markers that can be used to distinguish protein allergens from non-sensitising proteins. The same technologies (e.g. proteomics) could also be used to assess whether genetic transformation has caused any unintended changes in the level of expression of allergenic proteins. Cell-based models can certainly be used in assessing the allergenic potential of novel proteins, i.e. their ability to elicit clinical symptoms, but not in assessing sensitisation properties.

Conclusions

There is little doubt that GM products are highly scrutinised at many levels in science and society. Arguably, only pharmaceutical products are subjected to more extreme risk-assessment regimens. To date millions of individuals have eaten GM products with no proven ill effects, thus the risk-assessment procedures currently in place appear to be very effective. This situation does not mean that there should be any complacency, particularly as new GM traits come on line that might challenge the concept of 'familiarity' in relation to the crop species in question. For further reading the following texts are recommended, which provide a comprehensive overview outline of the concepts that surround the risk assessment of GM crops: ENTRANSFOOD (2003); Kuiper *et al*. (2004), which includes several keynote papers on GM safety testing; Task Force of the International Life Sciences Institute International Food Biotechnology Committee (2004); European Food Safety Authority (2004).

References

Cellini F, Chesson A, Colquhoun I, Constable A, Davies HV, Engel KH *et al.* (2004) Unintended effects and their detection in genetically modified crops. *Food and Chemical Toxicology* **42**, 1089–1125.

Codex Alimentarius Commission (2003) Report of the third session of the Codex *ad hoc* Intergovernmental Task Force on foods derived from biotechnology (ALINORM 03/34). Appendix IV. Proposed draft annex on the assessment of possible allergenicity of the draft guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants (advanced to steps 5 and 8 of the procedure). ftp://ftp.fao.org/codex/alinorm03/Al03_34e.pdf

ENTRANSFOOD (2003) European network safety assessment of genetically modified food crops. http://europa.eu.int/comm/research/quality-of-life/ka1/volume1/entransfood.htm

European Commission (2001) Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. *Official Journal of the European Communities* **L106**, 17.4.2001, 1–38.

European Commission (2003*a*) Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed products. *Official Journal of the European Communities* **L268**, 1–23.

European Commission (2003*b*) Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC. *Official Journal of the European Communities* **L268**, 24–28.

European Food Safety Authority (2004) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. http://www.efsa.eu.int/science/gmo/gmo_guidance/660/guidance_docfinal1.pdf

Food and Agriculture Organization/World Health Organization (2001) Evaluation of allergenicity of genetically modified foods. Report of a Joint FAO/WHO Expert Consultation on allergenicity of foods derived from biotechnology, 22–25 January 2001. ftp://ftp.fao.org/es/esn/food/allergygm.pdf

Hileman RE, Silvanovich A, Goodman RE, Rice EA, Holleschak G, Astwood JD & Hefle SL (2002) Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. *International Archives of Allergy and Immunology* **128**, 280–291.

International Life Sciences Institute Health and Environmental Sciences Institute (2001) *Allergenicity Assessment for Foods Derived from Genetically Modified Crops*. Washington, DC: ILSI HESI.

James C (2004) *Global Status of Commercialized Biotech/GM Crops: 2004. International Service for the Aquisition of Agri-Biotech Applications Briefs* no. 32. Ithaca, NY: ISAAA; available at http://www.isaaa.org/kc/CBTNews/press_release/briefs32/ESummary/Executive%20Summary%20(English).pdf

King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TAE & Thomas W (1994) Allergen nomenclature. *International Archives of Allergy and Immunology* **105**, 224–233.

https://doi.org/10.1079/PNS2005462 Published online by Cambridge University Press

Kuiper HA, Kleter GA, Konig A, Hammes WP & Knudsen I (editors) (2004) Safety assessment, detection and traceability, and societal aspects of genetically modified foods. European Network on Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD). *Food and Chemical Toxicology* **42**, 1043–1202.

Organisation for Economic Co-operation and Development (1986) *Recombinant DNA Safety Considerations*. Paris: OECD; available at http://www.oecd.org/pdf/M00032000/M00032689.pdf

Organisation for Economic Co-operation and Development (1993*a*) Safety considerations for biotechnology: Scale-up of crop plants. http://www.oecd.org/pdf/M00034000/M00034525.pdf

Organisation for Economic Co-operation and Development (1993*b*) Safety evaluation of foods derived by modern biotechnology: concept and principles. http://www.oecd.org/pdf/M00033000/M00033002.pdf

Task Force of the International Life Sciences Institute International Food Biotechnology Committee (2004) Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology. http://www.ift.org/pdfs/crfsfs/crfsfsv3n2p0035-0104ms20040106.pdf

World Health Organization/Food and Agriculture Organization (2000) Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO expert consultation on foods derived from biotechnology no. 29. http://www.fao.org/es/ESN/food/pdf/gmreport.pdf

US Office of Science and Technology Policy (1986) Coordinated framework for regulation of biotechnology. *Federal Register* **51**, 23302–23350.