ELSEVIER

Trends in Food Science & Technology 14 (2003) 277–293

TRENDS IN FOOD SCIENCE &TECHNOLOGY

8. The scientific basis for risk assessment and regulation of genetically modified foods

Harry A. Kuiper* and Gijs A. Kleter

RIKILT – Institute of Food Safety, PO Box 230, NL 6700 AE Wageningen, The Netherlands (tel.: +31-317-475422; fax: +31-317-417717; e-mail: harry.kuiper@wur.nl)

8.1. Introduction

The safety assessment of genetically modified (GM)[1] food (GMF) crops has attracted the attention of plant breeders, food scientists, risk assessors and regulators, and consumer and environmental organizations. A major concern is whether GM crops are so special in nature that new safety assessment strategies are needed, or whether safety evaluations can be carried out in the manner usually used for crops cultivated by traditional breeding techniques. This chapter examines the characteristics of GMF crops and the need for specific risk assessment models. The difficulties in safety testing of whole foods are also highlighted, and new approaches for the safety assessment of whole foods, taking advantage of modern molecular-biological, toxicological and analytical methods, are discussed. This is of particular interest for the safety and nutritional assessment of future GM crops with improved nutritional properties or properties beneficial to health.

8.2. Safety assessment principles for conventional foods

8.2.1. Safety assessment of food chemicals

Risk assessment of food chemicals, such as food additives and preservatives, and of food contaminants is based on general toxicological principles—hazard identification and characterization, and exposure assessment. Hazard is defined as the potential of an agent to cause harmful effect(s), and risk as a function of the probability that an adverse effect will occur owing to the presence of a hazardous compound and the severity of the effect (WHO, 1987, 1990). The safety evaluation of food chemicals is based on the establishment of a level of daily intake by humans that will not cause an appreciable risk (Acceptable Daily Intake, ADI). Most toxic effects induced by chemicals exhibit a threshold limit, that is, a dose level below which a toxic effect is not apparent. For the establishment of a 'safe' dose level (No Observed Adverse Effect Level, NOAEL) a series of (sub)chronic toxicity studies is performed in laboratory animals. Short-term studies are used for defining the acute reference dose (ARfD). If appropriate, genotoxicity and carcinogenicity studies and specific studies of immunotoxicity, reproduction and developmental toxicity are also carried out. Protocols for such studies are described in guidelines developed by the Organisation for Economic Co-operation and Development (OECD, 1993). From these studies a NOAEL is determined and, after application of an uncertainty factor, the ADI is derived. Normally an uncertainty factor of 100 is used to allow for differences in sensitivity between the test animal species and humans and for differences within the human population. Higher or lower safety

* Corresponding author.

[1] The definition of 'genetic modification' used in this chapter is limited to the insertion of genetic material into an organism by recombinant DNA techniques.

0924-2244/03/$ - see front matter
doi:10.1016/S0924-2244(03)00067-0

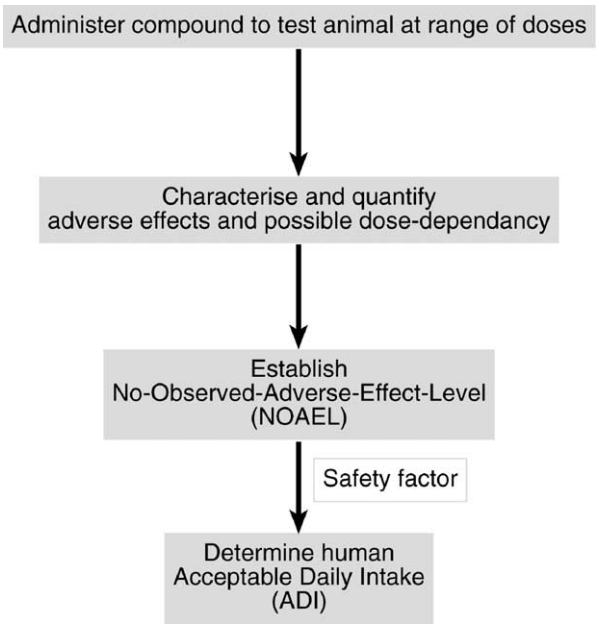

Fig. 8.1. Determination of an acceptable daily intake (ADI) of a compound for humans.

factors may be applied, depending on the chemical under study and the information available on the toxicological profile. For chemicals like genotoxic carcinogens, no threshold limits may be established, in which case a quantitative risk assessment is carried out, taking dose–response relationships into account. In general, the described strategy represents a 'conservative,' 'safety-first' approach, since relatively large safety factors are applied for calculation of the ADI (Fig. 1).

8.2.2. Safety assessment of whole foods

Since whole foods contain mixtures of macro- and micronutrients, and in many cases also antinutrients and naturally occurring toxins, a safety evaluation strategy similar to that described above for single chemicals is virtually impossible for whole foods. Testing of whole foods in laboratory animals has its specific problems. In general, amounts of foods that can be administered to animals are limited compared with amounts present in a human diet, owing to the bulk of foods, effects on satiety and possible negative interference with the nutritional status of the test animal. Feeding animals with whole foods at exaggerated dose levels may induce adverse effects that are not easily traceable to specific food components and may mask potential adverse effects of other components.

Most of the conventional foods currently on the market have never been assessed for possible adverse effects. The safety assessment of conventional crops, although not formally recognized or systematically carried out, is primarily based on a case-by-case analysis of the agronomic performance of the cultivated new crop and on a (limited) analysis of known macro- and micronutrients, antinutrients and toxicants. Food crops with an unusual agronomic performance or taste or harmful levels of specific compounds are rejected from traditional breeding programmes. Potatoes, for example, contain toxic glycoalkaloids, which may cause, depending upon the level of exposure, toxic effects in humans, ranging from gastrointestinal disorders to paralysis and mortality. Potato breeders nowadays screen varieties for their glycoalkaloid content, for which allowable threshold values have been defined. A level of 200 mg glycoalkaloids per kg fresh potatoes is generally considered the upper safety limit).[2] A newly developed variety of insect-resistant celery, for example, was removed from the market owing to the presence of high levels of UV-sensitive furanocoumarins, which caused skin-sensitization in humans following exposure to sunlight (Beier, 1990). Kiwi fruits, native to China and with no known history of inducing allergy, have been introduced into western markets, subsequently triggering allergy in some consumers (Fine, 1981).

In general, the safety evaluation of foods for human consumption is rather empirical and based on long-term experience. Even though these foods may contain antinutritional or toxic substances, this strategy has provided reasonable certainty that no harm will result from intended uses under anticipated conditions of consumption.[3]

8.3. Safety assessment principles for GM foods

Recognizing the difficulties of testing whole foods for their safety, an alternative approach has been developed for the safety assessment of GMFs, which is based on the concept of 'Substantial Equivalence' (OECD, 1996).[4,5]

[2] OECD (2002) *Consensus Document on Compositional Considerations for New Varieties of Potatoes: Key Food and Feed Nutrients, Anti-Nutrients and Toxicants* (Series on the Safety of Novel Foods and Feed, No 4; ENV/JM/MONO(2002)5), Paris, France, Environment Directorate,Organisation for Economic Co-operation and Development, available [November 2002] at http://www.olis.oecd.org/olis/2002doc.nsf/43bb6130e5e86e5fc12569fa005d004c/4091cf51091a9e4bc1256b3c00403dc7/$FILE/JT00119165.PDF.

[3] OECD (1993) *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles,* Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/pdf/M00033000/M00033002.pdf.

[4] OECD (1993) *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles,* Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/pdf/M00033000/M00033002.pdf.

[5] OECD (1998) *Report of the OECD Workshop on the Toxicological and Nutritional testing of Novel Foods* (Aussois, France, 5–8 March 1997; SG/ICGB(1998)1/FINAL), Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.olis.oecd.org/olis/1998doc.nsf/4cf568b5b90dad994125671b004bed59/2f17b887c5e79730c1256b6d004d9a24/$FILE/JT00121556.PDF.

The concept of substantial equivalence embodies the idea that conventional foods can serve as comparators for the properties of GMFs, since conventional foods are considered as safe, based on a history of safe use. The comparison takes the agronomical, morphological, genetic and compositional properties of the GMF and the traditionally produced food into account, and establishes the degree of equivalence between the GMF and the traditional counterpart. Identified differences are the subject of further toxicological, analytical and nutritional investigation.

Application of the concept of substantial equivalence does not lead to the establishment of the absolute safety of the GMF, but it does provide insight into whether the GMF can be considered as safe as the conventional counterpart. Application of the concept is not a safety assessment *per se*; it forms part of the safety assessment, by identifying similarities and potential differences between the existing food and the new GM product.

In order to obtain meaningful comparative data, particular attention should be paid to the choice of the comparator, the performance of field trials and statistical analysis of the generated data. The comparator should serve as the organism relative to which potential differences due to the genetic modification can be identified; it should preferably be the direct parent line used to generate the transgenic line. However, in many cases crops are developed using back-crossing, and consequently appropriate controls should be used (EU, 2002). Testing should be performed on GM plants and their comparators grown under identical environmental conditions, as environmental conditions may lead to phenotypic and genotypic differences not related to the specific transformation process. Protocols suggesting the number of locations, growing seasons, geographical spreading and replicates that should be used in testing have been described.[6]

Components that should be measured in the GM plants and control lines include the macro- and micronutrients, toxicants and antinutritional compounds, such as digestive enzyme inhibitors, that are typical for the crop under investigation. A fatty acid profile should be measured in oil-rich plants and an amino-acid profile should be measured in plants or seeds used as an important protein source. Additional analyses might be required, depending on the type of genetic modification or in order to investigate detected differences further. The OECD is in the process of making inventories of plant constituents that should always be analyzed in any new variety of the given crop. Consensus Documents[7] have been formulated for soybean, oilseed rape, sugar beet, potato, and maize, while others on wheat, rice, sunflower, cotton, barley, and forage legumes are in preparation. International harmonization and standardization is necessary to avoid differences in data requirements in different countries; such standardization would help prevent trade barriers.

Standard statistical analysis should be performed on the data, leading to the identification of differences with a pre-established degree of confidence. Completely randomized trials should be used to indicate whether the many experimental variables might be interactively linked to each other. The safety consequences of any observed changes would need further investigation; the extent of further investigation would be determined by the chosen parameter. If observed differences in composition between the GM organism (GMO) and its counterpart were to fall outside the observed ranges of natural variability, further studies on the potential safety impact might be requested. Even if observed changes were to fall within the range of natural variations, and thus not pose a safety concern, such changes might be indicative of other unknown changes in the metabolism of the modified organism; further investigations would, therefore, still be required.

8.4. Specific safety issues

Two principal advantages result from the genetic modification of organisms using recombinant DNA technology, in comparison with other technologies for genetic improvement. First, genetic modification allows for the transgression of genetic material across species borders, which would be impossible to do by natural breeding or propagation techniques. A striking example of this is a GM frost-resistant strawberry containing a fish gene that codes for an antifreeze protein (Firsov & Dolgov, 1998). Secondly, recombinant DNA techniques allow more targeted modification of the genetic material than do mutation or conventional breeding, in which many genes may be mutated or transferred to the target organism.

The safety evaluation of GMFs addresses several specific issues, as follows (Fig. 2):

- characterization of donor and host organism;
- description of the transformation process and of the genetic modification;
- stability of inserted recombinant DNA and expression of novel DNA;

[6] Food Standards Agency (2001) *Statistically Valid Data to support Applications for Safety Clearance of Crop Product under EC Regulation on Novel Foods and Novel Food Ingredients* 258/97, London, UK, Food Standards Agency, available [November 2002] at http://www.food.gov.uk/science/ouradvisors/novelfood/acnfppapers/validnoveldata.

[7] OECD (2002) *Consensus Documents for the Work on the Safety of Novel Foods and Feeds*, Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/EN/document/0,,EN-document-32-nodirectorate-no-27-24778-32,00.html.

- potential toxicity of newly expressed proteins;
- effects of the transformation on the composition, i.e. the occurrence of intended and unintended effects;
- toxicological and nutritional impact of alterations in composition of the new food;
- potential allergenicity of newly expressed proteins and altered allergenicity of the whole food;
- effects of processing and cooking;
- potential intake and dietary impact of the GMF; and
- potential for gene transfer from GMF crops to microorganisms of the human/animal gut flora and associated health impact.

These issues have been addressed in guidance documents prepared by national and international organizations (IFBC, 1990; OECD, 1993, 1996; EU, 2002).[8–14]

[8] FAO/WHO (1991) *Strategies for Assessing the Safety of Foods produced by Biotechnology: Report of a Joint FAO/WHO Consultation,* Geneva, Switzerland, World Health Organization, available [November 2002] at http://www.who.int/fsf/GMfood/bio1991repo.pdf.
[9] FDA (1992) *Statement of Policy: Foods Derived from New Plant Varieties* (Federal Register 57 (May 29, 1992), 22984-23005), Rockville, MA, US Food and Drug Administration, available [November 2002] at http://vm.cfsan.fda.gov/~lrd/bio1992.html.
[10] OECD (2002) *Report of the OECD Workshop on the Toxicological and Nutritional Testing of Novel Foods* (Aussois, France, 5–8 March 1997; SG/ICGB(1998)1/FINAL), Paris, France, Organisation for Economic Co-operation and Development, available [January 2003] at http://www.olis.oecd.org/olis/1998doc.nsf/LinkTo/sg-icgb(98)1-final.
[11] FAO/WHO (1996) *Biotechnology and Food Safety Report: Report of a Joint FAO/WHO Consultation* (30 September–4 October 1996) (FAO Food and Nutrition Paper 61), Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/es/esn/food/biotechnology.pdf.
[12] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.
[13] FAO/WHO (2001) *Evaluation of Allergenicity of Genetically Modified Foods: Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology*, (22–25 January, 2001), Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/es/esn/food/allergygm.pdf.
[14] FAO/WHO (2002) *Report of the Third Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology*, Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/alinorm03/Al03_34e.pdf.
[15] CFIA (1998) *Canada-U.S. Bilateral on Agricultural Biotechnology. Appendix I, Molecular Genetic Characterization Data,* Ottawa, Canada, Canadian Food Inspection Agency, available [November 2002] at http://www.inspection.gc.ca/english/plaveg/pbo/usda/usda03e.shtml.
[16] FAO/WHO (2002) *Draft Guideline for the Conduct of Food safety Assessment of Foods derived from Recombinant-DNA plants* (ALINORM 01/34A, Appendix III), Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/alinorm01/al0134ae.pdf.

8.4.1. Molecular characterization

Requirements for molecular data on the genetic modification process and on the transformed organism, for the purpose of risk assessment, are detailed in various guidelines and guidance documents (EU, 2002),[15,16] which include detailed descriptions of requirements for information on the following:

- characteristics of the donor and host organism(s);
- the specific method for transformation and the DNA used (genetic components, including marker genes and regulatory elements);
- inserted/deleted DNA sequences, number of insertion sites, organization of genetic material, open reading frame(s);
- expression of gene product(s) in the transformed organisms (level and site of expression, transfer of genes coding for known toxins or anti-nutrients);

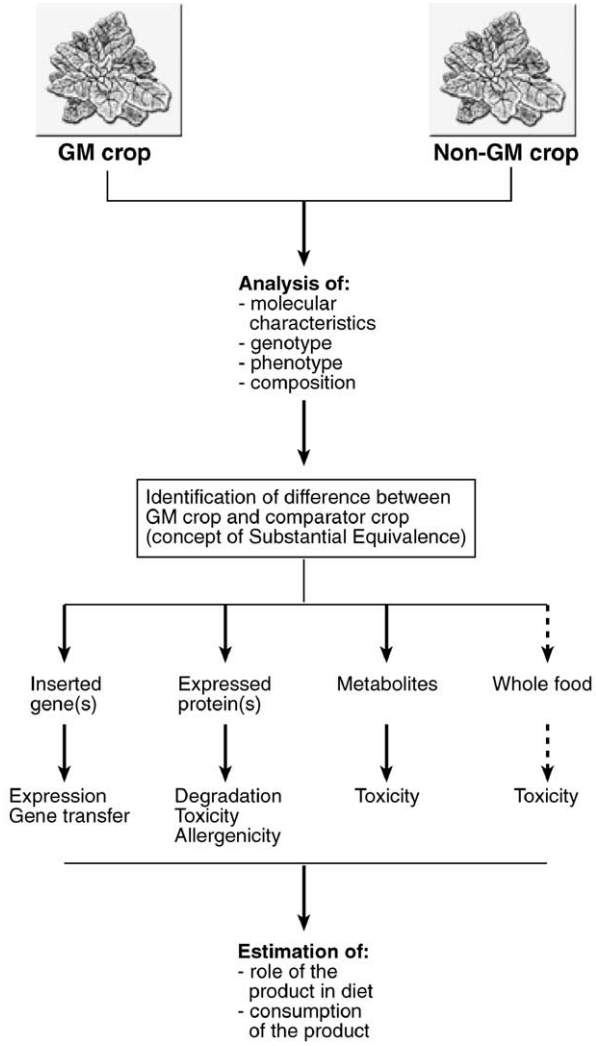

Fig. 8.2. Application of the concept of substancial equivalence in the risk assessment of GM crops: safety assessment strategy in crops.

- possible influence of the transformation process on the expression of inherent active or silent genes in the host organism; and
- possible expression of new fusion proteins.

The aim of the molecular characterization of the modification process and of the host organism is to provide the basis for the environmental safety and food/feed safety assessment of the GMO. The analysis of border sequences between foreign and host DNA also deserves special attention, both for safety assessment of GMOs and for the development of GMO-specific detection methods. For example, a recent study on the analysis of border DNA in GM soybeans, using the anchor polymerase chain reaction (PCR) method and adaptor ligation PCR techniques, demonstrated the insertion of additional and rearranged DNA next to the 3′ end of the inserted gene that was the target of the genetic modification (Windels, Taverniers, Depicker, Van Bockstaele, & De Loose, 2001).

8.4.2. Toxicological assessment

Safety assessment strategies for GMFs should be designed and carried out on a case-by-case basis, according to the degree of equivalence with the conventional counterpart. The safety of proteins expressed in the GMF but not the conventional product, other novel constituents, and natural constituents that have different levels as a result of the genetic modification should be assessed; each may need, by nature, different toxicological approaches.

Novel proteins

Before embarking on toxicity studies of novel proteins, molecular, biochemical, structural and functional characterization of the newly expressed protein should be carried out; such characterization should include: degradation under conditions of ingestion, processing and storage; biological response/immunological activity (receptor binding, enzymatic activity, substrate specificity etc); and sequence homology with proteins known to cause adverse effects.

Specific testing of a new protein should be undertaken in some circumstances: for example, when the novel protein has a specific biological function and/or mode of action, for instance like a protease inhibitor; when the protein is implicated in mammalian toxicity, for instance like a bacterial toxin; when human or animal exposure to the protein is not documented; when the primary structure of naturally occurring form(s) of the protein is modified; when estimated levels of intake of the new protein indicate adverse effects; and when the protein is not rapidly degradable.

A general problem is that it may not be possible to extract sufficient amounts of test protein from the GMO (e.g. the modified food plant); test proteins are, therefore, produced by cultures of over-expressing yeasts or bacteria. This means that a thorough comparative investigation should be made of the structural and functional properties of the test protein and the protein expressed in the food organism, as variations in, for example, protein folding and type and degree of glycosylation or phosphorylation may have an influence on the toxicological spectrum and potency of the protein. Comparative investigations should be made of electrophoretic behaviour of full-length as well as of trypsinated forms, immunoreactivity with poly-and/or monoclonal antibodies, patterns of post-translation modification, sequence similarity and functional characteristics.

The use of *in vitro* methods is recommended for studies on protein resistance to digestive enzymes; standardized and validated methods should be applied, such as the simulated gastric fluid model (Astwood, Leach, & Fuchs, 1996).

The predictive value of the comparative sequence homology approach is still in an exploratory phase, and better understanding is needed about the relationship between type and degree of homology and actual toxicity. Specific protein sequence databases (e.g. from the National Centre for Biotechnology Information, and SwissProt) and protocols for homology searches (e.g. minimal length of identical sequences) should be used.[17]

The toxicity of novel proteins is normally tested in laboratory animals, using appropriate dose-regimes and a duration of at least 28 days, and following standardized protocols (OECD, 1993). Preferably young laboratory animals, able to respond rapidly and sensitively to induced physiological or metabolic changes, should be used (EU, 2002). Depending on the results obtained, further investigations, possibly of longer duration, may be carried out, for example if indications of specific organ or endocrine toxicity are apparent.

Examples of new proteins expressed in GM crops are the Cry proteins from *Bacillus thuringiensis* (*Bt*) strains that have insecticidal properties for larvae of herbivoral insect species (Peferoen, 1997). The mechanism of action of Cry proteins is based on specific receptor binding, in susceptible insect larvae, in epithelial cells of the midgut, leading to pore formation, cell lysis, disintegration of the epithelium lining in the midgut and, eventually, to death of the larvae owing to starvation. The type of biological action that the newly introduced

[17] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

protein has directs further toxicity testing in mammals. The safety of a number of newly inserted proteins has been tested on a case-by-case basis (Kuiper, Kleter, Noteborn, & Kok, 2001). Studies have been undertaken on the binding of Cry1Ab5 and Cry9C proteins to tissues of the gastrointestinal tract of rodents and primates, including humans[18] (Noteborn *et al*., 1995). There is no evidence for the presence of specific receptors for these proteins in mammalian tissues, or for amino acid sequence homology with known protein toxins/allergens. Single and repeated dose toxicity studies of Cry1Ab5 and Cry9C did not indicate toxic effects in the rat, and histopathological analysis did not show binding of the Cry proteins to the intestinal epithelium of rodents or tissues of other mammals. In contrast to Cry1Ab5, Cry9C showed resistance to proteolysis under simulated human gastric conditions ($pH \geqslant 2.0$). Since there seems to be a relationship between protein resistance to proteolysis and allergenic potential (Astwood *et al*., 1996), this characteristic needs further investigation (see Section 8.4.3).

Other consituents

New constituents other than proteins need to be evaluated using traditional toxicological testing strategies and procedures as described in Section 8.2.1 (WHO, 1987, 1990). If the composition of the GMF is altered substantially, or if there are uncertainties about the occurrence of unintended effects arising from the genetic modification, the whole food may be tested for safety. Decisions about safety testing should be made on a case-by-case basis. In general an animal feeding trial with a minimum duration of 90 days is considered necessary to demonstrate the safety of long-term consumption of the food, but studies of longer duration are deemed necessary if results of a 90-day study indicate adverse effects, for example proliferative changes in tissues. A Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation recognised that the potential long-term effects of any food on the consumer are hard to detect, given the wide genetic variability within the human population and constantly changing dietary intake patterns.[19] It is unlikely that epidemiological studies will be able to identify effects related specifically to GMFs against the natural background of effects due to food consumption in general. The premarket assessment of a GMF should provide sufficient evidence that it is as safe as the conventional counterpart.

Examples of feeding trials with whole GMFs and GM feeds have been reviewed (Kuiper *et al*., 2001). No relevant toxic effects were observed in several studies on different food/feed crops into which genes derived from *Bt* species, coding for insecticidal proteins, or genes coding for enzymes that are insensitive for herbicide action had been inserted. Other studies on GMF crops have indicated signs of toxicity, but experimental flaws have been noted. For example, Ewen and Pusztai (1999) reported proliferative and antiproliferative effects in the gut of rats fed GM potatoes containing GNA lectin. The effects were presumed to be due to alterations in the composition of the transgenic potatoes, rather than to the newly expressed gene product. However, various shortcomings of the study, such as protein deficiency of the diets and the lack of control diets, make the results difficult to interpret (Kuiper, Noteborn, & Peijnenburg, 1999). Similar criticisms have been made by the UK Royal Society (1999).

There is an absolute need for a harmonized design for feeding trials in animals to test the safety of GMFs. Moreover, since feeding trials with whole foods are of limited value, as discussed above, new tools need to be developed to assess long-term effects resulting from the consumption of new foods. To this end genomics and proteomics may increase the diagnostic potential, specificity and sensitivity of whole food tests. The principles and methods for hazard identification and hazard characterization for non-GM whole foods have recently been reviewed (FOSIE, 2002).

8.4.3. Allergenicity assessment

The potential allergenicity of GMOs has attracted broad interest, not least because the prevalence of allergies has increased among people in the western world in recent decades (UCB Institute of Allergy, 1997). Food allergies are adverse reactions to a food or food component that involve abnormal immune responses in humans or animals; such responses range from mild intestinal discomfort to anaphylactic shock. The prevalence of food allergy in the European population is approximately 2–3% (UCB Institute of Allergy, 1997); there is limited information on the prevalence of food allergy in developing countries. The most common type of food allergy is mediated by immunoglobulin class E (IgE) antibodies. True food allergies also comprise delayed cell-mediated hypersensitivity reactions, for which mechanisms of action are less clear. The most

[18] EPA (2000) Assessment of scientific information concerning StarLink corn Cry9C Bt corn plant-pesticide. (Federal Register 65 (31 October 2000)): 65246-65251, Arlington, US Environmental Protection Agency, available [December 2002] at http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2000_register&docid=00-28076-filed.

[19] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

common reaction is celiac disease, a gluten sensitive enteropathy, which might affect 1 in 100–300 individuals (Farrell & Kelly, 2001).

A list of common allergenic foods associated with IgE mediated reactions established by the Codex Alimentarius Commission includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat and tree nuts (the 'big eight'), but at least 160 food items have been associated with allergenic reactions, albeit more sporadically[20] (Hefle, Nordlee, & Taylor, 1996), and allergens have also been identified in fruits and vegetables (Ortolani, Ispano, Pastorello, Bigi, & Ansolani, 1988).

Besides allergenic effects induced following ingestion of certain food proteins, allergy may also be induced through inhalation. This is a cause of concern, as foreign proteins may be expressed in plant pollen, and occupational exposure may result from dust produced from crops during crop processing.

The potential allergenicity of newly expressed proteins is not one specific predictable parameter; it is dependent on the genetic predisposition and variability of IgE responses in atopic humans. It is therefore necessary to obtain as much information as possible, from several biological and physical criteria, which should all be taken into account in risk assessment. A stepwise, case-by-case approach is usually applied in order to assess the potential allergenicity of newly expressed proteins. The International Food Biotechnology Council (IFBC) and the International Life Sciences Institute (ILSI) have designed a decision tree approach, which focuses on evaluation of the gene source, sequence homology of the newly expressed protein with known allergens, IgE binding in serum of individuals allergic to the transferred material and physicochemical properties of the new protein (Metcalfe *et al*., 1996). FAO/WHO has adopted a similar approach[21] and has further concluded that, in case the source of the genetic material is not known to be allergenic, the decision-tree approach should also include criteria like level and site of expression and assessment of the functional properties of the new protein.[22]

FAO/WHO have proposed decision tree[23] and integrated stepwise, case-by-case[24] approaches for the assessment of the possible allergenicity of newly expressed proteins. Essential elements for the assessment are as follows.

- Characterization of the source of the foreign gene with respect to information on allergenicity.
- Sequence homology comparison with known allergens—potential linear IgE-binding epitopes should be looked for using known algorithms, with the size of contiguous identical amino acids based on a scientific rationale that minimises false negative or false positive results. In addition, cross-reactivity should be considered when more than 35% identity in a segment of 80 or more amino acids has been identified. Absence of sequence homology is not conclusive since these searches have their limitations: they are limited to sequence analysis of known allergens that are available in the databases; and they are not capable of detecting non-linear epitopes binding IgE antibodies.
- Specific *in vitro* screening in sera from patients allergic to the source—sera from individuals with a clinically validated allergy to the protein source should be used.
- Targeted *in vitro* testing in sera from patients allergic to materials that are broadly related to the source of the original gene—these tests should be considered for proteins that do not exhibit sequence homology with known allergens. As stated above, this does not exclude the possibility that homologous allergens may exist; therefore more targeted screening may be appropriate.
- Testing of the expressed protein for pepsin resistance—since a certain correlation seems to exist between pepsin resistance and allergenic potential, the degradation of the protein in the presence of pepsin should be studied under appropriate conditions. It should be noted that foods might contain protease inhibitors or other substances that might influence protein degradation. FAO/WHO have proposed a specific protocol, but alternative protocols, such as the one described in the United States

[20] FAO/WHO (2001) *Codex General Standard for the Labelling of Prepackaged Foods*. Rome, Italy, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/standard/en/CXS_001e.pdf.

[21] FAO/WHO (1996) *Biotechnology and Food Safety Report: Report of a Joint FAO/WHO Consultation* (30 September–4 October 1996) (FAO Food and Nutrition Paper 61), Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/es/esn/food/biotechnology.pdf.

[22] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

[23] FAO/WHO (2001) *Allergenicity of Genetically modified Foods: Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology* (22–25 January) Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/es/esn/food/allergygm.pdf.

[24] FAO/WHO (2002) *Draft Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA plants* (ALINORM 01/34A, Appendix III), Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/alinorm01/al0134ae.pdf.

Pharmacopoeia, may also be used. The relationship between the survivability of a protein in the gastrointestinal tract and its ability to provoke allergic sensitization should be further explored.

- Immunogenicity in animal models—for additional assessment of the potential allergenicity of newly expressed proteins, FAO/WHO recommend the use of animal models, like the Brown Norway rat model, using oral sensitization (Knippels, Penninks, Spanhaak, & Houben, 1998), or the mouse model, with intraperitoneal administration (Dearman & Kimber, 2001). The potential allergenicity of the newly expressed protein should be ranked against well-known strong and weak allergens and non-allergenic proteins. It is emphasized that these models provide additional information but they do not reflect all aspects of IgE-mediated food allergies in humans.

The approach described above will indicate a high or low probability for the allergenic potential of a newly introduced protein. However, it is emphasized that no single type of experiment and no single criterion is sufficient to predict allergenicity; moreover, the methodology to be used in this field is rapidly evolving.

If the host organism is known to be allergenic, any potential change in allergenicity of the whole food should be evaluated as well, taking the traditional counterpart as the comparator. An illustrative case of risk assessment of a potential allergenic GMF is maize in which the bacterial gene *Cry9Ca1*, derived from *Bt tolworthi*, has been expressed. Specific modifications of the gene resulted in the expression in maize of a truncated Cry9C protein (molecular weight 68 kDa); modification of the protein at amino acid position 123, by substituting the arginine residue with lysine, resulted in an increased resistance to pepsin degradation. Based on the relative stability of the protein, maize containing Cry9C (Starlink yellow maize) was restricted to animal feed use only in the USA.[25]

However, in 2000, traces of Starlink maize were found in taco shells[26] and, subsequently, a number of consumers reported allergic reactions after eating maize products. The US Environmental Protection Agency (EPA) Scientific Advisory Panel (SAP) on the Federal Insecticide Fungicide and Rodenticide Act reviewed the potential allergenicity of Starlink corn.[27] Cry9C protein might be considered as a potential allergen because of (i) resistance to gastric proteolytic degradation and to heat and acid treatment, (ii) the capacity to induce a positive IgE response in the Brown Norway rat, (iii) bioavailability in the bloodstream of Brown Norway rat, (iv) probable glycosylation and (v) molecular weight of allergens (10–70 kDa). It was concluded that Cry9C had a medium likelihood of being an allergen.

Exposure to Cry9C in food for the highest expected exposure group, that is Hispanic children, was estimated to be 17 μg/day (95th percentile). This amount was considered to be low by the EPA SAP panel, but little is known about the doses and frequencies of exposure needed to sensitize people and to trigger allergic reactions in those who are immunosensitized.

The Food and Drug Administration (FDA) with the assistance of the Centers for Disease Control and Prevention (CDC) evaluated 28 consumer complaints allegedly linked to Starlink maize consumption. Analysis of collected serum samples by enzyme-linked immunosorbent assay (ELISA) revealed that no Cry9C-specific IgE antibody could be detected.[28] However, these results are not entirely conclusive, since the IgE-specific ELISA test did not include the Starlink-derived Cry9C protein, but the recombinant Cry9C expressed in *Escherichia coli* as antigen. Furthermore, a specific goat antiserum against Cry9C was used, as no human serum was available that contained the IgE antibody to Cry9C. Thus, the possibility of lack of specificity for human anti-Cry9C IgE cannot be entirely dismissed.

Moreover non-food exposure, such as exposure through inhalation or dermal contact, was not included in the risk assessment by EPA, neither was cumulative exposure to other *Bt* proteins or the potential for cross-reactivity addressed (Bucchini & Goldman, 2002). These issues should be quantitatively assessed before large-scale introduction of GMFs containing this type of stable proteins on the market.

An example of transfer of a gene from an allergenic source that codes for an allergenic protein is that of the Brazil nut (*Bertholetta excelsa*) 2S albumin expressed in soybean. This protein is rich in methionine and would therefore increase the nutritive value of soybeans for

[25] EPA (2000) *EPA Preliminary Evaluation of Information Contained in the 25 October 2000 Submission from Aventis Cropscience: Executive Summary*, Arlington, US Environmental Protection Agency, available [October 2002] at http://www.epa.gov/scipoly/sap/2000/november/prelim_eval_sub102500.pdf.

[26] EPA (2000) *EPA Preliminary Evaluation of Information Contained in the 25 October 2000 Submission from Aventis Cropscience: Executive Summary*, Arlington, US Environmental Protection Agency, available [October 2002] at http://www.epa.gov/scipoly/sap/2000/november/prelim_eval_sub102500.pdf.

[27] FIFRA SAP (2000) *A Set of Scientific Issues being considered by the Environmental Protection Agency regarding: Assessment of Scientific Information concerning StarLink™ Corn* (SAP Report No. 2000-06), Arlington, US Environmental Protection Agency, available [October 2002] at http://www.epa.gov/scipoly/sap/2000/november/one.pdf.

[28] CDC (2001) *Investigation of Human Health Effects associated with Potential Exposure to Genetically Modified Corn*, Atlanta, GA, USA, Centers for Disease Control and Prevention, available [October 2002] at http://www.cdc.gov/nceh/ehhe/Cry9CReport/.

animal feed. However, it was found that the newly expressed protein in soybean was reactive towards sera from patients who were allergic to Brazil nut. This observation blocked further development of the GM product (Nordlee, Taylor, Townsend, Thomas, & Bush, 1996).

8.4.4. Unintended effects

The possible quality and safety impacts of potential unintended or unexpected changes in food composition as a result of genetic modification should be addressed. Random integration of genes may result in disruption of endogenous gene functions, which may lead to changes in levels and activities of inherent enzymes, nutrients and metabolites, or the production of new proteins, or toxins. Recent reviews have dealt with these issues (Kuiper *et al.*, 2001), which are also being covered by the European Network Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD—a project sponsored by the European Commission in the 5th Framework programme).[29]

Unintended effects may sometimes be predictable on the basis of what is known about the insertion place(s) of the transgenic DNA, the function of the disrupted gene(s) and the function of the inserted trait and its involvement in metabolic pathways; however, other effects are still unpredictable, owing to limited knowledge about gene regulation and gene–gene interactions.

The occurrence of unintended effects is not unique to genetic modification using recombinant DNA technology, it also occurs frequently in conventional breeding; examples are well documented of high levels of glycoalkaloids in classically bred potatoes and of furanocoumarins in celery (Beier, 1990).

Different strategies can be followed to identify unintended effects. A targeted approach can be used to measure single known nutritional or toxic compounds present in the GMF and its corresponding parent. However, changes in function or phenotype of a plant that result from genetic modification may not be reflected in changes in a preselected, limited number of single compounds, and detection of unknown toxicants and antinutrients is not possible. Moreover, the number of validated detection methods for single, biologically active compounds is limited. These limitations are of particular importance for food plants with no history or a limited history of (safe) use, and for plants that have been modified extensively through multiple gene insertions.

A non-targeted approach for the detection of unintended effects makes use of profiling methods, which may provide information on potential changes in the physiology of the modified host organism at different cellular integration levels, that is at the level of mRNA expression and protein translation, and at the level of plant metabolism. New techniques, such as DNA/RNA microarray technology, proteomics and hyphenated analytical techniques, enable an integrated and simultaneous analysis of gene expression, gene product and metabolite formation, respectively (Figs. 3–5).

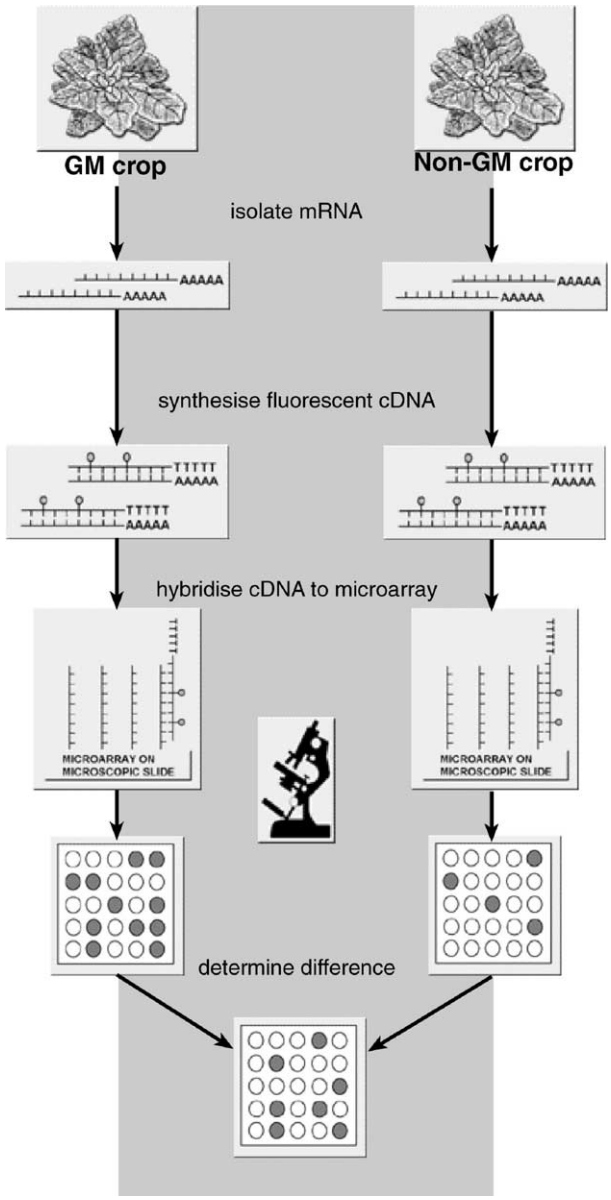

Fig. 8.3. Techniques with potential for non-targeted profiling of GM crops: gene expression analysis with cDNA microarrays.

Targeted single compound analysis

Key nutrients should be measured, such as protein, carbohydrates, fats, fatty acids, vitamins and other

[29] *ENTRANSFOOD,* Wageningen, RIKILT, available [November 2002] at http://www.entransfood.com/.

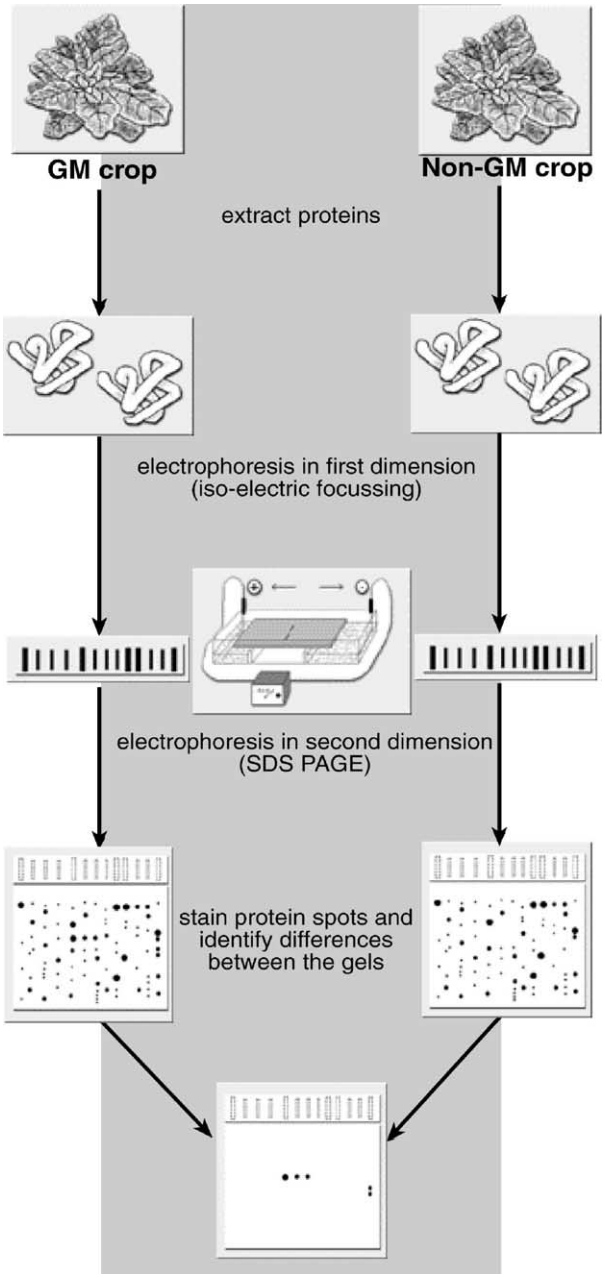

Fig. 8.4. Techniques with potential for non-targeted profiling of GM crops: protein expression profiling by two-dimensional gel electrophoresis.

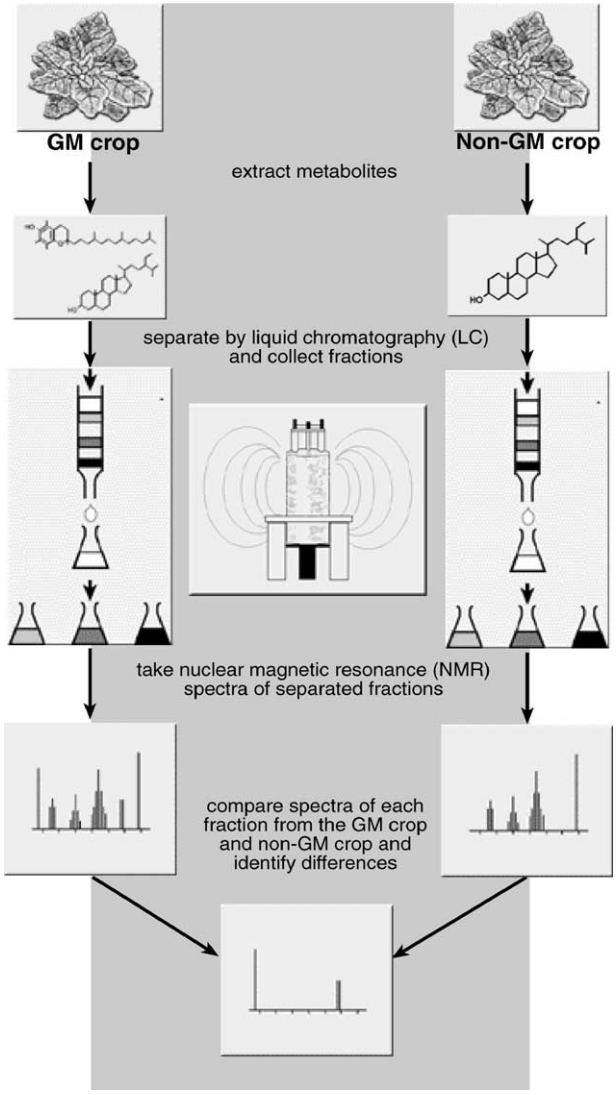

Fig. 8.5. Techniques with potential for non-targeted profiling of GM crops: metabolite analysis by liquid chromatography combined with offline nuclear magnetic resonance.

nutritional/antinutritional compounds that may impact on nutritional value and safety. Selection of key nutrients and toxicants for analysis needs to take into account the target species, structure and function of the inserted gene(s), and possible interferences in metabolic pathways. As noted above (Section 8.3) OECD has drawn up a number of Consensus Documents with information on the agronomical and compositional properties of widely cultivated crops.[30]

Differences in composition resulting from genetic modification may fall within or outside ranges of natural variability; effects on functionality, toxicity, efficacy and bioavailability should be evaluated. Differences in composition between the GMO and its counterpart that fall outside ranges of natural variability do not necessarily constitute a threat to human health.

[30] OECD (2002) *Consensus Documents for the Work on the Safety of Novel Foods and Feeds,* Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/EN/document/0,EN-document-32-nodirectorate-no-27-24778-32,00.html.

Non-targeted profiling analysis

Gene expression analysis. The study of gene expression using microarray technology, in which mRNA samples are hybridised to an array of DNA probes, allows small-scale analysis of the expression of a large number of genes at the same time, in a sensitive and quantitative manner (Fig. 3; Schena, Shalon, Davis, & Brown, 1995; Schena *et al*., 1996). Gene expression profiles of GM crops and parent lines under different conditions can be compared, and analysis of observed differences may be linked to the genetic modification process. Such technology and the related field of bioinformatics are still in development (Van Hal *et al*., 2000). It is possible that in future such methods may effectively be used to screen for altered gene expression and, at the same time, provide information on the nature of detected alterations—that is whether such alterations may affect the safety or nutritional value of the food crop under investigation. Further studies are being carried out with GM potatoes and tomatoes within the framework of a European Union (EU) sponsored project—GMOCARE.[31]

Proteomics. High-resolution two-dimensional gel electrophoresis in combination with mass spectrometry has increased the possibility of identifying and characterizing proteins involved in metabolic processes (Fig. 4). The technique is commonly employed for the study of protein profiles—'proteomics'. Proteomics can be used for identification of proteins and their post-translational modifications, quantification of the variation in protein contents and studies of protein–protein interactions.

When searching for unintended changes, proteomes of the GM and control lines should be compared. If differences in protein profiles are detected, normal variations should be looked at. It will probably be very difficult to detect differences specifically related to genetic modification, given the many proteins that are not involved in such changes and the many changes that may occur as a result of different environmental conditions. Restrictive conditions for the selection of proteins involved in important metabolic pathways may, therefore, reduce the number of confounding factors and yield more informative proteomes. Construction of protein microarrays (for instance of antibodies) may offer interesting opportunities to identify metabolic changes that may have an impact on the safety of GMF crops.

Chemical fingerprinting. A multicompositional analysis of biologically active compounds (i.e. nutrients, antinutritional factors, toxicants and other relevant compounds—the so-called metabolome) in GM and nonmodified plants may indicate whether intended and/or unintended effects have taken place as a result of genetic modification. The three most important techniques that have emerged for chemical fingerprinting are gas chromatography (GC), high performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR). These methods (e.g. Fig. 5) are capable of detecting, resolving and quantifying a wide range of compounds, in a single sample, derived from GM and non-GM plants grown under identical or different environmental conditions. The strength of these profiling methods is that they allow an 'unbiased' approach to identifying unexpected events resulting from genetic modification.

In conclusion, it is still too early to apply profiling methods for the detection of unintended effects in GMF crops on a routine basis. There is a need to (i) standardize sample collection, preparation and extraction procedures, (ii) standardize and validate measurements, (iii) generate information on profiles of plant extracts and on natural variations, and (iv) develop bio-informatic systems to treat large data sets. Data analysis is of great importance, and univariate and multivariate statistics, such as chemometric or pattern recognition techniques, facilitate the identification of relevant changes in metabolite patterns.

The above-mentioned approaches, including functional genomics, proteomics and metabolite profiling, are being further explored in the GMOCARE project.[32] The identification and human and animal safety assessment of unintended effects in GM crops are topics in the ENTRANSFOOD project.[33]

8.4.5. Effects of processing and dietary impact

Processing may remove or destroy transgenic products, such as foreign genes and proteins, in the final

[31] *About GMOCARE,* available [October 2002] at http://www.entransfood.com/RTDprojects/GMOCARE/aboutgmocare.html.

[32] *About GMOCARE,* available [October 2002] at http://www.entransfood.com/RTDprojects/GMOCARE/aboutgmocare.html.

[33] *ENTRANSFOOD,* Wageningen, RIKILT, available [November 2002] at http://www.entransfood.com/.

[34] SCF (1999) *Opinion on a Request for Consent to place on the Market a Tomato Fruit Genetically Modified to Down-regulate the Production of Polygalacturonase (PG), and solely intended for Processing*, Brussels, Belgium, Scientific Committee on Food, European Commission, available [November 2002] at http://europa.eu.int/comm/food/fs/sc/scf/out42_en.pdf.

[35] EPA (2000) *A Set of Scientific Issues being considered by the Environmental Protection Agency Regarding: Assessment of Scientific Information Concerning StarLink Corn*. Arlington, USA, FIFRA Scientific Advisory Panel, US EPA, available [December 2002] at http://www.epa.gov/scipoly/sap/2000/november/one.pdf.

GMF product. This is particularly important if the focus of a safety evaluation is the processed food. It has been noted, for example, that heat processing destroyed the antibiotic resistance gene in a GM tomato.[34] In contrast, new antigenic epitopes may be exposed by protein denaturation or splitting during processing[35] (Hefle, 1996).

In addition, GMF crops or derived food ingredients with modified composition may display different processing characteristics; for example GM High Oleate soybean oil is less prone to oxidation than conventional soybean oil, which is higher in polyunsaturated fatty acids (Kinney & Knowlton, 1998).

The Starlink case provides an example of the role that food processing may play in the evaluation of potential adverse effects from a GMF. As noted above, the Starlink maize had only been approved for animal feed use but was commingled with food. Data on Starlink evaluated by EPA included the level of Cry9C in foods and the estimated intake of Cry9C by American consumers. Cry9C is of toxicological interest owing to its relative stability during exposure to simulated human digestion. Levels of Cry9C were found to be reduced, by as much as 40%, by wet milling and dry milling of maize kernels, while additional processing, like alkaline cooking, decreased the protein content to 0.1–0.2% of the original protein content).[36,37] Information on the effects of processing was used to estimate the dietary intake of Cry9C by American consumers. Estimates of the dietary impact of Starlink corn, paying specific attention to the intake of Cry9C from foods containing Starlink, were based on a worst-case scenario. Factors considered included the area of cultivation of Starlink maize (including buffer zones), the level of Cry9C in Starlink maize kernels, the effects of processing on Cry9C, and human consumption (including consumption by subpopulations) of maize-derived products.

8.4.6. Gene transfer

Although the possibility for gene transfer from plants to gut microorganisms or human cells is a concern, particularly for genes coding for antibiotic resistance, it is considered a rare possibility owing to the many complex and unlikely events that would need to occur consecutively. Transfer of plant DNA to bacteria would include the following stages: release of the DNA containing the gene from ingested food into the gastrointestinal tract; escape from degradation; uptake of the foreign DNA by bacteria; incorporation into the bacterial chromosome or recircularization to an extrachromosomal plasmid; and stable inheritance. Thus, the probability of gene transfer from GM plants to bacteria is likely to be extremely low, but cannot be completely discounted (Jonas *et al.*, 2001).[38–40]

The use of marker genes to enable the selection of GMOs has been questioned, in particular marker genes that code for antibiotic resistance. Transfer of such genes from GM crop material to pathogenic microorganisms in the intestines of humans and animals consuming these crops could render pathogenic microorganisms resistant to antibiotics, and could thus compromise human or animal therapy.

Assessment of the consequences of gene transfer and expression in transformed cells would have to take into account the clinical and veterinary relevance of the antibiotic in question, the levels of natural resistance and the availability of effective alternative therapies. It has been recommended that antibiotic resistance genes that encode for clinically important antibiotics should not be present in widely disseminated GMOs.[41] The EU recently introduced legislation (Directive 2001/18/EC) that bans the use of antibiotic resistance markers in GMOs that may have adverse effects on human health and the environment. (Some alternatives to the use of antibiotic markers are described in Chapter 3 (Section 3.3.7).

Besides the potential transfer of antibiotic resistance genes from GM plants to bacteria in the gastrointestinal tract, another concern is the possible uptake of trans-

[36] EPA (2001) *White Paper on the Possible Presence of Cry9C Protein in Processed Human Foods made from Food Fractions produced through the Wet Milling of Corn*, Arlington, FIFRA Scientific Advisory Panel, US Environmental Protection Agency, available [November 2002] at http://www.epa.gov/scipoly/sap/2001/july/wetmilling.pdf.

[37] EPA (2000) *A Set of Scientific Issues being considered by the Environmental Protection Agency Regarding: Assessment of Scientific Information Concerning StarLink Corn*. FIFRA Scientific Advisory Panel, Arlington, USA, US EPA, available [December 2002] at http://www.epa.gov/scipoly/sap/2000/november/one.pdf.

[38] FDA (1998) *Guidance for Industry: Use of Antibiotic Resistance Marker Genes in Transgenic Plants. Draft Guidance*, Rockville, MD, Office of Premarket Approval, Center for Food Safety and Applied Nutrition, U S Food and Drug Administration, available [November 2002] at http://vm.cfsan.fda.gov/~dms/opa-armg.html.

[39] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

[40] FAO/WHO (2002) *Draft Guideline for the Conduct of Food safety Assessment of Foods derived from Recombinant-DNA Plants* (ALINORM 01/34A, Appendix III), Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/alinorm01/al0134ae.pdf.

[41] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

genic DNA by mammalian cells under conditions of dietary exposure. Schubbert and co-workers, for example, fed DNA from bacteriophage M13 to mice. Contrary to the notion that DNA would be degraded in the gastrointestinal tract, they found a fraction of the M13 DNA sustained in the gastrointestinal tract had actually been transferred to gastrointestinal tract epithelial cells and linked to DNA from lymphocytes, liver cells and spleen cells of the mice (Schubbert, Renz, Schmitz, & Doerfler, 1997). However, these results were later criticized by Beever and Kemp (2000) who concluded that the observed linkages of M13 DNA to mouse and bacterial DNA were cloning artefacts.

The experiments of Schubbert and co-workers have been extended to domestic animals fed GM crops. The uptake of small DNA fragments from *Bt* maize in various samples from cattle and chicken has been determined with the use of PCR (Einspanier *et al.*, 2001). Most of the larger fragments (532 bp) of maize chloroplast DNA were degraded during ensilage of the *Bt* maize prior to feeding; in contrast, smaller fragments (199 bp) appeared to be stable. The smaller fragments of chloroplast DNA were apparently taken up from animal feed into the blood cells and milk of cows and various tissues of chickens, but were not taken up by bulls. However the uptake of even smaller fragments (189 bp) of the foreign *Bt* gene could not be detected. The fate of foreign DNA from transgenic *Bt* maize, which was fed to chickens, was also investigated in another study (Chambers, Duggan, Heritage, & Forbes, 2002). Two DNA fragments of the transgenic antibiotic resistance gene *bla* and a maize mitochondrial gene could not be detected beyond the chicken stomach after uptake of GM *Bt* maize feed.

Another concern is the potential recombination of viral promoter sequences introduced into GMFs with those of human viruses. Ho, Ryan, and Cummins (2000), for example, expressed concerns that the 35S cauliflower mosaic virus promoter, commonly used in many commercial GM crops, might recombine with human hepadnaviruses. Concerns were founded on the assumption that the cauliflower mosaic virus and hepadnaviruses both belong to the class of pararetroviruses. In addition, the 35S promoter sequence would be a hotspot for recombination and could therefore ultimately trigger the pathological expression of human genes after its transfer to human DNA. In contrast, Hull, Covey, and Dale (2000) suggested that the different replication cycles of the cauliflower mosaic virus and human hepadnaviruses, as well as the different nucleotide sequences of the viruses, would preclude recombination. In addition, activation of human genes by the transfer of the 35S promoter would require more than only a recombination hotspot, namely a double strand break at both ends of the promoter and its insertion at a site within genome that allows for control of a non-active human gene proximate to the insertion site.

8.4.7. Safety assessment of GM animals

It has been considered that the principles for the safety assessment of GM plants can be applied to the safety assessment of GM animals, although more elaborate guidance may be required (Health Canada, 2001). The following additional items have been suggested:

- The well being of the transgenic animal.
- Compositional (nutritional analysis)—to include analysis of nutrients and bioactive compounds, sampling of raw and cooked material and the whole ground carcass, meat quality impact.
- Toxicology—to include screening for unintended effects by molecular profiling techniques and study of the GM animals' behaviour and development, attention to the possible consequence of under and over expression of genes.
- Disease susceptibility.

The potential risks associated with the use of retroviral sequences, including the risk of recombination with wild-type viruses, have been emphasized (Jones, 1998). An additional safety consideration has been the possible alteration of levels of intrinsic antinutrients and toxins in particular animal species, such as thiaminase in certain fish species (Kleter & Kuiper, 2002).

8.5. Review of strategies developed by international bodies

Years before the first GMFs entered the market, discussions had been going on in national and international fora on how to assess the safety of such foods. In 1990, IFBC published a compilation of papers on this issue. The recommended approach was to compare the composition of a GMF with the composition of conventional foods that have a safe history of use, and to determine the potential toxicity of the inserted foreign traits (IFBC, 1990). These recommendations were further developed by international organizations, such the OECD, FAO/WHO and the EU (see Section 8.4). Consensus has been reached on issues such as the comparison between a GMF and its conventional counterpart ('substantial equivalence') and allergenicity. ILSI has also dealt with the safety evaluation of GMFs; the evaluation of the safety of novel foods, for example, is discussed in a document generated by the European branch of ILSI (Jonas *et al.*, 1996).

8.5.1. Organization for economic co-operation and development

In 1993, OECD defined the principle of 'substantial equivalence', which has since become a central tenet in

the safety assessment of GMFs.[42] Application of the principle was further elaborated during OECD workshops held at Oxford (1994) and Aussois (1997). As explained above, the properties of a GMF are compared with those of a conventional counterpart in order to establish the degree of equivalence. Substantial equivalence testing is a starting point for the safety assessment, but not the safety assessment as such. Identified differences are the subjects of further toxicological or nutritional evaluations. The OECD Task Force for the Safety of Novel Foods and Feeds has contributed substantially to the field, through its consensus documents on the baseline characteristics of the agronomical and compositional properties of food crops.[43]

8.5.2. Food and Agriculture Organization/World Health Organization

The FAO and WHO, both of the United Nations, have jointly been active in the field of safety assessment of GMFs since 1990. A parallel can be drawn between the work of the FAO/WHO and the activities of the OECD in this field. In the 1996 publication of an FAO/WHO expert consultation, for example, the principle of substantial equivalence as a guiding tool for safety assessment was endorsed.[44]

Joint FAO/WHO Expert Consultations were held recently to evaluate current GMF safety assessment strategies and experiences, as well as the topic of allergenicity.[45,46] The outcome of these consultations provided input for the Codex *ad hoc* Intergovernmental Task Force on Foods Derived from Biotechnology, which agreed on guidelines for food safety assessment and for the assessment of allergenicity.[47] However, the regulatory activities of the FAO/WHO and the OECD are different. The Codex Alimentarius Committee of the FAO/WHO develops food safety standards that have a legal status. In addition, the World Trade Organization may refer to these standards in the case of a trade dispute over the safety of internationally traded foodstuffs. In addition, principles for the risk analysis of GMFs are being prepared, including aspects of risk assessment, risk management (decision-making), and risk communication (see also Chapter 10).

8.5.3. European Union

The EU Regulation 258/97[48] on novel foods and novel food ingredients, which came into force in the EU in 1997, distinguishes six categories of novel food products; two of them refer directly to products derived from GMOs. The concept of substantial equivalence is fully endorsed in the European approach. It is stated that testing of substantial equivalence is an analytical process, where the GMF is compared to the most appropriate food already on the market. Thirteen decision trees are added to the regulation to guide the producers to the data that need to be supplied to establish the safety of an individual novel food product (Commission Recommendation 97/618/EC).[49] Recently, a Joint Working Group of the Scientific Committees on Plants, Food, and Animal Nutrition has issued a new proposal for guidelines for food and environmental safety of GM plants (EU, 2002). For the nutritional assessment it may in some cases be necessary to set up post-launch monitoring programmes. The EU largely follows international consensus reports on allergenicity, requiring that potential allergenicity should be investigated with the available means, to avoid the introduction of new allergens in the food supply.

8.6. Post-market surveillance

There is continuing debate about the identification and assessment of unanticipated long-term effects of GMF crops on the environment and on human and animal health. Requirements for market permission for novel foods in the EU[50] demand a post-market surveillance plan, to be designed by the manufacturer, which

[42] OECD (1993) *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles,* Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/pdf/M00033000/M00033002.pdf.

[43] OECD (2002) *Consensus Documents for the Work on the Safety of Novel Foods and Feeds,* Paris, France, Organisation for Economic Co-operation and Development, available [November 2002] at http://www.oecd.org/EN/document/0,,EN-document-32-nodirectorate-no-27-24778-32,00.html.

[44] FAO/WHO (1996) *Biotechnology and Food Safety Report: Report of a Joint FAO/WHO Consultation* (30 September–4 October 1996) (FAO Food and Nutrition Paper 61), Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/es/esn/food/biotechnology.pdf.

[45] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

[46] FAO/WHO (2001) *Allergenicity of Genetically Modified Foods: Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology,* (22–25 January, 2001), Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp/fao/org/es/esn/food/allergygm.pdf.

[47] FAO/WHO (2002) *Report of the Third Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology,* Rome, Italy, Food and Agriculture Organization, United Nations, available [December 2002] at ftp://ftp.fao.org/codex/alinorm03/Al03_34e.pdf.

[48] OJ L43, 14.02.97, pp. 1–7.

[49] OJ L253, 29.07.97, pp. 1–36.

[50] OJ L253, 29.07.97, pp. 1–36

would enable the identification and assessment of the nutritional and adverse effects of a GMF once it had entered the market. However, it should be noted that very little is known about the potential long-term effects of any food, and many chronic health effects are multifactorial.[51] Therefore, safety evaluation must provide sufficient assurance of safety, in order to preclude long-term adverse effects after marketing.

Different strategies for post-marketing surveillance of newly introduced foods have been discussed (Kuiper *et al*., 2001). For food products that can easily be traced and identified, methods vary from direct consumer feedback to the repurchase of products in order to determine the quality of the product on the shelf. These strategies will, in most cases, not be directly applicable to GM-derived food products, since most GM products will be used in end products in which recipes have been slightly modified relative to the non-GM product. The consumer will only be able to trace potential adverse effects back to consumption of a particular GM product if such products are clearly labelled. Acute effects associated with intakes of a particular substance are likely to be identifiable by post-market surveillance; long-term and/or rare effects usually require targeted epidemiological techniques, beyond any normal post-marketing data collection.

The UK Food Standards Agency started a feasibility study in 1999 to determine whether long-term monitoring of novel foods is possible (Baynton, 1999). Data on household consumption patterns and supermarket sales in local authority districts in the UK are being collected. If variation in food purchasing and consumption can be detected at the district level, it may be possible to link such variation with health outcomes also at the district level. The results of the study will lead to recommendations about future surveillance of novel foods.

Examples of post-market surveillance related to food constituents include the food additive aspartame, a high-intensity sweetener, and Olestra, a fat substitute used in snack foods (Butchko, Tschanz, & Kotsonis, 1994; Cooper *et al*., 2000; Thornquist *et al*., 2000).

Studies on the human health effects of mycoprotein consumption have been carried out with the meat-substitute Quorn, which was introduced to the UK market in 1983. Quorn consists of mycoprotein, which is a filamentous protein produced by the fungus *Fusarium venenatum*. An 8-week study in human subjects who consumed cookies containing mycoprotein showed that mycoprotein caused a decrease in low-density lipoprotein (LDL) cholesterol, the 'bad' serum cholesterol. (Turnbull, Leeds, & Edwards, 1992). Reports of adverse reactions to mycoprotein among consumers in the UK and Europe have also been recorded (Miller & Dwyer, 2001).

From these examples it can be concluded that post-market surveillance may be informative where clear-cut questions are the basis for the surveillance. Novel (GM) food products should not be placed on the market if any question associated with negative health effects is left unanswered by the assessment. Questions about unpredictable adverse effects and alterations in the nutritional status of consumers, as a result of the marketing of a particular novel food, may be answered by post-market surveillance, if informative reporting systems are available.

8.7. Assessment of future crops

GMF plants with improved food-quality traits are under development and will enter the market soon. Food plants have been genetically modified in order to increase the levels of essential amino acids, unsaturated fatty acids, vitamins like beta-carotene, minerals like iron, and lycopene or flavanoids, or to lower the content of allergenic proteins[52] (Kuiper *et al*., 2001). Some of these plants are obtained through extensive genetic modification, by insertion of multiple genes coding for entire metabolic pathways.

The safety assessment of this 'second generation' of GMF crops should follow the comparative safety assessment approach outlined above, in order to establish the degree of equivalence (Kuiper *et al*., 2001). The unmodified host organism may function as the relevant comparator for testing the degree of equivalence; however, a safety assessment of the new food *per se* may be necessary. This may be the case for GM crops in which existing metabolic pathways have been extensively modified or new ones added, or for GM plants with decreased levels of naturally occurring toxins relative to the conventional plant, which could not previously be used as a food source. Safety assessment strategies should be designed on a case-by-case basis.

[51] FAO/WHO (2000) *Safety Aspects of Genetically Modified Foods of Plant Origin: Report of a Joint FAO/WHO Expert Consultation on Foods derived from Biotechnology* (29 May–2 June, 2000), Geneva, Switzerland, World Health Organization, available [January 2003] at ftp://ftp.fao.org/es/esn/food/gmreport.pdf.

[52] Kleter, G.A., Noordam, M.Y., Kok, E.J., Kuiper, H.A. (2000) *Novel Developments in Crop Plant Biotechnology and Their Possible Implications for Food Product Safety* (RIKILT Report 2000.004), Wageningen, The Netherlands, RIKILT – Institute of Food Safety, available [November 2002] at http://www.rikilt.nl/Publications/Publications/Tekstrapport2000%20004.htm.

8.8. Conclusions

Safety assessment of GMFs is carried out on a case-by-case basis, taking the specific modification features into account, and comparing the properties of the new food with those of the traditional counterpart. This comparative approach is valid since conventional foods are generally considered as safe for consumption, based on a history of use. Identified differences between the GMF and its counterpart are assessed with respect to their safety and nutritional implications for the consumer. The concept, as developed by OECD and endorsed by FAO/WHO, contributes to an adequate safety assessment strategy. Increasing knowledge of the safety and nutritional properties of foods will provide a more scientific basis for the presumed safety of traditional foods.

Safety testing of whole (GM) foods in laboratory animals is problematic and needs improvement. The use of specific *in vitro* models and of new molecular methods, such as DNA microarray technology, may contribute to the elucidation of mechanisms of action and interactions of biologically active compounds present in foods at the molecular level. This may lead to new concepts in risk–benefit analyses of foods.

Present approaches to the detection of expected and unexpected changes in the composition of GMF crops are primarily based on measurements of a limited selection of single compounds (targeted approach). In order to increase the possibility of detecting secondary effects, new profiling methods, using gene expression technologies, proteomics and metabolomics, should be further developed and validated (non-targeted approach).

The use of post-marketing surveillance as an instrument to gain additional information on long-term effects of foods or food ingredients, either GM-derived or traditional, should not be overestimated, given the multifactorial origin of many food-related diseases and the variability in genetic predisposition of the human population. Routine application in the food sector may yield limited information and will be costly. Only in cases with specific biological end-points, for example allergenicity or food intolerance, or when exposure assessment is hampered by insufficient insight into the diets of specific consumer groups, do post-marketing surveillance strategies seem to be useful. Pre-market safety assessment of GMFs must provide sufficient safety assurance.

Acknowledgements

The authors gratefully acknowledge financial support from the Netherlands Ministry of Agriculture, Nature Management and Fisheries and from the EU Thematic Network ENTRANSFOOD.

References

Astwood, J. D., Leach, J. N., & Fuchs, R. L. (1996). Stability of food allergens to digestion in vitro. *Nature Biotechnology*, *14*, 1269–1273.

Baynton, C. (1999). *Surveillance and post market monitoring of potential health effects of novel foods: feasability study*. London: Food Standards Agency.

Beever, D. E., & Kemp, C. F. (2000). Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutrition Abstracts and Reviews, Series A: Human and Experimental, 70*, 197–204.

Beier, R. C. (1990). Natural pesticides and bioactive components in foods. *Reviews of Environmental Contamination and Toxicology*, *113*, 47–137.

Bucchini, L., & Goldman, L. R. (2002). Starlink corn: a risk analysis. *Environmental Health Perspectives*, *110*, 5–13.

Butchko, H. H., Tschanz, C., & Kotsonis, F. N. (1994). Postmarketing surveillance of food additives. *Regulatory Toxicology and Pharmacology*, *20*, 105–118.

Chambers, P. A., Duggan, P. S., Heritage, J., & Forbes, J. M. (2002). The fate of antibiotic resistance marker genes in transgenic plant feed material fed to chickens. *Journal of Antimicobial Chemotherapy*, *49*, 161–164.

Cooper, D. A., Curran Celentano, J., Ciulla, T. A., Hammond, B. R. Jr., Danis, R. B., Pratt, L. M., Riccardi, K. A., & Filloon, T. G. (2000). Olestra consumption is not associated with macular pigment optical density in a cross-sectional volunteer sample in Indianapolis. *Journal of Nutrition*, *130*, 642–647.

Dearman, R. J., & Kimber, I. (2001). Determination of protein allergenicity: studies in mice. *Toxicology Letters*, *120*, 181–186.

Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R., Schwägele, F., Jahreis, G., & Flachowsky, G. (2001). The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *European Food Research and Technology*, *212*, 129–134.

EU. (2002). *Guidance document on the information needed for the risk assessment of genetically modified plants, food and feed derived from genetically modified plants (Joint Working Group on Novel Foods and Genetically Modified Organisms, GM-NF WG/Guide/005-final)*. Brussels: European Commission.

Ewen, S. W. B., & Pusztai, A. (1999). Effect of diet containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. *Lancet*, *354*, 1353–1354.

Farrell, R. J., & Kelly, C. P. (2001). Diagnosis of celiac sprue. *American Journal of Gastroenterology*, *96*, 3237–3246.

Fine, A. J. (1981). Hypersensitivity reaction to kiwi fruit (Chinese gooseberry, Actinidia chinensis). *Journal of Allergy and Clinical Immunology*, *68*, 235–237.

Firsov, A. P., & Dolgov, S. V. (1998). Agrobacterial transformation and transfer of the antifreeze protein gene of winter flounder to the strawberry (abstract). *Acta Horticulturae*, *484*, 581–586.

FOSIE. (2002). Food Safety in Europe (FOSIE): risk assessment of chemicals in food and diet. *Food and Chemical Toxicology*, *40*, 137–424.

Health Canada. (2001). *Technical workshop on food safety assessment of livestock animals and fish derived from biotechnology—report of key findings*. Ottawa: Health Canada,

Burea of Microbial Hazards Evaluation Division, Health Protection Branch.

Hefle, S. L. (1996). The chemistry and biology of food allergens. *Food Technology*, *50*, 86–92.

Hefle, S. L., Nordlee, J. A., & Taylor, S. L. (1996). Allergenic foods. *Critical Reviews in Food Science and Nutrition*, *36*, S69–S89.

Ho, M. W., Ryan, A., & Cummins, J. (2000). Hazards of transgenic plants containing the cauliflower mosaic viral promoter. *Microbial Ecology in Health and Disease*, *12*, 6–11.

Hull, R., Covey, S. N., & Dale, P. (2000). Genetically modified plants and the 35S promoter: assessing the risks and enhancing the debate. *Microbial Ecology in Health and Disease*, *12*, 1–5.

IFBC. (1990). Biotechnologies and food: assuring the safety of foods produced by genetic modification. *Regulatory Toxicology and Pharmacology*, *12*, S1–S196.

Jonas, D. A., Antignac, E., Antoine, J. M., Classen, H. G., Huggett, A., Knudsen, I., Mahler, J., Ockhuizen, T., Smith, M., Teuber, M., Walker, R., & De Vogel, P. (1996). The safety assessment of novel foods—guidelines prepared by ILSI Europe Novel Food Task Force. *Food and Chemical Toxicology*, *34*, 931–940.

Jonas, D. A., Elmadfa, I., Engel, K. H., Heller, K. J., Kozianowski, G., König, A., Müller, D., Narbonne, J. F., Wackernagel, W., & Kleiner, J. (2001). Safety considerations of DNA in food. *Annals of Nutrition and Metabolism*, *45*, 235–254.

Jones, D. D. (1998). Advisory considerations on the scientific basis of the food safety evaluation of transgenic animals. In A. Holland, & A. Johnson (Eds.), *Animal biotechnology and ethics* (pp. 265–275). London: Chapman and Hall.

Kinney, A. J., & Knowlton, S. (1998). Designer oils: the high oleic acid soybean. In S. Roller, & S. Harlander (Eds.), *Genetic modification in the food industry* (pp. 193–213). London: Blackie.

Kleter, G. A., & Kuiper, H. A. (2002). Considerations for the assessment of the safety of genetically modified animals used for human food or animal feed. *Livestock Production Science*, *74*, 275–285.

Knippels, L. M. J., Penninks, A. H., Spanhaak, S., & Houben, G. F. (1998). Oral sensitization to food proteins: a brown Norway rat model. *Clinical and Experimental Allergy*, *28*, 368–375.

Kuiper, H. A., Noteborn, H. P. J. M., & Peijnenburg, A. A. C. M. (1999). Adequacy of methods for testing the safety of genetically modified foods. *Lancet*, *354*, 1315–1316.

Kuiper, H. A., Kleter, G. A., Noteborn, H. P. J. M., & Kok, E. J. (2001). Assessment of the food safety issues related to genetically modified foods. *Plant Journal*, *27*, 503–528.

Metcalfe, D. D., Astwood, J. D., Townsend, R., Sampson, H. A., Taylor, S. L., & Fuchs, R. L. (1996). Assessment of the allergenic potential of foods derived from genetically engineered crop plants. *Critical Reviews in Food Science and Nutrition*, *36*(Suppl.), S165–S186.

Miller, S. A., & Dwyer, J. T. (2001). Evaluating the safety and nutritional value of mycoprotein. *Food Technology*, *55*, 42–47.

Nordlee, J. A., Taylor, S. L., Townsend, J. A., Thomas, L. A., & Bush, R. K. (1996). Identification of a Brazil-nut allergen in transgenic soybeans. *New England Journal of Medicine*, *334*, 668–692.

Noteborn, H. P. J. M., Bienenmann-Ploum, M. E., van den Berg, J. H. J., Alink, G. M., Zolla, L., Reynaerts, A., Pensa, M., & Kuiper, H. A. (1995). Safety assessment of the *Bacillus thuringiensis* insecticidal crystal protein CRYIA(b) expressed in transgenic tomatoes. In K.-H. Engel, G. R. Takeoka, & R. Teranishi (Eds.), *Genetically modified foods—safety aspects, ACS Symposium Series 605* (pp. 134–147). Washington, DC: American Chemical Society.

OECD. (1993). *OECD guidelines for the testing of chemicals*. Paris: Organisation for Economic Co-operation and Development.

OECD. (1996). *Food safety evaluation*. Paris: Organisation for Economic Co-operation and Development.

Ortolani, C., Ispano, M., Pastorello, E., Bigi, A., & Ansaloni, R. (1988). The oral allergy syndrome. *Annals of Allergy*, *61*, 42–47.

Peferoen, M. (1997). Progress and prospects for field use of Bt genes in crops. *Trends in Biotechnology*, *15*, 173–177.

Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, *270*, 467–470.

Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O., & Davis, R. W. (1996). Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. *Proceedings of the National Academy of Sciences of the USA*, *93*, 10614–10619.

Schubbert, R., Renz, D., Schmidz, B., & Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leucocytes, spleen and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proceedings of the National Academy of Sciences of the USA*, *94*, 961–966.

The UK Royal Society. (1999). *Review of data on possible toxicity of GM potatoes*. London: Royal Society Press Office.

Thornquist, M. D., Kristal, A. R., Patterson, R. E., Neuhouser, M. L., Rock, C. L., Neumark Sztainer, D., & Cheskin, L. J. (2000). Olestra consumption does not predict serum concentrations of carotenoids and fat-soluble vitamins in free-living humans: early results from the sentinel site of the Olestra Post-Marketing Surveillance Study. *Journal of Nutrition*, *130*, 1711–1718.

Turnbull, W. H., Leeds, A. R., & Edwards, D. G. (1992). Mycoprotein reduces blood lipids in free-living subjects. *Journal of Clinical Nutrition*, *55*, 415–419.

UCB Institute of Allergy. (1997). *European allergy white paper: allergic diseases as a public health problem in Europe*. Brussels: UCB Institute of Allergy.

Van Hal, N. L. W., Vorst, O., Van Houwelingen, A. M. M. L., Kok, E. J., Peijnenburg, A., Aharoni, A., Van Tunen, A. J., & Keijer, J. (2000). The application of DNA microarrays in gene expression analysis. *Journal of Biotechnology*, *78*, 271–280.

WHO. (1987). *Principles for the safety assessment of food additives and contaminants in food (International Programme on Chemical Safety, Environmental Health Criteria, 70)*. Geneva: World Health Organization.

WHO. (1990). *Principles for the toxicological assessment of pesticide residues in food (International Programme on Chemical Safety, Environmental Health Criteria, 104)*. Geneva: World Health Organization.

Windels, P., Taverniers, I., Depicker, A., Van Bockstaele, E., & De Loose, M. (2001). Characterisation of the Roundup Ready soybean insert. *European Food Research and Technology*, *231*, 107–112.