

Assessment of the safety of foods derived from genetically modified (GM) crops

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Abstract

This paper provides guidance on how to assess the safety of foods derived from genetically modified crops (GM crops); it summarises conclusions and recommendations of Working Group 1 of the ENTRANSFOOD project. The paper provides an approach for adapting the test strategy to the characteristics of the modified crop and the introduced trait, and assessing potential unintended effects from the genetic modification. The proposed approach to safety assessment starts with the comparison of the new GM crop with a traditional counterpart that is generally accepted as safe based on a history of human food use (the concept of substantial

Abbreviations: ADI, Acceptable Daily Intake; ADME, absorption, distribution, metabolism, and excretion; ALLERGEST, EU-project on the effect of gastrointestinal digestion on the allergenicity of foods; APHIS, Animal and Plant Health Inspection Service; Bt, *Bacillus thuringiensis*; cDNA, DNA complementary to an RNA strand; CP4 EPSPS, *Agrobacterium* sp. CP4-derived enolpyruvylshikimate-3-phosphate synthase; DAFNE, Data Food Networking; DNA, deoxyribonucleic acid; EDI, estimated daily intake; EFG, Euro Food Group; EFSA, European Food Safety Authority; ENDB, European Nutrient Database; ENTRANSFOOD, European network safety assessment of genetically modified food crops; EPA, US Environmental Protection Agency; EPIC, European Prospective Investigation into Cancer and Nutrition; EPSPS, enolpyruvylshikimate-3-phosphate synthase; EU, European Union; FAO, Food and Agriculture Organisation of the United Nations; FDA, US Food and Drug Administration; FOSIE, European Concerted Action Food Safety in Europe; FSANZ, Food Standards Australia New Zealand; GEMS/FOOD, Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme; GM, genetically modified; GMO, genetically modified organism; IFBC, International Food Biotechnology Council; ILSI, International Life Sciences Institute; INFORMALL, EU-project about communicating about food allergies and information for consumers, regulators, and industry; INVITTOX, databank of in vitro techniques in toxicology; MAFF, Japan Ministry of Agriculture, Food, and Fisheries; MHLW, Ministry of Health, Labour, and Welfare; MOS, margin of safety; NOAEL, No Observed Adverse Effect Level; ODE, US FDA Office of Device Evaluation; OECD, Organisation for Economic Co-operation and Development; PCR, polymerase chain reaction; Protall, EU-project on food allergens of plant origin and the relationship between allergenic potential and biological activity; RDA, recommended daily allowance; RNA, ribonucleic acid; SAFOTEST, EU-project on new methods for the safety testing of transgenic food; SGF, simulated gastric fluid; TMDI, theoretical maximum daily intake; UK, United Kingdom; UK ACRE, UK Advisory Committee on Releases into the Environment; US, United States; USA, United States of America; USDA, US Department of Agriculture; WHO, World Health Organisation of the United Nations.

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equivalence). This case-focused approach ensures that foods derived from GM crops that have passed this extensive test-regime are as safe and nutritious as currently consumed plant-derived foods. The approach is suitable for current and future GM crops with more complex modifications. First, the paper reviews test methods developed for the risk assessment of chemicals, including food additives and pesticides, discussing which of these methods are suitable for the assessment of recombinant proteins and whole foods. Second, the paper presents a systematic approach to combine test methods for the safety assessment of foods derived from a specific GM crop. Third, the paper provides an overview on developments in this area that may prove of use in the safety assessment of GM crops, and recommendations for research priorities. It is concluded that the combination of existing test methods provides a sound test-regime to assess the safety of GM crops. Advances in our understanding of molecular biology, biochemistry, and nutrition may in future allow further improvement of test methods that will over time render the safety assessment of foods even more effective and informative.

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1. Introduction

Approaches to the regulation and safety assessment of genetically modified (GM) crops have been developed in a very proactive manner. The first international and national provisions for the safety assessment and regulation of genetically modified organisms (GMOs), including GM crops and derived foods were drawn up by scientific experts in the mid-1980s (OECD, 1986; US OSTP, 1986). This was nearly a decade before the first regulatory approval of a genetically modified crop in 1995. Since then, the global area of commercial cultivation of such crops has risen to 58.7 million hectares in 2002 (James, 2002). Commercially cultivated GM crops include soybean, maize, cotton, canola, potatoes, and tomatoes. At present, the most widely grown GM crops contain new genes that confer herbicide tolerance or insect resistance. Other crops are being developed that have improved nutritional characteristics for their food or feed use; GM soybeans and oil seed rape with altered fatty acid profiles, for example, have already undergone regulatory review. Future advances in genomic sciences promise the discovery of new genes conferring desirable characteristics to crops that may fundamentally alter a crop's metabolic functions, promising further nutritional enhancement and resistance to abiotic stresses. It is important that we should continue to proactively assess whether current approaches to safety assessment are appropriate also for future GM crop products with more complex traits.

This paper presents a systematic approach for combining different test methods to assess the safety of foods derived from a specific GM crop. It provides guidance on how to tailor the test strategy to the characteristics of the modified crop and the introduced trait and identifying potential unintended effects from the genetic modification. The approach builds on internationally agreed guidelines and principles, and is suitable for current and future GM crops with more

complex modifications. The remainder of this paper is divided into four sections. Section 2 provides an overview of regulations and internationally agreed principles and guidelines for risk assessment of chemicals and foods derived from GM crops. Section 3 reviews existing test methods developed for chemicals and food additives, and examines their suitability for testing the safety of foods and food constituents derived from GM crops. Section 4 systematically sets out how to determine whether the GM crop is 'as safe as' a suitable comparator with a history of human consumption. It provides guidance on how to compile information on the parent crop and on the genetic modification. This information in turn guides the choice of test parameters and methods in the analysis of any introduced substance and of the whole GM crop. Any significant differences that are identified in this systematic comparison of the GM crop and the comparator then are subject to further investigation as to whether this difference might have implications for human health. Section 5 discusses implications of advances in molecular biology and the development of in vitro and in vivo test methods for the future refinement of food safety assessment strategies.

The paper provides detailed guidance for anyone involved in risk assessment and regulation of GM crops. The paper emphasises that this systematic approach to food safety assessment of GM crops offers a high level of safety assurance: this iterative and case-focused design of safety testing strategies ensures that all tested and approved foods derived from GM crops are as safe and nutritious as currently consumed plant-derived foods. The paper also considers how our continuously improving understanding of molecular biology, biochemistry, and nutrition will over time facilitate the development of new crop varieties and their safety assessment. The conclusion provides recommendations on priorities for research and development of test methods and strategies.

2. Food safety of GM crops: regulation, principles, and guidelines

2.1. Regulatory frameworks for GM crops and derived foods

Food safety systems, comprising institutions, policies, laws, and guidelines for assessments, continually evolve over time. The evolution of food safety systems in individual jurisdictions is affected both by science and society: Scientific advances improve our understanding of health implications of foods and lead to adoption of new agri-food production technologies, some of which require regulatory oversight. Changing societal values can lead to shifts in emphasis in consumer protection policies and regulatory and institutional change. Regulation in turn can affect both innovation and risk perception. The Organisation for Economic Co-operation and Development (OECD) recently compiled descriptions of national food safety systems of its twenty-nine Member States; these descriptions also specifically address national approaches to the regulation and assessment of foods derived from GM crops (OECD, 2000, 2003).

Two types of regulatory frameworks for foods derived from GM crops can be distinguished. Some jurisdictions enacted specific ‘process-based’ legislation for the regulation of all genetically modified organisms, these include the European Union (EU) and Australia. In contrast, other regulatory systems are ‘product-based’, focusing on the resulting product characteristics and use, and not on the process of genetic modification, as for instance those in the United States of America (USA) and Canada.

In the European Union (EU) the regulation of foods derived from genetically modified organisms has undergone two significant changes since it first was instituted in the early 1990s. These changes occurred in tandem with more fundamental changes in the governance of food safety in the EU. First, a horizontal, process-based law regulating all genetically modified organisms to be released into the environment came into force in 1990. Directive 90/220/EEC governed experimental releases and marketing authorisation of all genetically modified organisms (European Commission, 1990). The Directive set out an approval process requiring the case-by-case assessment of the potential risks to human and animal health and the environment of all genetically modified organisms or products consisting of or containing a GMO (except for pharmaceuticals, which are regulated separately). Partly in response to the public debate on GM crops, the Directive was revised to strengthen the existing requirements for risk assessment and the decision-making process (European Commission, 2001). The revised Directive 2001/18/EC on the deliberate release of genetically modified organisms, which entered

into force on 17 October 2002, introduces mandatory labelling and traceability requirements. It also limits approvals to a period of 10 years; furthermore, applicants have to provide post-market monitoring plans for some categories of products.

Since 1997 a separate approval procedure for foods derived from genetically modified organisms exists. Regulation EC No 258/97 concerning novel foods and food ingredients in 1997 (hence forth Novel Foods Regulation) (European Commission, 1997a) covers all foods that have not hitherto been used for human consumption to a significant degree within the European Community. The European Commission has published guidelines for data and information to be included in applications by petitioners (European Commission, 1997b). The Novel Foods Regulation requires the risk assessment and pre-market approval of novel foods, and also specifies labelling requirements for certain categories of novel foods. The Novel Foods Regulation gave the European Commission a clear role in the governance of food safety in the European Union.

This role was strengthened with the publication of the Regulation EC No 178/2002 on the general principles of food law and the establishment of the European Food Safety Authority (hence forth the General Food Law) (European Commission, 2002; see also European Commission, 2000a). The General Food Law provides the legal basis for the establishment of a fully integrated system for food safety legislation and controls covering all aspects of food production and the establishment of an independent European Food Safety Authority (EFSA). The General Food Law provides an integrated approach to ensuring food safety across the EU Member States and across the food and feed sectors. General principles of EU food law state that risk analysis is based on scientific risk assessment conducted by the recently instituted European Food Safety Authority and establish an EU-level authorisation procedure. Other general principles include the protection and informing of consumers through comprehensive labelling schemes; provisions for traceability—that is the ability to trace back to the origin and to understand the distribution of foods and food ingredients; and the application of the precautionary principle in instances of significant uncertainty in the risk assessment. Furthermore, the new law clarifies accountability of all legal entities involved in food production and regulation in the EU by describing general food safety requirements that are imposed on both the Member States and business operators.

The General Food Law provides for one decision-making procedure for all products that require EU-level approvals, such as food additives, pesticide residues in food, novel foods, and genetically modified organisms. The procedure is as follows: The European Commission Directorate for Health and Consumer

Protection administers the review process. The European Food Safety Authority reviews the risk assessment submitted by applicants intending to place a Novel Food on the European market. It is up to the administrators in the European Commission Directorate General for Health and Consumer Protection to draft proposals based on the risk assessment and other broader considerations that may affect choice of policy options. A regulatory committee of representatives of Member States competent authorities then decides whether to accept the Commission proposal through a weighted voting system. If the regulatory committee's opinion is not in accordance with the proposed measure or if no opinion is delivered, the question is referred to the Council of Ministers. The Council of Ministers can approve or reject a Commission proposal given a qualified majority of member States support the position. If rejected, the European Commission has to prepare a new proposal. If the Council of Ministers takes no decision within three months, or does not reach a qualified majority indicating that it opposes the proposal, the European Commission shall adopt the proposal.

In June 2003 the European Council of Ministers adopted two new Regulations specific for foods and feeds derived from genetically modified organisms. Regulation (EC) No 1829/2003 on genetically modified food and feed provides the legal basis for the approval procedure for genetically modified organisms as specified in the General Food Law. The safety of foods derived from genetically modified organisms is assessed by the European Food Safety Authority's Scientific Panel on genetically modified organisms (European Commission, 2003a). [The panel assesses the food safety, environmental and animal health aspects of genetically modified organisms ('one-door-one-key' principle).] Regulation (EC) No 1831/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms requires traceability and labelling of genetically modified organisms and derived products (European Commission, 2003b); this regulation also provides a legal basis for case-by-case decisions on post market monitoring requirements where deemed necessary.

The US regulatory framework for GM crops was laid out in the 1986 'Coordinated Framework for Regulation of Biotechnology' (US OSTP, 1986). Existing laws for the regulation of plant pests, pesticides and foods were amended, resulting in a vertical, product-based regulatory framework for GM crops and derived foods. Three principal regulatory agencies conduct science-based assessments of risks to human health and the environment: the United States Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA).

The USDA regulates the import, interstate movement, field trial release, and commercial release of GM crops under the Federal Plant Pest Act and the Plant Quarantine Act, which are administered by the Animal and Plant Health Inspection Service (APHIS). Prior to approval for unrestricted release, as in commercialisation, the USDA/APHIS must determine that the GM crop is not a plant pest. There is no Federal regulation requiring the registration of new plant varieties. The EPA has regulatory oversight for all GM crops that produce a plant pesticide. Plant-integrated pesticides are regulated according to the same procedures as other pesticides.

The FDA has authority over human food and animal feed safety and the wholesomeness of all plant products, including those produced via genetic modification, under the Federal Food Drug and Cosmetic Act. The FDA has concluded that food and feed derived from GM crops pose no unique safety concerns and, therefore, that the food and feed products derived from these plants should be regulated no differently than comparable products derived from traditional plant breeding or any other genetic modification approach (US FDA, 1992). Labelling is only mandated for foods that present a health risk to subgroups of the population, such as allergenic foods; the FDA does not mandate process-based labelling informing consumers for instance on a food's content of genetically modified organisms. Partly in response to demonstrations by activists against GM crops in Seattle, Washington in 1999, and to three public hearings, the FDA decided to adopt measures to strengthen the scientific basis and transparency of its decision-making process. The FDA proposed to modify its voluntary process so as to establish mandatory pre-market notification and to make its decision process more transparent. The agency also developed draft guidance for food manufacturers who wish to label their foods voluntarily.

Other systems that present variations of either the US or the EU approach are Canada, Australia, and Japan. In Canada all plants with novel traits are regulated, regardless of whether a plant with novel traits was produced by conventional breeding, mutagenesis, or recombinant DNA techniques (Health Canada, 1994; CFIA, 1998). Foods derived from GM crops are considered novel foods under the Food and Drugs Act (CFIA, 1998). The Canadian Biotechnology Advisory Committee recently reviewed the Canadian regulations of GM foods; its recommendations include that research be carried out in order to monitor for hypothetical long-term health effects (CBAC, 2002).

In Japan, the Ministry of Agriculture, Food, and Fisheries (MAFF) and the Ministry of Health, Labour, and Welfare (MHLW) administer the regulation of food safety of GMOs, including GM crops and other foods and food additives that contain organisms or have been

obtained through recombinant DNA techniques. The food safety assessment of genetically modified organisms is mandatory under the Specifications and Standards for Food and Food Additives and Other Related Products and is conducted according to guidelines published by the Ministry of Health and Welfare (Japan MHLW, 2000). The definition of recombinant DNA pertains to the introduction of foreign DNA from sources other than the host; “self cloning” is exempt from the assessment (Japan MAFF, 1995).

In Australia and New Zealand, the Food Standards Australia New Zealand (FSANZ) has regulatory oversight over food safety, including the safety of foods derived from genetically modified organisms. Food Standard 1.5.2 specifically regulates the marketing of foods derived using recombinant DNA techniques (FSANZ, 2000). The standard also provides for the possible post-market monitoring requirements for foods derived from genetically modified organisms on a case-by-case basis, in particular for foods derived from GM crops the nutritional characteristics of which were modified (FSANZ, 2001).

Whilst regulatory frameworks differ across jurisdictions, the approaches to the safety assessment of foods derived from GM crops are similar in most countries, as they are based on general principles for risk analysis and international guidelines for the safety assessment of foods derived from genetically modified organisms.

2.2. General principles of risk analysis

The general principles for risk analysis were first established for evaluation of health effects from potentially toxic chemicals. Risk is defined as the likelihood that, under particular conditions of exposure, an intrinsic hazard will represent a threat to human health. Risk is thus a function of hazard and exposure. Hazard is defined as the intrinsic *potential* of a material to cause adverse health effects; implicit in the definition is the concept of severity and adversity of the effect. This definition is consistent with internationally accepted principles (FAO/WHO, 1995; FAO/WHO, 1997; Codex Alimentarius Commission, 2003a).

The international principles and guidelines, as well as most European policy documents on risk analysis and food safety (see for example European Commission, 2000b), draw a distinction between science-based risk assessment usually conducted by experts, risk management, and risk communication. Risk management is defined as “the process of weighing policy alternatives to mitigate risks in the light of risk assessment and, if required, selecting and implementing appropriate control options, including regulatory measures” (FAO/WHO, 1995; FAO/WHO, 1997). Risk management strategies include authorisation, and implementation of risk management measures to minimise or prevent the

risk. Examples of risk management for conditional approvals include labelling requirements to inform the target group at risk, as done for food products that contain major allergens. Risk communication is defined as the exchange of information and opinion on risk between risk assessors, risk managers, other interested parties, and the general public (FAO/WHO, 1995, 1997).

Some critics voice concerns that the separation of risk assessment and risk management neglects that risks are also a product of societal circumstances; the salience of expert advice to concerns of policy makers and the public is thus potentially reduced (Jasanoff, 1990; NRC, 1994, 1996; Presidential/Congressional Commission on Risk Assessment and Risk Management, 1997; James et al., 1999).

The focus of this paper is on risk assessment, defined as the evaluation of the probability of known or potential adverse health effects arising from human or animal exposure to the identified hazards (FAO/WHO, 1995, 1997). Such evaluation will always be a central part in the regulation of health risks, regardless of who frames the questions and how broad the assessment is. Risk assessment involves combining information on severity of the consequences of exposure to a hazard and expected degree of exposure. The first stage in risk assessment is to identify the hazards posed by a substance, by establishing a cause-effect relationship between the hazard and the product or process using toxicological experiments, modelling and/or epidemiological methods. It establishes the intrinsic potential of a substance, such as a chemical, protein, or food, to cause adverse health effects.

Hazard characterisation aims to evaluate in qualitative and quantitative terms the nature of the identified intrinsic hazard. This usually involves an analysis of the dose-response relationship of harmful effects in the target organism or an appropriate surrogate species and characterisation of the severity of the effect. In routine toxicological studies animals are usually administered three different doses, including very small doses and doses that exceed anticipated human exposures by several orders of magnitude. The purpose of these studies is the establishment of the highest dose level at which no adverse effect occurs—the No Observed Adverse Effect Level (NOAEL). Animal-based toxicological methods for hazard identification and characterisation have recently been reviewed by Barlow et al. (2002). The NOAEL in the most sensitive animal species in which tests were conducted is the basis for establishing best estimates of a safe exposure level for humans. This estimation takes into account variations in susceptibility between animal species, and between individuals within the human population. The observed NOAEL is divided by uncertainty factors to establish a margin of safety; the default uncertainty factors to account for

variation between species and individuals are both ten. The NOAEL is therefore often divided by 100 to establish the margin of safety. This information then constitutes the basis for a determination of a reference dose, exposure to which is deemed safe. The amount of a substance exposure to which over a life time is deemed safe is the Acceptable Daily Intake (ADI). Risk assessment requires judgements on what data are considered sufficient and what uncertainties need to be taken into account.

Information on the quantity and distribution of a potentially hazardous substance in the environment is then required in order to determine where populations are expected to come into contact with the substance; for foods, dietary intake assessments of populations are required for exposure estimates. Particular attention is paid to expected average and worst-case exposure levels of the most sensitive subgroups of a population. This information is used to determine the population groups that may be at risk and the distribution of such risks. These are the elements that allow estimation of the probability that harm will occur. Exposure assessment often needs to take into account important societal factors necessary to anticipate behaviour of a wide range of individuals that might affect their exposure.

Risk characterisation then combines information on hazard and exposure. This includes the probable extent, nature, and duration of exposure with considerations of hazard characteristics and relevance of those hazards for humans in order to estimate the likely risk to human health. Any uncertainties inherent in the risk assessment should be highlighted. If the expected exposure exceeds the established reference dose that was deemed to be safe, this has implications for risk management decisions: risk mitigation measures for chemicals can include measures such as prescribing use of personal protective equipment or restrictions on conditions of use of the chemical; risk management measures for foods can include labelling, as is the case for allergenic foods. The general principles of risk analysis as described above apply to the safety assessment of foods derived from GM crops.

2.3. The concept of safety assessment of foods derived from GM crops

The techniques of molecular biology allow the transfer of genes from one organism to another without sexual reproduction and across species. This process allows desirable alterations to be introduced into plant genomes in a more specific and controlled manner than can be achieved through conventional breeding and selection of crops. International panels of experts deemed that there are no risks inherent in the use of recombinant DNA technologies (OECD, 1986; Royal Society, 1998, 2002), as all DNA is chemically and

structurally the same and as the transfer of genetic material across species barriers occurs not only in laboratories, but also has been a major driving force in evolution. Hence, concepts were developed to focus the safety assessment of GM crops on any functional and chemical changes that result from the genetic modification.

Foods prepared and used in traditional ways, and consumed under anticipated conditions are generally regarded as safe based on their history of human consumption, even though they may contain natural toxins or anti-nutritional substances, such as neurotoxic glycoalkaloids in potatoes, or carcinogenic coumarins in courgettes. The assessment of novel foods, including foods derived from a GM crop, relies on the use of a food generally recognised as safe as a comparator (FAO/WHO, 1991). The term ‘assessment of substantial equivalence’ describes this comparative assessment approach. This term was first coined by the Office of Device Evaluation (ODE) of US FDA in the context of the evaluation of new medical devices that have a comparable function to existing medical devices (Miller, 1999). Authorities and agencies involved in food safety assessment in most countries have based their safety assessment strategies and guidelines on this approach (UK Department of Health, 1991; US FDA, 1992; Health Canada, 1994; Japan MHLW, 2000; European Commission 1997b).

Application of the concept of substantial equivalence requires the comparison of the GM crop and an appropriate ‘safe’ comparator according to the agronomical and morphological characteristics, and the chemical composition, including macro- and micro-nutrients, key toxins, and key anti-nutrients. This allows identification of significant differences between the GM crop and the comparator, usually the traditionally-bred parent crop (OECD, 1993a). Compositional parameters are then selected that are typical for the crop that is assessed and representative of the main metabolic pathways. Significant changes in these parameters are expected to be indicative of any more fundamental changes in the crop that need to be evaluated for their potential to have adverse consequence to human health.

The hazard identification and characterisation of GM crops therefore is conducted in four steps: (i) Characterisation of the parent crop and any hazards associated with it; (ii) characterisation of the transformation process and of inserted recombinant DNA (the potential consequences of any gene transfer event of the recombinant DNA to microbes or humans should also be assessed); (iii) characterisation of the introduced proteins (their potential toxicity and allergenicity) and metabolites; and (iv) identification of any other targeted 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. The exposure assessment includes estimating the dietary intake of the new food derived from GM crops and anticipating the effect of food processing on any of the introduced changes.

The successful application of the concept of substantial equivalence largely depends on three critical elements: the availability of an appropriate comparator and an understanding of the range of variation to be expected within the measured characteristics of that comparator; the choice of parameters in the single constituent compound analyses, the number and type of which will strongly influence the validity of any conclusions on comparative safety; and the ability to discriminate between differences in the GM crop and the comparator that result from the genetic modification and those differences in the plant's germplasm, some of which may be attributed to soma-clonal variation introduced during tissue culture, and environmental or cultivation conditions. All such changes that might have health implications warrant further investigation, even if they can not only be attributed to the genetic modification, unless they are not manifested in subsequent generations foreseen for commercial cultivation.

Any identified differences are then further assessed as to whether they might have adverse implications for human health in the range of exposure scenarios. The concept of substantial equivalence is thus the starting point and guiding concept for the safety assessment, *not* its conclusion (FAO/WHO, 2000; Codex Alimentarius Commission, 2003b). If there are no significant differences between the GM crop and the comparator or if there are differences that will, with reasonable certainty, not adversely affect health, the GM product is considered 'as safe as' its counterpart. This approach also applies to GM crops with more complex metabolic modifications, where no single parent crop might be a suitable comparator, but where single widely consumed substances, food constituents, ingredients, or other whole foods that are deemed safe under representative conditions of use may serve as comparators.

Critics of the concept of substantial equivalence claim that current testing approaches do not sufficiently address putative unintended and unexpected effects and can not rule out the occurrence of potential long-term effects that result from sustained human exposure to such crops that might have subtle compositional changes that may be difficult to detect (Millstone et al., 1999). Furthermore, some critics maintain that there is a lack of detailed international standards guiding the choice of parameters to be measured in the comparative analysis and in the application of rigorous statistical analysis, reducing the quality of individual assessments (SBC, 2001). Groups of international experts have reviewed the concept of substantial equivalence in the light of these criticisms. It was concluded that the concept still

represents the best available assessment paradigm; no alternative approaches for the safety assessment of foods derived from GM crops have been proposed (FAO/WHO, 2000; Codex Alimentarius Commission, 2003b). Detailed international guidelines for choice of comparators and for best practices in statistical analysis have been and are being established under the auspices of the Organisation for Economic Coordination and Development (see also Section 4.1).

In summary, the concept of substantial equivalence is widely accepted by international and national agencies as the best available guidance for the safety assessment of new GM crops. The approach recognises that foods are complex matrices containing tens of thousands of individual constituents, and that their safety assessment therefore requires a comparative approach focusing on those parameters deemed indicative of the normal functioning of the plant and its metabolism (including biosynthesis of any compound that might affect human health). As with all scientific concepts, the concept of substantial equivalence is evolving and, together with guidelines, making its application more systematic. The assessment helps to determine whether the GM crop is 'as safe as' its conventional counterpart. Dialogue between experts and civil society will contribute over time to further clarify and structure risk analysis strategies to improve the salience of assessments to address concerns of policy makers and the public (Tait, 2001; Jasanoff, 2000; Schauzu, 2000).

3. Methods for toxicity testing

Regulatory requirements for chemicals such as food additives and pesticides, many of which were first instituted in the 1970s, have led to the development of a battery of tests to assess the safety of chemicals in foods. Strategies for assessing the food safety of chemicals often combine three approaches: investigation of the structure/function relationship for indications of potential toxicity and allergenicity; in vitro assays with enzymes, receptor proteins, or cultured cell lines; and in vivo animal studies. The selection of animal studies is based on considerations including a molecule's structure, function and in vitro toxicity results, as well as ethical criteria. Of these three distinct approaches, evidence from animal tests is usually most indicative of potential toxic effects of a test substance in humans. However, the extrapolation of results from animal tests to humans is uncertain, unpredictable differences can include inter-species and inter-individual differences in metabolism, physiological processes, and lifestyle. These uncertainties are usually addressed through the use of uncertainty factors (see Section 2.2). Individual toxicological tests can be designed to be specific to test an hypothesis of a molecule's toxic effect on one particular

organ, a combination of specific endpoints, or they can be broad and non-targeted.

A combination of database screening, in vitro, and in vivo testing approaches is therefore deployed in most food safety testing strategies and provides assurance that the tested food will be as safe as other foods that are routinely consumed. The characterisation of the test substance's physico-chemical properties and structure-activity relationship that might be indicative of the presence or absence of potential adverse health effects helps to frame and focus the testing approach. Several tests established for chemicals are applicable to, or have been adapted for testing purified recombinant proteins or other substances contained in foods derived from GM crops. Some animal methods have also been adapted to gain information pertaining to the safety of whole foods derived from GM crops. The following section will provide a brief overview on the three distinct approaches developed for chemicals, highlighting which of these have been deployed in strategies to assess the safety of foods derived from GM crops.

3.1. Investigating the relationship between structure and activity for indications of potential adverse effects

The investigation of a substance's structure-activity relationship starts with a description of the defining physico-chemical properties. Computers help to assess whether a molecule shares characteristics of known toxicants through screening databases with information on structural and physico-chemical properties of known toxicants. Some toxicants show a clear structure-activity relationship and their mechanism of toxicity is fully understood; other classes of toxicants just share common structural elements or physico-chemical properties that may be indicative of toxicity. Some physico-chemical or structural characteristics of molecules are therefore indicative of a potential toxic effect. A molecule's physico-chemical properties help for instance the prediction of its propensity to intercalate in DNA and interfere with its replication (Barratt, 1998). Databases also exist listing proteins with (food) allergenic properties (see Table 1). Computer-based methods have also been developed for the comparison of the primary amino acid sequences of proteins allowing identification of contiguous epitopes that might mediate an allergenic effect. Limitations of the method include that non-continuous epitopes cannot be identified, and false positive matches through epitopes that are similar but do not mediate allergenic effects. Any indication of toxicity or allergenicity obtained through computer screening has to be confirmed through other studies.

A computer-assisted search for similarity to proteins known to cause adverse effects, such as protein toxins or allergens, forms part of the current recommendations

for assessing the likely allergenicity of a novel protein (FAO/WHO, 2001; Codex Alimentarius Commission, 2003c; Kleter and Peijnenburg, 2002). The merits and limitations of this approach for assessing the safety of novel proteins introduced into GM crops are described in more detail in Section 4.3.4.

3.2. In vitro methods

In vitro methods contribute to the safety assessment of chemicals, including food additives; they can in some cases serve as indicators for specific toxic effects of discrete molecules and substances. There are several different types of in vitro methods; these include the in vitro simulation of digestive systems to assess stability; bioassays of the activity of purified enzymes; immortalised cultured cell lines; or in vitro reconstructions of receptor or membrane systems. The methods can serve either as screening systems to assess potential toxicity of a compound, or for investigations of a toxicological mechanism underlying a specific effect observed in vivo or predicted from the structure of a molecule. Such assays can serve for instance to assess whether molecules bind to, inhibit, or stimulate proteins with specific functions. In vitro tests include tests that are claimed to be indicative of specific organ toxicity. An overview on the status of many in vitro methods in relation to the assessment of acute toxicity endpoints has been published (Walum, 1998). A databank of in vitro techniques in toxicology, called INVITTOX, has also been established (INVITTOX, 2003). A critical discussion of the merits and limitations of in vitro methods is provided in Eisenbrand et al. (2002).

Few in vitro tests are however validated formally, and extrapolation of results from in vitro tests to in vivo situations is often challenging, often their predictive value has not been systematically assessed; uncertainties have to be clearly stated. Advantages of the use of in vitro methods include that they are relatively cheap and high-throughput. In vitro test results may be indicative of toxic effects; test systems relying on reconstituted purified protein or cell components, or immortalised laboratory cultures of cell lines are, however, not representative of the functioning of such cell components or cells in living organisms. In vivo tests are therefore required to confirm observations on toxicity from in vitro tests. The use of in vitro methods may become of greater importance with developments in the area of genomic research and microarray systems to monitor changes in gene expression. Future developments in this area are discussed in more detail in Section 5.

The US Pharmacopeia describes simulated gastric and intestinal fluid preparations that have been used to assess the stability of proteins (US Pharmacopeia, 1990; Astwood et al., 1996). The test is used as one indicator

as to whether the recombinant protein shares the characteristic of stability to digestion under these conditions that is common to many allergens (see [Section 4.3.4](#)).

3.3. Animal tests

A variety of tests with laboratory animals have been developed for identifying and characterizing health hazards associated with exposure to single well-defined chemicals and food additives. Tolerance studies are sometimes used to confirm the absence of adverse effects at expected levels of exposure. Ethical considerations should be an important driver in decisions on whether to conduct *in vivo* studies, in the choice of test species, and in the design of the study protocol. Both toxicological animal tests and tolerance studies are discussed in more detail below.

Animals can be used for the determination of acute toxicity of a substance, usually involving administration of a large single dose followed by a short period of observation. Sub-chronic toxicity, chronic toxicity, or carcinogenicity is tested in animals over prolonged periods of one or several months, or the lifetime of an animal. The use of these methods has recently been reviewed in detail by the European Concerted Action Food Safety in Europe (FOSIE) (Barlow et al., 2002). They form the basis of toxicity testing of chemicals, and have provided valuable information for this purpose (Kroes and Kozianowski, 2002). In addition to the standard tests listed in [Appendix A](#), other studies could be carried out where deemed necessary to address immuno-toxicity; endocrine toxicity; individual organ toxicity; and toxico-kinetic investigations on absorption, distribution, metabolism, and excretion (ADME) of a substance. Most of these methods have been standardised and OECD guidelines are available for their conduct and interpretation (see [Appendix A](#); OECD, 1993b).

These animal methods, if deemed necessary, can be adapted to test for potential adverse effects of recombinant proteins or novel compounds or metabolites introduced into crops through genetic modification (see [Section 4.3](#)). It can however be difficult to obtain sufficient quantities of purified recombinant proteins for testing in animals over prolonged periods of time.

Adapting OECD guidelines and principles for testing specific well defined chemicals to the assessment of whole foods, including whole foods derived from GM crops, presents, however, several challenges. First, animal tests for chemicals are usually designed to identify a dose response relationship from which the consequences of exposure to low doses, typical of human exposure, can be extrapolated. The highest administered dose is normally expected to produce some observable adverse effect. It is, however, often not possible to administer

whole foods to animals at doses that are large multiples of the expected human exposure. Frequently, in feeding trials with whole foods at the highest administered dose, no adverse effect is observed. In animal studies where no adverse effect is observed, and where administered doses do not significantly exceed expected human exposure levels, it is not possible to account for uncertainties regarding variations of susceptibility between species and between individuals within a population. Furthermore, the food matrix in which the test material is administered needs careful consideration: it may be difficult to ensure nutritional balance when diets contain high proportions of novel foods or food extracts. Inadvertent changes in nutritional status may result in adaptive changes that may mistakenly be attributed to adverse effects from intake from the GM crop and can be attributed to changes resulting from the genetic modification.

Animal feeding studies with whole foods that take account of these difficulties through careful design and description of conditions under which the tests are carried out and any remaining uncertainties have, however, been used to complement other tests for the safety assessments of foods derived from GM crops. Such tests may provide useful information, in particular if doses that are multiples of anticipated human exposure can be administered to animals (Hammond et al., 1996; Kuiper et al., 2001).

Studies in which animals (or, all be it rarely, human volunteers) are administered the expected level of intake or low multiples of that level, so-called tolerance studies, can be used to complement other safety testing approaches. Well-established protocols for tolerance studies of pharmaceuticals are available and can be adapted for this purpose. The highest dose used must be without adverse nutritional effects and must respect the appropriate balance of nutrients required by the test species. A classical range of parameters such as growth rate and feed efficiency are measured to identify possible adverse effects on specific endpoints. The use of appropriate controls is an important determinant of the validity of the results. For instance, if the novel component is normally present in a particular matrix, then that matrix would be an appropriate control. For the tolerance assessment of GM crops controls should include animal groups using the corresponding comparator. Similar methods can be used for animal and human studies (see also [Section 4.4.3](#)).

3.4. Post-market monitoring

Post-market monitoring systems have been established by several food companies for certain food products to act as early warning systems and to facilitate product recall in the event where health concerns might be associated with a specific food. The organisation of post-market monitoring is primarily the responsibility

of the manufacturer of the food. Methods vary from establishing channels of communication in the firm to receive direct consumer feedback on the product, to the repurchase of products to determine the quality of the product on the supermarket shelf.

Post-market monitoring programmes may serve to confirm the absence of specific adverse health effects of certain products after they have been marketed. The feasibility and validity of post-market monitoring depends on the health endpoint of interest and on the way the product is marketed. First, post-market monitoring of manifestation of adverse effects from intake of specific foods is most feasible if it is driven by an hypothesis on a potential and specific adverse effect that is acute and has distinctive symptoms. Acute adverse effects generally associated with relatively high intakes of a substance, or allergic reactions, are likely to become apparent by post-market monitoring for spontaneous events. However, long-term or rare effects require generally a more targeted and intrusive study design. Randomised controlled human trials could be used to investigate possible medium/long term effects, but the wide variation in diets and dietary components from day to day and year to year should be recognised. While clinical studies in humans may provide wider assurance of safety of whole foods, they cannot fully reproduce the diversity of the populations who will consume the marketed product. The possibility therefore remains that unpredicted side effects may occur in some sectors of the population, such as those with certain disease conditions or with particular genetic characteristics. In addition, risk assessment relies on an estimate of exposure to the food, which is variable and subject to uncertainty before the food is marketed. Critical issues to the success of a post-market monitoring programme relating to the marketing approach of the foods are the ability to estimate exposure with reasonable accuracy (traceability) and to match any reported effects to the consumption of the material (Wal et al., 2003). For identity-preserved products this may be feasible, whereas for commodities it is much more difficult, if not impossible.

To date, no GM crop has been placed on the market for which post-market monitoring was deemed necessary. The current safety testing strategy has been considered sufficiently predictive for these approvals. Problems limiting the interpretation of post-market monitoring of foods derived from commodity crops have been highlighted (FAO/WHO, 2000). The United Kingdom Food Standards Agency has commissioned a study to examine the feasibility of using supermarket and household survey data for post-market monitoring of novel foods. This study assesses the government's ability to detect variations of food purchasing and consumption at the district level in Great Britain, as this is seen as an indicator for the feasibility to detect

and to link such variations to health outcomes (FSA, 2002). Possible requirements for post-market monitoring of GM crops with more complex modifications, including altered nutritional value, are discussed in Section 4.6.

4. The safety assessment of foods derived from GM crops

Safety considerations for foods derived from GM crops are fundamentally the same as those for conventional foods or other types of novel foods (Cockburn, 2002). Since the genetic improvement of crops has always been the aim of plant selection and breeding, 'traditional' approaches are appropriate in order to assess the safety of foods derived from all GM crops, regardless of the crop species or the trait introduced by genetic modification.

This section sets out a systematic stepwise approach on how to select appropriate combinations of test methods to assess the safety of foods derived from GM crops (Figs. 1 and 2). The objective of the assessment is to determine whether these new foods are at least as safe as foods produced from conventional crops; this approach hence offers a high level of safety assurance.

The outlined approach serves to structure case-by-case assessments of specific products; it provides guidance on how to design a test programme for the safety assessment of a GM crop that is tailored to the specific characteristics of the parent crop and the introduced trait(s). The safety assessment focuses on the new gene product(s) and of whole foods derived from the GM crop. Both intended and potential unintended effects from the genetic modification are taken into account. The assessment involves the following steps: (i) characterisation of the parent crop; (ii) characterisation of the donor organism(s) from which any recombinant DNA sequences are derived, the transformation process, and the introduced recombinant DNA sequences; (iii) safety assessment of the introduced gene products (proteins and metabolites); and (iv) food safety assessment of whole food derived from, or edible part of, the GM crop.

The assessment focuses on any changes introduced through the genetic modification, including introduced genes and gene products, and potentially altered levels of endogenous compounds or the formation of new metabolites. Methods for the detection of unexpected changes in the composition due to the genetic modification process are discussed and evaluated in the paper by Cellini et al. (2004). Possible consequences of transfer of the recombinant sequences to gastrointestinal microflora or to humans should also be assessed and are evaluated in the paper by Van den Eede et al. (2004). The assembled information may also help to identify

where further information might be needed to assess safety.

4.1. Information on the parent crop

The description of the parent crop should include information on the origin, genotype, phenotype, diversity, and history of safe use of the parent crop and, where possible, of other related traditionally-bred varieties and species. The characterisation of the parent crop then guides the choice of test parameters for the comparison of the GM crop to a close comparator, which is usually the non-modified parent crop. Test parameters mostly include indicators of the crop's development, phenotype, and agronomic performance, as well as its main endogenous nutrients, and potential anti-nutrients or biologically active substances, toxins,

and food allergens, where applicable. An understanding of the natural variation of the main agronomic and compositional characteristics of the crop in different geographies and under different cultivation conditions is essential to interpret data comparing the GM crop to the chosen comparator. Whole food and substance-specific laboratory animal or farm animal studies that may already have been conducted on the parent crop may also provide valuable additional information relevant to planning the safety assessment strategy for the GM crop. The Organisation for Economic Cooperation and Development is compiling consensus documents for certain crop species that provide the information considered of most relevance of the characterization of the parent crop. Two types of consensus documents are available for the world's major food crops: the first describes a crop's biology, focusing on attributes that

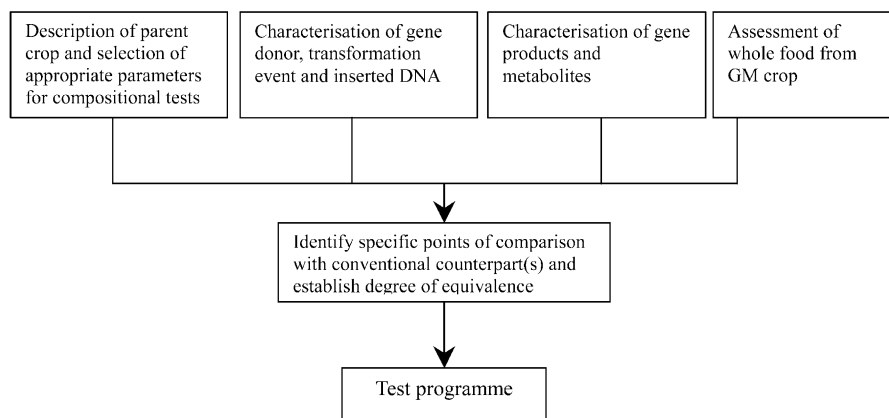


Fig. 1. Designing a test programme.

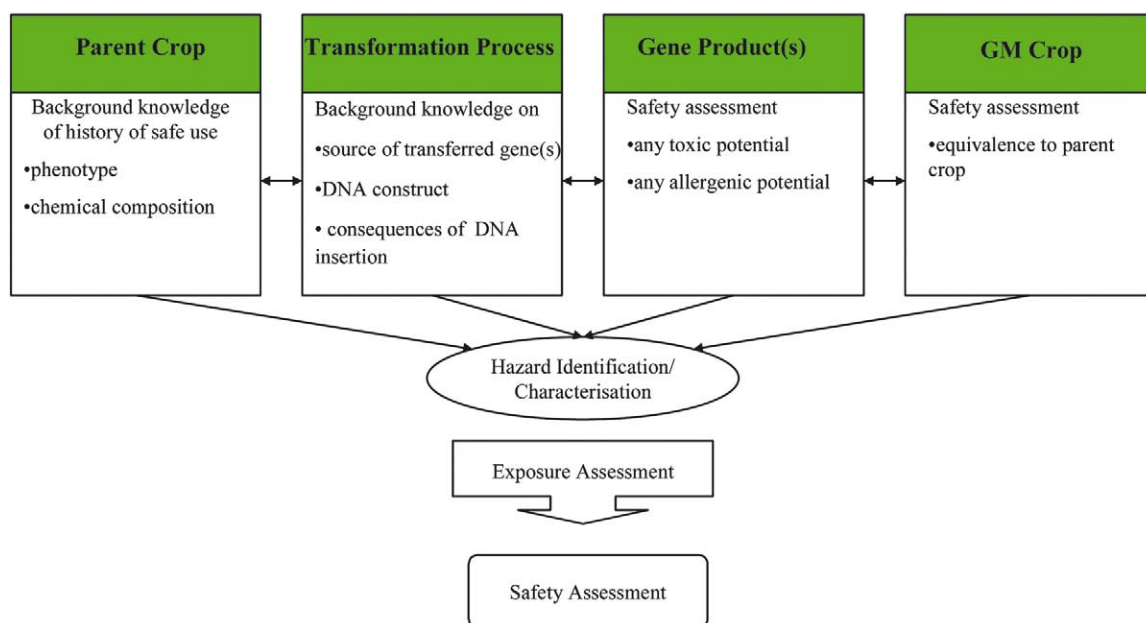


Fig. 2. The elements in safety assessment of food derived from a GM crop.

are relevant to the environmental safety assessment, and the second describes a crop's compositional characteristics that are of most importance for the food safety assessment. (For potatoes see for example OECD, 1997, 2002b).

The type of information on the parent crop that should be gathered at the outset of the assessment is shown in Fig. 3.

4.1.1. Identity, phenotypic and agronomic performance

Taxonomic identity of the parent crop should be established to referenced and internationally acceptable principles, including complete name, family name, genus, species, subspecies, cultivar/breeding line, common name, and sexually compatible wild relatives. Specification of the chemical proximate composition and key nutrients and anti-nutrients of the host plant is also required; the parent crop or conventional counterpart usually serves as the reference point in the safety assessment of the GM crop, unless in future, the new trait(s) extensively modify the composition and nutritional characteristics such that another comparator, possibly a combination of non-GM crops and/or derived foods with a history of human exposure, have to be used as safety standard.

4.1.2. Geographical distribution/source

Information about the geographical distribution of cultivation of the parent plant identifying usual climatic and soil conditions for its growth should be given. Knowledge of the native distribution centre of origin and history of domestication provides valuable information relevant to a crop's cultivation and the occurrence of wild relatives that are potentially sexually compatible and to which cross-pollination may occur.

4.1.3. History of use

The novelty, sometimes referred to as 'exoticness', of food plants is determined by their documented history of use in the food supply. It has to be recognised that many plant-derived foods that are common in one part of the world may not be part of the food supply in other

parts. Any known nutritional, anti-nutritional, toxicological, or allergenic characteristics of the food crops, or intolerance to foods derived from this crop should be highlighted and become an important element of the subsequent safety assessment.

In addressing the history of use and the importance of the plant-derived foods in the diet, any particular preparation, processing, or cooking practices should also be identified. The safe use of plant-derived foods sometimes depends on special pre-treatment established by 'custom and practice' that renders it palatable and safe. For example, red kidney beans require boiling before consumption to render the lectins, protease inhibitors, and haem-agglutinins inert. In general, the adverse effects to health of most anti-nutrients are caused by the consumption of raw plant material. Most anti-nutrients are not heat stable or are not evenly distributed in the plant; they can hence be inactivated or removed by measures such as heating, soaking, peeling, or germination.

4.1.4. Compositional analysis

Regulators widely agreed that the compositional assessment should include analyses of the key nutrients, toxins, allergens, anti-nutrients, and biologically active substances that are known to be associated with the crop. International consensus continues to develop on which key components should be analysed in order to perform a fully comprehensive compositional analysis of specific food crops. The OECD provides a platform for discussions amongst experts representing the Member States. The results of these discussions are published in the form of a series of consensus documents on compositional considerations for new varieties of crops. Consensus documents on rapeseed (canola), soybean (OECD, 2001a,b), potato, sugar beet, and maize (OECD, 2002a,b,c) have been published; consensus documents for wheat, rice, sunflower, cotton, and barley are in preparation. Third party papers exist on a number of other key crops such as cotton (Berberich et al., 1996; Nida et al., 1996).

The composition of individual plants grown in the same field can differ considerably due to natural biolo-



Fig. 3. Description of the parent crop.

gical variation, since the plant's development and metabolism are influenced by a range of biotic and abiotic factors, including pathogen infection, state of ripening, growing conditions (location, climate, heat/drought, soil quality), and storage. The International Food Biotechnology Council (IFBC; IFBC, 1990) has noted that there may be significant natural variation in the composition between samples from individual plants or composite samples from fields of the parent crop line that are grown in two different locations; the same holds true for samples from a GM crop line.

A thorough knowledge of the composition of the parent crop should always be established both from the literature and from analytical data resulting from field trials. Samples should be taken from a range of different varieties from the same crop. This represents the reference point for subsequent comparison with samples from the specific GM crop line that is tested. Any significant differences in composition between the GM crop line that fall outside of the range of natural variation in levels across a range of varieties of the same crop that are reproducible then become the focus of further evaluation.

There is a great need for continued international standardisation and harmonisation in this area to provide peer reviewed databases from which the range of natural variation of the levels of tested parameters can be deduced; such databases may then become the main reference or standard to determine the significance of observed differences in the comparative compositional analysis of a GM crop line and the comparator. The OECD and the International Life Sciences Institute (ILSI) provide platforms for the development of such consensus databases.

4.1.5. *Nutrients, anti-nutrients, toxins, and allergens*

The assessment should focus on those nutrients, toxins, anti-nutrients, allergens, and bioactive constituents in the host plant or in its close relatives, changes in the levels of consumption of which might affect human health and nutrition. Nutrients are components in a particular food that may have a substantial nutritional impact on the consumer or animal. These may be macro-nutrients (fats, proteins, carbohydrates) or micro-nutrients (minerals and vitamins).

Anti-nutrients are substances that inhibit or block important pathways in the human metabolism, or impair digestion. Anti-nutrients may reduce nutrient utilisation, typically proteins, vitamins, or minerals, thus decreasing the nutritive value of foods (Watzl and Leitzmann, 1995). Toxins are food components that may have a substantial negative impact on human health, including adverse effects other than those related to metabolism and digestion. Food toxins such as the neurotoxic solanine, cyanogenic glycosides, erucic acid, lectins, and trypsin inhibitors are found in a wide

variety of foods and often function as the plant's own natural pesticides.

Food allergens are proteins that induce allergic sensitisation in susceptible individuals, such that subsequent dietary exposure to the same protein may provoke an adverse reaction. Exposure of sensitised individuals to relevant allergens in food may result in serious adverse effects.

Plants are also a rich source of medicines, which have been widely exploited for therapy by the science of pharmacognosy. It follows that many crops consumed by humans contain powerful bioactive substances with pharmacological activity. Examples include phytosterols, caffeine, and theobromine.

It is therefore important to ensure that no new toxins, anti-nutrients, allergens, or bioactive substances are inadvertently introduced, up- or down-regulated as a consequence of the genetic modification. It is therefore necessary to ensure that transformation does not introduce new compounds of this type or affect negative changes in the levels or characteristics of endogenous compounds that may impact human health and that are already present in the crop plant.

4.2. *Information on the donor, transgene(s), and delivery process*

Molecular characterisation of the recombinant DNA in GM crops is usually done in accordance with broad international guidelines on the safety assessment of GM crops. In Europe, there are additional legal requirements for the molecular characterisation of GM crops in Directive 2001/18/EC (European Commission, 2001). The Directive requires that the inserted genetic material is well characterised and safe for humans and the environment under the conditions of the release of the GM crop. Further guidance on data to be generated has been given by the European Commission's Scientific Steering Committee (European Commission, 2003c) and the United Kingdom Advisory Committee on Environmental Releases (UK ACRE; UK ACRE, 2001). Both advisory committees emphasise the need to describe the cloning and transfer vectors and the recombinant DNA inserted into the GM crop. The information and data described in Fig. 4 needs to be provided in order to identify and characterise the potential hazards resulting from plant transformation.

4.2.1. *Description of the donor(s)*

The description of the donor organism(s) should include the classification and taxonomy to international standards (see Section 4.1.1) and should also address any evidence of potential toxicity, allergenicity or pathogenicity. A list of naturally occurring toxins, allergens, bioactive substances, and anti-nutrients

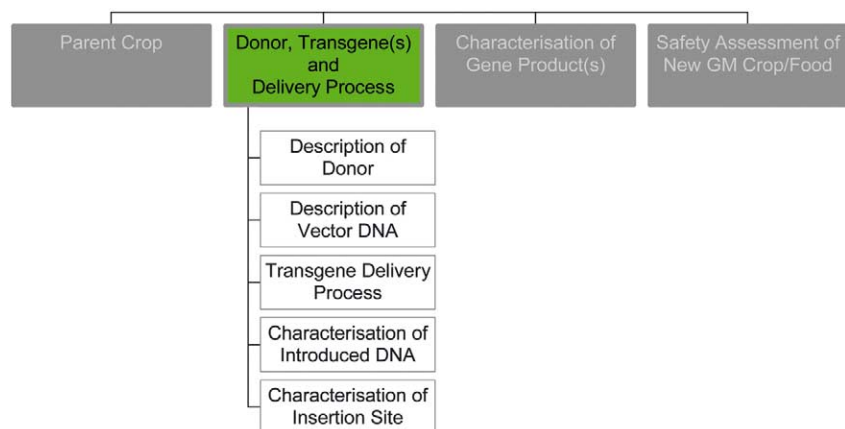


Fig. 4. Description of the donor(s), transgene(s) and delivery process.

contained in the donor organism should be drawn up. Documentation on the history of use of and exposure to the donor organism should be cited, where possible. This information, together with a detailed understanding of the function of any recombinant DNA sequences used in the transformation process, facilitates the hazard identification of the 'novel elements' that are transferred to the crop (see also 4.3.2). The introduced DNA should be shown to be unrelated to any characteristics of the donor organisms that could be harmful to human health. If the donor is known to be allergenic, transferred genes are assumed to encode allergens until evidence to the contrary has been obtained. Assessment of the potential allergenicity of recombinant proteins is described in more detail in Section 4.3.4.

4.2.2. Description of vector DNA

A step-wise description of the construction of the transformation vector should provide details on all organisms used for the amplification of vector DNA. It should also provide information on the function of all genetic elements of transformation vectors, including coding sequences, promoters and termination signals. A vector map with relevant restriction enzyme sites should also be made available (European Commission, 2003c). Subsequent proof of absence of vector fragments not intended to be transferred is requested as well. The provision of nucleotide sequence information of the vector is also considered helpful (UK ACRE, 2002).

4.2.3. Transgene delivery

Recombinant DNA is in most cases inserted into plant cells using *Agrobacterium tumefaciens* or a particle gun. *Agrobacterium* strains usually contain one vector encoding DNA mobilisation and transfer functions and a separate vector with the recombinant DNA intended for transfer and a recognition site for the transfer-mediating gene products. Using *Agrobacterium*, the risk of transfer of random DNA to the plant is relatively small (Gelvin, 2000; Hellens and Mullineaux, 2000). The

Agrobacterium donor strain and any plasmids contained in that strain should, however, be described to assess the risks of the presence of other sequences that might be recognised by the transfer-mediating gene products.

Using methods of direct transformation of plants, such as particle guns, the preparation of the DNA used for plant transformation should be checked for contaminating sequences of bacterial chromosomal DNA or other plasmid DNA. Restriction fragment-preparations obtained using gel purification should be checked for contaminating vector sequences using, for instance, Southern blots.

4.2.4. Characterisation of introduced DNA sequences

Thorough characterisation of inserted DNA sequences through Southern blotting or polymerase chain reaction (PCR) techniques is standard practice. The number of insertion sites and the copy number of introduced DNA sequences have to be determined. Characterisation of the inserted DNA merely using PCR is not sufficient, as it does not unambiguously reveal the number of insertion sites and the copy number of inserted genes. Either Southern blots or a combination of PCR and Southern blotting yields better results. Inserts at one site may be concatemers of the same sequence. In particular, the ends of the inserts adjacent to plant genomic DNA have to be carefully analysed to determine whether any truncated open reading frames start within the insert or the plant genomic DNA that might yield transcripts that span plant genomic DNA and might also produce fusion proteins. The UK ACRE guidelines recommend considering to sequence the recombinant DNA fragments introduced in to the plant genome, involving the construction of genomic libraries of each transformed plant line that is to be so characterised (UK ACRE, 2002). This recommendation is further discussed in Section 5.1 of this paper.

Evidence for the absence of vector backbone not intended for transfer is also required using Southern

blotting complemented in some cases by PCR. The presence of sequences that potentially mediate transfer or confer instability (repeated sequences, transposon-recognition sites, origins of replication) should be addressed. If present, potential consequences should be explored. Stability of the transgene insertion should be verified over five or more generations.

Assessment of the likelihood and potential consequences of transfer of inserted DNA sequences to gut bacteria should be provided, where applicable, for instance for antibiotic resistance markers (see Van den Eede et al., 2004).

4.2.5. Characterisation of insertion site

The provision of sequence information on the junction of the inserted recombinant DNA and the plant genome is required under Directive 2001/18/EC for the development of transformation-event specific detection methods (European Commission, 2001). Such sequences may also provide clues on the site of recombinant DNA insertion in the plant genome, i.e. they may help to characterise the insertion locus to predict if important plant endogenous genes might have been disrupted through the genetic insertion event. The UK ACRE guidelines recommend that where insertion has occurred within a gene of the plant genome, the associated complete gene should be sequenced beyond its ends, and any data on the potential role and identity of its product should be presented (UK ACRE, 2002). At present, this is not a routine regulatory requirement, as it is held that any such effects that might be of adverse consequence to human health or the environment, and hence relevant to the risk characterisation are identified through studies of agronomic, physiological, and compositional characteristics of the plant. The provision of sequence data of recombinant and adjacent plant genomic DNA is further discussed in Section 5.1 of this paper.

DNA sequence information of plant genome/insert junctions should, however, be required where truncated

open reading frames or promoters exist within the DNA insert that might give rise to fusion proteins.

4.3. Information on the gene products: recombinant proteins and/or metabolites

The recombinant gene(s) transferred into a host plant are usually translated into proteins, certain exceptions include anti-sense DNA or sequences encoding ribozymes. Secondary gene products, such as metabolites, may result from the recombinant proteins' regulatory or enzymatic functions. Hazard identification in assessments of GM crops should focus on the intended changes from the genetic modification, that is, the primary or secondary gene products (Fig. 5).

Proteins are not generally associated with safety concerns when consumed orally, as evidenced by the high protein content of our normal diet. Nevertheless, because there are some known specific proteins that are associated with toxic, anti-nutrient, pharmacological, or allergenic effects, any recombinant protein's potential to exert adverse effects on human health should be investigated. The amount of new test data required for a recombinant protein should depend on whether the protein has already been consumed or is structurally and functionally similar to proteins known to be safe for use as they are routinely ingested by humans without documented adverse effects (see Section 4.2.1). In this case, the safety concerns and new data requirements would be lower than in the case of a protein with no history of human exposure. As almost all food allergens are proteins, it is also necessary to assess the possibility of allergenic potential using a weight of evidence approach.

4.3.1. Characterisation of proteins and/or metabolites

The levels of the recombinant protein in various plant tissues, with a focus on the edible parts of the plant, should be determined; these levels are often very low; in

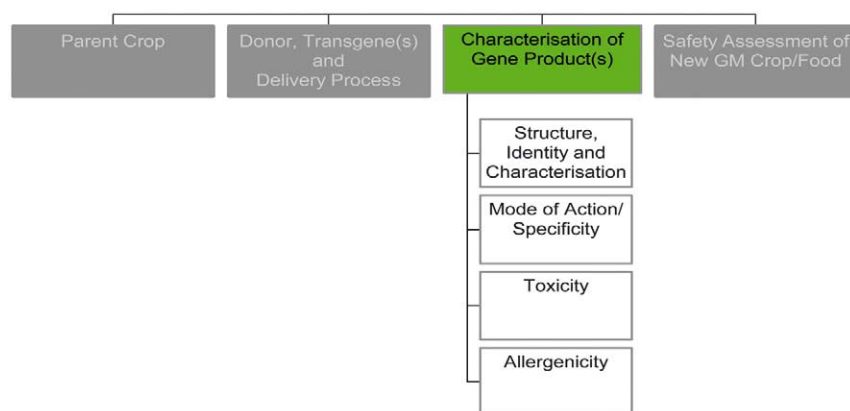


Fig. 5. Description of the gene product(s), proteins and/or metabolites.

GM crops that to date have entered the agri-food chain they are usually in the range of 0.01–0.1 percent of the total protein (Betz et al., 2000). Toxic effects of the protein might therefore not be detectable when testing plant material itself. The assessment of protein toxicity therefore may require either purifying sufficient amounts of the heterologous protein from the GM crop or from other hosts (e.g. bacteria, yeasts) that have been genetically modified to over-express the protein for testing.

Expression of genes in different organisms (plants or bacteria) can potentially result in differences in folding or post-translational modification of proteins; these need to be taken into account in the assessment (Jonas et al., 1996). Typical parameters considered in demonstrating the equivalence between a protein that is produced in a plant and the same protein produced by bacteria include molecular weight, amino acid sequence similarity, post-translational modification (e.g. level of glycosylation or phosphorylation), immuno-equivalence, activity and specificity of the reaction when the gene product is an enzyme.

The purified proteins and/or metabolites should be thoroughly investigated using classical approaches for defined chemical substances described in Section 3 of this paper, provided such data does not already exist. Knowledge of the primary amino acid sequence of the recombinant protein allows screening of computer databases for any sequence similarities with known protein toxins and allergens. A protein's physical stability and its stability in simulated digestive conditions should also be assessed. If proteins are rapidly degraded to peptides and amino acids, there will probably be less opportunity for the induction of allergic responses and/or adverse health effects. The safety assessment of recombinant proteins is described in more detail in Sections 4.3.3 and 4.3.4.

The inserted gene(s) may also be designed to result directly or indirectly in altering the levels of the crop's inherent micro- or macro-nutrients. Furthermore, pathway engineering or the insertion of a combination of new genes can also result in the intentional production of entirely new substances in that crop; one example is 'golden rice' that was modified to produce pro-vitamin A (Ye et al., 2000). The identity and characterisation of these substances will determine whether toxicological or other testing needs to be performed. If the substances are well characterised and human exposure to anticipated levels through GM crop intake is deemed safe through reference to a documented history of use, this may be sufficient to prove their safety. If the substance(s) are new and no data exist, full safety assessment may be required as in the case of any new defined single substance for human consumption, see Section 4.3.3.

Any significant unexpected changes in levels of substance(s) detected during compositional analysis will

require identification, characterisation, and safety assessment. In all cases, expression levels need to be established to ensure that the exposure levels lead to the conclusion of 'no harm under anticipated conditions of use.' If fusion proteins are expressed, these would need to undergo the same safety assessment as intentionally introduced recombinant proteins.

4.3.2. Mode of action and target specificity

An adequate hazard assessment can only be performed if the mechanism of action of the substance under investigation, i.e. the protein or secondary metabolite is known. A good example is the class of proteins from *Bacillus thuringiensis* (Bt-proteins) which are toxic to selected insects, but not to mammals. Mechanistic studies have shown that these proteins bind to specific receptor proteins on the insect gut wall with differing selectivity and at alkaline pH, but do not bind to cell surfaces in the human gut (Noteborn et al., 1995; Faust et al., 1974). Not only specificity is an important consideration. If enzymes are introduced into GM crops, an overview on all relevant metabolic pathways that could be affected by the enzymes' presence or altered levels or substrate specificity needs to be provided. The *Agrobacterium* sp. CP4-derived enzyme enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) for example confers tolerance to the herbicide glyphosate by supplementing the crop's own endogenous EPSPS enzyme, that is sensitive to the herbicide. EPSPS enzymes are part of the biosynthetic pathway of aromatic amino acids of all plants and micro-organisms. The CP4 EPSPS transferred to GM crops is not inhibited by the herbicide. The EPSPS enzyme is not a rate-determining step of the overall pathway, hence, an increase in level of this enzyme through the presence of the plant's endogenous EPSPS and the recombinant CP4 EPSPS is not expected to affect the rate of synthesis of the specific aromatic amino acids (Padgett et al., 1996; Taylor et al., 1999). This is confirmed in the analysis of levels of the aromatic amino acids in the crops, that is part of the compositional analysis. Both the fact that plants already contain the protein (the protein is highly conserved across species), and the role it plays in this particular metabolic pathway, are considered in the safety assessment.

4.3.3. Assessment of toxicity

The need for toxicity tests for introduced proteins and metabolites should be considered on a case-by-case basis. Choice of test methods should be based on the toxicological profile of the substance. The extent of testing should also depend on whether there has been documented human exposure to the substance that is similar to expected intake levels from use of the GM crop. Understanding of the mechanism of action or toxicity of the new protein(s) or metabolite(s) should

guide the toxicological testing programme and interpreting the data. If a recombinant protein or new metabolite (e.g. vitamins, omega-3 fatty acids) is identical to existing compounds, which have been safely consumed and for which adequate data exists, the safety assessment can be done by ‘bridging’, that is, by citing existing studies and explaining their relevance in the assessment of the substance that is contained in the GM crop.

The novelty of a protein or a secondary metabolite and its structure and function will determine the need for more rigorous toxicity testing programmes. The choice and design of toxicological tests must be proportionate to the nature of the substance under investigation. Different *in vitro* and *in vivo* test systems are used, often applied sequentially and in combination in order to elucidate the potential of compounds to cause harm as evidenced from the analysis of a broad range of biological and toxicological endpoints (see Section 2 of this paper, and Barlow et al., 2002). Chronic toxicity, carcinogenicity, and reproduction studies can be used to detect long-term effects, where applicable. Acute animal toxicity studies with the purified test substance are routinely conducted. Acutely toxic proteins typically elicit their adverse effects almost immediately upon consumption of relatively high dose levels of the protein (Sjoberg et al., 1992; FAO, 1995; FAO/WHO, 2000). A single dose oral toxicity study with proteins, however, may not identify potential adverse effects that might only appear upon repeated dosing. No dietary proteins are known to date to be directly associated with teratogenic, mutagenic, or carcinogenic effects in animal models (Pariza and Foster, 1983; Pariza and Johnson, 2001).

The safety assessment of novel recombinant proteins therefore needs careful consideration based on a protein’s prevalence and similarity to proteins that are routinely consumed by humans. The guidelines on data requirements for testing genetically modified organisms of the European Commission’s Scientific Steering Committee state that “in the case of novel proteins with insufficient database, and in particular if the available data suggest the existence of any cause for concern, a repeated dose study should be performed using laboratory animals” (European Commission, 2003c).

4.3.4. Assessment of allergenicity

Food allergy is an important health issue, which is caused by certain food proteins, including proteins derived from plants. Sensitisation develops when a susceptible individual is exposed to an amount of protein sufficient to induce an immune response. If the now sensitised individual is exposed subsequently to the same allergenic protein, then an adverse reaction may be provoked. Such reactions may be mild and local or severe, systemic, and fatal. There is therefore a need to

ensure that the products of novel genes introduced into GM crop 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.

Key considerations for the assessment of allergenicity of foods derived from GM crops are as follows. 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)?

The first systematic approach to the assessment of the sensitising activity of novel gene products was a decision tree jointly prepared by ILSI and the International Food Biotechnology Council (IFBC). Central to this approach was assessment of whether the protein of interest was the product of a gene from a source known to be allergenic (Metcalf et al., 1996). In case genes were derived from an allergenic source, several *in vitro* and *in vivo* tests, using allergenic patients and/or their serum, were suggested. In cases where genes were not derived from allergenic sources, a hierarchical testing cascade was proposed that classified proteins according to whether there was primary amino acid sequence similarity between the introduced protein and known protein allergens, and according to their stability in a simulated gastric fluid and under food processing conditions (pH). The joint Food and Agriculture Organisation and World Health Organisation (FAO/WHO) Expert Consultation further developed this decision tree (FAO/WHO, 2001) and proposed to include structural similarity with known allergenic proteins, a reduction of the contiguous amino acid sequence similarity from eight to six amino acids, additional serum screens (targeted serum screens to study cross-reactivity), and the inclusion of animal models. The Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology agreed on the elements which should be assessed with respect to allergenicity. They proposed an approach based on weight of evidence, rather than a decision tree approach. On the question of epitope screening of data bases, the Task Force indicated that that ‘the smaller the peptide used in the comparison, the larger the likelihood of false positives, inversely, the larger the peptide sequence used, the greater the likelihood of false negatives’. The Task Force also proposed to further develop and evaluate methods of targeted serum screening (i.e. the assessment of cross-reactivity of allergens through IgE binding assays), the establishment of international serum banks, and the examination

of introduced recombinant proteins for T-cell epitopes and structural motifs associated with allergens (Codex Alimentarius Commission, 2003c).

If the protein is derived from a source that is associated with human allergic disease, it is necessary to determine whether that protein in itself is a cause of sensitisation. Specific serum screening can determine whether IgE antibodies that recognize the protein of interest are detectable in the serum of individuals sensitised to the source material. In some circumstances, even if the source material is not implicated in human allergic disease, it may be prudent to determine whether individuals sensitised to related organisms have IgE antibodies cross-reactive with the protein of interest (targeted serum screening). However, no serum bank exists that can supply well documented sera from allergic patients and the positive predictive ability of the targeted serum screening is not yet known.

In order to determine whether the recombinant protein shares any amino acid sequence similarity with known food allergens, various publicly available databases (King et al., 1994; Table 1) may be used 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, 2003c). There is currently some debate on whether identity of 6 or 8 con-

tiguous amino acids between the novel gene product and a known allergen should signal a potential concern. Available data suggest that classifying as potential allergens proteins that have a linear sequence homology with a known allergen of 6 contiguous amino acids, as proposed by the FAO/WHO Expert Consultation (FAO/WHO, 2001), would result in a great number of false positive predictions. A contiguous 8 amino acid search is probably more effective in order to detect immunogenic epitopes (ILSI HESI, 2001; Hileman et al., 2002). Amino acid sequence searches can, however, not identify discontinuous or conformational allergenic epitopes that depend upon the tertiary structure of the protein (Metcalf et al., 1996).

Astwood et al. demonstrated a correlation between the resistance of proteins to proteolytic digestion in a simulated gastric fluid and their allergenic potential (Astwood et al., 1996). Such associations are not absolute. Recent work comparing the digestibility showed that food allergens are not necessarily more stable to in vitro digestion than non-allergenic proteins and that there does not seem to be a correlation between the digestibility of a protein measured in vitro and its allergenic potential. Many proteins with unproven allergenicity exhibit high stability (Fu et al., 2002). Nevertheless, examination of resistance to proteolysis in simulated gastric fluid, or by pepsin, is still considered useful information as part of the weight of evidence. Current strategies for the assessment of potential allergenicity of GM crops have proven successful for the identification of potential allergens (Nordlee et al., 1996) and has led

Table 1
Allergen databases on the Internet^a

Name	Web address	Allergen type	Information
Agmobiol	http://ambl.lsc.pku.edu.cn	Food, pollen	Protein databank accessions Literature references
CSL	http://www.csl.gov.uk/allergen (free registration required)	All	Protein databank accessions Epitopes Literature references
Farrp	http://www.farrp.org/ (free registration required)	All	Protein databank accessions of 658 allergens
NCFST	http://www.iit.edu/~sgendel/fa.htm	All, plus celiac	Protein databank accessions
Protall	http://www.ifr.bbsrc.ac.uk/protall	Plant	Protein databank accessions Biochemical and clinical data
SDAP	http://129.109.73.75/SDAP/	All	Protein databank accessions Allergenic protein sequences Search facility for identical and similar peptide sequences
SwissProt	http://us.expasy.org/cgi-bin/lists?allergen.txt	All	Protein databank accessions
WHO/IUIS	http://www.allergen.org	All	Nomenclature Protein databank accessions
Allergome	http://www.allergome.org	All	Protein databank accessions
Internet Symposium on Food Allergens	http://www.food-allergens.de (subscription required)	Food	Allergen data collections Articles

^a Adapted from Kleter and Peijnenburg (2002).

to discontinuation of the development of product concepts that might involve transfer of potential allergens.

There has been considerable interest in developing appropriate animal models that will provide additional assurance with respect to the ability of a novel protein to cause allergic sensitisation. Various approaches have been proposed, although it must be recognised that none of these has yet been evaluated fully or validated. The most promising methods are based on assessment of immune responses induced in experimental animals (usually rodents) following exposure to the protein of interest. The important measurement is whether the test protein can provoke the production of IgE antibody. This is further discussed in [Section 5.2](#) of this paper.

Exposure to novel proteins, a key step in the assessment of risk of allergenicity is considered in more detail in [Section 4.5](#).

4.4. Information on the whole GM crop

The overall objective of studies on the whole GM crop is to determine whether the GM crop or derived food is ‘as safe as’ its traditional counterpart(s). The assessment of the whole GM crop or food derived from it then serves to confirm the food safety of the intended changes and that there are no unintended and unexpected effects from the genetic modification that potentially have adverse health impacts.

Different mechanisms have been proposed that could result in unintended changes in the levels of metabolites and thus in the chemical composition of a GM plant, regardless whether the modification resulted from genetic engineering or conventional breeding ([Koschätzky and Maßfeller, 1994](#)). These include increased biosynthesis of metabolite(s) through altered enzymatic activity or concentrations of substrates or altered levels of biosynthetic enzymes, decreased synthesis of catabolic enzymes via gene deactivation/silencing, or reduced decomposition of a substance.

The concept of substantial equivalence guides the comparison of the GM crop to its counterpart, focusing on parameters such as agronomic performance, phenotype, compositional, and nutritional value that are

deemed to be indicative of any further differences that might have implications for human health. The comparison is typically made to the parental line and/or other edible lines of the same species. In addition, it can build on a comparison of the derived food product (e.g. oil) with the analogous conventional food product. The data required to enable detailed comparison with the traditional counterpart(s) may come from a variety of sources, in the first place from field trials with GM crops grown side by side with conventional crops under a diversity of environmental conditions representative of those typical for planned commercial growing. Furthermore, existing food compositional databases, chemical analyses, results from animal feeding studies, and toxicology data are potential sources of information.

To structure the questions to be asked and experiments to be performed an iterative approach is recommended to characterise the safety properties of the new GM crop/food ([Fig. 6](#)).

4.4.1. Identity, phenotypic, and agronomic analysis

Plant breeders routinely assess phenotypic appearance and agronomic performance of new candidate varieties. Similar assessments are conducted for the GM crop. [Table 2](#) shows some of the parameters typically assessed for corn. These parameters are expected to be affected by perturbations in the crop’s metabolism and potential pleiotrophic effects from the genetic modification. GM crops must therefore be phenotypically similar to their traditional counterpart, unless they had been intentionally modified to differ from those, and match on stringent performance criteria, including those that are routinely employed by plant breeders. Differences that can be reproducibly confirmed in any parameter in comparison with the traditional counterpart warrant further investigation.

4.4.2. Compositional analysis

The compositional analysis serves to assess whether the GM crop, or a food product derived from it, has a similar composition of nutrients, toxins, allergens, and anti-nutrients compared with the parent, except for the intentional alterations that change relevant characteristics.



Fig. 6. Description of the new GM crop.

Table 2
Phenotypic and agronomic characteristics used to evaluate equivalence (corn example)

Morphological and agronomic characteristics	
Stand Establishment	Early plant vigour
Leaf orientation	Leaf colour
Plant Height	Root strength (lodging)
Silk date	Silk colour
^a Ear height	Ear shape
^a Ear tipfill	Tassel colour
Tassel size	Reaction to fungicides/herbicides
Dropped ^a ears	Late season staygreen/appearance
Stalk rating	Susceptibility to pathogens/pests
Above ^a ear intactness	Yield

^a Ear, the corn cob per se.

The compositional analysis of the GM plant or the derived food should thus encompass the following elements: the proximate analysis of macro-nutrients such as protein and amino acid profile, fat and fatty acid profile, carbohydrates (fibre, starch), ash, and moisture; the analysis of micro-nutrients; and the analysis of inherent toxins, allergens, anti-nutrients, and bioactive substances (see Section 4.1.5). Their selection for analysis may be influenced by knowledge of the functionality of the inserted gene(s). For specific crop plants, lists with recommended parameters for compositional analysis are being developed (Nordic Council of Ministers, 1998; OECD, 2001a,b, 2002a,b,c).

The compositional analysis aims to assess whether the genetic modification has decreased the level of key nutrients or increased the levels of endogenous allergens, anti-nutrients, food toxins, or other biologically active substances present in the traditional cultivar.

Different consumption patterns and dietary practices across cultures need to be considered in the definition of key nutrients and anti-nutrients in a crop. This requires access to standard food consumption data for all regions in which the crop is to be commercialised. Additionally, it is necessary to consider potentially sensitive groups such as infants or nursing mothers (COMA, 1996). Another purpose of the compositional analysis and establishment of key nutrients and anti-nutrients in a crop is to guide the design of the animal diet for an in vivo safety assessment of the whole food derived from genetically modified crops (see Section 4.4.3).

It is essential to have a full understanding of the typical content of endogenous plant substances and their natural variation in the isogenic line across different geographies before commencing the safety assessment. This provides the baseline and range, effectively the 'control' against which the GM variety may then be compared, and any differences detected, measured, and evaluated for biological significance, become an essential part of the hazard characterisation process. The analytical methods applied should of course be stan-

dardised and validated to ensure the quality, consistency, and statistical confidence of the data.

Compositional analysis should be performed on samples of the GM crop and the comparator grown side-by-side in a variety of geographies and field trial locations. Typically, four sites with four replicates per site are employed over two growing seasons to assess the natural variability resulting from different abiotic as well as biotic factors, such as disease burden. Measurements from such samples are then compared to information on the natural range of variation of individual parameters for related varieties in the literature or databases. If significant differences are observed in ranges found in conventional crops, beyond natural variability, the deviations become the focus of further investigation.

4.4.3. Safety and nutritional analysis and the use of animal test methods

The purpose of the safety assessments of GM crops is to compare the overall safety of genetically modified plants with the safety of the traditionally bred food plants. Thus the task is to establish whether the food derived from a GM crop is *as safe and nutritious as* its conventional counterpart based upon its predicted usage.

Animal tests of whole foods can present challenges because of the need to prevent dietary imbalance associated with administration of large quantities of test diets with specific whole foods and low margins of safety compared with those that may be achieved with single chemical substances (see Section 3.3). If the composition of a GM food crop is modified substantially or if there are any uncertainties on the equivalence of its composition to a traditional counterpart, the whole food derived from a GM crop should be tested. For this purpose a dietary sub-chronic rat study is recommended as an appropriate study to demonstrate the overall safety of the food (FAO/WHO, 2000; see also Section 2). Sub-chronic dietary studies with rats serve as an indicator that there are no unintended changes in foods derived from GM crops that might render it less safe than the comparator.

The design of a sub-chronic rat feeding study should in principle follow the OECD guidelines for rodent feeding studies regarding the parameters to measure (OECD, 1993b). The study should typically be preceded by a pilot study to ensure palatability of the diet including the traditional non-modified crop and to make sure that the dietary inclusion levels are not likely to interfere with the interpretation of the results. The highest dose level used both for the parental crop and for the GM crop should be the maximum achievable without causing nutritional imbalance, whilst the lowest level should be approximated to the anticipated human intake. The diets used in these studies, as with all dietary studies, require careful attention to ensure

stability following dietary inclusion as well as nutritional equivalence between control diet and all the test diets used.

Uncertainties on potential adverse effects of inherent toxins and anti-nutrients in the extrapolation of results from animal tests to humans are not ordinarily of concern as long as the food derived from the GM crop is used in the same way as its conventional counterpart that is considered safe to eat. The utility of such a comparative approach depends upon the existence of reliable, comprehensive databases of the levels of toxins and anti-nutrients that occur naturally in commonly consumed foods (Duke, 1977; IFBC, 1990; Kessler et al., 1992; Pariza, 1996; Harborne and Baxter, 1996; OECD, 2001a,b, 2002a,b,c).

Animal feed performance (nutritional) studies in species other than rats and mice can in some cases also contribute to the overall judgement that GM crops are as safe for consumption as the chosen comparator. The rationale for this approach is to identify unintended nutritional effects during repeat dose dietary feeding studies (duration ranges from 42 to 120 days). An extremely sensitive study protocol to assess negative health impact due to nutritional or other factors is the 42 day broiler chicken study, in which one-day old chicks weighing as little as 35 g are grown to marketable weights of ~2.2 kg (Sidhu et al., 2000; Brake and Vlachos, 1998). Sensitivity to altered nutritional or toxicological properties of GM crop derived feed is known to be greatest under conditions of very rapid growth. In addition, very high dietary incorporation rates (up to 70% of the total diet) of the test material (e.g. corn feed) may be achieved in broiler studies. Endpoint measurements for animal nutrition studies also include quality measures, which relate to the economic value of the resulting farm produce. These measures include, in the case of broiler chicken studies, proximate analysis of meat, yield, and chill weight, as well as fat pad, wing, thigh, and drum weight.

A broad array of broiler, dairy cattle, beef cattle, sheep, and swine studies have been performed with no biologically or economically relevant differences observed between feeds derived from specific GM crops in comparison to feeds derived from traditional crop counterparts (Clark and Ipharraguerre, 2001). In addition, while animal nutrition studies are not generally viewed as an essential, sensitive, and specific element of the safety assessment of food, feed, or processed fractions derived from genetically modified crops, they provide significant further evidence of tolerance that can be taken into account in the overall assessment of safety.

The need for further toxicity tests should be considered on a case-by-case basis taking into account the observed toxicological profile of the substance/food being studied. This paper's recommendations on the conduct of animal toxicity testing with whole crops

include that protocols for animal feeding trials should be further standardised across individual laboratories conducting such tests. In particular, the design and preparation of the test and control diets, and the amount and duration over which they are administered would benefit from standardisation, to allow for better comparison of results from feeding trials in different laboratories. Furthermore, animal studies for the safety assessment of foods/food components are usually performed in rodents; studies in young fast-growing animals such as broilers should, however, also be considered for routine investigations of potential effects of whole foods on the growth rate of individuals. The use of broilers is not intended as a replacement, but rather to complement classical toxicological studies in rodents and other routinely used laboratory animals.

It is expected that advances in molecular biology, biochemistry, and methodological development, as described in Section 5, will further our understanding of animal and human dietary requirements; enhance the value of the information gained from animal trials; and help to further improve the sensitivity of various animal test methods. Working towards continued improvement of our effectiveness in gaining salient information on food safety from animal studies, aiming at minimising numbers of animals used and test time, is also imperative from an ethical perspective.

Fig. 7 provides an overview on how to aggregate information on the GM crops to a systematic *hazard identification and hazard characterisation* of food derived from a GM crop.

4.5. Exposure assessment

Risk is a function of the type of hazard and the levels and frequency of exposure. The primary objective of a food exposure assessment is to estimate the aggregate intake levels of that particular food or food constituent. This includes the determination of estimated daily intake (EDI) and the theoretical maximum daily intake (TMDI) per capita. Approaches to estimating exposure to GM crops depend on the introduced trait: some traits, such as improved agronomic characteristics may not alter a crop species' consumption patterns, if the GM crop partially or wholly replaces the traditional counterpart; other traits, such as the nutritional enhancement of crops, may change the overall dietary intake levels of the crop in a given population, as the food has another, or an additional function. As adoption of GM crops is far from uniform, fractions of GM crop contents in commodities may vary considerably across regions and countries.

Exposure assessments of specific foods often lack precision because of wide inter-individual variation in food consumption within and across different populations. In consequence, it is important to gather infor-

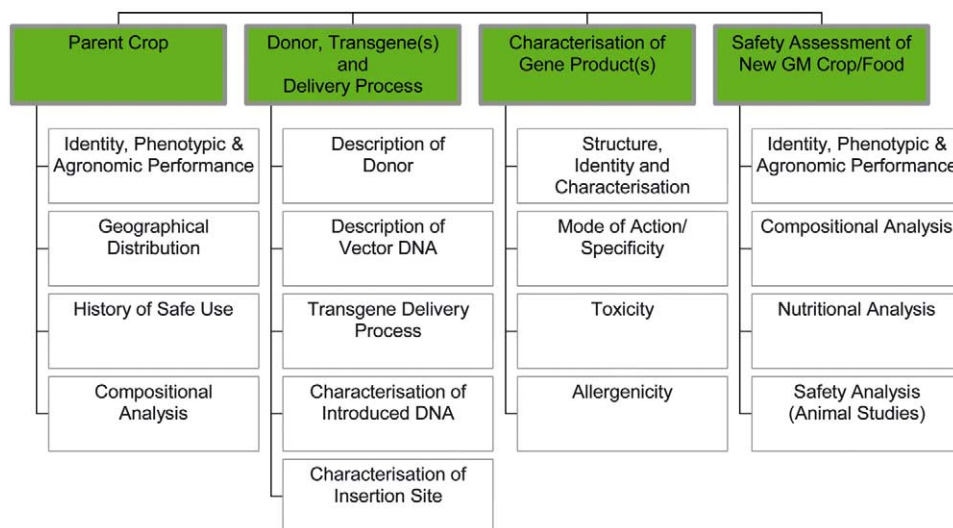


Fig. 7. A fully integrated approach to the hazard assessment and characterisation of all elements involved in producing a new GM variety.

mation on food consumption both at the population level and at the per capita level (WHO, 1999). Such estimates should take into account variation amongst demographic subgroups of a population; for purposes of dietary intake assessments of specific foods, populations are often stratified according to age, gender, socio-economic status, location, and ethnic origin.

Furthermore, specific subgroups of a population might be more sensitive to a potential hazard associated with a food. More susceptible subgroups of a population to certain risks often include infants, nursing mothers, and possibly the elderly. If there are significant uncertainties in exposure assessments, it is common practice to resort to 'worst case' scenarios, assuming maximum intake levels across the population; available information can then be used to refine the worst case estimates, and to create alternative scenarios for exposure assessments. This section provides an overview on how to compile and interpret data for exposure assessments to food crops and food constituents required for safety assessments of GM crops.

4.5.1. Data sources used to estimate food consumption

Several complementary sources of information for food exposure assessments have been developed in Europe and North America. Principally, information on the levels of consumption of specific food crops is derived from three types of sources: food supply data, generally referred to as food balance sheets; data on household expenditure; and results from food consumption surveys of individual consumers. Food balance sheets provide an approximate estimate of consumption across a population; information on household expenditure and from surveys of individuals provides complementary data with more information on the range of variation in consumption levels amongst individuals. As assessments of exposures to GM crops need to consider that some

products may be imported, but not cultivated in some countries, information from import statistics (see for example Eurostat [2002]) may therefore be required to estimate the proportion of a GM crop in relation to the traditional counterpart.

Food balance sheets and household expenditure surveys provide information on both food availability and consumption data. Food balance sheets compiled by intergovernmental organisations, such as the FAO Food Balance Sheets (FAO, 2002), WHO documentation on GEMS/FOOD Regional Diets (Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme; WHO, 1998), and DAFNE (Data Food Networking; DAFNE, 2003) are relatively imprecise and are known to overestimate actual consumption. They are, however, very useful for comparisons of consumption patterns of specific foods across countries (WHO, 1998). Descriptions and classification systems for foods vary amongst different international repositories; the FAO Food balance sheets and the WHO GEMS/Food Regional Diets, for instance, describe food at the commodity level, whereas the DAFNE classification system categorises food items at the raw ingredient level.

National repositories are largely based on consumer surveys (Verger et al., 2002). Data obtained from national surveys generally only focus on actual consumption. Such national repositories include, in the United Kingdom, the Dietary and Nutritional Survey of British Adults (Gregory et al., 1990) and the National Diet and Nutrition Survey of Children (Gregory and Lowe, 2000), and, in the United States, the National Health and Nutrition Examination Survey (NCHS, 2001).

The development of data repositories and methods that allow comparison of nutrient intake levels across populations are also desirable. It has been recognised that comparisons of food intakes across European

countries using national repositories are facilitated if data for food ingredients was also solicited in surveys. In the European Prospective Investigation into Cancer and Nutrition (EPIC), for example, country-specific questionnaires were employed to collect information on individual dietary intakes of food ingredients. An additional interesting part of the EPIC study involves the compilation of a European Nutrient Database (ENDB) and software to evaluate and catalogue survey results. National data sets are chosen as inputs for the ENDB; the Eurofoods Recommendations for Data Interchange guide the design of the database (Schlotke et al., 2000; Ireland et al., 2002). The Euro Food Group (EFG) is drawing up a food classification system and food compositional databases with a focus on food ingredients in raw foods using the software developed under EPIC (Slimani et al., 2000; Slimani and Valsta, 2002) for a list of 33 food groups. An appreciable amount of work remains to be done, especially in the vegetable, semi-manufactured, and processed food categories prior to system launch (Ireland et al., 2002; Verger et al., 2002).

4.5.2. Calculating the exposure to a new or altered level of a food constituent

Combining estimates of the daily per capita intake of a specific food and data on the levels of recombinant proteins and/or novel metabolites in that crop allows calculation of the estimated daily intake of the new intended plant constituent(s). Food crop intakes are estimated using databases described above in Section 4.5.1.

Typically, newly introduced proteins are expressed in the range of 0.01–0.1 per cent of the total protein content of a GM crop (Betz et al., 2000). Taking the dietary consumption figures into account together with the percentage GM derived material being consumed, based on grain importation and usage statistics, estimated human daily intake figures are often in the range of 0.017 mg/kg/day to 0.07 mg/kg/day. Data on NOAELs from single dose or longer term studies in laboratory animals, or from human experience, can then indicate the approximate safety factors. Based on published acute toxicity data on a range of proteins introduced into commercial crops, where the lowest NOAEL is >100 mg/kg/day (this corresponds to the maximum level that could be fed to the animal, at which still no adverse effects were observed) there is often a minimum 5 million-fold margin of safety over the intake a human would be expected to consume in a 24 h period (Chassy, 2002). For new or increased levels of metabolites such as pro-vitamin A it is straightforward to compare the daily intake with the recommended daily allowance (RDA) to show whether the dietary exposure and hence intake remains within the RDA.

The NOAEL observed in animal tests can also be a starting point for calculations on margins of safety for both the gene product and whole-food. This approach

does not only take into account the presence in GM crops of new trait(s) but also any other possible consequences of the transformation. For instance, a 90 day feeding trial with rats fed tomatoes containing the insecticidal protein CRYIA(b) did not show adverse effects compared to animals receiving a diet containing unmodified tomatoes. The average daily intake of tomato powder corresponded to 200 g/kg body weight/day, equivalent to a human consumption of 13 kg tomatoes (Noteborn et al., 1995). Calculating margins of safety from a 90 day animal feeding study with a NOAEL divided by the EU anticipated mean per capita dietary intake by adults and toddlers affords in general a typical margin of safety (MOS) of greater than 100 fold. Appropriate information on anticipated exposures for a range of different demographic groups, which may be coupled with calculations on margins of safety, provides helpful information with which to progress to the overarching safety assessment.

The overall assessment of the nutritional implications of a novel food should consider any impact at expected customary (normal) dietary intakes and at maximum levels of consumption. This is of particular relevance in the case of nutritionally enhanced foods derived from GM crops. In this context, it is necessary to refer to the appropriate human nutritional and dietary intake data for different substances and foodstuffs. In the case of such products attention should also be paid to the particular physiological characteristics and metabolic requirements of potentially sensitive groups such as infants, children, pregnant and lactating women, the elderly, and those with chronic diseases (European Commission, 1997b).

Precise exposure assessments to food constituents in GM crops may, however, be complicated for three reasons: first, the GM crop may only be a small fraction of the total commingled seed; secondly, food ingredients derived from commodity crops such as corn and soybean enter a very wide variety of products in the food chain requiring aggregate assessments of routes of exposures through various food products to obtain individual consumption estimates; thirdly, food products are often processed into ingredients and/or incorporated in formulated processed food products; processing operations using heat or wet- or dry-milling can degrade and denature proteins, and degrade or inactivate metabolites. Whilst obtaining the highest level of safety assurance, any loss or degradation through these reasons is often not considered, resulting in overestimated exposure levels.

4.6. Considerations for GM crops with complex modifications

The preceding section sets out a systematic stepwise approach on how to tailor combinations of test methods

to assess the safety of foods derived from a specific GM crop, focusing on the characteristics of the modified crop species and the new trait. The safety assessment strategy for foods derived from GM crops as described here is robust, offers a high level of safety assurance, and can be tailored to all crop-trait concepts, including future GM crops with more complex traits. With the chosen approach differences between the new food and its traditional counterpart are identified. Any identified differences are examined as to their potential impact on human and animal health. Assessments are conducted on a case-by-case basis. Combining the outcome of the hazard characterisation with exposure level and frequency, the actual *risk* may be estimated that describes the probability of harm occurring under anticipated conditions of use of the product (See Fig. 8). Risk characterisation then combines information about the probable extent, nature, and duration of exposure with considerations of hazard characteristics and relevance of those hazards for humans into an integrated view of the likely risk to human health. The risk assessment of GM crops makes relative statements on whether the GM crop is as safe as the comparator that is generally accepted as safe. Any uncertainties inherent in the risk assessment will be highlighted. This information then constitutes the basis for a determination of a safe intake level of the new food. If expected exposure exceeds that level, risk mitigation measures should be considered,

possibly in the form of conditional regulatory decisions. The assessment also serves to identify whether specific risk management measures may be required when a GM crop is commercialised ([Codex Alimentarius Commission, 2003a](#)).

The safety assessment of GM crops that are designed to be compositionally different, including nutritionally enhanced crops, needs to pay particular attention to the choice of an appropriate comparator for the safety determination, and to estimating the anticipated exposure. The safety assessment of these foods derived from GM crops is based on the comparison of the new or increased components in the GM crop with the composition of the traditional food it is intended to substitute.

Examples of such crops that are currently under regulatory review include GM canola that contains high levels of lauric acid (C12:0), a fatty acid not normally found in canola oil. The product was developed as a substitute for tropical oils in certain food applications ([FAO/WHO, 1996](#)). Another example is a soybean oil developed to have high levels of oleic acid (C18:1) instead of linoleic acid (C18:2 n-6) ([OECD, 1998](#); [Health Canada, 2000](#)). In these cases, not the parent crop, but rather the food product for which the GM crop derived food will be the substitute is the appropriate comparator. Regarding exposure assessment, in the case of high-laurate canola oil, it has been estimated that the total intake of lauric acid in the diet would not

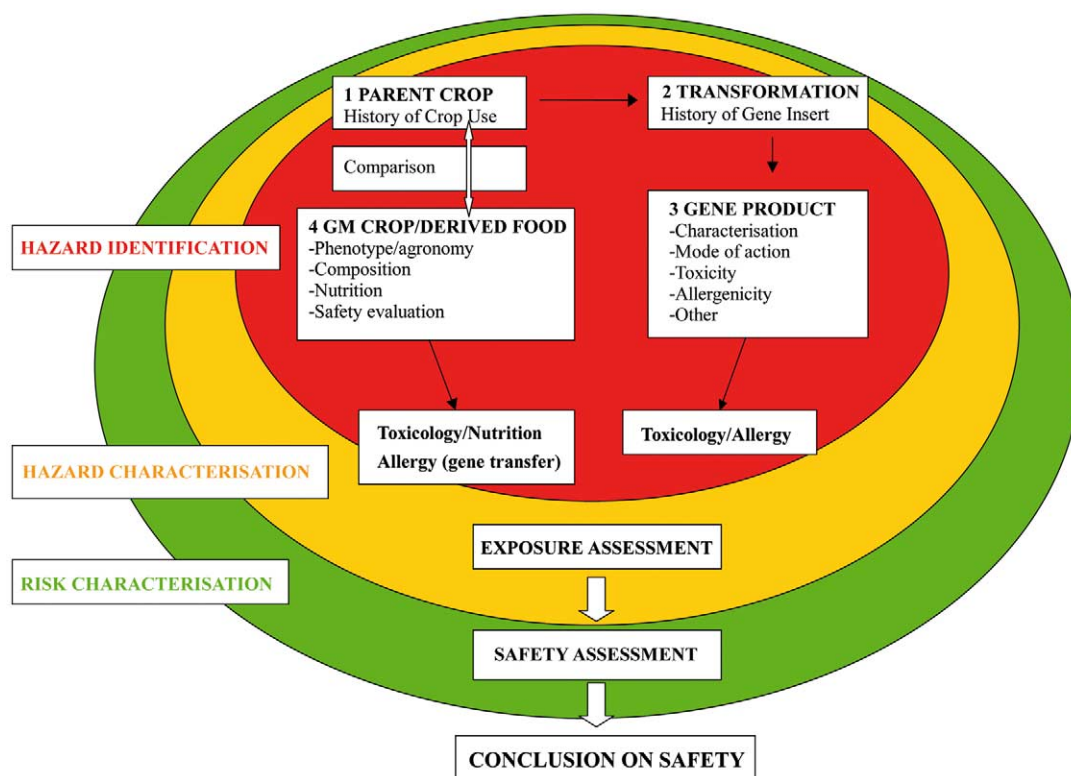


Fig. 8. Safety assessment approach for GM crops and derived food or feed.

change significantly by the substitution of this product for the conventionally used tropical oils in the anticipated food applications (Voelker, 1997).

An important element in the testing of foods derived from GM crops with nutritional benefits is toxicological and nutritional testing in animals, and in particular, the optimised and balanced composition of the animal diet. Most ordinary animal diets used for conventional toxicological studies on defined chemical substances contain a *surplus* of macro- and micro-nutrients, which normally do not disturb the outcome of the toxicity testing, because there is no overlap between the mechanisms and endpoints of toxicity and of nutritional impact. Rich diets may however mask the toxicity of the chemical. Therefore a basic rodent diet used for the testing of GM plant foods with enhanced nutritional or health benefits will need to be a *de minimis* diet, just maintaining normal growth, development, and well-being in the young growing rats. If the GM plant food replacing part of the basic diet does not disturb the delicate balance for normal growth and development and does not change the biochemical and pathological parameters normally measured, it can be considered nutritionally neutral.

For certain novel foods, including specific foods derived from future GM crops where the genetic modification was aimed at major changes of functional properties or the consumption pattern, systematic post-market monitoring might be considered in order to complement the safety assessment programme undertaken before marketing (Wal et al., 2003). International guidelines also suggest that post-market monitoring of certain foods derived from GM crops might be considered as an appropriate risk management measure in specific circumstances (Codex Alimentarius Commission, 2003a). This approach may be chosen to address the following questions: (i) is the product used as predicted/recommended, (ii) are known effects and recognised side-effects as predicted, and (iii) does the product induce unknown or unexpected side effects.

The value of a post-market monitoring of foods derived from GM crops depends on several factors, similar to those described for post-market monitoring of all foods, above: Relevant data may be obtained in the case of monitoring of a branded product, in which the product was effectively the sole route of intake of the ingredient of interest. Strategies with respect to detection and traceability of GM foods are discussed in the paper by Miraglia et al. (2004). Post-market monitoring might be of little value if the material to be monitored was a commodity type food used in a wide variety of products, the consumption of which would not be mutually exclusive. Intakes of the same food component from different sources may be difficult to deal with, as each company can only monitor its own products.

Monitoring of adverse effects will be more successful for certain types of effect than others. Ideally, the effect to be monitored should be manifested early and with clear symptoms (e.g. an allergic reaction) and therefore readily associated with consumption of the food of interest. Conversely, monitoring for longer-term effects poses major problems.

Other considerations for the safety assessment of GM crops with more complex compositional modifications are discussed in Section 5.

5. Future developments in the safety assessment of foods

Existing methods are deemed adequate for the safety assessment of foods derived of the GM crops that are cultivated now; this has been borne out by regulatory bodies, all supported by expert advice, and in guidelines on food safety assessment and biotechnology elaborated by national and intergovernmental organisations. With the rapidly expanding understanding of the molecular biology of food crops and how they interact with their environment, opportunities arise to engineer crops with more complex traits that will contribute to improving public health and natural resource management in agri-food production; such traits include enhanced nutritional value and tolerance to abiotic stresses. Science policy should ensure that advances in molecular biology, biochemistry, and nutrition not only serve as a basis to facilitate the development of new crop varieties with new traits, but also their safety assessment.

This chapter attempts to predict how progress in genomic research, the development of new transformation methods, and animal and cell-based methods, will contribute to the design of more refined approaches for the safety and nutritional assessment of foods derived from GM crops. The three sections will describe in more detail: (1) advances in molecular biology and improvements of the characterisation of plant derived foods and their health effects, and the transformation process; (2) developments of new *in vitro* and *in vivo* methods for the allergenicity assessment of the gene products; and (3) contributions of genomics towards more informative and effective combinations of nutritional and toxicological tests.

5.1. Advances in molecular biology

Genomic research adds a new dimension to our understanding of biology and provides powerful new tools to study induced changes in gene expression. Genomic sciences will also contribute to the characterisation of plants and other organisms that may be sources of useful novel traits, and, in fact, lead to the identification of new traits. Concomitantly, the tools for

the transfer of genes into plants are also being improved. Implications of these developments for the characterisation of the parent crop and the transformation event, and improvements of transformation technologies that will facilitate the safety assessment of GM crops by minimizing the introduced recombinant DNA sequences are explored in more detail below.

5.1.1. Characterisation of the parent crop

Results from large scale sequencing projects are rapidly increasing our understanding of plant genomes, and of their evolution, regulation, and plasticity. The recent completion of the first draft sequences of the rice genome (Yu et al., 2002; Goff et al., 2002) and the availability of the *Arabidopsis* sequence information now allow whole genome comparisons between monocots and dicots (Riechmann et al., 2000). More than 80% of the genes that were annotated in *Arabidopsis* were also found in rice (Benetzen, 2002). Apart from elucidating differences in monocots and dicots, and evolutionary biology, reverse genetics provides important information about the functions of individual genes. The establishment of international systems for improved access to rapidly evolving crop genome databases and latest bio-informatics methods in order to facilitate and harmonise the future analysis of such data is key.

5.1.2. Characterisation of the transformation event

The availability of increasing amounts of plant genomic sequences and the development of cheaper and quicker sequencing methods will facilitate the molecular characterisation of the introduced DNA and the characterisation of the insertion site.

Requirements for the molecular characterisation of a GM crop include provision of a map of inserted recombinant sequences; sequence information of the inserted genetic elements; and sequence information of the sequence that bridges the introduced DNA and the plant genome (see Section 4.2). The molecular characterisation methods of Southern and Northern blotting as well as reverse transcriptase polymerase chain reaction and DNA sequencing, used currently, already provide fair predictions on the introduced recombinant DNA, open reading frames, and the possible presence of fusion proteins. As plant genome cloning and sequencing methods become increasingly automated, and thereby cheaper and quicker, sequence information of the introduced recombinant DNA and its genomic flanking regions may be obtained more effectively on a routine basis. This will also further simplify the prediction of unintended fusion proteins resulting from genetic rearrangements that link promoters and gene fragments of different genes. The future combination of improved and more effective sequencing methods, access to plant genome sequence information, and bio-

informatics methods will also facilitate prediction of whether important plant endogenous genes might have been disrupted through the genetic insertion event, and to anticipate putative consequences of such disruptions.

Decisions on whether more detailed information on DNA sequence and gene expression levels should be required in routine risk assessments of GM plants are not straightforward. Taking into account that changes in plant gene expression levels are at least as likely to result from natural processes effecting genome rearrangements and the use of other (non GM) breeding technologies, and that very few such changes may adversely impact human health, raises the question whether such information should be obtained on a routine basis in food safety assessment in general.

5.1.3. Improved gene delivery methods and site-directed mutagenesis

One important criterion for the selection of plant lines for commercialisation from all transformed plant lines is the nature of the introduced recombinant DNA sequence. Simple single copy inserts with the gene of interest are preferred, as this facilitates the safety assessment as the molecular characterisation is more straightforward, and as chances of fusion proteins or unintended effects through the gene insertion are minimised. Further improvements in methods for crop transformation can potentially further simplify and reduce uncertainties in the safety assessment of GM crops if they reduce the amount of recombinant DNA and recombinant proteins introduced into the GM crop, and be beneficial for developers if they help to increase the proportion of transformants with simple single inserts. Multiple inserts both increase the risk of recombination events between repeated sequences, hence increasing the risk of unintended effects from the genetic modification, and result in gene silencing-mediated instability of the trait, which also raises uncertainties on the relevance of the data presented in the safety assessment. This is hence not only a product-performance related issue, but is of relevance in safety assessment. Several recent policy documents have advocated minimising recombinant DNA inserted in GM crops to facilitate the safety assessment (UK ACRE, 2001; European Commission, 2003c; FAO/WHO, 2000).

DNA transferred to GM crops can be reduced to a minimum by avoiding insertion of multiple copies of recombinant DNA sequences at multiple insertion sites and by avoiding use of or removing selectable marker genes. Improvements in methods for the delivery of recombinant DNA into plant cells, including *Agrobacterium*-mediated transformation, the use of particle guns, and more recently developed microinjection and plant mutagenesis methods, all of which enhance control and allow minimising transferred DNA, are described in turn.

Improvements in transformation methodology over the past two decades have resulted in greater control over the DNA sequences that are transferred to the plant genome. Particle gun-mediated transformation protocols were developed that, at least in some crop species, yield a higher proportion of GM crops with single simple inserts. This contrasts to the previous approach of transforming plants with circular plasmids that contained undesired DNA; moreover, use of circular plasmids yielded high numbers of transformants with multiple insertions at several sites in the plant genome. For the risk assessment, this is undesirable, as it renders the molecular characterisation of the recombinant DNA transferred to the GM crop more difficult, and as repeated sequences may result in recombination and DNA rearrangement at the insert, potentially giving rise to unintended effects that indirectly result from the genetic modification. Gene silencing effects that have been observed where there are several copies of the same recombinant promoter and/or termination signal can introduce uncertainty on stable gene expression.

In *Agrobacterium*-mediated transformation, the use of double border vectors, where the 'transfer DNA' that is targeted for insertion into the plant genome is flanked by two 'border sequences', allows the transfer of only the necessary genes that are required to express the desired trait and for the selection of transformed plant cells (Martineau et al., 1994). The system is imperfect, as the border sequences occasionally are overridden, resulting in the insertion of sequences outside these borders; it is, however, still considered more reliable and more controlled than gene delivery to plant cells using the particle gun. *Agrobacterium*-mediated transformation methods are being developed for use on cereals (see, for example, Chan et al., 1992). However, even with these improvements of plant cell transformation methods over time, the degree of control over the transformation process varies amongst crop species. In the transformation of potatoes and oil seed rape, for instance, large proportions of the transformed cells contain multiple inserts; it can therefore be difficult to obtain transformed cells with single inserts of the desired sequences only, in particular for those developers operating on a small scale.

A novel DNA microinjection method has been developed to introduce heterologous DNA sequences into single plant cells or organelles (Knoblauch et al., 1999). Using a newly developed miniaturised cell injection system, defined transgene expression cassettes composed of only the promoter, gene, and terminator sequences can be injected into plant cells. The technique, once developed for routine deployment in plants, will avoid the presence of marker genes, and will hopefully be optimised to produce crops with single inserts. The transformation efficiencies have to be considerably improved

before the method can be considered for routine use in commercial research and development activities.

New forms of oligo-nucleotides that are resistant to cellular digestion are being developed to obtain improved tools for the *in situ* mutagenesis of plant cells (Culver et al., 1999; Rice et al., 2001; Majumdar et al., 1998), whilst avoiding the introduction of new recombinant material and hence immensely facilitate the safety assessment by targeted modification of existing alleles. An imidazolinone-tolerant maize plant has been developed successfully using chimeric RNA/DNA nucleotides (Zhu et al., 2000). The method is however only applicable to engineer new traits that can be introduced through point mutations in endogenous plant genes.

5.1.4. Methods for elimination of selectable markers

In spite of foreseeable future improvements in methods for transgene delivery, transformation systems for most crops will still depend on the use of selectable markers for some years to come. Potential benefits from marker elimination include improved and simplified safety assessments through reducing recombinant sequences and gene products present in the transgenic crop that would need to be characterised. Other advantages of marker elimination in product development include the possibility of re-transforming transgenic crops using the same marker gene to insert additional traits. If multiple new traits are combined in one transgenic crop line using conventional breeding methods, the removal of marker genes reduces the risk of gene silencing that can occur due to the presence of several identical gene regulatory or coding sequences and may affect the stability of the expression of the trait (see, for example, Vance and Vaucheret, 2001). The avoidance of repeated recombinant DNA sequences also reduces the risks of unintended effects from recombination between such sequences. Methods that hold the most potential for allowing routine specific removal of DNA sequences, such as marker genes, from transgenic crops are homologous recombination, co-transformation, and recombinase-mediated excision (reviewed in König, 2003).

Homologous recombination relies on the occurrence of base pairing between identical sequences that are in close proximity during the DNA replication process. This can lead to the excision of DNA sequences that are located between the two repeated DNA sequences (Zubko et al., 2000; Peterhans et al., 1990; Reiss et al., 1996). At present recombination frequencies are, however, too low for the routine use of this method in product development (Zubko et al., 2000). Research on improved control over homologous recombination in plants may in the long term help the development of gene targeting techniques allowing greater control over the insertion locus and expression of recombinant

sequences. This may help to reduce uncertainties due to random insertion events, further simplifying the safety assessment of GM crops. Careful investigation of the stability of the remaining insert after recombination will, however, always be required in safety assessments of GM crops, and in particular those that may be developed relying on recombination, as high recombination frequencies may be indicative of transgene insertion in recombination hotspots or other less stable areas of chromosomes (Puchta, 2000).

Agrobacterium-mediated co-transformation may be used to obtain plant cells in which the gene(s) of interest and the marker gene integrate into unlinked genomic locations to allow segregation of the two insertions by breeding (Depicker et al., 1985; De Framond et al., 1986). A significant advantage of this method is that it provides marker-free transgenic crop for commercial cultivation, obviating the need to provide a food safety assessment of any selectable marker gene and gene product that might have been used in the transformation process. The method has already been deployed successfully in the development of commercial products. It is, however, much less effective as in experiments to date only a maximum of 25% of the transformation events contained single copies of both the marker and the gene of interest in separate genomic locations that were hence suitable for further development. Moreover, the method is patented and access to its use is therefore restricted, or comes at a cost. In addition the associated need for increased laboratory space and time can be prohibitive for use of the method in smaller laboratories.

Site-specific enzyme-mediated excision systems rely on a recombinase enzyme that specifically cuts DNA at two short parallel DNA recognition sites, and then reseals the two DNA strands after the intervening DNA between the two sites has been removed. In GM crops in which marker removal is foreseen through use of the recombinase-mediated excision, the respective gene needs to be flanked by the specific recombination target sequences. A subsequently introduced recombinase enzyme precisely excises the marker sequence from the genome. Recombination then results in marker-free plants (Kilby et al., 1995; Onouchi et al., 1991; Dale and Ow, 1991; Zuo et al., 2001). Use of the method requires licensing. No transgenic crop developed with this method has to date been approved for commercialisation, and no guidelines exist on what data are required for the risk assessment of such crops. Use of the method is unsuitable in transgenic crops with multiple inserts, as recombination might occur. The likelihood of unintended recombination in transgenic crops with single insertion events will need to be assessed through comprehensive study of the binding site-specificity of recombinases and the occurrence of potential recombinase-recognition sites in plant genomes. Such unintended rearrangements are, however, likely to occur at

only low frequency compared with natural mechanisms for rearrangements of plant genomes (König, 2000).

The use of automated high-throughput molecular characterisation technologies instead of selectable markers to identify genetically modified cells may in future also allow to phase out the use of selectable markers. As very high transformation efficiencies are required, this will, however, in the medium term only be feasible for two or three crop species that are already highly amenable to transformation such as potatoes and oil seed rape. Future developments in the areas of site-directed mutagenesis, microinjection, and high-throughput molecular characterisation technologies may in the long term (decade(s)) obviate the need for marker-based selection in some crops altogether.

5.2. Protein allergenicity: future targets

The overarching aim of research in this area is to improve our understanding of the cellular and molecular bases for the development of allergic sensitisation to proteins in the context of food safety, and the prospective identification and characterisation of potential protein allergens. This requires: (i) further elucidation of the relationships between protein structure and function and sensitising activity; (ii) improved understanding of the relationships that exist between the resistance of proteins to proteolytic digestion and sensitising potential; and (iii) the development, evaluation and validation of new in vitro methods and animal models that will provide a more direct and more holistic assessment of the inherent sensitising potential of proteins.

5.2.1. Relationships between protein structure and function and allergenic potential

The development of robust methods for identifying and characterising the potential of proteins to cause IgE-mediated allergic sensitisation would be facilitated greatly by a more detailed appreciation of the features that confer on proteins allergenic activity. Although certain physicochemical characteristics and functional properties are known to influence the ability of proteins to induce allergic sensitisation (Aalberse, 2000; Bredehorst and David, 2001; Bufe, 1998; Huby et al., 2000), a more detailed understanding is needed. Such would also provide the foundation for the definition of structure-activity relationships. In theory, this in turn might also permit consideration of designing out the allergenic properties of proteins. The requirement is, therefore, for research examining further the relationship between the biological, biochemical, and physicochemical properties of proteins and their sensitising activity. A concerted action programme (PROTALL) funded by the European Union (EU) Fifth Framework Programme has addressed some of these issues. The overall aim of this project was to determine whether, and to what extent,

relationships exist between the sensitising activity of plant proteins and their structural and functional properties. The successor programme in the EU Fifth Framework is INFORMALL (INFORMALL, 2003), the main aim of which is to enhance communication by provision of credible sources of information on food allergy for all stakeholders.

Many assays are performed using proteins expressed by *Escherichia coli* or *Pichia sp.* Bacteria- and yeast-derived proteins may differ from the product of the transgene expressed in the plant. Among the possible differences are folding and function, as well as post-translational modifications, such as glycosylation and phosphorylation. There is a need to understand in greater detail the ways in which such variables may affect the ability of a protein to induce allergic sensitisation to allow extrapolation between bacteria- and yeast-derived proteins and those expressed in the GM crop.

A component of protein safety assessment is consideration of whether the new protein of interest shares amino acid sequence homology with known protein allergens (Gendel, 1998a,b; Metcalfe et al., 1996). There is currently some debate on whether identity of 6 or 8 contiguous amino acids between the novel gene product and a known allergen should signal a potential concern. The Codex ad hoc Intergovernmental Task Force on Foods derived from Biotechnology recommended that “the size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false positive and false negative results” (Codex Alimentarius, 2003c, see also Section 4.3.4). It is clear that the approach currently is partly empirical and there is a need for a more detailed understanding of what degree of sequence similarity signals a likely hazard. Progress is being made in the design and application of novel bio-informatic and bio-computational approaches to align sequence data and protein folding with allergenic potential (Aalberse, 2000; De Groot et al., 2002; Kauppinen et al., 1999; Rost, 2001; Singh et al., 1999; Snow et al., 2002; Zorzet et al., 2002), and there is a need to exploit these opportunities fully.

The requirement is for continued research into the relationship between amino acid sequence identity, and overall structural homology, and sensitising properties. Moreover, the sequence and structural requirements for immunological cross-reactivity and particularly cross-allergenicity need further clarification. In this context there is some interest in defining the structural motifs of T and B lymphocyte epitopes on proteins. The challenge here is to identify epitopes that are associated with the initiation of allergic (IgE antibody) responses, and not of immunogenic (for instance, IgG antibody) responses per se (the vast majority of food proteins are foreign and therefore potentially immunogenic in man, but only

a fraction of these are associated with allergic sensitisation). The requirement is therefore to determine whether there are specific lymphocyte epitopes that are associated with an increased likelihood of allergic sensitisation, by for instance favouring the development of IgE antibody responses. The application of advanced bio-informatic methods will be necessary to meet this challenge.

5.2.2. Protein stability and allergenicity

Another strand of current safety assessments is determination of the stability (or otherwise) of proteins in a simulated gastric fluid (SGF) or their resistance to digestion by pepsin (Metcalfe et al., 1996). Recently, ILSI has sponsored an inter-laboratory evaluation of this method with a view to establishing a standard protocol for conduct of the assay.

Although a correlation between resistance to proteolytic digestion and allergenic potential has been proposed (Astwood et al., 1996), the association is not absolute (Fu et al., 2002). It cannot be concluded that allergenic food proteins are necessarily more resistant to proteolytic digestion, and certainly there are stable proteins that are not associated with sensitisation (and vice-versa). Presently, measurement of resistance to digestion in SGF cannot and should not be regarded as a stand-alone method for the safety assessment of novel proteins, as recognised by international Expert Consultations (e.g. FAO/WHO, 2001). Moreover, it has been reported that partial proteolytic digestion may increase IgE binding (Haddad et al., 1979) and that tryptic digestion may reveal one or more immunodominant linear IgE binding sites located within hydrophobic regions of the parent protein (Maynard et al., 1997; Spuergin et al., 1996). Sensitisation capacity of food proteins appears also to be influenced by exposure, or dose. Although thresholds of both sensitisation and elicitation are challenging to assess, recent studies describe progress in attempting to establish thresholds for elicitation of food allergic reactions that should be expanded upon (Taylor et al., 2002; Bindeslev-Jensen et al., 2002; Wensing et al., 2002a,b).

There is a need, therefore, for a more detailed understanding of the extent to which there exists a relationship between allergenic potential and resistance to proteolytic digestion and exposure. The requirement is for biochemical and immunobiological research focused on the way in which protein digestibility influences sensitising potential. This is one objective of an EU RTD research project (ALLERGEST) funded within the European Union Fifth Framework Programme (ALLERGEST, 2003). It is hoped that a greater understanding of the relationship between sensitising potential and resistance to proteolytic digestion, linked with an appreciation of the relevant physicochemical properties of sensitising proteins (see above), will provide a

more detailed definition of the characteristics of food allergens.

5.2.3. Animal models

There is a growing consensus that an approach to the safety assessment of food from GM crops will require the use of appropriate animal models for characterisation of allergenic potential (FAO/WHO, 2001; Kimber and Dearman, 2001). In recent years several models have been proposed and some of these show promise either as methods for safety assessment and/or for more mechanistic studies (Adel-Patient et al., 2000, 2003; Dearman et al., 2001; Dearman and Kimber, 2001; Ermel et al., 1997; Helm et al., 2002; Kimber and Dearman, 2002; Knippels et al., 1998; Penninks and Knippels, 2001). However, none has yet been formally evaluated or validated. The requirement is for the most promising and most appropriate animal models for allergenicity to be evaluated fully with a range of sensitising (weak and strong) and non-sensitising proteins so that their sensitivity and selectivity can be assessed.

At present animal models for predicting and characterising protein allergenicity are based upon assessment of induced antibody responses and/or the frequency of responders in the test groups. However, it should be possible soon to consider alternative or supplementary endpoints based on a more detailed understanding of the immunobiological basis for sensitisation and an appreciation of why proteins differ in their sensitising potential. There is a need also to define the ways in which the food matrix in which a protein allergen is encountered may impact upon sensitising potential. Research in these areas will undoubtedly be facilitated by the availability of microarray and proteomic technologies that should aid in the definition of appropriate markers. The requirement is for continued research into the immuno-biology of protein allergy with particular emphasis on the identification of molecular markers that can be used to distinguish protein allergens from non-sensitising proteins. The same technologies may be appropriate also for determining whether transformation has caused any unintended changes in the level of expression of allergenic proteins endogenous to the host plant.

5.2.4. Cell-based models

Finally, there is some interest in the development of cell-based methods for the *in vitro* assessment of sensitising properties. This is a very challenging objective. One approach that has attracted some attention is the use of mast cells, or mast cell lines, passively sensitised with antibody (Fritsche and Bonzon, 1990; Hoffmann et al., 1997). This approach may be of some value in examining serological cross-reactivity between proteins, but will not be relevant for determination of the sensitising potential of novel proteins.

5.3. Safety and nutritional testing of foods derived from GM crops

5.3.1. Identification and assessment of unintended effects

The need for development of more effective testing protocols to improve the assessment of the safety of whole foods has been highlighted by several expert groups, in particular for the assessment of GM crops with more complex modifications, such as complicated nutritional enhancements (NRC, 2000; Royal Society of Canada, 2001; Hollingworth et al., 2003). First, it has to be emphasised however, that the targeted testing approach described above in Section 4 remains the fundament to any test regime. Furthermore, traditional ADME (absorption, distribution, metabolism, and excretion) studies, with a focus on bioavailability (see Section 3.3) will play a greater role in the nutritional assessment and verification of benefits claims of nutritionally enhanced crops. Advanced analytical “profiling” methods are currently being developed, by which profiles are created of substances present in a sample in an indiscriminate manner (substances need not be identified on beforehand). Examples of such profiling methods include gene expression analysis by microarrays (functional genomics), protein analysis by two-dimensional gel electrophoresis followed by mass spectrometry (proteomics), and analysis of chemical compounds by, among others, liquid chromatography followed by nuclear magnetic resonance (metabolomics). The use of profiling methods in food safety assessment has also been suggested as an additional approach that might in future complement the targeted approach, for the detection of compositional differences between GM crops and parent crops that might not be detected using the targeted comparative approach (FAO/WHO, 2000; Kuiper, 2000; Kuiper et al., 2001; Cellini et al., 2004).

The use of cDNA micro array technology, proteomics, and the coupling of methods to analyse mixtures of chemical compounds, such as nuclear magnetic resonance spectrometry and chromatography, allows for a broad screening of possible changes in the physiology and metabolism of the modified host organism at different integration levels of cells or tissues in a non-selective, unbiased manner. The approaches that are being developed for profiling of food crops are potentially powerful, but much work has still to be done with respect to sampling and extraction procedures, standardisation and validation of methods and the generation of background data on natural variations in profile patterns within crop species. A serious limitation is the interpretation of potentially observed differences with respect to their biological relevance and toxicological significance. A better understanding of the natural variation of levels of compounds in crops needs to be developed. For this purpose, interconnected databases

containing gene transcript, protein, and metabolite profiles that contain data for specific crop species at different developmental stages and grown in diverse environmental conditions would be helpful. Thus, application of these techniques on a routine basis for identification of unintended effects in GM organisms may therefore only be an option in the longer term. If, in future, interpretation of results from profiling studies will be possible, this approach will be welcome to complement the targeted test approach. The overall aim to identify possible differences between traditionally used and novel crops that might have adverse effects on human health is the same. The success of developing profiling methods to complement the targeted test approach may be of particular relevance for the next generation of GM food crops with improved nutritional characteristics, obtained, for instance, through insertion of multiple metabolic pathways.

5.3.2. Further development of models and methods for safety and nutritional tests for foods

The aim of safety and nutritional tests should be to assess whether the safety and nutritional properties (wholesomeness) of the novel food are similar or improved compared to those of traditional plant-derived foods. Profiling methods might contribute to improving the efficacy of animal testing methods in that more information on the safety and nutritional value of test substances relating to a range of different endpoints can be gained from a single animal test. But as emphasised above, due to difficulties of data interpretation, the added value of use of profiling methods in food safety assessment still remains to be proven.

One interesting research project in this area develops a testing strategy for whole GM foods by combining in vivo animal models, in vitro toxicological systems, and the use of selected profiling methods (Knudsen, personal communication). The SAFOTEST project investigates GM rice containing lectins. Ninety-days rat feeding trials are performed with diets containing parent rice, GM rice, or GM rice spiked with the lectin at a relatively high dose level that is known to be toxic. Extended clinical and histopathological analyses are carried out, the endpoints of which include immunotoxicity. These experiments are paralleled by in vitro experiments on the digestibility and cytotoxicity of the recombinant proteins. The cytotoxicity of the proteins and their proteolysis fragments is assessed in intestinal epithelial cell lines derived from humans and rats. Changes in gene expression profiles in rat and human intestinal epithelial cell lines upon exposure to sub-cytotoxic concentrations of the lectins and their peptic-tryptic digests are determined using DNA microarrays. These in vitro profiles are then compared with the expression profiles in intestinal samples taken from live rats exposed to the same proteins during feeding

experiments. This allows the comparison of results obtained from in vitro and in vivo systems of animal and human origin.

The use of profiling methods might also help to identify new biomarkers for adverse effects. DNA micro arrays for example may help to identify biomarkers that help to extend dose response curves into lower dose ranges. Particular sets of genes, and changes in expression levels thereof, may serve as biomarkers for specific toxicological, nutritional, or disease endpoints. If reliable protocols for DNA micro array systems that allow the detection of such subtle changes can be developed and validated, such methods may help in future to increase the sensitivity of both in vivo and in vitro test systems, and establish more precisely cellular threshold levels at which specific molecules produce observed effects, extrapolation factors and safety margins through a more detailed understanding of interspecies and inter-individual variations of susceptibility. The identification of sets of genes that may serve as biomarkers may be possible by studying the reaction to well-known toxicants and nutrients in such systems.

The safety assessment of foods derived from GM crops with more complex modifications conferring nutritional benefits may in some cases require the development of improved in vivo dietary studies of whole foods. It is important to develop animal models that are very sensitive to the detection of toxic and anti-nutritive effects and intended positive nutritive effects.

6. Conclusions

This paper provides detailed guidance on how to assess the safety of a GM crop for anyone involved in risk assessment and regulation. The 1990s have demonstrated that agricultural practices can be improved by applying the methods of modern biotechnology to genetically modify traditionally used crops. New characteristics that have been conferred to crops through genetic modification include protection against fungal-, viral-, and bacterial diseases and insects, and tolerance to selected herbicides as well as improvements in yield, flavour, nutritional value, and characteristics for feed use. The paper sets out a systematic stepwise approach on how to tailor appropriate combinations of test methods to the safety assessment of foods derived from a specific GM crop, focusing on the characteristics of the modified crop and the introduced trait. The approach builds on internationally agreed guidelines and principles, and is suitable for current and future GM crops with more complex modifications.

Over the last two decades, individual governments and intergovernmental organisations have designed strategies and protocols for safety assessment of foods/feed derived from GM crops (FAO/WHO, 1991, 1996,

2000, 2001; Codex Alimentarius Commission, 2003b; NAS, 1987; NRC, 1989; OECD, 1993a, 1996, 1998, 2002d). Experts who have contributed to the debate at national and international level generally agree that potential risks arising from foods produced through modern biotechnology are no different from those associated with new varieties of plants produced through conventional breeding (OECD, 1986). Furthermore, there are no indications that there may be adverse effects from the consumption of GM crops, like the herbicide-tolerant soybean and insect-protected maize. All GM crops that have been approved for commercialisation and entered the agri-food chain have undergone extensive testing. The safety testing has been conducted on a case-by-case basis and is tailored to the specific characteristics of the modified crop and the changes that are introduced through genetic modification.

The corner stone of the safety assessment of novel foods, including foods derived from GM crops, is the concept of substantial equivalence. This concept serves to structure the safety assessment procedure, but does not describe the outcome of the safety assessment per se. Comparison of the GM crop to a suitable comparator with a long history of use according to predetermined parameters allows the identification of any significant differences that might impact human health. Any such differences then become the focus of further analyses. The safety assessment comprises four steps: The characterisation of the parent crop; the characterisation of the genetic modification process, the transferred genes, and the source organism of the recombinant DNA. The subsequent assessment focuses on the recombinant proteins and new or existing metabolites, the levels of which were altered. The comparison of the GM crop to the parent crop then serves to confirm the food safety of the intended changes and demonstrate that the genetic modification has not produced unintended and unexpected effects with potential adverse health impacts.

The approach is also suitable for future GM crops with more complex changes in the metabolism and/or developmental pathways, such as nutritionally enhanced crops. In these cases, the choice of a suitable comparator that is generally regarded as safe under present use conditions is critical. The assessment strategy may rely on the use of several comparators, including the food(s) that the new GM crop is intended to replace. Exposure estimates anticipating usage patterns of such novel food products derived from nutritionally enhanced crops are also critical.

Our increasing understanding of whole genomes and the development of new molecular biology tools to study induced changes in gene expression in plants will facilitate the characterisation of recombinant DNA introduced into GM crops. Methods for the transfer of genes into plants are being improved to reduce recombinant DNA transferred into GM crops to a minimum,

and thus decrease the burden of evidence to be provided for safety assessment. One conclusion from the review of the development of new transformation methods and molecular characterisation methods is that more systematic efforts are required to compare the relative risks, costs, and benefits of established and potential new technologies before novel technologies are endorsed in policy documents.

Available toxicological test methods are used to provide a sufficient degree of safety assurance for new components introduced into foods derived from genetically modified plants and for testing potential changes in the composition. At present, *in vitro* studies are not well-suited for the study of whole foods. However, future developments in genomics and microarray technologies hold the promise of providing sets of genes that serve as biomarkers for a cell's responses to toxins and allergens; if these promises are realised, and assays can be developed that are reliable, reproducible, and validated, microarrays with human genes will assist in the testing of whole foods.

Current strategies for assessing the potential allergenicity of GM crops are based on a weight of evidence approach. The overarching aim of further research in the area of allergenicity is to develop methods to better predict the allergenic potential of food constituents. To do this, we need to improve our understanding of the cellular and molecular bases for the development of allergic sensitisation to proteins in the context of food safety, and the prospective identification and characterisation of potential protein allergens. This requires: (i) further elucidation of the relationships between protein structure and function and sensitising activity; (ii) improved understanding of the relationships that exist between the resistance of proteins to proteolytic digestion and sensitising potential; and (iii) the development, evaluation and validation of new *in vitro* methods and animal models that will provide a more direct assessment of the inherent sensitising potential of proteins.

Classical toxicity studies in animals have been adapted for the assessment of whole foods, including foods derived from GM crops. Challenges in the use of animal models for testing whole foods include the importance of ensuring nutritional balance when diets contain high proportions of novel foods or food extracts. Furthermore, in cases where adverse effects might be observed, it is difficult to establish whether these are consequential to the genetic modification. Where such difficulties are successfully accounted for, such feeding studies meaningfully contribute to the safety assessment of foods derived from GM crops. Animal studies for the safety assessment of foods/food components are usually performed in rodents; studies in young fast-growing animals such as broilers should however also be considered for routine investigations of potential effects of whole foods on the growth rate of individuals. This allows the

Table A1
OECD guidelines for toxicity testing of chemicals

Hazard endpoint	OECD G/L ^a	Applicability to assessment of purified transgene product (protein) e.g. insecticidal protein
Acute toxicity: establishes toxicity of a single high dose	401, 420, 423, 425	Yes
Repeated dose toxicity (28/90 days): establishes toxicity profile of material	407, 408	Yes - but should be associated with studies on lability in GI tract
Chronic toxicity, incl. Carcinogenicity: establishes whether long-term use can lead to adverse effects	451, 452, 453	Yes - but information obtained may not be much more than from sub-chronic studies
Genotoxicity: demonstrates whether material has mutagenic activity in vivo	474, 475, 478, 486	Yes
Reprotoxicity tests: establish whether test material impairs reproductive function and/or damages offspring	414, 415, 416, 421	Yes
Allergenicity	N/A ^b	no validated models, limited assessment of hazard ILSI/IFBC (Metcalfé et al., 1996) and/or FAO/WHO (2001)

^a G/L = guideline.

^b N/A = not applicable.

identification of early signs of metabolic disturbances. The use of broilers is, however, not intended as a replacement, but rather to complement classical toxicological studies in rodents and other routinely used laboratory animals. Progress in molecular biology, toxicology, biochemistry, and nutrition will allow further improvement of these methods.

In conclusion, the food safety assessment paradigm as described in this paper, under which any differences in the new food are identified and any hazards and risks characterised, relative to the conventional food or product, clearly establishes whether the test food derived from a GM crop is as safe as the conventional counterpart. It can even be argued that foods from GM crops are better characterised than other non-regulated plant-derived foods, due to the additional rigour in the current regulatory requirements and testing regime compared to that for conventionally-bred crops. Advances in molecular biology, toxicology, biochemistry, and nutrition will provide novel biomarkers and methodologies, which could be developed into new safety assessment tools. These new tools will facilitate the development and assessment of GM crops with more complex traits that will contribute to improving human and animal health or natural resource management in agri-food production.

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Appendix A

Animal testing

The Organisation for Economic Cooperation and Development (OECD) has developed guidelines for animal- and in vitro-testing of chemicals. An overview of the guidelines for testing in animals is provided by Table A1.

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- ome. This DNA causes the host cell to grow into a tumour-like structure that synthesizes specific opines that only the pathogen can metabolize. This DNA-transfer mechanism is exploited in the genetic engineering of plants.
- Agrobacterium tumefaciens-mediated transformation*: The process of DNA transfer from *Agrobacterium tumefaciens* to plants, that occurs naturally during crown gall disease, and can be used as a method of transformation.
- Allergen*: any substance that causes an allergic reaction
- Antibiotic resistance marker gene*: (Abbreviation: ARMG). Genes (usually of bacterial origin) used as selection markers in transgenesis, because their presence allows cell survival in the presence of normally toxic antibiotic agents. These genes were commonly used in the development and release of first generation transgenic organisms (particularly crop plants), but are no longer favoured because of perceived risks associated with the unintentional transfer of antibiotic resistance to other organisms.
- Antisense DNA*: The DNA strand complementary (hence “anti”) to the mRNA, i.e. the non-transcribed strand. However, there is not universal agreement on this convention, and the preferred designations are coding strand for the strand whose sequence matches that of the mRNA, and non-coding strand or template strand for the complementary strand (i.e. the transcription template).
- Antisense gene*: A gene that produces an mRNA complementary to the transcript of a normal gene (usually constructed by inverting the coding region relative to the promoter).
- Antisense RNA*: An RNA sequence that is complementary to all or part of a functional mRNA molecule, to which it binds, blocking its translation.
- Biolistics*: A technique to generate transgenic cells, in which DNA-coated small metal particles (tungsten or gold) are propelled by various means fast enough to puncture target cells. Provided that the cell is not irretrievably damaged, the DNA is frequently taken up by the cell. The technique has been successfully used to transform animal, plant, and fungal cells, and even mitochondria inside cells. *Synonym*: microprojectile bombardment.
- Backcross*: Crossing an individual with one of its parents or with the genetically equivalent organism. The offspring of such a cross are referred to as the backcross generation or backcross progeny.
- Biotechnology*: 1. “Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use” (Convention on Biological Diversity). 2. “Interpreted in a narrow sense, ... a range of different molecular technologies such as gene manipulation and gene transfer, DNA

Glossary

Biotechnology terms

Agrobacterium tumefaciens: A bacterium that causes crown gall disease in some plants. The bacterium characteristically infects a wound, and incorporates a segment of Ti plasmid DNA into the host gen-

typing and cloning of plants and animals” (FAO’s statement on biotechnology).

Breeding: The process of sexual reproduction and production of offspring.

Codex Alimentarius Commission: An international regulatory body (part of FAO) responsible for the definition of a set of international food standards. The Commission periodically determines, then publishes a list of food ingredients and maximum allowable levels (the *Codex Alimentarius*) deemed to be safe for human consumption.

Coding sequence: That portion of a gene which directly specifies the amino acid sequence of its product. Non-coding sequences of genes include introns and control regions, such as promoters, operators, and terminators.

Construct: An engineered chimeric DNA designed to be transferred into a cell or tissue. Typically, the construct comprises the gene or genes of interest, a marker gene and appropriate control sequences as a single package. A repeatedly-used construct may be called a cassette.

Conventional counterpart: An existing acceptable food or food component, which is sufficiently close in terms of composition, structure, function, and use to the new food component so as to provide a safety standard against which to assess the new food component.

Critical nutrients/toxins: Chemical compounds present in a food or food component, which are known to have nutritional or toxicological effects in humans and which, if present in the new food at levels which are significantly different from those in traditional foods or food components, could be expected to have consequences for human health bearing in mind the likely patterns of consumption of the food or food component.

Cry proteins: A class of crystalline proteins produced by strains of *Bacillus thuringiensis*, and engineered into crop plants to give resistance against insect pests. These proteins are toxic to certain categories of insects (e.g. corn borers, corn rootworms, mosquitoes, black flies, armyworms, tobacco hornworms, some types of beetles, etc.), but are harmless to mammals and most beneficial insects. *Synonym:* delta endotoxins.

Disease resistance: The genetically determined ability to prevent the reproduction of a pathogen, thereby remaining healthy. Some resistances operate by pathogen exclusion, some by preventing pathogen spread, and some by tolerating pathogen toxin.

Donor: The organism from which the gene(s) are selected to make the new trait

Edible cultivar: Those cultivars of a particular plant species that are used as human food or as a source of human food or food components.

Expression: Not all genes are active. When a gene is read and the product of the gene (always including RNA and usually a protein) is produced, the gene is said to be expressed.

Event: See transformant.

Flanking region: The DNA sequences extending either side of a specific sequence.

Food component: A constituent fraction of a food that is capable of being identified and characterised and may include major or minor nutrients and natural toxins as well as macro components such as oil, protein, or starch.

Gene: The basic unit of heredity transmitted from generation to generation during sexual or asexual reproduction; an ordered sequence of nucleotide bases, comprising of a segment of DNA. A gene contains the sequence of DNA that encodes an individual protein or RNA.

Gene construct: See: construct.

Gene expression: The process by which a gene produces mRNA and protein, and hence exerts its effect on the phenotype of an organism.

Gene gun: See: biolistics.

Gene insertion: The incorporation of one or more copies of a gene into a chromosome.

Gene product: A RNA or a protein (e.g. an enzyme) the production of which, in a living plant, is directed by the corresponding gene.

Gene transfer: See: transformation.

Genetic engineering: Modifying genotype, and hence phenotype, by transgenesis.

Genetic transformation: See: transformation.

GM food: Abbreviation for genetically modified food. Food that contains above a certain legal minimum content of raw material obtained from genetically modified organisms.

GMO: Abbreviation for genetically modified organism.

Herbicide resistance: The ability of a plant to remain unaffected by the application of a herbicide.

Inserted gene: A piece of DNA that has been inserted into a plant using recombinant DNA technology and that contains sufficient heritable information to direct the production of a particular gene product in that living plant.

Marker: An identifiable DNA sequence that is inherited in Mendelian fashion, and which facilitates the study of inheritance of a trait or a linked gene.

Marker gene: A gene of known function or known location, used for marker-assisted selection or genetic studies.

Metabolomics: The study of the complement of metabolites present in a single cell/tissue under specified conditions.

Modern biotechnology: Techniques, based on molecular biology, for making specific modifications to the

genome of plants, which have been made possible by scientific advances in the understanding of the nature and function of DNA and in the use of recombinant DNA technology.

Molecular characterisation: Includes DNA sequence data and the mapping of particular functions on the plant chromosome and on the inserted DNA.

Mutagenesis: Induction of heritable change(s) in the genetic constitution of a cell through alterations to its DNA.

Open reading frame: (Abbreviation: ORF). A sequence of nucleotides in a DNA molecule that has the potential to encode a peptide or protein: comprises a start triplet (ATG), followed by a series of triplets (each of which encodes an amino acid), and ending with a stop codon (TAA, TAG or TGA). The term is generally applied to sequences of DNA fragments, for which no function has yet been determined. The number of ORFs provides an estimate of the number of genes transcribed from the DNA sequence.

Parent: The recipient crop for the donated gene(s).

Phenotypic: The appearance or other characteristics of an organism, resulting from the interaction of its genetic constitution with the environment.

Pleiotrophic effects: A phenomenon in which a single genetic alteration affects multiple phenotypic characteristics (e.g. a change in a metabolic pathway that affects multiple end products of that pathway or metabolic effects of a new gene product on the overall behaviour of a modified plant).

Post-translational modification: The addition of specific chemical residues to a protein after it has been translated. Common residues are phosphate groups (phosphorylation) and sugars (glycosylation).

Primer: A short oligonucleotide annealed to a template of single-stranded DNA, providing a doubled-stranded structure from which DNA polymerase will synthesize a new DNA strand to produce a duplex molecule.

Promoter: 1. A short DNA sequence, usually upstream of (5' to) the relevant coding sequence, to which RNA polymerase binds before initiating transcription. This binding aligns the RNA polymerase so that transcription will initiate at a specific site. The nucleotide sequence of the promoter determines the nature of the enzyme that attaches to it and the rate of RNA synthesis. 2. A chemical substance that enhances the transformation of benign cells into cancerous cells.

Proteomics: An approach that seeks to identify and characterize complete sets of protein, and protein-protein interactions in a given species.

Recombinant-DNA plant: A plant in which the genetic material has been changed through in vitro nucleic acid techniques, including recombinant deoxy-

ribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

Reference characteristics: Parameters, which may be agronomic, molecular, or chemical, and which are useful in making the comparison of a new food with its conventional counterpart.

Risk analysis: A process consisting of three components: risk assessment, risk management, and risk communication performed to understand the nature of unwanted, negative consequences to human and animal health, or the environment.

Risk assessment: A scientifically based process consisting of the following steps: (i) hazard identification; (ii) hazard characterisation; (iii) exposure assessment; and iv) risk characterisation.

Risk communication: The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors, and risk perceptions, among risk assessors, risk managers, consumers, industry, the academic community, and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk management: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Secondary metabolism: The production by living organisms of substances not essential for primary metabolic functions or physiology. Their role is associated with interaction with the environment, for example for defence, as elicitors, or as attractants. Some of these have useful pharmacological or nutritional properties, while others are toxic.

Silencing: Loss of gene expression either through an alteration in the DNA sequence of a structural gene, or its regulatory region; or because of interactions between its transcript and other mRNAs present in the cell (See: antisense RNA).

Substantial equivalence: A concept that embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety for human consumption of a food or food component that is new or is modified.

Transformant: A cell or organism that has been genetically altered through the integration of a transgene(s). Primary: the first generation following the transformation event. Secondary: progeny of the primary transformant.

Transformation: 1. The uptake and integration of DNA in a cell, in which the introduced DNA is intended to change the phenotype of the recipient organism in a predictable manner. 2. The conversion, by various means, of cultured animal cells from controlled to uncontrolled cell growth, typically through infection with a tumour virus or transfection with an oncogene.

Transgenic: Adjective describing an organism in which a foreign DNA gene (a transgene) is incorporated into its genome.

Wholesomeness: The property of being favourable to health and which embraces both the nutritional and toxicological aspects of a food under the anticipated patterns of consumption.