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Safety evaluation of genetically modified foods

Abstract The concept of substantial equivalence has been accepted as the cornerstone of the health hazard assessment of genetically modified (GM) foods (OECD 1993). Substantial equivalence is the most practical approach to address the safety of foods or food components derived from GM crops and is based on comparison of the phenotypic and compositional characteristics of the parent crop and the GM crop. Basically, three categories of GM crops can be considered (FAO/WHO 1996; EU 1997): (a) GM crops which have the same composition as the parent crop, (b) GM crops which have the same composition as the parent crop with the exception of a well-defined trait, and (c) GM crops which are different from the parent crop. For the safety assessment of the first category of GM foods only a molecular characterisation of the genetic insert is sufficient, whereas for the second category a safety assessment of the expressed protein(s) is also required. For the last category an extensive evaluation including bioavailability and wholesomeness studies are required, beside the molecular characterisation and safety assessment of the expressed protein(s) and their products. By molecular characterisation is meant the position, nature, stability and number of copies of the inserted DNA. Substantial equivalence is established by the determination of the phenotypic characteristics (e.g. resistance against diseases, agronomic properties) and the complete chemical composition of the plant including nutrients, toxicants, antinutrients, and allergens. The toxicity of the expressed protein(s) is assessed by their homology with known protein toxins, degradation in the gastro-intestinal tract, stability to food processing and acute toxicity in rodents. The possible allergenicity of the

expressed proteins is evaluated by comparison of their amino acid sequence with that of known allergens and determination of their stability to digestion and food processing. If the source of the genetic insert is allergenic then the use of solid-state immunoassays, skin prick tests and even food challenge tests can be considered.

Key words GMO · Genetically modified foods · Genetically modified crops · Safety evaluation · Substantial equivalence · Toxicity testing · Allergenicity testing

Introduction

Recombinant DNA technology has been applied in plant breeding to improve compositional, processing and agronomic characteristics of crops through the introduction of a well-characterised trait. Unlike classic breeding techniques this technology allows for the introduction of genes in a very specific, selective and controlled manner. Examples of genetic modifications in food crops are delayed softening and improved ripening of tomatoes; virus resistance of squash and papaya; insect resistance of corn, soybean, cotton and potato; herbicide tolerance of corn, canola, cotton and soybean and altered fatty acid content of canola and soybean.

Various national and international organisations and the biotechnology industry have been working together since 1990 to design a safety assessment strategy for genetically modified (GM) crops and their derived products. The organisations involved in the process are the International Food Biotechnology Council (IFBC) and the International Life Sciences Institute (ILSI) [1], the organisation for Economic Cooperation and Development (OECD) [2, 3], the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) [4, 5, 6].

Until 1997, there was no uniform legislation in the EU on the placing of GM foods on the market. In May

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1997, the EU Regulation concerning novel foods and novel food ingredients (EC/258/97) was issued [7] describing the procedure that should be followed for the registration of GM foods in the EU. On July 29, 1997 a Commission Directive (97/618/EC) [8] was published with recommendations on the scientific aspects of information necessary to support applications for the placing of novel foods and their ingredients on the market. In this Directive, six classes of novel foods are defined. Three classes pertain to GM foods, i.e. GM plants and their products (class 3), GM animals and their products (class 4) and GM micro-organisms and their products (class 5). The principles of safety assessment of GM foods as developed by the OECD and FAO/WHO were adopted. The basis of the safety assessment is the principle of substantial equivalence. When substantial equivalence is established between the GM plant and its traditional counterpart then the GM plant can be treated in the same manner with respect to safety [2].

The safety assessment paradigm for GM foods that will be further discussed here subdivides the safety assessment approaches for GM foods according to the degree of substantial equivalence: (1) foods which are completely substantially equivalent, (2) foods which are equivalent with the exception of the introduced trait, and (3) foods which are not substantially equivalent. The first category concerns the type of foods which have been derived from the GM plant but do not contain anymore the gene product (e.g. oil from the glyphosate-tolerant soybean).

The other components of the safety assessment for each of the three categories are genetic characterisation, protein safety assessment, nutritional studies and further ad-hoc safety studies (Fig. 1).

Substantial equivalence

Plants are considered substantially equivalent when there are no differences in the phenotypic characteristics and composition, taking natural and geographic variation into account, and when the intended use as well as the quantity and frequency of human consumption is the same. Aspects of phenotypic analysis are morphology and the agronomic properties of the plant such as growth rate, production of biomass, resistance to pests and disease, tolerance to soil composition (acid, salt, minerals), tolerance to herbicides and organoleptic characteristics. The most elaborate part of the determination of substantial equivalence is the compositional analysis of the plant. This chemical analysis needs to be carried out on the GM plant as well on the traditional counterpart grown in geographical areas with different soil compositions and climatologic conditions. The data obtained on the GM plant are then compared against those from the parental plant and the compositional databases of the crop. Before being able to conclude substantial equivalence, there should be sufficient

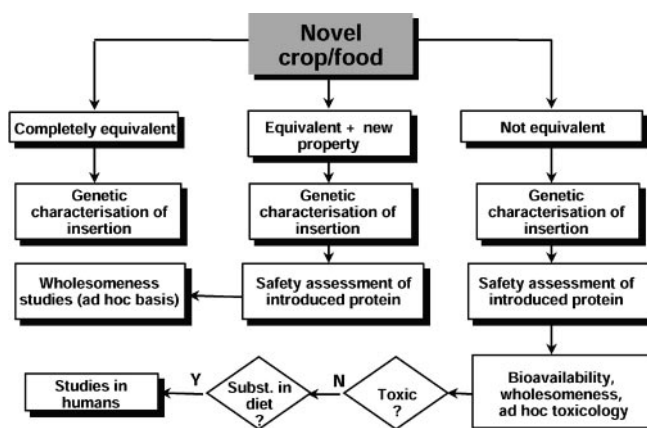


Fig. 1 Safety assessment paradigm for GM foods applied in the EU and based on the principle of substantial equivalence

knowledge about the natural variation of the concentration of the constituents analysed. Chemical analysis can be used for the determination of substantial equivalence of the whole plant, specific parts of the plant (e.g. roots, leaves, seed) or products that have been derived from the plant through processing (e.g. oil, sugar). The chemical analysis consists of various parts and these may differ according to the composition and nutritional value of the plant and the likely influence of the genetic insert on plant metabolism (pleiotropic effects). The part which is common for all plants is the determination of macronutrient composition, which concerns total carbohydrate content, total protein, total fat, ash, fiber and water. These data can be supplemented with protein electrophoresis patterns and amino acid and fatty acid analyses. Analyses which are more specific to the plant are those for natural plant toxins such as phytoestrogens (soybean), glucosinolates (oil seedrape), coumarins (celery) and solanins (potato); allergens such as glycinin (soybean) and 2S-albumin (Brazil nut); anti-nutrients such as lectins and trypsin inhibitors (soybean); vitamins such as vitamin C and β -carotene (squash) and specific carbohydrates such as glucose, sucrose, lactose and fructose (squash).

Genetic characterisation

Genetic characterisation is an essential part of the safety assessment of the three categories of novel foods (Fig. 1). It enables the applicant to understand which part of the genome of the plasmid has been transferred from the vector (e.g. agrobacterium) to the host plant and how it is inserted in the plant genome. The data elements that have to be reported by the applicant in the registration dossier are: the description of the gene transfer technique (particle gun, agrobacterium, electroporation), the position and nature of the inserted DNA sequence, the number of inserts, the number of copies, the level of expression of the inserted gene and the stability of the gene over several generations.

Protein safety assessment

The safety assessment of the newly introduced protein is a cornerstone of the GM food safety assessment paradigm. This consists of identification, homology determination with existing proteins and toxicity and allergenicity testing.

The identity of the newly introduced protein is characterised by determination of its amino acid sequence, 3D structure and degree of glycosylation. Also, the function of the protein (e.g. CP4 5-enolpyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS) in glyphosate-tolerant soybeans) and specificity for certain substrates are important. Since the newly introduced protein is present in the GM plant in very low concentrations (ppm range) in many cases a GM micro-organism (e.g. *Escherichia coli* carrying the glyphosate-tolerant gene) has to be developed to produce enough protein for complete characterisation and safety testing.

Another aspect of the safety testing of the newly introduced protein is determination of its homology with proteins commonly present in our food and living environment and to which humans have been sufficiently exposed, e.g. CP4 EPSPS from glyphosate-tolerant soybean with EPSPS from petunia or *E. coli*. Homology determination is based on comparison of the amino acid sequence of the GM protein and the naturally occurring proteins. One can conclude sufficient homology between two proteins when, for example, 50% similarity and 30% identity of the total amino acid sequence has been obtained.

As part of the toxicity evaluation homology of the protein is also checked against the amino acid sequence of known toxins. To this effect several protein databases (e.g. PIRprotein, Swissprot, Genpept) containing more than 1900 known protein toxins are consulted. Normally no homology should exist between the newly introduced protein and known protein toxins. The homology testing against known proteins is only a minor part of the toxicity evaluation, and the only thing that can be concluded from this comparison exercise is that there are no amino acid sequences present in the protein that are also present in toxins. This does not mean that the protein is devoid of any toxicity; therefore more tests are required such as stability at low pH and to digestive enzymes, stability to food processing and acute toxicity in mammals. In the first test the protein is dissolved in artificial gastric fluid and artificial intestinal fluid at body temperature. The concentrations of the protein employed are several orders of magnitude greater than those present in the GM plant. The composition of the gastrointestinal fluid is the same as described in the US Pharmacopoeia XXII for the testing of gastric and intestinal degradation of drug forms. Just after the addition of the protein, samples are taken to measure the concentration of the remaining protein or its fragments. The technique which is normally used is gel electrophoresis along with specific protein staining or immu-

noblotting techniques. Sometimes degradation of the protein can be monitored by making use of the specific enzymatic (e.g. EPSPS activity) or toxic (e.g. Bt toxin effect on insect larvae) activity.

Sensitivity to temperatures used in the food processing of certain GM crops means an important contribution to the safety of the GM food. The protein responsible for glyphosate tolerance is completely inactivated when heated at 65 °C for 15 min. The processing of soybean meal occurs at 66–107 °C for a period of 38 min, which means that all present newly introduced protein has been inactivated.

The last part of the toxicity testing of the protein is the acute toxicity test in the mouse. This is the classic LD₅₀ test in which the protein is administered by gavage to 10 mice/sex per dose. The dose levels used are as high as 1000 times the maximum daily human consumption of GM crops containing the same protein. Bovine serum albumin serves as the control in this test. The animals are observed for clinical signs, body weight gain, food consumption, mortality and gross pathology changes.

The allergenicity testing decision tree of GM foods which is currently followed by industry and which is in line with the EU recommendation is based on the scheme that has been developed by the ILSI/IFBC (1) (Fig. 2).

The ILSI scheme is composed of three testing flows which are selected on the basis of the allergenic properties of the donor organism of the gene and the occurrence of the allergen. When the donor organism is not allergenic then the amino acid sequence of the gene product is compared against that of more than 550 known allergenic proteins selected from protein databases (e.g. Genbank, EMBL, PIRprotein, Swissprot). The allergenic proteins which are used for comparison are plant allergens and gliadins, animal allergens, pollen, fungi, animal, insect and mite aero-allergens, venoms and salivary allergens, contact allergens and nematode and worm allergens. In a second phase the correspondence of eight consecutive amino acids can be searched for the presence of IgE-binding epitopes. When correspondence in amino acid sequence has been detected the GM food containing the protein will be further tested as if the donor organism were allergenic. When no correspondence of amino acid sequence is found with known allergens the stability of the newly introduced protein is tested in artificial gastric and intestinal fluids and at processing temperatures. These results are normally available from the toxicity testing programme. When the protein is quickly digested (e.g. degradation half-life time of less than 10 s) and is completely deactivated at processing temperatures then the GM food containing the protein should not be labelled as allergenic. If resistance against digestion is noted then all the available data should be reviewed together with the responsible authorities as to whether no label is warranted or more tests must be conducted.

When the donor organism is allergenic then the most elaborate part of the testing scheme is followed. Differ-

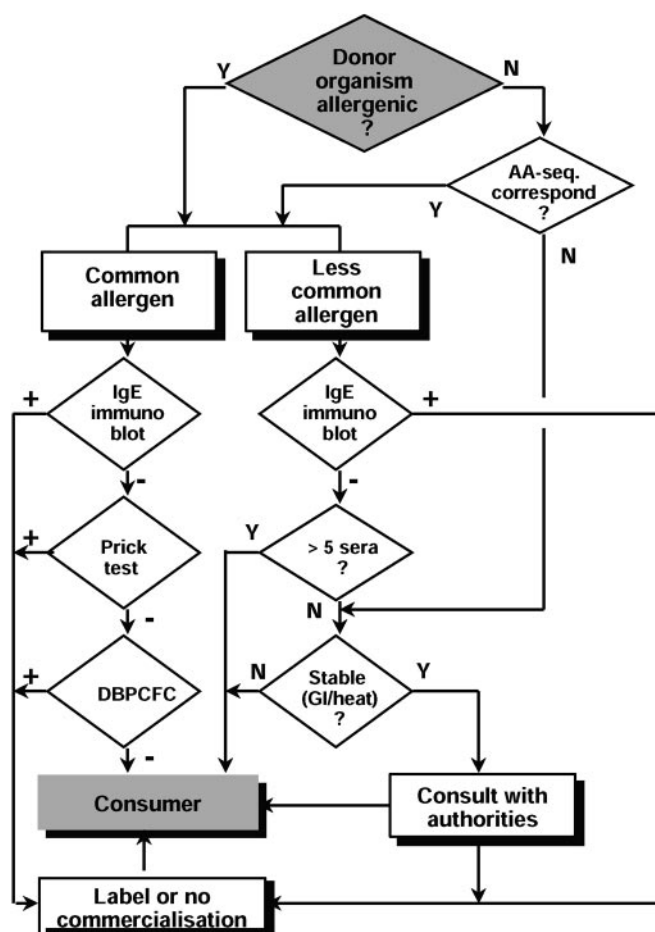


Fig. 2 Decision tree for allergenicity testing of GM foods based on the scheme developed by IFBC/ILSI)

ence is made between common allergens (e.g. tree nuts, peanuts, soybeans, wheat, fish, eggs, milk) and less common allergens (e.g. apple, peach, rice, Brazil nut) because of the difference in availability of sera. The test which is most used in a first stage is gel electrophoresis of the protein extract with detection of the allergen by means of IgE-immunoblotting. At a later stage in the development of the GM food other tests can also be employed such as RAST and ELISA. In the case of a common allergen, sera from at least 14 different people allergic to that protein should be used in the immunoblotting technique. For less common allergens, where less people are available, a minimum of sera from five allergic people is necessary to decide whether the GM food should be labelled as an allergen or not. When the result of the IgE-immunoblotting assay is positive then the GM food should be labelled as an allergen. In the event of less common allergens it may even be decided to stop the further development of the GM food (e.g. 2S-albumin from Brazil nut in soybean). If the result of the immunoblotting technique is negative for the less common allergen and sera from more than five people are available then no label should be proposed. If less sera are available then a supplementary test is required to

establish stability against digestive enzymes and processing heat. If not found stable then no label is required. If no sufficient degradation of the protein is observed then a review of all the data with the responsible authorities is proposed before deciding to label or not. When the immunoblot assay of the common allergen is negative more tests are required before deciding not to label the GM food as an allergen. These tests are the skin prick test and the double-blind placebo-controlled food challenge test (DBPCFC). These tests are conducted with fully informed human volunteers and in compliance with all regulatory and ethical provisions regarding human experimentation in the country of testing. Only when these two tests are negative for the GM food, should it not be labelled as an allergen. Despite this rigorous testing scheme it should be recognised that post-launch human monitoring is a useful tool to further assure safe consumption of the GM food.

Nutritional studies

Nutritional studies, also called wholesomeness studies, are conducted on a case-by-case basis and should not be regarded as sub-acute toxicity studies. Wholesomeness studies are designed to detect changes in the nutritional value of the GM food and can be considered as a complementary assay to confirm substantial equivalence with the parental crop. Normally these assays are performed on laboratory animals (e.g. rat) and large domestic animals (e.g. cattle, sheep, goat) with an exposure time of 28 days. Other species can also be selected because of the sensitivity of their growth rate for changes in the nutritional value of their diet (e.g. chicken, catfish). The parameters which are normally recorded are the general clinical condition, food consumption, body weight change, changes in gross necropsy and organ weight. In ruminants the influence of the GM food on the digestion process is also monitored. In these assays it is extremely important to maintain the intake of essential nutrients in the whole diet when it is substantially replaced (up to 35%) by the GM food. The practical difficulty of these assays is the tolerance of the test animal for food constituents which are not normally part of their diet. Examples are anemia in dogs and rats fed with onions [9], development of the megacolon syndrome in rats as a result of high concentrations of modified starch [10, 11] and damage of the duodenal mucosa of rats due to administration of a chili pepper solution [12].

Further safety assessment studies

In the case of GM crops which belong to the third category (Fig. 1), more tests are needed than those mentioned for the two other categories of GM foods. The tests may include bioavailability and sub-chronic toxicity tests of the protein in rodents. The possibility also exists to conduct human feeding studies if the quantities

of consumed food are important and if there are no indications of toxicity. More specialised toxicology studies like long-term assays, reproduction toxicity and developmental toxicity may also be considered on an ad-hoc basis. It is important to note that the lack of availability of the protein and/or the intolerance of the test species to the GM food may constitute practical problems which need to be overcome.

Conclusion

This paper has attempted to give a representative overview of the principles of safety evaluation followed by the producers of GM crops to assure safe consumption crops by humans and animals. The safety assessment paradigm that has been followed so far has given satisfactory results for GM crops and novel foods which are substantially equivalent with their traditional counterparts (1st and 2nd category of novel foods). Much less experience is available with the safety evaluation of crops which are not substantially equivalent or crops containing new proteins. The most typical example demonstrating that the system works is 2S-albumin, which was introduced in the soybean to increase protein sulfur and which was found to be the allergen of Brazil nut. This discovery led to the discontinuation of the development of this GM crop. In the meantime continuous improvements have been made to the test systems which are part of this safety assessment scheme and efforts are underway to propose satisfactory safety testing schemes for GM foods which are not substantially equivalent with their traditional counterparts. In any event, the scrutiny by which the safety testing of GM crops is carried out provides much more assurance of safety to human health than that for crops which have been obtained through traditional breeding techniques.

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