



FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

SAFETY ASSESSMENT OF GENETICALLY MODIFIED FOODS

Guidance Document

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FORWARD

Applications to amend the *Australia New Zealand Food Standards Code* are required before a new food produced using gene technology can be approved in Australia and New Zealand.

A food produced using gene technology is defined as ‘a food which has been derived or developed from an organism which has been modified by gene technology’, where gene technology is defined as ‘recombinant-DNA techniques that alter the heritable genetic material of living cells or organisms.’ FSANZ typically refers to such foods as genetically modified or GM foods.

FSANZ is required to assess the safety for human consumption of each GM food prior to giving approval. Approved GM foods are listed in Standard 1.5.2 – Foods produced using Gene Technology. The safety assessment is applied to the food derived from a GM organism, and is not applied directly to the organism itself, except in so far that the organism is itself the food.

FSANZ assesses the safety of GM foods in accordance with internationally established scientific principles and guidelines developed through the work of the Organisation for Economic Cooperation and Development (OECD), Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO) and the Codex Alimentarius Commission. These guidelines, which are intended to apply to a broad range of foods, provide both rigour and flexibility to the assessment. Flexibility is needed to allow GM foods to be assessed on a case-by-case basis and to take into consideration future scientific advances.

This document describes FSANZ’s approach to the safety assessment of GM foods and is intended to be read in conjunction with Section 3.5.1 of the *FSANZ Application Handbook*, which outlines the information required to support an application for approval of a GM food.

While this guidance document focuses primarily on foods derived from GM plants, it also has general applicability to foods derived from GM micro-organisms and GM animals. Additional issues requiring specific consideration in relation to GM micro-organisms and GM animals are outlined in separate sections of this document.

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1. BACKGROUND

FSANZ's approach to the safety assessment of GM foods applies the concepts and principles outlined in the Codex General Principles for the Risk Analysis of Foods Derived from Biotechnology (Codex 2004a) developed by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology. These principles state that the risk analysis process applied to GM foods should be consistent with the Codex Working Principles for Risk Analysis (Codex 2005).

While the Codex risk analysis principles can in general terms be applied to whole foods including GM foods, it is recognised that the approach, particularly that for risk assessment, was elaborated specifically to address chemical hazards, and was not intended to apply to whole foods, such as GM foods. The traditional toxicological approach (i.e. animal toxicity tests) for assessing substances such as food additives, pesticides and contaminants cannot easily be applied to whole foods. In fact, few conventional foods safely consumed today have been assessed scientifically in a manner that would fully characterise all risks associated with the food. In addition, many foods contain substances (e.g. natural toxicants) that would likely be found harmful if subjected to conventional approaches to safety testing. Because of this, a multidisciplinary approach is considered appropriate, where the safety of the whole food is evaluated (Codex 2004b).

This approach is based on the concept that the safety of GM foods can be assessed, to a large extent, by comparison to the conventional counterpart¹ having a history of safe use, and taking into account both intended and unintended changes. The objective is to identify new or altered hazards relative to the conventional counterpart. Any identified hazards then become the focus of further assessment.

The basic principle for the comparative assessment of GM foods – known as the concept of substantial equivalence – was first established through a Joint FAO/WHO Consultation in 1991 (WHO 1991) and was then further elaborated by the OECD (OECD 1993). A Joint FAO/WHO Expert Consultation on Safety Aspects of Genetically Modified Foods of Plant Origin re-evaluated the usefulness of the concept of substantial equivalence and concluded that 'there were presently no alternative strategies that would provide a better assurance of safety of GM foods than the appropriate use of the concept of substantial equivalence' (WHO 2000). This approach has also been endorsed for use with foods derived from GM micro-organisms (FAO/WHO 2001) and GM animals (FAO/WHO 2003).

2. INTRODUCTION

The safety assessment of a GM food is conducted within the established risk assessment framework used by FSANZ. In the case of GM food, the primary purpose is: (i) to identify new or altered hazards associated with the food as a result of the genetic modification; (ii) to assess whether there is any risk associated with these hazards under the intended conditions of use; and (iii) to determine if any new conditions of use are needed to enable safe use of the food.

¹ Defined by Codex as 'a related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food' (CAC/GL 45-2003)

The safety assessment is characterised by:

- 1. Case-by-case consideration of GM foods**
Case-by-case assessment is necessary because the key issues requiring consideration in a safety assessment will often depend on the type of food being evaluated and the nature of the genetic modification. Application of the safety assessment guidelines therefore needs to remain flexible in order to address the specific and unique issues that can arise as a result of different genetic modifications.
- 2. Consideration of the intended and unintended effects of the genetic modification**
The intended effects of the genetic modification relate to the direct consequences of the introduction of new DNA into an organism, e.g. the result of the expression of a new protein in the food or an increased concentration of end-products generated through the biological action of the protein. There may also be other effects associated with the genetic modification that were unintended (e.g. compositional changes to the food) and which may impact on the health and safety of the population. These must also be considered in detail in the safety assessment.
- 3. Comparisons with conventional foods having an acceptable standard of safety**
Such a comparative approach focuses on: (i) the identification of similarities and differences between the GM food and an appropriate comparator; and (ii) a characterisation of any of the identified differences in order to determine if they may raise potential safety and nutritional issues. A number of different comparators may be used for these purposes.

The goal of the safety assessment is not to establish the absolute safety of the GM food but rather to consider whether the GM food is comparable to the conventional counterpart food, i.e. that the GM food has all the benefits and risks normally associated with the conventional food.

Use of the comparative approach relies on: (i) consideration of the molecular characterisation of the genetic modification; (ii) phenotypic characterisation of the new organism, compared with an appropriate comparator²; and (iii) compositional analysis of the new food or the specific food product.

The application of the comparative approach does not, by itself, constitute a safety assessment. Rather, it is a tool that is used to facilitate the identification of similarities as well as differences (either intended or unintended) in the food. It is these defined differences that then become the focus of further scrutiny. The extent of this further scrutiny will depend on the nature of the identified differences, and could range anywhere from further comparisons with relevant conventional foods to the undertaking of traditional nutritional, toxicological or immunological testing.

² Phenotypic characterisation is of particular relevance in the case of GM animals, where the health of the animal is an important element of the food safety assessment.

3. SELECTION AND USE OF COMPARATORS

Comparators are used throughout the safety assessment and are fundamental to the effective use of the comparative approach. They are used in the molecular characterisation, the compositional analyses and may also be used to characterise any nutritional changes to a food. Depending on the types of study, comparators may also be used for allergenicity assessment and assessment of potential toxicity.

In principle, there are two conditions that should be met when selecting an appropriate comparator. The first is that the comparator should have a history of safe use as food, in order that it may serve as a suitable benchmark for comparison. The second is that the comparator should be as closely related as possible to the line or strain from which the GM food is derived, so that it may serve as a suitable experimental control and thus ensure the comparison is sensitive enough to identify relevant differences. The comparator should therefore ideally be the near isogenic line to that from which the GM food is derived. That is, genetically identical, except for the introduced trait.

In the case of GM plants, it is common practice for more than one comparator to be used for the safety assessment, with the various comparators serving different purposes. For the molecular characterisation, usually only one comparator is necessary and this should be the near isogenic line. The use of the near isogenic line ensures the extent of the genetic modification can be appropriately characterised.

For compositional analysis, a number of conventional varieties with a similar, although not identical, genetic background, are often grown alongside the GM line and included in the comparison, along with the near isogenic non-GM line. These additional or secondary comparators are used to provide a reference range for comparison and also assist in interpreting any identified differences between the GM line and its near isogenic comparator. Further comparisons to literature or historical ranges are made to determine the range of natural variation and to establish the biological significance of any identified differences. Through this process it is possible to determine if the identified differences are what would typically be expected on the basis of normal biological variation, and therefore whether further analysis is required.

In practice, the selection of an appropriate comparator may not always be straightforward. It can sometimes be difficult to identify a comparator that has both a history of safe use as food, and which is also closely related to the line from which the GM food is derived. This is increasingly the case as more complex genetic modifications are being introduced, and as more complicated breeding programs are used to generate the food-producing line. Some scenarios where it may be necessary to depart from the usual practice for the selection and use of comparators are outlined below:

1. *Where the parental line, which had been transformed in order to derive the GM food, is itself a GM line (i.e. a double transformation).* In this case, the parental GM line, as the near isogenic line, is likely to be the most appropriate comparator to use as the control for the molecular characterisation as well as to identify relevant differences in the compositional analysis. However, because this line may not have a history of safe use, it would be important to also include an additional comparator in the compositional analyses, which ideally would be the original non-GM parental line.

As is standard with compositional analyses, it would also be important to include further comparators representing conventional counterpart foods already in the food supply;

2. *Where the breeding process used following the transformation event is so complex that the parental (non-GM) line may no longer be closely related to the line from which the GM food is derived.* Comparison to the parental non-GM line in such instances would be of little value for detecting relevant differences. In these circumstances, at least in the case of plants, negative segregants have been selected for use as comparators as they are often the only lines available that are close enough to the GM line to serve as a suitable control. However, as such comparators may be considered to be a product of gene technology, even though they do not themselves exhibit a detectable genetic modification, it will be important to include additional conventional varieties in the analysis to satisfy the condition for history of safe use;
3. *Where it is not possible to maintain a genetically 'pure' line because of a high frequency of inbreeding depression.* Inbreeding depression, which can affect both plants and animals, refers to the lowered fitness or vigour of inbred individuals compared with their non-inbred counterparts. In this case the comparator will likely consist of a population of genetically similar but not identical (isogenic) individuals.

Because of the complexity of the technology being applied, it is not possible to envisage all potential scenarios for the selection and use of comparators. Ultimately, the comparators that are used should be those best suited to the particular situation in question. FSANZ will assess the appropriateness and acceptability of the selected comparators on a case-by-case basis.

4. UNINTENDED EFFECTS

The genetic modification of an organism by the insertion of DNA sequences to achieve a specific phenotype (the intended effect) carries with it the possibility that unintended effects may occur. An unintended effect could be deleterious, beneficial or neutral with respect to health of the organism or the safety of the foods derived from it. Unintended effects are not restricted to the use of gene technology but can also occur with the use of conventional techniques of genetic modification such as traditional breeding.

Many unintended effects are detected and eliminated in the early stages of research and development, particularly if they impact on the health or fitness of an organism. GM lines exhibiting adverse unintended effects are typically discontinued and would rarely, if ever, be considered for commercialisation. For example, in the case of plants, one of the approaches to manage the problem of unintended effects is to select and discard plants with unusual or undesirable phenotypic and agronomic characteristics early in the breeding process. The practice of successive backcrossing in plants is also a strategy that is used to eliminate undesirable characteristics.

Many unintended effects will also be predictable based on knowledge of the genetic modification and its physiological impacts in the organism or knowledge of the site of insertion, which means it would be possible to devise specific tests to screen for such effects.

Over time, as knowledge of biological systems and genomes increases, and as techniques of genetic modification become more advanced, the predictability of unintended effects is likely to increase, making it easier to screen for such effects or to prevent or minimise their occurrence.

The safety assessment of GM foods involves methods to identify and detect unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. As no individual test can detect all possible unintended effects or identify with certainty those relevant to human health, a variety of data and information is necessary to assess unintended effects. These data and information are considered together in order to assess the likelihood or otherwise of an unintended effect.

A thorough understanding of the genetic modification, as well as the characteristics and function of any novel proteins, is essential to ensure that the most appropriate comparative analyses are undertaken and hence identify any important differences that may need to be investigated before the safety of a food can be established. Where unintended differences are observed, their biological significance should be assessed. If the differences exceed natural variations in conventional foods, further assessment may be required.

Profiling techniques, which can be used at different levels, e.g. genomic, proteomic or metabolic profiling, may enable the detection of differences in a more extensive way than targeted chemical analyses such as is used for the compositional analyses. While such approaches could potentially provide an integrated analysis of gene expression, protein translation and metabolite formation, there remain a number of unresolved questions regarding their ability to detect and highlight differences which are likely to impact on the safety or nutritional value of the food. Additionally there is not yet enough information available on the extent of natural variation to enable the utility of profiling techniques to be fully assessed. Further research is required to provide answers to these fundamental questions before profiling techniques are ready for validation and acceptance procedures to enable them to become a routine data requirement for the safety assessment of GM foods.

5. MOLECULAR CHARACTERISATION

The molecular characterisation is critical to the food safety assessment as it provides an understanding of the genetic material introduced into the host genome and helps to frame the subsequent parts of the safety assessment.

Molecular characterisation typically addresses three main aspects:

- the transformation method together with a detailed description of the DNA sequences introduced to the host genome;
- a characterisation of the inserted DNA including any rearrangements that may have occurred as a consequence of the transformation;
- the genetic stability of the inserted DNA and any accompanying expressed traits.

The main purpose of the molecular characterisation is to define the inserted DNA, and with it the intended effect.

The intended effect is usually one of the most important differences that will be identified, and a determination of the impact of the intended effect on the safety of the food comprises the main part of the overall safety assessment.

While molecular characterisation is the starting point for describing the intended effect, it may also be useful for providing important clues about possible unintended effects. For example, it might be possible to determine the site of insertion and therefore whether any host genes have been disrupted; or whether a mutation has occurred in the coding region of the introduced gene during insertion, which could alter the function and specificity of any encoded protein.

6. SAFETY ASSESSMENT OF NOVEL PROTEINS

There are many examples of GM food plants that express a novel protein as a result of the transfer of new genes. Examples include viral proteins used to protect plants against virus infection, bacterial toxins used to protect plants against insect attack, various enzymes responsible for detoxifying a number of different herbicides, and bacterial enzymes used to alter nutrient composition.

In considering the safety of such proteins it is important to consider that a large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects, although a small number have the potential to impair health e.g. because they are allergens or anti-nutrients. As proteins perform a wide variety of functions in organisms, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutritional and allergenic effects.

To effectively identify any potential hazards requires knowledge of the characteristics, concentration and localisation of all novel proteins expressed in the organism as well as a detailed understanding of their biochemical function and phenotypic effects. For example, in some GM food plants, there is no human exposure to the novel proteins because they are expressed in the non-food parts of the plant. It is also important to determine if the novel protein is expressed as expected, including whether any post-translational modifications have occurred.

6.1 Assessment of Potential Toxicity

Protein is an essential component of the diet. A large number and diverse array of proteins exist in nature and their consumption as part of food is not typically associated with toxic effects. The combination of the physical conditions (e.g. pH) and proteolytic enzymes in the digestive system produces an environment that denatures and degrades the structural integrity and functional activity of most dietary proteins. Protein consumed in the diet therefore has a predictable metabolic fate, where it is typically broken down into its constituent amino acids and small peptides and then readily absorbed. All orally-consumed proteins are subject to the same digestive processes, irrespective of their source or function, including novel proteins expressed in GM foods. Even if a protein were to survive the digestive process, most dietary proteins are sufficiently large that they are unlikely to be absorbed intact by the gastrointestinal tract.

A small number of proteins are however known to be harmful to humans. These proteins are often more resistant to digestive processes, enabling them to exert toxic effects directly on the gastrointestinal tract or to be absorbed into the systemic circulation. The majority of these proteins are produced by micro-organisms and are toxic at low levels. There are also some plant-produced proteins, such as ricin, that are toxic to humans. In addition, some proteins, while not strictly toxins, may have anti-nutritional effects. Therefore, while the vast majority of proteins are innocuous, a small number may be harmful to health.

If the GM food differs from the conventional counterpart food by the presence of one or more novel proteins, these proteins should be assessed for their potential toxicity. The main purpose of an assessment of potential toxicity is to establish, using a weight of evidence approach, that the novel protein will behave like any other dietary protein.

An assessment of potential toxicity of a novel protein should consider the following:

- whether the novel protein has a prior history of safe human consumption, or is sufficiently similar to proteins that have been safely consumed in food;
- whether there is any amino acid sequence similarity between the novel protein and known protein toxins and anti-nutrients; and
- whether the novel protein is resistant to heat or processing and/or to digestion.

If a novel protein is found to have no significant sequence similarity to known protein toxins, is not stable to heat and/or processing, is sufficiently similar to proteins that have been safely consumed in food and is readily digested in conditions that mimic mammalian digestion, it can be reasonably concluded that the protein is non-toxic to humans and no further investigations would be required.

Appropriate oral toxicity studies in animals may also be considered, particularly where results from the biochemical, bioinformatic, digestibility or stability studies indicate a concern. The need for, and nature of such studies, should be discussed with FSANZ prior to submitting an application. Such toxicity tests in animals are done using purified protein that is administered at dose levels considerably higher than the levels normally encountered by humans. Ideally, the protein to be tested should be that which has been directly purified from the new organism. Where this is not possible, usually because it is difficult to obtain sufficient quantities of purified protein, it is essential to ensure that the protein tested is biochemically and functionally equivalent to that expressed in vivo in the GM food.

As part of the assessment of the potential toxicity of a novel protein it is important to also determine if the activity of the novel protein in the organism is likely to produce any secondary effects, such as the accumulation of other substances. If other substances are found to accumulate as a result of the activity of a novel protein, e.g. the accumulation of a metabolite as a result of the detoxification of a herbicide in a plant, it is important to also include an assessment of the potential toxicity of such substances.

6.2 Assessment of Potential Allergenicity

The evaluation of the potential allergenicity of GM foods was the subject of a Joint FAO/WHO Expert Consultation in 2001 (FAO/WHO 2001). The outcome of this consultation was subsequently used by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology to develop guidance on the assessment of possible allergenicity for foods derived from GM plants and micro-organisms, and more recently GM animals.

Food allergy, defined as an adverse immune response to food proteins, affects as many as 6% of young children and 3% to 4% of adults (Sicherer and Sampson 2006). Food-induced allergic reactions are responsible for a variety of symptoms involving the skin, gastrointestinal tract, and respiratory tract and are caused by both IgE-mediated and non-IgE-mediated (cellular) mechanisms. Both FSANZ³ and the Codex Alimentarius Commission have adopted a list of the most common allergenic foods associated with IgE-mediated reactions that includes peanuts, soybeans, milk, eggs, fish, crustacea, wheat, and tree nuts. These foods account for over 90% of all moderate to severe allergic reactions to foods⁴. The most common cell-mediated reaction is known as celiac disease or gluten-sensitive enteropathy. Gluten-sensitive enteropathy is a T-cell mediated response triggered by gluten, a protein found in some cereals, and affects genetically predisposed individuals.

All IgE-mediated food allergens are proteins, but only a small fraction of the many proteins found in food are allergenic. Therefore, even though foods can contain tens of thousands of different proteins, relatively few are allergenic. However, as the use of gene technology can result in additional protein diversity being added to the food supply, the potential allergenicity of any novel protein present in food should be a part of the safety evaluation. This should include consideration of whether:

- a novel protein is one to which certain individuals may already be sensitive; and
- a protein new to the food supply is likely to induce allergic reactions in some individuals.

It should be noted that additional protein diversity could also be introduced into the food supply through conventional breeding techniques. For example, the conventionally bred kiwi fruit has proven to be an additional source of food allergens.

The prediction of allergenic potential of novel proteins is not a simple matter and no single test is currently available that is fully predictive of potential allergenicity. While a number of animal models are currently being investigated for the purpose of predicting allergenicity (Prescott and Hogan 2006, Knippels and Penninks 2005), they are still experimental and are yet to be widely accepted or validated, making them unsuitable for safety assessment purposes at this stage.

³ Table to Clause 4 in Standard 1.2.3 – Mandatory Warning and Advisory Statements and Declarations of the Australia New Zealand Food Standards Code

⁴ Allergic reactions to fresh fruits and vegetables are also common but these have not been included on the Codex list because the symptoms are typically mild and confined to the oropharyngeal region (the oral allergy syndrome) and the allergens are unstable to heating and digestion (Hefle *et al* 1996).

Because of the absence of a suitable single test for predicting allergenicity, it has been recommended that an ‘integrated, stepwise, case-by-case approach’ be used in the assessment of possible allergenicity of novel proteins (Codex 2004b and 2004c). This weight of evidence approach takes into account evidence derived from several types of information and data, since no single criterion is sufficiently predictive. This assessment strategy is not however applicable to assessing whether a novel protein is capable of inducing gluten-sensitive or other enteropathies.

The assessment approach includes consideration of the following⁵:

1. *Source of the novel protein* – it is important to determine if the source of the novel protein is known to cause allergic reactions in humans;

Genes derived from sources known to be allergenic should be assumed to encode an allergen unless demonstrated otherwise.

2. *Amino acid sequence similarity of the novel protein to known allergens* – the amino acid sequences of many allergens are readily available through public domain databases.

Bioinformatic analyses can be done to determine the overall level of similarity. IgE cross-reactivity between the novel protein and a known allergen should be considered a possibility when there is greater than 35% identity in a segment of 80 or more amino acids (FAO/WHO 2001, Codex 2004b and 2004c). Contiguous amino acid segment searches may also be done to identify sequences that potentially represent linear epitopes. The appropriate length of amino acid segment to use should be scientifically validated in order to minimise the occurrence of both false negative and false positive results.

3. *Structural properties of the novel protein* – this includes, but is not limited to, its susceptibility to digestion, heat stability and/or acid and enzymatic treatment;

Resistance to hydrolysis by digestive proteases has been observed in several food allergens (Astwood et al 1996). The standard test that has been advocated to determine susceptibility to digestion is pepsin hydrolysis (Codex 2004b and 2004c, Thomas *et al* 2004). Some allergens however are sensitive to pepsin hydrolysis (Moneret-Vautrin *et al* 1997) therefore, a lack of resistance to pepsin by itself does not exclude that possibility that the novel protein could be a potential allergen. The presence of any post-translational modifications, e.g. glycosylation, and its impact on the allergenic potential of the novel protein would also be a relevant consideration. Glycosylation may affect the susceptibility of a protein to processing and proteolysis and may introduce glycan epitopes, which are known to be highly cross-reactive (FAO/WHO 2001).

⁵ Previously, the level of expression of a novel protein was thought to be an important factor to consider in assessing potential allergenicity (Metcalf et al 1996), however very little information currently exists on threshold levels of proteins required for sensitisation and subsequent elicitation of an allergic response. As a consequence, it is currently not possible to consider the level of expression of a novel protein as a relevant factor in the assessment of potential allergenicity. This may change in the future as knowledge improves.

4. *Specific serum screening* – this should be undertaken where a novel protein is derived from a source known to be allergenic or has amino acid sequence similarity with a known allergen.

Specific serum screening involves evaluating the immunoreactivity of the novel protein with IgE antibodies from the sera of individuals allergic to the known allergen. Such tests are contingent on the availability of good quality sera from well characterised patients. Additional testing, for example using skin prick tests or *ex vivo* protocols, may be necessary to confirm a negative result from the serum screening.

The exposure to the novel protein and the effects of relevant food processing will contribute to an overall conclusion about the potential human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing which would be applied and its effects on the presence of the protein in the final food product.

In cases where the introduced genetic material is obtained from wheat, rye, barley, oats or related cereal grains, any novel proteins should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy.

If the assessment of potential allergenicity results in a conclusion that the novel protein is a potential allergen then FSANZ would take appropriate regulatory action to manage the risk to susceptible population groups. The appropriate risk management approach would need to be determined on a case-by-case basis, however it is unlikely that approval would be given for a new, allergenic food that is likely to be widely distributed throughout the food supply.

7. COMPOSITIONAL ANALYSES

The main purpose of compositional analysis is to determine if any unexpected changes in composition have occurred to the food and to establish its nutritional adequacy.

Compositional analysis can also be important for evaluating the intended effect where there has been a deliberate change to the composition of food. Analysis of the composition of the food in these circumstances helps to confirm that the trait is being expressed appropriately, and also helps to quantify the magnitude of the change, which may be important for assessing safety.

Compositional analysis of food is not a simple matter and is often limited by the complexity of the food matrix being evaluated, and by available analytical methodologies. It is therefore important that appropriate analytical methods are used for the compositional analysis and that the sensitivity of such methods is documented.

The classic approach to the compositional analysis of GM food is a targeted one; rather than analysing every single constituent, which would be impractical, the aim is to analyse only those constituents most relevant to the safety of the food or that may have an impact on the whole diet. Important analytes therefore include the key nutrients, toxicants and anti-nutrients for the food in question. The key nutrients and anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates or enzyme inhibitors as anti-nutrients) or minor constituents (minerals, vitamins).

Key toxicants are those toxicologically significant compounds known to be inherently present in an organism, such as compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes)⁶.

Depending on the nature of the genetic modification, or the characteristics of a novel protein, additional constituents may need to be analysed. This will need to be determined on a case-by-case basis. For example, if a gene is transferred which results in increased production of a particular nutrient (e.g. the amino acid lysine), the levels of metabolic products resulting from that nutrient should also be determined and compared to an appropriate comparator; or introduction of an invertase into potatoes could influence carbohydrate metabolism, therefore this should be thoroughly investigated.

Analyses of concentrations of key components of the GM food should be compared with an equivalent analysis of food derived from an appropriate comparator (usually the non-GM counterpart) produced under the same conditions. Ideally, the comparator used for this assessment should be food derived from a near isogenic line. This may not be feasible in all cases, in which case a line as close as possible should be chosen. The relevance of any observed differences should be assessed in the context of the range of natural variation for that parameter to determine its biological significance. Varieties assessed should include those with a history of safe use as food.

It is important to recognise that food composition is not just determined by the genetics of an organism but can also be influenced by environmental factors. For example, the mineral composition of many plant derived foods is heavily influenced by soil type and fertiliser practices. In the case of animals, diet is known to influence the composition of food products. For example, supplementation of the diet of chickens with omega-3 fatty acids has been used to increase the omega-3 content of eggs. Studies for the collection of compositional data should be designed and conducted in such a way to control for these environmental influences.

For GM plants, field trial sites should be representative of the range of environmental conditions under which the plant would normally be grown. The number of trial sites should be sufficient to allow accurate assessment of phenotypic/agronomic characteristics over this range and an adequate number of plants should be sampled. Each trial site should include replicates. In designing field trials, a comparison with the GM plant grown under its expected agronomic conditions may need to be considered. For example, in the case of herbicide tolerant plants, comparisons should be done between the comparator plant and both herbicide-treated and untreated GM plants.

For GM animals, the comparator used for the assessment should ideally be matched in housing and husbandry conditions, breed, age, sex, parity, lactation, or laying cycle (where appropriate), although this may not be feasible in all cases. It is recognised that, particularly in the case of certain species, the available number of samples for compositional analysis may be limited and there is likely to be large variation between animals, even those bred and raised under the same husbandry conditions.

⁶ The OECD has developed a series of Consensus Documents to aid in the compositional analysis of foods derived from GM plants. These documents provide information on the key constituents for particular crops and also provide baseline data on the concentration range for each constituent.

8. NUTRITIONAL CONSIDERATIONS

At present, there are no GM foods on the market that have been developed specifically with the aim of improving human health, however, several plants with intentionally altered nutrient composition have been developed using gene technology. For example, soybean oil with increased levels of oleic acid has been produced which is reported to have improved cooking properties as well as possible nutritional benefits (Kinney and Knowlton 1998). Other GM foods, not yet commercialised, include: 'golden rice', a genetically modified variety of rice producing the vitamin A precursor, β -carotene (Ye *et al* 2000); cottonseed oils with altered fatty acid profiles (Liu *et al* 2002); tomatoes with increased lycopene content (Mehta *et al* 2002); and rice with elevated iron levels (Goto *et al* 1999, Lucca *et al* 2002).

If the compositional analysis indicates biologically significant changes to the levels of certain nutrients in the GM food, additional nutritional assessment should be undertaken to assess the consequences of the changes and determine whether nutrient intakes are likely to be altered by the introduction of such foods into the food supply. This assessment should include consideration of the bioavailability of the modified nutrient.

If necessary, FSANZ will undertake a dietary exposure assessment for the nutrients in the GM food using a custom-made computer program, DIAMOND, which combines food consumption data from the latest Australian and New Zealand National Nutrition Surveys together with food nutrient composition data. This dietary exposure assessment will be used to assess the nutritional implications of the altered nutrient profile and to determine the potential for any adverse nutritional outcomes.

When the modification results in a food with a composition that is significantly different from the conventional counterpart food, it may be appropriate to use other conventional foods as comparators that are better matched in terms of nutrient composition to assess the nutritional impact of the food. Such comparators should have a history of safe use, but they do not need to be closely related as, in this instance, they are being used to further characterise a change that has already been identified through earlier comparisons.

It should be noted that significant nutritional changes to food can also be introduced using conventional breeding techniques. An assessment of nutritional impact is important for all significant dietary changes and is not specific only to the introduction of GM foods.

9. GENETICALLY MODIFIED MICRO-ORGANISMS

The safety assessment of foods derived from GM micro-organisms was the subject of a Joint FAO/WHO Expert Consultation in 2001 (FAO/WHO 2001). The outcome of this consultation was subsequently used by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology to develop the Guideline for the Conduct of Food Safety Assessment of Foods Produced Using Recombinant-DNA Micro-organisms (Codex 2004c).

While the safety assessment of food produced using GM micro-organisms can generally be conducted using the same approach and framework applied to food from GM plants, a number of additional issues require special consideration (FAO/WHO 2001, Codex 2004c).

Of particular importance is the need to establish whether the GM micro-organism itself will become a component of the food, as would be the case for starter cultures and probiotics, or whether the GM micro-organism will be subsequently removed from the food, e.g. through filtration. The presence or absence of viable organisms in the food will determine the range of issues to be considered in the safety assessment.

Where viable organisms remain in the final food (e.g. yoghurt) the additional issues that should be considered are:

1. antibiotic resistance and gene transfer;
2. immunological effects;
3. potential pathogenicity; and
4. viability and residence in the digestive tract.

9.1 Antibiotic Resistance and Gene Transfer

There are well-known mechanisms for transfer of genetic material between micro-organisms, such as transduction and conjugation. The probability of gene transfer in the digestive tract has to be assessed in the light of the nature of the GM micro-organism and the characteristics of the gene construct. Possible consequences of a transfer event should be assessed based on the function of the transferred gene.

The likelihood of maintenance of the transferred gene in a recipient micro-organism increases if the gene confers a selective advantage to the micro-organism. Factors that may enhance the selective advantage over other organisms or the colonisation ability include: phage resistance, virulence, adherence, substrate utilization or production of bacterial antibiotics. If the function of the gene suggests that survival of the recipient organism would be enhanced, the possible health consequences need to be assessed, based on the function and specificity of the gene.

The design of gene constructs for micro-organisms should be directed towards minimizing intrinsic traits that allow them to transfer genetic information to other micro-organisms.

Selectable marker genes that encode resistance to clinically useful antibiotics should not be used in micro-organisms intended to be present as living organisms in food. Food components obtained from micro-organisms that encode such antibiotic resistance genes should be demonstrated to be free of viable cells.

Strains in which antibiotic resistance is encoded by transmissible genetic elements should not be used where such strains or these genetic elements are present in the final food. Any indication of the presence of plasmids, transposons, and integrons containing such resistance genes should be specifically addressed.

The transfer of plasmids and genes between the resident intestinal microflora and ingested recombinant-DNA micro-organism may occur. The possibility and consequences of gene transfer from GM micro-organisms and food products produced by GM micro-organisms to gut micro-organisms or human cells should also be considered. Transferred DNA would be unlikely to be maintained in the absence of selection pressure. Nevertheless, the possibility of such events cannot be completely discounted.

The possibility of gene transfer can be minimised through:

- chromosomal integration of the inserted genetic material;
- avoidance of genes that may provide a selective advantage to recipient organisms in the event of gene transfer;
- avoidance of sequences that may mediate integration of the introduced genetic material into other genomes.

9.2 Immunological Effects

Micro-organisms that remain viable in foods may interact with the immune system in the gastrointestinal tract. The need to examine these interactions in the case of GM micro-organisms will depend on the nature of the genetic modification as well as any identified differences between the GM micro-organism and its conventional counterpart.

9.3 Potential Pathogenicity

GM micro-organisms intended for use as food or in food processing should be derived from organisms that are known, or have been shown by appropriate tests in animals, to be free of traits that confer pathogenicity.

9.4 Viability and Residence in the Digestive Tract

Permanent, life-long colonisation of the digestive tract by ingested micro-organisms is rare, however, the possibility remains that an ingested GM micro-organism may influence the gastrointestinal microflora of the human host (FAO/WHO 2001). For this reason, the viability and residence of the GM micro-organism may need to be examined. The need for such an assessment should be based on the presence of the conventional counterpart in the food, and the nature of the intended and any unintended effects of the genetic modification.

If processing of the final food eliminates viable micro-organisms (such as baking), or if accumulation of end-products toxic to the micro-organism (such as alcohol or acids) eliminates viability, then viability and residence of the GM micro-organism in the digestive tract need not be examined.

Where the GM micro-organism remains viable in the final food products, such as would be the case for some dairy products, the viability or residence time of the micro-organism alone and within the respective food matrix in the digestive tract and the impact on the intestinal microflora should be determined using appropriate systems. The nature of the intended and unintended effects of the genetic modification and the extent of any differences from the conventional counterpart will determine the extent of any such studies.

10. GENETICALLY MODIFIED ANIMALS

The safety assessment of foods derived from GM animals has been the subject of two separate Joint FAO/WHO Expert Consultations; one in 2003 (FAO/WHO 2004), which examined strategies applicable to the food safety assessment of foods derived from GM animals; and one in 2007 (FAO/WHO 2007), which addressed specific technical questions raised by the Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology.

The 2003 consultation concluded that the food safety assessment of foods derived from GM animals can largely be performed along the lines that have already been established for food from GM plants, using a comparative safety assessment approach.

One of the key differences between plants and animals is that animals that have a history of safe use as sources of food generally do not contain genes encoding for toxic proteins (or producing toxic end-products). This is in contrast to plants which can be ‘healthy’ yet still produce compounds (e.g. natural toxicants, anti-nutrients) that are harmful to human health. Because of this, the health of a conventional animal has traditionally been used as a useful indicator of the safety of derived foods. The practice of only allowing animals with known and acceptable health status to enter the human food supply is an essential step to ensuring safe food. This practice can also be applied to GM animals.

In assessing the safety of food derived from GM animals the approach should therefore take into account: (i) the nature of the DNA construct and its expression products, if any; (ii) the health status of the GM animal; and (iii) the composition of foods derived from the animal, including key nutrients.

11. OTHER CONSIDERATIONS

11.1 Whole Food Toxicity Studies

Toxicity testing in animals is routinely used in chemical risk assessment, where its primary purpose is to characterise the intrinsic toxicity of chemical substances (e.g. food additives, pesticides, chemical contaminants) in certain laboratory animal species, and thereby assist in predicting adverse effects in humans.

The chemical substances that are the subjects of such testing regimes are usually well characterised, of known purity, have no particular nutritional value and human exposure levels tend to be relatively low. Such substances can therefore be fed to animals at a range of doses, some several orders of magnitude greater than the expected human exposure levels, to identify any potential adverse effects of relevance to humans. In most circumstances, it is possible to determine levels of exposure at which adverse effects are not observed, and so set safe upper limits by the application of appropriate safety and/or uncertainty factors.

In contrast, using animal toxicity testing for assessing the safety of whole foods presents a number of difficulties, which are well recognised and described (FAO/WHO 2000, Codex 2004b and 2004c). Foods are complex mixtures of chemical substances, varying widely in both their composition and nutritional value. Due to their bulk and effect on satiety, they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. A key factor to consider in designing and conducting toxicity studies on whole foods is the nutritional value and balance of the diets used. If these are not carefully selected, it can lead to nutritional imbalances in the animals, which in turn can induce a range of adverse effects not related directly to the food itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can in practice be extremely difficult.

FSANZ considers that a scientifically-informed comparative assessment of GM foods with their conventional counterparts can generally identify any potential adverse health effects or differences requiring further evaluation.

In the majority of circumstances, animal toxicity studies with whole foods are not likely to contribute any further useful information to the safety assessment and are therefore not warranted. As a consequence, FSANZ does not require that animal toxicity studies with GM foods be undertaken on a routine basis.

FSANZ acknowledges there may be future GM applications, particularly for foods with intentional modifications to composition, where the results of animal toxicity studies may be informative. FSANZ will therefore continue to assess the need for whole food studies on a case-by-case basis, taking into account the nature of the genetic modification and the results of the comparative assessment. Potential applicants are encouraged to discuss their application with FSANZ prior to submission to clarify whether such information may be required in the application.

While FSANZ does not routinely require animal toxicity studies to be undertaken, where such studies already exist, Applicants are expected to provide these to FSANZ to evaluate as additional supporting information.

These guidelines are consistent with the recommendations of an expert panel convened by FSANZ to develop guidance and recommendations on the role of animal feeding studies in the safety assessment of whole GM foods. The expert panel met in June 2007 and made a number of recommendations, including that FSANZ continue the case by case assessment of GM foods on the basis of best available science. The panel noted that whole-food animal feeding studies may be informative in some limited circumstances, but that any potential adverse health effects can generally be identified by a scientifically-informed comparative assessment of the GM food against its conventional counterpart. The panel further recommended that, where available, FSANZ evaluate the results of relevant animal feeding studies, while considering the potential limitations in interpretation of the results. A report of the workshop is available on the FSANZ web site.

11.2 Horizontal DNA Transfer

Horizontal DNA transfer is the non-sexual transfer of DNA from one organism to another (Nielsen *et al* 1998).

For GM foods, the following two issues are frequently raised in relation to horizontal DNA transfer:

1. that novel DNA from GM foods could transfer to human cells; and
2. that antibiotic resistance genes from GM foods could be transferred to micro-organisms inhabiting the human digestive tract.

In considering both issues it is important to note that humans have always consumed large amounts of nucleic acid as a normal component of food. Any concerns over the presence of novel DNA in GM foods must take into consideration that this DNA would represent only a minute fraction of the total DNA consumed on a daily basis. Thus, on this basis alone, the probability of transfer of novel DNA from GM foods to human or microbial cells would be extremely low.

The major concern in relation to horizontal DNA transfer relates to the use of antibiotic resistance genes. In particular, whether antibiotic resistance genes present in some GM foods could transfer to disease-causing bacteria in the human digestive tract thus compromising the therapeutic use of antibiotics.

Antibiotic resistance genes can be intentionally linked to the gene responsible for conferring the desirable trait, e.g. insect-protection. Their purpose is to provide a selective advantage to the cell that harbours the gene of interest so that only those cells will grow and divide in the presence of the antibiotic, and so are known as ‘selectable marker genes’.

In assessing whether there is likely to be any impact on human health from the use of an antibiotic resistance gene as a selectable marker, two issues should be considered. The first consideration should be the probability that the gene would be successfully transferred to microbial cells under normal circumstances of dietary exposure. The second, and perhaps more important, consideration should be the potential impact on human health should such a transfer occur.

Horizontal DNA transfer of antibiotic resistance genes from food products to gut micro-organisms is regarded as a rare possibility because of the many complex and unlikely events that would need to occur consecutively (see for example WHO 1993, Nielsen *et al* 1998 and WHO 2000)⁷. Furthermore, the transfer of antibiotic resistance genes from GM food to bacteria has not been observed under natural conditions. (Gay and Gillespie 2005). While horizontal DNA transfer would be expected to be extremely rare, its occurrence cannot be completely ruled out, therefore the potential human health impact, should such a transfer occur, needs to be considered in any safety assessment.

The human health considerations will largely depend on the nature of each particular antibiotic resistance gene and must be assessed on a case-by-case basis. However, in the case of the commonly used kanamycin, ampicillin and streptomycin resistance genes, it is unlikely that their transfer to bacteria in the human digestive tract would have any significant health impact because bacteria harbouring resistance to these antibiotics are already widespread in nature or are found to naturally inhabit the human digestive tract (Smalla *et al* 1993, Calva *et al* 1996, Shaw *et al* 1993, Smalla *et al* 1997). Furthermore, kanamycin/neomycin and streptomycin are rarely used clinically because of unwanted side effects (WHO 1993) and ampicillin has now largely been replaced by more potent forms of β -lactam antibiotics or is only used in combination with drugs that work to inactivate β -lactamase (Walsh 2000). As a precaution, however, the use of marker genes encoding resistance to antibiotics with significant public health uses (e.g. vancomycin) should be avoided.

However, it should be recognised that not all GM foods contain antibiotic resistance genes. In some cases, these genes are removed after successful transformation. In other cases alternative selectable marker genes may be used (e.g. herbicide tolerance genes are frequently used for selection purposes in plants). With time, it is anticipated that the presence of antibiotic resistance genes in GM foods will become less commonplace.

⁷ This does not apply in the case where the food is or contains a viable GM microorganism carrying an antibiotic resistance gene. There are well-known mechanisms of transfer of genetic material between microorganisms, such as transduction and conjugation, therefore the probability of horizontal DNA transfer will be significantly increased. This special case is discussed in the section on GM microorganisms.

If alternative marker genes are used, they also need to be evaluated for their safety in the same way as for any other novel gene.

11.3 Post-market Monitoring

Post-market monitoring is often suggested as a means of demonstrating whether long-term adverse health effects may be associated with the consumption of GM foods.

It has been recognised internationally that the use of pre-market safety assessment already provides assurance that a GM food is comparable to its conventional counterpart in relation to health risks and benefits, therefore the likelihood of identifying long-term effects specifically attributable to GM foods would be very low (WHO 2000). Moreover, the practicality of using post-market monitoring to assess the long-term human health impacts of consuming GM foods has not been established.

Many chronic health problems have complex causes and it is unlikely that observational epidemiological studies could identify such effects specifically related to GM foods. The same also applies to the identification of potential long-term beneficial health effects.

In general, therefore, FSANZ does not consider post-market monitoring to be a practical, enforceable or effective risk management option. This is particularly the case where passive monitoring or general health surveillance, which does not address a specific hypothesis, is proposed.

Nevertheless it is recognised that post-market monitoring may be an appropriate risk management measure in certain circumstances. Post-market monitoring may be useful in situations where a GM food has been developed specifically to produce a nutritional effect in the population. In these cases it may be desirable to monitor changes in nutrient intake levels in order to confirm assumptions made during the risk assessment and evaluate their potential effect on the nutritional and health status of the population.

The need for post-market monitoring following approval of a GM food will be considered by FSANZ on a case-by-case basis, taking into account the unique characteristics of the GM food and the feasibility of undertaking such a study.

11.4 Animal Feeds

Many animal feeds are derived from the same GM commodities that are used for human consumption and concerns are occasionally expressed that this practice may pose an indirect risk to humans through consumption of the meat, milk and eggs derived from such animals.

The OECD has produced a paper as part of their series on the safety of novel foods and feeds entitled 'Considerations for the safety assessment of animal feedstuffs derived from genetically modified plants' (OECD 2003). This paper specifically examined potential hazards to humans from the presence of novel DNA and protein in animal feedstuffs. They noted that both DNA and protein are extensively digested when consumed by animals.

It was concluded that while fragments of plant genomic (non-GM) DNA have been detected in animal food products such as milk, there is no basis to suppose that recombinant DNA poses hazards any different to other sources of DNA and the possibility of incorporation of functionally intact DNA or protein derived from GM feeds into animal products is extremely remote.

In addition to the above report, an overview was conducted of regulatory assessments of GM foods and to summarise empirical data generated for assessing the safety of meat, milk and eggs derived from animals fed GM crops that express agronomic input traits (e.g. herbicide tolerance) (CAST 2006). It was concluded that there are no effects from feeding GM plant material to livestock and poultry on the nutritional value or safety of the meat, milk and eggs derived from those animals. Moreover, because most components of feeds are broken down into smaller components during digestion by the animal, proteins and DNA derived from the GM plants cannot be detected in milk, meat or eggs.

The European Food Safety Authority has also recently prepared a literature survey on the fate of recombinant DNA or proteins in meat, milk and eggs from animals fed with GM feed (EFSA 2007). On the basis of this literature survey, EFSA concluded that: (i) DNA and protein are common constituents of foods and feeds and such substances are rapidly degraded into small fragments upon digestion by animals and humans; and (ii) a large number of experimental studies with livestock have shown that recombinant DNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible products of farm animals or other livestock.

In relation to foods and feeds derived from GM plants, the current approach taken by FSANZ is to avoid 'split use' approvals. A 'split use' approval is where a GM plant receives approval for use as animal feed but not for human food. This approach is also practiced in the United States and Canada, which are sources of imported GM foods and food ingredients into Australia and New Zealand. The practice of not allowing 'split-use' approvals arose following an incident in the United States where traces of a GM corn (known as StarLink™ corn), which had been approved for animal feed only, were found in human food products. The incident caused widespread consumer concern and significant disruption to trade and highlighted the impracticality of a split approval process without well-developed identity preservation and segregation systems. To prevent similar incidents occurring in the future it is now common practice for GM plants intended primarily for feed use to also undergo food safety assessment and approval for human food use. This minimises the risk of unassessed and unapproved products entering the food supply as a result of inadvertent co-mingling of grain/seeds during transport and storage, and also ensures that their use as feed will not pose indirect risks to humans.

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