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Assuring the safety of genetically modified (GM) foods: the importance of an holistic, integrative approach

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Abstract

Genes change continuously by natural mutation and recombination enabling man to select and breed crops having the most desirable traits such as yield or flavour. Genetic modification (GM) is a recent development which allows specific genes to be identified, isolated, copied and inserted into other plants with a high level of specificity. The food safety considerations for GM crops are basically the same as those arising from conventionally bred crops, very few of which have been subject to any testing yet are generally regarded as being safe to eat. In contrast a rigorous safety testing paradigm has been developed for GM crops, which utilises a systematic, stepwise and holistic approach. The resultant science based process, focuses on a classical evaluation of the toxic potential of the introduced novel trait and the wholesomeness of the transformed crop. In addition, detailed consideration is given to the history and safe use of the parent crop as well as that of the gene donor. The overall safety evaluation is conducted under the concept known as substantial equivalence which is enshrined in all international crop biotechnology guidelines. This provides the framework for a comparative approach to identify the similarities and differences between the GM product and its comparator which has a known history of safe use. By building a detailed profile on each step in the transformation process, from parent to new crop, and by thoroughly evaluating the significance from a safety perspective, of any differences that may be detected, a very comprehensive matrix of information is constructed which enables the conclusion as to whether the GM crop, derived food or feed is as safe as its traditional counterpart. Using this approach in the evaluation of more than 50 GM crops which have been approved worldwide, the conclusion has been that foods and feeds derived from genetically modified crops are as safe and nutritious as those derived from traditional crops. The lack of any adverse effects resulting from the production and consumption of GM crops grown on more than 300 million cumulative acres over the last 5 years supports these safety conclusions.

Keywords: Safety; Assessment; Biotechnology; Genetic modification; Novel food

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1. Introduction

He who has bread may have troubles,
He who lacks it has only one,
Old Byzantine proverb

Food and water are fundamental to life. Both have been key drivers in our evolution as hunter-gatherers. Lurking never far away have been the ongoing threats of hunger, drought, crop loss and famine. Migrations and wars have often been initiated by inadequate food and feed supply and mankind has continuously sought to improve food security by experimenting by trial and error with the foods provided by nature, both plant and animal. Some have proved safe and wholesome, others inedible or toxic. Medicinal properties have also been discovered and many products of this biodiversity have been utilised for healing. The consumption of many foods is often deeply rooted in traditions which may require special preparation processes such as selection, cooking or slaughtering to render them safe or acceptable. Domestication of crops over centuries has today resulted in our knowledge of those crops which are generally regarded as safe to eat based on a long tradition and history of safe use.

Nevertheless, the demands of a rapidly growing world population has exerted increasing pressure on the earth's resources and more and more land has been taken into cultivation. The world population at the beginning of the last century was just under 2 billion and had tripled to 6 billion by the year 2000. Estimates predict that the population will increase by half as much again to 9 billion by the year 2040 or 2050 (OECD, 2000). New approaches will be required to expand food production from ca. 5 billion tons per year by at least another 50% over the next 3 or 4 decades, while maintaining or indeed improving environmental diversity and respecting social structures.

Nature evolves by the process of natural selection of the fittest organisms that survive in a given environment. Organisms vary one from the other as a consequence of natural mutation and genetic recombination. In mans' quest for food security, farmers and their forebears have accelerated genetic modification (GM) through recurrent selection of the most high yielding, hardy and pest resistant plants. Since Gregor Mendel's seminal work in the 19th century, a greater understanding of genetics has further accelerated this process. More recently, radiation and chemical mutagenesis have been used to increase the number and variety of crops which might have desirable traits. One of the most popular malting barleys, 'Golden Promise' was produced in 1957 using radiation mutagenesis (Technical Brochure, 1970). This became the leading malting barley for many years.

Following the discovery of the structure of DNA by Watson and Crick in 1953, developments in molecular biology have been rapid in both the plant and animal kingdoms. Work on plant and human genomes has proceeded in parallel and progress in the former is leading to a greater understanding of agronomic performance and phenotypic appearance through studies of genomics, proteomics and metabolomics. This has allowed breeders to identify the genes associated with specific desirable traits which would provide major opportunities for crop improvement. Recombinant DNA methods then enable the transfer of single genes from close or distant species. Such knowledge also allows the identification and potential removal of undesirable traits, such as those responsible for peanut allergy or coeliac disease. To date, modifications have resulted in benefits mainly orientated to the farmer and the environment with only indirect benefit to consumers resulting from, for example, pesticide reduction (Gianessi and Carpenter, 2001). However, a number of second generation genetically modified products with consumer related benefits, including nutritional and other health related characteristics, are just starting to make an appearance. For example, stearic acid content has been increased in corn and canola oils to make foods that are suitable for certain applications without the need for chemical hydrogenation and the production of trans fatty acids (Riley and Hoffman, 1999; Mazur et al., 1999). Other traits likely to result from agricultural biotechnology are new varieties with greater tolerance to drought, water-logging, salinity, heat and cold, each of which, by using poor agricultural land, will be valuable to meet the growing food demand and to maintain wilderness and hence biodiversity for the future.

The first genetically improved crop was the Flavr Savr™ tomato which was approved for sale in the United States of America in 1994. Since then the uptake of agricultural biotechnology forfood, feed and fibre production has been prodigious, with probably the most rapid adoption of new varieties in the history of agriculture. Between 1996 and 2000 transgenic plantings increased globally from 4.2 to 104.7 million acres (James, 2000) an area approximately twice the size of the United Kingdom (Fig. 1).

The introduction of this new technology resulted in appropriate regulatory requirements and guidelines to provide the framework for the development, testing and safe use of these 'so-called' genetically modified organisms (GMOs), as well as protection for people and the environment. Notwithstanding, there has been a high level of public concern emanating from a number of sources and varying qualitatively and quantitatively from region to region. The debate normally rotates on two major considerations—safety and concerns over technology access resulting from intellectual property ownership. This paper focuses on the former topic.

2. Evolution of safety assessment procedures for whole foods and defined chemical substances in the diet

Today, very few traditional varieties of food crop that are consumed have been subject to systematic toxicological evaluation, yet because of their history of use, they are generally regarded as safe to eat. The OECD addressed this in 1991 and concluded that a food is safe if 'there is reasonable certainty that no harm will result from its consumption under anticipated conditions of use', (FAO/WHO, 2000). In contrast, single, defined chemical substances in the diet are required to undergo classical toxicological testing, case by case using in vitro and in vivo techniques as surrogates for man. Aspects of both approaches are incorporated into testing procedures for GM crops, which results in a very robust safety assessment process.

2.1. Testing of whole foods

It is generally not possible to apply a typical toxicological evaluation exploring the effect of 50–100 times the normal dietary level because of the natural bulkiness of food, the need to

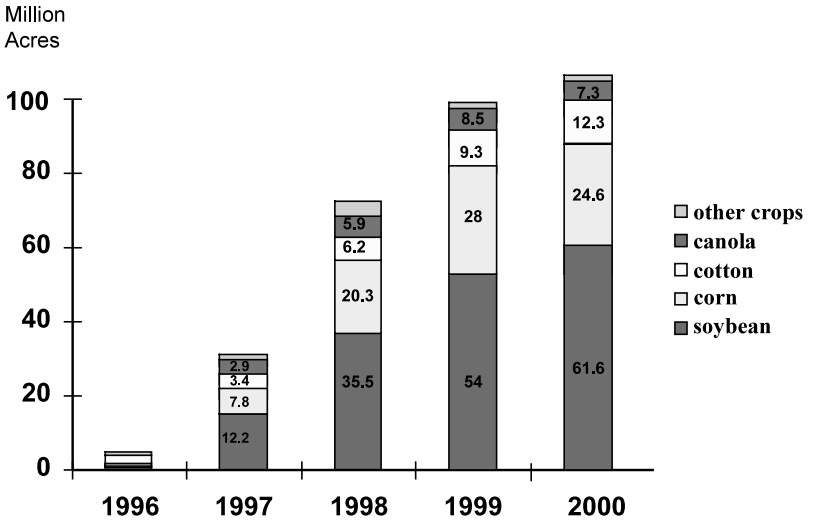


Fig. 1. Global area of GM crops grown since 1996. Source: ISAAA (2000).

Table 1
Whole food toxicological testing—limitations

Foods	Defined single substances, e.g. chemicals
Complex mixture small and large molecules	Single small molecules
Identity of most molecules unknown	Identity known, high purity
'Batch' variation in composition (ripeness, location etc.)	Batch consistency
Nutritional component	None
Bulk from biomass	None
Dose limitations due to bulk	None
Difficulty of pinpointing toxicants	Usually straightforward
Viable	Non-viable
Safety assessment	Risk assessment

maintain nutritional balance and its effects on satiety. In consequence, at the resulting low multiples that can be achieved it is difficult to detect toxicological effects reliably, and this has been well recognised (OECD, 1996; WHO, 1995) (Table 1). In consequence it was necessary to develop a new methodology for GM crops built on established science-based precepts.

It has been argued that each food constituent should be individually assessed. However, as each food contains thousands of natural substances, most of which are safe and many of which are unidentified, this would be a virtually impossible, uneconomic and unnecessary task. Therefore, it has been recommended internationally that efforts should focus on targeting defined substances of specific interest, for example those identified as important from a nutritional, anti-nutritional or toxic perspective and assess any impacts if their levels are changed.

2.2. Testing of chemical substances in the diet

Chemicals in foods fall into several categories and include pesticide residues, antibiotic residues, animal growth promoters, food additives, food supplements, mycotoxins, antinutrients as well as substances migrating into the food from packaging material. Each of these categories of defined chemical substances is separately regulated and evaluated using the traditional risk assessment process which relies on toxicity testing of the individual chemicals in animals at intake levels many times higher than is likely in humans.

This is necessary to identify any potential adverse effects and the results are taken into account for establishing the safe level for man. Typically the dose level at which no observed adverse effects are seen, the no-observed-adverse-effect-level (NOAEL) in the most sensitive test species, is used together with exposure data to define the 'acceptable daily intake' for man using appropriate factors, known as Margins of Safety, usually $\times 100$ to generate very large margins of safety for human use.

This approach (Fig. 2), used for single defined chemical substances in the diet, is relevant to the direct safety assessment of the gene product(s) in the case of GM crops.

3. Safety assessment for GM crops and foods

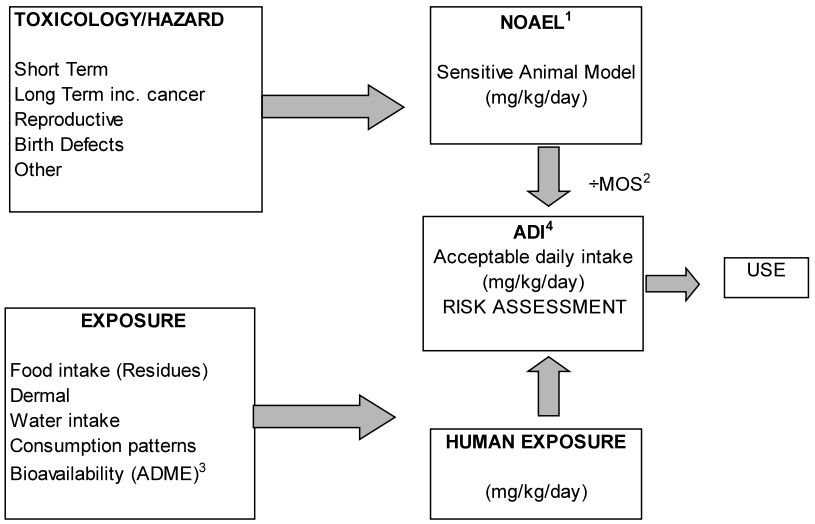
Food safety is a shared responsibility of industry, farmers, and regulatory authorities. As there is normally no history of safe use for a novel food or food derived from a GM crop, but may be available for both the conventional food and the introduced protein, science based assessment is necessary. In considering the requirements, it is essential that the testing procedures should be proportionate to the nature and magnitude of the risk while maintaining a high level of safety assurance for the consumer. This is not only important to gain consumer confidence but also to facilitate regulatory harmonisation, to move towards the principle of subsidiarity, that is the mutual acceptance of data, as a minimum within European Member States but ideally between major global regulatory authorities. The issues associated with applying traditional testing methodologies to conventional crops and whole food are added to in the case of GM crops by the presence of a novel component(s), the inserted trait. This has resulted in the development of a novel paradigm which is essentially a hybrid of the safety evaluation process for single defined dietary chemical sub-

stances, in this case the introduced trait, and the whole food derived from the GM crop containing the trait (Fig. 3). Due to the complexity of whole foods, the goal of the assessment is not to prove absolute safety, but to establish whether the food from the GM crop is as safe as its traditional counterpart.

In considering how a novel paradigm for the safety assessment of novel foods might be structured, it was necessary to take into account the fact that like traditional breeding, GM has the potential to alter the safety of foods. The newly introduced protein might theoretically be associated with toxicity or allergenicity and changes could occur resulting from insertion or pleiotrophy leading to relevant toxicological effects. The potential for insertional effects is not new and is known from traditional breeding. Moreover, enormous random changes can result from chemical and irradiation mutagenesis which is also traditionally used for crop breeding. In contrast to both these techniques, where perhaps curiously there are no formal food safety assessment requirements, rigorous testing is undertaken for crops derived from modern agricultural biotechnology.

4. Basic principles for designing an holistic integrated novel safety testing paradigm for GM crops

In developing a safety assessment paradigm for foods derived from GM crops it is essential to proceed sequentially through a number of steps to ensure a holistic approach to safety assessment which is fully integrated and draws on



[1]NOAEL no-observed-adverse-effect-level

[2]MOS margin of safety, usually X100

[3]ADME absorption, distribution, metabolism and excretion

[4]ADI acceptable daily intake

Fig. 2. Hazard and risk assessment for a defined single substance, e.g. food ingredient or pesticide.

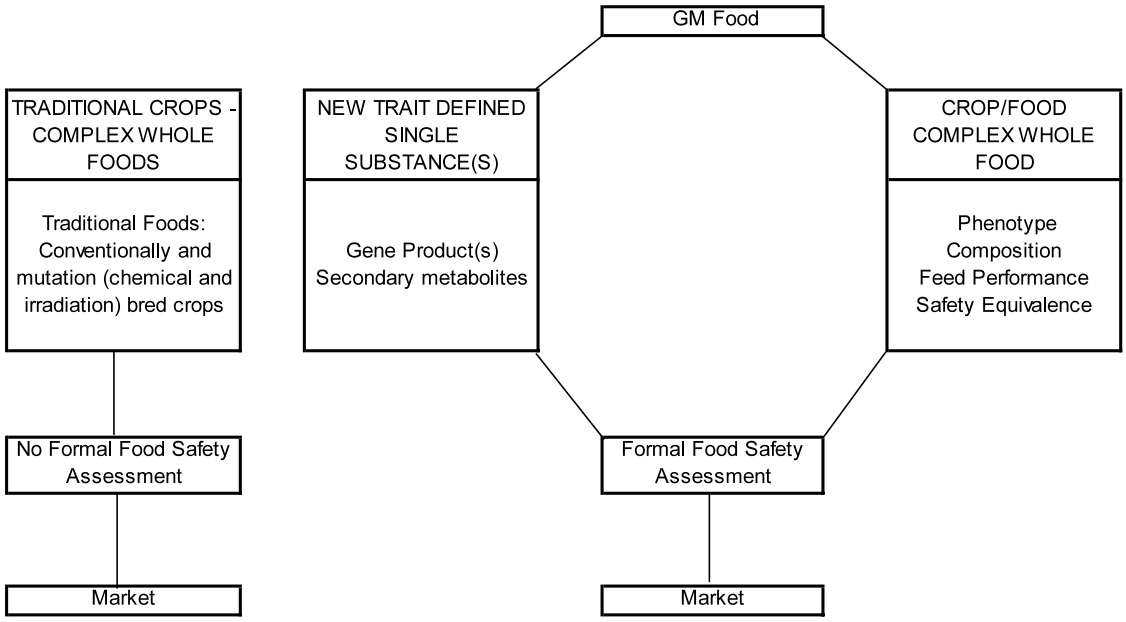


Fig. 3. Evolution of food safety assessment procedures for genetically modified crops.

the extensive experience gained from food, drug and chemical safety evaluation over the last 50 years or so.

4.1. Consideration of sources of potential hazard

As a starting point it is essential to map the sources of potential hazard involved in the process of transforming a crop by genetic engineering. The sources of hazard derive from the four principal components of the transformation process:

- The parent (host) traditionally bred crop.
- The gene donor.
- The primary gene product(s) and any resulting novel secondary metabolites.
- The transformed crop.

4.2. Consideration of types of potential hazard

Hazard, which may be defined as the intrinsic potential of a material to cause adverse health effects, may theoretically result from: (1) toxicity including allergenicity; (2) nutritional change or antinutrient effects; and (3) the remote possibility of gene transfer to bacteria or mammalian cells.

4.3. Consideration of the targets for the potential hazards

The scope of this paper is restricted to safety assessment of genetically modified crops and their use for human and animal food and feed. For this reason environmental impact is not covered. In consequence, the principal targets for the potential hazards are:

- Farmers.
- Processors.
- Consumers.
- General public.
- Food producing (farm) animals.

Nevertheless, it needs to be recognised that none of these potential targets become a risk from the hazard unless exposure takes place. Risk is defined as the likelihood that, under particular conditions of exposure, an intrinsic hazard will represent a threat to human health, the relationship is summarised as:

$$\text{Risk} = f(\text{hazard} \times \text{exposure}),$$

where, exposure is quantified in terms of dose, intensity, duration and route; hazard is deter-

mined and characterised by toxicological evaluation.

4.4. Safety assessment process

It is essential to assess the potential hazards that could be contributed to the newly transformed variety by evaluation of each potential source in turn as described above. This involves a four step process utilising an array of techniques which includes literature searches, in vitro, in vivo, and in silico toxicology, molecular characterisation, estimation of protein expression/exposure levels, bioinformatics, field trials, animal feeding studies and nutritional assessment. Observed differences from the traditional counterpart crop or other comparator then becomes the focus for further evaluation.

4.5. Step 1. The parent (host) crop

As the starting material for the transformation process it is essential to know as much as possible about the genotype, phenotype, diversity and history of safe use of the parent crop. The safety assessor needs information on the compositional analysis (and its relative consistency in different geographies and under different growing conditions), the presence of known antinutrients or biologically active substances such as phytoestrogens as well as any overt toxins or food allergens. Whole food laboratory animal or farm animal studies that may already have been conducted also provide valuable background data on safety, wholesomeness and performance. There is a great need for international standardisation and harmonisation in this area to provide peer reviewed databases to facilitate the determination of compositional equivalence. The OECD and ILSI are playing major roles in developing such databases consensus.

4.6. Step 2. The gene donor and construct

The gene donor contributes the 'novel element' to the traditional crop during the transformation process. This 'novel element' or transgene(s) normally expresses one or more new constituents (typically a protein) in the plant or may change the expression of existing constituents as in the case of antisense technology, either upwards or downwards. Clearly, knowledge on the history of use of the gene product, if available, is of considerable reassurance.

The systematic process that has been developed was designed to identify the theoretical mechanisms by which GM might affect human health and by taking these into account to conduct tests on a case by case basis to assess any such potential. For example, new genes might theoretically produce a harmful gene product such as a toxic or allergenic protein. Alternatively, new genes might inactivate an endogenous gene or switch on a silent gene whose effects we might know little about.

4.7. The gene construct

First of all it is important to know the source of the gene donor and whether the source is associated with any known toxic or allergenic history which might conceivably be transferred during the transformation process. If such products exist in the donor, analysis should be conducted to assure that these are not transferred. The gene insert typically contains a promoter sequence, the coding sequence of the gene and a terminator sequence. In the case of *Agrobacterium* transformation, the so-called 'T-DNA borders' are also included to facilitate the insertion process.

Depending on the regulatory sequences, expression of the gene can be constitutive, time or tissue specific or triggered by external abiotic factors. Two different transformation methods, microparticle bombardment and *Agrobacterium* transfection, are typically used. Both result in random integration and can lead to rearrangement of the DNA to be inserted and the introduction of small DNA fragments at additional secondary sites. This occurred in the case of Roundup Ready® soya in which it was established that the additional DNA was not expressed (Monsanto Company Reports, 2000) and thus had no impact on the original safety assessment. Clearly such molecular characterisation of the GM crop is critical

from the safety perspective. The DNA components of the construct to be used for plant transformation should be fully characterised before transformation. The transformation vector may contain one or more genes, which encode the trait of interest, plus typically a marker gene to enable selection of the transferred DNA. Overall the use of recombinant technology permits relatively precise introgression of the new gene(s) which contrasts with new so-called conventional breeding techniques such as alteration of ploidy, somaclonal variation, embryo rescue, anther culture and mutation breeding using chemicals or irradiation, which result in major uncharacterised genetic alterations.

4.8. Safety of viral DNA

As described, genetic elements such as promoters and protein coding sequences derived from plant viruses are used in the construction of plant transformation vectors.

The cauliflower mosaic virus 35S promoter (CaMV 35S) (Odell et al., 1985) and its homologues are strong constitutive promoters that are positioned at the 5′ end of a transgene protein coding sequence that result in the production and accumulation of transgene messenger RNA (mRNA) molecules. Since all transgenes must have a promoter, the CaMV 35S promoter is used in many types of transgenic plants that display a wide range of agronomic enhancements although it is not transcribed. Alternatively, plant virus protein coding sequences may be specifically used to confer virus resistance to transgenic plants and these are transcribed. Recent reports have suggested that the use of these viral DNA elements may confer untoward characteristics on plants that are transformed with such vectors. For instance, because of a proposed 'recombination hotspot', it has been suggested that the consumption of transgenic plants that contain the 35S promoter may result in 'inappropriate over-expression of genes' leading to cancer in humans, or that recombination may lead to the reactivation of 'dormant viruses' or the creation of 'new viruses' (Ho et al., 1999). It has also been proposed that viral infection of transgenic plants containing viral protein coding sequences may result in the "generation of novel viruses with biological properties distinct from those of the parental (virus) strain" (Aaziz and Tepfer, 1999). Although viral DNA and the resultant mRNA transcripts that may be derived from this DNA are capable of recombination (just as non-viral DNA and/or RNA are capable of recombination), there is no evidence that if such events occur, they occur at any different rate or produce any unique end products that would lead to human health consequences. Moreover, intact and unencapsidated plant viruses have been consumed safely (Bouhida et al., 1993; Harper et al., 1999; Ndowora et al., 1999; Hull et al., 2000) for thousands of years by man and animals. Due to virus copy number per cell versus transgene copy number per cell, the consumption of virus infected plant tissues may result in up to a 100,000-fold greater dosage of CaMV 35S promoter per gram of tissue than would be obtained by consuming transgenic plant tissues (Hull et al., 2000). In short, there is no scientific evidence for, and no biologically plausible mechanism by which the consumption of food or feed containing the 35S promoter may lead to adverse health effects in animals or humans.

4.9. Safety of consumed novel DNA

Horizontal gene transfer across the gut wall of man or animals has to be considered even though any risks associated with the consumption of DNA have been shown to be non-existent since all non-processed and most processed foods that we eat contain DNA which is highly sensitive to inactivation and degradation (Jonas et al., 2001). In this context it has been estimated that as all foods contain DNA and RNA, human dietary intake will vary in the range from 0.1 to 1.0 g per day (Doerfler and Schubbert, 1997). Allowing for typical levels of transgenic DNA in plants only 1:10,000–1:100,000 or less of the total DNA of a transgenic plant is the transgene DNA depending on the event. The high dietary digestibility of DNA renders the probability of gene transfer from GM plants to mammalian cells to be extremely low. Also there is no scientific reason that the transgenic DNA will be any more likely to be

transferred than the plant DNA. Furthermore, recent analyses of human genome DNA through the Human Genome project has shown that there is no evidence of DNA transfer from either bacteria or plants to humans (Stanhope et al., 2001; Salzberg et al., 2001). The United Nations Food and Agriculture Organisation and the World Health Organisation (FAO/WHO, 1991), the U.S. Food and Drug Administration (US FDA, 1992) and the U.S. Environmental Protection Agency (US EPA, 2000) have each stated that the consumption of DNA from all sources is safe. There is no inherent difference between traditional and transgenic DNA.

The transfer of plant DNA into mammalian or microbial cells under normal circumstances of dietary exposure would require all of the following events to occur (FAO/WHO, 2000):

- the relevant gene(s) in the plant DNA would have to be released, probably as linear fragments;
- the gene(s) would have to survive nucleases in the plant and in the gastrointestinal tracts;
- the gene(s) would have to compete for uptake with dietary DNA;
- the recipient bacterial or mammalian cells would have to be competent for transformation and the gene(s) would have to survive their restriction enzymes; and
- the gene(s) would have to be inserted into the host DNA by rare repair or recombination events.

In a series of experiments by Schubbert et al. (1994, 1997, 1998) high doses of M13mp18 duplex circular DNA and bacterial plasmid DNA were fed to mice with apparent incorporation into bacterial and mouse cells. The significance of these findings has been seriously questioned (Beever and Kemp, 2000). The overall conclusion was that the data do not demonstrate that transgene DNA can be transferred to and stably maintained by mammalian cells. Using a natural scenario, feeding soyabeen leaves to mice, it was found that the Rubisco gene or fragments of it remained in the gut for up to 121 h after consumption. While Rubisco gene specific products have been found in spleen and liver DNA there is no evidence for expression as assessed by reverse transcriptase PCR methodology. Moroever, mice have been continuously fed daily with green fluorescent protein (GFP) DNA for eight generations without any evidence of the transgenic state in blood or internal organs when DNA was assayed by PCR (Hohlweg and Doerfler, 2001). In the unlikely event that a transgene(s) might have properties that would lead to health concerns should transfer take place, data would be required to clarify the possibility of such an event. To date, transgenic plant DNA and proteins introduced into GM crops approved for consumption as foods and feeds have not been detected in animal products (milk, pork, chicken, beef and eggs) using the most sensitive detection methods available (FASS Facts, 2000; Glenn, 1999). In 1998, Klotz showed that the highly sensitive method of PCR followed by Southern blotting could detect a small fragment of a highly abundant endogenous chloroplast gene in white blood cells but not in milk. In the same study the CP4 EPSPS gene of Roundup Ready® soya was not detectable (Klotz and Einspanier, 1998). More recent studies by Einspanier et al. (2000) investigated the fate of conventional or Bt-maize DNA in normally fed farm animals using cattle and chickens. Bt gene specific constructs originating from Bt-maize were not detectable in any of the organs or samples investigated from either species.

4.10. Antibiotic resistance marker genes

Safety assessment must include a consideration of the potential for horizontal transfer of antibiotic resistance marker genes to microorganisms in the gut of humans or farm animals or indeed in the soil, and the resultant consequences. With the pre-existing levels of resistance, even in the extremely remote situation of horizontal gene transfer of ampicillin or kanamycin resistance genes, it would not add significantly to the current high frequency of resistant bacteria in humans and animals (Nap et al., 1992; Kresken et al., 1999). Notwithstanding, supportive reviews by the U.S. Food and Drugs Administration concerning the *nptII* gene which confers kanamycin resistance, and the EU Commission's Scientific Committees on Food (SCF) and Scientific Committees on

Animal Nutrition (SCAN) concerning the use of ampicillin resistance genes, the EU has issued notice that antibiotic resistance markers in GMOs which may have adverse effects on health should be phased out by 31st December 2004 in the case of GMOs approved according to part C and by 31st December 2008 in the case of GMOs authorised under part B (Directive, 2001/18/EC). Antibiotic resistance markers which are not used for clinical or veterinary purposes should be allowed for continued use. Alternative approaches are also being developed but it is important that these do not raise scientific concerns.

4.11. Step 3. The gene product

The possible direct toxicological effects of gene insertion resulting in the expression of one or more new constituents in the plant can be grouped into five possible categories:

1. The inserted gene results in a product that has a history of safe consumption or is highly homologous to a product (protein) with a history of safe use.
2. The inserted gene results in a product that has no known history of safe consumption.
3. The inserted gene results in a down regulation of expression or gene knockout occurs.
4. The inserted gene results in an up regulation of an existing gene in the host crop.
5. The inserted gene(s) leads to modified metabolism and hence secondary metabolites.

Appropriate tests for hazard identification and characterisation of the gene product(s), secondary metabolites or other components will be discussed at the end of this section.

4.11.1. Gene products with a history of safe consumption

An example from the recent past is the use of the Bt gene *Cry-1A* from *Bacillus thuringiensis* coding for an insecticidal Bt protein. This gene has been inserted into several varieties of maize to render them resistant to attack from the European corn borer and other insect pests.

B. thuringiensis strains are found in nearly every environment and are thus ubiquitous in soils. Moreover, Bt microbial cultures containing the insecticidal toxin have been used widely for some 40 years as biological pesticides on a variety of plants and Bt protein has thus been consumed without any history of adverse effects (US EPA, 1988). Furthermore the United States EPA have stated that "since the introduction of microbial formulations containing Cry proteins in 1961, no reports of allergy have occurred" (US EPA, 1995). For Bt, the insecticidal protein has been thoroughly investigated for mode of action (MOA), specificity and toxicity and is thus highly characterised in addition to having a history of safe consumption.

A second example is the gene that confers resistance to glyphosate which works by expressing enolpyruvylshikimate-3-phosphate synthase (EPSPS). EPSPS is present in the shikimic acid pathway for the biosynthesis of aromatic amino acids in all plants and microorganisms. Inhibition of this enzyme by glyphosate leads to a deficiency in the production of aromatic amino acids and lack of growth in plants. The aromatic amino acid biosynthetic pathway is not present in mammalian, avian or aquatic life forms, which explains the selective activity of glyphosate in plants and glyphosate's low mammalian toxicity. The glyphosate tolerant modified EPSPS (mEPSPS) protein used to confer glyphosate tolerance in corn is only two amino acids different from the EPSPS protein naturally produced in corn (amino acid homology 99.3%). EPSPS is a member of the class of proteins found ubiquitously in plants and microorganisms.

Another factor to be taken into account is the quantitative level of exposure. Reassurance from the intake history can only be used as a decisive element when the level of intake of the gene product does not exceed significantly the level previously regarded as safe. The remaining consideration is route of exposure. Typically, for foods, we are considering oral consumption and in the case of a gene product where there has been a prior safe history of human intake, we also consider that this has been by mouth. In the case of fibres, exposure may be dermal or mucosal. Alternatively, it may be that the gene product is expressed at significant levels in the plant pollen or other vegetative components other than the

traditional food itself which will result in a different route of exposure for which there is no history of safety e.g. by inhalation. In this case additional studies may be required based on risk assessment considerations.

4.11.2. The gene product has no known history of safe consumption

Where there is no history of safe use of the expressed protein or a protein sharing a similar amino acid homology to the expressed protein, it is self evident that the gene product must be fully evaluated for safety. In this situation the starting point for the safety assessment is to understand the MOA of the gene product in the gene donor and then by analogy in higher species where homologous proteins may also exist. Pending this information, a detailed safety assessment for each of the newly expressed proteins should be conducted.

4.11.3. The inserted gene results in down regulation of protein expression or gene knockout occurs

Typically, this would appear to present less of a toxicological concern as there will be less of the gene product present than was formerly the case. However, if that substance has a key controlling role as a cofactor or antioxidant and contributes to metabolic balance in another way, such as a nutritional value, there may be an overall impact which would be detected at the level of testing the food derived from the GM plant. The potential for accumulation of metabolites preceding the step in a potential pathway where the gene/protein was down regulated would also be assessed as described in point 5 below. Unintended down regulation or knock-out is no more likely to occur with GM than with conventional breeding. Gene silencing of transgenes containing a viral promoter has been observed following crop infection with a virus homologous to the transgenic promoter (Al-Kaff et al., 1998, 2000).

4.11.4. The inserted gene results in up regulation of an existing gene(s) in the host crop

Such an effect could either be an intended or unintended consequence of gene insertion or manipulation. For example, increasing vitamin content or other nutrients by de-controlling regulatory genes or by upregulating other genes will normally lead to quantitative rather than qualitative changes. Compositional analysis may then be used to determine whether levels remain in the normal range. If not, classical risk assessment will need to be followed to establish the margins of safety compared with conventional exposure levels.

4.11.5. The modified gene(s) leads to a changed metabolic pathway or a new pathway

This could occur as the consequence of altered regulation of an existing pathway or the introduction of a de novo pathway.

High oleic soyabean was developed by insertion of the *GmFad 2-1* gene. In this case sense/antisense techniques were used to reduce or prevent gene expression (Kinney, 1998). While this typically only leads to RNA production which is deemed inherently non-toxic as is the case for DNA, there is the small possibility that the inserted gene may be translated into a protein. Full analysis of the gene for open reading frames, ribosome-binding sites and analysis for the predicted protein are necessary to confirm a lack of toxic potential. Moreover, the metabolic economy of the cell may be altered upstream or downstream of the targeted change in the pathway affecting the overall nutritional and or toxicological profile of the crop. Once again this would be detected at the point of testing the whole crop and is discussed in the next main section.

Gene insertion to lead to the expression of an enzyme which will moderate a metabolic pathway via a shunt can redirect the metabolic flow to increase an existing or create a new product. The gene product will require evaluation as in subsection (1) or (2) above and the secondary metabolite (new product) will require hazard assessment if it is a new substance or risk assessment if it is present at a quantitatively higher level than was formerly the case.

4.12. Direct toxicological assessment of the gene product(s)

The gene product as a defined chemical substance can be evaluated for hazard potential using conventional toxicological methodologies. Knowing the nature and identity of the substance coded by the gene it is then possible to develop a suitable testing programme. As the vast majority of gene products per se will be proteinaceous it is important to know their relative digestibility in simulated gastric and intestinal fluid. Physical stability clearly increases the opportunity for a protein to be absorbed and cause systemic effects such as toxicity or allergenicity.

Typically most proteins are readily digested into amino acids and peptides to facilitate assimilation for the purposes of nutrition. In this situation their opportunity to impact safety is generally far reduced compared with the situation for a fully stable protein, or for that matter a chemical substance. The extent of testing depends on the source, characteristics and expression level of the gene product.

4.13. Acute oral toxicity studies

Such studies, normally performed in mice, assess the possibility of adverse effects following a single exposure to the introduced protein. High dose levels can be employed subject to the availability of the protein. If significant treatment related toxicity is observed further toxicological assessment would be necessary. The protein is normally derived from fermentation using recombinant bacteria coding the substance. In this situation it is essential to show chemical and functional equivalence to the plant derived material. Practically it can be virtually impossible to obtain sufficient quantities of the plant derived protein for meaningful testing because of extremely low expression levels—usually less than 0.1% total protein. A dose which corresponds to at least 100-fold the anticipated human exposure by oral consumption, taking into account the typical dietary food consumption, is typically tested. In the case of pesticidal proteins, these are normally tested at a limit dose as proposed by EPA (Table 2).

If the inserted trait is well known with a history of safe human intake the study may be unnecessary. However, a novel proteinaceous gene product would normally require to be tested in this way.

4.14. Repeat dose toxicity studies

In the case of significant adverse effects in the acute toxicity study, a stable protein or where the gene product has clear pharmacological activity, repeat dose toxicological testing must be considered. The tests required depend on the qualitative nature of the gene product(s). The potential range of 'classical' toxicity studies cited for a food additive which are selected case by case, would need to be considered for a completely novel substance which lacked a history of safe consumption.

4.15. Computer searching for lack of homology with known toxins

A number of different protein sequence data banks exist, for example Swiss-Prot, where it is possible to assess relationships between the introduced protein, or segments of the protein in comparison with known protein toxins.

4.16. Allergenicity evaluation

Since foods derived from GM crops normally contain traits expressed as new proteins, safety

Table 2
Acute oral toxicity: NOAELs in mice

Protein	Crop	Dose (mg/kg)
Cry 1A(c)	Cotton, tomato	4200
Cry 1A(b)	Corn	4000
Cry 2Aa	Cotton	4011
Cry 2Ab2	Corn/cotton	1450
Cry 3A	Potato	5200
CP4 EPSPS	Soybean, cotton, canola, sugarbeet	572
mEPSPS	Corn	46
NPTII	Cotton, potato, tomato	5000
ACC deaminase	Tomato	602

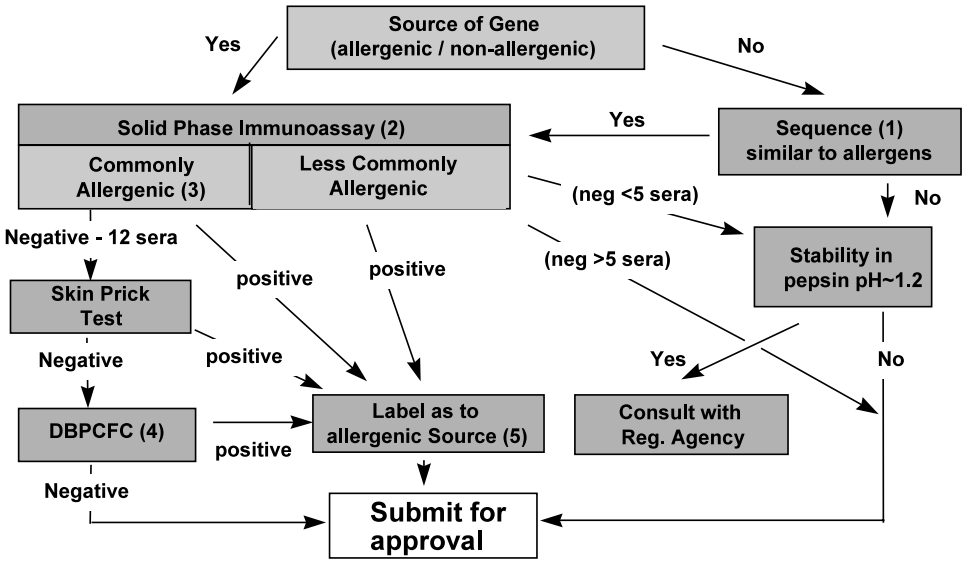


Fig. 4. Decision tree: assessment of the allergenic potential of proteins encoded by genes transferred to genetically modified crops (FAO/WHO, 1996; Metcalfe et al., 1996). (1) Amino acid sequence comparison with known allergens, positives are eight or more contiguous identical amino acids, or sufficient similarity to indicate homology. (2) Patients used for serum tests (solid phase immunoassays), or in vivo are individuals who have been diagnosed with clinically relevant allergies. (3) Commonly allergenic sources include any of the 'big eight' food allergens: peanuts, soybeans, milk, eggs, wheat, crustacea, fish, tree nuts; as well as common environmental allergens (aeroallergens). (4) DBPCFC, double blind placebo controlled food challenge. (5) In practice, no products which are positive in clinical tests have been approved.

assessment must include an evaluation of protein allergenicity potential to ensure that the novel crop or derived food is as safe as the traditional counterpart. To date there are no validated models (in silico, in vitro or in vivo) for the accurate prediction of human food allergy, unless that protein has been derived from an allergenic source, which is not typically the case. Therefore, the scientific guidance has been to utilise an holistic indirect weight-of-evidence approach based on a decision tree (Fig. 4). This procedure was elaborated at workshops by OECD (1995), WHO (1995) and integrated in 1996 by the International Food Biotechnology Council (IFBC) and the Allergy and Immunology Institute of the International Life Sciences Institute (ILSI) (Metcalfe et al., 1996). This allergy assessment strategy has been widely adopted and has proved efficacious. The best case study was the discovery during the safety assessment process, that a gene transferred from Brazil nuts into soyabeans encoded a clinically relevant Brazil nut allergen (Nordlee et al., 1996). Subsequent development of the soyabean was discontinued. In 2001 an FAO/WHO consultation was called to evaluate the overall process and to determine if changes might be made to improve the predictive value of the existing 1996 scheme (FAO/WHO, 2001). The consultation resulted in some recommendations that would be considered to improve the process but also some specific criteria that are not likely to add to the positive predictive value of the current scheme. For example, the recommended use of six amino acid epitope searching would result in an increase in the number of false positive matches resulting in decreased predictive value. Moreover, the recommendations for multiple targeted serum screens and the use of animal models will require considerable definition and validation prior to implementation. It will be essential to have universally agreed criteria to evaluate the utility and diagnostic potential of any new model that may be presented as a potential predictive tool.

Due to the fact that there is no single general

characteristic that all food allergens share (Kimber et al., 1997) concerns remain regarding both the positive and negative predictive value of the revised decision tree. Endpoints are now directly focussed towards the probability of allergenicity from 'likely' to 'low' (FAO/WHO, 2001) (Fig. 5). Further developments are anticipated to enhance approach.

The strategy for assessing the probability of a given protein possessing allergenic potential utilises a weight-of-evidence approach which focuses on the following considerations:

1. History of the parent crop and source of the gene(s). (Is the source of the gene allergenic?).
2. *Sequence homology*: Is the amino acid sequence similar to any known allergen? The amino acid sequence of many allergens is readily available (King et al., 1994). The amino acid sequence of the introduced protein can therefore be searched using FASTA or BLAST for broad homology to known allergens, checking for any eight or more contiguous amino acids that are identical to any segment of any known allergen, to identify any short sequence that might represent an allergenic epitope. However, this criterion cannot identify discontinuous or conformational epitopes that depend upon the tertiary structure of the protein (Metcalfe et al., 1996).
3. *Immunoreactivity of the newly introduced protein*: If the new protein is derived from a known allergenic source or if it has sequence homology with a known allergen, then the reactivity of this novel protein with IgE from the blood serum of appropriate allergic individuals is determined (It is unlikely that such a protein would be progressed.).
4. *Effect of pH and/or digestion*: Most allergens are resistant to gastric acid and to digestive proteases, such as pepsin (Fuchs and Astwood, 1996; Astwood, et al., 1996) (Table 3), whereas common plant proteins and introduced proteins are not (Table 4).
5. *Heat or processing stability*: Labile allergens in foods that are eaten cooked or undergo other processing before consumption are of less concern.
6. *The level of expression of the introduced protein is important*: Major food allergens normally represent $>1\%$ of total plant protein (Yunginger, 1990).

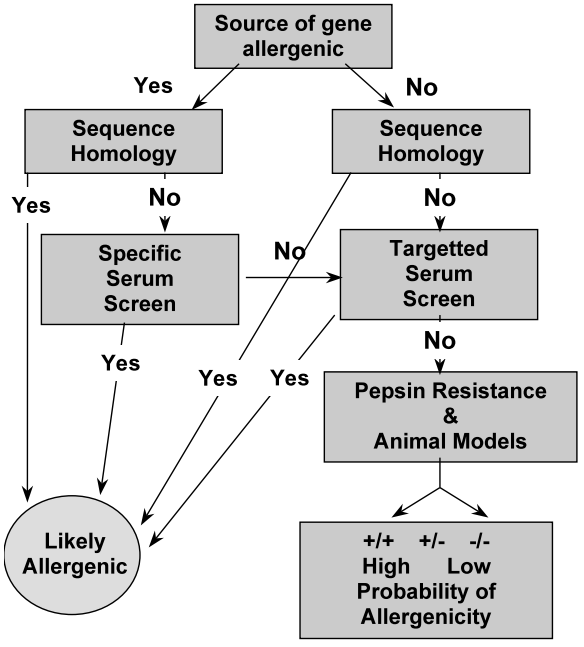


Fig. 5. Assessment of the allergenic potential of foods derived from biotechnology (FAO/WHO, 2001).

Other parameters, such as protein functionality, molecular weight (range 10–40 kDa) (Metcalfe, 1997; Taylor, 1997), and glycosylation have also been considered as possible factors in the weight-of-evidence assessment but are generally considered of lower diagnostic potential.

Notwithstanding the foregoing, the major question relevant to the whole issue of food allergens remains what characteristic(s) per se confer on some proteins the ability to induce allergy (Kimber et al., 1997).

4.17. Step 4. Novel crop or derived food

Formal safety assessment is required in the European Union (EU) for the "placing on the market of crops, foods or food ingredients which have not hitherto been used for human consumption to a significant degree within the community". This includes GMOs within the meaning of the Council Directive 90/220/EEC, which are

defined as, ''Organisms in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination''. In the EU the main legislation is based on complementary documentation, Directive 90/220/EEC covering plants and Regulation (EC) 258/97 which relates to novel foods or food ingredients. The Directive has been modified to improve the monitoring of GMO releases and to have a common methodology among Member States for carrying out risk assessment of GMOs. The revised Directive was approved by the European Parliament on 17 April 2001 and is known as Directive 2001/18/EC.

In the USA, GM crops are regulated by the Food and Drugs Administration (FDA) and the United States Department of Agriculture (USDA) except where a plant pesticide is expressed in which situation the Environmental Protection Agency (EPA) is involved to evaluate the safety of the pesticidal component. In Japan the Ministry of Agriculture, Forestry and Fisheries (MAFF) Innovative Technology Division is involved together with the Ministry of Health, Labour and Welfare (MHW).

Table 3
Tendency for stability of known allergens to digestion by simulated gastric fluid (SGF) (Astwood et al., 1996)

Protein	Stability (min)	% Total protein
Egg and milk allergens		
Egg ovalbumin (Gal d 2)	60	54
Milk β-lactoglobulin	60	9
Egg ovomucoid (Gal d 1)	8	11
Milk casein	2	80
Milk BSA	0.5	1
Milk α-lactalbumin	0.5	4
Egg conalbumin (Gal d 3)	0	12
Seed allergens		
Soy β-Conglycinin (b)	60	18.5
Soy Kunitz trypsin inhibitor	60	2–4
Peanut Ara hII	60	6
Mustard Sin a I	60	20
Mustard Bra j IE	60	20
Soy lectin	15	1–2
Peanut lectin	8	1.3

4.18. The concept of substantial equivalence (SE)

The difficulties of applying traditional toxicological testing to whole foods meant that an alternative approach was required for the safety assessment of GM foods. This led to the development of the concept of SE (FAO/WHO, 2000). By determining any differences in the GM crop or derived food from its traditional counterpart which is regarded as safe, the differences become the focus of detailed safety evaluation. This concept is known as SE and the goal of the assessment is to identify similarities and any differences between the GM crop and conventional plant variety which then became the focus for detailed evaluation. Substantial equivalence is a concept that provides guidance by helping to identify the questions to be asked during the safety assessment of new foods, feeds or processed fractions, it is not an endpoint. If the process of assessing SE leads to the conclusion that the food, feed or processed fraction from a crop developed via biotechnology is compositionally equivalent to, and as safe as, that of the traditional counterpart except for the introduced trait(s), then the safety of the trait(s) (gene product(s)) becomes the focus of direct assessment, which may require in vitro and/or in vivo studies. Since the concept of SE was first developed by the FAO/WHO (1991), numerous international meetings and symposia have discussed and refined its use and application (OECD, 1993; WHO, 1995; FAO/WHO, 1996; OECD, 1996; FAO/WHO, 2000; OECD, 2001). Most national regulations are based on the use of the SE approach (US FDA, 1992; Health Canada, 1994; Japanese Ministry of Health and Welfare, 1996; EU Novel Foods, 1997) and many other governments around the world have adopted the approach as well.

4.19. Controversy over the concept of SE

Millstone et al. (1999) and others have criticised the concept of SE as pseudo-scientific and hence inadequate for the assessment of food safety. The

argument relates to their belief that plant genetics are insufficiently understood in terms of GM and downstream impact on composition and hence toxicological profile. This argument which aligns with the inability to prove a negative is equally applicable to so-called conventionally bred crops and is therefore disproportionate and specious: it makes no sense to single-out GM crops.

The FAO/WHO Joint Expert Consultation identified that some of the criticism related to the mistaken perception that the determination of SE was the end point of the safety assessment rather than the starting point (FAO/WHO, 2000). Moreover, further disagreement may have developed from reference to three outcomes, implying end points, of SE, namely *substantially equivalent*, *substantially equivalent apart from defined differences* (e.g. new trait) and *not substantially equivalent* (intentionally or unintentionally) (FAO/WHO, 1996).

Table 4
Relative instability of common plant proteins and introduced proteins to simulated gastric fluid (Astwood et al., 1996)

Protein	Stability (s)	% Total protein
Common plant proteins		
Rubisco LSU (spinach leaf)	<15	25
Rubisco SSU (spinach leaf)	<15	25
Lipoxygenase (soybean seed)	<15	<1
Glycolate reductase (spinach leaf)	<15	<1
PEP carboxylase (corn kernel)	<15	<1
Acid phosphatase (potato tuber)	<15	<1
Sucrose synthetase (wheat kernel)	<15	<1
β-Amylase (barley kernel)	<15	<1
Introduced protein		
Cry 1A(c)	30	<0.01
CP4 EPSPS	<15	<0.1
mEPSPS	<15	<0.05
Glyphosate oxidoreductase (GOX)	<15	<0.01
ACC deaminase (ACCd)	<15	0.4
β-D-Glucuronidase (GUS)	<15	0.01
NPTII	<10	<0.01

The concept of SE continues to be recognised by scientific and regulatory experts as the most appropriate foundation for the assessment of the safety of foods from crops developed via biotechnology (FAO/WHO, 2000). Substantial equivalence achieves a central public health objective, which is the assurance that no undeclared or unexpected alterations in dietary nutrients, antinutrients, toxins or allergens are introduced into the food or feed supply. In addition, by sampling the biochemistry through compositional analysis of food, feed or processed fractions derived from genetically modified crops in comparison to traditional crops, unexpected effects due to genetic insertions (i.e. pleiotropy) are assessed in depth. In combination with specific safety evaluations of introduced traits, it can be assured that foods, feeds or processed fractions derived from genetically modified crops are as safe and nutritious as their traditional counterparts.

4.20. How to use the SE concept to plan the programme of safety evaluation

As mentioned above, SE is a guiding principle to be used before the safety assessment programme begins. This facilitates the planning of an appropriate test strategy on a case by case basis. This takes into account the nature of the transformation and hence predicted similarities and differences between the proposed genetically modified food and the relevant comparator that has a history of safe use. A final check for any unexpected or unpredicted effects is made by thorough assessment of the new whole food. The FAO/WHO consultation further stated that compositional changes were not the sole basis for determining safety. This can only be determined when the results of aspects under comparison are fully integrated. The comprehensive procedure for assessing overall safety of the whole food is described below.

Procedure for determining whether the genetically modified crop or whole food is 'as safe as' its traditional counterpart. In Step 4 of the assessment, the aggregated knowledge is integrated from the three prior stages, i.e. Steps 1, 2 and 3.

- History of safe use of the parent (host) crop composition, nutrition, toxicants, antinutrients, etc.
- History of use of the gene donor, the molecular characterisation of the gene cassette and insert into the host genome, and consideration of horizontal gene transfer and DNA safety.
- Hazard assessment data on the gene product(s) (trait) from a toxicological and allergenicity perspective.

Theoretically, having evaluated the safety profiles of the 'starting materials' in depth together with their integration in the transformation process, the outcome, i.e. the phenotypic characteristics of the novel crop, may be predicted with reasonable certainty. However, as further reassurance to ensure the new crop is 'as safe as' its traditional counterpart, up to four overarching assessments are undertaken where equivalence to the traditional counterpart or comparator must be shown:

- Phenotypic and agronomic equivalence.
- Compositional equivalence.
- Safety equivalence.
- Nutritional and feed performance equivalence.

4.21. Phenotypic equivalence

It is essential to demonstrate that there are no unexpected biological effects of the introduced trait. The basic question is: does the biotech crop fit within the usual definition of the crop, for example, does Bt corn still possess the expected plant performance of traditional corn. There are two key components:

- Morphology.
- Agronomic traits.

Table 5 shows the parameters assessed for corn. These are very sensitive indices of perturbations in metabolism and potential genetic pleiotrophy and hence robust indicators of equivalence. Genetically modified crops must therefore be phenotypically equivalent to their traditional counterpart on stringent performance criteria.

4.22. Compositional equivalence

Apart from evaluating the safety profile of the introduced trait it is essential to know that the transformed novel crop has the same composition of macro- and micronutrients as the parent, unless intentional alterations have been made to impact one or more of these components. This provides overall confidence that the GM has not increased the levels of natural allergens, antinutrients or even food toxicants present in the traditional cultivar.

Compositional analysis is performed by sampling the food (often grain) grown in a variety of geographies to assess the natural variability resulting from different abiotic factors as well as biotic factors such as disease burden. The goal is to establish whether components of the new food or feed fall within the generally accepted ranges of traditional varieties. The composition of Roundup Ready® corn line GA21, in comparison with literature and historical ranges in terms of fibre, mineral, proximate, amino and fatty acid composition is shown in Tables 6–8.

As can be seen 50 or more biochemical components were measured which provides important information in two key areas:

- The biochemistry of the plant is 'sampled' as a sensitive index for pleiotropic (unexpected effects) caused by the genetic insertion.
- The potential for nutritional and/or anti-nutritional impact can be assessed which could have implications for man.

Typically equivalence with the ranges found in conventional crops is the expected outcome. If significant differences are observed, beyond natural variability, the deviations become the focus of further investigation; other considerations come into play if the crop was designed to be compositionally different. One example is a canola oil high in lauric acid (C12:0), a fatty acid not normally found in canola oil, which was developed to serve as a substitute for tropical oils in certain food applications (FAO/WHO, 1996), together with a soybean oil has been developed to have

Table 5
Phenotypic and agronomic characteristics used to evaluate equivalence (corn example)

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

high levels of oleic acid (C18:1) at the expense of linoleic acid (C18:2 n-6) (OECD, 1998). However, the concept of SE still can be applied to assess the safety of these components in the whole food (i.e. oil) by comparing them to the composition of foods for which they substitute and by comparing their anticipated intake versus intake of the component from other foods in the diet. For example, in the case of high laurate canola oil, it was demonstrated that the total intake of lauric acid in the diet would not change significantly by the substitution of this product for tropical oils in the anticipated food applications. Additionally, it is well-recognised that tropical oils have a long history of safe use. This example illustrates how the concept of SE can be applied to the evaluation of the safety of whole foods. The traditional counterpart of a food from a crop developed via gene technology need not be the appropriate comparator; rather, there will be instances where the product for which it substitutes is the appropriate comparator.

4.23. Safety and wholesomeness equivalence

The safety profile of the new crop, food or feed can be built up by integrating the findings from the hazard assessment of the trait and or its secondary metabolites, its phenotypic appearance, the compositional analysis of the novel crop paying particular attention to the levels of natural antinutrients, toxicants and allergens and the results of animal feed performance studies which also have the potential to display untoward effects. If this evaluation shows that the biotech food product is substantially equivalent to the traditional counterpart with the exception of the introduced trait, further testing should focus on the safety of the introduced trait (FAO/WHO, 2000; OECD, 2000; ANZA, 2000).

Assessing the safety of the whole food via animal testing has been discouraged WHO 2000 unless there remains any unresolved questions relative to safety for which animal studies with the whole food are deemed most appropriate to answer the outstanding questions. In this case it is generally considered (FAO/WHO, 2000) that a subchronic study of 90 days duration is the appropriate study to demonstrate the safety of repeated consumption of a foodstuff in the diet. Such a study may need to be preceded by a pilot study to ensure palatability of the diet including the traditional crop in the control diet at the highest inclusion level to make sure that natural substances do not interfere with the outcome. For example, rats are very sensitive to polyols and develop caecal enlargement. This could give a false positive outcome for a novel product in the absence of traditional controls. The highest dose level used in any animal study should be the maximum achievable without causing nutritional imbalance whilst the lowest level should approximate to the anticipated human intake. The need for additional toxicity tests should only be considered on a case by case basis taking into account the outcome of the 90-day study and other findings. Similarly case specific in vitro toxicology studies could be envisaged for a protein having potential pharmacological activity.

4.24. Nutritional equivalence

Animal feed performance (nutritional) studies may also contribute to the overall judgement of the SE of biotech crops. The rationale for this approach has been that in addition to the potential to identify unexpected nutritional effects during these feeding studies (duration ranges from 42 to 120 days), important economic conse-

Table 6
Fibre, mineral and proximate composition of grain from Roundup Ready® corn line GA21

Component[c]	1996[a]		1997[b]			Literature (range)[h]	Historical[g] (range)[h]
	GA21 mean (range)[h]	Control[d] mean (range)[h]	GA21 mean (range)[h]	Control[e] mean (range)[h]	Commercial lines[f] mean (range)[h]		
Protein	10.05 (9.39–11.00)	10.05 (9.17–11.19)	11.05 (9.48–14.06)	10.54 (9.70–12.92)	10.87 (7.8–14.20)	(6.0–12.0)[k] (9.7–16.1)[l]	(9.0–13.6)
Total fat	3.51 (2.94–3.72)	3.55 (2.76–3.93)	3.90 (3.04–4.63)	3.98 (3.30–4.81)	3.69 (2.48–4.81)	(3.1–5.7)[k] (2.9–6.1)[l]	(2.4–4.2)
Ash	1.27 (1.06–1.45)	1.27 (1.21–1.40)	1.38 (1.06–1.80)	1.56 (1.07–3.09)	1.79 (0.89–6.28)	(1.1–3.9)[k]	(1.2–1.8)
ADF[i]	3.73 (3.35–3.99)	3.72 (3.52–4.05)	6.35 (2.73–9.47)	6.35 (3.00–9.33)	6.06 (2.75–11.34)	(3.3–4.3)[k]	(3.1–5.3)
NDF[i]	10.82 (10.06–11.88)	11.70 (9.40–13.58)	9.33 (7.51–11.57)	9.8 (8.03–11.58)	10.12 (7.58–15.91)	(8.3–11.9)[k]	(9.6–15.3)
Carbohydrates	85.15 (84.00–86.11)	85.15 (83.71–86.14)	83.66 (80.57–84.97)	83.79 (81.69–85.26)	83.68 (77.41–87.16)	Not reported in this form	(81.7–86.3)
Calcium	0.0026 (0.0020–0.0031)	0.0027 (0.0024–0.0033)	0.0039[j] (0.0027–0.0056)	0.0043 (0.0033–0.0058)	0.0040 (0.0022–0.0208)	(0.01–0.1)[k]	(0.0029–0.006)
Phosphorus	0.299 (0.28–0.32)	0.299 (0.28–0.31)	0.326 (0.303–0.350)	0.326 (0.292–0.349)	0.330 (0.208–0.411)	(0.26–0.75)[k]	(0.288–0.363)
Moisture	14.15 (7.44–22.60)	14.40 (7.24–23.00)	16.86 (9.57–23.10)	16.21 (8.67–24.70)	16.30 (8.18–26.20)	(7–23)[k]	(9.4–15.8)

[a] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide.
[b] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide.
[c] Percent dry weight of sample, except for moisture.
[d] Non-transgenic negative segregant.
[e] Parental control line.
[f] Commercial lines; local hybrids planted at each site.
[g] Range for control lines planted in Monsanto Company field trials conducted between 1993 and 1995.
[h] Range denotes the lowest and highest individual value across sites for each line.
[i] ADF, acid detergent fibre; NDF, neutral detergent fibre.
[j] Statistically significantly different from the control at the 5% level ($P<0.05$).
[k] Watson (1987),
[l] Jugenheimer (1976), both cited by Sidhu et al. (2000).

Table 7
Amino acid composition of corn grain from Roundup Ready® corn line GA21

Amino acid[a]	1996[b]		1997[c]			Literature[g] (range)[i]	Historical[h] (range)[i]
	GA21 mean (range)[i]	Control[d] mean (range)[i]	GA21 mean (range)[i]	Control[e] mean (range)[i]	Commercial lines[f] mean (range)[i]		
Alanine	7.62 (7.34–7.81)	7.64 (7.45–7.84)	7.64 (7.49–7.86)	7.62 (7.50–7.97)	7.78 (7.44–8.98)	(6.4–9.9)	(7.2–8.8)
Arginine	4.13 (3.72–4.34)	4.30 (4.05–4.51)	4.48 (3.74–4.93)	4.51 (4.11–4.90)	4.36 (3.67–5.34)	(2.9–5.9)	(3.5–5.0)
Aspartic acid	6.71 (6.46–6.87)	6.78 (6.35–6.83)	6.63 (6.17–7.05)	6.65 (6.22–7.08)	6.57 (6.14–7.35)	(5.8–7.2)	(6.3–7.5)
Cystine	2.10 (1.85–2.36)	2.11 (1.91–2.24)	2.22 (1.73–2.49)	2.28 (2.06–2.57)	2.19 (1.63–2.62)	(1.2–1.6)	(1.8–2.7)
Glutamic acid	19.27 (18.70–19.71)	19.06 (18.61–19.64)	18.78 (18.12–19.45)	18.70 (18.04–19.43)	19.17 (17.83–20.53)	(12.4–19.6)	(18.6–22.8)
Glycine	3.72 (3.44–3.95)	3.78 (3.48–3.96)	3.83 (3.44–4.27)	3.89 (3.52–4.14)	3.71 (3.05–4.29)	(2.6–4.7)	(3.2–4.2)
Histidine	2.81 (2.72–2.99)	2.84 (2.75–2.93)	2.67 (2.36–2.87)	2.74 (2.46–2.86)	2.80 (2.36–3.20)	(2.0–2.8)	(2.8–3.4)
Isoleucine	3.60 (3.48–3.66)	3.58 (3.44–3.70)	3.53 (3.06–3.85)	3.57 (3.13–3.92)	3.75 (3.13–4.14)	(2.6–4.0)	(3.2–4.3)
Leucine	13.11 (12.32–13.71)	12.90 (12.37–13.49)	12.98 (12.33–13.96)	12.87 (12.26–13.69)	13.32 (11.99–15.19)	(7.8–15.2)	(12.0–15.8)
Lysine	3.02 (2.68–3.30)	3.09 (2.69–3.27)	3.11 (2.59–4.04)	3.02 (2.66–3.33)	2.96 (2.20–3.50)	(2.0–3.8)	(2.6–3.5)
Methionine	1.98 (1.78–2.24)	2.03 (1.85–2.28)	2.16 (1.80–2.34)	2.17 (1.67–2.44)	2.02 (1.53–2.44)	(1.0–2.1)	(1.3–2.6)
Phenylalanine	5.15 (4.88–5.31)	5.17 (4.98–5.30)	5.31 (5.03–5.63)	5.33 (4.96–5.76)	5.36 (4.88–6.10)	(2.9–5.7)	(4.9–6.1)
Proline	8.69 (8.41–8.92)	8.69 (8.49–9.10)	8.98 (8.22–9.38)	9.00 (8.62–9.23)	9.16 (8.08–9.94)	(6.6–10.3)	(8.7–10.1)
Serine	5.33[j] (5.25–5.49)	5.27 (5.17–5.43)	5.17 (4.43–5.60)	5.03 (3.82–5.63)	4.64 (2.87–5.63)	(4.2–5.5)	(4.9–6.0)
Threonine	3.77 (3.64–3.88)	3.73 (3.58–3.85)	3.59 (3.33–3.74)	3.54 (3.08–3.71)	3.43 (2.61–3.89)	(2.9–3.9)	(3.3–4.2)
Tryptophan	0.62 (0.55–0.66)	0.57 (0.53–0.61)	0.61 (0.52–0.75)	0.61 (0.43–1.04)	0.59 (0.41–1.04)	(0.5–1.2)	(0.4–1.0)
Tyrosine	3.81[j] (3.68–3.99)	3.95 (3.88–4.10)	3.73 (3.06–4.20)	3.77 (2.78–4.32)	3.48 (2.37–4.32)	(2.9–4.7)	(3.7–4.3)

Table 7 *(continued)*

Amino acid[a]	1996[b]		1997[c]			Literature[g] (range)[i]	Historical[h] (range)[i]
	GA21 mean (range)[i]	Control[d] mean (range)[i]	GA21 mean (range)[i]	Control[e] mean (range)[i]	Commercial lines[f] mean (range)[i]		
Valine	4.58 (4.40–4.74)	4.64 (4.45–4.73)	4.57 (4.15–5.18)	4.62 (4.00–5.00)	4.79 (3.93–5.40)	(2.1–5.2)	(4.2–5.3)

[a] Values expressed as percent of total amino acids for statistical comparisons. These values are slightly higher when expressed as percent of total protein, e.g. alanine = 7.8% for GA21 (1996).

[b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide.

[c] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide.

[d] Non-transgenic negative segregant.

[e] Parental control line.

[f] Commercial lines; local hybrids planted at each site.

[g] Watson (1982). Values are percent of total protein [10.1% total protein ($N \times 6.25$)].

[h] Range for control lines planted in Monsanto Company field trials conducted between 1993 and 1995; values are percent of total protein.

[i] Range denotes the lowest and highest individual values across sites.

[j] Value statistically significantly different than the control at the 5% level ($P < 0.05$).

Table 8
Fatty acid composition of corn grain from Roundup Ready® corn line GA21

Fatty acid[a]	1996[b]		1997[c]			Literature[g] (range)[i]	Historical[h] (range)[i]
	GA21 mean (range)[i]	Control[d] mean (range)[i]	GA21 mean (range)[i]	Control[e] mean (range)[i]	Commercial lines[f] mean (range)[i]		
Arachidic (20:0)	0.40 (0.36–0.48)	0.41 (0.39–0.46)	0.37 (0.32–0.44)	0.36 (0.33–0.41)	0.40 (0.31–0.57)	(0.1–2)	(0.3–0.5)
Behenic (22:0)	0.16 (0.14–0.18)	0.17 (0.16–0.18)	0.16 (0.12–0.24)	0.15 (0.13–0.16)	0.18 (0.13–0.24)	(Not reported)	(0.1–0.3)
Eicosenoic (20:1)	0.28 (0.27–0.31)	0.29 (0.28–0.30)	0.30 (0.28–0.34)	0.30 (0.28–0.36)	0.30 (0.19–0.45)	(Not reported)	(0.2–0.3)
Linoleic (18:2)	58.56 (54.20–64.70)	58.72 (53.40–65.60)	61.40 (58.2–63.4)	61.51 (59.7–63.0)	59.18 (46.9–64.3)	(35–70)	(55.9–66.1)
Linolenic (18:3)	1.10 (1.07–1.13)	1.08 (0.98–1.16)	1.14 (0.92–1.24)	1.14 (1.04–1.20)	1.11 (0.77–1.55)	(0.8–2)	(0.8–1.1)
Oleic (18:1)	27.50 (22.10–31.30)	27.40 (21.40–32.40)	24.2 (22.4–26.0)	24.1 (22.9–26.0)	26.2 (21.3–39.2)	(20–46)	(20.6–27.5)
Palmitic (16:0)	9.94 (9.59–10.40)	9.92 (9.60–10.40)	10.70 (10.30–11.40)	10.72 (10.40–11.40)	10.58 (8.75–13.30)	(7–19)	(9.9–12.0)
Stearic (18:0)	1.87 (1.52–2.11)	1.86 (1.46–2.11)	1.68 (1.44–2.04)	1.67 (1.59–1.86)	1.88 (1.36–2.65)	(1–3)	(1.4–2.2)

[a] Value of fatty acids expressed as % of total fatty acid. The method included the analysis of the following fatty acids which were not detected in the majority of samples analysed: caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), gamma linolenic (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). Palmitoleic acid (16:1) was observed at levels of ~0.17% of total fatty acids in grain samples collected in 1996 but was not detected in the majority of grain samples collected in 1997.

[b] Data from five U.S. sites; GA21 grain harvested from plants not treated with Roundup herbicide.

[c] Combined data from four non-replicated E.U. sites, six U.S. non-replicated sites and one U.S. replicated site; GA21 grain harvested from plants treated with Roundup herbicide.

[d] Non-transgenic negative segregant.

[e] Parental control line.

[f] Commercial lines; local hybrids planted at each site.

[g] Watson (1982). Values expressed as % of total fat except for palmitic acid (16:1) which is expressed as % of triglyceride fatty acids.

[h] Range for control lines planted in Monsanto Company field trials conducted between 1993 and 1995; values are expressed as % of total fatty acids.

[i] Range denotes the lowest and highest individual values across sites.

quences (i.e. disadvantages) can accrue to farmers who experience lower feed efficiency, lower yield or lower quality products as a result of using feeds derived from biotech crops when compared to traditional crops. The most robust study design implemented to date has been the 42-day broiler chicken study in which 1 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 feed is believed to be greatest under these conditions of very rapid growth and in this species relative to other potential study designs. In addition, very high diet incorporation rates (up to 70% of the total diet) of the test material (e.g. corn feed) in broiler studies may be achieved. 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, chill weight, 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 genetically modified crops in comparison to feeds derived from traditional crop counterparts (Clark and Ipharraguerre, 2001). In addition, while animal nutrition studies are not viewed as an essential element of the safety assessment of food, feed or processed fractions derived from genfletically modified crops, it can be fairly said that the equivalence of the meat, dairy and poultry products derived from animals fed genetically modified crops have been extensively corroborated and re-confirmed in these highly sensitive long term feeding trials which are relevant to farmers and consumers.

In conclusion, establishing the equivalence of the GM crop against robust, measurable endpoints such as morphological and agronomic performance, composition, nutritional performance and overall safety equivalence provides a powerful, systematic and comprehensive process for demonstrating that the novel crop is 'as safe as' its traditional counterpart.

4.25. Safety evaluation—second generation (quality) traits

While the modifications made to date have been relatively simple, more complex modifications are already under development, which incorporate multiple traits or the manipulation of metabolic pathways to produce novel crops such as 'Golden Rice' and 'Golden Mustard' (Ye XuDong et al., 2000; Potrykus, 2001; Shewmaker et al., 1999; Clark and Ipharraguerre, 2001). In the case of Golden Rice, levels of beta-carotene and iron are enhanced which will help to supplement the intake of these essential micronutrients for populations in Asia and India. As new foods with extensive GM(s) are developed, the core concept of defining similarities and differences from the appropriate comparator and then focussing on the differences is likely to remain as the cornerstone of the safety assessment strategy. While new procedures are in the early stages of research it is probable that classical testing procedures whose efficacy have been shown over time and as described in this paper, will continue to be used in the main. This targeted approach to specific constituents contrasts with the research into profiling methods (non-targeted approach). Such methods are being considered for the simultaneous screening of potential changes in the physiology of the modified host organism at the level of the genome (genomics) during gene expression and protein synthesis (proteomics) and at the levels of metabolic pathways (metabolomics) (Kuiper et al., 2000).

It has been suggested that such methods may also have value for highlighting unintended effects which may occur due to random insertion. However, the potential occurrence of unintended effects is not specific to the use of recombinant DNA techniques. Rather, it is an inherent phenomenon that can occur in conventional breeding and is handled by discarding plants which are not phenotyp-

ically or agronomically typical or by back-crossing.

In either situation these new methods are still in the research phase and require further development followed by validation. Most challenging of all will be the ability to differentiate between differences that are relevant from a toxicological perspective, compared with those which are not. It can be predicted that bioinformatics will prove an essential component if the application of these new methodologies is to prove of practical utility.

4.26. The role of post-launch monitoring (surveillance)

Despite the rigorous iterative food safety assessment described and the requirement for full data review and approval by competent authorities prior to environmental release and marketing, it has been proposed that unexpected effects could occur in some sectors of the population or that exposure and consumption patterns might change from those anticipated prior to marketing. In consequence post-launch monitoring has now been called for in the revised Directive 2001/18/EC. The two possibilities are either through epidemiological studies, for which there are several possible approaches, or by randomised controlled clinical trials. Finding suitable population groups where one has been consuming a wide range of foods containing GM components and an equally matched group that has not is almost impossible to imagine. Moreover, foods already contain GM elements from fermentation production which have a long history of safe use, e.g. chymosin, gums, food additives, flavourings, etc. Such surveillance needs to be proportionate to the very low probability of risk which is predicted from the rigorous testing performed and observed with the developing history of safe use for GM crops and foods in the United States where such products have been consumed widely since the mid 90s.

5. Conclusion

The food safety considerations for GM crops produced by techniques that modify the heritable traits of the food plant or crop, are basically the same as those arising from other ways of altering the genome such as conventional breeding (FAO/WHO, 1996). However, public concerns in Europe have led to a demand for much higher safety levels than is the case for traditionally bred crops or indeed those produced via alternative 'breeding' methods such as chemical or irradiation mutagenesis.

The consequent safety assessment procedure which has been developed for GM crops, foods and derived products is extremely robust and comprehensive. It integrates a classical evaluation of the direct toxicity of the inserted novel trait (gene product(s)) with an overall safety assessment of the resultant genetically modified food. It is necessary to proceed step wise and case by case, based on prior consideration of the modification intended and working within a generally agreed framework known as 'SE'. This is a guiding principle and not an end point per se. The concept of SE recognises that existing foods have a long history of safe use and can therefore serve as a basis or reference point for comparison when assessing the safety of foods or feeds that have been genetically modified. The comparison is holistic as it takes into account not only the traditional crop or comparator as the benchmark but also all sequential processes and changes involved in transforming it to the new GM variety, hence:

- characteristics of the parent crop or comparator;
- characteristics of the gene donor and transformation process;
- characteristics of the newly expressed gene product(s);
- characteristics of the new crop, food, or processed product including possible secondary effect of the GM.

Such a 'hybrid' approach focussing in particular on both the new gene product and the novel crop (or derived food) is not only scientifically sound, but also recognises that foods are bulky and complex in nature making traditional toxicological testing impractical due to limitations of food intake, satiety, nutritional effect and dietary balance.

By building a safety profile of each component and process involved in the modification and by noting similarities and differences a very comprehensive matrix of information is constructed which permit the conclusion that the novel GM crop or food either is or is not 'as safe as' its traditional counterpart. For comparative purposes this should be the most closely related transgenic plant or food (Custers, 2001). However, it should be understood that isogenic controls (e.g. controls in which the only difference is the introduced DNA and encoded proteins) are not available and hence there are genetic differences which must be taken into account in the safety assessment. It is also important that the traditional plants used for comparison are grown under typical conditions, for example, sprayed with herbicide, if a crop with an herbicide resistance trait is under investigation, and grown in a similar location to the GM crop.

An ongoing concern of the public has been the potential for long term effects even though very little is known about the potential long term effects of many if not most existing foods (FAO/WHO, 2000). However, on the basis that any GM food will have been tested to ensure that it is 'as safe as' the traditional version which, by definition, has a history of safe use, this makes the possibility of long term effects highly unlikely. Furthermore, the expressed protein(s) will often have a long history of safe use as well. Post-launch monitoring or epidemiological studies though laudable in aim, appear unlikely to detect untoward effects against the background of natural effects from conventional foods.

In determining whether a new GM crop or feed is as safe as its comparator, it has been stated that up to four equivalence endpoints should be assessed and integrated, phenotype, composition, safety and wholesomeness and feed/nutritional performance. Concerning composition there is a strong need to gain international harmonisation on the quality of data relating to natural variability. This is all the more important because of the natural potential for wide variability and the need for robust reference data. Indeed, even in the case of observed differences in composition between a new crop and its counterpart there is not automatically a health concern. This depends on qualitative and quantitative considerations with regard to the substance(s) showing the deviation, as it is not feasible to define a priori the degree of difference which is acceptable and that which is not.

For second generation GM products where quality traits are likely to result from potentially extensive GM it will be possible to construct appropriate safety testing programmes case by case drawing from the classical methodologies referred to in this paper. It is not yet clear the extent to which profiling methodologies using genomics, proteomics, and metabolomics (Kuiper et al., 2000) will become involved as a non-targeted approach. Due to a number of practical limitations it is clear that a high level of validation and bioinformatics to highlight and discriminate those changes of potential significance will be required.

As a very high level of safety assurance has been achieved through a scientific approach to the development and testing of GM crops, consumers should be informed of these standards compared with traditional foods, where only anecdotal evidence of safety exists.

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