Comparative safety assessment for biotech crops

Harry A. Kuiper dedicates this paper to the memory of Prof. Eraldo Antonini, unforgettable friend and teacher of biochemistry at the University of Rome La Sapienza, who passed away prematurely 20 years ago on March 19th, 1983.

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Since the first discussions on strategies to assess the food safety of genetically modified (GM) crop plants, assessment of GM plants and derived tissues has been based on comparisons with their traditionally bred counterparts. This was termed the Principle of Substantial Equivalence. However, implementation of the principle led to controversy and hampered the precision of the actual safety assessment. Here, we propose the principle be rephrased into the Comparative Safety Assessment strategy. This describes the analytical nature of the first step of the entire (GM) food safety assessment in combination with consecutive toxicological and nutritional evaluations. Further development of advanced analytical methods will help to improve the efficacy of assessment strategies.

Genetically modified (GM) food crops were introduced commercially in 1994. The first commercial GM crop plant, which was introduced in the USA, was the FLAVR SAVR™ tomato that had delayed ripening characteristics. Since then, adoption of GM food crops has increased continuously, achieving a cultivated area of 58.7 million hectares worldwide [1] (and see Fig. 1). Crops that are cultivated today carry foreign traits introduced by genetic modification that are predominantly of agronomic importance. The best-known examples include herbicide-resistant soybeans and insect-resistant maize, which have their own weed and insect control, respectively.

Currently, several cultivated GM crops have been modified with traits that affect the functional properties of the final product. For example, long-ripening tomatoes have favourable post-harvest texture characteristics for processing into tomato paste. Oilseed crops have a modified oil composition, including soybeans that are high in oleic acid (more stable during frying), and canola that is high in lauric acid (a desirable physical property). It is anticipated that, in the near future, more GM-crop-derived foods will have traits that are beneficial in food processing or that might positively influence the nutritional and health status of the crop for consumers and animals [2] (Table 1). Recently, GM crops have been designed – or are under development – to combat certain nutritional deficiencies. Well-cited examples include 'Golden Rice', in which provitamin A is introduced into the kernels [3], and iron-fortified GM rice [4]. The aim of these GM rice modifications is to alleviate vitamin A deficiency and/or anaemia in developing countries where rice is the staple crop. These modifications have been achieved through the insertion of genes encoding entire non-native metabolic pathways, or through targeted alterations in existing pathways.

There is now a trend towards high-expression levels of foreign or endogenous proteins with an enhanced content of essential amino acids (e.g. high-lysine corn). Moreover, plants can be designed as 'protein factories' that serve as a medium for purification of a protein of interest, or to produce high levels of insecticidal proteins that decrease resistance development in insects. High protein expression levels have been achieved by plastid transformation; in one example, 45.3% of soluble leaf protein was transgenic [5].

Safety assessment strategies for GM-crop-derived foods

From the very first initiatives to establish globally agreed guidelines for the safety assessment of foods and food ingredients derived from GM organisms, comparison with the characteristics of relevant traditionally bred plant varieties was the leading principle [6]. The underlying assumption was – and still is – that traditional crop plant

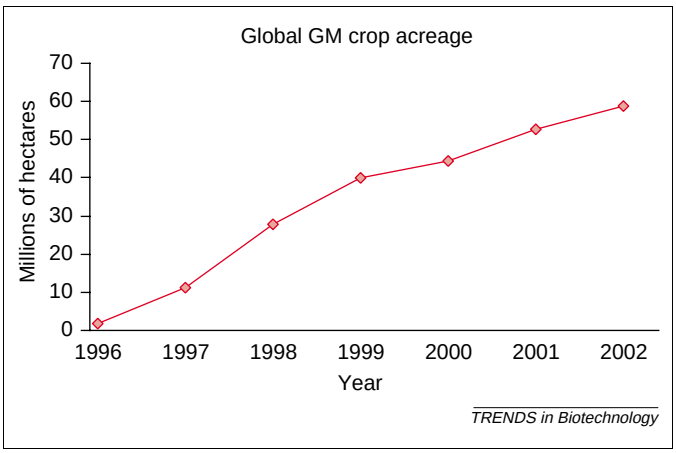

Fig. 1. Commercial genetically modified (GM) crop cultivation from 1996 until present [1].

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Table 1. Experimental biotech food crops with potential benefits for processors and consumers

Aim	Introduced trait	Crop	Refs
Nutritional			
Elevated levels of micronutrient	Synthesis of provitamin A from geranylgeranyldiphosphate (naturally present in kernels) by transgenic enzymes	Rice	[3]
	Iron-binding protein (ferritin) and two proteins for promotion of intestinal iron bioavailability (phytase, metallothionein)	Rice	[4]
Improved protein nutrition	Transgenic protein with favourable amino acid composition	Potato	[35]
Processing			
Bread baking	Transgenic glutenin protein associated with favourable dough characteristics	Tritordeum (cross between wheat and barley)	[36]
Less contamination of seed oil	Decreased synthesis of chlorophyll in seeds by antisense suppression	Canola	[37]
Improved starch degradation during malting	Transgenic amylase in kernels	Barley	[38]
Medical			
Edible vaccine	Polypeptides of heat-labile enterotoxin of *E. coli* that raise immunity against diarrhoea-causing bacterial toxins	Maize	[39]

varieties currently on the market have not been elaborately tested in the laboratory before being marketed. However, because they have been consumed (after appropriate processing) for decades, they have gained a history of safe use. This history of safe use can be used as a baseline for the safety assessment of new GM plant varieties derived from established plant lines. The comparative concept for the safety evaluation of foods derived from GM crops has further been elaborated by the Organisation for Economic Cooperation and Development (OECD) and crystallised in the so-called Principle of Substantial Equivalence [7].

Food safety evaluation issues of foods derived from GM crops comprise:

- Molecular characterization of the introduced genetic fragment and resulting new proteins or metabolites (in addition, an increasing number of European member states routinely ask for characterization of the insertion point of the transgenic fragment);
- Analysis of the composition of the relevant plant parts with respect to key nutrients and anti-nutrients, including natural toxins and potential allergens;
- Potential for gene transfer of specific genes from the GM food to – particularly – microorganisms in the human and animal gastro-intestinal tract;
- Potential allergenicity of the new gene products, or alteration of the intrinsic allergenicity of the GM food organism;
- Estimated intake levels of the newly introduced proteins as well as of the final product, including any altered constituent;
- A toxicological and nutritional evaluation of the resulting data; and
- Additional toxicity testing (of the whole food) where necessary.

With regard to the last point, toxicity testing of the whole crop or derived plant products might be required. For example, cases where the composition of the whole crop has been changed significantly compared with the traditional counterpart, or where there is a need to further investigate potential unintended side effects of the genetic modification, warrant additional toxicity testing.

Specific guidance on these issues has been provided by: (1) the OECD [8], (2) the European Scientific Committee on Foodstuffs (SCF) [9], (3) the United Nations Food and Agriculture Organisation/World Health Organisation (FAO/WHO) [10–12], and (4) Codex [13]. A detailed overview of safety assessment practices relating to GM food crops has been published by Kuiper and colleagues [14]. A tiered approach for data generation and subsequent assessment is shown in Figure 2.

Application of the Substantial Equivalence Principle needs to be improved

The approach of first comparing the GM line with the parent line and then with other traditionally bred varieties already on the market was predominantly formalized by the OECD [7]. The Principle of Substantial Equivalence was introduced with the aim of establishing a scientifically sound approach that would meet global acceptance. However, it soon became clear that the principle left much scope for individual (and national) interpretations. Further concerns established that the principle could only be applied on the basis of a thorough compositional analysis of the varieties under scrutiny (the GM line and its traditional counterpart). In addition, the compositional comparison is the starting-point of the food safety evaluation and not – as was misinterpreted in some publications [15,16] – an end-point in itself. Once differences in composition have been identified between the GM food plant and its appropriate comparator, targeted toxicological and nutritional studies should be carried out to assess the safety and nutritional impact on humans. Thus, toxicological and nutritional testing is an essential part of the safety assessment model for foods derived from GM crops. The Principle of Substantial Equivalence is merely a tool to identify potential differences and is part of a comprehensive comparative safety assessment approach. This issue was extensively discussed by the FAO/WHO Expert Consultation held in 2000 [11].

The OECD took up the challenge to formulate consensus documents on individual crop plants. This included

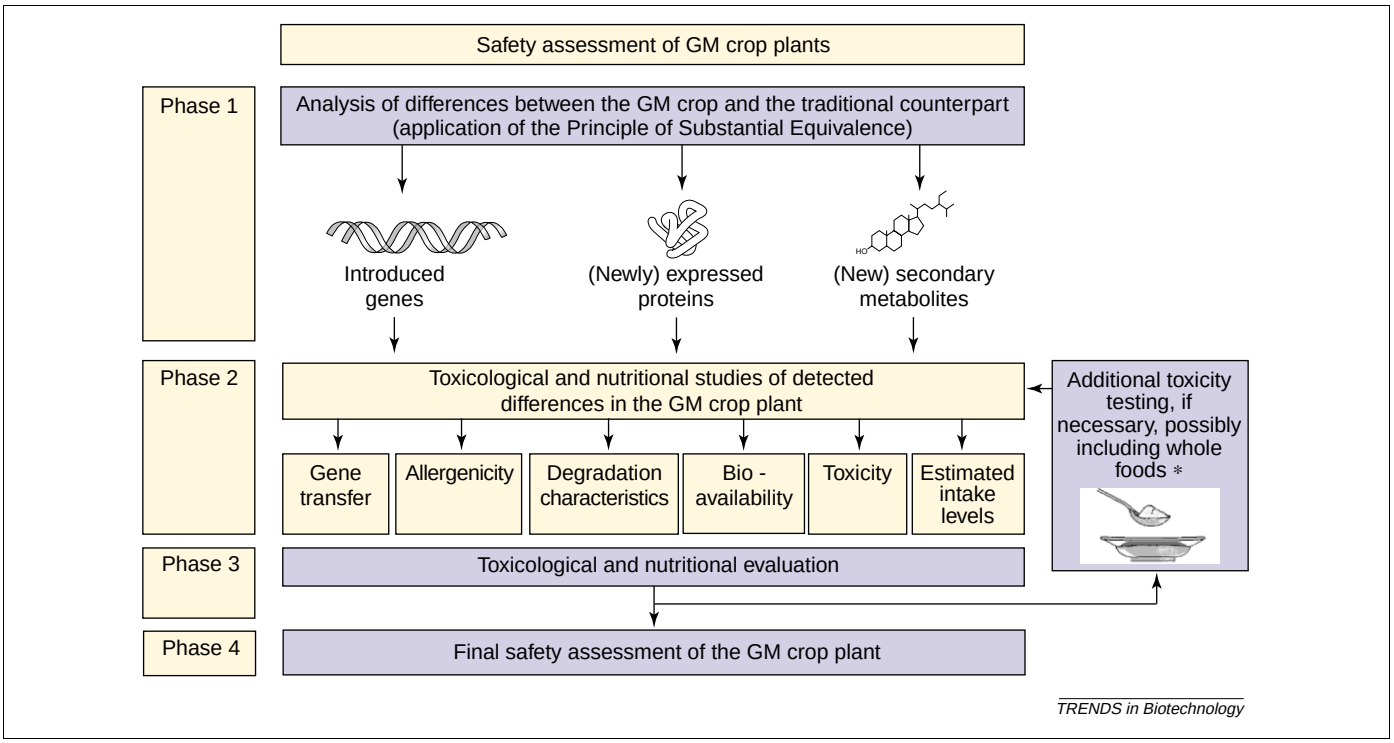

Fig. 2. Safety assessment strategies for genetically modified (GM)-crop-derived foods. Tiered approach for data generation and subsequent safety assessment of genetically modified (GM)-derived foods.

an overview of the key macro- and micronutrients, as well as anti-nutritional factors, natural toxins and (where reported in the literature) their background values, for the different food crops [17]. This proved a difficult task because our knowledge, for example, of the levels and toxicity of anti-nutritional factors in crop plants is often fragmentary, especially in crops that are less economically important. Therefore, specific attention should be given to the quality and validation status of the analytical methods used to generate specific compositional data. In addition, the crop varieties and analytical methods used to generate the data might now be outdated, compared with present crops and methods. The ILSI Crop Composition Database, which has recently become available on the Internet, contains quality-controlled data and could be a valuable supplement to the OECD consensus documents.

Another complicating factor is the selection of plants to be analysed. The comparator of the GM line should preferably be the direct parent line. However, this line might no longer be available (e.g. it could be in possession of another breeding company). Furthermore, analysis of the plant line that will actually be marketed might reveal substantial changes to the parent line that are unrelated to the genetic modification. This is because, in general, a whole breeding programme separates the initial modification event from the breeding of the final genotype that will be marketed. Therefore, although comparison to several relevant lines is recommended, the data obtained might be less informative, and a proper analysis of these compositional data will be more complicated.

Environmental conditions also influence the physiology of the plant. It is therefore important that GM and non-GM plants to be analysed are grown under identical environmental conditions. In addition, it might be helpful to analyse plants grown under a range of environments and climates, which would influence the activity rate of individual metabolic pathways. However, it is unclear how much extra information could be obtained in this way; more unclear is how many environmental and climatological conditions should be assessed to improve significantly the food safety assessment of novel plant varieties by these extra analyses. All proposed conditions for the performance of field trials outlined in national and international guidelines thus far, are arbitrary and based on practical (breeding) experience with conventional crops, rather than on scientific evidence. Any extra information gained might therefore be limited.

Methods to detect and assess unintended effects of a genetic modification

Concerns that unintended and unexpected side effects might occur in GM organisms (GMOs) as result of the genetic modification process, thereby impacting on human and animal health, has attracted attention from both scientific and public groups. However, the potential occurrence of side effects in non-GM organisms must also be highlighted [14]. Compositional analyses of the GM plant and its traditional counterpart, in addition to the notion that relevant unintended side effects might remain undetected when analysing only specific compounds or intermediates in important nutritional and anti-nutritional pathways, are complicated issues. It was therefore encouraged that more general, unbiased methods of analysis be developed to detect relevant changes in a much larger part of the physiology of the plant [8,11,18]. This could be of particular importance for GM plants that have multiple genes inserted, which possibly have a higher occurrence of unexpected and unintended effects (Table 1).

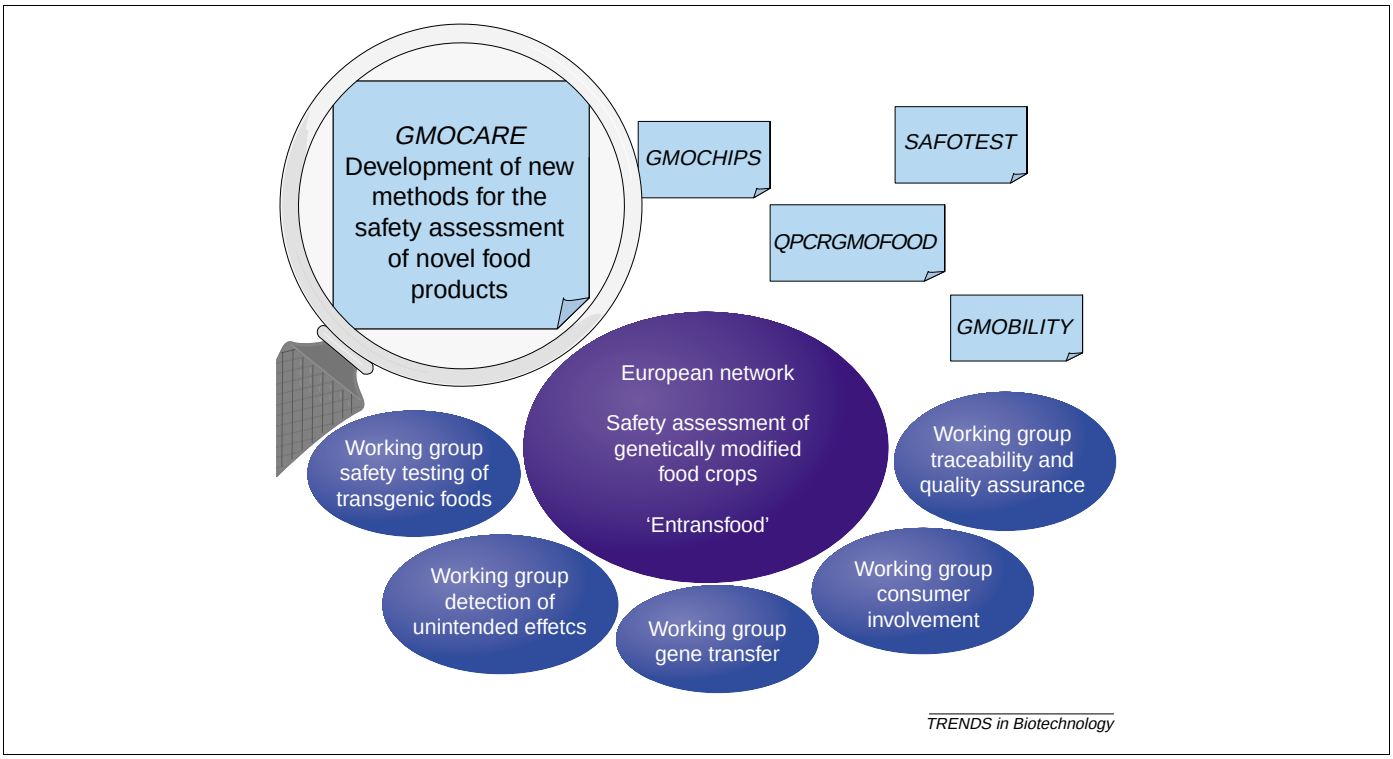

Fig. 3. Organization of Entransfood, the European Thematic Network on the Safety Assessment of Genetically Modified Food Crops.

As a result, specific projects were initiated to develop more informative, unbiased methods for different levels of integration of the physiology of the plant on mRNA, protein and metabolite levels.

The European Thematic Network, Entransfood, covers most of the current initiatives to develop new approaches for the food safety assessment of GM varieties (http://www.entransfood.com). The network serves as an umbrella project for five research groups and five working groups (Fig. 3). Three of these are directly related to the food safety assessment of genetically modified organisms (GMOs). First, GMOCARE focuses on the development of new tools based on the unbiased analysis of the relevant plant tissues using fingerprinting techniques in the fields of genomics, transcriptomics, proteomics, metabolomics and glycomics. Second, SAFOTEST focuses on the development of new toxicological approaches to assess the safety of consumption of novel food products. Third, GMOBILITY investigates the possibility of gene transfer in the human gastrointestinal tract using model systems. The remaining two projects relate to the detection, identification and quantification of GMOs in the food production chain. QPCRGMOFOOD focuses on the development of identification and quantification methods in the food production chain. GMOCHIPS aims to develop a chip-based approach for the screening of large numbers of GMO varieties in a single assay. Besides Entransfood, there is another important British initiative: the GO2 Programme on the Safety of Novel Foods. This began in 2001 and includes projects that aim to investigate the potential of new methods for the safety assessment of novel food products (http://foodstandards.gov.uk/science/research/NovelFoodsResearch/g02programme).

Unbiased fingerprinting approaches at the level of DNA, gene expression, proteins, metabolites and their secondary structures, could potentially provide a more thorough insight into any unpredicted changes in the physiology of the plant that might go undetected when focusing on single compounds [19]. For example, it is possible to introduce entirely new metabolic pathways, without any obvious phenotypic change [20]. Nevertheless, it is unlikely that new metabolic pathways do not affect existing pathways. Fingerprinting techniques might be a more efficient method of identifying such alterations. However, significant research will be required before these techniques produce confirmed and validated information. Fundamental setbacks that need to be addressed before these techniques can be included in a routine, integrated evaluation protocol are outlined in the following sections.

DNA level

Owing to the large sequencing projects of recent years, sequencing of large DNA stretches is now routine. Sequence analysis of the insertion point of the genetic fragment might be significant to evaluate whether it is possible to identify any potential side-effects, for example, based on the interruption of regulatory or gene sequences, or the presence of any such sequence in the vicinity. However, there is still limited knowledge of the genetic code of the organisms under investigation [21,22]. Additional knowledge, especially for regulatory elements, is crucial for the correct interpretation of DNA sequencing results.

Gene expression level

Microarrays enable altered gene expression to be screened in large numbers of genes simultaneously. However, correct interpretation of the resulting data is both difficult

and dependent on many different factors. These include experimental set-up, available equipment, software, and knowledge of the organism under investigation [23,24].

Protein level

Given that altered gene expression levels might not correlate directly to shifts in protein levels [25], the most direct method of investigating unpredicted alterations is proteomic analysis of the tissues of interest. Considerable expertise in 2D gel electrophoresis has enabled the simultaneous screening of large numbers of proteins, with subsequent characterization by mass spectrometry (MS) [26]. However, there are several important setbacks. Setting up an informative system for a single tissue is time-consuming. Furthermore, reliable quantification remains problematic, despite the availability of advanced software. The sensitivity of such an approach is affected by slight changes in isolation conditions, which, in turn, might profoundly affect the behaviour of the proteins under investigation. Protein microarrays can theoretically expand more easily on the basis of increasing knowledge of the proteome. This could reduce the time-consuming set-up of new protein analysis systems, and increase reproducibility and potential for quantification. Current issues relating to array production and assay performance still need to be addressed [27,28].

Metabolite level

Another direct approach is analysis of the secondary metabolites. Informative systems have been set-up for different organisms using gas and liquid chromatography (GC/LC) in combination with MS [29,30] or nuclear magnetic resonance (NMR) [31,32]. In theory, identification of large numbers of constituting compounds can be achieved using a combination of these techniques. However, in practice, there are several important drawbacks. These include a lack of reliable data on profile variation for relevant compounds in different matrices of the organism under study, and standardization of extraction procedures and measurement protocols.

Despite the technical hurdles, it is clear that these new developments have the potential to give increased insights into relevant changes in the physiology of plant products resulting from genetic modification or from the application of new and existing food processing techniques.

Concluding remarks

Although the Principle of Substantial Equivalence has received comments from all types of stakeholders (producers, regulators, consumers, evaluators, etc.) [15,33], the basic idea behind the principle remains untouched. When evaluating a new or GM crop variety, comparison with available data on the nearest comparator, as well as with similar varieties on the market, should form the initial part of the assessment procedure. The term 'substantial' has provoked interesting discussions, but has also led to misinterpretations. Therefore, the principle should be rephrased as the 'Comparative Safety Assessment (CSA)' approach. This phrase better outlines the comparative nature of the assessment, while avoiding the idea that it is a safety assessment in itself. Nutritional and toxicological assessment should be performed on the basis of the CSA, and might require additional safety tests.

Even where the idea of acceptable safety of conventional foods has gained worldwide approval, underlying assumptions of relative safety can still be questioned. For example, traditional plant breeding practices such as chemical mutagenesis might lead to a higher rate of mutations compared with genetic changes induced by recombinant DNA technology [34]. Only in exceptional cases will a safety assessment of the resulting plant-lines be demanded. It is debatable whether the results of such generally accepted breeding practices should serve as the baseline for the safety assessment of new or GM plant-lines.

Perhaps it is time to rethink our philosophy on the safety of foods produced by different agricultural methods. This would result in a more-balanced universal risk analysis system and basic safety assessment protocol for all novel food crop varieties. In all cases, a CSA of available data on crop plant varieties with a history of safe use should serve as a starting-point for the consumer safety assessment. A system of reliable databases and informative profiles on individual compounds will provide significant progress towards a safe food supply, even as the concept of third-generation GMOs becomes reality.

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