

Review Article

The concept of substantial equivalence in safety assessment of foods derived from genetically modified organisms

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Abstract

Foods derived from genetically modified organisms have been on the market in many countries, predominantly in the USA and in Canada, for more than five years now without any reports of adverse effects on human health. Safety assessment criteria have been the subject of early discussions among competent international and national organisations and institutions and have led to the development of guidelines. Common to all guidelines is the principle of substantial equivalence as a reasonable approach to identifying differences between novel foods and their traditional counterparts. In order to safeguard the food supply, differences are subject to further analyses with regards to their impact on human health. There has been a consensus among competent authorities and the scientific community, with respect to food safety, that the placing on the market of novel foods, regardless of whether they are a substantial equivalent or not, is only acceptable if they are as safe as the existing traditional products with which they can be compared. However, the concept of substantial equivalence itself has also been subject to criticism that calls for further discussion.

Introduction

The first successful genetic modification of a plant was reported in 1983. It was a tobacco plant in whose genome a foreign gene had been inserted which resulted in an antibiotic resistant phenotype (Horsch *et al.*, 1984). The first genetically modified food – the famous Flavr Savr™ tomato – was placed on the USA market in 1994. Currently more than fifty genetically modified crop plant varieties have been commercialised, among them different types of genetically modified tomatoes, soyabeans, maize, rapeseed and potatoes. Their new traits are mainly of agronomic interest, i.e., herbicide or insect tolerance. However, usage of herbicides and pesticides are attracting the interest of consumers as well. More consumer relevant developments are oilseed plants with a modified fatty acid composition or tomatoes with prolonged shelf life and improved flavour.

Preceding the first commercialisation of a food derived from genetically modified organisms (GMOs), criteria for the assessment of its safety have been discussed intensively by those international and national organisations with competence in food safety issues. Guidelines for the safety assessment of foods derived from modern biotechnology have been elaborated by the World Health and the Food and Agriculture Organisations (WHO and FAO), the Organi-

sation for Economic Co-operation and Development (OECD), the International Food Biotechnology Council (IFBC), the International Life Science Institute (ILSI), the US Food and Drug Administration (FDA), the UK Advisory Committee on Novel Foods and Processes (ACNFP), the Nordic Council, the German Research Community (DFG) and other national bodies. These organisations base their recommendations for the safety assessment of a modified organism on the comparison with the non-modified counterpart in order to identify equivalencies and differences.

History and definition of substantial equivalence

The term substantial equivalence was first mentioned in connection with food safety in a report of the OECD Group of National Experts on Safety in Biotechnology (OECD, 1993). The members of the group agreed that the most practical approach to determining the safety of foods derived by modern biotechnology is to consider whether they represent a substantial equivalent to analogous traditional products. The term substantial equivalence and the underlying approach were “borrowed from the US Food and Drug Administration’s (FDA) definition of a class of new medical devices that do not differ materially from their predecessors and thus, do not raise new regulatory concerns” (Miller, 1999).

According to the OECD definition, the concept of substantial equivalence is based on the idea that existing products used as foods or food sources can serve as a basis for comparison when assessing the safety and the nutritional value of a food or food ingredient that has been modified by modern biotechnological methods or is new. It implies that if a novel food or novel food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety. No additional safety concern would be expected. If a novel food or novel food ingredient has not been found to be substantially equivalent to its conventional counterpart, this does not imply that it is unsafe. It is then to be evaluated on the basis of its unique composition and properties (OECD, 1993).

The term substantial equivalence is also referred to in the Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients which came into force in the member states of the European Union on 15 May 1997. According to this Regulation, it is sufficient to notify to the European Commission of the placing on the market of novel foods which are substantially equivalent to existing products as regards their composition, nutritional value, metabolism, intended use and level of undesirable substances contained therein. The notifier has either to provide the European Commission with the scientific evidence available and generally recognized or to ask a competent authority of a member state in which the product is to be launched with the necessary information, including the known results of all studies which have been carried out as well as any other information which is available to demonstrate the safety of the food.

This procedure does not apply to novel foods containing, or consisting of, GMOs. For the placing on the market of this category of novel foods, authorisations are mandatory, even if the result of the safety assessment may prove their substantial equivalence to conventional foods. The applicant has to provide the competent authority of the member state in which the product is to be launched with the necessary information, including the known results of all studies which have been carried out as well as any other information which is available to demonstrate the safety of the food.

In addition, as GMOs can reproduce and transfer their genetic material, they must be subjected to an environmental risk assessment according to Directive 90/220/EEC on the Deliberate Release of GMOs into the Environment in order to prove that their release will not cause any harm to human health and the environment. It is the same Directive according to which the placing on the market of the first two GMOs, Monsanto's herbicide tolerant soyabean in 1996 and Novartis' insect tolerant maize in 1997, and derived foods, was authorized in the European Union before the Novel Foods Regulation came into force.

Under the Novel Foods Regulation the placing on the market of novel foods requires specific labelling in order to inform the consumer of any characteristic or food property, such as composition, nutritional value or nutritional effects, or intended use of the novel food, which renders it no longer equivalent to an existing food or food ingredient.

Although the Novel Foods Regulation discriminates between equivalence and substantial equivalence, it gives no clear definition of the term "substantial". Both, equivalence and substantial equivalence are described in terms of composition, nutritional value or nutritional effects, and intended use. The term "nutritional effect" is replaced by "metabolism" and "level of undesirable substances contained in the food" is added in the list of terms related to substantial equivalence.

Since a genetic modification is aimed at introducing new traits into organisms, the result will always be a different composition of genes and proteins. The most reasonable interpretation therefore is that a food derived from a GMO is considered substantially equivalent to

its traditional counterpart if the genetic modification has not resulted in intended or unintended alterations in the composition of relevant nutrients and inherent toxicants of the organism, and that the new genes and proteins have no adverse impact on the dietary value of the food and do not therefore pose any harm to the consumer or the environment.

The concept of substantial equivalence can thus be considered no more but also no less than a reasonable tool to assess the nutritional composition and safety of a novel food in relation to the nutritional composition and safety of its traditional counterpart. This is consistent with the Novel Foods Regulation's requirements that foods and food ingredients falling within its scope must not present a danger to the consumer, must not mislead the consumer and do not differ from food or food ingredients which they are intended to replace to such an extent that their normal consumption would be nutritionally disadvantageous for the consumer.

Application of the concept of substantial equivalence in safety assessment

The concept of substantial equivalence was applied for the first time to a GMO in the safety assessment of the Flavr SavrTM tomato before it was placed on the USA market in 1994. Data collected from field trials and from analyses of the molecular and chemical composition showed that the genetically modified tomato was equivalent to the non-modified parent plant, with the exemption of the newly introduced traits which were then the subject of further studies in order to establish food safety. The data were presented and the food safety of the genetically modified tomatoes was accepted in consultation with the FDA.

In the following years, we gathered a lot of experience with the safety assessment of a large variety of genetically modified plants. In the European Union, food ingredients derived from herbicide tolerant soyabeans and from several insect and/or herbicide tolerant maize lines, and refined oils derived from several herbicide tolerant rape seed lines, were registered and approved according to the legal requirements that have been in place since 1990 and 1997, respectively. These foods may be placed on the market in member states of the European Union (Table 1).

The safety assessment is always based on a comparison of the modified food to its traditional counterpart in terms of molecular, compositional, toxicological and nutritional data. The level of detail of the analyses required depends on the degree to which the new product differs from its traditional counterpart. Thus, the extent of analyses may differ and therefore there is no general checklist that could be followed by those who are responsible for allowing the product to be placed on the market. However, recommendations were published by the European Commission in September 1997 in order to provide guidance to both applicants and competent authorities concerning the scientific aspects and the presentation of the information necessary to support applications for the placing on the market of novel foods and novel food ingredients and for the preparation of initial assessment reports under Regulation (EC) No 258/97.

According to the European Commission's recommendations, the applicant has to provide the competent authorities with information about the product as well as about the process specifications and the dietary exposure. The genetic construct and the vector used to transfer the foreign DNA sequences into the host organism must be identified, in order to evaluate environmental risks and health hazards. It must also be demonstrated which new genes have been integrated into the genome and which new proteins are expressed from the genetically modified organism. The latter have to be analysed as to their structure and function and their potential toxicity or allergenicity.

Table 1: Notified/authorized GMO derived foods and food ingredients in the European Union

| Applicant/ Notifier | GMO and derived products | Date of Authorisation (A)/ Notification (N) | Legislation/ Competent Authority |
|-----------------------|--|---|--|
| Monsanto | Foods and food ingredients from herbicide tolerant soyabean | A: April 1996 | Directive 90/220/EEC/ European Commission |
| Novartis | Foods and food ingredients from insect tolerant maize Bt 176 | A: February 1997 | Directive 90/220/EEC/ European Commission |
| Monsanto | Foods and food ingredients from insect tolerant maize MON 810 | N: December 1997 | Regulation (EC) No 258/ 97 UK ACNFP |
| AgrEvo | Foods and food ingredients from herbicide tolerant maize T 25 | N: January 1998 | Regulation (EC) No 258/ 97 UK ACNFP |
| Novartis | Foods and food ingredients from insect and herbicide tolerant maize Bt 11 | N: January 1998 | Regulation (EC) No 258/ 97 UK ACNFP |
| Pioneer | Foods and food ingredients from insect and herbicide tolerant maize MON 809 | N: October 1998 | Regulation (EC) No 258/ 97 UK ACNFP |
| AgrEvo | Refined oil from herbicide tolerant rapeseed TOPAS 19/2 | N: June 1997 | Regulation (EC) No 258/ 97 UK ACNFP |
| PGS | Refined oil from male sterile and herbicide tolerant rapeseed MS1XRF1, MS1XRF2 | N: June 1997 | Regulation (EC) No 258/ 97 UK ACNFP |
| Monsanto | Refined oil from herbicide tolerant rapeseed GT 73 | N: November 1997 | Regulation (EC) No 258/ 97 UK ACNFP |
| AgrEvo | Refined oil from herbicide tolerant rapeseed Liberator L 62 | N: October 1999 | Regulation (EC) No 258/ 97 BgVV, Germany |
| AgrEvo | Refined oil from herbicide tolerant rapeseed FALCON GS 40/90 | N: October 1999 | Regulation (EC) No 258/ 97 BgVV, Germany |
| Plant Genetic Systems | Refined oil from herbicide tolerant hybrid rapeseed MS8XRF3 | N: October 1999 | Regulation (EC) No 258/ 97 BgVV, Germany |

In order to detect any unintended modifications which could have resulted from the insertion of the newly introduced DNA sequences due to the interruption or enhancement of regular gene functions, the chemical composition as well as the phenotypic characteristics of the genetically modified organism need to be checked.

Chemical composition

Analytical studies of the composition of the novel food are of crucial importance not only for the establishment of substantial equivalence, but also as a prerequisite for nutritional and toxicological assessments. The compositional analyses should focus particularly on the determination of the content of critical nutrients and any critical toxicants and anti-nutritional factors, which might be either inherently present or process-derived.

The crucial importance of compositional analyses and a thorough knowledge of the nutritional properties of the novel food as a prerequisite for toxicological studies can be demonstrated by the genetically modified potatoes expressing the lectin *Galanthus nivalis* agglutinin (GNA) which had been fed to rats in a disputed study undertaken by Stanley W.B. Ewen, University of Aberdeen, UK, and by Arpad Pusztai at the Rowett Research Institute in Aberdeen, UK, in order to examine whether the GNA gene insertion had affected the nutritional and physiological impact of potatoes on the mammalian gut. The researchers observed that the diets containing the genetically modified potatoes had variable effects on different parts of the rat's gastrointestinal tract, particularly on the small intestine and caecum. This was not observed in control rats, which were fed non-modified potatoes or non-modified potatoes supplemented with GNA. The authors assume that not only the GNA in the potatoes but also other parts of the vector or the transformation itself could have contributed to the overall effects (Ewen and Pusztai, 1999). Unfortu-

nately, apart from other shortcomings in this study, data on the composition of the different GM potato lines used in the diet are not reported in the publication. Some details released by Arpad Pusztai on the internet (Pusztai, 1999) indicate that the composition of the genetically modified potato lines differed substantially from that of the parental line. However, it remains uncertain whether critical toxicants were affected. The results of the study were assessed by the UK Royal Society, which found the data to be inadequate because they were not clear about the differences in chemical composition between strains of non GM and GM potatoes and invalid because of technical limitations of the experiment and the incorrect use of statistical tests (The Royal Society, 1999). In spite of the wide public attention these researches have attracted, the use of their results is limited, to say the least.

Toxicity and allergenicity of new proteins

New proteins have to be characterized with regards to their toxic or allergenic potential. Under these procedures, existing immunological tests are being applied to the foreign proteins if they are expressed by genes derived from a source known to be associated with food allergy. For new proteins with no history of food use, the procedure requires that the properties of the new proteins are compared with those of known toxicants and allergens. As a first step, a comparison of the amino acid sequences of the new proteins with known toxins and allergens will reveal homologies. Another important factor is the quantification of the new protein contained in the food.

Food allergens share further specific characteristics. They are quite stable against high temperatures, low pH and proteolytic degradation. One of the most relevant tests here is a model of *in vitro* digestion for measuring resistance to proteolysis. Applied to the main known food allergens, this test shows that they are stable, whereas the new proteins expressed in genetically modified plants

are rapidly degraded. So it can be assumed with a reasonable amount of certainty that the GMOs which contain these new proteins do not represent an extra allergenic risk. But then, of course, the available tests do not guarantee non-allergenicity.

Further toxicological test requirements need to be considered on a case-by-case basis, taking into account the source, familiarity and characteristics as well as the amount of the new protein contained in the food and the results of the compositional analyses of the food.

If no equivalence to conventional food components can be established, the products have to be subjected to an extensive nutritional and toxicological assessment including studies on toxicogenetics, genotoxicity, and reproductive and developmental toxicity, as well as short-term and long-term carcinogenicity feeding studies with rodents. The necessity for other toxicity studies, including studies with a second species, will depend on the concern level, which is determined by the chemical structure and the intended level of human exposure (Jonas *et al.*, 1996).

Antibiotic resistance genes

The safety assessment of antibiotic resistance genes which have been used in many genetically modified plants to identify and select those plant cells which have been transformed successfully has to consider the potential for horizontal gene transfer to micro-organisms in the human gastrointestinal tract and its consequences. If the plant cell expresses the enzyme conferring resistance to antibiotics for therapeutic reasons it has to be secured that antibiotic therapies are not compromised.

The latter can be excluded, either if the antibiotic resistance gene is under control of a bacterial promoter and therefore cannot be expressed in plant cells, or if the antibiotic which the transferred gene confers resistance to is not used in human therapeutics.

A horizontal gene transfer can of course not be excluded with absolute certainty. But there is no evidence for a transfer of plant DNA to prokaryotic organisms, and the probability is, due to several stringent requirements, considered to be very low. In the case of ampicillin or kanamycin resistance genes, which are present in some of the GMOs and derived products which may be placed on the European Union's market, it is known from clinical studies that a high frequency of resistance can be observed in Enterobacteriaceae and other clinically relevant bacterial strains, e.g. *Escherichia coli* (Nap *et al.*, 1992; Kresken *et al.*, 1999). Even the rare event of a horizontal gene transfer would not add significantly to the already considerable number of ampicillin and kanamycin resistant bacteria in humans and animals. The European Commission's Scientific Committees on Food (SCF) and on Animal Nutrition (SCAN) therefore concluded, in their reviews of the safety of the ampicillin resistance gene which has been introduced into the genome of a maize plant, that there is no evident risk of ampicillin resistance for the bacteria of the human or animal digestive tract from foods or feeds derived from genetically modified maize.

Controversy about the concept of substantial equivalence

The principle of substantial equivalence has been criticized as being pseudo- or even anti-scientific and therefore as inadequate to assess the safety of foods derived from genetically modified organisms. Millstone *et al.* (1999) argue that plant genetics is not sufficiently understood and the relationship between genetics, chemical composition and toxicological risks are unknown. According to them the principle of substantial equivalence should therefore be replaced by safety and toxicological testing to the same extent as it is required for pharmaceuticals, pesticides or food additives, including the setting of 'acceptable daily intakes' (ADI).

As suitable as this strategy may be for the safety testing of isolated substances, it is not suitable for examining the safety of such a complicated and many-structured item as, for example, a potato, or other complex foodstuff, which may contain only one new but well characterized protein in a concentration of less than 0.1% of the total protein content among more than 5000 plant proteins. In traditional toxicological testing, e.g., of food additives, the aim is to determine a No-Observed Adverse Effect Level (NOAEL), which is the highest tested dose at which no adverse effects are observed. By applying a safety factor or uncertainty factor, usually of 100, to the NOAEL, a tolerable dose or ADI can be estimated (WHO, 1987). With complex foods it would be difficult to feed the amount of the food which might be necessary to obtain high enough concentrations of the possibly relevant ingredients in the diet without causing nutritional imbalances.

It should also be kept in mind that unintended effects can also result from conventional breeding techniques or from introducing exotic foods on the European market. It hardly makes sense to single out GMO-derived foods as the sole subjects of painstaking analyses in pursuit of their potential health hazards and ecological risks.

Conclusion

The term "substantial equivalence" has been introduced into the concept of safety assessment of novel foods only recently, but the underlying strategy of comparing newly developed products or techniques to existing ones has been applied for a long time – not only in agriculture, but in many other fields of science and technology when new developments were introduced. Establishing substantial equivalence is not a safety assessment in itself, but is a pragmatic tool to analyse the safety of a new food. It goes without saying that in the testing of new foods, use has to be made of the latest scientific methods. We have to make all conceivable efforts to protect consumers from health risks. At the same time, however, consumers should be adequately informed about the real extent of risks and hazards from novel foods in relation to traditional foods.

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