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Challenges in food toxicity testing

Safety Assessment of Genetically Modified (GM) Foods

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Summary
Modern biotechnology enables a transfer of genes between species that would not occur naturally. Safety assessment of genetically modified (GM) foods is primarily based on the concept of substantial equivalence, a comparison with conventional counterparts and similar varieties as a starting point. Nutritional and toxicological studies should be based on the outcome of the comparison, and additional safety tests should be carried out as required. The limitations of animal studies, particularly in assessing the safety of whole GM foods may be addressed by the new technologies that brought us GM foods in the first place. While animal studies may still have a role in the testing of GM food components, the advent of technology such as "genomics" offers a real and possibly superior alternative.

Keywords: modern biotechnology, genetically modified foods, substantial equivalence, genomics

Introduction

A genetically modified organism (GMO) is any living organism that has been manipulated at the level of its genome in a way that would not happen in nature. In theory, the DNA of any living organism can be manipulated, though most experience has been obtained through the genetic engineering of microorganisms (bacteria and viruses) and plants. However, a number of GM fish and GM animals have been developed and are awaiting authorisation for commercialisation. While microorganisms have been genetically manipulated to produce medicines, pharmaceuticals and food ingredients for some time, consumer opposition to GMOs became more widespread when crop plants were engineered to improve aspects of agricultural food production. While the reasons for this bias are multi-factorial, the food industry and regulators alike acknowledge the negative public perception and are endeavouring to improve the quality and transparency of food safety assessments. Such is the sensitivity to public perception that commercialisation of many modern biotechnology innovations in some parts of the world, including GM salmon, GM wheat and terminator technology, have been put on hold due to negative public opinion.

Science is an ever-evolving discipline and while the methods and means to produce new foods are being constantly renewed, so too must the tools used to assess the safety of new foods. The science that gave us modern biotechnology and GM food has also yielded technology that allows us to examine the safety of food at the molecular level. This new technology provides an option to reduce or possibly replace many of the animal studies routinely used in the safety assessment of GM foods where only minor differences from the parent plant are identified. An example of such a new methodology is toxigenomics, the combination of toxicology and genomics that allows the mapping of gene expression using microchip technology. Regardless of the methodology used to assess the safety of a food, a "completely safe" evaluation is not possible as no food is risk-free. This view is particularly salient given what we now know about the toxins, allergens and even carcinogens naturally present in many staple foods. In line with this, GM foods that are allowed on the EU market are classed

"as safe as their non-GM counterpart" without the guarantee of absolute safety, which does not even apply to the parent or non-GM variety.

Safety assessment of GM food

Guidelines for the safety assessment of GM food have been developed by international organisations (OECD, 1996; FAO/WHO, 1996, 2000, 2001, and 2002; and WHO, 2005), by the European Scientific Committee for Food (EU, 1997) and by the European Food Safety Authority (EFSA, 2004a). Standard GM food safety assessments are designed to identify characteristics such as nutrition, toxicity and allergenicity that can all impact on human health. Animal studies have played a significant role in GM food safety assessments and have been considered central to the identification of risk to human health. Animal studies are effective in identifying and quantifying the potential effects of chemical or microbial entities on a biological system where a cause and effect correlation is feasible. However, animal testing of whole foods such as maize or soya yields data of limited value, as the effects observed, if any, may be attributable to any of a number of the food constituents, while the consequences of minor constituents may go undetected. In addition, animal feeding studies are usually carried out on small animals such as rats, mice or chickens that have a relatively short life cycle, and the physical limitations of these animals may preclude testing for dose responses due to the potential for nutritional imbalance. Even using animals with a relatively short life cycle still requires weeks or months of tests, while biological and environmental variations demand that significant numbers of animals be tested with any results being subjected to statistical analysis. Assessment of the results of these tests can also lead to conflicting interpretations with respect to the significance of identified effects, as recently experienced by the European Food Safety Authority where the interpretation of a rat feeding study on a GM crop was challenged (EFSA, 2004b).

The majority of GM foods available on the market are derived from GM plants that differ from the parental variety only by the presence of one or a small number of introduced genes that ultimately result in the production of one or a small number of proteins or associated metabolites. Some examples are herbicide-resistant and/or insect-resistant soybeans, maize, cotton and oilseeds. The potential for introduced proteins possessing toxic or allergenic properties can be assessed *in vitro* and by using bioinformatics to compare the amino acid sequences of the new protein to known allergenic or toxic amino acid sequences stored in established databases. The insertion of a new gene into a plant genome can also result in unintended effects such as disruption in the expression or regulation of a plant gene. However, these unintended effects can generally be predicted and characterised through bioinformatic investigation of the insertion site. Unintended effects that are less predictable are possible however, and include interactions between an introduced protein and other proteins, DNA, RNA or metabolites, all of which may go unnoticed regardless of the method used.

Substantial equivalence

Fundamental to the international consensus on the safety assessment of GM food is the concept of substantial equivalence. First proposed by the OECD in 1993, (OECD, 1993) it has been developed and accepted as a first step in the safety evaluation of GM foods (FAO/WHO, 2004). The application of the principle of substantial equivalence is not a safety assessment in itself but is a framework for identifying similarities and differences between existing foods and new products. The properties of GM foods are compared with similar existing foods that have a long history of safe use, taking into account both intended and unintended effects. Detected differences are subjected to additional analyses to understand their significance and potential impact, which can ultimately be used in the final safety assessment. The OECD Task Force for the Safety of Novel Foods and Feeds, which first convened in 1999, develops and publishes "Consensus Documents" relating to important crop plants such as soybean, maize, potatoes, sugar beet and others (OECD, 2001-2005). These science-based consensus documents, which are mutually acceptable among Member States, contain what is considered to be key information on nutrients, anti-nutrients and toxicants for use in the regulatory assessment of new foods.

The concept of substantial equivalence has been challenged by various groups as vague and ill defined, and the term "substantial" has been misinterpreted in the past (Kok and Kuiper, 2003). These authors suggest that the principle should be rephrased as a "Comparative Safety Assessment", which describes the basic principle of comparing the new product with conventional counterparts, the relative safety of which are accepted.

Alternatives to animal testing for GM food safety assessment

Modern biotechnology has not only introduced new foods but also new techniques with which to characterise foods at the molecular level, which in turn provides an opportunity to reduce the reliance on animal testing. In particular, the advent of technology such as genomics, transcriptomics, proteomics and metabolomics offers new ways of examining the molecular details of food and thereby identifying even minor differences between GM plants and the non-GM parent. Genomics technology involves gene chips or microarrays that allow the analysis of thousands of genes at one time and which can identify genes that are expressed or switched off and whether expression is up or down regulated. Proteomics, including 2–dimensional polyacrylamide gel electrophoresis offers a direct window into a cell, revealing the presence or absence and levels of thousands of proteins on a single gel. Metabolomics utilises a number of standard techniques, such as GC, HPLC, MS and others, to analyse for and compare the levels of chemicals and metabolites. These techniques, when used to elucidate nutritional profiles, are termed "nutrigenomics" and for toxicology studies "toxigenomics", etc. This

technology presents advantages over animal studies in that a relatively short time scale is involved, it is more sensitive, being inclusive of minor constituents, and is reliable in that results are less reliant on statistical interpretation compared to animal studies. While genomics provides a more efficient comparative tool, it has also enabled the furnishing of a wealth of information to a number of accessible databases that can be interrogated to predict the toxicological or allergenic properties of uncharacterised proteins.

Evaluation of any significant differences and safety assessment

Once significant differences are identified between a GM plant and its non-GM parent, more detailed analyses of these constituent proteins or metabolites can be undertaken with a view of elucidating nutritional, toxicological or allergenic characteristics. While many of these studies can be carried out *in vitro* or using predictive bioinformatics, animal studies may be appropriate to determine dose responsiveness and empirical biological consequences. The significance of the observed differences can then be evaluated from the information provided at this stage and fed into the safety assessment, which may also take into account other factors such as potential intake.

Future GM foods

While the development and production of new GM foods within the EU is relatively insignificant, GM crop development and production in the rest of the world is progressing. Comparative safety assessment methodology, or substantial equivalence, could have a role to play in the safety assessment of GM foods into the future, even those with multiple added traits. However, as new, more complex and less benign GM foods are developed, assessing their safety may require a new approach. The engineering of food crops to produce additional nutrients, pharmaceuticals, medicines and even non-food constituents such as "bioplastics" promises to challenge the industry and regulators yet again to provide sufficient assurances about their safety to consumers.

Conclusions

The European Regulation (EC, 1996) on harmonised rules for the protection of animals used for experimental purposes obliges Member States to refrain from using animals where other testing methods are as effective and to promote the development of alternative experimental techniques. Numerous analytical techniques bases on "-omic" technologies exist or are under development and provide a new approach to understanding the effects of gene manipulation or chemical exposure. These advances will have significant implications for the safety assessment of GM foods and regulatory decision-making. Data generated from studies using toxigenomics, nutrigenomics and others will in future provide an objective way of assessing surrogate systems for reporting or predicting adverse effects of chemicals in humans that can replace or reduce the reliance on animal testing.

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