Lead Article

Risk assessment of genetically modified crops for nutrition and health

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The risk assessment of genetically modified (GM) crops for human nutrition and health has not been systematic. Evaluations for each GM crop or trait have been conducted using different feeding periods, animal models, and parameters. The most common result is that GM and conventional sources induce similar nutritional performance and growth in animals. However, adverse microscopic and molecular effects of some GM foods in different organs or tissues have been reported. Diversity among the methods and results of the risk assessments reflects the complexity of the subject. While there are currently no standardized methods to evaluate the safety of GM foods, attempts towards harmonization are on the way. More scientific effort is necessary in order to build confidence in the evaluation and acceptance of GM foods.

INTRODUCTION

Plant foods produced through genetic engineering, including staples such as soybean, maize, canola, rice, and potatoes, have already reached the consumer marketplace. This technology aims to express novel and desirable traits, which offer some advantages for the producer or the consumer, over conventional crops. Using modern techniques of genetic engineering (or biotechnology), it is possible to introduce specific genetic material derived from any species of plant, animal, or microorganism, or even synthetic material, into different species of plants. The resulting plants are commonly known as genetically engineered or genetically modified (GM) plants; when used as food sources, they are known as GM plant foods or GM foods.

The use of biotech crops has been rising since their commercialization in 1996. After more than a decade, the global area of planted biotech crops has increased more than 80-fold, from 1.7 million hectares in six countries in 1996, to 143 million hectares in 23 countries in 2007. The world's top six producers – the United States, Argentina, Brazil, Canada, India, and China – account for more than 90% of global GM production, with more than 50% being produced in the United States alone. GM soybean has been the principal biotech crop, occupying 51% of the global biotech area in 2007, followed by maize (31%), cotton (13%), and canola (5%).[1]

GM crops are currently classified in generations, according to the objective of the trait being introduced. The first generation of GM crops refers to seeds that have been biotechnologically derived to increase production, but the crops themselves are not substantially different from their conventional counterparts. In other words, these are similar for consumers either in appearance, taste, or nutritional value. These seeds have specific resistance mechanisms to combat herbicides, pests, diseases, or viruses. Some examples of the first-generation GM crops are the herbicide-resistant (glyphosate) soybean,[2] insect-resistant maize,[3] and herbicide- and insect-resistant potato.[4] These crops are currently planted on millions of farmland hectares.[1]

The second generation of GM plants consists of crops with new traits of direct value to consumers. It offers to the processor, end-user, and consumer, benefits such as increased levels of protein, modified and healthier fats, modified carbohydrates, improved flavor characteristics, or increased levels of micronutrients or other

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phytochemicals. Some examples of these GM plants are rice with beta-carotene or higher iron and zinc levels;[5–7] tomato with enhanced levels of carotenoids, flavonoids, and phenolics;[8–10] maize with increased vitamin C levels;[11] soybean with improved amino acid composition,[12] or potato with enhanced calcium content.[13]

A third generation of GM plants is emerging from the research pipeline. Some of the genetic modifications in these plants are designed to confer to plants a greater ability to resist abiotic stress such as drought, high temperatures, or saline soils. Other modified crops provide food with additional health benefits or renewable raw materials. This third generation also includes "pharma-plants", which are used as biological production systems for manufacturing high-grade active pharmaceutical ingredients.

Since the development of GM crops, many discussion forums, studies, and publications have been devoted to them. Topics like the advantages to developing countries, economic issues, environmental impact, ethical and social considerations, and public confidence in regulatory procedures for GM crops, are discussed repeatedly. However, the most frequent topic in the current debate over GM crops is whether or not they are safe for the environment or for human health. From the beginning, there were concerns about the possibility of unintentionally creating plant species that are super-resistant to herbicides or antibiotics or with unintended effects for human health. Among the potential risks to human health that have been listed are toxicity, allergenicity, the instability of the inserted gene, and negative effects on nutrition.[14] The concern is not about the technology but about its possible consequences. Although the environmental risks associated with GM crops are not discussed any further in this review, other excellent reviews on this topic can be consulted.[15–20] The present review focuses on the safety assessments of GM food consumption as it relates to nutrition and health.

WHY CONSUMERS ASK FOR SAFETY EVALUATION

The controversy surrounding GM foods can be traced back to the summer of 1998. Prior to that time, the production and commercialization of GM crops in the United States, the United Kingdom, and other countries had been proceeding smoothly. GM cheese was produced using GM enzymes, tomato paste was produced from GM tomatoes, and processed foods containing herbicide- or insect-resistant soybean and maize were sold at marketplaces. Cheese and tomato paste had large labels advertising that they were made from or contained GM ingredients and there was no apparent hostility towards these products. Labeling allowed consumers to choose between GM and traditional varieties. At the same time, the field of genetic engineering was moving ahead, scientists were actively performing experiments and publishing their results on plant transformation.[21,22]

In 1998, Dr. Pusztai, a senior nutrition scientist, announced on television in the United Kingdom that GM potatoes expressing a protein against pests were toxic to rats and affected their immune systems.[23] As a result, media attention became focused on GM crops and this was the catalyst for the negative reaction to GM crops and foodstuffs. Soon afterwards, the detection of GM soybean in foods not carrying appropriate labeling accentuated fears of the UK population, which had been sensitized to safety threats in the food supply following the 1996 outbreak of "mad cow disease". People had become cognizant of the relationship between some diseases and foods and were thus concerned about food safety. In subsequent years, various facts and myths related to GM foods and health were reported by the media, which also affected the public's opinion.[22] While the current food crisis could modify the public's perception of GM foods, in light of the need to increase food production, there could be a problem if the risk of each new development is not evaluated thoroughly.

RISK ASSESSMENT OF GENETICALLY MODIFIED FOODS

Since the introduction of recombinant DNA technology in plant breeding, it has been necessary to define internationally standardized guidelines for assessing the safety of foods derived from GM crops. These guidelines have been improved to obtain broad international consensus among experts on food safety evaluation, but the interpretations may be divergent. According to some specialists, safety assessment is based on scientific principles and rigorous testing, and the requirements have been more demanding for GM plants than for any other foods.[24–28] However, according to others, it is based on very little scientific evidence in the sense that the testing methods recommended are not adequate to ensure safety.[29–31] In general, it is recognized that any single method of safety assessment has strengths and weaknesses and its strength depends on the aggregate sensitivity and robustness of the evidence provided by different combined methods.

When Pelletier[32,33] analyzed the safety assessment process for GM foods, some deficiencies were revealed. The first guidelines were originally designed to regulate the introduction of GM microbes and plants into the environment with no attention being paid to food safety concerns. However, they have been widely cited as adding authoritative scientific support to food safety assessment. Additionally, the Statement of Policy released by the Food and Drug Administration of the United States, presumptively recognizing the GM foods as GRAS (generally recognized as safe), was prepared while there were critical

gaps in the scientific knowledge concerning the compositional effects of genetic transformation and the severe limitations of the methods for safety testing.

Another pitfall in the safety assessment of GM foods is the concept of substantial equivalence. Initially, it was formulated by Organisation for Economic Co-operation and Development in 1993, based on the idea that existing foods could serve as a baseline for comparing the properties of a GM food with its conventional counterpart. A Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Consultation on Biotechnology and Food Safety concluded the following: "When substantial equivalence is established for an organism or food product, it is regarded to be as safe as its conventional counterpart and no further safety consideration is needed." Some practical implications of considering that the traditional food supply is the appropriate safe reference have been highlighted. The correct manner should be to make a comparison between the new variety and the parental variety grown under the same conditions or with the range of values for all untransformed varieties grown under varying conditions. It is also important to select key compounds and the genotypic and phenotypic variations of components to include in comparative analyses.[34] However, some low-content compounds of plants with biological activity may be unknown. Therefore, methods to evaluate the overall effects independent of the composition are required.

A main issue in the risk assessment of GM foods has been the consideration that unintended consequences appear no more likely in GM crops than in conventional crops, as if GM technology is an extension of traditional plant breeding.[33] However, unintended changes in GM crops may affect other metabolites differently than those directly related to the transgene.[35] Examples of these changes in some GM crops are a higher lignin content in Bt maize than in non-Bt maize,[36] depleted plant flavonoids in herbicide-tolerant soybeans,[37] and others reviewed by Kuiper et al.[38] Therefore, substantial equivalence is not an acceptable method for GM evaluation because of its inability to detect unintended effects. Theoretically, unintended changes can be predicted from information about the insertion site of the genetic construct, gene regulation, gene-gene interactions, and possible interferences in metabolic pathways. Thus, appropriate detection methods such as DNA analysis, DNA/mRNA microarray hybridization, and proteomics and chemical fingerprinting (metabolomics) are needed. These methods were not available at the beginning of GM production and are still not widely applied to its risk evaluation.

It is currently accepted that substantial equivalence is not a safety assessment *per se*; rather, it helps in the identification of similarities and differences between conventional and GM crops for further analyses. Since 1996, guidelines prepared by the International Life Sciences Institute Europe[28] and FAO/WHO[39] recommend that safety evaluation should be based on the concept of substantial equivalence, considering parameters such as molecular characterization, phenotypic characteristics, key nutrients, toxicants, and allergens.

Since 2003, official standards for food safety assessment have been published by the *Codex Alimentarius* Commission of FAO/WHO.[40] Published reviews with around 25 peer-reviewed studies have found that despite the guidelines, the risk assessment of GM foods has not followed a defined prototype.[29,38,41,42] The present review summarizes 31 published studies of safety assessment of GM crops in Table 1, where animal models, parameters, and main effects are shown. Differences in the methodological designs among studies are also acknowledged.

It is apparent that no standardized design to test the safety of GM foods yet exists.[38,42] However, there is consensus that the safety assessment should be carried out on a case-by-case basis before a GM product is introduced to the market. Moreover, there is a need for standardization and harmonization of the design and analysis of animal feeding trials, as well as a particular need for appropriate statistical analysis of the data. The current improvement of studies has produced a tendency to use more sensitive indicators, such as transcriptomics, proteomics, and metabolomics into the experimental risk assessment approach.

Case study 1: glyphosate-tolerant soybean

The glyphosate-tolerant soybean (GTS) event 40-3-2 (Roundup Ready™) has been widely studied; however, it continues to generate controversy. Earlier, it was demonstrated that there were no differences in composition between GTS and its isogenic line.[43] Also, no toxicity occurred with the novel expressed protein (5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, EPSPS-CP4)[44] and, finally, feeding value was not altered by genetic modification.[45] An early compositional study[43] was conducted using unsprayed Roundup Ready™ soybeans, and subsequent studies with soybean treated with glyphosate confirmed the initial results.[46,47] However, the toxicity assay was done using EPSPS-CP4 protein expressed in bacteria and not in soybean. Although the gene is the same, processing is different; while bacteria do not add carbohydrates to proteins, plants do.[29] Glycosidic moieties of glycoproteins are involved in recognition events in the immune system.[48] Therefore, the response evaluated for the bacterial EPSPS-CP4 could be different than that of the plant-produced EPSPS-CP4.

Even though the genetic construct introduced into GTS was described, no information was available for

Table 1 **Studies of safety assessment performed with genetically modified crops.**

GM food	Inserted protein or trait	Animal model	Sample size (each diet)	Parameters	Effects	*P* value*	Reference
Potato	GNA lectin, insect-resistant	Rat	6	Gut histopathology	Gastric mucosa proliferation, thinner cecal mucosa	<0.05	Ewen and Pusztai. (1999)[23]
Potato	Cry1, insect-resistant	Mouse	5	Light and electron microscopic structure of ileum	Several villi with abnormally large enterocytes, hypertrophied and multinucleated		Fares and El-Sayed. (1998)[82]
Potato	PAT, gluphosinate-tolerant	Rat	50	Body weight, food consumption, reproductive performance, and organ weight	Mating and fertility index, development and viability of pups, final body weight, relative organ weight, skeletal and visceral alterations in fetuses	>0.05	Rhee et al. (2005)[84]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	50	Toxicity, food consumption, body weight, gross pathology	Minor pathological findings in female mice, such as corneal opacity, kidney and pituitary lesions, and hydrometra of the uterus randomly distributed among all groups	>0.01 for body weight; pathological findings not considered treatment related	Harrison et al. (1996)[44]
Soybean	CP4-EPSPS, glyphosate-tolerant	Rat	10	Growth, feed conversion, histological changes	Differences ($P < 0.05$) in relative organ weights and pathologic findings observed for males fed GTS or parental line ground soybeans (unprocessed) compared with control-diet males.	Not considered related to genetic modification	Hammond et al. (1996)[45]
Soybean	CP4-EPSPS, glyphosate-tolerant	Broiler	120	Growth, feed conversion, breast muscle, fat pad weights	No differences among conventional and GM diets	>0.05	Hammond et al. (1996)[45]
Soybean	CP4-EPSPS, glyphosate-tolerant	Catfish	100	Growth, feed consumption, fillet composition	No differences among conventional and GM diets	>0.05	Hammond et al. (1996)[45]

Soybean	CP4-EPSPS, glyphosate-tolerant	Dairy cattle	12	Milk production, milk composition, rumen fermentation, nitrogen digestibility	No differences among conventional and GM diets	>0.05	Hammond et al. (1996)[45]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	12	Ultrastructural, morphometrical and immunocytochemical analyses of exocrine pancreas	Decreased total area, percentage, and granule area of zymogen	<0.05	Malatesta et al. (2002)[53]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	12	Ultrastructural, morphometrical and immunocytochemical analyses on hepatocytes	Irregularly shaped nuclei, higher number of nuclear pores	<0.05	Malatesta et al. (2002)[55]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	12	Structural and molecular modifications of nucleoplasmic and nucleolar constituents on pancreatic acinar cell nuclei	Lowering of nucleoplasmic and nucleolar splicing factors, perichromatin granule accumulation	<0.05	Malatesta et al. (2003)[54]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	12	Ultrastructural analysis of testes	Decreased Sm antigen, hnRNPs, SC35, and RNA polymerase II at 2 and 5 mo.	<0.05	Vecchio et al. (2004)[56]
Soybean	CP4-EPSPS, glyphosate-tolerant	Mouse	8	Fetal, postnatal, pubertal, and adult testicular development	No differences among conventional and GM diets	>0.05	Brake and Evenson. (2004)[51]
Soybean	CP4-EPSPS, glyphosate-tolerant	Salmon	300	Intestinal somatic indices, histology, and cell proliferation	Cell proliferation in distal intestine with respect to diet control	<0.05	Sanden et al. (2005)[60]
Corn	Cry1Ab, resistance to European corn borer (MON810)	Salmon	300	Intestinal somatic indices, histology, and cell proliferation	Lower cell proliferation	<0.05	Sanden et al. (2005)[60]

Table 1 **Continued**

GM food	Inserted protein or trait	Animal model	Sample size (each diet)	Parameters	Effects	*P* value*	Reference
Soybean	CP4-EPSPS, glyphosate-tolerant	Salmon	300	Feed utilization, whole body, liver and muscle proximate compositions, muscle fatty acid profiles, relative sizes of organs	Decreased spleen and distal intestine somatic index	<0.05 and <0.02, respectively	Hemre et al. (2005)[52]
Soybean	CP4-EPSPS, glyphosate-tolerant	Rabbit	10	Determination of several specific enzymes	Increased LDH1 in kidney and heart	<0.05	Tudisco et al. (2006)[58]
Soybean	CP4-EPSPS, glyphosate-tolerant	Salmon	600	Histological, digestive, metabolic, hormonal, and immune response	Moderate inflammation in the distal intestine and increased head kidney lysozyme activity	<0.05	Bakke-McKellep et al. (2007)[59]
Soybean	CP4-EPSPS, glyphosate-tolerant	Rat	17 (two bioassay)	Nutritional evaluation, plasma amylase levels, histological and gene expression pancreatic response	Zymogen-granule depletion, acinar disorganization, acute increase of PAP mRNA	<0.05	Magaña-Gómez et al. (2008)[57]
Soybean	CP4-EPSPS, glyphosate-tolerant	Salmon	300	Growth parameters, histological screening, enzyme activities, transport activity, and protein expression in intestinal brush border membrane vesicles and endocrine pancreatic and immune response	Intestinal Na+-dependent D-glucose uptake, and SGLT1 protein level in the pyloric cecal region	<0.01	Bakke-McKellep et al. (2008)[64]
Corn	Cry1Ab, resistance to European corn borer (MON810)	Salmon	300	Growth parameters, histological screening, enzyme activities, transport activity, and protein expression in intestinal brush border membrane vesicles and endocrine pancreatic and immune response	No differences among conventional and GM diets	>0.05	Bakke-McKellep et al. (2008)[64]

Corn	Cry1Ab, resistance to European corn borer (Bt176)	Chicken	NS	Overall health, nutritional parameters, gross appearance of organs	Significant increase in breast skin and *Pectoralis minor* yield	NS	Brake and Vlachos. (1998)[80]
Corn	Cry1A, resistance to European corn borer (Bt176)	Sheep	NS	Body weight gain, feeding value	No differences among conventional and GM diets	>0.05	Barriere et al. (2001)[61]
Corn	Cry1A, resistance to European corn borer (Bt176)	Cows	24	Body weight gain, feeding value	No differences among conventional and GM diets	>0.05	Barriere et al. (2001)[61]
Corn	Cry9c, resistance to European corn borer (CBH351)	Chicken	128	Body weight gain, feed conversion, biochemical and hematological values	No differences among conventional and GM diets	>0.05	Yonemochi et al. (2002)[70]
Corn	Cry1Ab, resistance to European corn borer (MON810)	Dairy cattle	12 and 16 (two experiments)	Feed intake, body weight change, ruminal digestion, milk production and composition	No differences among conventional and GM diets	>0.05	Donkin et al. (2003)[71]
Corn	CP4-EPSPS, glyphosate-tolerant (GA21)	Dairy cattle	12	Feed intake, body weight change, ruminal digestion, milk production and composition	No differences among conventional and GM diets	>0.05	Donkin et al. (2003)[71]
Corn	CP4-EPSPS, glyphosate-tolerant (GA21)	Steer	175	Feedlot steer performance, carcass characteristics	No differences among conventional and GM diets	>0.05	Erickson et al. (2003)[72]
Corn	CP4-EPSPS, glyphosate-tolerant (nk603)	Steer	196 and 200	Feedlot steer performance, carcass characteristics	No differences among conventional and GM diets	>0.05	Erickson et al. (2003)[72]
Corn	CP4-EPSPS, glyphosate-tolerant (nk603)	Cows	4	Milk production and composition, body weight	No differences among conventional and GM diets	>0.05	Ipharraguerre et al. (2003)[85]

Table 1 **Continued**

GM food	Inserted protein or trait	Animal model	Sample size (each diet)	Parameters	Effects	*P* value*	Reference
Corn	Bt endotoxin, insect-resistant (Bt176)	Mouse testes	10	Germ cell populations	No differences among conventional and GM diets	>0.05	Brake et al. (2004)[65]
Corn	CP4-EPSPS, glyphosate-tolerant (nk603)	Rat	200	Overall health, body weight, food consumption, clinical pathology parameters, organ weights, gross and microscopic appearance of tissues	No differences among conventional and GM diets	>0.05	Hammond et al. (2004)[67]
Corn	Cry3Bb1, resistance to corn root worm (MON863)	Rat	200	Food consumption, hematology, blood biochemical indices, organ weights, and histopathology	Slight increase in male white blood cells and glucose, decrease in chloride and kidney tubule mineralization, increase of focal inflammation and tubular regenerative changes in kidneys, decreased cardiomyopathy	<0.05	Hammond et al. (2006)[68]
Corn	Cry1Ab, resistance to European corn borer (MON810)	Salmon	270	Selected stress- and immune-response biomarkers at the gene transcript (mRNA) and protein level	Small changes in stress protein levels and activities, changes in white blood cell level associated with an immune response	<0.05	Sagstad et al. (2007)[69]
Peas	α-amylase inhibitor	Rat	4	Weight gain, tissue weights, nutritional evaluation	Decrease of nutritional value at the higher (650 g) inclusion level	<0.05	Pusztai et al. (1999)[81]
Sweet pepper	Coat protein, resistance to cucumber mosaic virus	Rat	40	Growth, body weight gain, hematology, and biochemical indices, acute toxicity, genotoxicity	No differences among conventional and GM diets	>0.05	Chen et al. (2003)[11]

Tomato	Coat protein, resistance to cucumber mosaic virus	Mouse	40	Growth, body weight gain, hematology and biochemical indices, acute toxicity, genotoxicity	No differences among conventional and GM diets	>0.05	Chen et al. (2003)[11]
Rice	Cry1Ab, insect-resistant (KMD1)	Rat	32	Animal behavior, weight gain, hematological and biochemical parameters, macroscopic and histopathological examinations of organs	Higher sodium, urea, and glucose levels, reduced protein and adrenal levels, lower MCH and white blood cell count, increased relative and absolute weight of testis and absolute weight of uterus	<0.05	Schroder et al. (2007)[77]
Rice	GNA lectin, insect-resistant	Rat	32	Clinical, biological, immunological, microbiological, and pathological parameters	Lower potassium, protein, albumin, creatinine, MCHC, and large unstained cells, increased alanine aminotransferase level and PLT, increased weight of small intestine and adrenals	<0.05	Poulsen et al. (2007)[76]
Rice	PHA-E lectin, insect-resistant	Rat	16	Biological, biochemical, microbiological, and pathological parameters	Increased weight of small intestine, stomach, and pancreas	<0.05	Poulsen et al. (2007)[74]

* In all reviewed studies, $P < 0.05$ was considered significantly different, except in the study of Harrison et al. (1996)[44] where $P < 0.01$ was statistically significant.
Abbreviations: LDH1, lactic dehydrogenase 1; MCH, mean corpuscular hemoglobin; MCHC, mean cell hemoglobin concentration; NS, not specified; PAP, pancreatitis-associated protein; PLT, platelet count.

some years about the exact DNA sequences following the endpoints where it was inserted. It was recently reported that integration of the inserted DNA produced several rearrangements at the 3′ NOS junction and that the genomic plant DNA at the pre-integration site may have been rearranged.[49] An additional 250-bp fragment of the *epsps* gene localized downstream of the NOS terminator is processed further, resulting in four different RNA variants, which might code for (as yet unknown) EPSPS fusion proteins.[50]

Several studies of GTS have been conducted to evaluate health and nutritional risks; 13 of them are summarized in Table 1. No differences have been found between GTS and its conventional counterparts in animal growth parameters, organ weight, and appearance.[45,51–53] In spite of this, adverse effects were detected at ultramicroscopic and molecular levels. The effects of the chronic ingestion of GTS soybean were studied in Swiss mice.[53–55] Pregnant mice were fed a diet containing 14% GTS or wild soybean and the respective litters were also fed with the same parental diet from the ages of 1 to 8 months. Body weight, pancreas, and liver macroscopic appearance were similar between control and GTS-fed animals. However, statistically significant differences were found after analyses of ultrastructural microscopy and immunohystochemistry results. The livers of the GTS-fed mice had irregularly shaped nuclei, suggesting a high metabolic rate, and higher numbers of nuclear pores, indicating intense molecular trafficking. Similarly, the nucleoli had typical signs of increased metabolic rate. In pancreas, the zymogens content, total zymogens area, percentage of cytoplasmic area occupied by zymogens, and zymogens granule size were always smaller in the GTS-fed mice than in the control mice. Thus, it seems that the GTS diet influenced the synthesis and processing of the zymogens. Analysis of the mice testes suggested that, during the 2–8-month interval, a transient transcriptional decrease occurred in the GTS-fed mice.[56] While it cannot be ruled out that traces of the herbicide glyphosate possibly played a role, the exact cause of these modifications remains unspecified and further investigation is recommended.

Another study analyzed the acinar pancreatic histology and the expression of pancreatitis-associated protein (PAP) and trypsinogen mRNAs in rats fed GTS protein. Wistar rats were distributed into two groups fed with either non-GM or GTS protein (18% protein) from 0 to 30 days. No differences were found in the nutritional performance of the diets. The GTS diet induced significant depletion of zymogens granules and acinar disorganization 5 days after feeding, with these parameters increasing until day 15 and returning to normal levels after day 30. Levels of PAP mRNA increased significantly in the early days and decreased to the basal level by day 15. The authors concluded that GTS protein intake affected pancreatic function, as evidenced by the increased levels of early acute PAP mRNA and cellular changes in the pancreas, followed by regeneration at 15 days and full recuperation of acinar cells after 30 days.[57]

GTS was also analyzed for metabolic effects in rabbits.[58] The animals received a diet containing 20% soybean meal from GTS or conventional soybeans (representing around 65% of the total protein requirements for rabbits[58]) for 40 $\pm$ 5 days. No effects were detected on body and organ weight, but a significant increase in the level of lactic dehydrogenase 1 was found in the kidney and heart, suggesting potential alteration in the local production of the enzyme due to an increase in cell metabolism. The methodology used introduces enzymatic analysis as an additional tool to evaluate the risks of GM consumption on cell metabolism, even in the absence of clinical and biochemical signs.

GTS has also been evaluated in the Atlantic salmon *Salmo salar* L, and the results were published in three peer-reviewed publications.[52,59,60] Post-smolt salmon were fed a diet containing 130 g/kg of GTS protein for 3 months. The GTS diet was compared with a commercial hybrid non-isogenic soybean line (non-GM) diet and with a standard fish-meal diet without soybean protein. This comparison is relevant, because both fish-meal diets are common commercial products. A pitfall was the slight difference in anti-nutrient levels among the diets. However, similar to other evaluations, no significant differences were found in feed utilization, whole body, and liver and muscle fatty acid profiles. The relative sizes of the kidney, liver, and brain were similar in all dietary groups, while the spleen was larger in the GTS-fed group. Soybean (from GTS or control diets) reduced the size of the distal intestine. The incidence of moderate inflammation and head kidney lysozyme activity was statistically significantly higher in the GTS-fed fish, compared to the non-GM-soybean-fed fish. The last effect is a possible indicator of phagocytic activity or of phagocyte presence in the tissue.

Brake and Evenson,[51] studied the effects of GTS soybean in mouse testes. Pregnant mice were fed with GTS or a non-transgenic diet during gestation and lactation. After weaning, male litters were maintained on the respective parental diets. At 8, 16, 26, 32, 63, and 87 days after birth, the testes were surgically removed, and the percentage of germ cell populations was measured by flow cytometry. The results showed no differences between the mice fed the GTS diet and those fed the conventional diet. It was concluded that GTS had no measurable or observable effect on fetal, postnatal, pubertal, or adult testicular development or body growth.

The absence of negative effects attributable to glyphosate-tolerant soybean intake in gross indicators of nutrition and health was constant in all studies. However,

a tendency towards microscopic and molecular changes was observed, suggesting some kind of cell damage. These studies should be used to support further experiments using profiling techniques to screen for potential changes at different cellular levels: gene expression, protein translation, or metabolic pathways. In addition, obstacles such as difficulty acquiring the non-GM parent line of the GTS crop for evaluation, assuring nutritional equivalence among diets, and identifying the best animal model must be surmounted. It is clear that evaluation of the potential health risks associated with GTS intake is not yet definitive.

Case study 2: GM maize

In 2007, GM maize became the second most important biotech crop after GM soybeans,[1] and the first one to have a wider rariety of genetic modifications than GTS. The traits of GM maize have been evaluated for compositional and agronomic features.[61,62] Additionally, in vivo studies have been conducted to test the health safety of different transgenic maize events.[63–70] It has been concluded that some of the GM maize traits are substantially equivalent to their conventional counterparts.

Barriere et al.[61] evaluated the GM Bt176 maize hybrid (Rh208Bt) in comparison to its isogenic line (Rh208) in three separate feeding trials, in Texel sheep, Holstein cows, and midlactation multiparous Holstein cows for 1, 13, and 3 weeks, respectively. No differences were found between GM Bt176 maize and its isogenic line in terms of the coagulation properties of the produced milk, as well as the digestibility of organic matter, crude fiber, and neutral detergent fiber. The authors concluded that cattle could be fed equally well with GM Bt176 or conventional maize silage.

Donkin et al.[71] evaluated the effects of feeding silage and grain from maize resistant to European borer (Bt-MON810, experiment 1 and 2) and glyphosate-tolerant Roundup Ready™ maize (RR-GA21, experiment 3) in dairy cattle. Diets contained 42–60% maize silage and 20–34% maize grain from Bt-MON810, RR-GA21, or the appropriate non-transgenic counterpart. The treatments were applied using a switchback design of three periods of 21 days in experiment 1 and 28 days for experiments 2 and 3. There were no differences in feed intake, ruminal digestion, and milk production between Bt-MON810 or RR-GA21 maize and its counterpart. A weakness of this study was that although the total protein content in the diets was adequate, the maize protein content was limited.

Erickson et al.[72] carried out three experiments to test the effect of Roundup-Ready™ events GA21 or nk603 maize on steer performance and carcass characteristics. The assay periods were 92, 94, and 144 days, including a 20-day diet adjustment period in experiments 1 and 2 and a 28-day adjustment period for experiment 3. The final diets contained a maximum of 75% maize. Performance and carcass characteristics were not affected by Roundup Ready™ maize, and the authors concluded that it was similar to the non-transgenic maize at the end of the feeding trials. These experiments demonstrate once again that genetic modification does not affect macroscopic health and nutrition indicators.

Hammond et al.[67] presented the results of a 13-week feeding study in rats with diets containing 11% and 33% Roundup Ready™ maize (nk603) or controls (non-GM corn). Overall health, body and organ weights, food consumption, hematology, blood chemistry, and urinalysis were analyzed. Organ weights and the gross and microscopic appearance of tissues were comparable between the groups fed each Roundup Ready™ maize diet and its control. This study involving macro- and microscopic indicators confirms that nk603 maize is as safe and nutritious as existing commercial maize hybrids. Although the diets were well balanced, the total level of maize protein was as high as 3.3%, and the presence of the GM protein, which was several times lower, could potentially not be enough to induce any adverse reaction.

A special case of risk assessment was the one carried out on the YieldGard® Rootworm maize (MON 863).[68] Rats were fed for 90 days with diets at the same levels (11% or 33%, w/w) of MON 863 in comparison to its non-GM near-isogenic control line. Additionally, six groups of rats were fed diets containing grain from different conventional (non-GM) reference varieties. Evaluated parameters were overall health, body weight gain, food consumption, clinical pathology parameters, organ weight, gross and microscopic appearance of tissues. Male rats fed the 33% MON 863 diet presented a slightly elevated white blood cell count, lymphocyte count, and number of absolute basophiles. Also, a slight increase in glucose (females) and decrease in chloride (males) was observed in rats fed the MON 863 diet. These changes were considered within the variability of the reference population. A statistically significant minor incidence of kidney tubule mineralization (females) and high incidences of focal inflammation and tubular regenerative changes in the kidneys (males) were also shown for rats fed the 33% MON 863 diet. In spite of these differences, the authors considered that most of the microscopic findings were of minimal severity, incidental, and not treatment related.

In the previously described MON 863 study, none of the pathological findings were considered to be attributable to the tested crop. Later, the database of the results was released and reanalyzed.[73] When appropriate statistical analyses were applied, slight but dose-related significant variations in growth were observed. Signs of hepatorenal toxicity were revealed by chemistry measure-

ments; triglycerides increased in females and urine phosphorous and sodium excretions were diminished in males. Therefore, the two main organs of detoxification, the liver and kidney, were disturbed. It is important to note that effects were evident at a very low level of GM protein. Perhaps other non-protein, unintentionally expressed compounds affected the animals. Thus, reassessment is advised before concluding that MON 863 is a safe food for animals or humans.

Bt176 maize was analyzed similarly to the Roundup Ready™ soybean study[51] to test the potential toxic effects on mouse testes as a sensitive form of biomonitoring.[65] The authors concluded that ingestion of Bt176 maize in a nutritionally balanced diet by the mother during pregnancy and lactation and later by litters had no negative effect on fetal, postnatal, pubertal, or adult testicular development or body growth.

Case study 3: GM rice

To solely evaluate the in vivo testing approach proposed within the European project titled "New methods for the safety testing of transgenic food" (SAFOTEST), a type of rice was genetically modified with a gene encoding an insecticidal protein from the kidney bean, the *Phaseolus vulgaris* lectin E-form (PHA-E lectin). This lectin is known to possess high mammalian toxicity when tested in its raw uncooked form.[74] For evaluation, the animals in three experimental groups containing 16 females each were randomized and stratified. The rats were fed purified diets containing 60% parental rice, or PHA-E rice, or PHA-E rice + 0.1% PHA-E lectin for 13 weeks. Biological, biochemical, microbiological, and pathological parameters were examined. Significant differences were seen between groups in the weights of the small intestine, stomach, and pancreas and in plasma biochemistry, with effects seen in the rats fed the PHA-E rice whether or not it was spiked with PHA-E lectin. Although no dose-response relationships were found, for most of the changes, the differences were either statistically significant or showed a tendency for the effects to be more prominent in the group fed PHA-E rice spiked with PHA-E lectin. According to the authors, this suggests that the majority of effects seen in the 90-day study were caused by the presence of the gene product and not by secondary effects of the genetic modification.

The former evaluation model also included analyses for chemical composition and molecular characterization as well as the construction and production of the recombinant PHA-E lectin for a preceding assay in order to evaluate the toxicity. Therefore, the 90-day study was improved in design and could be a valuable tool for evaluating the safety of GM foods. However, it is difficult to obtain the purified expressed protein for spiking the diet and also to design the best model and method to pre-evaluate toxicity. In the case of the PHA-E lectin, there were several previous studies, conducted principally by Pusztai et al., providing antecedents for the study design.[75]

Poulsen et al.[76] used an experimental design to assess the safety of a rice variety expressing the snowdrop *Galanthus nivalis* (GNA lectin), which was similar to one they used to evaluate the rice expressing the PHA-E lectin without including the previous in vivo determination of toxicity of the pure lectin.[74] Ranges of clinical, biological, immunological, microbiological, and pathological parameters were examined. There was no statistically significant difference in food consumption between groups. However, a significantly higher relative water intake was seen in rats fed the GNA-rice diet. The authors attribute some hematological differences to this increased water intake. A statistically significant increase in the relative weight of the small intestine (+10%) was observed in female rats fed on GNA rice, as well as an increase in the absolute and relative weight of the adrenals. A significantly higher level of alanine aminotransferase was observed in females fed GNA rice and could indicate some kind of effect on the liver.

Schroder et al.,[77] tested the transgenic KMD1 rice expressing the Cry1Ab protein (Bt rice), compared to its non-transgenic parental wild type. No differences in weight gain were observed during the study. Histopathological examination revealed minor changes, but these were reportedly not attributable to KMD1 rice.

Other cases

Chen et al.,[11] assessed the safety of GM sweet pepper and tomato expressing the cucumber mosaic virus (CMV) coat protein (CP) gene. Acute toxicity assay (LD50), micronucleus test, sperm aberration test, and Ames test were performed in addition to a 30-day feeding period. Results of the micronucleus test, sperm aberration test, and Ames test revealed that GM sweet pepper and tomato were not genotoxic either in vitro or in vivo. Rats fed a diet of sweet pepper or tomato were not affected in growth, body weight gain, food consumption, hematology, blood biochemical indices, organ weights, and histopathology in comparison with those fed the non-GM diet. The authors concluded that the CMV-resistant sweet pepper and tomato were as safe as their non-GM counterparts.

Other GM crops have also been evaluated for safety. Examples include the Canola GT200 and GT73 tested in rainbow trout[78] and peas tested in mice.[79] None of these studies found differences in the assayed animals that could be attributable to the genetically modified crop under investigation.

OVERVIEW OF FINDINGS

Several feeding trials have been reported that tested GM maize, potatoes, rice, soybeans, and tomatoes for different periods, and parameters such as body weight, feed consumption, blood chemistry, organ weight, and histopathology have been measured. Over time, studies assessing the risk of GM foods have improved, and publications have begun including sensible and specific indicators about the safety of GM food consumption in accordance with changes in the guidelines. In publications reviewed by Pryme and Lembcke,[42] as well as in those mentioned in this review, the conclusions have varied from no alteration of the nutritional value of the GM food tested,[11,45,51,61,65,67,71,80] to minimal detrimental effects on the nutritional value,[81] to in vivo submicroscopic effects in different animal species.[23,54,55,57,82]

Animal models used to test GM foods have been diverse, including rats, mice, cattle, fish, and poultry, and the assay periods have also varied. Some of the studies did not use microscopic, biochemical, or in vivo indicators to test for effects in the animals.[59,61,71] These studies only analyzed the performance of the animals in terms of body weight, food ingested, or milk production. Additionally, metabolic alterations or classic indicators of stress or some kind of adverse effects should be searched. Some researchers analyzed the effects at the level of inheritance or testicular development, but not in organs or specific tissues previously reported to show evidence of alteration due to GM foods.[51,65] The most common result has been that there were no effects at the macroscopic level; however, organelles and other subcellular structures are clearly affected, as shown at ultramicroscopic levels.[52–58,59,68,73,74,76]

The necessity of testing GM crops case by case has been established. Therefore, efforts should be directed towards finding the best experimental design, taking into consideration the inclusion levels of GM food and the appropriate animal model to detect effects that can occur in a human organism. One drawback is that animal studies are performed without prior identification of all the possible harmful substances; therefore, it could be difficult to achieve a sufficient dose in experimental diets. Some whole foods, such as grain and other staple crops, can provide adequate nutrition for some test animals when consumed as a high percentage of the diet. However, the methods have not been validated for foods containing substantial amounts of harmful substances or those with limited nutritional value to serve as a major constituent of the test-animal diet, as is required in order to obtain sufficient exposure to detect problems in animals. Furthermore, testing a single animal model does not seem to be sufficient to assure health safety since the metabolic differences among species could mask or hide adverse effects. The methodology developed previously by Poulsen et al.[74] to determine the LOAEL of tested GM molecule resulted in an interesting strategy. If no effects are observed between groups, it can be debated whether this was due to a lack of real effects or a lack of sensitivity and specificity of the study.

Another important point is the level of inclusion of the tested food. It is well known that the evaluation of a whole food poses complications for the diet formulation. Some imbalance could result from the inherent components of the GM food. Although many GM crops have been approved for direct human consumption, the byproducts have arrived at the food chain, especially from the the soybean. This grain is processed industrially and used in several forms, as the protein concentrates and isolates. Therefore, the evaluation of different industrial products must be considered. An additional problem is the difficulty of acquiring the non-GM parent line of the tested GM crop. Without adequate controls, it is difficult to attribute the differences between the non-GM and GM groups to the GM food or to the different nutrient and anti-nutrient compositions of each crop that are caused by genetic and/or environmental influences. Natural variations in large numbers of plan genes and proteins, the functions of many of which may not yet be known, could obscure the biological significance of such changes.

The detection and characterization of unintended effects of genetic modification continues to be an issue requiring more research. Inferences about the statistically significant changes observed in in vivo studies need to be based on more than just chemical analyses of single macronutrients and micronutrients and known anti-nutrients or toxins. The newly developed methods of screening for potential alterations in the metabolism of the modified organism such as analysis of gene expression (microarrays, mRNA fingerprinting), overall protein analysis (proteomics), and secondary metabolite profiling should be integrated in the risk assessment process. Thus, the biological relevance and significance of expression profile alterations and significant changes in animal studies could be integrated to facilitate more reliable interpretation.

The in vitro methods can serve either as screening systems to assess the potential toxicity of a compound or for studying a toxicological mechanism underlying a specific effect observed in vivo or predicted from the structure of a molecule.[83] The nutritional status indicators are not sufficiently sensitive to detect changes in the organism. Therefore, the safety assessment must integrate advances in genomics, nutrition, toxicology, and new technological developments to thoroughly investigate the possible effects of GM foods. These methods should be applied in the evaluation of second- and third-generation GM crops, which intentionally induce compositional

changes. Therefore, the substantial equivalence principle is not applicable and should be assessed on a case-by-case basis with focus placed on the modified metabolic pathways.

CONCLUSION

The controversy about the health safety of GM foods is complex and good science and its communication are required in order to find solutions. The current guidelines for the safety assessment of GM foods have broad evaluation criterions but no detailed methodologies for testing safety or thorough guidelines.

In order to prove the safety of transgenic foods, it is necessary to exhaust the available possibilities, not discard the previous studies. The advantages of transgenic foods could provide solutions for many problems, but it is first necessary to prove that these foods will not cause other problems. Although numerous advancements can improve the reliability of GM food safety assessment, additional research in other important areas are needed in order to develop new and more effective methods. Advances in molecular biology, toxicology, biochemistry, and nutrition hold the promise of providing sets of genes and methodologies that serve as biomarkers for a cell's responses to toxins, allergens, or other compounds. They will facilitate the development of new tools to facilitate the advancement and assessment of GM crops. The scientific priority is to contribute to the improvement of human and animal health or natural resource management without compromising public safety. More scientific effort and investigation is needed to ensure that consumption of GM foods is not likely to provoke any form of health problem. The next step in GM crop safety assessment is to have regulatory agencies adopt the developments and recommendations that have been made by advisory committees convened by regulatory agencies and science organizations and put forth in scientific publications.

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