

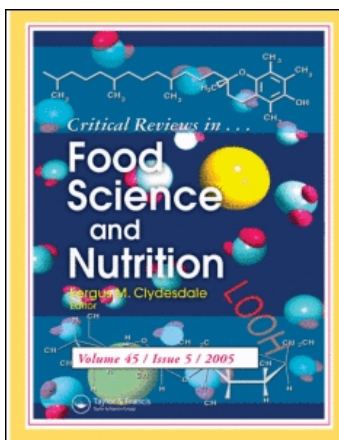
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## Toxicity Studies of Genetically Modified Plants: A Review of the Published Literature

José L. Domingo <sup>a</sup>

<sup>a</sup> Laboratory of Toxicology and Environmental Health, School of Medicine, "Rovira I Virgili" University, San Lorenzo, Reus, Spain

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# Toxicity Studies of Genetically Modified Plants: A Review of the Published Literature

JOSÉ L. DOMINGO

Laboratory of Toxicology and Environmental Health, School of Medicine, “Rovira I Virgili” University, San Lorenzo 21, 43201 Reus, Spain

*According to the information reported by the WHO, the genetically modified (GM) products that are currently on the international market have all passed risk assessments conducted by national authorities. These assessments have not indicated any risk to human health. In spite of this clear statement, it is quite amazing to note that the review articles published in international scientific journals during the current decade did not find, or the number was particularly small, references concerning human and animal toxicological/health risks studies on GM foods. In this paper, the scientific information concerning the potential toxicity of GM/transgenic plants using the Medline database is reviewed. Studies about the safety of the potential use of potatoes, corn, soybeans, rice, cucumber, tomatoes, sweet pepper, peas, and canola plants for food and feed were included. The number of references was surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce these products. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe?*

**Keywords** genetically modified (GM) plants, toxicity, safety, health risks, DNA

## INTRODUCTION

The World Health Organization (WHO) defines genetically modified organisms (GMOs) as those organisms in which the genetic material has been altered in a way that does not occur naturally (WHO, 2002). The technology used allows selected individual genes to be transferred from an organism into another, and also between non-related species. Such methods are used to create genetically modified (GM) plants, which are then used to grow GM food crops. The GM crops currently on the market are mainly aimed at an increased level of crop protection through the introduction of resistance against plant diseases caused by insects or viruses, or through increased tolerance towards herbicides.

Taking into account that different GMOs include different genes inserted in different ways, the WHO indicates that individual foods and their safety should be assessed in a case-by-case basis, and that it is not possible to make general statements on the safety of all GM foods. In general terms, the safety assessment of GM foods should investigate:

- a) toxicity,
- b) allergenicity,
- c) specific components thought to have nutritional or toxic properties,
- d) stability of the inserted gene,
- e) nutritional effects associated with genetic modification, and
- f) any unintended effects which could result from the gene insertion (WHO, 2002).

Although the WHO declares that the GM products that are currently on the international market have all passed risk assessment conducted by national authorities, in a review on the scientific literature performed in 2000, we were not able to find sufficient published information concerning that assessment (Domingo and Gómez, 2000). In particular, the lack of published toxicological studies on adverse health effects was evident. Although a considerable number of commentaries, general news, and letters to the Editor were published in reputable international journals, papers about experimental investigations on the safety of GM foods were surprisingly very scant. We concluded that if data on toxicological assessment of GM foods were obtained, these were not reported in scientific journals and subjected to the scientific judgment (Domingo, 2000; Domingo and Gómez, 2000).

Address correspondence to Dr. Jose L. Domingo, School of Medicine, URV, San Lorenzo 21, 43201 Reus, Spain. Tel.: +34 977 759380; Fax: +34 977 759322; E-mail: joseluis.domingo@urv.cat

An important problem seems to be related to the safety assessment of new GM foods, which is initially based on the use of the concept of “substantial equivalence.” This concept is based on the following principle: “if a new food is found to be substantially equivalent in composition and nutritional characteristics to an existing food, it can be regarded as being as safe as the conventional food” (SOT, 2003). Although application of the concept is not a safety assessment per se, it enables the identification of potential differences between the existing food and the new product, which should then be investigated further with respect to their toxicological impact. It is a starting point rather than an end point (Kuiper et al., 2002).

Which is the current situation concerning health risks of GM foods six years after our previous revision was performed (Domingo and Gómez, 2000)? The scientific literature on the potential adverse health/toxic effects of GM/transgenic foods has been again reviewed using the Medline database (available at <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>). The search covered the period January 1980-October 2006. The following “key terms” (number of references in parenthesis) were used: genetically modified foods (686), GM foods (3498), transgenic foods (4127), toxicity of transgenic foods (136), health risks of transgenic foods (23), adverse effects of genetically modified foods (170), toxicity of genetically modified foods (38), health risks of GM foods (38), health risks of genetically modified foods (72), toxicity of GM foods (120), adverse effects of GM foods (276), and adverse effects of transgenic foods (199). It can be seen that citations corresponding to general “key terms” such as: genetically modified foods, GM foods, and transgenic foods are quantitatively very important. However, references concerning specific risk assessment are much more limited. Moreover, most references corresponding to the key terms “adverse effects,” “toxicity” and “health risks,” did not directly correspond to the main topic of the search. A review of the published studies directly related with health risks (including toxicity) of GM plants consumed as food and/or feed is here presented. Information and details are given according to the specific plant. A summary of results concerning the most relevant studies are summarized in Table 1. With only a few exceptions, studies concerning allergenicity of GM plants were not included here. However, a system of food allergy vigilance encompassing the full range of foods consumed is clearly essential (Moneret-Vautrin et al., 2004). Those GM crops that are specifically related to food sensitivity (e.g., wheat, peanuts) are of special concern.

## GM PLANTS

### Potatoes

In the mid 1970s, the WHO and other international institutions initiated studies on the development of existing and new biological control agents for pest controls. The most popular of these agents are strains of *Bacillus thuringiensis*. Among

these, *Bacillus thuringiensis* var. *kurstaki* was proven to produce an effective toxin against lepidopteran insects. In recent years, transgenic potatoes were produced in which the CryI gene of *Bacillus thuringiensis* var. *kurstaki*. The gene was transmitted into the plant cells via a shuttle plasmid vector after cloning in *E. Coli*. Fares and El-Sayed (1998) investigated the effect of feeding transgenic potatoes, which carry the CryI gene of *Bacillus thuringiensis* var. *kurstaki* strain HD1, on the light and electron microscopic structure of the mice ileum, in comparison with feeding potatoes treated with the “delta-endotoxin” isolated from the same bacterial strain. The microscopic architecture of the enterocytes of the ileum of both groups of mice revealed certain common features such as the appearance of mitochondria with signs of degeneration and disrupted short microvilli at the luminal surface. However, in the group of mice fed on the “delta-endotoxin,” several villi appeared with an abnormally large number of enterocytes. Fifty percent of these cells were hypertrophied and multinucleated. Basal lamina along the base of the enterocytes was damaged at several foci. Several disrupted microvilli appeared in association with variable-shaped cytoplasm fragments. Some of these fragments contained endoplasmic reticulum, as well as ring-shaped annulate lamellae. In addition, the Paneth cells were highly activated and contained a large number of secretory granules. These changes might suggest that delta-endotoxin-treated potatoes resulted in the development of hyperplastic cells in the mice ileum. The authors concluded that the appearance of several multinucleated and hypertrophied enterocytes, as well as several associated cytoplasmic fragments with highly recognized annulate lamellae suggested the possible participation of feeding on the delta-endotoxin-treated potatoes in the hyperplastic development in the mice ileum. They recommended that in order to avoid any potential risks to the consumers, new types of heredity and new genetic structures must be evaluated before releasing for marketing new transgenic foods.

Because of the wide controversy and international repercussions of the results, especially remarkable was the publication of the study by Ewen and Pusztai (1999), who investigated the effects of diets containing GM potatoes expressing *Galanthus nivalis* lectin on rat small intestine. It was found that these diets had variable effects on different parts of the rat gastrointestinal tract. Some effects such as the proliferation of the gastric mucosa, were mainly attributed to the expressions of the *Galanthus nivalis* agglutinin (GNA) transgene. However, the authors suggested that other parts of the construct or the genetic transformation (or both) could also have contributed to the overall biological effects of the GNA-genetically modified potatoes, particularly on the small intestine and caecum. It was concluded that there would exist the possibility that a plant vector in common use in some GM plants could affect the mucosa of the gastrointestinal tract and exert powerful biological effects. It might also apply to GM plants containing similar constructs, particularly those containing lectins, such as soybeans or any plants expressing lectin genes or transgenes. The main concern in relation to this study was the short experimental period, 10 days. Would this

**Table 1** A summary of experimental studies concerning dietary administration of a number of genetically modified plants to various animal species

Plant/crop	Animal species	Length of the study	Main adverse effects	Reference
<b>Potatoe</b>				
GM (delta-endotoxin treated)	mice	2 weeks	Mild changes in the structural configuration of the ileum. Potential hyperplastic development of the ileum	Feres and El-Sayed (1998)
GM	Rats	10 days	Proliferation of the gastric mucosa. Effects on the small intestine and caecum	Ewen and Pusztai (1999)
GM	Rats	4 weeks	Absence of pathologic symptoms and histopathological abnormalities in liver and kidney	Hashimoto et al. (1999a)
GM	Rats	5 weeks	Increase in the number of bacteria phagocytized by monocytes, percentage of neutrophils producing ROS, and oxygen-dependent bactericidal activity of neutrophils	Winnicka et al. (2001)
GM	Rats	10 weeks prior to mating	No adverse effects on the multigeneration reproductive-developmental ability	Rhee et al. (2005)
<b>Maize/corn</b>				
Transgenic Event 176 Bt	chickens	38 days	No deleterious effects were noted	Brake and Vlachos (1998)
GM	pigs	Growing phase	Toxicity was not assessed	Spencer et al. (2000a,b)
GM (Bt)	pigs	91 days (growing period)	Side effects were not observed. However, the studies did not indicate the performance of toxicological tests	Reuter et al. (2002a,b)
GM (CBH351)	rats and mice	13 weeks	No immunotoxicity was detected. No other specific toxicity tests were included	Teshima et al. (2002)
Roundup Ready®	rats	13 weeks	No adverse effects were reported on overall health, body weight, food consumption, clinical pathology parameters, organ weights, and gross and microscopic appearance of tissues	Hammond et al. (2004)
<b>Soybeans</b>				
Glyphosate-tolerant	rats, broiler chickens, catfish and dairy cows	4 weeks (rats and cows), 6 weeks (broilers) and 10 weeks (catfish)	No significant effects in the concentrations of nutrients and antinutrients	Hammond et al. (1996)
GM 40-3-2	rats	5 months	The hepatocyte membrane function and enzymatic activity were modified within physiological standards	Tutel'ian et al. (1999)
Glyphosate-tolerant	rats and mice	15 weeks	No adverse effects on growth and the histopathology of immune-related organs. No immunotoxic activity	Teshima et al. (2000)
Glyphosate-tolerant	pigs	growing period	The studies did not indicate the performance of toxicological tests	Cromwell et al. (2002)
Glyphosate-tolerant	rats	13 weeks	No adverse effects of GM soybean meal were seen even at levels as high as 90% of the diet	Zhu et al. (2004)
Glyphosate-tolerant	mice	gestation and lactation periods	No negative effects on fetal, postnatal, pubertal or adult testicular development	Brake and Evenson (2004)
<b>Rice</b>				
Transgenic (soybean glycinin gene)	rats	4 weeks	No adverse effects on the blood count, blood composition or internal organ weights. No pathological symptoms. No histopathological abnormalities in liver and kidney	Momma et al. (2000)
Transgenic (anti-herbicide gene(BAR))	mice and rats	30 days	No adverse effects on body or histopathological alterations were noted	Wang et al. (2000)
Transgenic (cowpea trypsin inhibitor)	rats	period from lactation to sexual maturation	No maternal toxicity, embryotoxicity and teratogenicity were noted	Zhuo et al. (2004a)
Transgenic (cowpea trypsin inhibitor)	rats	90 days	Some alterations on hematological parameters	Zhuo et al. (2004b)

(Continued on next page)

**Table 1** A summary of experimental studies concerning dietary administration of a number of genetically modified plants to various animal species (*Continued*)

Plant/crop	Animal species	Length of the study	Main adverse effects	Reference
Transgenic (cowpea trypsin inhibitor)	mice	30 days	No immunotoxic effects were observed. No other toxicity tests were performed	Chen et al. (2004)
Transgenic	rats	90 days	Not enough evidences were found to conclude that transgenic rice had adverse effects on the rat	Li et al. (2004b)
Transgenic KMD1	rats	90 days	Although only minor changes were detected, additional tests group(s) are required	Schroder et al. (2007)
<b>Cucumber</b>				
Transgenic	rats	5 weeks	No adverse effects on the growth and health status	Kosieradzka et al. (2001)
<b>Tomatoes</b>				
GM (Bt)	rats	90 days	Body weights and food consumption were normal. Microscopy examination of tissues did not show adverse effects	Noteborn et al. (1995)
GM (CMV)	rats and mice	30 days	No significant differences with rats fed non-GM tomatoes	Chen et al. (2003)
<b>Sweet pepper</b>				
GM (CMV)	rats and mice	30 days	No significant differences with rats fed non-GM sweet peppers	Chen et al. (2003)
<b>Peas</b>				
Transgenic	rats	10 days	No harmful effects on growth, metabolism and health were observed	Pusztai et al. (1999)
<b>Canola</b>				
Transgenic (GFP)	rats	26 days	No general health risks were detected including a low allergenicity	Richards et al. (2003)

period be sufficient to detect relevant toxicological changes on rats small intestine?

Hashimoto et al. (1999a) confirmed that transgenic potatoes with native and designed soybean glycinins were safe based on their almost equivalent composition to that of non-transgenic and the ready digestibility of native and designed glycinins expressed in the transgenic potatoes. However, these authors indicated that this safety was based only on the concept of "substantial equivalence." Consequently, in a subsequent investigation, laboratory animal feeding experiments were included (Hashimoto et al., 1999b). Four groups of rats fed:

- (I) only a commercial diet,
- (II) the diet plus non-transgenic potatoes,
- (III) the diet plus transgenic potatoes with native glycinin, and
- (IV) the diet plus transgenic potatoes with designed glycinin.

Rats were fed 2,000 mg/kg-weight potatoes every day by oral administration. During the period tested, rats in each group (groups II, III, and IV) grew well without marked differences in appearance, food intake, body weight, or in cumulative body weight gain. No significant differences were found in blood count, blood composition, and in internal organ weights among the rats after feeding potatoes (groups II, III, and IV) for four weeks. Necropsy at the end of the experiment indicated neither pathologic symptoms in all rats tested nor histopathological abnormalities in liver and kidney. Except for a small increase in sodium levels in serum of group III rats, in general terms there were no significant differences between rats fed non-

transgenic and transgenic potatoes. In conclusion, the transgenic potatoes with glycinins were confirmed to have nearly the same nutritional and biochemical characteristics as the non-transgenic ones. Despite this conclusion, the authors remarked:

- 1) that the safety assessment with laboratory animals is often influenced by many undefined factors,
- 2) that it is also difficult to feed a relevant dose of transgenic crops,
- 3) that previously to extrapolate the safety of GM plants to humans, long-term feeding animals experiments (including the capability to induce malformations, alterations on the reproductive function, mutagenicity and carcinogenicity), as well as the use of cultured human cell systems are clearly necessary (Hashimoto et al., 1999b; Momma et al., 2002).

The effect of feeding GM potatoes on selected indices of non-specific resistance was investigated in rats (Winnicka et al., 2001). Genetic modification of potatoes consisted of repressing the gene encoding ADP-ribosylation factor (ARF) of protein and intensification of the 14-3-3 protein synthesis (Wilczynski et al., 1997). Two semi-synthetic iso-protein diets containing potatoes, non-modified (control diet), or subjected to genetic modification (GM, experimental diet), were used. Initial mean body weight of rats was 150 g and animals fed during 5 weeks. Feeding GM potatoes increased the number of bacteria phagocytized by monocytes, the percentage of neutrophils producing reactive oxygen species (ROS), and the oxygen-dependent bactericidal activity of neutrophils. The authors concluded that a

determination of the precise mechanism of inducing the phagocytic activity observed was required. We would add the necessity to prolong the period of feeding, which in that study was probably too short.

El-Sanhoty et al. (2004) evaluated in rats the composition, nutritional and toxicology safety of GM potato Spunta lines compared to that of conventional potato Spunta. A feeding study was done for 30 days. Four groups of rats were used.

- Group (I) was fed on control basal diet,
- Group (II) was fed on control diet plus 30% freeze-dried non-GM potato Spunta,
- Group (III) was fed on control diet plus 30% freeze-dried GM potato Spunta, and
- Group (IV) was fed on control diet plus 30% freeze-dried GM potato Spunta GMO G3.

During the period tested, rats in each group (I, II, III, IV) grew well without marked differences in appearance. No significant differences were found in food intake, daily body weight gain, and feed efficiency. However, there was a slightly significant difference in finally body weight between the control and the experimental groups. No significant differences were found in serum biochemical values between groups, and also between relative organ (liver, spleen, heart, kidney, testes) weights. Although the results of this safety evaluation did not show significant differences among groups, our main concern regarding the potential extrapolation to humans of the results is again the short duration of the feeding study. Moreover, since detoxification systems in rodents are largely different from those in humans in activity and amount, as well as in the detoxification enzyme species, there would have been some additional difficulties in extrapolation of the results of animal experiments to humans (Momma et al., 2002). This comment would be appropriate not only for the study by El-Sanhoty et al. (2004), but also for any of the above studies in rodents.

A multigeneration reproductive and developmental toxicity study of the bar gene inserted into GM potatoes was recently performed in rats (Rhee et al., 2005). In each generation, animals were fed a solid pellet containing 5% GM potato and non-GM potato for 10 weeks prior to mating. In the multigeneration study, there were no GM-potato related changes in body weight, food consumption, reproductive performance, and organ weight. In each generation, the litter-related indexes did not show any GMO-related changes.

### **Maize/Corn**

The first-commercial-scale plantings of insect-protected field corn hybrids, commonly referred to as "Bt" corn, occurred in 1996, following regulatory review by USA and Canadian authorities. These first field corn hybrids derived from a genetic modification designated "Event 176," which expresses a gene that enables the plants to produce an insecticidal protein, Cry1Ab,

similar to that produced in the nature by certain subspecies of the common soil bacterium *Bacillus thuringiensis*. To determine whether transgenic Event 176-derived corn had an adverse effect on broiler chicken performance, Brake and Vlachos (1998) performed a 38-day feeding study in males and females. No statistically significant differences in survival and body weight were observed between animals reared on mash or pelleted diets prepared with transgenic corn and similar diets prepared using control corn. Broilers raised on diets prepared from the transgenic corn exhibited significantly better feed conversion ratios and improved yield of the Pectoralis minor breast muscle. Although it was not evident whether this enhanced performance was attributable to the transgenic corn per se, or due to possible slight differences in overall composition of the formulated diets, in that study that the transgenic corn had no deleterious effects.

A genetically modified corn hybrid homozygous for the *lpa1* allele, containing low phytate (LP), and its nearly isogenic equivalent hybrid (normal) were compared in two experiments with growing-finishing swine (Spencer et al., 2000a). In the first experiment, 210 barrows (27 kg) were allotted to one of six dietary treatments with two corn hybrids (LP and normal) and three phosphorus (P) feeding regimens. Pigs fed the LP corn diet without added P had greater body weight gain, feed efficiency, breaking load (BL), and ash content of the fourth metacarpal than pigs fed the normal corn diet without added P. Performance was similar between pigs fed the LP diet without added P and pigs fed LP and normal corn with added P. In a second experiment with different diets, no significant differences in growing-finishing performance or BL among treatments were noted. However, pigs fed diets containing LP corn possessed carcasses with less back fat and a higher percentage of lean. These results confirmed that the P in LP corn was available to the pig and suggested that pigs fed diets containing this GM corn would have more desirable carcasses. In turn, these results corroborated previous findings of the same research group, which showed that low-phytate corn contained at least 5 times as much available P as normal corn (Spencer et al., 2000b), and suggested that low-phytate corn diets with no supplemental P might be adequate for growing-finishing swine. No toxicity experiments were included in these short-term investigations.

Studies with Bt maize in pig nutrition were also performed by Reuter et al. (2002a,b). In a first study, the composition of parental and transgenic (Bt) maize grain and its digestibility and nutritional value of both maize lines in pigs were investigated (Reuter et al., 2002a). It was concluded that from the point of view of a nutritional assessment, the GM maize could be regarded as substantially equivalent to the parental maize line. In a second study, a grower-finisher performance trial was designed to compare the growth performance of pigs fed diets containing either GM Bt-maize (NX6262) or its parental maize (Prelude) line. During a 91 days growing period, the pigs of both groups recorded equal performance in daily weight gain depending on equal amounts of feed intake (parental vs. transgenic). These results confirmed equal performance among growing-finishing pigs fed parental or GM maize containing diets. It was concluded

that diets containing a high proportion of either GM Bt maize or its non-modified parental counterpart could be fed to growing-finishing pigs without significant differences on feed consumption, daily weight gain, and energy efficiency. Unintended or unexpected side effects of the GM maize grain were not observed (Reuter et al., 2002b). However, it is important to note that there was no indication about the performance of toxicological tests in those studies.

Subchronic animal feeding studies to examine the effect on the immune system of genetically modified corn CBH351, which contains the Cry9C protein derived from *Bacillus thuringiensis* subspecies *tolworthi*, were conducted in female BN rats and B10A mice by Teshima et al. (2002). The studies were designed to compare the effect of a line of genetically modified corn CBH351 (GM corn) with that of isoline corn (non-GM corn). The study duration was 13 weeks. The following results were obtained:

- (1) no remarkable compositional differences in fatty acids, amino acids or phytate were found between the GM and non-GM corns,
- (2) no significant differences in growth, food intake, or weight of the thymus, spleen, and liver were found between animals fed the non-GM and GM lines,
- (3) the histological findings in thymus, spleen, mesenteric lymph nodes, Peyer's patches, small intestines, liver, kidney, and bone marrow were similar in animals fed GM and non-GM lines, and
- (4) no evidence of production Cry9C-specific IgE (specific marker of allergenicity) or IgA antibodies were detected in the serum of either group, whereas a minor increase of Cry9C-specific IgG (marker of exposure to the new protein) was found in the serum of rats fed 50% GM corn, but not in those fed 5% GM corn.

In conclusion, no immunotoxic activity was detected in the GM-corn-fed rats and mice in this subchronic dietary study. Although this was an extensive study concerning immunotoxicity of GM corn, again no specific toxicity tests were included.

One of the few published investigations performed by the biotechnology companies involved in commercially available GM foods is that reported by Hammond et al. (2004). These authors carried out a 13 week feeding study in rats with grain from Roundup Ready® (Monsanto, USA) corn which is tolerant to the herbicide glyphosate. The responses of rats fed diets containing Roundup Ready corn grain were compared to those of rats fed diets containing non-transgenic grain (controls). All diets were nutritionally balanced and conformed to Purina Mills, Inc. specifications for Certified LabDiet 5002. There were 400 rats in the study divided into 10 groups of 20 rats/sex/group. Overall health, body weight, food consumption, clinical pathology parameters (hematology, blood chemistry, and urinalysis), organ weights, and gross and microscopic appearance of tissues were comparable between groups fed diets containing Roundup Ready

and control corn grain. The no-observed-effect level (NOEL) was equal to the highest dietary level (33%) of Roundup Ready corn grain fed to rats. According to the authors, this study complements extensive agronomic, compositional, and farm animal feeding studies with Roundup Ready corn grain, confirming it is as safe and nutritious as existing commercial corn hybrids. Although the study is extensive and seems to be well-elaborated, a potential limitation is the relatively short time of GM corn administration, 13 weeks.

On the other hand, the mineral and phytic acid contents of a low-phytic acid "flint" maize (LPM) and its parent, wild-type strain (WTH), were evaluated. Iron absorption from tortillas prepared with each type of maize and from a reference dose of ferrous ascorbate were also measured (Mendoza et al., 1998). It was found that consumption of genetically modified, low-phytic acid strains of maize, might improve iron absorption in human populations that consume maize-based diets, including those that are dependent primarily on plant-derived diets.

### Soybeans

In 1996, Padgett and co-workers reported the results of extensive compositional analyses that demonstrated that glyphosate-tolerant soybeans (GTS) seeds were substantially equivalent to the commercial parental soybean variety. In another study of the same research group, the safety of the protein expression product of the cloned gene, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. Strain CP4 (CP4 EPSPS), which is highly resistant to inhibition by glyphosate, was determined in mice (Harrison et al., 1996). There were no treatment-related adverse effects in animals given CP4 EPSPS protein by gavage at dosages up to 572 mg/kg of body weight. This dose represents a significant (greater than 1,000 fold) safety margin relative to the highest potential human consumption of CP4 EPSPS protein and assumes that the protein is expressed in multiple crops. However, these results showed that the CP4 EPSPS protein was not toxic to mammals only following acute exposure.

Although the compositional studies confirmed the equivalence of GTS to commercial soybean varieties, animal feeding trials were undertaken to provide further support for this new soybean variety. Animal feeding studies were conducted with rats, broiler chickens, catfish, and dairy cows as part of a safety assessment program. Two GTS lines and a parental variety were utilized in all animal feeding studies. The growth and gain-to-feed performance of animals fed GTS meal sources was comparable to those of animals fed parental-line soybeans. No meaningful differences between the parental and GTS lines were noted in the concentrations of important nutrients and antinutrients (Hammond et al., 1996). However, although the authors concluded that the introduced protein was safe, the period of administration was probably too short to draw convincing conclusions, as it ranged from 4 weeks for rats and dairy cows to 10 weeks for catfish. Moreover, typical toxicological parameters

were not evaluated. On the other hand, Shirai et al. (1998) reported that GTS formed approximately 1.1% of the commercial soybeans, when commercially available soybeans were cultivated and the number of soybeans resistant to glyphosate was found. This level was somewhat lower than an estimated value announced officially on the basis of the cultivation area of the GTS.

Tutel'ian et al. (1999) fed rats with albuminous concentrate from the genetically modified soybean 40-3-2 (Monsanto Co., USA), 1.25 g/rat/day for 5 months. Blood, urine, and liver were investigated to measure total protein and glucose levels, amino-transferase and alkaline phosphatase activities in blood, pH, relative density and creatinine level in the urine, and hepatic enzyme activity of the I and II phases of xenobiotic metabolism, as well as the whole and non-sedimentated lysosomal enzyme activities. It was found that the addition of the GM soybean to the diet of rats modified the hepatocyte membrane function and enzymatic activity within physiological standards, while it was not harmful to the adaptation systems.

The effect of GM and non-GM soybeans on the immune system of BN rats and B10A mice was investigated by Teshima et al. (2000). The studies were designed to compare the feeding value of a line of GM GTS to that of closely-related and one-parent same cultivar (non-GM soybeans). The study duration was 15 weeks. Growth, feeding value, and the histopathology of immune-related organs showed no significant differences between animals fed GM and non-GM lines. The production of soybean-specific IgE was not detected in the serum of any group, and the increase in soybean-specific IgG was identical in the GM and non-GM groups. No immunotoxic activity was found in GM-soybean-fed rats or mice. Some limitations of that study are the reduced number of animals per group, five, as well as the relatively short experimental period, 15 weeks.

Phipps et al. (2002) fed a GM crop to lactating dairy cows to determine if GM DNA could be detected in the milk produced by those cows. In study weeks 4–12 the total mixed ration of forage (non-GM grass and maize) was replaced by soybean meal at 26.1% of the total diet in weeks 4–5, and 13.9% of the total diet in weeks 6–12. Weekly milk samples were taken from all cows. The results showed that transgenic DNA could not be detected in milk from cows receiving up to 26.1% of their diet as herbicide glyphosate-tolerant soybean meal. The detection limits for the test was established at 7.5  $\mu\text{g/l}$  of milk. It was suggested that an extensive degradation of DNA occurred, which would be attributed to the aggressive and extensive digestion process in the dairy cow, which was reviewed by Beever and Kemp (2000). The authors remarked that even if fragments of transgenic DNA had been detected in their study, it must be taken into account that the WHO (1993) concluded that there was no inherent risk in consuming DNA, including that from GM crops.

Recently, the health safety of transgenic soybeans (glyphosate-tolerant or Roundup Ready) was studied using the mammalian testis (mouse model) as a sensitive biomonitor of potential toxic effects (Brake and Evenson, 2004). Pregnant mice were fed a transgenic soybean or a non-transgenic (conventional)

diet through gestation and lactation. After weaning, the young male mice were maintained on the respective diets. At 8, 16, 26, 32, 63, and 87 days after birth, three male mice and an adult reference mouse were killed, the testes surgically removed, and the cell populations measured by flow cytometry. Multigenerational studies were conducted in the same manner. In comparison with animals fed the conventional diet, no adverse effects on macromolecular synthesis or cell growth and differentiation were observed in mice given the transgenic soybeans. Moreover, no differences between groups were noted in litter size and body weights. The authors concluded that the transgenic soybeans did not cause negative effects on fetal, postnatal, pubertal or adult testicular development, or body growth in the mouse. Zhu et al. (2004) did not find adverse effects of glyphosate-tolerant soybean meal in rats at levels as high as 90% of the diet.

Any of the above studies reported results concerning potential endocrine effects of the GM soybeans. Information about it, as well as on the composition of GM soybeans is important taking into account that this crop has been used for preparation of soymilk and other products recommended as health food. With respect to the composition of GM soybeans, Cromwell et al. (2002) showed that Roundup Ready soybean meal was essentially equivalent in composition and nutritional value to conventional soybean meal for growing-finishing pigs. In turn, McCann et al. (2005) concluded that the composition of commercial glyphosate-tolerant soybeans over 3 years of breeding into multiple varieties remained equivalent to that of conventional soybeans. On the other hand, according to Kim et al. (2006) the allergenicity of wild type and GM soybeans extracts was identical in adults. However, other authors concluded that to assess the allergenicity of GM soybean and other GM food, more research, including a selection of controlled sample materials and immunoassays of qualified sera, is needed (Yum et al., 2005; Cantani, 2006).

### *Rice*

Wang et al. (2000) investigated the safety of the anti-herbicide gene(BAR) transgenic rice. Acute toxicity studies, mutation tests and a 30-day feeding study were conducted in rats and mice. The oral LD<sub>50</sub> in both species of mammals was >21.5 g/kg of body weight, while no mutations were found. Rats consuming 16.3 and 64 g/kg of body weight had a normal growth and development at the 30-day feeding test. Neither adverse effects on body weight nor histopathological alterations were noted.

Momma et al. (1999) showed that accompanying the higher protein level in GM rice with the soybean glycin gene, the contents of almost all amino acids including lysine were higher (20% more) in the GM rice. The high-level expression of the desired proteins had the possibility to provoke not only nutritional changes but also metabolic disturbances in the host crops. Therefore, the authors remarked that the safety assessment based on "substantial equivalence" would not be always enough to apply to the safety assessment of GM crops thus created. Thus, in

order to assess the effects of these metabolic fluctuations, this research group conducted in rats feeding studies on rice genetically modified with soybean glycin for four weeks. The administered amount was 10 g/kg-rat/day, which is ten times higher than that prescribed for the safety assessment of food additives. During the experimental period, no differences were noted in appearance, food intake, body weight, and cumulative body weight gain. There were also no significant differences in the blood count, or in the biochemical parameters determined in plasma. No abnormalities of organs were observed regarding weight, shape and function (Momma et al., 2000). In spite of these results, the authors concluded that the potential risks of unknown toxins in the GM rice, and the capability to induce malformations, reproductive disorders, mutagenicity, and carcinogenicity of the GM rice could not be confirmed by this short-term experiment (Momma et al., 2000). We absolutely agree with this conclusion, as most studies on potential health risks of transgenic foods are only short-term studies. In a subsequent investigation of the same research group, no biochemical, nutritional, or morphological abnormalities were detected in long-term chronic toxicity experiments (Momma et al., 2002). However, to date data on the ability of GM rice to induce mutagenicity, teratogenicity, and carcinogenicity are not available from the scientific literature.

A research group of the Institute of Nutrition and Food Safety of Beijing (China) recently reported a series of studies to assess in rodents the potential adverse effects of GM rice, which expressed insecticidal protein CpTI (cowpea trypsin inhibitor). Despite the evident scientific interest of these investigations, the results were only published in Chinese. One of these studies investigated if the transgenic rice possessed potential teratogenicity in weanling rats. Animals were divided into four groups: transgenic rice group, non-transgenic rice group, and negative and positive control groups. The diet of the non-transgenic rice group contained 74.7% of non-transgenic rice, which was the parent line of the transgenic one. When the sexual maturation period of rats arrived, conventional teratogenicity tests were performed. Body weight of pregnant rats, and body weight, body length, and tail length of fetuses were significantly higher in the transgenic rice group than in the positive control group, whereas the malformation rate of fetuses was significantly lower in the transgenic rice group. The transgenic rice modified with CpTI was considered to have neither maternal toxicity nor embryotoxicity/teratogenicity (Zhuo et al., 2004a). In turn, Li et al. (2004a) evaluated the effects of genetically modified rice with Xa21 on the development of rat embryos. Weanling rats were divided into four groups: transgenic rice group, non-transgenic rice group, AIN93G negative control group, and MATDA positive control group. The rats were fed with corresponding food for 90 days and mated. The development of maternal rats and embryos was observed. Body weight gain of pregnant rats, as well as body weight, body length, and tail length of fetuses in the transgenic rice group were significantly increased in comparison with those in the positive control group. The number of deaths and reabsorbed embryos, and the malformation rates (external, visceral, and skeletal) were lower in the transgenic rice

groups than in the positive control group. Compared with the non-transgenic rice, transgenic rice modified with Xa21 gene did not show significant differences in rat pregnancy rate and embryo development.

The nutrition effects between transgenic and non-transgenic rice were also investigated in rats. Following 28 days of exposure, with the exception of the liver weight/body weight ratio, which in male rats was higher in the transgenic rice group than in the non-transgenic rice group, all other indicators did not show significant differences. In females, liver weight/body weight ratio, blood calcium and bone density were higher in the transgenic rice group than in the non-transgenic one. It was concluded that transgenic rice had good nutritional effects on rat development, while no adverse/toxic effects were observed in the transgenic rice group (Li et al., 2004b). It is important to note that the slight differences noted should not be underrated, especially taking into account that the experimental period was only 28 days. A semichronic study was also performed in weanling rats by the same research group (Zhuo et al., 2004b). Animals were divided into three groups: T, N, and C group. The diet of T group contained 78.3% of transgenic rice, while the diet of N group contained 74.7% of non-transgenic rice which was the parent line of transgenic one. The diet of C group was the standard diet AIN93G. Rats were fed for 90 days. In general, no significant nutritional differences among the three groups could be found, whereas no histopathological damage was noted. At the end of the first month, the male rats' body length of the T group was longer than that of the other two groups, while at the end of the test period, the male rats' blood glucose and ALT were lower than those in the other two groups. In the middle of the test period, the female rats' red blood cell number and hemoglobin were higher than those in the other two groups, while at the end of the test period, the female rats' monocyte number was higher than that found in the other two groups. However, all these results were in the normal range. Therefore, the authors concluded that the results of the 90 days feeding test of transgenic rice on rats did not reveal any signs of toxic and adverse effects. However, this was not a toxicological study, and therefore, the data are irrelevant from the toxicological point of view.

Recently, Schroder et al. (2006) reported the results of a 90-day safety study of GM rice (KMD1) expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. The KMD1 rice contained 15 mg Bt toxin/kg. No adverse effects on animal behavior or weight gain were observed during the study. A few hematological and biochemical parameters were different from those considered as standard for Wistar rats, but all within the normal reference intervals for rats of this breed and age, and consequently not considered treatment related. Upon sacrifice, only minor changes were observed in a large number of organs on weight, macroscopic, and histopathological examinations. In spite of these results, Schroder et al. (2006) concluded that the safety assessment for unintended effects of a GM crop could not be done without additional test group(s).

To assess the potential immunotoxicologic effects of transgenic rice, a short-term feeding study was conducted in mice

(Chen et al., 2004). Animals were fed with food composed by transgenic rice (into which cowpea trypsin inhibitor gene was introduced) or non-transgenic rice (which had the same gene composition as the transgenic rice except for the cowpea trypsin inhibitor gene) for 30 days. At the end of this period, immunotoxicologic indexes of each group were compared (body weight, guts index, blood routine test, lymphocyte sort, serum antibody titer, plaque forming cell, delayed hypersensitivity response, and macrophage function test). No significant differences between transgenic rice and non-transgenic rice groups were observed. It was concluded that transgenic rice was substantially equivalent to non-transgenic rice in relation to immunotoxicologic effects.

### **Cucumber**

Kosieradzka et al. (2001) examined in rats the effects of feeding diets with a considerable proportion of transgenic cucumber on growth parameters, relative organ weights, and nutrient digestibility. These effects were compared with those of feeding the fruits in balanced diets. The genetic modification consisted of introducing the gene coding a sweet protein, thaumatin, and the marker gene of resistance to kanamycin. The experiment was conducted for 5 weeks on 3 groups of male rats with an initial mean body weight of 150 g. Isoprotein diets containing 0 or 15% lyophilized transgenic or non-transgenic cucumbers did not affect weight gain, apparent health status, or relative organ weights of animals. Protein digestibility was slightly but significantly lower (89.2 vs. 90%) in diets containing transgenic cucumbers than in those contained non-transgenic cucumbers, whereas digestibility of crude fiber was higher in the group given non-transgenic cucumbers (28.2% vs. 15%). In turn, digestibility of fat and N-free extractives did not differ. Consequently, consumption of transgenic cucumbers for 28 days did not affect the growth and health of rats, although it did slightly affect nutrient digestibility. We agree with the conclusion of the authors noting that the influence of feeding transgenic plants on animal organisms requires more thorough and longer studies.

### **Tomatoes and Sweet Pepper**

Noteborn et al. (1995) assessed in weanling rats the safety of the *Bacillus thuringiensis* insecticidal Crystal Protein CRY1a(b) expressed in transgenic tomatoes. During 90 days, rats ate tomato-diets, which on average corresponded to 20 g of fresh tomatoes per day. Percent survivals, final body weights, and organ (liver, kidneys, testes) weights, as well as macroscopic and microscopic examination of organs and tissues did not reveal significant differences between consumption of GM tomatoes and the unmodified parent.

In the early 1990s, a coat protein gene (*cp*) from a cucumber mosaic virus (CMV) Chinese isolate was cloned (Hu et al.,

1990) and a genetic transformation system was established for sweet pepper and tomato plants. In order to assess the safety of GM sweet pepper and tomato with CMV-*cp* gene as food, Chen et al. (2003) conducted the following tests in rats and mice: acute toxicity assay, micronucleus test, sperm aberration test, Ames test, and 30-day animal feeding study. The LD<sub>50</sub> for the two GM products was considered to be greater than 10 g/kg for rats and mice, indicating that lyophilized GM powders were as innocuous as their non-GM counterparts. No genotoxicity either in vitro or in vivo by the micronucleus test, sperm aberration test, and Ames test were detected. Animal feeding studies did not show significant differences in growth, body weight gain, food consumption, hematology, blood biochemical indices, organ weights, and histopathology between rats or mice of either sex fed with either GM sweet pepper or tomato diets compared with those given non-GM diets. According to the authors, these results demonstrated that the CMV-resistant sweet pepper and tomato would be comparable to the non-GM counterparts in terms of food safety.

### **Peas**

Pusztaï et al. (1999) evaluated the effect of expression of bean alpha-amylase inhibitor (alpha-AI) transgene on the nutritional value of peas in pair-feeding rats diets (10 days) containing transgenic or parent peas at 300 and 650 g peas/kg, respectively, and at 150 g protein/kg diet, supplemented with essential amino acids to target requirements. The results were also compared with the effects of diets containing lactalbumin, with or without 0.9 or 2.0 mg bean alpha-AI, levels equivalent to those in transgenic pea diets. The weight gain and tissue weights of rats fed either of the two pea diets were not significantly different from each other or from those of rats given the lactalbumin diet even when this was supplemented with 0.9 g alpha-AI/kg. The digestibilities of protein and dry matter of the pea diets was slightly, but significantly lower than that of the lactalbumin diet. The nutritional value of diets containing peas at the higher (650 g) inclusion level was less than that of the lactalbumin diet. However, the differences between transgenic and parent pea lines were small, possibly because neither the purified recombinant alpha-AI nor that in transgenic peas inhibited starch digestion in the rat small intestine in vivo to the same extent as did bean alpha-AI. In conclusion, this short-term study indicated that transgenic peas expressing bean alpha-AI gene could be used in rat diets at 300 g/kg level without major harmful effects on their growth, metabolism and health, raising the possibility that transgenic peas might also be used at this level in the diet of farm animals. However, the authors remarked that at that stage, the results of their nutritional study could not be taken as a proof that transgenic peas were fit for human consumption. More specific risk assessment testing procedures, which must be designed and developed with human consumers in mind, would be clearly necessary. To date, and according to the literature, these studies have not been conducted yet.

### **Canola Plants**

To evaluate the potential toxicity and allergenicity of green fluorescent protein (GFP), Richards et al. (2003) fed pure GFP and diets containing transgenic canola plants expressing GFP to weaned male rats for 26 days. GFP has become a valuable tool in biotechnology because it has unparalleled effectiveness as a real-time marker of promoter activity and gene expression in vivo. Animals were fed either AIN-93G (control), control diet plus 1.0 mg of purified GFP daily, modified control diet with 200 g/kg canola (*Brassica rapa* cv Westar), or control diet with 200 g/kg transgenic canola containing one of two levels of GFP. Ingestion of GFP did not affect growth, food intake, relative weight of intestine or other organs, or activities of hepatic enzymes in serum. A comparison of the amino acid sequence of GFP to known food allergens revealed that the greatest number of consecutive amino acid matches between GFP and any food allergen was four, suggesting the absence of common allergen epitopes. Moreover, GFP was rapidly degraded during simulated gastric digestion. These data indicated that GFP had a low allergenicity risk and provided preliminary indications that GFP would represent a minimal risk for the food supply. However, in their conclusions the authors remarked that this short-term study was not sufficient to guarantee the lack of potential health risks, and consequently, long-term feeding studies were required. These data are not currently available from the scientific literature.

### **GENETICALLY MODIFIED DNA IN FOOD**

Humans typically consume a minimum of 0.1 to 1 g/day of DNA in their diet (Doerfler, 2000). Therefore, the transgene in a genetically engineered plant is not a new type of material to our digestive system, and it is present in extremely small amounts. There is no compelling evidence for the incorporation and expression of plant-derived DNA, whether as transgene or not, into the genomes of consuming organisms (SOT, 2003). Although much remains to be learned about the fate of dietary DNA in the mammalian systems, the possibility of adverse effects arising from the presence of transgenic DNA in foods, either by direct toxicity or gene transfer, would be minimal according to the WHO (2002) and other international regulatory organisms. Jonas et al. (2001) reviewed whether the consumption of DNA in approved novel foods and novel food ingredients derived from genetically modified organisms (GMOs) could be regarded as safe as the consumption of DNA in existing foods. It was concluded that the probability of transfer and functional integration of DNA from ingested food by gut microflora and/or human cells was minimal.

However, not all the investigators are in agreement with these conclusions. For example, the same WHO indicates that gene transfer from GM foods to cells of the body or to bacteria in the gastrointestinal tract would cause concern if the transferred genetic material adversely affects human health, which would

be particularly relevant if antibiotic resistance genes, used in creating GMOs, were to be transferred (WHO, 2002). Although intact foreign DNA is not thought to be available for transfer into human cells, there is a remote possibility that DNA fragments may be taken up by bacteria in the gut (Donaldson and May, 1999). DNA fragments, after passing through the intestinal wall, might be actively removed by cells of the gut immune system or they might enter the circulation (Jonas et al., 2001). In relation to this, Schubbert et al. (1997) demonstrated that food-ingested foreign DNA was not completely degraded in the gastrointestinal tract of mice. Orally administered M13mp18 DNA could be recloned from spleen DNA in linkage to DNA with 70% homology to the mouse IgE receptor, whereas the DNA recloned from spleen also contained bacterial DNA possibly transported from the gut through the intestinal wall by a route akin to M13mp18 test DNA. In summary, foreign DNA ingested by mice might reach peripheral leucocytes, spleen, and liver via the intestinal-wall mucosa (Schubbert et al., 1997). Therefore, a gene that has been transferred might be incorporated in an unpredictable place in the genome (Godfrey, 2000). In the UK, a report on the health implications of GM foods concluded that "there is no current evidence that GM technologies used to produce food are inherently harmful; this is true, but one cannot conclude that all application will be harmless" (<http://www.doh.gov.uk/gmfood.htm>).

The results of a study on the implications for the possible transfer of genes from GM food (Chiter et al., 2000) raised also some uncertainties. It was demonstrated that the treatment of plant tissues at temperatures of 95°C or above for more than a few minutes was sufficient for degradation of DNA to take place to the extent that it should be incapable of transmitting genetic information. However, materials that had not been subjected to such treatments not only had non-fragmented DNA but also retained specific polymerase chain reaction (PCR)-detectable sequences suggesting that DNA was intact. It would imply that stringent conditions are needed in the processing of GM plants for food consumed by animals and humans to eliminate the possibility of transmission of transgenes. Similar conclusions were also drawn by Chowdhury et al. (2003), who tried to detect maize DNA fragments in the intestinal contents of pigs fed GM maize (atarlink CBH351) or non-GM maize by PCR. These authors suggested that ingested DNA was not totally degraded, but rather was present in a form detectable by PCR.

On the other hand, Duggan et al. (2003) using the PCR technique, investigated the fate of a transgene in the rumen of sheep fed silage and maize grains from an insect-resistant maize line. Free DNA survived in a functional state for a significant amount of time in the ovine oral cavity, suggesting that DNA released from the diet might transform competent oral bacteria. By contrast, the chances of microbial transformation in the rumen and lower regions of the ovine digestive system would be likely low due to a high level of nuclease activity. Nevertheless, a rare transformation event would be significant if the donor DNA is an antibiotic resistance gene and the recipient is a human or animal pathogen. The authors concluded suggesting that the use of GM crops harboring antibiotic resistance genes, in particular

the use of unprocessed grains in animal feed, deserved further evaluations.

In their investigations on GM maize (Bt-maize) in pig nutrition, Reuter and Aulrich (2003) also showed that feed-ingested DNA was partially resistant to the mechanical and enzymatic activities of the gastrointestinal tract and was not completely degraded. Small DNA fragments derived from feedstuff could pass the gut wall and might enter organs and tissues of pigs.

## CONCLUSIONS

In recent years, three reviews on similar topics than that of the current paper have been published. Zdunczyk (2001) concluded indicating that for a safe use of transgenic food, evaluation of the concordance of the chemical composition of transgenic and conventional crops ("substantial equivalence") would not be sufficient. Subchronic *in vivo* studies, as well as a comparison of the nutritional equivalence of transgenic and conventional crops are advisable. These actions would be justified not only by the possibility of undesirable transgenic effects, but also by the consumer's right to explicit information on food safety.

In a wide review of the scientific literature on the potential adverse health effects of genetically modified crops, Bakshi (2003) indicated that these were generally safe their consumption being not associated with serious health problems. However, this author remarked that because genetic engineering of crops was a new technology in its embryonic stages, scientists still had an incomplete understanding of physiology, genetics, and nutritional value of genetically engineered crops. It leads to the inability to predict everything that can go wrong, including many risks that have not been identified. Some concerns are that GM crops may contain allergenic substances due to the introduction of new genes into crops, or that genetic engineering often involves the use of antibiotic-resistance genes as "selectable markers," which could lead to production of antibiotic-resistant bacterial strains that are resistant to available antibiotics. The genetically modified crops might contain other toxic substances (such as enhanced amounts of heavy metals) and the crops might not be "substantially equivalent" in genome, proteome, and metabolome compared with unmodified crops.

Pryme and Lembcke (2003) reviewed literature published *in vivo* studies on possible health consequences of genetically modified food and feed where the ingredients in question consisted of genetically modified plant materials. According to a Norwegian report "Gen-mat" (NOU 2000:29), and a more recent search in Medline and Citations Index, they only found a total of ten studies on the health effects of GM-foods and feeds. The authors concluded that much more scientific effort and investigation would be necessary before guaranteeing that eating foods containing GM material in the long-term will not be a probable cause of health problems. They considered essential to test in a transparent manner each individual GM product before its introduction into the market.

The conclusions of the current review are quite in agreement with those of Zdunczyk (2001), Bakshi (2003), and Pryme and Lembcke (2003), which are in the same line than those also suggested in our previous review (Domingo and Gómez, 2000). One of our main concerns is related with the use of the principle of "substantial equivalence" to guarantee the safe use of GM/transgenic plants. Why must it be thought that two plants (GM and non-GM) with the same nutritional capacity should also imply similar health risks (or absence of risks)? Why a similar principle is not authorized, for example, for chemical substances that are going to be commercialized such as pesticides, drugs, food additives, etc.? It is currently admitted that this principle is a starting point rather than an end point. If this seems to be quite clear, why the published information is so scant, taking into account that the debate about the safety of GM plants generates a great controversy?

In summary, the above seems to indicate that regulatory agencies reduce the concern for human health risks derived from the potential tendency to provoke gene transfer following consumption of GM foods. However, experimental studies carried out by independent researchers do not underrate the possibility that a transgene could be itself toxic or be transferred to the genome of the consumer. Recent investigations have concluded suggesting the necessity of further investigations on this important issue. With respect to this, in 1999, Ewen and Pusztai emphasized two potentially relevant concerns:

- (1) the scant attention that has been given to people with abnormal digestion as a result of chronic gastrointestinal disease, and
- (2) the possibility of allowing unexpected enhancement of intercurrent viral infection, taking into account the widespread mucosal accessibility to food viral DNA, a hot spot of DNA recombination.

Similarly, in countries where HIV-1 infection is endemic, the assumption that a viral component of GM food is harmless might be misplaced.

The main goal of the present paper has been to review critically the published scientific literature concerning potential toxic effects/health risks of GM plants. It has been noted that experimental data are very scarce. As shown throughout the paper, most investigations correspond to short-term studies, mainly nutritional studies, with very limited toxicological information (Filip et al., 2004). Where are long-term toxicological studies that should guarantee the safety of the transgenic plants for animal and human consumption? (Patel et al., 2005). Because of the importance that the consumption of GM foods has acquired, as well as its enormous potential in the near future, the performance of a complete case-by-case study seems would be advisable (Weil, 2005). Long-term studies are clearly necessary. This review can be concluded raising the following question: where is the scientific evidence showing that GM plants/food are toxicologically safe, as assumed by the biotechnology companies involved in commercial GM foods?

## REFERENCES

- Bakshi, A. (2003). Potential adverse health effects of genetically modified crops. *J. Toxicol. Environ. Health, B* **6**:211–225.
- Beever, D. E. and Kemp, C. F. (2000). Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutr. Abstr.*, **70**:175–182.
- Brake, J. and Vlachos, D. (1998). Evaluation of transgenic event 176 “Bt” corn in broiler chickens. *Poultry Sci.*, **77**:648–653.
- Brake, D. G. and Evenson, D. P. (2004). A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. *Food Chem. Toxicol.*, **42**:29–36.
- Cantani, A. (2006). Benefits and concerns associated with biotechnology-derived foods: can additional research reduce children health risks? *Eur. Rev. Med. Pharmacol. Sci.*, **10**:197–206.
- Chen, Z. L., Gu, H., Li, Y., Su, Y., Wu, P., Jiang, Z., Ming, X., Tian, J., Pan, N., and Qu, L. J. (2003). Safety assessment for genetically modified sweet pepper and tomato. *Toxicology*, **188**:297–307.
- Chen, X., Zhuo, Q., Piao, J., and Yang, X. (2004). Immunotoxicologic assessment of transgenic rice. *Wei Sheng Yan Jiu*, **33**:770–80 (in Chinese).
- Chiter, A., Forbes, J. M., and Blair, G. E. (2000). DNA stability in plant tissues: implications for the possible transfer of genes from genetically modified food. *FEBS Lett.*, **481**:164–168.
- Chowdhury, E. H., Mikami, O., Nakajima, Y., Hino, A., Kuribara, H., Suga, K., Hanazumi, M., and Yomemochi, C. (2003). Detection of genetically modified maize DNA fragments in the intestinal contents of pigs fed StarLink CBH351. *Vet. Hum. Toxicol.*, **45**:95–96.
- Cromwell, G. L., Lindemann, M. D., Randolph, J. H., Parker, G. R., Coffey, R. D., Laurent, K. M., Armstrong, C. L., Mikel, W. B., Stanisiewski, E. P., and Hartnell, G. F. (2002). Soybean meal from Roundup Ready on conventional soybeans in diets for growing-finishing swine. *J. Anim. Sci.*, **80**:708–715.
- Doerfler, W. 2000. *Foreign DNA in Mammalian Systems*. Wiley-WCH, Weinheim.
- Domingo, J. L. (2000). Health risks of GM foods: many opinions but few data. *Science*, **288**:1748–1749.
- Domingo, J. L. and Gómez, M. (2000). Health risks of genetically modified foods: a literature review. *Rev. Esp. Salud Pública*, **74**:255–261 (in Spanish).
- Donaldson, L. and May, R. (1999). Health implications of genetically modified foods (available at <http://www.doh.gov.uk/gmfood.htm>).
- Duggan, P. S., Chambers, P. A., Heritage, J., and Michael-Forbes, J. (2003). Fate of genetically modified maize DNA in the oral cavity and rumen of sheep. *Br. J. Nutr.*, **89**:159–166.
- El-Sanhoty, R., El-Rahman, A. A., and Bogl, K. W. (2004). Quality and safety evaluation of genetically modified potatoes spunta with Cry V gene: compositional analysis, determination of some toxins, antinutrients compounds and feeding study in rats. *Nahrung*, **48**:13–18.
- Ewen, S. W. and Pusztai, A. (1999). Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet*, **354**:1353–1354.
- Fares, N. H. and El-Sayed, A. K. (1998). Fine structural changes in the ileum of mice fed on delta-endotoxin-treated potatoes and transgenic potatoes. *Nat. Toxins*, **6**:219–233.
- Filip, L., Miere, D., and Indrei, L. L. (2004). Genetically modified foods. Advantages and human health risks. *Rev. Med. Chir. Soc. Med. Nat. Iasi.*, **108**:838–842 (in Romanian).
- Godfrey, J. (2000). Do genetically modified foods affect human health? *Lancet*, **355**:414.
- Hammond, B. G., Vicini, J. L., Hartnell, G. F., Naylor, M. W., Knight, C. D., Robinson, E. H., Fuchs, R. L., and Padgett, S. R. (1996). The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *J. Nutr.*, **126**:717–727.
- Hammond, B., Dudek, R., Lemen, J., and Nemeth M. (2004). Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. *Food Chem. Toxicol.*, **42**:1003–1014.
- Harrison, L. A., Bailey, M. R., Naylor, M. W., Ream, J. E., Hammond, B. G., Nida, D. L., Burnette, B. L., Nickson, T. E., Mitsky, T. A., Taylor, M. L., Fuchs, R. L., and Padgett, S. R. (1996). The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *J. Nutr.*, **126**:728–740.
- Hashimoto, W., Momma, K., Katsube, T., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S., and Murata, K. (1999a). Safety assessment of genetically engineered potatoes with designed soybean glycinin: compositional analysis of the potato tubers and digestibility of the newly expressed protein in transgenic potatoes. *J. Sci. Food Agric.*, **79**:1607–1612.
- Hashimoto, W., Momma, K., Yoon, H. J., Ozawa, S., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S., and Murata, K. (1999b). Safety assessment of transgenic potatoes with soybean glycinin by feeding studies in rats. *Biosci. Biotechnol. Biochem.*, **63**:1942–1946.
- Hu, T. H., Wu, L., Liu, W., Mi, J. J., Pan, N. S., and Chen, Z. L. (1990). cDNA cloning of gene encoding coat protein of cucumber mosaic virus infecting tobacco in China. *Chin. Sci. Bull.*, **35**:1209–1214 (in Chinese).
- Jonas, D. A., Elmadfa, I., Engel, K. H., Heller, K. J., Koziowski, G., König, A., Müller, D., Narbonne, J. F., Wackernagel, W., and Kleiner, J. (2001). Safety considerations of DNA in food. *Ann. Nutr. Metab.*, **45**:235–254.
- Kim, S. H., Kim, H. M., Ye, Y. M., Nahm, D. H., Park, H. S., Ryu, S. R., and Lee, B. O. (2006). Evaluating the allergic risk of genetically modified soybean. *Yonsei Med. J.*, **47**:505–512.
- Kosieradzka, I., Sawosz, E., Pastuszewska, B., Szwacka, M., Malepszy, S., Bielecki, W., and Czuminiska, K. 2001. The effect of feeding diets with genetically modified cucumbers on the growth and health status of rats. *J. Anim. Feed Sci.*, **10**(suppl 2):7–12.
- Kuiper, H. A., Kleter, G. A., Noteborn, H. P., and Kok, E. J. (2002). Substantial equivalence—an appropriate paradigm for the safety assessment of genetically modified foods? *Toxicology*, **181–182**:427–431.
- Li, Y., Piao, J., Zhuo, Q., Chen, X., Mao, D., Yang, L., and Yang, X. (2004a). Study on the teratogenicity effects of genetically modified rice with Xa21 on rats. *Wei Sheng Yan Jiu*, **33**:710–712 (in Chinese).
- Li, Y., Piao, J., Zhuo, Q., Chen, X., Chen, X., and Yang, X. (2004b). Subchronic toxicity test of transgenic rice. *Wei Sheng Yan Jiu*, **33**:575–578 (in Chinese).
- McCann, M. C., Liu, K., Trujillo, W. A., and Dobert, R. C. (2005). Glyphosate-tolerant soybeans remain compositionally equivalent to conventional soybeans (*Glycine max* L.) during three years of field testing. *J. Agric. Food Chem.*, **53**:5331–5335.
- Mendoza, C., Viteri, F. E., Lönnnerdal, B., Young, K. A., Raboy, V., and Brown, K. H. (1998). Effect of genetically modified, low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.*, **68**:1123–1127.
- Momma, K., Hashimoto, W., Ozawa, S., Kawai, S., Katsube, T., Takaiwa, F., Kito, M., Utsumi, S., and Murata, K. (1999). Quality and safety evaluation of genetically engineered rice with soybean glycinin: analyses of the grain composition and digestibility of glycinin in transgenic rice. *Biosci. Biotechnol. Biochem.*, **63**:314–318.
- Momma, K., Hashimoto, W., Yoon, H. J., Ozawa, S., Fukuda, Y., Kawai, S., Takaiwa, F., Utsumi, S., and Murata, K. (2000). Safety assessment of rice genetically modified with soybean glycinin by feeding studies on rats. *Biosci. Biotechnol. Biochem.*, **64**:1881–1886.
- Momma, K., Hashimoto, W., Utsumi, S., and Murata, K. (2002). Safety assessment of genetically modified rice with soybean glycinin. In: *Molecular Methods of Plant Analysis*. pp. 139–151. Jackson, J. F., Linskens, H. F., and Inman, R. B., Eds., Springer-Verlag, Berlin.
- Moneret-Vautrin, D. A., Kanny, G., Morisset, M., Rance, F., Fardeau, M. F., and Beaudouin, E. (2004). Severe food anaphylaxis: 107 cases registered in 2002 by the Allergy Vigilance Network. *Allerg. Immunol.*, **36**:46–51.
- Noteborn, H. P. J. M., Bienenmann-Ploum, M. E., van den Berg, J. H. J., Alink, G. M., Zolla, L., Reynaerts, A., Pensa, M., and Kuiper, H. A. (1995). Safety assessment of the *Bacillus thuringiensis* insecticidal Crystal Protein CRY1A(b) expressed in transgenic tomatoes. In: *Genetically Modified Foods. Safety Aspects*. pp. 134–147. Engel, K. H., Takeoka, G. R., and Teranishi, R., Eds., ACS Symposium Series 605, Washington, DC.
- Padgett, S. R., Taylor, N. B., Nida, D. L., Bailey, M. R., MacDonald, J., Holden, L. R., and Fuchs, R. L. (1996). The composition of glyphosate-tolerant

- soybean seeds is equivalent to that of conventional soybeans. *J. Nutr.*, **126**:702–716.
- Patel, R., Torres, R. J., and Rosset, P. (2005). Genetic engineering in agriculture and corporate engineering in public debate: risk, public relations, and public debate over genetically modified crops. *Int. J. Occup. Environ. Health*, **11**:428–436.
- Phipps, R. H., Beever, D. E., and Humphries, D. J. (2002). Detection of transgenic DNA in milk from cows receiving herbicide tolerant (CP4EPSPS) soyabean meal. *Livestock Produc. Sci.*, **74**:269–273.
- Pryme, I. F. and Lembcke, R. (2003). *In vivo* studies on possible health consequences of genetically modified food and feed—with particular regard to ingredients consisting of genetically modified plant materials. *Nutr. Health*, **17**:1–8.
- Pusztai, A., Bardocz, G. G., Alonso, R., Chrispeels, M. J., Schroeder, H. E., Tabe, L. M., and Higgins, T. J. (1999). Expression of the insecticidal bean alpha-amylase inhibitor transgene has minimal detrimental effect on the nutritional value of peas fed to rats at 30% of the diet. *J. Nutr.*, **129**:1597–1603.
- Reuter, T. and Aulrich, K. (2003). Investigations on genetically modified maize (Bt-maize) in pig nutrition: fate of feed-ingested foreign DNA in pig bodies. *Eur. Food Res. Technol.*, **216**:185–192.
- Reuter, T., Aulrich, K., Berk, A., and Flachowsky, G. (2002a). Investigations on genetically modified maize (Bt-maize) in pig nutrition: chemical composition and nutritional evaluation. *Arch. Tierernahr.*, **56**:23–31.
- Reuter, T., Aulrich, K., and Berk, A. (2002b). Investigations on genetically modified maize (Bt-maize) in pig nutrition: fattening performance and slaughtering results. *Arch. Tierernahr.*, **56**:319–326.
- Rhee, G. S., Cho, D. H., Won, Y. H., Seok, J. H., Kim, S. S., Kwack, S. J., Lee, R. D., Chae, S. Y., Kim, J. W., Lee, B. M., Park, K. L., and Choi, K. S. (2005). Multigeneration reproductive and developmental study of bar gene into genetically modified potato on rats. *J. Toxicol. Environ. Health*, **68**:2263–2276.
- Richards, H. A., Han, C. T., Hopkins, R. G., Failla, M. L., Ward, W. W., and Stewart, C. N. Jr. (2003). Safety assessment of recombinant green fluorescent protein orally administered to weaned rats. *J. Nutr.*, **133**:1909–1912.
- Schroder, M., Poulsen, M., Wilcks, A., Kroghsbo, S., Miller, A., Frenzel, T., Danier, J., Rychlik, M., Emami, K., Gatehouse, A., Shu, Q., Engel, K. H., Altosaar, I., and Knudsen, I. (2007). A 90-day safety study of genetically modified rice expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats. *Food Chem. Toxicol.* **45**:339–349.
- Schubbert, R., Renz, D., Schmitz, B., and Doerfler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA*, **94**:961–966.
- Shirai, N., Momma, K., Ozawa, S., Hashimoto, W., Kito, M., Utsumi, S., and Murata, K. (1998). Safety assessment of genetically engineered food: detection and monitoring of glyphosate-tolerant soybeans. *Biosci. Biotechnol. Biochem.*, **62**:1461–1464.
- SOT (Society of Toxicology). (2003). The safety of genetically modified foods produced through biotechnology. *Toxicol. Sci.*, **71**:2–8.
- Spencer, J. D., Allee, G. L., and Sauber, T. E. (2000a). Growing-finishing performance and carcass characteristics of pigs fed normal and genetically modified low-phytate corn. *J. Anim. Sci.*, **78**:1529–1536.
- Spencer, J. D., Allee, G. L., and Sauber, T. E. (2000b). Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate corn for pigs. *J. Anim. Sci.*, **78**:675–681.
- Teshima, R., Akiyama, H., Okunuki, H., Sakushima, J., Goda, Y., Onodera, H., Sawada J., and Toyoda, M. (2000). Effect of GM and non-GM soybeans on the immune system of BN rats and B10A mice. *J. Food Hyg. Soc. Japan*, **41**:188–193.
- Teshima, R., Watanabe, T., Okunuki, H., Isuzugawa, K., Akiyama, H., Onodera, H., Imai, T., Toyoda, M., and Sawada, J. (2002). Effect of subchronic feeding of genetically modified corn (CBH351) on immune system in BN rats and B10A mice. *Shokuhin Eiseigaku Zasshi*, **43**:273–279.
- Tutel'ian, V. A., Kravchenko, L. V., Lashneva, N. V., Avren'eva, L. I., Guseva, G. V., Sorokina, E. I., and Chernysheva, O. N. (1999). Medical and biological evaluation of safety of protein concentrate from genetically-modified soybeans. *Biochemical studies. Vopr. Pitan.*, **68**:9–12 (in Russian).
- Wang, Y., Lai, W., Chen, J., and Mei, S. (2000). Toxicity of anti-herbicide gene (BAR) transgenic rice. *Wei Sheng Yan Jiu*, **29**:141–142 (in Chinese).
- Weil, J. H. (2005). Are genetically modified plants useful and safe? *IUBMB Life*, **57**:311–314.
- WHO. (2002). Foods derived from modern technology: 20 questions on genetically modified foods (available at: <http://www.who.int/fsf/GMfood/>).
- Wilczynski, G., Kulma, A., Sikorski, A. F., and Szopa, J. (1997). ADP-ribosylation factor (ARF) regulates cAMP synthesis in potato. *J. Plant Physiol.*, **151**:689–698.
- Winnicka, A., Sawosz, E., Klucinski W, Kosieradzka, I., Szopa, J., Malepszy, S., and Pastuszewska. 2001. A note on the effect of feeding genetically modified potatoes on selected indices of nonspecific resistance in rats. *J. Anim. Feed Sci.*, **10**(suppl 2):13–18.
- Yum, H. Y., Lee, S. Y., Lee, K. E., Sohn, M. H., and Kim, K. E. (2005). Genetically modified and wild soybeans: an immunological comparison. *Allergy Asthma Proc.*, **26**:210–216.
- Zdunczyk, Z. (2001). *In vivo* experiments on the safety evaluation of GM components of feeds and foods. *J. Anim. Fed. Sci.*, **10**(suppl 1):195–210.
- Zhu, Y., Li, D., Wang, F., Yin, J., and Jin, H. (2004). Nutritional assessment and fate of DNA of soybean meal from Roundup Ready or conventional soybeans using rats. *Arch. Anim. Nutr.*, **58**:295–310.
- Zhuo, Q., Chen, X., Piao, J., and Han, C. (2004a). Study on the teratogenicity effects of genetically modified rice which expressed cowpea trypsin inhibitor on rats. *Wei Sheng Yan Jiu*, **33**:74–77 (in Chinese).
- Zhuo, Q., Chen, X., Piao, J., and Gu, L. (2004b). Study on food safety of genetically modified rice which expressed cowpea trypsin inhibitor by 90 day feeding test on rats. *Wei Sheng Yan Jiu*, **33**:176–179 (in Chinese).