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Review

A literature review on the safety assessment of genetically modified plants

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ABSTRACT

In recent years, there has been a notable concern on the safety of genetically modified (GM) foods/plants, an important and complex area of research, which demands rigorous standards. Diverse groups including consumers and environmental Non Governmental Organizations (NGO) have suggested that all GM foods/plants should be subjected to long-term animal feeding studies before approval for human consumption. In 2000 and 2006, we reviewed the information published in international scientific journals, noting that the number of references concerning human and animal toxicological/health risks studies on GM foods/plants was very limited. The main goal of the present review was to assess the current state-of-the-art regarding the potential adverse effects/safety assessment of GM plants for human consumption. The number of citations found in databases (PubMed and Scopus) has dramatically increased since 2006. However, new information on products such as potatoes, cucumber, peas or tomatoes, among others was not available. Corn/maize, rice, and soybeans were included in the present review. An equilibrium in the number research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was currently observed. Nevertheless, it should be noted that most of these studies have been conducted by biotechnology companies responsible of commercializing these GM plants. These findings suggest a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies. All this recent information is herein critically reviewed.

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1. Introduction

In recent years, the use and release of genetically modified organisms (GMOs) has been an issue of intense public concern and, in the case of foods, products containing GMOs or products thereof carry the risk of consumer rejection. The World Health Organization (WHO) defines GMOs as those organisms in which the genetic material has been altered in a way that does not occur naturally (WHO, 2002). As genetically modified (GM) foods are starting to be present in our diet concerns have been expressed regarding GM food safety (Dona and Arvanitoyannis, 2009). Although the WHO declares that the GM products that are currently on the international market have all gone through risk assessment by national authorities, the risk assessment of GM foods in general, and crops in particular for human nutrition and health, has not been systematically performed as indicated in the scientific literature (Domingo, 2007; Magaña-Gómez and de la Barca, 2009). 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 to a

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certain extent (Magaña-Gómez and de la Barca, 2009). Diversity among the methods and results of the risk assessments reflects the complexity of the subject.

Among the different GMOs, in recent years GM plants have attracted a large amount of media attention. However, the general public remains largely unaware of the real notion of GM plants or what advantages and disadvantages the technology has to offer, particularly with regard to the range of applications for which they can be used. From the first generation of GM crops, two main areas of concern have emerged, namely risk to the environment and risk to human health. As GM plants are gradually being introduced into the European Union it is likely that public concern regarding potential health issues will arise. Although it is now commonplace for the press and media to adopt 'health campaigns', the information they publish is often unreliable and unrepresentative of the available scientific evidence (Key et al., 2008).

Approximately 15 years have passed after the introduction of genetic modifications in food, and new GM products are currently added to the existing list of foods. However, 10 years ago we already noticed that there was no sufficient published information concerning safety of GM foods in general, and GM plants, in particular. Specifically, the lack of published toxicological studies on adverse health effects was evident (Domingo, 2000; Domingo-Roig and Gómez-Arnáiz, 2000). In 2006, 6 years after our initial review was published, we carried out a new review of the scientific literature on the potential adverse health/toxic effects of GM/transgenic plants (Domingo, 2007). 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 in that review. The number of references found in the databases was yet surprisingly limited. Moreover, most published studies were not performed by the biotechnology companies that produce/commercialize these products. However, as it also occurred with our first review (Domingo, 2000), we found a considerable number of references concerning commentaries, general news, and letters to the Editor (published in reputable international journals). Notwithstanding, papers about experimental investigations on the safety of GM foods/plants were very scant. Hence, the conclusion from our 2006 review (Domingo, 2007) was, for the second time, that if data on toxicological assessment of GM foods/plants existed, these had not been reported in scientific journals, and therefore, they were not available to the general scientific judgment.

Probably, one of the most important problems related with the lack of studies (at least not published in the scientific literature) on the safety assessment of GM foods/plants was the use of the "substantial equivalence" concept. This notion is based on the 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 further investigated with respect to their toxicological impact. 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 used, for example, for chemical substances of commercial interest such as pesticides, drugs, food additives, etc.? In fact, the "substantial equivalence" principle is a starting point rather than an end point (Kuiper et al., 2002). If this seems to be reasonably obvious, and taking into account the great controversy generated by the debate about GM plants safety, why the published information is so scarce?

The conclusions of our 2006 review concerning the doubts on the use of the principle of "substantial equivalence" in GM plants, as well as the lack of toxicological studies (Domingo, 2007), were quite in agreement with the conclusions of other reviews (Zduńczyk, 2001; Bakshi, 2003; Pryme and Lembcke, 2003), as well as with those of our previous review (Domingo, 2000; Domingo-Roig and Gómez-Arnáiz, 2000). In a recent paper (Dona and Arvanitoyannis, 2009), it was reported that the results of most studies with GM foods indicated that they might cause some common toxic effects. There is no doubt that one of the main issues concerning GM food safety assessment is based upon detection of their potentially toxic properties, which could provoke unintended effects of the genetic modification (Tyshko et al., 2007).

2. Risk assessment of GM plants

In our previous two reviews (Domingo, 2000, 2007), as well as in the current one, the scientific literature on the potential adverse health/toxic effects of GM/transgenic foods/plants was reviewed using the PubMed database (available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). In our first review, the search covered the period January 1980–May 2000, while the second review covered the period January 1980–October 2006. The current one covers the period January 1980–August 2010. We initially used the following "key terms": genetically modified foods, GM foods, transgenic foods, toxicity of transgenic foods, health risks of transgenic foods, adverse effects of genetically modified foods, toxicity of genetically modified foods, health risks of GM foods, health risks of genetically modified foods, toxicity of GM foods, adverse effects of GM foods, and adverse effects of transgenic foods. Citations corresponding to general "key terms" such as: genetically modified foods, GM foods, and transgenic foods were, not surprisingly, quantitatively the most important. After this preliminary screening, our search was focused in these four terms: (a) genetically modified foods, (b) toxicity of transgenic foods, (c) adverse effects of transgenic foods, and (d) health risks of transgenic foods. The number of citations has dramatically grown in recent years. Thus, in 2000, 2006 and 2010, those numbers were respectively: 101, 686 and 2879 for (a); 44, 136 and 376 for (b); 67, 199 and 504 for (c), and 3, 23 and 75 for (d) (Fig. 1). In spite of the notable increase in the number of citations, those concerning specifically to studies focused on demonstrating the health safety of GM foods remain very limited. Given that mentioned earlier, it is noteworthy that search terms such as "substantial equivalence" were not considered herein aiming to avoid any misleading information on the possible toxicological/safety concerns of GM crops to human health.

The present review, as our previous one (Domingo, 2007), was focused on GM plants only, a group of GMOs for which an especial interest exists for their potential use in food and feed. In addition to PubMed (Pub), we have also used Scopus (Sc) as database for the present online search. The number of references found between January 1980 and August 2010 were the following: for toxicity of genetically modified plants, 508 (Pub) and 339 (Sc), for adverse effects of genetically modified plants, 702 (Pub) and 156 (Sc), and for health risks of genetically modified plants, 168 (Pub) and 321 (Sc) (Fig. 1). Comparing the citations related to genetically modified potatoes, cucumber, tomatoes, sweet pepper, peas and canola, with those corresponding to the same products in our previous review (Domingo, 2007), it must be noted that no new toxicological/adverse effects/health risks studies references are available. In contrast, new information (October 2006–August 2010) was found concerning corn, soybean and rice, which is next reported.

2.1. Corn/maize

In the last few years, one of the most active research groups focusing its investigations on GM maize is that of Dr. Séralini and coworkers from the University of Caen (Caen, France). These authors reanalyzed data from a 90-day toxicity study performed in rats under the responsibility of Monsanto Company with a transgenic corn MON

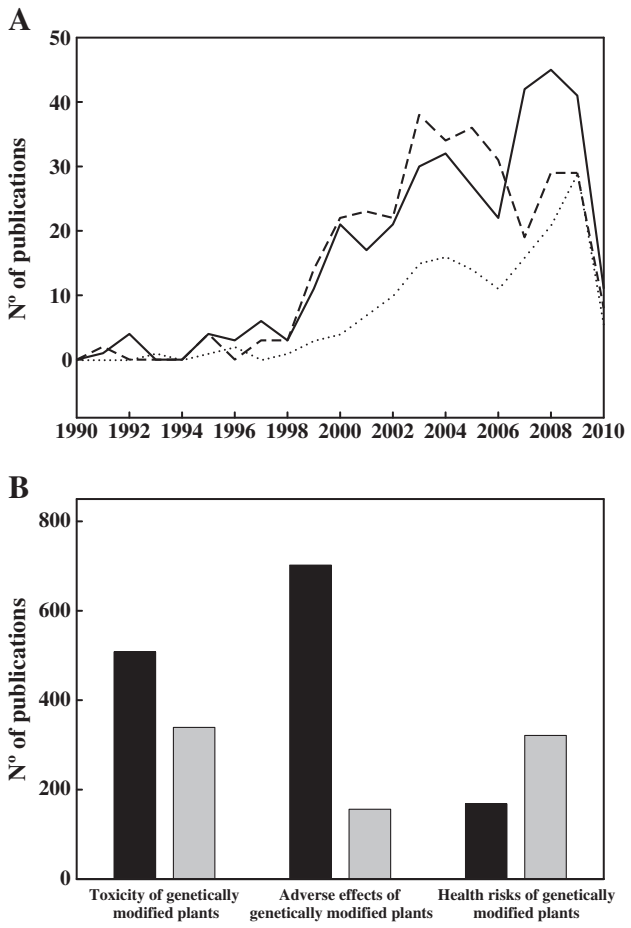

Fig. 1. (A) Number of publications per year, from 1990 to present, referring to (—) toxicity of genetically modified plants, (···) adverse effects of genetically modified plants and (- -) health risks of genetically modified plants, using the Scopus database. (B) Comparison between total number of publications using different keywords with Scopus (▒) and PubMed (■) databases.

863 (a genetically engineered corn variety that contains the gene for modified *Bacillus thuringiensis* (Bt) Cry3Bb1 protein to protect against corn rootworm). MON 863 had been subjected to questions from regulatory reviewers in Europe, where it was finally approved in 2005. Séralini et al. (2007) reported that after the consumption of MON 863, animals showed slight but dose-related significant variations in growth for both sexes, resulting in 3.3% decrease in weight for males and 3.7% increase for females. Moreover, signs of hepatorenal toxicity, marked also by differential sensitivities in males and females, were also noticed, while triglycerides increased by 24–40% in females (either at week 14, dose 11% or at week 5, dose 33%, respectively). In turn, urine phosphorus and sodium excretions diminished in males by 31–35% (week 14, dose 33%), being the most important results significantly linked to the treatment in comparison to seven diets tested. It was concluded that longer experiments were essential in order to indicate the real nature and extent of the possible pathology. It was remarked that based on the Monsanto data, it could not be concluded that GM corn MON 863 was a safe product (Séralini et al., 2007).

An Expert Panel (Doull et al., 2007) was subsequently convened to assess the original study results as analyzed by the Monsanto Company, and the reanalysis conducted by Séralini's group. The Expert Panel concluded that the reanalysis conducted by Séralini et al. (2007) provided no evidence to indicate that MON 863 was associated with adverse effects in the 90-day rat study. In each case, statistical findings reported by both Monsanto and Séralini et al. (2007) were considered to be unrelated to treatment or of no biological or clinical importance because they failed to demonstrate a dose–response relationship, reproducibility over time, association with other relevant changes (e.g., histopathology), occurrence in both sexes, difference outside the normal range of variation, or biological plausibility with respect to cause-and-effect. In a recent review (Séralini et al., 2009), the authors assumed that the methodology used in their previous paper (Séralini et al., 2007) was appropriate to discriminate potential false positive and GM-linked effects, avoiding to some extent false negative results, in the best manner it may be done for somehow too limited protocols already in use for commercialized GMOs (Séralini et al., 2007). Accordingly, the authors (Séralini et al., 2009) declared that GM-linked effects in the 90 days feeding studies were signs of toxicity rather than proofs of toxicity by itself. Besides, it was pointed out, that the biological plausibility of a subchronic or chronic side effect of the GM diet, either linked to the new toxin in the mammalian regimen or due to the mutagenesis effect of the genetic modification itself, was consequently non negligible (Séralini et al., 2009).

Recently, de Vendômois et al. (2009) performed, for the first time, a comparative analysis of blood and organ system data from trials with rats fed three main commercialized GM maize (NK 603, MON 810 and MON 863). The authors found for the 3 GMOs new side effects linked with GM maize consumption, which were sex- and often dose-dependent. Effects were mostly associated with the kidney and liver, the dietary detoxifying organs, although different between the 3 GMOs. Other effects were also observed in heart, adrenal glands, spleen and hematopoietic system. It was concluded that these data highlighted signs of hepatorenal toxicity, possibly due to the pesticides specific to each GM corn (glyphosate and AMPA in NK 603, modified Cry1Ab in MON 810 and modified Cry3Bb1 in MON 863). In addition, unintended direct or indirect metabolic consequences of the genetic modification could not be excluded. To date, and to the best of our knowledge, this study has not been scientifically questioned. Statistically significant effects of GM diets, or of residues of pesticides containing GMOs, have been also previously observed in some (Malatesta et al., 2002a, 2003; Vecchio et al., 2004), but not in all studies (Brake and Evenson, 2004; Brake et al., 2004) enlightening the necessity of a case-by-case approach and that toxicological studies are quite limited, up to date, for this approach (Domingo, 2007). For the Séralini's group it seems unbelievable that a risk assessment carried out only on forty rats of each sex receiving GM rich diets for 90 days (yielding results often at the limits of significance) has not been repeated and prolonged independently.

With regard to the above, it is important to note that according to a recent report of the EFSA GMO Panel working group on animal feeding trials (EFSA, 2008), the aim of the 90-days rodent feeding study with the whole GM food and feed is mainly focused on assessing potential unintended effects of toxicological and/or nutritional relevance and to establish whether the GM food and feed is as safe and nutritious as its traditional counterpart rather than determining qualitative and quantitative intrinsic toxicity of defined food constituents. A 90-day animal feeding trial has a large capacity (sensitivity and specificity) to detect potential biological/toxicological effects of single well defined compounds (Knudsen and Poulsen, 2007). Therefore, it should be possible to model the sensitivity of the rat subchronic feeding study for the detection of hypothetically increased amount of compounds such as anti-nutrients, toxicants, or secondary metabolites. However, with respect to the detection of potential unintended effects in whole GM food and feed, the EFSA GMO Panel also indicates that it would be unlikely that substances present in small amounts, and with a low toxic potential, could result in any observable (unintended) effects in a 90-day rodent feeding study, as they would be below the no-observed-effect-level (NOEL), and thus of unlikely impact to human health at normal intake levels (EFSA, 2008). It is worthy of being mentioned that the EFSA GMO Panel employs the term “unlikely” a couple of times in a few lines, which may suggest certain potential

limitations in the conclusions of 90-day rodent feeding studies performed with GM food and feed.

In contrast to the concern raised in the studies by Séralini and coworkers, other investigators reported that various GM maize grains were as safe as conventional maize grains. The most active group of researchers supporting this is headed by Dr. Delaney, who has published a notable number of papers on this topic since 2007. The conclusions of these studies are next summarized. MacKenzie et al. (2007) performed a subchronic (approximately 90 days) feeding study in Sprague–Dawley rats fed diets containing 1507 maize grain. Maize line 1507 is a GM maize plant that expresses the cry1F gene from Bt sbsp. aizawai and the phosphinothricin-N-acetyltransferase (pat) gene from *Streptomyces viridochromogenes* throughout the plant including the grain. Expression of the Cry1F protein confers to the plant resistance to the European corn borer and other lepidopteron pests. No significant differences were observed in the nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology between any pair of treatment groups. In turn, when compared to control groups, Malley et al. (2007) did not find adverse diet-related differences in rats fed given 59122 maize grain with respect to body weight/gain, food consumption/efficiency, clinical signs of toxicity, mortality, ophthalmology, neurobehavioral (FOB and motor activity) assessments, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), and pathology (organ weights and gross and microscopic pathology). 59122 is a transgenic maize line containing event DAS-59122-7 that expresses the corn rootworm (CRW) specific pesticidal Cry34Ab1 and Cry35Ab1 proteins from Bt Berliner strain PS149B1 and the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes* for tolerance to the herbicidal ingredient glufosinate-ammonium. According to the authors, the results of their studies indicated that 1507 and 59122 maize grains were nutritionally equivalent to and as safe as conventional (non-GM) maize grain (MacKenzie et al., 2007; Malley et al., 2007).

In Sprague–Dawley rats, Appenzeller et al. (2009a) conducted a subchronic feeding study to evaluate the potential health effects of long-term consumption of a rodent diet containing 1507 × 59122 maize grains compared with a diet containing maize grain from its near-isogenic control (091). 1507 × 59122 maize is a GM hybrid that confers resistance to lepidopteran and coleopteran pests and tolerance to the herbicidal active ingredient glufosinate-ammonium. Diets were fed *ad libitum* for at least 92 days. No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophthalmology, clinical pathology (hematology, clinical chemistry, coagulation, and urinalysis), organ weights, and gross and microscopic pathology between rats in the 091 and 1507 × 59122 treatment groups. In another 13-week feeding study by the same authors (Appenzeller et al., 2009b) also conducted in Sprague–Dawley rats, the potential health effects from consumption of a diet formulated with grain from GM herbicide-tolerant maize DP-Ø9814Ø-6 (98140; trade name Optimum GAT) were evaluated. Maize event 98140 expresses the GAT4621 (glyphosate acetyltransferase) and ZM-HRA (modified version of a maize acetolactate synthase) proteins. The first one, encoded by the *gat4621* gene, is responsible for confering plant tolerance to glyphosate-containing herbicides by acetylating glyphosate and thereby rendering it non-phytotoxic whereas the ZM-HRA protein, encoded by the *zm-hra* gene, confers tolerance to the ALS-inhibiting class of herbicides (Appenzeller et al., 2009b). Compared with rats fed diets containing grain from the conventional near-isogenic control maize, no adverse effects were observed in animals fed diets containing grain from 98140 or 98140 + Gly/SU (treated with herbicides containing the active ingredients glyphosate and nicosulfuron plus rimsulfuron) maize with respect to standard nutritional performance metrics and OECD 408-compliant toxicological response variables. In both studies (Appenzeller et al., 2009a,b), the authors concluded that 1507 × 59122 maize grain and Optimum GAT were as safe and nutritious as non-GM maize grain.

In mice, Juberg et al. (2009) did not find evidence of acute toxicity following oral exposure to either the Cry34Ab1 or Cry35Ab1 proteins individually or concomitantly. Similarly, no adverse effects were observed in a repeated dose (28 day) dietary toxicity study that incorporated these proteins into diets at concentrations corresponding up to 1000-fold greater than the highest estimate of human exposure based on the concentrations of these proteins expressed in 59122 maize grains (Juberg et al., 2009). According to the authors (Juberg et al., 2009), these studies demonstrated that the Cry34Ab1 and Cry35Ab1 proteins did not represent a risk to human health and supported previous studies indicating that 59122 maize grain is as safe and wholesome as non-GM maize grain. Expression of the Cry34Ab1 and Cry35Ab1 proteins from Bt Berliner strain PS149B1 in GM maize (event DAS-59122-7) protects the crop from damage due to feeding by *Diabrotica* larvae including the western corn rootworm (*Diabrotica virgifera* virgifera). On the other hand, other researchers (McNaughton et al., 2007) did not observe statistically significant differences in mortality, growth performance variables, or carcass and organ yields between broilers consuming diets containing transgenic maize grains from event DP-Ø9814Ø-6 (Optimum GAT), near-isogenic control maize grain, or commercial reference maize grains. It must be noted that in this study adverse/toxic effects of the transgenic maize were not investigated given that the study was mainly conducted to mimic some variables that would be normally measured by commercial poultry producers.

Recently, two 90-days feeding studies (He et al., 2008, 2009) were conducted in Sprague–Dawley rats, to which grain from corn rootworm resistant transgenic DAS-59122-7 maize, and transgenic lysine-rich maize grain (Y642) were given. The results were compared with those obtained from rats given non-transgenic maize. In the first study (He et al., 2008), significant differences were observed in certain hematology and serum chemistry response variables between rats consuming diets formulated with 59122 compared to AIN93G diet (a commercial diet used as control). However, the authors concluded that these differences were related to consumption of diets containing high concentrations of maize flour (compared to AIN93G diets) regardless of source, rather than to consumption of flour from 59122 maize grain. Therefore, it was concluded that 59122 maize grain was as safe as non-transgenic maize grain (He et al., 2008) and hence in accordance with that reported by Malley et al. (2007) although using different experimental designs.

On a similar approach, following studies (He et al., 2009) showed no adverse diet-related differences in body weights, feed consumption/utilization, clinical chemistry, hematology, and absolute and relative organ weights between rats consuming diets with Y642 maize grain compared with rats consuming diets containing Nongda 108 maize grain (near-isogenic non-GM quality protein maize). Maize event Y642 has kernels enriched in lysine content primarily aiming to improve monogastric animal nutrition whereas Nongda 108 maize, used in the above-mentioned study as a control, is a high-lysine corn obtained by conventional breeding. No differences in gross or microscopic pathology were observed and according to the authors (He et al., 2009), these results demonstrate that Y642 lysine-rich maize was as safe and nutritious as conventional quality protein maize.

Other groups of investigators have also evaluated the safety of GM maize/corn grains. For instance, Healy et al. (2008) performed a 13-week rat feeding study with grain from MON 88017 corn (brand name YieldGard VT Rootworm/RR2), protected from feeding damage caused by corn rootworm and tolerant to glyphosate, the active ingredient in Roundup agricultural herbicides. MON 88017 was formulated into rat diets at 11 or 33% (w/w) levels with its near-isogenic control at a level of 33% (w/w). Additionally, six diets containing grain from different

conventional (non-biotechnology-derived), reference hybrids were formulated, each at 33% (w/w) levels of one of six reference grains. No adverse health effects were noted. Consistent with agronomic, compositional and farm animal feeding studies, the 90-day rat study did not detect unintended effects. The authors concluded that MON 88017 was as safe and nutritious as conventional corn hybrids. Other researchers (Herouet-Guicheney et al., 2009) assessed the potential safety concerns related to the transgenic 2mEPSPS (5-enol pyruvyl-shikimate-3-phosphate synthase), a protein with a lower binding affinity for glyphosate, which is highly resistant to the inhibition by glyphosate, and thus allows sufficient enzyme activity for the plants to grow in the presence of herbicides that contain glyphosate. The safety evaluation supported that the expressed protein was innocuous. The 2mEPSPS enzyme did not possess any of the properties associated with known toxins or allergens, including a lack of amino acid sequence similarity to known toxins and allergens, a rapid degradation in simulated gastric and intestinal fluids, and no adverse effects in mice after intravenous or oral administration (at 10 or 2000 mg/kg body weight, respectively). It was concluded that there was a reasonable certainty of no harm resulting from the inclusion of the 2mEPSPS protein in human food or in animal feed.

In the scientific literature, there also exist various references concerning studies performed by Russian investigators (Tutel'ian et al., 2008, 2009; Tyshko et al., 2008, 2009). These authors assessed medical and biological safety of GM maize rootworm *Diabrotica* spp.-protected event MIR604 and rootworm *Diabrotica* spp.-protected and glyphosate-tolerant maize event MON 88017. Analysis of morphological, hematological and biochemical parameters and system (sensitive) biomarkers did not reveal any toxic effect of maize event MIR604 and MON 88017 (Tutel'ian et al., 2008, 2009), while analysis of damages of DNA and structural chromosome aberrations and assessment of the allergenic potential and immunoreactive properties did not show any genotoxic, allergenic and immunotoxic effect of those GM corns (Tyshko et al., 2008, 2009). Nevertheless, and considering that these four references (Tutel'ian et al., 2008, 2009; Tyshko et al., 2008, 2009) are in Russian, only information from the abstracts was included in the present review.

2.2. Rice

The most recent studies concerning safety of GM-rice have been performed as a part of the SAFOTEST project by the group headed by Dr. Knudsen from the Danish Institute for Food and Veterinary Research. SAFOTEST is an EU project designed to develop scientific methodologies for assessing the safety of GM crops, being the 90-day animal study the core study for the safety assessment of GM foods (Poulsen et al., 2007a). Accordingly, in a 90-day feeding study on Wistar rats (Schrøder et al., 2007), the authors compared the transgenic KMD1 rice expressing Cry1Ab protein (Bt toxin) to its non-transgenic parental wild type, Xiushui 11. The KMD1 rice contained 15 mg Bt toxin/kg, and based on the average feed consumption, the daily intake was 0.54 mg Bt toxin/kg body weight. No adverse effects on animal behavior or weight gain were observed during the study. A few hematological and biochemical parameters (analyzed from blood samples collected 1 week prior to sacrifice) were significantly different. Nonetheless, all were within the normal reference intervals for rats of this breed and age, and consequently not considered treatment related. Upon sacrifice, a number of organs were weighed, and macroscopic and histopathological examinations were performed. Only minor changes were observed (Schrøder et al., 2007). Although the results showed no adverse or toxic effects of KMD1 rice when tested in the 90-day study, the authors indicated that based on the experiences from that investigation, safety assessment for unintended effects of a GM crop could not be done without additional test group(s). In another feeding study conducted by the same research group (Poulsen et al., 2007b), Wistar rats were given a purified diet containing either 60% of a rice variety expressing the snowdrop *Galanthus nivalis* lectin (GNA lectin), or parental rice for 90 days. A range of clinical, biological, immunological, microbiological and pathological parameters were examined, with a number of significant differences observed between groups fed the two diets. Although none of them was considered to be adverse, the authors remarked that the design of their study was not able to conclude on the safety of the GM food. As in an earlier study (Schrøder et al., 2007), it was suggested that additional group(s), where the expressed gene products have been spiked to the diet, should be included in order to be able to distinguish whether the observed effects were due to the GNA lectin per se or to secondary changes in the GM-rice. Besides, as part of the SAFOTEST project, the immunomodulating effect of Cry1Ab protein from Bt and *Phaselous vulgaris* lectin agglutinin E-form (PHA-E lectin) from kidney bean was examined in 28- and 90-day feeding studies in Wistar rats. Animals were fed control rice, transgenic rice expressing Cry1Ab protein or PHA-E lectin, or transgenic rice spiked with the purified recombinant protein (Kroghsbo et al., 2008). Total immunoglobulin levels, mitogen-induced cell proliferation, T-dependent antibody response to sheep red blood cells, and the antigen-specific antibody response in serum were examined at the end of the studies. A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days indicating a local effect of PHA-E in the intestine. No adverse effects of Cry1Ab protein were found, while an anti-PHA-E and anti-Cry1Ab antibody response was induced both after inhalation (control groups) and after inhalation/ingestion (groups fed recombinant protein alone or together with transgenic rice). In conclusion, only PHA-E lectin was found to have an immunomodulating effect when feeding rats for 90 days with approximately 70 mg PHA-E/kg body weight per day.

Recently, Domon et al. (2009) reported the results of the first oral long-term safety assessment of transgenic plant products containing 7Crp (seven major human T-cell epitopes derived from Japanese cedar pollen allergens, which might be exploited to control pollen allergy in humans) using nonhuman primates (*Cynomolgus macaques*) over 26 weeks. Specifically, monkeys were orally administered a high or low dose of transgenic rice containing 7Crp or the non-transgenic control by gavage every day. No adverse effects on general behavior or body weight of animals were observed during the study, while analysis of blood from primates administered for 26 weeks showed that, with few exceptions, there were no significant differences in hematological or biochemical values between them. Moreover, neither pathological symptoms nor histopathological abnormalities were seen. It was concluded that oral administration of transgenic rice containing T-cell epitopes from Japanese cedar pollen allergens had no adverse effects and were safe when eaten every day (Domon et al., 2009).

2.3. Soybeans

With respect to recent studies on safety assessment of GM soybeans, the scientific literature shows rather contradictory results. Two research groups have been especially active in relation to those investigations. One of them, headed by Dr. Delaney from Pioneer Hi-Bred International, Inc. (Johnston, IA, USA), has reported data showing that various GM soybeans were safe. In contrast, the group headed by Dr. Malatesta from the University of Verona (Verona, Italy) has shown notable concerns. A summary of recent studies is next presented.

In Sprague–Dawley rats, Appenzeller et al. (2008) conducted a subchronic feeding study with the herbicide-tolerant soybean DP-356Ø43-5 (356043). Diets were fed to young adult animals for at least 93 days. Compared with rats fed the isoline control or conventional reference diets, no biologically-relevant, adverse effects were observed in rats fed diets containing 356043 soybean with respect to body weight/gain, food consumption/efficiency, clinical signs, mortality, ophthalmology, neurobehavioral assessments (sensory response, grip strength and motor activity), clinical pathology (hematology, coagulation, serum chemistry and urinalysis), organ weights, and gross and

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

Plant/crop	Animal species	Length of study	Main adverse effects	Reference
Corn/maize				
MON 863	Rats	90 days	Slight but dose-related weight variations in both males (3.3% reduction) and females (3.7% increase). Signs of hepatorenal toxicity, increased triglycerides in females (24–40%) and urine phosphorus and sodium excretions diminished in males (31–35%)	Séralini et al. (2007)
MON 863[a]	Rats	90 days	No evidence of adverse effects	Doull et al. (2007)
NK 603, MON 810 and MON 863	Rats	14 weeks	Sex- and dose-dependent side effects linked with consumption of 3 GMOs and mostly associated with hepatorenal toxicity. Other adverse effects were also detected in heart, spleen, adrenal glands and hemopoietic system	de Vendômois et al. (2009)
Maize 1507	Sprague–Dawley rats	90 days	No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophthalmology and clinical pathology, organ weights and gross and microscopic pathology between treatment groups	MacKenzie et al. (2007)
Maize 59122	Rats	90 days	No adverse diet-related differences in body weight, food consumption, clinical signs of toxicity, mortality, ophthalmology, neurobehavioral assessments, clinical pathology and pathology	Malley et al. (2007)
Maize 1507 × 59122	Sprague–Dawley rats	92 days	No significant differences were observed in nutritional performance variables, clinical and neurobehavioral signs, ophtalmology and clinical pathology, organ weights and gross and microscopic pathology between treatment groups	Appenzeller et al. (2009a)
Maize DP-Ø9814Ø-6	Sprague–Dawley rats	13 weeks	No adverse effects were observed in nutritional performance variables and OECD 408-compliant toxicological response variables	Appenzeller et al. (2009b)
Maize 59122[b]	Mice	28 days	No signs of acute toxicity or adverse effects due to diets containing high concentrations of Cry34Ab1 or Cry35Ab1 proteins, individually or concomitantly, were found at concentrations nearly 1000-fold greater than those found in 59122 maize grains	Juberg et al. (2009)
Maize DP-Ø9814Ø-6	Broilers	42 days	No significant differences in mortality, growth performance variables or carcass and organ yields. Adverse-toxic effects of the transgenic maize were not assessed.	McNaughton et al. (2007)
DAS-59122-7	Sprague–Dawley rats	90 days	Significant differences in certain hematology and serum chemistry response variables, but attributed to diets containing high maize flour (compared to control diets). It was concluded that 59122 maize grains were as safe as non-transgenic maize diets	He et al. (2008)
Y642 (lysine-rich)	Sprague–Dawley rats	90 days	No adverse diet-related adverse effects in body weight, feed consumption, clinical chemistry, hematology, and absolute and relative organ weights	He et al. (2009)
MON 88017	Rats	13 weeks	No adverse health effects were noticed.	Healy et al. (2008)
Maize (2mEPSPS)	Mice	–	The safety evaluation concluded that the protein was innocuous and hence could be included in human food or animal feed.	Herouet-Guicheney et al. (2009)
MIR 604, MON 88107	–	–	Analysis of morphological, hematological and biochemical parameters and system sensitive biomarker did not reveal any toxic effect.	Tutel'ian et al. (2008, 2009)
MIR 604, MON 88107	–	–	Analysis of DNA damage and structural chromosome aberrations, assessment of allergenic potential and immunoreactive properties did not show any genotoxic, allergenic and immunoreactive effects.	Tyshko et al. (2008, 2009)
Rice				
KMD1	Wistar rats	90 days	No adverse effects on animal behavior or weight gain. Few hematological and biochemical parameters were significantly different between treatment diets. However, all were within the normal reference intervals for rats of this breed and age. Minor changes were observed in organs weight and macroscopic and histopathological examinations.	Schrøder et al. (2007)
Rice expressing GNA lectin	Wistar rats	90 days	No adverse effects were observed. However, a range of clinical, biological, i mmunological, microbiological and pathological parameters were significantly different between diet groups. The authors remarked that the design of their study was not able to conclude on the safety of the product.	Poulsen et al. (2007a,b)
Rice expressing Cry1Ab protein or PHA-E lectin	Wistar rats	28- and 90-days	A dose-dependent increase in mesenteric lymph node weight and total immunoglobulin A was seen when feeding PHA-E transgenic rice alone or spiked with 0.1% purified PHA-E lectin for 90 days. No adverse effects of Cry1Ab protein were found.	Kroghsbo et al. (2008)
Rice containing 7Crp	*Cynomolgus macaques*	26 weeks	No adverse effects on general behavior or body weight, hematological and biochemical variables. No pathological symptoms or histopathological abnormalities.	Domon et al. (2009)
Soybeans				
DP-356Ø43-5	Sprague–Dawley rats	>93 days	No adverse effects on body weight/gain, food consumption, clinical signs, mortality, ophthalmology, neurobehavioral assessment, clinical pathology, organ weights and gross and microscopic pathology	Appenzeller et al. (2008)
DP-356Ø43-5	Broilers	42 days	No adverse effects were found. It was concluded that GM 356Ø43 was nutritionally equivalent to non-GM soybean with comparable genetic background	McNaughton et al. (2008)
DP-3Ø5423-1	Sprague–Dawley rats	–	No adverse effects on body weight/gain, food consumption, and mortality, clinical signs of toxicity or ophthalmological observations, neurobehavioral assessments, organ weights or clinical and anatomic pathology	Delaney et al. (2008)
HRA	Mice	28 days	No adverse effects	Mathesius et al. (2009)
Soybean expressing CP4 EPSPS gene	Mice	–	Several proteins belonging to hepatocyte metabolism, stress response, calcium signaling and mitochondria were differentially expressed in	Malatesta et al. (2008a)

(continued on next page)

Table 1 (*continued*)

Plant/crop	Animal species	Length of study	Main adverse effects	Reference
			GM-fed mice indicating a more marked expression of senescence markers in comparison to controls. GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate	
GM	Mice	–	No morphological differences in embryos of GM and non-Gm soybean-exposed groups. Microscopic and ultramicroscopic cellular changes attributed to GM soybean intake	Cisterna et al. (2008)
SUPRO 500E	Wistar rats	30 days	No adverse effects in nutritional performance. Altered pancreas function evidenced by the early acute PAP mRNA increased levels and pancreas cellular changes	Malatesta et al. (2002a,b)
Glyphosphate tolerant	F344 rats	52 weeks	No adverse effect in gross necropsy findings, hematological and serum biochemical parameters, organ weights and pathological findings	Sakamoto et al. (2007)
Glyphosphate tolerant	F344 rats	104 weeks	No adverse effect in gross necropsy findings, hematological and serum biochemical parameters, organ weights and pathological findings	Sakamoto et al. (2008)

[a] Expert panel convened to assess the original study results analyzed by Montsanto Company and the reanalysis conducted by Séralini et al. (2007).
[b] Oral exposure to either the Cry34Ab1 or Cry35Ab1 proteins found in 59122 maize.

microscopic pathology. In a 42-day feeding trial study conducted in broiler chickens (McNaughton et al., 2008), it was also concluded that 356043 soybean was nutritionally equivalent to non-transgenic control soybean with a comparable genetic background. Delaney et al. (2008) carried out in Sprague–Dawley rats a subchronic feeding study of high oleic acid soybeans (Event DP-3Ø5423-1). DP-3Ø5423-1 (305423) is a GM soybean produced by biolistic insertion of a gm-fad2-1 gene fragment and the gm-hra gene into the germline of soybean seeds. Compared with rats fed the non-GM control diet, no biologically-relevant differences were observed in animals fed the 305423 diet with respect to body weight/gain, food consumption/efficiency, mortality, clinical signs of toxicity, or ophthalmologic observations. In addition, no diet-related effects were noted on neurobehavioral assessment, organ weights, or clinical or anatomic pathology. Based on the results of these studies, the authors concluded that 356043 and 305423 soybeans were as safe and nutritious as conventional non-GM soybeans (Appenzeller et al., 2008; Delaney et al., 2008). Also related to GM soybeans, Mathesius et al. (2009) assessed the safety of a modified acetolactate synthase protein (GM-HRA) used as a selectable marker in GM soybeans. The authors (Mathesius et al., 2009) did not find adverse effects in mice following acute oral exposure to GM-HRA at a dose of at least 436 mg/kg of body weight, or in a 28-day repeated dose dietary toxicity study at doses up to 1247 mg/kg of body weight/day. It was concluded that GM-HRA protein is safe when used in agricultural biotechnology.

In contrast to the above results, in a long-term study on female mice fed a GM modified soybean (insertion of the bacterial CP4 EPSPS gene to confer a high level of tolerance to glyphosate), focused on assessing the effects of this diet on liver of old animals (until 24 months of age) and to elucidate possible interference with aging, Malatesta et al. (2008a) found that GM soybean intake could influence the liver morpho-functional features during the physiological process of aging. Several proteins belonging to hepatocyte metabolism, stress response, calcium signaling and mitochondria were differentially expressed in GM-fed mice, indicating a more marked expression of senescence markers in comparison to controls. Moreover, hepatocytes of GM-fed mice showed mitochondrial and nuclear modifications indicative of reduced metabolic rate. In previous studies on hepatocytes from young and adult (2–8 months of age) female mice fed GM soybeans, nuclear modifications involving structural constituents of the transcription and splicing properties pathways were seen (Malatesta et al., 2002a). Although the cause(s) of the observed alterations could not be conclusively established, it was noted that these modifications disappeared when GM soybean was replaced by a non-GM one in the diet (Malatesta et al., 2005). Since the GM soybean used was tolerant to glyphosate and was treated with the glyphosate-containing herbicide Roundup, the effects observed might be due to herbicide residues. Accordingly, and aiming to verify this hypothesis, Malatesta et al. (2008b) treated rat hepatoma tissue culture (HTC) cells with 1–10 mM Roundup and analyzed cellular features by flow cytometry, fluorescence, and electron microscopy. Under these experimental conditions, the death rate and the general morphology of HTC cells were not affected, as well as most of the cytoplasmic organelles. However, in HTC-treated cells, lysosome density increased and mitochondrial membranes were modified indicating a decline in the respiratory activity. In addition to the above, nuclei underwent morpho-functional modifications suggesting a decreased transcriptional/splicing activity. The authors did not exclude that factors other than the presence of the herbicide residues could be responsible for the cellular modifications described in GM-fed mice. However, they indicated that the concordance of the effects induced by low concentrations of Roundup on HTC cells suggested that the presence of Roundup residues could be one of the factors interfering with multiple metabolic pathways.

Cisterna et al. (2008) investigated the ultrastructural and immunocytochemical features of pre-implantation embryos from mice fed either GM or non-GM soybean in order to verify whether the parental diet could affect the morpho-functional development of the embryonic ribonucleoprotein structural constituents involved in pre-mRNA pathways. Morphological observations revealed that the general aspect of embryo nuclear components were similar in the GM and non-GM soybean-exposed groups. However, immunocyto-chemical and in situ hybridization results suggested a temporary decrease of pre-mRNA transcription and splicing in 2-cell embryos and a resumption in 4–8-cell embryos from mice fed GM soybean. In addition, pre-mRNA maturation seemed to be less efficient in both 2-cell and 4–8-cell embryos from GM-fed mice than in non-GM-fed animals. In a previous ultrastructural analysis of testes from mice fed GM soybean conducted by the same research group (Vecchio et al., 2004), it was found that the immunolabelling for Sm antigen, hnRNPs, SC35 and RNA Polymerase II was decreased in 2 and 5 month-old GM-fed mice, and was restored to normal at 8 months. In GM-fed mice of all ages considered, the number of perichromatin granules was higher and the nuclear pore density lower. Moreover, enlargements in the smooth endoplasmic reticulum in GM-fed mice Sertoli cells were also observed. Consequently, the studies by the Malatesta's group (Malatesta et al., 2005, 2008b; Cisterna et al., 2008) at the microscopic and ultramicroscopic levels showed cellular changes attributable to GM soybean intake.

Magaña-Gómez et al. (2008) conducted a study in Wistar rats, in which the hypothesis was that the intake of GM (SUPRO 500E) soybean could induce pancreatic stress or injury by analyzing the expression of pancreatitis-associated protein (PAP) and trypsinogens

by qRT-PCR in rats fed GM soy protein for 30 days. The hypothesis was based on the results of previous investigations showing that mice chronically fed since gestation with GM had problems in synthesis and processing of zymogens by pancreatic acinar cells and reduced nucleoplasmic and nucleolar and perichromatin granule accumulation on pancreatic acinar cell nuclei (Malatesta et al., 2002b, 2003). Magaña-Gómez et al. (2008) did not find differences in nutritional performance among rats fed non-GM and GM diets. The GM diet induced significant zymogen-granule depletion after 15 days feeding, returning to normal levels after 30 days. Acinar disorganization started as early as 5 days after initiation of the GM diet and it recovered after 30 days. Levels of PAP mRNA significantly increased in the GM diet between day 1 and day 3 and decreased to the basal level by day 15. In turn, trypsinogen mRNA peaked at two different times: at day 1 and at day 15, decreasing to basal levels after 30 days, while plasma amylase levels remained unchanged at all times. The authors indicated that GM soy protein intake affected pancreas function, evidenced by the early acute PAP mRNA increased levels and pancreas cellular changes followed by recuperation of acinar cells after 30 days. In Japan, Sakamoto et al. (2007, 2008) conducted 52-week and 104-week feeding studies of genetically modified soybeans in F344 rats. Although in both studies several differences in animal growth, food intake, serum biochemical parameters and histological findings were observed between rats fed the GM (glyphosate-tolerant) soybeans and those fed a commercial diet, body weight and food intake were similar for the rats fed the GM and non-GM soybeans. Gross necropsy findings, hematological and serum biochemical parameters, organ weights, and pathological findings showed no meaningful differences between rats fed the GM and non-GM soybeans. These results indicate that long-term intake (54 and 104 weeks) of GM soybeans at the level of 30% in the diet had no apparent adverse effect in rats.

3. Final remarks

In the same line of our previous papers (Domingo, 2000, 2007; Domingo-Roig and Gómez-Arnáiz, 2000), the main purpose of this review-article was to critically revise the published scientific literature on potential toxic effects/health risks of GM plants. It was noticed that the total number of general references on GMOs in general, and GM foods/plants in particular, found in the databases PubMed and Scopus has considerably increased between our 2006 search (Domingo, 2007) and the current one. In spite of this, the number of studies specifically focused on safety assessment of GM plants is still limited. However, it is important to remark that for the first time, a certain equilibrium in the number of research groups suggesting, on the basis of their studies, that a number of varieties of GM products (mainly maize and soybeans) are as safe and nutritious as the respective conventional non-GM plant, and those raising still serious concerns, was observed. Moreover, it is worth mentioning that most of the studies demonstrating that GM foods are as nutritional and safe as those obtained by conventional breeding, have been performed by biotechnology companies or associates, which are also responsible of commercializing these GM plants. Anyhow, this represents a notable advance in comparison with the lack of studies published in recent years in scientific journals by those companies (Domingo, 2007). The scientific community may finally be able to critically evaluate and discuss all that information, which was not possible until now. Scientists know quite well how different may be the information published in reputed international journals, which has been submitted to peer-review processes, from those general comments/reports not submitted to this selective procedure.

A relatively remarkable finding of the present review is that the published scientific literature between October 2006 (Domingo, 2007) and August 2010 (current review) on edible GM plants, concerns only to three products: corn/maize, soybeans, and rice, rice being comparatively the less abundant. We have not been able to find citations involving investigations on GM potatoes (except a review by Arvanitoyannis et al., 2008), peas, tomatoes, pepper, etc., after October 2006. A summary of experimental studies (October 2006–August 2010) concerning dietary administration of those products to various animal species is shown in Table 1. With respect to corn/maize, various studies have concluded that the transgenic varieties 1507 (MacKenzie et al., 2007), 59122 (Malley et al., 2007; Juberg et al., 2009; He et al., 2008), 1507 × 59122 (Appenzeller et al., 2009a), 98140 (Appenzeller et al., 2009b; McNaughton et al., 2007), Y642 (He et al., 2009), and MON 88017 (Healy et al., 2008) were as safe as conventional quality protein maize. In contrast, Séralini's group raised concern regarding some commercialized GM maize (NK 603, MON 810 and MON 863) (Séralini et al., 2007, 2009; de Vendômois et al., 2009). Similarly, scientific controversy is also present in relation to the safety of GM soybeans. While it has been reported that 356043 (Sakamoto et al., 2007) and 305423 (Delaney et al., 2008) soybeans were as safe as conventional non-GM soybeans, some authors are still concerned by the safety of GM soybeans and recommend to investigate the long-term consequences of GM diets and the potential synergistic effects with other products and/or conditions (Malatesta et al., 2008a,b; Cisterna et al., 2008; Magaña-Gómez et al., 2008).

In the period here revised, October 2006–August 2010, a few reviews on health risks of GM foods/plants have been also published (Dona and Arvanitoyannis, 2009; Magaña-Gómez and de la Barca, 2009; Key et al., 2008). In general terms, all these authors agree in remarking that more scientific efforts are clearly necessary in order to build confidence in the evaluation and acceptance of GM foods/plant by both the scientific community and the general public. Especially critical is the recent review by Dona and Arvanitoyannis (2009), who remarked that results of most studies with GM foods would indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects, and might alter the hematological, biochemical, and immunologic parameters. These authors also concluded that the use of recombinant GH or its expression in animals should be re-examined since it has been shown that it increases IGF-1 which, in turn, may promote cancer. A harsh response to that review was recently published in the same journal (Rickard, 2010). This is indeed only an example on the controversial debate on GMOs, which remains completely open at all levels.

Finally, we would like to indicate that the review on allergenicity of GM plants has not been included herein. European legislation stipulates that GMOs have to be monitored to identify potential adverse environmental effects (Reuter et al., 2010). The European Food Safety Authority (EFSA) has recently published a Scientific Opinion regarding assessment of allergenicity of GM plants and microorganisms and derived food and feed (EFSA, 2010). Detailed information on this important issue is available at http://www.efsa.europa.eu/en/scdocs/scdoc/1700.htm.

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