

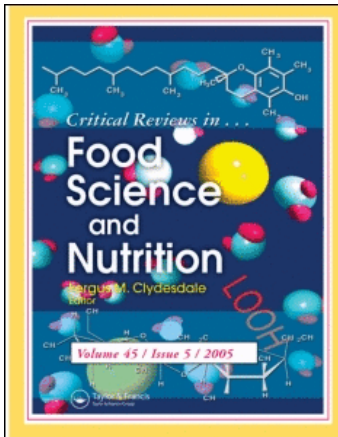
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Health Risks of Genetically Modified Foods

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As genetically modified (GM) foods are starting to intrude in our diet concerns have been expressed regarding GM food safety. These concerns as well as the limitations of the procedures followed in the evaluation of their safety are presented. Animal toxicity studies with certain GM foods have shown that they may toxically affect several organs and systems. The review of these studies should not be conducted separately for each GM food, but according to the effects exerted on certain organs it may help us create a better picture of the possible health effects on human beings. The results of most studies with GM foods indicate that they may cause some common toxic effects such as hepatic, pancreatic, renal, or reproductive effects and may alter the hematological, biochemical, and immunologic parameters. However, many years of research with animals and clinical trials are required for this assessment. 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 may promote cancer.

Keywords Allergenicity, antibiotic resistance, food safety, genetically modified, health risks, recombinant growth hormone, toxicity

INTRODUCTION

Nearly fifteen years have passed after the introduction of genetic modifications (GM) in food and new GM food are added in the existing list of foods. Who could imagine that there would come a day when the pig would be as “fat –free healthy food” as a fish or that the ice cream our children eat would contain a protein from the fish? Are GM safe to human health? Studies concerning their safety are still few when one considers the toxicity studies that must accompany the application of any novel drug for approval by the corresponding drug administration. The results from most toxicity studies available in literature are reviewed and the significance of these findings is discussed. In the absence of adequate safety studies, the lack of evidence that GM food is unsafe cannot be interpreted as proof that it is safe. Furthermore, if they are not considered safe for human consumption why should they be approved for animals? Humans can inadvertently consume foods that contain GM products fed to animals, i.e., crops modified for enhanced productivity in animals. This

was the case when traces of a StarLink GM crop, restricted for use only in feed, were found in taco shells already in the market. One has to wonder what will happen if we start consuming food crops contaminated with GM crops containing genes for the production of drugs and industrial chemicals that have never been assessed for their toxicity? (Margulis, 2006). The debate over its safety continues. One should not forget that every single GM food through the food chain will eventually reach the consumer. Issues such as the concern of the public for possible hazards due to the consumption of a GM food have already been discussed, but there is always something to add. However, prior to discussing these issues one must take into account in brief the regulation of testing for GM food safety.

THE STANDARDS AND REGULATION OF TESTING FOR FOOD SAFETY

In Europe, the placing on the market of genetically modified foods is covered by Regulation (EC) 1829/2003 on genetically modified food and feed. Multiple guidelines for the safety assessment process of GM foods have been developed (FAO/WHO, 2000; EFSA, 2005) and the new approach designed by ENTANSFOOD to guide the choice of test methods for this safety

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assessment requires compositional analyses of key nutrients and anti-nutrients in GM crops (Kuiper et al., 2004).

Another issue of great importance for the EU consumer following the carried out consumer studies is the GM (novel food) area which lead to a confrontation between USA and EU. A novel food is defined as a food or food ingredient which does not have a significant history of consumption within the EU prior to May 1997. All novel foods are subject to a pre-market safety assessment under the Novel Foods Regulation (EC) No. 258/97. The Food Standards Agency Board is satisfied that the current safety assessment procedures for GM foods are sufficiently robust and rigorous to ensure that approved GM foods are as safe as their non-GM counterparts, and pose no additional risk to the consumer. Each GM food is assessed for safety, including its toxicological, nutritional, and allergenic potential, on a case by case basis before it can be approved for marketing (Arvanitoyannis et al., 2005). The EU and US legislation focused on GMOs is given in Table 1.

The cultivation of new GM crop events also remains far on the horizon in the EU. On 7 December 2005, the EFSA adopted a first positive opinion for cultivation of the GM potato event EH92-527-1. However, its cultivation will be restricted to a closed loop system of contractors (EFSA, 2006). Moreover, the European adoption rate of previously approved GM crops for cultivation was slow (Demont and Tollens, 2004). With the registration of seventeen MON810 hybrids in the common seed catalogue on 8 September 2004, the GM maize cultivation area increased in France, Germany, and Spain, and expanded to the Czech Republic and Portugal in 2005. Nonetheless, in 2005, the European cultivation area of GM maize was approximately 55000 ha, whilst globally 21.2 million ha was reached (Devos et al., 2006).

The results are evaluated based on the principle of "substantial equivalence" criticized by Millstone et al. (1999) as "being created to provide an excuse for not requiring biochemical and toxicological tests." Moreover, Burlingame (2004) states that existing food composition databases do not necessarily reflect the complete natural variation since it was shown that the protein content may be different for both the transgenic and the parental line. Although genomics, proteomics, and metabolomics will provide a "global" overview of gene expression and have the potential for generating massive amounts of data, the possibility of predicting toxicity would still remain low due to complex metabolic pathways (Cellini, 2004). Taking into consideration the possibility that an analytical method might give false negative results for a toxic substance that may be produced in a GM food, this principle should not be the limiting step in evaluating GM crop safety. "Substantial equivalence" may provide some theoretical points background in predicting toxicity, but in practice the only reliable way to evaluate the toxicity of a GM food is through toxicity tests on animals. Furthermore, it has been argued that GM foods should be subjected to the same testing and approval procedures as medicines (i.e., clinical trials) since they must be adequate to ensure that any possibility of an adverse effect on human health from a GM food can be detected.

HAZARDS OF GM FOOD

Possible hazards of GM food for animals and populations exposed to a diet containing GM products include the potential for pleiotropic and insertional effects, effects on animal and human health resulting from the increase of anti-nutrients, potential effects on human health resulting from the use of viral DNA in plants, possible transfer of antibiotic resistant genes to bacteria in gastrointestinal tract, and possible effects of GM foods on allergic responses.

The Potential For Pleiotropic and Insertional Effects

Concern has been expressed about the above potential effects which might cause the silencing of genes, changes in their level of expression or, potentially, the turning on of existing genes that were not previously being expressed (Conner and Jacobs, 1999). This interaction with the activity of the existing genes and biochemical pathways of plants, may lead to disruption of metabolism in unpredictable ways and to the development of new toxic compounds or an increase of the already existing ones as it happened with two genetically produced foods, tryptophan and g-linolenic acid (Hill et al., 1993; Sayanova et al., 1997). Moreover, research into epigenetics has also revealed that genes account for only a part of the control of the biochemistry of organisms, and organisms have a level of control above genes that interact with genes explaining why genetic engineering is so unpredictable, with different results produced by each attempt and why the products are often unstable. The possibility that an unidentified compound may be present in the GM food makes crucial that each transgenic food as whole food and not as a single protein should be tested directly for toxicity in animals, although as Kuiper et al. (2004) state there are limitations in establishing dose-response relationships.

Possible Effects on Animal Health Resulting from the Increase of Anti-nutrients

The insertion of a new gene can sometimes lead to increase in existing levels of anti-nutrients, some of which cannot be reduced with heat treatment (Bakke-McKellep et al., 2007). One of the most widely available commercial GM products nowadays glyphosate-resistant Roundup Ready[®] soybean may display an increase in anti-nutrients (Padgette et al., 1996). Heat-stable anti-nutrients such as phytoestrogens, glucinins, and phytic acid were also found to cause infertility problems in sheep and cattle (Liener, 1994), allergenic reactions and binding to phosphorus and zinc thereby making them unavailable to the animal respectively (Adams, 1995). An increase in the anti-nutrient level should not be accepted since a GM food may be consumed as raw material.

Table 1 EU Directives and Regulations and US Acts (main points and comments) for GMOs

Title	Main points	Comments
EU legislation		
Directive 90/219/EEC (entry into force 23/10/1991) Contained use of G.M. Microorganisms	<ul style="list-style-type: none"> ▪ Measures for limited use of GM micro-organisms. ▪ Not applicable to certain techniques of genetic modification. ▪ Measures for avoidance of adverse effects in human health and environment. 	➤ Directive 98/81/EC amended this Directive (entry into force 5/12/1998)
Directive 90/220/EEC (entry into force 23/10/1991) Deliberate release into the environment of GMOs	<ul style="list-style-type: none"> ▪ Protective measures for human health and environment. ▪ Not applicable to certain techniques of genetic modification. ▪ Activities of Member States for deliberate release into the environment of GMOs for research, development and market placing purposes. 	➤ Directive 97/35/EC And Regulations (EC) No.258/97 and No.1139/98 amended this Directive
Directive 2001/18/EC (entry into force 17/4/2001) Deliberate release into the environment of GMOs	<ul style="list-style-type: none"> ▪ Measures of authorization of the release and disposal on the market of GMOs. ▪ Obligatory controls after the disposal of GMOs on the market. ▪ Consultations with the public and labelling of GMOs. 	➤ The last amendment of this Regulation (EC) No.1830/2003 (entry into force 7/11/2003)
Directive 2004/204/EC (entry into force 23/3/2004) Arrangements for the operation of the registers for recording information on genetic modifications in GMOs	<ul style="list-style-type: none"> ▪ Lists of information of genetic modification in GMOs. ▪ Lists should contain detailed report of documents. ▪ Lists are public available. 	
Directive 2004/643/EC Placing on the market of a maize product (<i>Zea mays</i> L. line NK603) GM for glyphosate tolerance	<ul style="list-style-type: none"> ▪ Product should be as safe as conventional (equivalence principle). ▪ Obligatory recordation of the code MON-00603-6 (unique). ▪ Measures for labelling and traceability in all stages of the market promotion. 	
Directive 2004/657/EC Placing on the market of a sweet corn from GM maize line Bt11 as a novel food or novel food ingredient	<ul style="list-style-type: none"> ▪ Product should be as safe as conventional. ▪ Obligatory labelling as "GM sweet corn." ▪ Obligatory recordation of the code SYN-BTø11-1 (unique). 	
Regulation (EC) No.258/97 (entry into force 14/5/1997) Novel food and novel food ingredients	<ul style="list-style-type: none"> ▪ Placing on the market within the Community of foods and food ingredients which have not been used for human consumption to a significant degree within the Community before. ▪ Not applicable to food additives, flavourings and extraction solvents. ▪ Specific procedure for foodstuffs containing GMOs. 	
Regulation (EC) No.1139/98 (entry into force 1/9/1998) The compulsory indication of the labelling of certain foodstuffs produced from GMOs	<ul style="list-style-type: none"> ▪ Application to food and food ingredients which are produced from GM soybean or GM corn. ▪ No application to food additives and condiments. ▪ No application to products which are legally produced, labelled and imported, commercialized in the Community. 	➤ Regulations (EC) No.49/2000 and No.50/2000 amended this one.
Regulation (EC) No.1829/2003 (entry into force 7/11/2003) GM food and feed	<ul style="list-style-type: none"> ▪ Measures for human and animal health protection, Community procedures of approval, inspection and labelling of GM food and feed. ▪ Approvals are applicable for 10 years with the potential of renewal. 	
Regulation (EC) No.1830/2003 (entry into force 7/11/2003) Traceability and labelling of GMOs and traceability of food and feed products produced from GMOs	<ul style="list-style-type: none"> ▪ Traceability of products consisting of, or containing GMOs and foodstuffs, feed produced from GMOs. ▪ Application for all stages of disposal on the market. ▪ Specific demands on labelling. 	
Regulation (EC) No.65/2004 (entry into force on the date of its publication in the <i>Official Journal of the European Union</i>) Establishment of a system for the development and assignment of unique identifiers for GMOs	<ul style="list-style-type: none"> ▪ Unique identifier for each GMO which is placed on the market. ▪ Not applicable to pharmaceuticals intended for human and veterinary use. 	
Regulation (EC) No.641/2004 (entry into force 18/4/2004) The authorization of new GM food and feed, the notification of existing products and adventitious or technically unavoidable presence of GM material which has benefited from a favorable risk evaluation	<ul style="list-style-type: none"> ▪ Transformation of applications and statements in the applications. ▪ Requirements of input on the market of certain products. ▪ Transitional measures for adventitious or technically unavoidable presence of GM material which has benefited from a favorable risk evaluation. 	
Proposal for a Regulation COM/2002/0085 – COD 2002/0046 (entry into force 27/10/2002) The transboundary movement of GMOs	<ul style="list-style-type: none"> ▪ Establishment of a notifying system and exchanging information on the exports of GMO to third countries. ▪ No application for pharmaceuticals for human use. ▪ Surveillance, submission of reports, and imposition of sanctions for any infringement. 	

(Continued on next page)

Table 1 EU Directives and Regulations and US Acts (main points and comments) for GMOs (*Continued*)

Title	Main points	Comments
	US legislation	
Genetically Engineered Food Safety Act, 2003	<ul style="list-style-type: none"> ▪ Definitions (genetically engineered organism, genetically engineered material etc) ▪ Federal determination of safety of genetically engineered food, regulation as food additive ▪ Rulemaking, effective date, previously unregulated marketed additives 	
Genetically Engineered Crop and Animal Farmer Protection Act, 2003	<ul style="list-style-type: none"> ▪ Definitions (genetically engineered plant, genetically engineered animal, genetically engineered material etc.) ▪ Contract limitations regarding sale of genetically engineered seeds, plants, and animals ▪ Prohibition on labelling certain seeds as non-genetically engineered 	
Genetically Engineered Food Right to Know Act, 2003	<ul style="list-style-type: none"> ▪ Definitions (genetically engineered organism, genetically engineered material etc.) ▪ Requirements for labelling regarding genetically engineered material ▪ Misbranding of food with respect to genetically engineered material 	
Genetically Engineered Pharmaceutical and Industrial Crop Safety Act, 2003	<ul style="list-style-type: none"> ▪ A pharmaceutical crop or industrial crop is a plant that has been genetically engineered to produce a medical or industrial product, including a human or veterinary drug, biologic, industrial, research chemical, or enzyme. ▪ Definitions (genetically engineered plant, genetically engineered animal, genetically engineered material etc.) ▪ Report to Congress on alternative methods to produce pharmaceutical and industrial crops 	

Potential Effects on Human Health resulting from the use of Viral DNA in Plants

Most of the manipulated crops utilize the Cauliflower Mosaic Virus 35S promoter (CaMV35S) to switch on the introduced gene. There has been a lot of controversy concerning whether the highly infectious CaMV35S can be horizontally transferred and cause disease, carcinogenesis, mutagenesis, reactivation of dormant viruses and even generation of new viruses (Hodgson, 2000). According to Ho et al. (2000), CaMV found in normal foods is not highly-infectious and cannot be absorbed by mammals. In contrast others believe that although humans have been ingesting CaMV and its 35 s promoter at high levels it has never been shown to cause disease in humans or to recombine with human viruses (Paparini and Romano-Spica, 2004). The transient expression in mammalian cells of transgenes transcribed from the CaMV35S promoter reported by Tepfer et al. (2004) raised the possibility that genes controlled by the 35S promoter have the potential for expression in animals. On the contrary, in recent studies Paparini and Romano-Spica (2006) failed to detect DNA transfer in mice and CaMV35S transcriptional activity with real time polymerase chain reaction (PCR), although they do emphasize the need for further studies.

Possible Transfer of Antibiotic Resistant Genes to Bacteria in the Gastrointestinal Tract

An area of concern focuses on the possibility that antibiotic resistance genes used as markers in transgenic crops may

be horizontally transferred to pathogenic gut bacteria, thereby reducing the effectiveness of antimicrobial therapy. Although this probability is considered to be low (Halford and Shewry, 2000) other marker genes, such as the jellyfish green fluorescent protein (GFP) gene have been utilized. The only study assessing toxicity and allergenicity of GFP in male rats for 26 d, concluded that GFP exhibits a low allergenicity risk (Richards et al., 2003). It should be emphasized that only one transgenic plant (canola) containing GFP has been tested for toxicity. Every transgenic organism containing a new marker gene should be tested for toxicity with long term studies, since GM food will be consumed for a life time.

Possible Absorption of Genes Introduced in a GM Plant from the Gut

One concern associated with GM foods is the possibility that genes introduced into the plant might be taken up by the gut and become incorporated into the genetic make-up of consumers. In recent studies, Jennings et al. (2003 and 2003b) failed to detect fragments of the glyphosate resistant in a variety of tissue samples from pigs, fed glyphosate-tolerant soybeans and of transgenic and endogenous plant DNA in the chicken breast muscle. These findings are in contrast with those of Schubert et al. (1994), who reported that orally administered naked M13 phage DNA was detected in the mice blood. Moreover, short DNA fragments of GM plants have been detected in white blood cells and in milk of cows and in chicken and mice tissues that had been fed GM corn and soybean, respectively (Beever and

Kemp, 2000; Einspainer et al., 2001; Hohlweg and Doerfler, 2001; Phipps and Beever, 2001). Furthermore, fragments of recombinant cry1Ab gene were detected in the gastrointestinal tract of *Bacillus thuringiensis* (Bt)11 corn-fed pigs but not in the blood (Chowdhury et al., 2003). Therefore, it seems plausible that small amounts of ingested DNA are not broken down under physiological digestive processes. The fact that fragments of transgenic genes may not be detected in blood but can be detected in tissues of animals by PCR, underlies that they are in quite low levels in circulation and more sensitive methods of detection are needed (Puztai 2001). Moreover, Murray and his coworkers (2007) showed that not all PCR assays can detect DNA in extractions of shortly cooked corn, making the interpretation of the results from PCR even more difficult. These limitations in the detection of GM DNA should make us reconsider the view that gene transfer cannot occur, which falls in agreement with the findings of Netherwood et al. (2004) that transgene from GM soya survived passage through the small bowel in human ileostomists. According to Flachowsky (2005) the uptake of GM DNA into cells of the gastrointestinal tract will normally have no biological consequences because the DNA will be degraded in the cell. The question is whether it can be degraded in patients with severe gastrointestinal diseases. In the unlikely event that the DNA is recombined into a host chromosome, the probability that it will exert any biological effect on that cell remains unknown.

Possible Effects of GM Foods on Allergic Responses

The introduction of novel proteins into foods such as a GM soybean variety expressing methionine from Brazil nut (Nordlee et al., 1996) and GE corn variety modified to produce a Bt endotoxin, Cry9C (Bernstein et al., 2003) may elicit potentially harmful immunological responses, including allergic hypersensitivity (Conner et al., 2003; Taylor and Hefle, 2002). Moreover, according to Prescott et al. (2005) the introduction of a gene expressing nonallergenic protein such as GM field pea, expressing alpha-amylase inhibitor-1, may not always result in a product without allergenicity. This study underlines the need to evaluate new GM crops on a case-to-case basis and to improve the screening requirements for GM plants.

Brassica juncea, another GM plant, expressing choline oxidase gene caused low IgE response in mice and a cross-reactive epitope search showed a stretch similar to Hev b 6 having some antigenic properties although according to Singh et al. (2006) it had no allergenicity. These findings should be more carefully interpreted and repeated in other animal series in order to elucidate whether IgE response may play a role in toxicity.

As for Bt expressed in many crops, farm workers exposed to Bt pesticide may develop skin sensitization and IgG antibodies to the Bt spore extraction (Bernstein et al., 2003). "Antifreeze" protein which is produced through GM yeast, expressing a protein derived from fish is being considered for use in foods such as ice creams. Bearing in mind that allergy to fish is well estab-

lished, a potential risk from such proteins to susceptible human beings exists although the only clinical study investigating this potential has shown that it does not possess allergenicity (Crevel et al., 2007).

Allergenicity Assessment

To evaluate allergenicity of GM foods the decision tree approach was developed in 1996 (Metcalf et al., 1996) has been revised (FAO/WHO, 2001, Metcalfe, 2003). Risk assessment of the whole GM plant must consider whether allergenicity or toxicity of the crop could be increased. This is particularly important when the non-GM host plant is known as allergen or toxin source. Toxicity testing most often includes a 90-day toxicity study in rodents; allergenicity testing is done by comparison of the allergen repertoire of the GM crop with that of the conventional non-GM variety. Another aspect that is of concern when considering the extrapolation of the whole GM crop or food/feed toxicology and allergenicity studies carried out with single GM events to the GM stacked event, are the potential interactions of the newly introduced genes, regulatory sequences, and proteins (or its metabolites) with the host genome of the GM stacked event. Given that the transgenic DNA sequences/proteins are brought into a different genetic background, namely the stacked genetic background, their interaction with the genome might change, particularly if regulatory proteins, such as in experimental stress-resistant crops described in literature, are involved (De Schrijver et al., 2007).

Criticism on this approach includes the limited predictive ability of the amino acid sequence analysis for sequence similarity to known allergens (Alinorm, 2003; Prescott and Hogan, 2005). In vitro assessing degradability has also been questioned whether it can be correlated with allergenicity (Bannon et al., 2003) and instead Puztai et al. (2003) proposed its replacement with in vivo (animal/human) testing. It has been emphasized that animal models used to assess the potential allergenicity of GM foods need to be validated. Studies with animals such as BALB/c mouse, HLA transgenic mouse, swine and atopic dog have shown that no single model can meet the requirements for an ideal model covering both the respiratory allergens as well as the gastrointestinal and dermatologic reactions (Tryphonas et al., 2003). Moreover, the model's ability to sensitize or alter endogenous protein expression may not be readily captured due to genetic differences across species (Germolec et al., 2003).

The questions in the area of human clinical data for the evaluation of protein allergenicity of GM foods have been discussed in detail (Germolec et al., 2003). Issues concerning human studies in individuals not only with an allergy history but with immunodeficiency problems as well should be included in a future discussion of the problem.

It has also been suggested that the oral consumption of a certain GM plant expressing a known allergen can help allergic individuals, since in rats GM lupine stimulates the development of a protective regulatory T-cell response and suppresses the development of allergic airways disease (Prescott and Hogan,

2005). One should consider whether this protective mechanism is stimulated in allergic immunodeficient patients. Moreover, it is not known whether the expression of an allergic reaction plays a protective role against other diseases that might have been caused by the exposure to this allergen.

POSSIBLE EFFECTS OF GM FOODS IN ANIMALS

Only recently a body of evidence is starting to emerge from a small number of animal feeding trials into the health effects. Ewen and Pusztai (1999) were the first to demonstrate the need to thoroughly test each GM plant product on animal models. The effects of most GM foods in animals are reviewed and include also the reanalysis of the controversial data reported by Monsanto's 90-Day feeding study on GM corn Mon863 (Seralini et al., 2007). As Varzakas et al. (2007) state, Member states should carefully scrutinize all applications, because companies try to hide information about the health impacts of GM. Although long-term feeding of high levels of individual "foods" to animals can result in nutritional imbalance (Varzakas et al., 2007) it should be stated that this is the only way that any substance can reveal its toxicity.

Effects on Growth

Body weight might be significantly altered as it has been shown with the consumption of Mon863 corn (Seralini et al., 2007) and GM rice on rats (Li et al., 2004).

Effects on the Gastrointestinal Tract

Stomach erosion and necrosis were reported in rats fed with flavr-savr™ GM tomatoes, while GM potatoes expressing *Galanthus nivalis* (GNA) lectin induced proliferative growth in their stomach which is of particular importance if one takes into consideration that glomerular stomach erosions can lead to life-threatening hemorrhage, especially in the elderly and patients on nonsteroidal anti-inflammatory agents (Pusztai et al., 2003). Intestines may also be affected by GM food consumption as it has already been shown with GM potatoes expressing Bt-toxin which caused the disruption, multinucleation, swelling, and increased degradation of ileal surface cells in rats (Fares and El-Sayed, 1998), GM potatoes expressing *gna* which induced proliferative growth in the small-large intestines (Ewen and Pusztai, 1999a) and GM soybean type Roundup Ready® which caused moderate inflammation in the distal intestine of salmon (Bakke-McKellep et al. 2007).

Recent work with gene transfer research has resulted in the production of the aquatic species with enhanced abilities in areas such as growth, cold tolerance, disease resistance, and metabolism of plant-based diets. Research with transgenic GHs has made the most progress, with the patented production of a

line of Atlantic salmon capable of increased growth and feed conversion efficiency. This product has been licensed to a major biotechnology company and is currently awaiting regulatory approval for commercial use in the United States and Canada. Although transgenic research with invertebrates is far behind that for vertebrates, there is much potential for generic improvements among commercial bivalve species. Recent advances include development of successful, patented gene transfer methods, and research into boosting disease resistance. Despite the potential for GMOs in aquaculture, a number of environmental and human health concerns remain. Major concerns include escapement of transgenic fish into the wild, where they could disrupt natural gene pools through breeding with wild species, and the possible detrimental effects of introducing transgenics into the human and aquatic food chains (Rasmussen and Morrissey, 2007).

Binding to surface carbohydrates of the mouse jejunum was also revealed with Cry1Ac protoxin of the Cry genes, the most common terminators applied in currently approved crops (Vazquez-Padron et al., 2000). According to Pusztai et al. (2003) since it is the genetic manipulation process itself which led to toxicity, similar hazards might be seen in animals or humans fed genetically-manipulated soya, canola, and corn over a long period of time (i.e., years or decades). The chronic inflammation and proliferative effect that may be caused by some GM plants on the gastrointestinal tract may lead after years to cancer.

As for the effects of GM food on liver there are only a few long-term studies. It has been found that GM soya can alter the cell structure and functioning of the liver in mice reversibly (Malatesta et al., 2002; 2003; 2005) and can cause changes in histomorphology (Ostaszewska et al., 2005) and the protein profile of the liver in rainbow trout (Martin et al., 2003). Alterations have also been observed in hepatic enzymes after consumption of raw rice expressing GNA lectin (Poulsen et al., 2007), GM Bt with vegetative insecticidal protein gene (Peng et al., 2007) and in DuPont's subchronic feeding study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007). These alterations in hepatocyte cells and enzymes may be indicative of hepatocellular damage. Consumption of Mon863 corn in rats led to increase in triglycerides in females (Seralini et al., 2007).

Pancreatic Effects

GM soybean has also an impact on pancreas, since changes occurred in pancreatic acinar cells of mice and a high synthetic rate of zymogen granules containing low amounts of α -amylase (Malatesta et al., 2003).

Another target organ of some GM crops is the kidney. Smaller kidneys were developed in DuPont's study in rats fed diets containing GM corn 1507 (MacKenzie et al., 2007), whereas consumption of Mon863 corn in rats led to lower urine phosphorus and sodium excretion in male rats. There were also small increases in focal inflammation and tubular degenerative changes

characteristic of a classic chronic progressive nephropathy (Seralini et al., 2007). Rats fed GNA rice had elevated creatinine plasma concentration either due to some kind of renal effect or the increased water consumption in order to excrete the excess iron in the GNA rice diet (Poulsen et al., 2007). Salmon fed GM soybean had higher head kidney lysozyme and higher acid phosphatase activities (Bakke-McKellep et al., 2007).

Alterations in Hematology

Response variables were observed in animals fed with GM crops. DuPont's study in rats fed diets containing GM corn 1507 showed a decrease in red blood cell count and hematocrit of females (MacKenzie et al., 2007) while GM corn Mon863 affected the development of blood with fewer immature red blood cells (reticulocytes) and changes in blood chemistry in rats (Seralini et al., 2007). Bt with VIP insecticidal protein gene caused a decrease in platelets, monocytes ratio in female rats, and an increase in the granulocytes ratio in male rats (Peng et al., 2007).

As for the effects of GM crops on the immune system an increase in the production of Cry9C-specific IgG and IgG1 in rats and mice fed with GM heat-treated corn CBH351 was observed (Teshima et al., 2002) because the Cry gene possesses immunogenic properties as it was shown by Vazquez-Padron et al. (1999). Serum IgG mediates the inhibition of serum-facilitated allergen presentation. The presence of enhanced IgG Abs activates the IgG response (van Neerven et al., 1999) thereby indicating the occurrence of an allergic reaction having occurred, although Germolec et al. (2003) suggest that antigen specific IgG does not correlate to clinical allergy.

Moreover, GM corn Mon863 caused higher white blood cell levels in male rats (Seralini et al., 2007). DuPont's subchronic feeding study in rats fed diets containing GM corn 1507 showed that eosinophils concentration in females was decreased (MacKenzie et al., 2007). Rats given a diet based on GNA rice showed enlargement of the lymph nodes, and decreased weight of the mesenteric and of the female adrenal lymph nodes which may be indicative of an immune toxic response (Poulsen et al., 2007).

Effects on Biochemical Parameters

Subchronic feeding of GNA rice in rats resulted in decrease in glucose, while cholesterol, triglyceride, and HDLD concentration were higher (Poulsen et al., 2007).

Mortality

An increased mortality was observed in rats fed with GM tomatoes since seven out of forty rats died within two weeks without any explanation (Pusztai et al., 2003).

Reproductive and Developmental Toxicity

Of particular concern is the exposure of infants and children to GM foods because of their possible enhanced susceptibility for untoward effects. Only a limited number of studies regarding this topic are available, quite a few studies concerning this subject exist. Food-ingested M13 DNA fed to pregnant mice, was detected in various organs of fetuses and newborn animals, suggesting a possible transfer through the transplacental route (Doerfler and Schubert, 1998). Maternally ingested foreign DNA could be a potential mutagen for the developing fetus.

Birthrates of piglets fed GM corn in Iowa country displayed an 80% fall due to high levels of Fusarium mold (Strieber, 2002), although it has been claimed that Bt corn expressing Cry proteins is less contaminated with mycotoxins (Weil, 2005). A Russian rat study reported very high death rates in the young of rats fed GM soya (56% died) in stunted growth in the surviving progeny (Ermakova, 2005). A study of GM rice expressing Xa21 on the development of rat embryos showed that there was an increase in the body weight gain of pregnant rats, the body weight, body length, and tail length of fetal rats (Li et al., 2004) whereas GM rice expressing cowpea trypsin inhibitor caused an increase in the male rats' body length and in the female rats' red blood cell number, hemoglobin, and monocyte number (Zhuo et al., 2004). The fact that no adverse effects have been observed in a reproductive and developmental study of bar gene inserted into GM potato may be due to the very low content of GM potato in food, so that the undesired effects are masked (Rhee et al., 2005).

GM food should be assessed for unexpected health effects in a vulnerable population such as children since after the first year their consumption is inevitable.

Finally, the consumption of products from Bt insect resistant plants raised some controversy regarding the possible long term effects of Bt on health. Although Betz et al. (2000) state that it has been used for over 40 years without causing adverse effects, the difference with GM plants is that Bt is not degraded in the plant and as a result both animals and humans may be exposed to this toxin (Aronson and Shai 2001).

Genotoxicity

Safety assessment for GM sweet pepper and tomato conferring resistance to cucumber mosaic virus showed no genotoxicity in animals (Chen et al., 2003). The use of lyophilized instead of raw GM food in this study may alter the toxicity results since there may be structural differences.

Pusztai's discipline of using animals with an acceptable starting weight range should be adopted in order to evaluate the toxic effects (Alliance for BioIntegrity website 1998). The results of most studies with GM foods indicate that they may cause hepatic, pancreatic, and renal effects and may alter the hematological, biochemical, and immunologic parameters the significance of which remains to be solved with chronic toxicity studies.

Not only plants but animals as well have been genetically altered. The problems that may arise from the consumption of such products are also discussed.

EFFECTS OF INJECTED RECOMBINANT BOVINE GROWTH HORMONE (RBGH) IN ANIMALS

The use of rbGH in dairy cattle in order to increase milk yield has caused large controversy. Problems occurring such as an increase in mastitis may pose a risk to human health since the increased antibiotic use leads to antibiotic residues in milk (Epstein, 1996). Adverse effects in cows have been observed including lameness, mastitis, subclinical ketosis, an increase in embryonic loss and abortion, a decrease in final pregnancy rates, as well as a decrease in birth rate (Dohoo et al., 2003). It should be noted that lameness has also been reported in studies with transgenic pigs genetically engineered to carry human and bovine growth hormone genes (Pursel et al., 1989).

POSSIBLE RISKS FOR HUMAN HEALTH FROM THE USE OF MILK FROM COWS TREATED WITH RBGH

The consumption of milk from cows injected rbGH leads to an increase in IGF-I in humans, since IGF-1 survives digestion (Xian et al., 1995). The oral free IGF-1 feeding studies in rats sponsored by Monsanto and Elanco looked at by the Joint Expert Committee on Food Additives (JECFA) in 1992 had ambiguous results since neither used IGF-1 associated with its binding proteins, which are resistant to acidic conditions and may enable IGF-1 to survive digestion in the stomach. Moreover, IGF-1 is protected from digestion by the major milk protein casein (Hansen et al., 1997) and the milks buffering effect (Xian et al. 1995). Moreover, Monsanto's 90-day rat study which had previously shown that rbGH "is not orally active in rats" was re-examined and it was found that rbGH elicited a primary antigenic response meaning that rbGH was absorbed intact from the gut (Eppard et al., 1997). The full significance of human exposure to rbGH and IGF-1 is unknown, particularly in the neonate, the subpopulation at greatest risk (Morris, 1999). According to Chan (1998), at least some of the absorbed IGF-I can effectively stimulate the proliferation of cancer cells. The increased levels of IGF-I in humans predict increased rates in colon, breast, and prostate cancer, since they stimulate the indolent slowly growing tumor cells that appear in an aging individual resulting in clinical cancer necessarily old. On the other hand, FDA states that this potential does not exist since any increase of IGF-I in milk is much lower than the physiological amount produced in the organism. These concerns about the consumption of milk from cows injected rbGH may be carried also to other animals such as pigs expressing human GH, pigs injected recombinant porcine somatotropin (rpST), and GH transgenic salmon.

PIGS EXPRESSING HUMAN GROWTH HORMONE AND PIGS INJECTED RPST

Transgenic pigs expressing human GH showed dramatic effects in growth rates, feed conversion, and body composition, but exhibited serious side effects that were attributable to the high level of GH expression (Pursel et al., 1989). Repeated injections of rpST can also produce altered lipid composition similar to that of the GH transgenic pigs (Solomon et al., 1997).

GH TRANSGENIC FISH

Although the potential effect of feeding GM feed to poultry and cattle has been studied quite extensively (Einspanier et al., 2001; Hohlweg and Doerfler, 2001), there are only two available publications (Padgette et al., 1995; Hammond et al., 1996) in the case of fish feed. In both publications the effect of using GM ingredients in catfish feed, in terms of final fish weight and other physiognomic parameters, was investigated. Their conclusions were similar since the feeding values of GM soybeans and conventional soybeans were not found to be different. A more recent publication (Sanden et al., 2004) was focused both on: i) the fate of selected GM soy DNA fragments from feed to fish and on their survival through the fish gastrointestinal (GI) tract and ii) whether the DNA could be traced in a variety of fish tissues. Fish were fed in three experimental diets for six weeks, which were formulated from defined components and represented either GM or non-GM materials (17.2% of the fish meal was replaced with either GM or non-GM soy). A control diet composed of fish meal as the only protein source was used for comparison purposes. The transgenic sequences (120 and 195 bp) and the lectin gene (180 bp) could be detected in the GM soy feed. In the fish GI tract, however, only the smaller DNA fragment (120 bp) could be amplified from the content of the stomach, pyloric region, mid-intestine, and distal intestine. No transgenic or conventional soy DNA fragments could be detected in liver, muscle, or brain tissues dissected from sacrificed fish. The sensitivity limit of the method was evaluated to be 20 copies. Their data indicated that though GM soy transgenic sequences may survive passage through the GI tract, they could not be traced in fish tissues (Exadactylos and Arvanitoyannis, 2006).

However, when the fish growth hormone (GM) gene is introduced in salmon may GH circulation may elevate by 40-fold, leading to enlarged skulls and impair feeding and respiration (Dunham and Devlin, 1999). Experiments should be conducted in animals being fed GH transgenic salmon and other fish in order to examine whether the consumption of GH transgenic fish expressing high levels of GH will increase the levels of IGF-I and lead to the same health risks as rbGH milk. It should be emphasized that as in milk there is a possibility that the presence of other proteins in the fish tissue may protect IGF-1 from digestion, which remains to be demonstrated in animal studies.

Table 2 Comparison of values relevant to GE crops and foods among EU, Japan, Canada, and the USA (Arvanitoyannis, 2006)

Values	Importance of food safety	Environmental consciousness	Approach to science and technology	Attitude towards risk technology	Attitude towards food supply and trade
EU	Highly important but occurrence of diseases and contamination undermined the public trust	Very strong	Cautious	Medium	Strong but heavily opposed by environmental awareness
Japan	Highly important and public supports regulatory agencies' actions	Very strong	Innovative	Medium	Strong and linked with environmental awareness
Canada	Highly important and public encourages regulatory agencies' actions	Strong	Positive	Strong	Strong but mitigated by environmental awareness
USA	Highly important and public favours regulatory agencies' actions	Moderate	Enthusiastic	Very strong	Strong

GM PIG

The experiment of Saeki et al. (2004) with pigs containing spinach desaturase gene which converts saturated fat into the unsaturated fat linoleic acid resulted in a high degree of mortality in founders and the F₁ generation. Increased mortality might have been due to a random integration process where the transgene can insert in and damage any active gene locus (insertional mutagenesis) or to the significant alteration in the embryonic lipid profile caused by the transgene. The porcine embryo is unique in its high intracellular lipid content, which is associated with its sensitivity against freezing or in vitro production (Niemann and Rath, 2001). We strongly believe that the same toxicity could occur if the pregnant pigs were fed only the new source of g-linolenic acid obtained from transgenic canola or of any future modified crop, since it alters the percentage of 18:2n-6 in liver (Palombo et al., 2000). We should be aware that any change in the lipid profile of liver can also result in changes in metabolism with unexpected consequences.

ETHICS

The lasting sceptical and/or ambivalent attitude of Europeans towards agro-food biotechnology and the continued controversies about the commercialization of transgenic agro-food products are illustrative of an ongoing legitimacy crisis. One could even interpret the stigma on agro-food biotechnology and its products as testifying to a "robust" societal disapproval: it signals a lack of trust in scientific institutions and expert systems, and voices a social response against the reduction of the complexity of the GMO issue to a solely scientific risk-based problem. Hence, a move from a merely scientific evaluation towards a socially more robust one—that addresses precaution and socio-ethical issues in a more "sensible" way, whilst making "sense" of the different stances taken in the GMO debate—is still sought after. It will be interesting to see whether new controversies show (triggered, for example, by GMO contaminations or traces of unapproved transgenic events in nontransgenic produces), how these will be communicated and developed in the societal climate, and how they will be interpreted and tackled by, and/or lead to new adjustments in the now running legal system (Devos et al., 2007). The comparison of values relevant to GE crops

and foods among EU, Japan, Canada, and the USA is given in Table 2.

CONCLUSIONS

From the review of the toxicity studies concerning GM foods one might see that although toxicity can be assessed, the duration of exposure is too short in order to fully evaluate any potential disruptions in biochemical parameters and to evidence possible signs of pathology within the limited subchronic exposure of animals. Moreover, a larger number of animals should be used in the toxicity tests. The toxicity tests should comply with the guidelines for toxicity testing of drugs. It should be emphasized that since these GM foods are going to be consumed by every human being they should be tested even more thoroughly than drugs and more experiments are required in order to study the possible toxicity and make any conclusions. Tests to determine how a GM food affects mutagenesis and carcinogenesis should be conducted as well. Finally, postmarketing surveillance should be part of the overall safety strategy for allergies, especially of high-risk groups such as infants and individuals in "atopic" families. Evaluation of protein allergenicity in man should also include studies in individuals not only with a history of allergy but with immunodeficiency as well. The use of recombinant GH in animals, such as cows or the expression of GH in animals such as salmon should be re-examined since it may promote cancer. The results of most of the rather few studies conducted with GM foods indicate that they may cause hepatic, pancreatic, renal, and reproductive effects and may alter hematological, biochemical, and immunologic parameters the significance of which remains unknown. The above results indicate that many GM food have some common toxic effects. Therefore, further studies should be conducted in order to elucidate the mechanism dominating this action. Small amounts of ingested DNA may not be broken down under digestive processes and there is a possibility that this DNA may either enter the bloodstream or be excreted, especially in individuals with abnormal digestion as a result of chronic gastrointestinal disease or with immunodeficiency.

Although intensive scientific effort is currently in progress to thoroughly understand and forecast possible consequences on humans, animals, and the environment, it is anticipated that

many years of careful, independent research with animals and clinical trials will be needed in order to accomplish this assessment.

ABBREVIATIONS

Bt	Bacillus thuringiensis
CaMV	Cauliflower Mosaic Virus
FAO	Food and Agriculture Organization of the United Nations
GFP	Green fluorescent protein
GM	Genetically modified
GNA	Galanthus nivalis
rGH	Recombinant growth hormone
WHO	World Health Organization

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