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Benefits and concerns associated with biotechnology-derived foods: Can additional research reduce children health risks?

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Abstract. – The development of techniques devised for the genetic manipulation of foods poses new risks for children with food allergy (FA). The introduction of foreign allergenic proteins from different foods into previously tolerated foods may trigger allergic reactions, often complicating with anaphylactic shock in a subset of allergic babies. Children with FA, even if subjected to preventative diets, always challenge the risk of developing allergic manifestations after unintentional intake of a non tolerated food in restaurant settings, with relatives or schoolmates, etc, where product labelling is necessarily lacking. The introduction of potentially allergenic proteins into foods generally considered safe for allergic children can be done deliberately, by either substantially altering the food ingredients, or by genetic manipulation which change the composition or transfer allergens, or unintentionally by qualitycontrol failures, due to contaminations in the production process, or to genetic mismanipulation. There is a controversy between multinationals often favored by governments and consumer association resistance, thus an equidistant analysis poses some unprecedented impediments. The importance of FA and the potential of transgenic plants to bring food allergens into the food supply should not be disregarded. The expression in soybeans of a Brazil nut protein resulted in a food allergen ex-pressed in widely used infant formulas, so paving the way to an often reported multinational debacle. Genetic engineering poses innovative ethical and social concerns, as well as serious challenges to the environment, human health, animal welfare, and the future of agriculture. In this paper will be emphasized practical concepts more crucial for pediatricians.

Key Words:

Transgenic foods, Genetic manipulations, Food allergy, Frankenstein food, Multinational companies, Consumers' apprehension, Romulus and Remus.

Introduction

Transgenic foods have gained in 1999 a world role when Doctor Pusztai, referring to his experiments on genetically modified (GM) potatoes, has defined them, without formality, "Frankenstein food". For this reason, Doctor Pusztai was stripped of his post, publicly humiliated as a person whose mind was badly confused, and openly accused of being incapable to carry out well designed works. His fate was similar to that of the JAMA Editor[1-3]. Food allergies are caused by abnormal immunological responses to substances in foods, usually naturally occurring proteins. Allergic reactions can be manifested by symptoms ranging from mild cutaneous or gastrointestinal problems to life-threatening anaphylactic shock reactions. Virtually all food allergens are proteins, but only a small fraction of the many proteins found in foods are allergenic. Since genetic modification results in the introduction of a segment of DNA containing one or more genes from one organism into a chromosome of another organism, the potential allergenicity of the newly introduced protein (NIP) should be a major component of the safety assessment process. An assessment of its allergenicity can be accomplished by evaluating the source of the gene, the NIP sequence homology to known allergens, the NIP immunochemical reactivity with immunoglobulin E (IgE) antibodies from the blood serum of individuals with known allergies to the source from which the genetic material was obtained, and the NIP physicochemical properties. The importance of FA and the potential of transgenic plants to bring food allergens into the food supply should not be minimized. Clearly, the determination of allergenicity of transgenic proteins by analogy to other food allergens is inadequate, and that tests must be developed that in-

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Table I. "Transgenic" modified by genetic engineering techniques.

Introduced protein	Crop products and targets
ACC deaminase , antisense PG, antisense ACC synthase	Delays without impairing the tomatonatural ripening and softening, to obtain a more concentrated juice
Phosphinothricin acetyltransferase	Renders corn tolerant to herbicides
Neomycin phosphotransferase II	Protects from insects potato and delays tomato natural ripening and softening
Glyphosate oxidoreductase	Renders corn tolerant to herbicides
Btt-HD1 insecticidal protein	Protects from insects corn and tomato
Btt-HD 73 insecticidal protein	Protects from insects potato
CP4 EPSPS synthetasr	Renders canola, corn, cotton, soy and sugarbeet tolerant to herbicides
β-D-glucuronidase	Renders soy tolerant to herbicides

Adapted from references[8,9]. Abbreviations: ACC = 1-amino-1-cyclopropane-carboxylic acid, Btt = *Bacillus thuringiensis subsp.tenebrionis*, Btk = *Bacillus thuringiensis subsp. kurstaki*, from strains HD-1, CP4 EPSPS = 5-enolpyruvylshikiimate-3-phosphate syn-thase from Agrobacterium strain CP4, PG = poligalatturonas. Notes: Recently there were controversies regarding transgenic soy and corn, some types of modified maize have been prohibited in Austria, France, Greece, Luxembourg. Denmark has interrupted both farming and selling of such crops, in response to public opinion and in the United Kingdom the cultivations were limited for 3 years, but large supermakets were forced to stop socking GMFs. However, the UE has authorized the import and selling of some varieties of transgenic soy and maize, experimentally produced in US and Canada. The Italian government, resuming two laws issued from UE on 16/2/1996 and 2/6/1998, has prohibited the use of such foods for the infantile alimentation (Decr PR 7/4/1999)[10]. Adapted from FoE Groups and Biotech Campaigning. Link 2000; 93: 21-23.

volve the interaction of the transgenic protein in question with the immune system. Given the extensive recent increases in our knowledge of this important system[4-6], the development of such tests would appear to be well within the capabilities of the scientific community. Multinational companies producers of GM organisms (GMO) have gained a world fame.

The Pros and Cons: Which Prevail?

For the first time in history, human beings are becoming the architects of life. The variety of traits introduced into crops is astonishing, including insect protection, delayed ripening, herbicide tolerance, modified oils, disease resistance and genetically altered foods. GMOs pay for a kind of "original sin": the allergenicity increase, such as the introduction of allergens from different sources via genetic manipulations. Such an approach was used recently to assess the possible allergenicity of a transgenic soybean with an inserted gene from Brazil nuts that expressed a high-methionine protein. Brazil nuts are known to be allergenic, and it was demonstrated that the high-methionine protein was indeed a major allergen from Brazil nuts. As a result of this assessment, commercial interest in this transgenic soybean variety was abandoned[7]. However, we stress that such experiments in the hands of not experts may pave the way to new mishaps.

Table I[8-10] shows the plant species transferred by genetic engineering or GMO, and Figure 1[8-10] those cultivated in the US and imported in Europe. We understand that such plants are geneticall-ly manipulated to obtain products with prolonged average life and better aspect and taste; however allergic patients can run the risk of anaphylaxis due to the introduction of new allergens even into wholly common foods?

With the development of techniques for genetic manipulations surprising results can be obtained, such as transfer into rice strains vitamin A

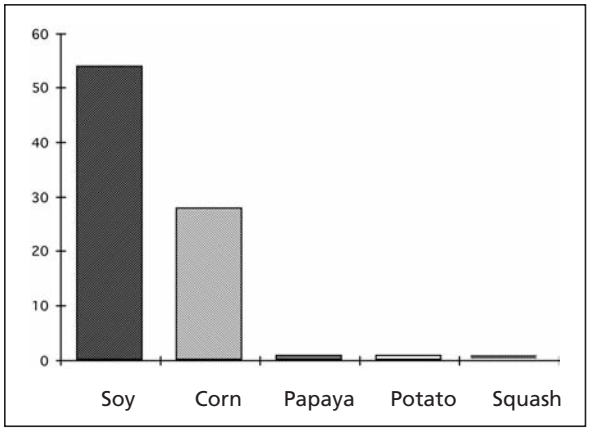

Figure 1. Frankenstein foods cultivated in the US and imported in Europe. Tomato is not included. From references[8-10].

present instead in the sleeves, to combat malnutrition[11], but also rice allergy in the Chinese and Japanese populations, eating a high daily quantity[12,13], not to mention the transgenic fishes deriving from monosexualization or doubling maternal DNA[14]. Similarly, the introduction of peanut genes into tomatoes and of fish proteins into potatoes, to enable storage of the vegetable below 00C, may cause serious anaphylactic reactions in children allergic to these foods[15,16]. Not only the toxin *Bacillus thuringiensis* (Bt) can kill the monarch butterfly, but also Bt toxin can bind to soil particles and persist in the soil for over 200 days, harming soil health. However, testing the effect of Bt insecticidal preparations on a number of human cell types denotes that spore-containing Bt products have an inherent capacity to lyse human cells in free and interactive forms and may also act as immune sensitizers[17].

Several methods exist to manipulate FA, but one side is to achieve a selection of strains with reduced allergenic content, and the other is to reduce the allergenic content by changing the relative ratio of the normal constituents of a food. However there is a great need for standardization of the methods employed for testing potential allergens, but such controlled program to assess allergenicity in manipulated foods should be settled within an international framework[15]. The greater problem is the high number of foods potentially interested by genetic engineering (Table II)[9] and the first place of France and the second of Italy among the European countries, regarding the number of fields where transgenic cultivations are experimented (Figure 2)[18].

Table II. Additional foods transformed with techniques for genetic manipulations.

Apricot	Melon
Asparagus	Mustard
Barley	Oats
Bilberry	Oil Seed Rape
Black currant	Orange
Broccoli	Papaya
Buckwheat	Pea
Cabbage	Peach
Carrot	Plum
Cauliflower	Potato
Celery	Raspberry
Chicory	Rice
Colza	Rye
Corn	Soybean
Eggplant	Strawberry
Fennel	Sugarbeet
Grape	Sweet potato
Horseradish	Tomato
Kiwi	Walnut
Lemon	Wheat
Lettuce	
Licorice	
Lotus	

Modified from reference[9].

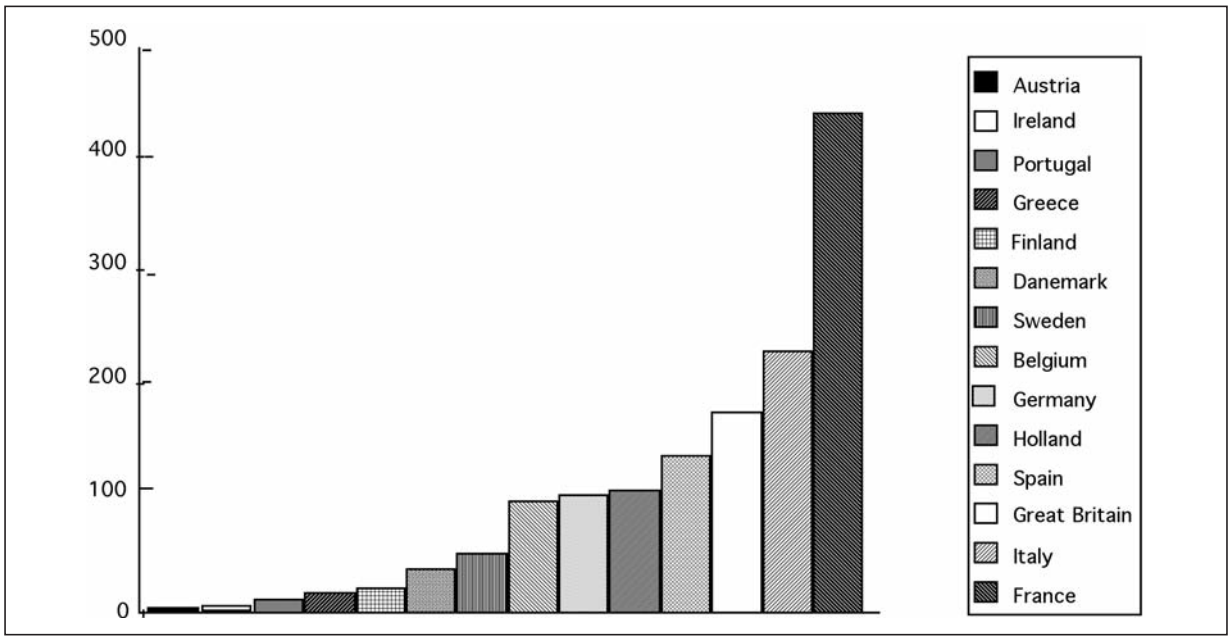

Figure 2. Number of fields where transgenic cultivations are experimented. From NIH data.

A recent debate on GMOs has triggered controversial, but not unfounded discussions. The European Union (EU) has decided that all GMOs totally or partly introduced into marked foods should be detailed on the label, but excluding such a guarantee for the consumers when the GMO level is ≤ 1%. A pediatrician can easily maintain that the core of the problem does not regard the 1% present in foods, but the missing prerequisite of precisely clarifying it on the label stating that they have been genetically engineered. A very strange procedure: who shall evaluate or check the exactness of this 1%? Who has so sophisticated weighing-machines to precisely measure 1%?, or has money enough to buy such precision instruments? We all remember that a similar exception was provided for chocolate bars containing instead of the usual cocoa butter, one deriving from inferior vegetable oils, hence denaturing the habitual taste. We have demonstrated that only one drop of cow's milk (CM) can trigger an anaphylactic shock in a baby[19]. When the hydrolysate formulas (HFs) were first commercialized, certain studies have claimed that reactions to HFs were a rare occurrence, however we have documented in two papers that HFs have provoked about 240 reactions until anaphylaxis, probably because of minimal traces of β-lactoglobulin, one of the most immunogenic CM proteins[20,21]. A drop is a big dose, the sensitizing substances are measured in μg, one μg is = 0,000001 g[22]. The Monsanto supporters claim that GMOs provoke no harm. Often the truth has been found only after several years, as it was the case of DDT, of thalidomide, of HFs: nobody could have foreseen such consequences.

An improper FDA directive has established that GMO marketing can be authorized on the basis of their "substantial" equivalence to foods, or to natural products, since the agency does not require that GMO be safety tested before it is marketed, thus without scheduling exhaustive verifications of its safety. Here we make a first remark: what does this neologism "substantial" mean in this setting? Is there an association with the 1%? However, "substantial" does not mean entirely equal to the original, thus if the product is only "substantially" equivalent to the natural food, this means that it is a different product, offering no guarantee. We return to the ambiguity of the term "hypoallergenic": in either case one falls into the error of a false security[20].

Diagnosis

Diagnosis is the appropriate means to ascertain whether parts of GMOs are present, with possible noxious implications for children's health. Thus is the more delicate moment, but also the more critical. Thereby, several organizations have tried to protect the consumers and to comply with the desiderata of the public opinion. In particular the Food and Agriculture Organization (FAO) of the United Nations has suggested new labelling procedures of foods of potentially allergenic nature[23]. However, their recommendations resulted even more liberal than those above mentioned of the EU, proposing that GMO present in concentrations less than 5% to 25% of the food needed not to be declared[23]. Consequently, HFs should be "exempted", whereas very sensitive children may react to even low amounts of residual epitopes in these HFs[20,21]. Therefore, new guidelines specific for GMOs have been selected. There it is stated that "the transfer of genes from commonly allergenic foods should be discouraged unless it can be documented that the transferred gene does encode the pertinent allergen[24]. Basically, three categories of GM crops can be considered:

1. GM crops which have the same composition as the parent crop,
2. GM crops which have the same composition as the parent crop with the exception of a well-defined trait,
3. GM crops which are different from the parent crop[24].

However, such guidelines denote a more marked interest for both allergens and databases than for GMOs and it is significant that other authors found it beneficial to compile lists of allergens[8], more useful for immunology textbooks.

Allergenic Sources of Genes

As it has been clearly indicated[15], if a gene transferred in one or more foods is obtained from a source commonly known for its allergenicity, data should establish that the gene does encode the allergen in question. Figure 3[15,16], above all critical for the identification and labelling of these foods, shows all the necessary approaches, including tests of proven validity:

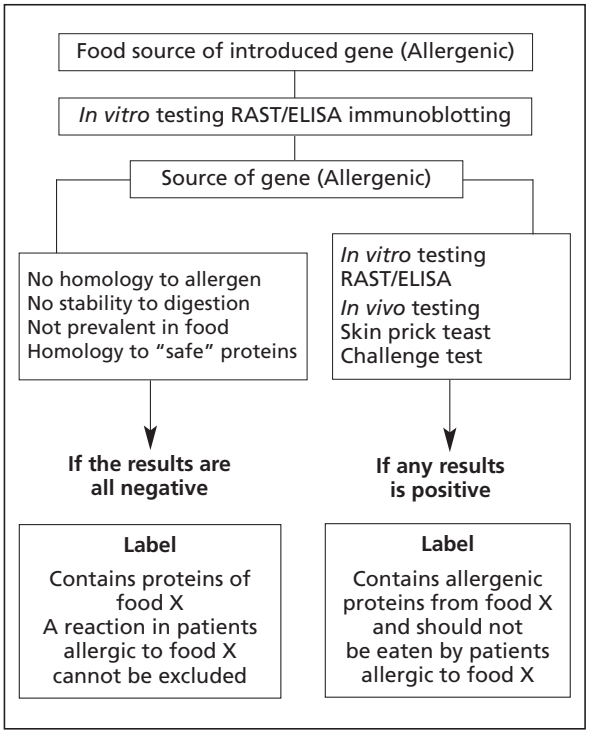

Figure 3. Allergenicity assessment of potential transgenic foods. Flow chart for investigation of genetically modified foods for potential allergenicity before their release on market, with suggestions about labelling of the pertinent foods. Modified from reference 15,16.

1. *In vitro* or first level tests, RAST (radioallergosorbent test)[25], ELISA (enzyme-linked immunosorbent assay)[26] and immunoblotting[27],
2. *In vivo* or second level tests, SPTs (skin prick tests)[25] and DBPCFC (double-blind, placebo-controlled food challenge)[25,28].

The diagnostic protocol shall conclude for the complete negativity if it can be established that the gene transferred did not encode, as previously alluded to, any foreign allergen and the product could be freely marketed, even if we cannot exclude reactions in children allergic to that food[16]. Instead, if whatever analysis results positive. the label should specify that the product contains allergenic proteins from the food under examination and should therefore not be eaten by children allergic to that food.

To assure a correct execution of the procedure, it is crucial that[16]:

1. Any GMO should be investigated following the method shown in Figure 3 before becoming commercially available,
2. Any test should be performed in independent laboratories (for example from multinationals) of recognized standard having access to sufficient numbers of patients previous diagnosed as allergic to the food(s) in question ("reference laboratories"),
3. Patients should be diagnosed with testing strictly adhering to EAACI guidelines[29].

Additional techniques, including studies on animal models and systems suited for determination of DNA or nRNA in foods may soon be developed and subjected to adequate procedures and may be included in the standard diagnostic program after sufficient testing[16]. Since 1996 it was requested to safety test on animal models bioactive proteins produced by transgenic organisms before adding them into formulas for infants[30].

Taylor et al suggest a somewhat different procedure[31]:

- The combination of tests involving allergic human subjects or blood serum from such subjects should provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small rate of the population allergic to the source material.
- Any positive results obtained in tests involving allergic human subjects or their blood serum as before would provide a high level of confidence that the novel protein was a potential allergen. Foods containing such novel proteins would need to be labelled to protect allergic children.
- A novel protein with either no sequence similarity to known allergens or derived from a less commonly allergenic source with no evidence of binding to IgE from the blood serum of a few allergic individuals ($n < 5$) but that is stable to digestion and processing should be considered a potential allergen. Further assessment would be necessary to address this uncertainty. The nature of the tests would be determined on a case-by-case basis.
- A novel protein with no sequence similarity to known allergens and that was not stable to digestion and processing would have no evidence of allergenicity. Similarly, a novel protein expressed by a gene obtained from a less commonly allergenic source and demon-

strated to have no binding with IgE from the blood serum of a small number of allergic individuals (n = >5 but <14) provides no evidence of allergenicity[31].

Nonallergenic Sources of Genes

We follow a clear assessment[15] to verify the nonallergenic sources of candidate genes: as yet there exists no single predictive assay to insure the identification of the allergenic potential of food proteins deriving from nonallergenic food sources. Viewing Figure 3, it is possible to compare the typical biological and physicochemical properties of a transferred protein with known allergenic proteins[8,31]. Although it is known that the analysis is very complex, it seems an urgent priority in this field to focus on two approaches, either searching the amino acid sequence homology of the transferred protein, or analyzing the physicochemical properties of the above protein[15].

Amino Acid Sequence Studies

As a first point. a comparison of the amino acid sequence homology between the introduced protein and allergenic protein is performed based on the reported sequences of several allergens, including food allergens[32]. Even if many T-cell, B-cell and IgE-binding allergenic epitopes have been mapped[33,34], the distinction between allergenic and nonallergenic epitopes remains to-date concealed[35]. Considering previous studies on the number of contiguous amino acids necessary to the binding of peptide fragments to T-cell epitopes of allergenic proteins, a sequence homology, to be immunologically relevant, requires at least 8 contiguous identical amino acids. However, further tests in this field yielded no results, since the genes introduced into the tested proteins do not encode known allergens or their homologues, and no such protein shares linear epitopes with known allergens[16], so they are T-cell epitopes to be better analyzed.

Studies on Stability to Digestion

The controversies related to food allergens can be overcome by testing for example their stability to digestion. The ability of such allergens of reaching and crossing the mucosal membranes of the bowel[36] is increased if the allergens succeed in maintaining their stability in the gut, characterized by the acidity and proteolysis there prevailing. Since different allergens exhibit proteolytic activity[8,31,36], the physicochemical properties favoring such stability can uncover the allergenic potential[15]. To evaluate the potential digestive stability of a number of common food allergens an experimental model was prepared with the objective of simulating the mammalian gastrointestinal fluids[37]. Test protein were so incubated in a solution of pepsin at acid pH with the results that food allergens were stable for at least 2 minutes, and the major allergens were stable for >60 minutes[15]. We easily contend that only one μg[22] is able to trigger an anaphylactic shock[19].

Is GMO Overestimated?

What we have hitherto discussed has an only meaning: the high interests of the multinationals. The Monsanto supporters claim that GMOs provoke no harm. Often the truth has been found only after several years, as were the discussed earlier cases: nobody could have anticipated such outcome. Once again the unaware consumer and parents of infants and young children must make a leap in the dark and/or eat boiled crow. We deem it very urgent that it is clearly specified whether foods to be sold in supermarkets are Frankenstein's food or not?

Certainly both WHO and EU look after the perfect correspondence between normal and GM foods. However the label should always indicate not only whether the food is or not genetically manipulated, but also the amount of GM food it contains. As yet the regulations fail to specify such characteristics on the product labels, a deficiency often stressed by us in other fields. As previously alluded to, among the UE directions there is even the exemption from the obligation of specifying on the label that it may contain a GMO. The confusion (and the damage to consumers) has been amplified by the recent discussion on the seven GM oils of which nobody knows whether they are to be banned and the resulting ascertainment that all processed foods potentially containing those oils should be removed from grocery shelves, without knowing, practically, which foods contain such oils as ingredients.

We pass over the damages laid on the citizens by biodiversity reduction, substituting it with a few standardized products, however the "own-goals" of the multinational industries producing GMOs are now countless: Monsanto only recently should have discovered that in the GM soybean produced seven years ago to the FDA to get the official permission to commercialize soy-

bean, there were two more genes in addition to the three that were denounced: a 166% increase. According to Monsanto such genes remained "dormant" during seven years, were completely inactive: not only we object, which analysis the Monsanto has done to affirm this truth, but also whether they have evaluated the world consequences, being soybean a natural ingredient of a myriad of GMOs. Which credit can be given, from now on, to the Monsanto, to all multinational companies interested in GMOs? The second own-goal of the multinationals is the discovery in April 2001 that the laboratories that should control the GMOs can identify ONLY SIX GMO SEEDS OUT OF 24 that are diffused throughout the world, a 433,3% reduction! Thus, the tests for GMOs may not be accurate. The third own-goal was reached with 550% of honey cans containing pollen traces, that were GM pollens[38], therefore contraindicated for children with respiratory allergy and/or oral allergic syndrome[33], and with GM canola transported by the wind on fields with biological farming[39]: the GMO supporters are served.

The zenith (of own-goals) was reached when the Royal Society of Canada has issued on 20.1. 2001 a document stating that 53 new procedures should be fulfilled before a new permission to cultivate GMOs could be released[40]. The Society stresses that lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of that living modified organism intended for direct use as food or feed, or for processing, in order to avoid or minimize such potential adverse effects. The safety assessment process, as it stands, is not adequate to pick out every GM crop harmful to human or animal health. The Royal Society also reported that "the use of substantial equivalence as a decision threshold by regulatory agencies is, in the Panel's view, scientifically unjustifiable when used to exempt new products from full scientific scrutiny". Substantial equivalence is the concept that underpins the safety assessment of GM crops around the world. The basic premise is that if a GM food is shown by composition analysis to be the same as a non-GM food then it should be considered to be as safe as the non-GM food. However, GM foods cannot be exactly the same as non-GM foods, by the very fact of the novel proteins they contain, and so it was determined that they would be considered as safe as normal foods if they were substantially equivalent to them[40].

But the risks have reached the climax when the GM corn destined to animal feeding was mistakenly mixed with maize prepared for human alimentation: The global marketing of GM food has been dealt a blow following reports of allergic reactions to Starlink corn, which was detected in corn food products. The case of allergic reactions reported after the consumption of products containing the GM maize Starlink was unusual simply because consumers were made aware that they were eating it. Starlink corn was not approved for human food, thus any food found to contain evidence of Starlink was recalled from the marketplace by the manufacturers. Unfortunately, the news media often portrayed Starlink as an allergen or a potential allergen, causing consumer concerns[31].

In the wide and variegated field of Frankenstein foods something appears to move. Suppliers of soy formulas to prevent atopy in at-risk neonates, and to cure atopic infants and children (Abbott, Dieterba, Milupa) have announced that their production shall utilize no GM soy. However, two producers, Nestlé and the aforesaid Dieterba were subjected to an inquiry, having introduced into their SPFs GM soy in the 1% proportion. The marketing of 4 types of GM corn has been discontinued (however excluding 3 types of GM rape oil). Moreover the Novartis multinational has stopped the production of GM foods (but not of the pertinent seeds) and the French government has decided to destroy the GM soy illegally introduced into the country.

Stimulating Challenges

The recent polemics related to the mad-cow hysteria has somewhat obscured another bewilderment linked with GM foods: they have no taste. A recent presse-prononciation of the great French "chefs" declares that these GMO represent the "nothing" in the gastronomic specialties at the point that the "chefs" are not able to find out pure ingredients anywhere in this field.

An additional presse-release regards not genetically but DNA-selected tomatoes which were given a gene to delay their ripening, so they may remain in a refrigerator without losing their characteristics, except the natural ta-

ste. When scientists tried to feed tomatoes to rodents, however, the animals wouldn't eat them. So it is all at the expense of our taste: we usually eat what we like, but the highest choice is for food palatability[41].

An estimated 3.5×10^{12} transgenic plants have been grown in the US. in the past 12 years, with over two trillion being grown in 1999 and 2000 alone (Figure 4)[42]. In a press-inquiry done in Italy on a sample of 1200 citizens, 67% virtually refused GMO, 75% judged less adequate or fully inadequate the laws in force in Italy, and 60% blamed that nobody warrants the consumers' food safety. Of those polled, 98% said yes, and 2% said no[43]. In fact, not only Europeans but also Americans have called for a recall of GM-foods on the market. GM food is still selling briskly on US stores, but probably only because GM foods are not labelled, so consumers have no idea what they are. GMO soybean growers in the US claim savings of $5 to $20 per acre (0.447 hectares) from reduced fuel and herbicide costs. However, the Americans are now becoming worried about GM foods, and the US secretary of agriculture has suggested the need for unbiased research on the safety of GM crops[44]. Problems for GM foods are arising in Australia, Austria, EU, Thailand; and in Canada. Should all GM foods be labelled? Can additional research reduce uncertainties and increase parent confidence? Certainly, Americans would continue their efforts to convince the Europeans to change their policies. Therefore, it is hoped that a collaboration between the EU and the US would give a rational basis for protection of children of both countries[45].

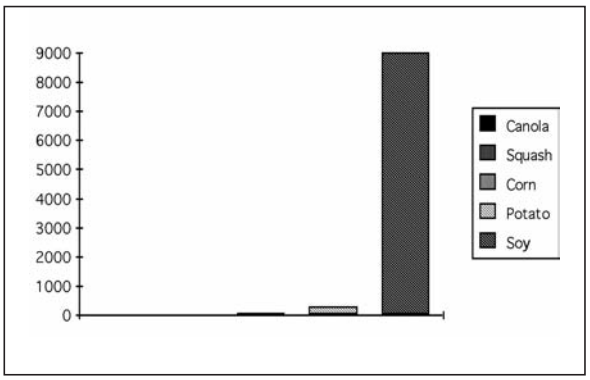

Figure 4. The more diffused GM foods cultivated in the US (2001). Figure × 104. Data from reference 42.

Future Frontiers

According to the legend of Romulus and Remus, the twins were abandoned by their mother on the river-bed of the Tiber river of Rome. A wolf took care of the babies and breastfed them. The twins survived in such a hostile environment as the Tiber river being fed with such different milk as the wolf's milk. However, they grew so strong as to enable them to build Rome. This fascinating legend teaches us that human newborns are able to overcome many difficulties. However, there is no doubt that the twins would fail to react in this way if they would have been fed GM foods in our times. US citizens blame that borrowing genes from various creatures and implanting them in others, scientists are creating super-fast growing GM salmon, trout and carfish, oysters that can withstand viruses[46].

Investigations of any potential FA risks associated with GM food are vital for consumer protection.

In conclusion, the safety assessment of GM crops should be subjected to full review in light of the suggestions of Table 3[47]: We ask therefore, who has established for sure the perfect correspondence of GMO and natural foods considering the resources of vitamins and minerals, especially of trace elements, as regards both nutrition and growth of children?

References

1) Cantani A, Micera M. Genetically modified foods and children potential health risks. Eur Rev Med Pharmacol Sci 2001; 5: 25-29.

2) Nylenna M. JAMA's editor sacked. World medical journal editors should establish an award for editorial integrity in Lundberg's name. Br Med J 1999; 318: 394.

3) Ewen SWB, Pusztai A. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. Lancet 1999; 354: 1353-1354.

4) Skripak JM, Matsui EC, Mudd K, Wood RA. The natural history of IgE-mediated cow's milk allergy. J Allergy Clin Immunol 2007; 120:1172-1177.

5) Eigenmann PA. The spectrum of cow's milk allergy. Pediatr Allergy Immunol 2007; 18: 265-271.

6) Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol 2006; 117: S470-S475.

7) NORDLEE JA, TAYLOR SL, TOWNSEND JA, THOMAS LA, BUSH RK. Identifica-tion of a Brazil-nut allergen in trans-genic soybeans. N Engl J Med 1996; 334: 688-692.

8) METCALFE DD, ASTWOOD JD, TOWNSEND R, SAMPSON HA, TAYLOR SL, FUCHS RL. Assessment of the allergenic potential of foods derived from geneti-cally engineered crop plants. Crit Rev Food Sci Nutr 1966; 36 (suppl): S165-S186.

9) DAY PR. Genetic modification of proteins in food. Crit Rev Food Sci Nutr 1996; 36 (suppl): S49-S67

10) GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA. Decreto del Presidente della Repubblica del 7/4/1999; 12/5/1999 n. 109, art. 3, p 6.

11) FRIEDRICH MJ. Genetically enhanced rice to help fight malnutrition. JAMA 1999; 282: 1508-1509.

12) BUCHHOLZ WG, CONNELL JP, KUMPATLA SP, HALL TC. Molecular analysis of transgenic rice. Methods Mol Biol 1998; 81: 397-415.

13) MATSUDA T, NAKASE M, ADACHI T, NAKAMURA R. Allergenic proteins in rice: Strategies for reduction and evaluation. In Anonimous, ed. Food al-lergies and intolerances. Bonn: DFG Senate Commission on the Evaluation of Food Safety 1995.

14) HEW CL, FLETCHER G. Transgenic Fish for Aquaculture. Intellectual Property & Biodiversity News 1997; 6/7 May 28.

15) ASTWOOD JD, FUCHS RL. Allergenicity of foods derived from transgenic plants. Monogr Allergy 1996; 32: 105-120.

16) BINDSLEV-JENSEN C. Allergy risks of genetically engineered foods. In van Ha-ge-Hamsten M, Wickman M, eds. 30 years with IgE. Copenhagen: Munksgaard 1998; 63-66.

17) TAYABALI AF, SELIGY VL. Human cell exposure assays of Bacillis thuringiensis commercial insecticides: Production of Bacillus cereus-like cyto-lytic effects from outgrowth of spores. Environ Health Perspect 2000; 108: 919-930.

18) CANTANI A, MICERA M. Transgenic foods, pesticides, dioxin, passive smoke: Conse-quences on breast milk. Minerva Pediatr 2001; 53: 199-210.

19) CANTANI A, GAGLIESI D. Severe reactions to cow's milk in very young infants at risk of atopy. Allergy Asthma Proc 1996; 17: 205-208.

20) CANTANI A, MICERA M. Immunogenicity of hydrolysate formulas in children (part 1). Analysis of 202 reactions. J Investig Allergol Clin Immunol 2000; 10: 261-276.

21) CANTANI A, MICERA M. Immunogenicity of hydrolysate formulas in children (Part 2) 41 case-reports. J Investig Allergol Clin Immunol 2001; 11: 21-26.

22) HOLT PG. Immunoprophylaxis of atopy: light at the end of the tunnel? Immunol Today 1994; 15: 484-489.

23) FAO/WHO CONSULTATION. Biotechnology and food safety. Report of a joint FAO/WHO consultation. FAO Food and Nutrition Paper 61. Roma, Italy: Food and Agriculture Organization of the United Nations; 1996.

24) FAO/WHO CONSULTATION. Safety aspects of genetically modified foods of plant origin. Report of a joint FAO/WHO consultation. Geneva, Switzerland: World Health Organization; 2000.

25) SAMPSON HA, ALBERGO R. Comparison of results of skin tests, RAST, and double-blind, placebo-controlled food challenges in children with atopic dermatitis. J Allergy Clin Immunol 1984; 74: 26-33.

26) BURKS AW, BROOKS J, SAMPSON HA. Allergenicity of major component proteins of soybean determined by enzyme-linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J Allergy Clin Immunol 1988; 81:1135-1142.

27) RESTANI P, PLEBANI A, VELONÀ T, CAVAGNI G, UGAZIO AG, POIESI C, MURARO A, GALLI CL. Use of immunoblotting and monoclonal antibodies to evaluate the residual antigenic activity of milk protein hydrolysed formulae. Clin Exp Allergy 1996; 26: 1182-1187.

28) BOCK SA. Food challenges in the diagnosis of food hypersensitivity. Nestlé Nutr Work-shop Ser 1996; 34: 105-117.

29) BRUIJNZEEL-KOOMEN C, ORTOLANI C, AAS K, BINDSLEV-JENSEN C, BJÖRKSTÉN B, MONERET-VAUTRIN D, WÜTHRICH B. Adverse reactions to food. European Academy of Allergology and Clinical Immunology Subcommittee. Al-lergy 1995; 50: 623-635.

30) LÖNNERDAL B. Recombinant human milk proteins - an opportunity and a challenge. Am J Clin Nutr 1996; 63: 622S-626S.

31) TAYLOR SL, HEFLE SL. Will genetically modified foods be allergenic? J Allergy Clin Immunol 2001; 107: 765-771.

32) WHO IUIS ALLERGEN NOMENCLATURE SUBCOMMITTEE. Allergen nomenclature. J Allergy Clin Immunol 1995; 96: 5-14.

33) CANTANI A. Allergologia e Immunologia Pediatrica: Dall'infanzia all'adolescenza. Roma: Verduci Editore 2000.

34) RUDENSKY AY, PRESTON-HURLBURT P, HONG S-C, BALOW A, JANEWAY CA JR. Sequence analysis of peptide bound to MHC class II molecules. Nature 1991; 353: 622-627.

35) O'HEHIR RE, GARMAN RD, GEENSTEIN JL, LAMB JR. The specificity and regulation of T-cell responsiveness to allergens. Annu Rev Immunol 1991; 9: 67-95.

36) GARDNER MLG. Gastrointestinal absorption of intact proteins. Annu Rev Nutr 1988; 8: 329-350.

37) Anonymous. The United States Pharmacopeia, vol XXII, NF XVII. Rockville: United States Pharmacopeial Convention 1990.

38) FoE EWNI. Funny honey. Link 2000; 93: 18.

39) Canadian canola contamination. Link 2000; 93: 30.

40) Royal Society of Canada. Elements of Precaution: Recommendations for the Regulation of Food Biotechnology in Canada. January 2001.

41) Weiss R. Washington Post 1999; 15 Aug.

42) Stewart CN Jr, Richards HA 4th, Halfhill MD. Transgenic plants and biosafety: science, misconceptions and public perceptions. Biotechniques 2000; 29: 832-836, 838-843.

43) Friends of the Earth press release. September 18, 2001.

44) Reichardt. US sends mixed message in GM debate. Nature 1999; 400: 298.

45) Godfrey. Do genetically modified foods affect human health? Lancet 2000; 355: 414.

46) Rachel's. Environment and Health Weekly, May 2000; 695.

47) Cantani A. Pediatric Allergy, Asthma and Immunology. Springer Verlag, 2008.