# Biosafety: evaluation and regulation of genetically modified (GM) crops in the United States

Richard E. Goodman
*Food Allergy Research and Resource Program,Dept. of Food Science and Technology,University of Nebraska-Lincoln,*143 *Food Industry Complex,Lincoln, NE* 68583-0955*, USA*

**Abstract** This review of the safety assessment of genetically modified (GM) crops is focused primarily on the process and progress in the United States (US). It reviews the development of the safety evaluation process from the Asilomar conference in 1975 considering issues relevant to recombinant DNA technology, to discussions between the US government, academic and industrial scientists between 1984 and 1994 when the first GM crops were being field tested and evaluated commercial release for food and feed production. International guidelines were also reviewed for consistency with the US system. The overall process includes consideration of information relating to history of safe or unsafe human and exposure to the gene source and expressed proteins. The primary considerations of safety for dietary proteins are whether or not some consumers are sensitized and have IgE antibodies against the protein encoded by the transgene or whether the transgene represents a risk of eliciting celiac disease. The process considers potential toxic effects of expressed proteins as well as potential impacts on human and animal nutrition. The process in the US is consistent with Codex Alimentarius recommendations. It follows a science based process based on justifiable hypotheses. To date there is no evidence that GM crops approved in the US have harmed human or animal consumers. The evaluation takes into account genetic and environmental variation in products produced by plant varieties and is intended to maintain the standard that foods developed from GM plants are intended to be as safe as non-GM genetically similar varieties.
**Keywords** genetically modified crops; food safety assessment; allergenicity; toxicity

## 1 Introduction

The safety of foods produced from genetically modified (GM) organisms, GMOs or GM crops is mandated by most countries including the US, China and countries who are members of the Codex Alimentarius Commission, an international food standards program within the World Health Organization and the Food and Agricultural Organization of the United Nations (www.codexalimentarius.org). The Codex includes 185 member countries plus the European Union (EU) and has 224 official observers (non-member countries plus non-governmental organizations (NGOs) and outlines guidelines for many important questions regarding food safety and international trade.

The overall process of evaluating the safety of foods produced from GMOs has been described in only a few documents. The primary food safety guidance for developers and regulators of GMOs is the combined documents of the Codex Alimentarius Commission, Second edition, "Foods Derived from Modern Biotechnology"[1]. The principles are outlined in the first chapter (CAC/GL 44-2003) and include definitions of organisms derived from "Modern biotechnology", or genetic modification and the risk assessment process that is intended to identify any new hazard or nutritional or safety concern presented by the new GM organism as well as risk management procedures if appropriate. Three major sections follow that are intended to define processes to evaluate the safety and nutritional properties of GM plants, GM animals and GM microbes. The assessment strategies are quite similar for all three. The food allergy assessment is outlined as a separate annex at the end of the chapters. Evaluation of potential risks of toxicity, celiac disease and nutritional equivalence are discussed in the section on substantial equivalence toward the front of each of the documents. The Codex[1] document is a guideline for individual countries that have to develop their own regulations. The intent of Codex is the signatory parties should develop regulations that are consistent with these guidelines unless differences are scientifically justified. Since the documents were written from 2001-2003, there have been some advances in the science

Received date:September 22,2014
E-mail: rgoodman2@unl.edu, Tel and fax: +1(402)472-0452; +1(402)472-0693

and those will be discussed along with commonalities. Newer evaluation steps will be discussed here. This paper will focus primarily on the assessment as performed in the US. However a review of some of the concerns and criticisms of people and organizations who opposed GMOs will also be discussed as it is important to understand whether there are legitimate safety issues that are not being addressed by regulatory bodies and developers.

The safety of foods derived from GMOs is a focus of some NGOs including Greenpeace, Friends of the Earth, Union of Concerned Scientists as well as personalities on popular television and the internet in the United States (US), European Union (EU) and China. We must recognize that it is natural for consumers to be concerned about food safety, especially regarding new foods or technologies that many individuals do not fully understand. Most consumers do not understand the scientific basis of allergens and allergies, toxins, nutrients and anti-nutrients in foods. Most consumers also do not understand the importance of genetic diversity in food crops to enable production in diverse environmental conditions[2]. Many consumers believe that all soybeans are identical unless they have been modified by genetic engineering. Scientists in other disciplines also do not understand the tremendous variation and complexity in the normal composition of proteins, oils, carbohydrates and metabolites of all food crops. A major focus of plant breeders is to introduce variation and if we consider the principles of genetic engineering and look closely at the changes, it is clear biotechnology introduces minimal uncertainty compared to the natural or induced mutations that breeders have relied upon to develop useful new varieties[2]. Can we explain realistic risks to consumers and also explain how the current safety assessment process minimizes risk?

An important concern that is often voiced by the opponents of GM crops and of the pharmaceutical industry is that the companies developing the products are the ones who test for safety. That concern seems reasonable, but needs to be considered in the context the entire legal framework, governmental and economic structure of each country. Scientists in the government of the US and many other countries do not perform safety testing for most products. It is worth noting that no government in the world has enough scientists with the right expertise or enough money to perform the appropriate safety tests for all potential products in a reasonable amount of time. Development of many important products would stop if developers had to wait for safety testing by their governments. The regulatory systems established by most, including the US is to have a number of quality scientists in the regulatory departments who can review safety data critically and make decisions based on protocols and guidelines. Governments like the US have legal mechanisms for consultations with academic experts to assist in the evaluations. The regulators also should have the ability to efficiently communicate with developers to ask for additional data or tell them what additional tests or questions must be answered to gain approvals.

I am most familiar with Monsanto as a major GM crop developer. They have approximately 600 college educated (BS, MS and PhD) individuals working in the regulatory division of the company. These scientists plan and conduct safety and environmental studies, archive and characterize test substances (plants, seeds, DNA constructs and proteins), perform tests, analyze data and write reports for submission to regulatory agencies. They have to grow plants in different environments and sometimes multiple countries in order to perform field and environmental tests. The regulatory process is extremely complex even for one product and development and regulatory approvals for each product often takes ten to fourteen years. Companies like Monsanto also have separate quality assurance units (QAU) that report to a different management team from the development and sales divisions. The QAU reviews protocols prior to study conduct and audit data and reports before they are submitted to regulators to ensure study adequacy and accuracy. Their scientists evaluate mountains of data and develop the dossiers that are submitted to multiple governments before a product is allowed to be grown commercially. Most will gain approvals in major trading countries (Australia, Canada, China, Japan, Korea, the US and Taiwan) before releasing seeds of a new GM product to farmers. International trade of commodities and foods and feed will only work if the developer is managing materials and data as they have the capacity and incentive to ensure timely and coordinated processes. In some cases regulatory studies are performed by contract laboratories, especially toxicology studies as there are regulations that are very strict that require specific tests to be performed following “Good Laboratory Practices” (GLP) as defined by the Environmental Protection Agency (EPA) of the US government. Few developers have the infrastructure to meet all GLP requirements. The toxicology contract companies specialize in meeting regulatory demands. Those studies can be audited by

EPA. There are strict rules about record keeping, ethics and integrity of data. It might be important to consider that if the government were to perform those studies, who would audit them and hold them accountable?

Some studies are performed by academic laboratories because neither the developer nor any contract GLP laboratory has the right expertise to perform the study. My laboratory at the University of Nebraska has performed a number of allergenicity studies (human serum IgE binding and bioinformatics studies) for biotech companies and non-profit agricultural organizations as well as food companies developing novel ingredients. We have collaborations with clinicians who arrange samples from specifically allergic patients who are willing to contribute serum samples to evaluate product safety. We develop protocols, perform the studies, evaluate data, write reports and maintain records related to the studies. We do those studies under contract with the developers under ethical standards managed by the University of Nebraska as well as ethical standards of any collaborator's institution.

I have been involved in designing, performing or reviewing safety studies on allergenicity, toxicology and nutritional qualities and performance of GM crops and novel food ingredients for 17 years. I was at the Codex Alimentarius Task Force Working Group meeting that was held in Vancouver Canada in 2001 that developed the allergenicity guideline[1]. I have been involved in safety studies and reviewing procedures for GMO safety for submission to governments of the US, Canada, Argentina, Brazil, the EU, India, South Korea and Taiwan and reviewed hundreds of publications on allergenicity, toxicity and potential horizontal gene transfer. In my career I have not seen any documented cases of adverse health problems in humans or agricultural animals caused by consuming approved GM crops and I believe that the safety assessment of GMOs is quite robust[2-3].

Of course I had to go through a learning process to gain an understanding and comfort level with the assessment process for GMOs because I am a born skeptic. My scientific career began during the early years of development of agricultural biotechnology. This paper reviews some of the history of development of the safety assessment and regulation of GM crops in the US. It includes the primary proven food safety hazards and risks and describes the process of evaluating safety of new GM crops prior to commercial release. It includes a description of the most significant case of a GM product that was approved and then withdrawn from the market because of uncertainties of safety data, not because of harm.

### 1.1 Real risks of foods vs. hypothetical risks

Many of the foods we eat today were initially consumed hundreds to thousands of years ago. The genes and exact nutritional composition of many crops have been changed from the earliest varieties using conventional breeding techniques. However, to a great extent commodity crops including wheat, rice, corn and soybeans as well as many of the fruits and vegetables are quite similar to the food materials humans have consumed safely from these plants for centuries. The experiences of using those crops have guided regulators in establishing a safety evaluation process that begins with considering whether humans have had experience and contact or consumption of the host plant (the gene recipient) and the donor organism (source of the gene to be transferred).

In the mid-1970s as I was earning a bachelor's degree in biology I was an active member of Greenpeace, Friends of the Earth and Union of Concerned Scientists. Details of techniques of recombinant DNA methods were first being described in college classrooms as we learned about potentially useful recombinant bacteria and plants that might come from the technology. At that time most students and many professors had a very superficial understanding of DNA, RNA, ribosomes and protein synthesis compared to our knowledge in the 1990s and certainly compared to information available even in high school classes in 2014. In the early 1970s Paul Berg, Walter Gilbert and Frederick Sanger (all future Nobel Laureates in chemistry) began discussing potential (hypothetical) risks that recombinant organisms might pose if certain viral DNA sequences from pathogens were introduced into bacteria using this technology. Maxine Singer and others called on the community of scientists to develop safety standards. Much of the concern was on the proposed use of the simian virus 40 (SV40) DNA elements in recombinant bacterial plasmids that were being transferred in culture into monkey cells to understand gene function as described by Cole et al[4]. In response Berg and others organized the Asilomar Conference in 1975 at the urging of the National Academy of Science (US) to establish guidelines for ensuring safety. The process and twenty years of experience of safety of recombinant DNA work since then were reviewed by Berg and Singer[5]. Essentially all recombinant DNA work was halted in the US for one year while the guidelines were developed. They detailed considerations based on perceived risks and

called for the establishment of institutional biosafety committees to review each new rDNA experiment in any institution or company that was performing genetic engineering research. The primary focus was the potential risk or safety of the new DNA elements based on mode of action and risk of the DNA donor organism. The guidelines have helped ensure that really hazardous organisms were not created using the technology. Relatively safe cloning experiments can be performed in a typical clean laboratory environment with few restrictions (Biosafety level 1 or 2). There are few places with extremely tight controls (Biosafety level 3 or 4) where recombinant experiments can be performed on highly lethal and infectious agents (http://en.wikipedia.org/wiki/Biosafety_level).

The safety issues related to foods derived from GM plants are of course different. Genetically modified plants are not infectious, potential risks of food safety for GMO are quite low compared to risks from microbes and risks are not different from those posed by non-GMO plants. There are of course specific risks from foods that must be evaluated such as the potential transfer of an allergen or a toxin from another organism into a food crop. Many hypothetical risks are the focus of discussion today rather than the finite and definable risks that should be evaluated based on our extensive knowledge of science and safety. The evaluation of a new product that has added one or a few new gene(s), new protein(s), or new metabolites to a crop that has 10 000 to 20 000 endogenous genes and has already been safely consumed should focus on the safety of the gene source, protein characteristics and metabolites if the protein is an enzyme. The risks would be presented by the other 10 000 plus genes and proteins would be the same risks that already occur from that crop. In addition, the types of risks the new gene and protein could present are definable based on our experiences with other foods. Most current non-GM food crops have specific allergenic proteins; a few may have toxins (solanine) or anti-nutrients (trypsin inhibitors). So the focus on the new proteins should be on evaluating potential allergenicity, toxicity and any anti-nutritional properties.

There is now a history of nearly 20 years of production and consumption of a few commonly grown GM crops, for example insect protected corn containing a specific protein from Bacillus thuringiensis or Bt-corn; herbicide tolerant soybeans with a gene from a soil bacterium and virus resistant papaya, without evidence of harm. Crops improved through biotechnology have shown benefits due of reduced pesticide applications or in some cases reduced plant pathogen impacts. A number of GM crops have improved agricultural practices in ways that minimize soil erosion, energy or water consumption.

Some might argue that the strong fears voiced against GMOs stimulate healthy debates about proper regulatory studies that have helped ensure a robust assessment process. Others suggest that many of the new regulatory demands developers face today are excessive and delay scientific progress in medicine, industrial development and agriculture. The truth probably lies between the extremes, but based on conversations with GM developers, commodity companies and food companies as well as review of regulatory guidelines of the EU and other countries it is clear that the global process of GM evaluation and approvals are slowing development and leading to global trade barriers over the past 10 (2004 to 2014) years. Because of the international nature of trade, agricultural companies have to wait many years before new products can be released in order to obtain approvals in the major world markets. It seems that regulators in all countries are becoming more precautionary as they are afraid of being blamed for approval of a GM crop that is not proven to be absolutely safe under all possible uses. The precautionary principle is counter to the policy of the Food and Drug Administration (FDA) of the US as outlined in 1994, which recognized that all foods pose some risks that can be evaluated and managed and that the standard of safety is that foods from GM crops must be as safe as conventional crops of similar types.

A searching of scientific literature today identifies many new study questions and designs that are being performed on potential GM crops using a variety of search terms (transgenic, GM, genetically engineered, toxicology, reproductive, cancer) that should only be performed if there is as testable hypothesis based on information about the crop or the gene and gene products. Few (if any) dietary proteins alter reproductive fitness, cause cancer, act as adjuvants or increase the prevalence of a broad range of autoimmune diseases.

Today regulators and politicians are being pressured by activists like Eric-Gilles Seralini, Terje Traavik, Vandana Shiva, Mae-Wan Ho and Jeffrey Smith as well as celebrities like Oprah Winfrey, Dr. Oz and Cui Yongyuan or by consumers who listen to these activists make unsubstantiated claims of health risks of GMOs on websites in books, in the news media and television. For example, Jeffrey Smith's website, the deceptive

"Institute for Responsible Technology (http://www.responsibletechnology.org/) claims that very diverse human diseases including autism, celiac disease, food allergies and cancers are dramatically increasing due to increased consumption of foods produced from GM crops. He takes small observations from a few poorly controlled animal studies that have not been validated to predict human disease and implies that humans will experience many complicated diseases from eating foods derived from GMOs. Mr. Smith offers a training program for "anti-GMO speakers" for a fee of $150 USD. Mr. Smith does not post credible peer-reviewed scientific studies to support his claims and generally cites correlations of increased GMO production and increases in these diseases that have highly diverse and uncertain causes. In fact the correlations usually do not match the introduction of most of the GMOs in the food chain. Yet many highly educated people take statements by Smith and other activists to be factual and they refuse to look more deeply for the many public and published studies that are available to demonstrate the approved GMOs have been evaluated for safety by scientifically sound studies. There are no studies that link consumption of insect-protected corn to celiac disease or food allergies, nor autism nor cancers. If coincidental changes in our lives and environment demonstrated causality; we should stop air-travel, shut off the internet, discard cell phones and television; ban processed foods, vaccines and prescription medications. We would need to live our lives as they were in 1914 when the world population was less than two billion, life was very different and the average life-expectancy less.

In considering risks from foods, it is highly doubtful that genetic diversity of our foods represents a food safety risk. We are omnivores and subsist on highly diverse diets. We consume foods that are markedly different in 2014 compared to those consumed in 1914 and certainly compared to 1514 before tomatoes, potatoes and peppers were transferred from South America to Europe, India and China. If there are significantly different risks associated with eating plants that have only minor genetic differences compared to the varieties we eat every day, then maybe we need very complicated testing methods. However, humans have been pretty good at evaluating food safety over thousands of years without highly complex scientific studies. One could argue the extended life-expectancy, relatively low infant mortality rates and general health status of humans in the US and China in 2014 provides pretty convincing evidence that the current GMOs are not likely to be harmful. It is important to focus on realistic risks of foods and the development of processes that help ensure that foods produced from GM crops are as safe as foods produced from similar non-GM crops.

### 1.2 Early development of the safety evaluation of GMOs

In the mid-1980s I had not considered the safety assessment process that might be performed on GM crops to evaluate food safety. I knew little about the process of using agrobacterium mediated transformation system to insert functional segments of DNA into plants[6]. As I learned more about biotechnology during training as a PhD student at the Ohio State University, cloning a cDNA of bovine lactoferrin for sequencing and expression I had to learn and comply with evaluations by institutional review committees at the university. I had to answer questions about the source of the gene, the encoded protein, the plasmid vectors and the host cells and organism that was to receive the cloned DNA. The training was reinforced during my work developing cDNA clones for rodent and human cytokines as I studied immunology at Cornell University and later at the University of Michigan. By the time I joined Monsanto as a regulatory scientist working on the safety assessment of GM plants in 1997, I stopped believing the statements by Greenpeace and others about many hypothetical risks of GM crops and statements that there were no safety evaluations and resigned my memberships in those organizations. Within two months of joining Monsanto I was thrust into the role of developing an animal model to evaluate the potential impact of a GM event to evaluate potential impacts on allergenicity. The tests were novel and unprecedented as no one had demonstrated that a rodent model could predict potential sensitization in humans. However the government of India demanded an animal model test for allergenicity. The approval process took nearly 7 years after the US had approved the same crop. India dropped the requirement to use animal models to evaluate potential risks of food allergy after bringing their guidelines into alignment of the Codex Alimentarius Commission (2003) guideline[1] in 2008. My work at Monsanto involved becoming familiar with the regulatory process in the US and other countries and learning the science of risk evaluation for potential allergenicity, toxicity and nutritional equivalence. I continued being involved in the regulatory evaluation process when I was hired at the University of Nebraska in 2004 and have become even more broadly involved through 2014. But I am still learning about the process

that led to the current assessment.

A review of publically available information shows that academic, industrial and government scientists have collaborated in many consultations to develop a useful and predictive safety assessment process for GM crops. The US government outlined a coordinated regulatory framework in 1986 that includes the Food and Drug Administration (FDA) the Environmental Protection Agency (EPA) and the US Department of Agriculture to evaluate and regulate GM crops (Office of Science and Technology Policy, 1986; http://www.aphis.usda.gov/brs/fedregister/coordinated_framework.pdf). That was eight years before the first GM crop approval. A group of academic and industrial scientists held meetings as the International Food Biotechnology Council (IFBC) in collaboration with the International Life Sciences Institute (ILSI) and developed a risk assessment guideline that was published as a supplement to volume 12 of Regulatory Toxicology and Pharmacology (1990)[7]. The IFBC-ILSI volume was prepared by 28 highly experienced scientists and legal experts. The volume presented methods of genetic modification, variable crop composition of traditional foods, safety evaluation of food ingredients derived from microorganisms, safety evaluation of single chemical entities, safety evaluation of whole foods and complex mixtures and legal and regulatory issues. The draft reports were reviewed by 150 experts in industry, government and academia from 13 countries prior to publication. The major issues were presented and discussed by 120 experts in an open symposium. The IFBC-ILSI document presented a number of key evaluation steps and decisions for whether further evaluations were necessary and also discussed the legal food safety regulatory framework in the US. They supported the decision by the US government that foods derived from GM products could be efficiently regulated within the existing regulatory framework as they found that generating the new varieties (e.g., transformation through biolistics or *Agrobacterium* constructs) were not different in terms of potential impacts on safety compared to traditional breeding methods. The panel concluded the focus should be on questions related to characterizing and evaluating the safety of the introduced DNA, proteins and any metabolic products of any new enzyme in the GMO.

### 1.3 US regulatory process for GMO evaluations

In 1992 the FDA issued a policy statement on the safety and evaluation process for foods derived from new plant varieties including those derived from recombinant DNA techniques under the Federal Food, Drug and Cosmetic act (FDA Federal Register vol. 57, No. 104, docket No. 92N-0139). The evaluation process was followed for the safety assessment of the first GM crop approvals in 1994-1995 and although more complex now, are consistent with the process followed in 2014. Under the unified regulatory system the US Department of Agriculture (USDA) Agricultural and Plant Health Inspection Service (APHIS) is responsible for oversight of regulated field trials of unapproved GM events, control through a permit system of GM organisms, plant pests and veterinary products. A different section of USDA, the Food Safety and Inspection Service (FSIS) is responsible for regulating the safety of meat and some poultry products. The FDA has authority of other food safety issues including evaluating the safety of GM crops and all milk and dairy ingredients. The Environmental Protection Agency (EPA) is the lead agency involved in evaluating GM plant incorporate pesticidal (PIP) genes (e.g. plants containing genes encoding crystal proteins from *Bacillus thuringiensis*, or Bt plants; plants including genes for viral resistance such as the Plum Pox Virus resistant plum tree) as well as regulating chemical herbicides and chemical insecticides. The EPA and FDA follow the same food safety guidelines and the normal process for a PIP includes consultations with the FDA and a full dossier submission to the EPA. Although the FDA consultation and data submission is in theory "voluntary", failure to consult with FDA and provide data to complete evaluation of potential allergenicity, toxicity and nutritional effects of a GM crop is likely to lead to mandatory recall and legal action if there is any suspicion of harm. Requirements by EPA and USDA are clearly mandatory. Both the EPA and the FDA expect similar evaluation processes and tests for food safety before a product goes to market.

### 1.4 FDA policy on food safety of GMOs: as safe as similar varieties of non-GMOs

The FDA and regulatory agencies from Australia, Brazil, Canada, Japan, the Netherlands and the United Kingdom governments were significant contributors to the Codex 2003 guidelines[1] that were established as part of the Codex system that is agreed to by the US and China. The process includes evaluation of the same types of risks presented by non-GMO sourced foods that are known to cause adverse health effects: food allergy, food toxicity and adverse nutritional effects including potential increases in anti-nutrients or inclusion of potential celiac eliciting proteins (glutens from wheat and near-wheat relatives). Developers

are expected to present documented information evaluating the history of safe human use (HOSU) or exposure of the gene source and protein or gene, as well as information showing adverse effects. The information must include characterization of the gene products (protein or RNA) and any metabolites of any introduced enzyme, dose of consumption of the protein or metabolites that will be expressed in the new GM plant food material based on consumption patterns of foods made from the host organism. If there is historical evidence showing potential risk from consumption of the gene donor, additional testing may be required.

The FDA recognized that a few endogenous ingredients of all foods pose some risks for consumers. Some risks are normally mitigated by food storage, preparation (cooking) or limiting consumption. For instance lectins, protease inhibitors and amylase inhibitors of legumes (beans) are inactivated by cooking prior to consumption. Cassava is soaked and pressed to remove hydrocyanic acid to prevent cyanide poisoning before manioc is made and consumed. Potato varieties are selected in breeding to ensure they have low concentrations of the glycoalkyloid solanine as it is a mild toxicant. Young, green potatoes are not consumed as the content of solanine is high at that stage. Humans have adapted the foods and processing to ensure safety. Those hazards affect essentially all consumers if not handled appropriately. Other hazards that affect everyone are from contamination by bacteria, fungi or chemicals.

It is important to recognize that the most common and severe risks of food ingestion are from contamination of food with exogenous materials. Contamination can occur on the farm, or during storage in restaurants or homes. Bacteria, viruses, fungi, parasites and chemicals including mycotoxins, heavy metals and pesticides are relatively common food contaminants. The most significant acute risks are presented by bacteria including *Escherichia coli* O157 and other toxin producing strains; *Listeria monocytogenes, Salmonella* sp., *Campylobacter* sp, and *Clostridium perfringens*. The Center for Disease Control (CDC) and USDA FSIS estimate that there will be approximately 3 000 deaths in 2014 in the US population of 310 million, and approximately 128 000 hospitalizations (www.foodsafety.gov/poisoning/causes). Some parasites are also commonly spread through food. Toxoplasmosis is caused by Toxoplasma gondii, the most common food borne parasite in the US causing hospitalization and some deaths. Some viruses are commonly spread through foods. Norovirus is the most common cause of acute gastroenteritis in the US. It is spread through contact with many foods due to unsanitary food handling in a given outbreak, but rarely causes fatalities. Hepatitis A can lead to death in susceptible individuals who go untreated. Mold contamination is rarely documented as a cause of significant food borne illness in humans with the exception occasional outbreaks of mycotoxin poisoning caused by moldy grains[8]. However, mycotoxins more commonly cause severe outbreaks in poultry and other agriculturally important species as they are often fed grain at high concentrations[8]. Mycotoxins are small to moderate molecular weight organic compounds that are typically polycyclic and are not easily detoxified by the liver of some individuals or species. A few of the substances that cause toxic reactions are proteins, such as botulinum which is produced by the bacteria *Clostridium botulinum* along with some other toxins while ricin from castor beans is one of the rare plant protein toxins known to affect mammals[9]. Interestingly GM plants expressing plant incorporated protectants such as Cry1A in corn can reduce fumonisin (a mycotoxin) levels, thus reducing the potential for toxicity in chickens, pigs and cattle. Future GM products are likely to include specific anti-fungal and anti-microbial proteins that will further enhance food safety.

While toxins and anti-nutrients often affect nearly every consumer, a few hazards in foods only affect a small percentage of the population. Specific food allergens affect less than 1% of the population, but can cause severe reactions or death in a very small percent of the population. Glutens (gliadins and glutenins) of wheat and closely related grains cause celiac disease (CD), a chronic autoimmune disease in less than 1.5% of the population. Celiac disease affects a genetically restricted subset of the population that includes over 25% of the total population and there are many other factors that are not completely understood. A major focus of the food safety assessment then is to evaluate and ensure that the transfer of a gene into a GM plant does not transfer an allergen or a CD eliciting gluten from the allergenic or CD eliciting source into another source.

### 1.5 Recognized risks of food allergy including celiac disease in non-GM crops

The most common endogenous risks of food consumption are IgE mediated food allergies[10] and cell-mediated celiac disease[11-13]. Food allergy to all sources may affect 2% to 6% of the population in the

US, with varied degrees of severity. Individuals are usually sensitive to between one and five allergenic foods. Allergens pose a significant risk to those who are already allergic to the specific proteins while they do not pose a risk for non-allergic consumers. Because food allergy is highly variable between subjects in terms of severity of disease and the complexity of food composition, the sources of allergy for an individual are not always obvious[14-15]. The methods used for diagnosing food allergy are not standardized in many medical facilities and few doctors are well trained to accurately diagnose food allergy[16-17]. Food allergies are specific because the patient has been sensitized and produces IgE antibodies that bind specifically to one or more proteins in the food. In IgE mediated food allergy, reactions occur because the individual has developed specific IgE antibodies to at least two epitopes (IgE binding sites) on a relatively abundant protein in the food. Their IgE antibodies are bound to FcεR1 receptors on the surface of mucosal mast cells and blood basophils. Upon subsequent ingestion of the food containing the allergenic protein, the protein or fragments of the protein are absorbed and bind IgE on the mast cells or basophils, stimulating signals within the cell. If a sufficient number of allergen-IgE binding events occur within a few minutes it triggers the release of histamine and leukotrienes from the mast cells and basophils, inducing vascular leakage and symptoms due to angioedema and nerve stimulation. Some individuals experience relatively mild oral itching and mild swelling (angioedema) in the mouth and throat, others get hives or urticaria. Some experience asthma with wheeze and shortness of breath. Others may vomit or have diarrhea. A few will experience hypotension (drop in blood pressure). Anaphylaxis is a severe, life-threatening systemic reaction that includes hypotension and breathing difficulty that usually requires immediate medical attention including injection of epinephrine, other medications and oxygen. Perhaps 150 to 200 highly allergic individuals in the US die each year due from anaphylaxis triggered by food allergy[18]. Most who died because they did not receive immediate medical treatment including an injection of epinephrine. Peanuts, a few tree nut species, milk and eggs are the most common causes of fatal anaphylaxis from food[19]. Although exposure to the allergen triggers an acute reaction in the allergic individual, once sensitized, the individual may remain allergic throughout their life. However, young children often become tolerant to their allergenic food (milk, soybeans or egg) five or more years after initial reactions through a process leading to immune tolerance.

Estimates of the prevalence of food allergy are approximations. The best estimates available for the US, Europe and Japan indicate that food allergy affects from between 1% and 2%, up to 10% of the general population in those countries[20-21]. The frequency of cases of severe, life-threatening reactions is not well established, but clearly some allergenic foods such as peanuts, some tree nuts, cow's milk and eggs account for more severe reactions than fruits and vegetables. In most countries including the US there has not been a standard reporting system for food allergy anaphylaxis. Epidemiologists at the US Centers for Disease Control reviewed hospital coding within the US system for a period of 1997-2007 using various resources and estimated that there are approximately 317 000 food allergy related hospital visits per year in the US (years 2003-2006), with more than 9 000 admissions due to severe reactions[22]. Anaphylaxis was usually attributed to peanuts, crustacean shellfish (shrimp), tree nuts, milk, eggs and fish.

Celiac disease is a genetically restricted autoimmune disease initiated by sensitization to specific wheat, barley and rye glutens (gliadins and glutenins) by activation of T helper 1 type CD4+ T cells[23]. The disease is chronic and lead to flattening of the villi in the upper small intestine, wasting disease and sometimes to specific cancers and other autoinmmune diseases. The genetic restriction is due to unusual protein sequences that are presented most effectively by those with Major Histocompatability Complex loci HLA-DQ2.5 or HLA DQ8[24]. However, while more than 25% of the US population has either HLA-DQ 2.5 or DQ-8, only an estimated 1% of US consumers are clearly diagnosed with CD, which is similar to the rate in Europe[25]. The rate of CD in China is not known, but one recent study suggests that it is more common than once believed[26]. There are uncertainties in prevalence due to the complexity of accurately diagnosing affected individuals as endoscopy with multiple biopsies are taken as the gold standard following consumption of glutens, but endomesial-specific or tissue transglutaminase-specific antibody tests in conjunction with HLA typing or associations with diagnosed near relatives are often used as sufficient evidence for diagnosis[27]. Specific peptides of glutens and gliadins have been identified as stimulating Th1 CD4+ T cell clones from MHC-restricted CD patients[28-30]. The only way CD patients manage their disease is through avoiding consumption of foods containing proteins from wheat, barley, rye and for some, oats[31]. There is

also a growing number of consumers who believe they have non-celiac gluten sensitivity, however the specific disease pattern is not uniform, the mechanism of reactions are not known and there is some disagreement between Gastroenterologists as to the authenticity of the disease[32].

### 1.6 Food allergens are specific proteins, not whole foods

Generally people describe food allergy as being a reaction to a whole food (e.g. milk, eggs or peanuts). But research over the past two decades has identified specific proteins in the foods as the causes of allergy. The International Union of Immunological Societies (IUIS) Allergen Nomenclature Subcommittee (www.allergen.org) lists 12 protein families as commonly allergenic. The most prominently named peanut proteins reported as the dominant allergens are the small molecular weight prolamins (14 to 18 kDa) including the abundant 2S albumins (Ara h 2 and Ara h 6) and higher molecular weight cupins (50 to 75 kDa) major seed storage proteins, Ara h 1 and Ara h 3. The cupins each account for more than 15% of the total protein content of the seeds. Subjects with substantial IgE concentrations to any of these four proteins are most at risk for severe reactions following ingestion of peanut[33-34]. The Ara h 2 and 6 proteins are highly cross-linked small molecular weight proteins with four intra-chain disulfide bonds making them relatively resistant to digestion by the stomach protease pepsin[35-36]. A few other proteins have been identified as allergens in peanuts but represent low abundance and/or low stability proteins which are considered to be minor allergens. Most people with clear IgE mediated allergy to peanut have IgE to the major allergenic proteins, it is not clear that IgE to the minor allergens cause significant clinical reactivity. A few proteins in some foods are nearly identical to homologous proteins in other foods or in pollen and are considered pan-allergens since the IgE of one subject may bind the homologous proteins from a wide variety of species. The pan-allergens do not cause serious reactions in most allergic subjects. Pan-allergens in peanuts include profilin (Ara h 5), pathogenesis related protein-10 family members (Ara h 8.0101 and Ara h 8.0201) and a lipid transfer protein (LTP), Ara h 9. The sequences of two defensin proteins Ara h 12 and Ara h 13 recognized by the IUIS nomenclature committee has not yet been published and the frequency and severity of induced allergic reactions are unknown. Individuals allergic to tree nuts including almonds, hazelnuts, pecans and walnuts usually have IgE antibodies that recognize similar 2S albumins and cupin seed storage proteins. In some cases there seems to be cross-reactivity among the tree nut proteins and even to peanut, but it is difficult to separate IgE cross-reactivity from de novo sensitization, where a subject is co-sensitized and co-reactive. Certainly though pecans and walnuts are very closely related and their allergenic proteins nearly identical.

A number of individual IgE-binding allergenic proteins from foods, inhalation sources (pollen, house dust mites and mold spores) and dermal (latex) or injection (venom, saliva of biting insects) sources have been characterized and studied in the past 25 years. The sequences of the proteins with published proof of IgE binding using sera from appropriately allergic subjects have been included in the AllergenOnline.org database managed by the Food Allergy Research and Resource Program at the University of Nebraska-Lincoln (www.AllergenOnline.org) to provide a bioinformatics tool for the GM safety assessment process. A number of the proteins included in the Allergen Online database have also been demonstrated to cause biological reactivity by skin prick tests of allergic subjects, basophil histamine release or basophil activation and those proteins are more reliably defined as allergens. The references used to categorize each allergenic protein group are listed in the database (www.AllergenOnline.org) along with an explanation of the process of classification. The database also provides sequence comparison algorithms to evaluate potential new GM or novel food proteins for potential risks of cross-reactivity.

It is not clear why people become allergic to certain proteins and foods rather than becoming tolerant to these generally innocuous proteins although there are genetic risk factors for IgE mediated allergy. It is clear that the prevalence of food allergy is rising in industrialized countries and it cannot be explained by changes in the genetics of consumers[37]. There are a number of proposed mechanisms including the "hygiene" hypothesis (lack of certain bacterial types from the environment or within the gastrointestinal tract) for sensitization (induction of specific IgE) and tolerance (suppression of IgE and allergy), but no single markers or hypothesis fits everyone[37]. Very likely multiple factors interact at the time of introduction of foods in the developing child, food processing methods, reduced vitamin D levels due to sedentary indoor lifestyles and reduced exposure to certain microorganisms or reduced parasite burden that together are contributing to increases in allergic

disease.

Celiac disease is elicited by a limited number of glutenins and gliadins from wheat, barley, rye and possibly oats, all members of the Pooideae subfamily of grasses. In order to provide a possible risk assessment tool for food safety assessment, we have gathered 1 016 peptides and 58 proteins f that have been found to stimulate CD restricted T cells into a CD-specific database for use in risk assessment (www.allergenonline.org/celiachome.shtml). We have also developed bioinformatics tools that help evaluate novel food proteins for identity matches to be able to flag potentially important proteins as possible risky proteins for those with CD to consume.

## 2 Assessment of GM crop safety in the US

### 2.1 History of safe use (HOSU)

The scope of the HOSU evaluation of the gene source, the gene recipient and the specific products of the gene includes determining whether there is documentation of direct contact with the protein or indirect contact with metabolites if the protein is an enzyme. Descriptions of appropriate allergenicity and toxicity assessments have been published by experienced scientists who have expertise in those areas[9,38]. In cases where the gene source is a common cause of allergy or toxicity, additional tests are likely to be required compared to sources without any history of allergy or toxicity. For example, peanuts and certain tree nuts (walnut, pecan, almond and hazelnut) are considered common causes of allergy. If a gene is transferred from one of the commonly allergenic sources, specific serum IgE testing is likely to be required similar to the study performed by Nordlee et al., for the Brazil nut 2S albumin[39]. If the gene source is castor bean (*Ricinus communis*), the *Closteridium botunlinum* bacterium or a wasp (*Vespula germanica*), regulators are likely to ask for additional specific toxicity tests to verify that the protein is not a toxin. Specific testing requirements will be dictated by the nature of the risk. If the source has neurotoxicity, then neurotoxicity tests are likely to be called for. The identifiable risks of the source would normally be discovered by searching published peer reviewed literature, although sometimes sources including searching Google may be useful. If there is a clear history of consumption of the source material, and the protein in question is proven to be expressed in the material that is consumed (e.g. the nut, fruit or herbaceous material), the lack of allergenicity or toxicity would aid in determining the protein is unlikely to present a risk. However, in many cases there will not be a history of safe consumption, which does not automatically mean additional tests are required, only that there may be slightly less certainty of safety.

Often there are clear, restricted risks associated with a given gene source. Apples contains two proteins that might be considered significant allergens, a non-specific LTP that is known to cause severe allergy in a very small number of consumers and a less potent, common cross-reactive protein Mal d 1. The Mal d 1 protein is a sequence similar homologue of an airway allergen Bet v 1 that is common in pollen of birch and related tree species. Other proteins from apple are expected to represent low or no risks of food allergy. Peanuts contain four potent allergens and a few additional minor allergens. Food labeling laws are written to differentiate risks of food allergy based on the prevalence and severity of allergy to the sources. In the US, Europe and Japan, peanuts are considered common and important sources of food allergy and any processed food that contains an ingredient from peanut must be labeled as to source. Apples are not considered to be common, potent sources of food allergy. The safety of proteins derived from a peanut gene would be more thoroughly evaluated than a protein from apples for potential risks of allergy.

The source of the insecticidal crystal proteins Cry1A, Cry2A and Cry3A is the bacterium *Bacillus thuringiensis*. Spores of this species have been used as microbial pesticides for 70 years without demonstration that they cause allergies or toxicity in mammals. The historical safe use of the organic pesticides provides assurance of HOSU for some Bt toxins, although that is true only for proteins that are demonstrated to be expressed by the bacteria used as microbial pesticides and not from all varieties of the species.

The developer is expected to provide documentation of the history of safe use of the gene source organism and if possible of the gene products. The description should also include evidence that the protein or other gene products are expressed in the materials encountered in food as well as a description of preparation of the food.

### 2.2 Characterizing the new protein and product attributes

The developer must describe the DNA or RNA sequence transferred in making the GMO. The source of other genetic elements (promoter and terminator) in the construct must be included. The method of transfer must be defined. Confirmation of copy number,

gene integrity and stability of the DNA through reproductive cycles of the organism must be verified. Any gene product should be quantitatively measured under conditions of normal use of the plant. In some cases mRNA size and accumulation in various plant tissues are also necessary to ensure the transcript is as expected. In most cases the gene encodes a protein. If the protein is an enzyme, any expected and measured metabolites must be described. The function of the gene and products must be disclosed. The sequence of the DNA and the protein are disclosed and data comparing the protein amino acid sequence to known toxins and allergens must be evaluated.

### 2.3 Potential allergenicity

Due to the importance of food allergies, the FDA has focused on preventing the transfer of allergens into a new food source as a primary concern for GM crops. A major risk for consumers with allergy to peanuts would be the transfer of a gene encoding a major peanut allergen into rice or corn. That possibility was demonstrated by the experience of Pioneer Hi-Bred when they transferred a gene encoding the 2S albumin from Brazil nut into soybean to improve feed quality for animals. Soybeans have a high concentration of protein, but are deficient in sulfur containing amino acids. The 2S albumin of Brazil nut is a small protein with a high concentration of methionine and cysteine amino acids. Pioneer Hi-Bred was preparing a dossier for submission for regulatory review for this potential product when they consulted with Dr. Steve Taylor at the University of Nebraska who suggested that since Brazil nut is known to cause food allergy in some consumers, the protein expressed by the transferred gene should be evaluated for potential allergenicity. In 1995 no one knew what the allergenic proteins were in Brazil nuts, but during studies described by Nordlee et al.[39], it became apparent that the 2S albumin is an important allergen. The results were published and Pioneer Hi-Bred stopped development of that potential product without submitting it to regulators. The experience helped validate the evaluation process that had been outlined in the FDA Federal Register in 1992. The experience also helped crystalize the evaluation process outlined by Metcalfe et al.[40], for evaluating potential allergenicity of GM proteins and eventually the Codex Alimentarius Commission guideline first published in 2003[1].

Food allergy is usually restricted to reactions mediated by antigen specific IgE antibodies and the mechanisms described can be found in any immunology text book. Most dietary proteins stimulate the immune system to become tolerant to contact with the protein. However, for those prone to allergies, their T helper cells and B cells may become educated to develop IgE immunoglobulin production because of the mixture of cytokines and cell surface signals provided by T-helper type 2 cells. The B cells differentiate into plasma cells or B memory cells expressing high levels of protein-specific IgE that becomes bound to the FcεRI high affinity receptors on mucosal or dermal mast cells and blood basophils. When the antigen is absorbed again in subsequent meals, it cross-links IgE antibodies on the receptors if at least two epitopes are bound and initiates a signal cascade. If a sufficient number of cross-links occur within a few minutes the mast-cells or basophils releases histamine, leukotrienes and proteases that elicit vascular leakage and inflammation. Symptoms may include angioedema, urticarial, asthma, emesis (vomit), hypotension (drop in blood-pressure) and in rare cases death due to systemic anaphylaxis. Since the IgE antibodies are specific in peptide epitope recognition, the symptoms are reproducible; same antigen, similar reactions. Generally allergic sensitivity is assumed to be life-long. Many dietary proteins also induce IgG and IgA antibodies, but those are not risk factors for acute food allergy. Production of these immunoglobulins by B cells also requires T cell help, but the responses and signals differ from those leading to IgE responses. The focus of the allergenicity evaluation is therefore on measuring IgE responses.

There are also T-cell mediated reactions to some dietary proteins, the major one being gluten-sensitive enteropathy or CD as discussed previously. Evaluating GM proteins for potentially eliciting CD is relatively straight-forward and will be discussed later. There are rare cases of T cell mediated food protein induced enterocolitis syndrome (FPIES), which is a severe reaction primarily to proteins in cow's milk or soybean but occasionally to proteins in rice or oats and a few other foods[41]. Individuals usually become tolerant to the responsible food within three to five years and no specific proteins have been identified as the causative agents. Therefore it is not possible to evaluate proteins as a possible cause of FPIES at this time.

There is credible evidence that the prevalence of food allergies and celiac disease are on the rise globally, although there is great uncertainty about the magnitude of the rate of increase and the cause. Part of the increase is likely due to increased consumer awareness of allergy and CD as well as more awareness and testing by doctors. There is much misinformation about prevalence and people are often incorrectly

diagnosed. Many individuals reported being food allergic, but a clinical evaluation demonstrates they are not food allergic in many cases.

The major risk for food allergy is acute, within minutes to hours after consumption of the allergenic food. The primary risk of food allergy from GM crops is the potential transfer of a protein that already causes allergy in specific consumers. If affected individuals consume a biotech crop that includes their allergen, reactions would likely be as severe as they would be to the natural source of the allergen. Thus the primary concern for GM crops is to avoid the transfer of a protein that already causes allergy (of any kind, contact, airway or food) into a food grade plant of another species.

The International Life Sciences Institute (ILSI)-Allergy and Immunology Institute and the International Food Biotechnology Council organized discussions and a series of scientific peer-reviewed publications to consider potential risks of food allergy from GM crops. The publications were presented in a special issue of Critical Reviews in Food Science and Nutrition (Vol. 36, Supplement, 1996). Panelists included scientists with expertise in biotechnology development and regulation or allergens and allergy. The first chapters explain allergy, food allergy, the biology of plant proteins the process of genetic modification of food plants and review allergenic foods known at the time. Two chapters provide the basis much of the background information that guided development of a science based assessment process to evaluate potential risks of food allergy for novel proteins[42-43]. The last chapter outlines an evaluation process to determine whether a protein expressed by a transgene would potentially present a risk of food allergy to consumers[40].

The evaluation process outlined by Metcalfe et al.[40] was consistent with the FDA recommendations of 1992, and included decision tree flow-chart beginning with evaluating the allergenicity of the source of the gene. However, the decision tree did not exactly match the description in the text and some things were not clear. Fig.1 represents my interpretation of the tree from the text[40]. If the gene is from a clearly defined allergenic source (food, airway or contact allergen), the next step would be to obtain sera from 14 humans allergic to the source and test for IgE binding to the GM protein using standard laboratory test methods. If fewer than 5 allergic donors are found for the test, then the protein is evaluated for stability to digestion by pepsin. All proteins regardless or source should be evaluated by sequence comparison to known allergens and a list of known food and respiratory allergens known in 1995 was included[40]. They recommended using FASTA to align the protein to known allergens and search for any contiguous 8 amino acid segment having an identical match to any allergen. In practice

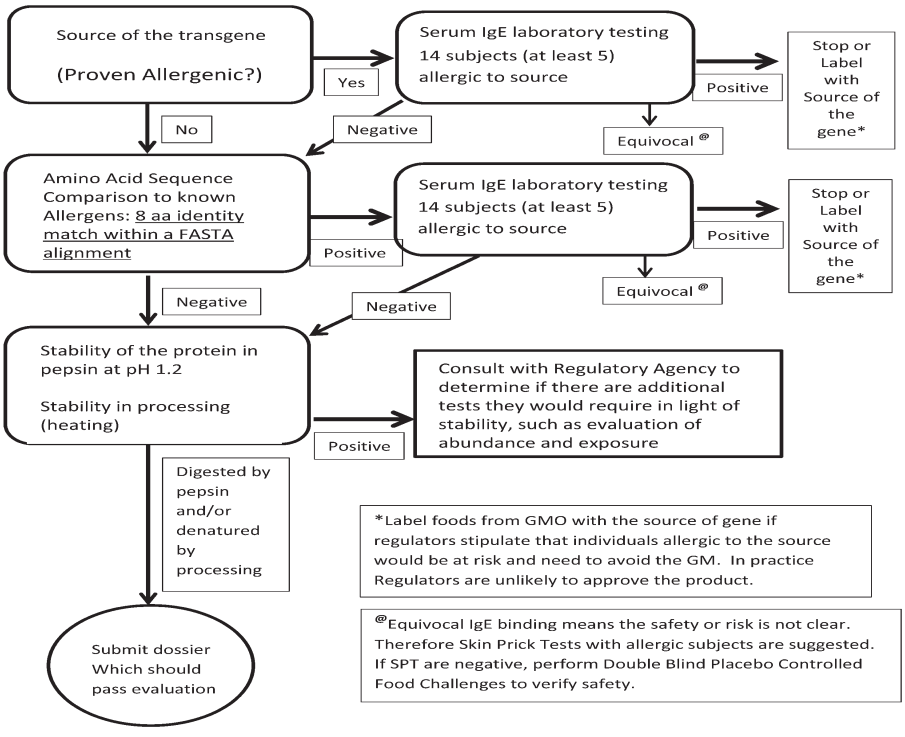


**Fig.1** Assessment of the allergenic potential of GM proteins, adapted from Metcalfe et al., 1996 to more accurately reflect the description in the text.

most bioinformatics matches were simply performed by a sliding "WORD" match of 8 amino acids. If the protein matches an allergen, sera from humans allergic to the source of the matched allergen would be tested for binding. If IgE from appropriately diagnosed allergic serum donors clearly binds to the protein, the developer is likely to stop development and not submit the dossier to regulators. However, if they would try to continue, the regulator would likely demand foods made from that GM event would have to be labeled as to the source to alert allergic subjects to avoid the food. In the case of inconclusive IgE binding, subjects allergic to the source would be tested by skin prick tests (SPT) with the protein and if all are negative, they would be asked to undergo a double-blind, placebo-controlled food challenge (DBPCFC), under ethical panel approval. In addition, the protein would be tested for stability in acid with pepsin (protease) and the time of disappearance would be graded to evaluate digestibility. The protein might be tested following typical food processing methods for that specific crop to determine if it denatures. However, processing stability is really only useful to understand if a risk of allergy or toxicity can be mitigated by normal food processing, similar to the inactivation of natural lectins and protease inhibitors in legumes during cooking. If the protein is stable to digestion by pepsin, the regulatory agency would be consulted for any requests for additional tests.

Most GM events and newly expressed proteins approved in the US meet the criteria for minimum risks regarding the allergenicity evaluation presented by Metcalfe et al.[40] and the Codex[1]. A literature search finds only one potential GM crop product that did not fit the history of safe use and upon testing for serum IgE binding using samples from the at-risk population of Brazil nut allergic subjects was found to bind IgE[39]. That potential product was not submitted to regulators and was terminated by the developer (Pioneer Hi-Bred). No currently approved product that I am aware of received a gene from a commonly allergenic organism. Thus the Brazil nut 2S albumin is the only one that would have presented a major risk of food allergy to a subset of consumers.

### 2.4 Bioinformatics for matches to allergens

In addition to evaluating the source of the gene the bioinformatics search for identity matches between the GM protein and any known or suspected allergen has become probably the most important tool to identify possible risk and a reason to do serum IgE testing[38,44-45]. The Codex[1] document calls for a FASTA or BLASTP search with the amino acid sequence of the GM protein against a database known allergens. The www.Allergenonline.org database is the most comprehensive peer-reviewed allergen database that I am aware of. The criteria of greatest emphasis is any match >35% identity over any segment of 80 or more amino acids. The Allergen Online database (www.AllergenOnline.org) was established in the Food Allergy Research and Resource Program at the University of Nebraska in 2004 and implemented an expert review process. It is updated annually to provide a curated database and search algorithms for risk assessment of allergenicity using bioinformatics tools[44].Version 14 of the database was released in January, 2014 and includes 1 706 sequences from 645 protein-taxonomic groups representing 290 species. In my opinion the search for >35% identity over any segment of 80 amino acids is quite conservative as described in a number of publications. There is little evidence of in vitro cross-reactivity for proteins sharing less than 45% identity by overall alignment (full-length). And in terms of shared allergic reactions due to cross-reactivity, very few proteins sharing less than 50% overall identity matches are cross-reactive[46]. Since 1996 there have been a number of scientific consultations and recommendations for "improving" the allergenicity assessment of GM crops. The FAO/WHO expert panel review[47] suggested including a number of changes that were not validated. The FAO/WHO recommendation was to use FASTA or BLASTP to identify any segment of 80 or more amino acids with an identity match of >35%; and to search for short identity matches of six contiguous amino acids (aa) rather than eight aa suggested by Metcalfe et al.[40]. But those precautionary criteria have not been validated. They were reviewed in previous publications[3,38,45]. A number of studies demonstrated that searches for six aa identity matches produce far more false positive matches than true positives. The eight aa matches are better, but still do not have a high predictive value. Others have also described the 80 amino acid searches as being overly conservative; however I have not identified many probable false positives using that criterion, but also did not miss any likely cross-reactive protein pairs[45]. There is some disagreement about the optimum algorithm for the 80 amino acid search as some bioinformaticians suggest searching for the best overall alignment by FASTA or BLASTP and then scoring the best match as a more reliable alternative[48-50]. Recently the European Food Safety Authority (EFSA)[51] dropped the recommendation

to search for short, contiguous identity matches and the European Commission[52] accepted that advice in dropping it from their regulations. There have been a few instances where short contiguous identity matches that occur by chance (no evidence of overall sequence homology), have led to requirements for serum IgE tests that were not needed[53]. There is a risk in performing such tests as in vitro IgE binding results can be ambiguous, with false positive binding that may inappropriately implicate a protein as a possible allergen[54]. Although there is little relevance of IgE binding to a single short segment of two non-homologous proteins means a risk of allergy in that situation is unlikely, some regulatory bodies would want the developer to continue the investigation and possibly demand *in vivo* testing in humans.

### 2.5 Serum IgE testing

Serum IgE tests are rarely warranted for evaluating the potential allergenicity of GM proteins. However, if serum IgE testing is warranted the assays must be well designed, the methods should be validated with known allergens for the allergic serum donors and the test subjects should be demonstrated to have the appropriate IgE sensitivities. Test materials should include purified GM protein, purified allergenic target (e.g. the sequence matched allergen), and the specificity of any IgE detection antibody must be verified, appropriate blocking solutions are needed. In some cases specific inhibition assays may be required. Critical factors in materials and assay design are presented in a number of publications[45,54-55]. If the protein may contain asparagine-linked carbohydrates, there is the possibility the plant might modify the protein with the addition of alpha-1,3 fucose or beta-1,2 xylose on the stem of the asparagine-linked glycan may bind IgE from many subjects, but there is little or no evidence for clinical reactivity[56-57]. Those structures are known now as cross-reactive carbohydrate determinants (CCD). If there is a signal peptide and an N-linked sequon (Asn-x-Thr/Ser), the protein should be tested for the presence of CCD. Inhibition studies may need to be performed to evaluate the relevance of any *in vitro* IgE binding. If there is evidence of IgE binding in vitro and there is a desire to continue with development of the product, the biological relevance of binding may be tested using basophil activation or basophil histamine release[58]. Alternatively, skin prick tests (SPT) or double-blind, placebo-controlled food challenges may be required using highly characterized test materials and subjects who are well informed and have consented to the challenge.

### 2.6 Potential de novo sensitization: stability in pepsin and abundance

If there is no evidence the protein is likely to be an allergen based on source and a lack of bioinformatics match, there is no justification for performing serum IgE tests. There is a low probability of risk and there is no at-risk population. The only other questions regarding allergenicity are whether the protein might sensitize de novo. As suggested by Metcalfe et al.[40], proteins that are stabile in pepsin in an *in vitro* digestion assay and are abundant, have a higher probability of being an important food allergen. However, the correlation is modest even though many major food allergens are stable or fractions of the protein are stable and abundant[59]. The correlation of stability in pepsin has been performed at pH 1.2 and 2.0 and the FAO/WHO, 2001 recommendation was to use both conditions to evaluate stability. We did not find any significant difference[59]. The EFSA[57] recommendation was to use more "physiological" pH (3.5), but that has the effect of markedly reducing pepsin activity and has not been investigated in terms of predictive value. The FDA continues to accept the use of either pH 1.2 or 2. There is also not a consensus on abundance although it is clear that the abundance of a number major allergenic proteins in plants used for foods is greater than 1% of the protein in the food fraction[60].

Most of the GM proteins have been found to be digested rapidly in pepsin at pH 1.2 or 2. However, the Bt protein Cry9C that was originally introduced into corn to protect against the European corn borer moth larvae by Plant Genetic Systems (PGS) in Ghent, Belgium was found to be quite stable in pepsin. The product was called StarLink corn. The company was purchased by AgrEvo, then Aventis CropScience which was finally acquired by Bayer CropScience. Food approval was withheld because the protein was stable in the pepsin digestion assay (described later) and regulators felt there was some risk the protein might eventually sensitize someone, predisposing them to allergic responses to Cry9C. StarLink corn was grown on ~ 122 000 hectares in the US in 1999, and some grain from the corn was accidentally, but illegally included in some human food products (corn chips and taco shells). Tests by an anti-GM NGO discovered the inclusion of StarLink corn event in some food products and notified the US government and news media. Interestingly the question was whether people might become allergic to the protein, which would take time to sensitize people. There is no indication that people

were pre-exposed to Cry9C, so sensitization would have been from exposure in the contaminated taco shells and chips. However, within two weeks of the announcement more than 100 consumers complained they had experience food allergic reactions following consumption of taco shells or corn chips. Since corn is one of the least allergenic of grains and the quantity of Cry9C was quite low in corn grain and the grain was grown for only one year, it is highly unlikely that anyone was sensitized to the protein. However, the Center for Disease Control of the US investigated each consumer report. Those individuals who claimed reactions that might be consistent with food allergy were asked if they would provide blood samples and 18 did[61]. None of those individuals had IgE specific for Cry9C[61]. Since the grain and corn seeds were released and in food without approval, the US government demanded recalls and monitoring. Foods, ingredients and corn seed were screened and those containing the Cry9C protein or the transgene were pulled from the market. It took six or more years to completely remove all traces of Cry9C from seed and grain stores. There have been rough estimates that total costs for removal may have exceeded $500 million. Yet we should remember that there is no proof that anyone was harmed by consuming Cry9C. There is clearly a different level of risk of allergy that might be present from a GM food crop such as StarLink than would be associated with an outbreak of Norovirus, hepatitis or *E. coli* O157. We might conclude that the regulatory response was not in proportion to the risk in the case of StarLink. However, the ability to remove a GMO from production was demonstrated by the recall of StarLink corn and it shows that you can remove a GMO from the agricultural and food system if there is a reason to do so. It just takes time and an enormous amount of money.

Another product that is not as rapidly digested in pepsin as Cry1Ab (in corn) or CP4 EPSPS (in herbicide tolerant soybeans) are the two proteins (Cry34Ab2/Cry35Ab1) in another insect protected corn event. The proteins have intermediate stability as reported by Dow, the developer[62]. The EPA did allow this product into the market as the abundance of the proteins is low in grain and the stability intermediate.

New proteins expressed in the GM crops approved so far have been expressed and accumulate at low levels in the food materials of the crop, often in the range of or less than a few micrograms per gram dry weight of seed (CERA GM Crop database, 2014; http://cera-gmc.org/index.php/GMCropDatabase). Thus all of the GM proteins accumulate at levels markedly below the concentration of most of the important dietary allergens (typically >1% of total protein).

There is no published evidence that an approved GM crop has caused allergies due to the presence of the transgenic protein. A study was performed to determine whether soybean allergic subjects might have IgE binding to the CP4 EPSPS enzyme that was introduced into soybean to provide tolerance to the herbicide glyphosate[55]. This was not a regulatory study, but was performed as a stewardship study to see if there was any evidence of sensitization years after the product entered the market. Serum samples were collected from soybean allergic subjects in Europe and South Korea and tested using common protocols and highly characterized test materials. The study did not find evidence of IgE binding to purified CP4EPSPS or to the protein in extracts of GM soybeans[55].

### 2.7 Potential improvements for evaluating IgE mediated allergenicity

The FAO/WHO panel[47] recommended using targeted human serum testing in an attempt to determine whether a protein that is not similar to any known allergen might pose a risk due to existing sensitization or cross-reactivity. Targeted testing was defined as in vitro IgE binding tests using sera from 50 subjects with allergy to sources that are broadly related to the source of the transferred gene. For genes from a dicotyledonous plant, individuals allergic to one or more other dicot species would be used for serum testing. There was an exemption for proteins from bacteria since there are almost no allergies to bacteria. The targeted serum testing has never been tested in a way that would demonstrate its predictive power and it is counter-intuitive based on our knowledge of cross-reactivity. Homologous proteins from even moderately related sources (family level) are rarely cross-reactive by in vitro tests and clinical reactivity is rarely shared. The only proteins that are so broadly cross-reactive in laboratory tests are profilins, PR-10 proteins (Bet v 1 homologues), lipid transfer proteins and tropomyosins from crustaceans and other invertebrates. Those are all easily identified by bioinformatics. The US does not recognize targeted serum testing as a useful tool for the assessment of novel proteins.

The FAO/WHO[47] also recommended performing sensitization tests using two species of animal models, or two routes of sensitization in one species to evaluate the allergenic potential of each new protein. While many laboratories have tested various animal models in an attempt to predict the allergenicity of proteins, there

are none that have proven predictive over a wide range of effective allergenicity (from mild or non- to strongly allergenic), as reviewed by Ladics et al.[63]. There has been research that shows some promise for evaluating mechanisms of allergy and immunotherapy[64] and for preliminary ranking of allergenic sources[65-66]. A few have tested purified or partially purified proteins[67], but have not been validated to rank new proteins in the context of potency or prevalence of allergens in the human population[63]. The US does not recognize animal models as being useful at this time for predicting the allergenicity of novel proteins.

The Codex guideline did incorporate the recommendation for testing the sequences using the FASTA or BLASTP search alignments to identify matches of >35% identity over any segment of 80 or more amino acids. Codex[1] also retained the language suggesting the use of a short identity match of 6 or 8, but suggested the evaluator must justify that choice. The US regulators now expect a comparison for identifying matches with >35% identity over 80 amino acids using a comparison like that available on www.AllergenOnline.org or a full-length comparison by BLASTP with evaluation of alignments to meet the same criterion. They do not seem focused on short-8 amino acid matches, but most (all?) developers have supplied that data.

The EC regulation[52], which was based to a large extent on recommendations from another expert panel review process by the EFSA[51] also includes a number of suggestions for unproven tests including: evaluation of potential adjuvanticity of the new protein; the use of proteomics to consider possible changes in the expression of endogenous allergenic proteins for commonly allergenic species (e.g. soybean, peanut); and the use of more physiological pH (3.5) for the pepsin digestion assay. Yet those test methods have not been validated to demonstrate they would improve the risk assessment and are not asked for by US regulators. The US regulators do not ask for additional tests such as potential adjuvanticity unless there is information that would reasonably support the hypothesis that a new protein may be a lectin or have some other adjuvant-like properties.

### 2.8 Celiac disease

Risks related to CD have only been found to involve certain glutens (gliadins and glutenins) from wheat and near wheat grain relatives. Codex[1] recommends and the US government would require an evaluation if a gene from wheat, barley, rye or possibly oats, is transferred into another species, such as corn, rice, or sorghum. As far as I know, no developer has submitted a potential product to US regulators using such a construct. While the Codex demands an evaluation for proteins from wheat or wheat relatives, they have not provided guidance on the process. My laboratory considered the problem in the context of what is currently known about CD and the glutens involved in and developed a celiac database to provide a bioinformatics tool to allow rapid identification of potential hazardous proteins. In order to develop the tool we reviewed published scientific information on CD.

Symptoms of malabsorption and diarrhea associated with diet of bread were first described nearly 2 000 years ago in medical writings from Greece[68]. But it wasn't until 1888 that a physician in the United Kingdom (UK) gave the name coeliac (or celiac) to those suffering intestinal distress associated with eating foods containing wheat. Those observations were lost on modern medicine until 1952 that physicians in the UK published descriptions associating the wasting and intestinal pathology with the consumption of wheat. In the 1990's gastroenterologists developed methods for endoscopy and developed antibody tests that demonstrated patients with CD were developing antibodies that bound to connective tissue in the intestine and had T cells that were activated upon binding wheat peptides from glutens in the context of specific major histocompatibility antigen resenting receptors. Recent studies have identified many peptides from glutenins and gliadins of wheat, barley and rye grains that are responsible for activating T cells in genetically susceptible individuals[30, 69]. As many of these discoveries were occurring in the mid-1990's and beyond, the evaluation of proteins in wheat, barley and rye that might be responsible for causing the T cell specific responses in the fraction of subjects with the correct MHC Class II for susceptibility (MHC DQ 2.5 and MHC DQ 8) were just emerging. Since then many studies have been published that identified peptide sequences that are responsible for binding to the right MHC and activating effector T cells in those with CD have emerged. While the Metcalfe et al.[40] and the Codex[1] recommendations do not recognize the predictive capabilities of bioinformatics to evaluate risks of celiac disease from wheat subfamily proteins, it is clear that a substantial number of proteins were being identified that might serve as a database of "risky" proteins. Metcalfe et al.[40] and the Codex[1] both suggest that genes taken from wheat or wheat relatives that encode proteins should be evaluated for

their potential to cause CD, they did not specify how. In 2011, Plaimein Amnuaycheewa, a PhD graduate student in my laboratory reviewed more than 50 available publications identifying peptides involved in T cell reactivity using cell samples from celiac patients and we developed a database of peptides that can be used to screen potentially hazardous peptides from proteins from the wheat and wheat relatives. We have constructed a database of peptides from wheat, barley and rye that cause T cell stimulation or intestinal epithelial pathology (www.allergenonline.org/celiachome.shtml). The database is part of the www.AllergenOnline.org database for bioinformatics evaluation of potential IgE mediated allergenicity for GM proteins. Currently it includes 1 016 peptides with published evidence of T cell reactivity using cells from CD patients in the context of MHC Class II DQ 2.5 or DQ8, or toxic effects in intestinal epithelial cells or pathology in intestinal villi from those with CD. The amino acids of proteins introduced into GM crops may be searched for exact matches to the peptides in the database, or the proteins can be searched by FASTA for meaningful matches to 68 whole proteins known to stimulate CD, using criteria of >45% identity over alignments of at least 100 amino acids as potentially stimulating CD. A total of 53 references are included to explain the selection of peptides and proteins that might cause CD in susceptible individuals. Similar to the allergenicity assessment, bioinformatics methods should are able to identify proteins that might represent a modest to clear risk of causing disease. If there is a desire to introduce a wheat sub-family protein into another crop e.g. rice or eggplant (brinjal), the amino acid sequences there should be screened using this database to consider risk. If a positive match is found, the protein should be tested using cells or challenges in CD subjects to evaluate risks using cell based assays or possibly food challenges in at least 10 consenting CD subjects to ensure minimal risk to the CD population as the "at-risk" group of consumers. The bioinformatics criteria we believe is predictive based on extensive simulations is any 100% identity match to one of the 1 016 peptides or a FASTA match of >45% identity with any segment of 100 aa or more having an E score of < 1x 10-15. Genes taken from plants outside of the Pooideae subfamily of grasses represent little risk of causing CD and therefore even if they are homologues of glutens that cause CD, they are highly unlikely to result in disease. Proteins that do not exceed these criteria should present little or no risk of inducing CD.

### 2.9 Potential toxicity

Few proteins are toxic when consumed and most of those act acutely (e.g. ricin)[70]. The HOSU evaluation is a key consideration in addition to a bioinformatics comparison of the amino acid sequence of any newly expressed protein to the NCBI protein database using a keyword limit of "toxin" or "toxic". Although it seems there is a lack of published data on how to perform a bioinformatics evaluation for potential toxicity for a GMO, all GM products submitted to the US FDA or EPA must undergo an evaluation[71]. I have performed the bioinformatics searches for a few potential GM crops and novel food ingredients for regulatory submissions using the general NCBI protein database using BLASTP with keyword limits of toxin or toxic to focus on potential risks. Usually additional sequence comparisons are needed using the new protein in the search but without keyword limits to provide a relative comparison of other proteins with a known history of safe use or safe human exposure and the query protein (GM protein) or novel food ingredient. The process also requires a careful evaluation of published scientific literature related to the closest sequence matched proteins as well as the protein of interest. While bioinformaticians often claim that proteins sharing greater than 25% identity over their full-length are homologues and often have similar functions, most proteins with such relatively low identities do not share specific toxic properties or exact enzymatic functions. Therefore bioinformatics evaluations must be evaluated relative to other proteins. The results should guide decisions regarding a need for any toxicology testing, and if needed, the target organs and tests that might be useful to evaluate risks. So far there is no evidence that any protein introduced into a GM plant approved in the US has had a toxic effect of humans or other mammals.

In the US regulatory system, if a protein introduced into a GM crop is intended to have toxic activity to insects, bacteria, a fungus or have anti-viral activity, such as the plant incorporated pesticidal Bacillus thuringiensis crystal proteins, the proteins must be tested by an acute mouse toxicity test. The OECD guideline for acute toxicity testing (E425, 2001) is the model followed in many studies. The protein is gavaged into adult mice using a dose that is typically 1 000 fold higher on a mg protein per kg body weight, expected for human food consumption. Sometimes the excess dose is not quite so high, but normally at least 100 fold higher. The dose is given on day 0 and the health of the animals is monitored along with control (mock-dosed) animals for 14 days. At that time

body weights, blood samples appearances and clinical observations are collected. The animals are euthanized and gross pathology and if needed histology samples are examined for abnormalities. Usually there are 10 animals per sex per treatment group. Quite often two doses are used as separate treatment groups to ensure that any abnormality has a dose-effect. While there may be some statistically different findings for a few measurements between groups for the GM and control animals, historical weights and measures of the same strain of mice should be available for that specific toxicology facility to be able to evaluate unexpected differences. Some studies describing the acute mouse toxicity tests for approval of some GM products have been published[72-74]. In rare circumstances longer term toxicology studies are called for by regulators or critics of the technology, but the scientific justification for extra testing is usually quite weak. It is important to consider that unlike a number of organic compounds or heavy metals, consumed proteins do not accumulate in the body of mammals and toxic effects are expected to be acute rather than chronic.

Some countries (e.g. within the European Union) require an acute mouse test as well as a subchronic, 90-day whole-food feeding study in rats, or repeat dose testing with high doses of whole protein. While the 90-day study design is detailed in the OECD guidelines and a few published studies have been performed, there is not a good justification and little proof that such a study will identify known hazards[75]. The 90-day rat feeding study is more of a hybrid toxicology-nutritional study. Some regulators and critics suggest that the 90-day study provides a tool to evaluate "unintended effects" that might arise due to the insertion site of the new gene into the genome of the crop. It should be worth considering that the host (recipient) crops are normally species that have been consumed for centuries with good history of safety and that genetic variation in naturally bred varieties and lines have introduced many unintended genetic changes without introducing adverse toxic properties in the food.

Two studies designed to test the predictive value of the 90-day rat whole food feeding study using experimentally designed recombinant rice gave somewhat conflicting results[76-77]. The first tested a GM rice expressing the snow-drop lectin from Galanthus nivalis (GNA) and the authors conclude that the study failed to show the potential toxicity of the lectin. The second experimental GM expressed high levels of the common bean phytohemagglutinin lectin PHA-E, which did show toxicity when the protein was fed in raw form at high concentration. My interpretation is that the raw, uncooked PHA gave significant toxicity as would be expected to occur in humans consuming raw kidney or navy bean. The GNA study seems to have had negative results because the protein expression was too low in concentration or the protein was heated in feed preparation. Since humans can consume cooked kidney and navy beans, but not raw beans, it seems the test results were predictive of the human experience. It might have been more appropriate to test raw and cooked samples as two separate treatments. The assay is not very sensitive and there are severe limitations to the dose that can be feed compared to the human diet, typically much less than the 100 fold safety factor typically used in toxicity studies. Many toxicologists have questioned the usefulness of the 90-day whole food feeding study[9]. While others claim even more detailed, complex and expensive studies are needed to fully test potential toxicity[78]. However, a recent peer reviewed evaluation of published safety, toxicology and whole grain rat feeding studies on current GM crops provides objective evaluation of the overall approach and concludes that in most cases a 90-day feeding trial is not needed to evaluate safety, but results are certainly consistent with safety[79]. Interesting at a time when animal welfare groups and even the institutional animal care and use committees in many institutions are calling for reduced animal testing, some scientists involved in regulation or testing are calling for more unproven animal studies.

### 2.10 Additional toxicology studies

Questions should be asked about any new proposed toxicity test, as well as existing testing methods. What types of hazard can be or has been identified with a given test protocol? What is the rate of false positive and false negative results for each test? And finally, are there more effective tests that could be used? A number of recent publications have discussed the pros and cons of using alternative computer based, cell-based, or tissue based methods, primarily for pharmaceutical toxicology evaluation[80-81]. They focus on having a scientifically sound hypothesis, validated methods and historical control data as essential criteria. Understanding the limitations and benefits of the different models are essential in making a determination about tests that might be useful for evaluating potential toxicity. In most countries including the US, there is a general requirement by animal care and use committees to show that the specific test on the specific test material has not been performed previously unless there is a reason to doubt the results. Therefore repeating

the same animal tests on the same GM crop event in multiple countries is deemed unethical.

The final conclusion of toxicity evaluations should be either the GM crop does not pose any additional significant risk of toxicity compared to similar non-GM varieties, or that it does pose a substantial new risk. The FDA and EPA have been able to reach those conclusions for many new GM products if the developer followed the standard assessment process. Unfortunately some regulatory bodies (e.g. EFSA and the European Commission) in Europe and regulators in India and China continue to raise new questions about hypothetical concerns including potential adjuvanticity, alteration of fertility or the potential to induce cancers even though there are very few examples that any dietary protein could have such an effect. Those regulators then fail to approve products for which there is no evidence of risk. The US regulatory agencies have emphasized the need to use proven methods to evaluate safety of novel proteins and GM products. They have not asked (so far) for additional studies that are not already demonstrated to help assess safety. However, if a developer provides data from a new evaluation, they will consider it, although it may delay approvals or acceptance.

## 3 Evaluting GM products for unintended effects

The methods and genetic modifications used to generate the herbicide tolerant or insect protected traits that have been widely adopted following regulatory approvals introduce relatively minor variations in the host plant genomes compared to those introduced through "natural processes" of mutations and reproduction. Interestingly those "unknown" natural changes are not characterized except by phenotypic variation and they have evolved to provide the diverse genetic background needed to allow plant survival with challenges of plant diseases and pests, and diversity of climactic conditions and soils. The GMOs on the other hand have been characterized in insertion site, copy number, gene sequence and encoded products. If the introduced gene encoded an enzyme, metabolites of the enzyme would have to be evaluated. Interestingly, a good portion of the maize (Zea mays) genome is made and modified by transposons that were described as "jumping genes" by Barbara McClintock from her studies in 1948. She was awarded the Nobel Prize in Physiology in 1983 for that discovery[82]. A recent study identifying genes in 503 genetically diverse lines of maize found ~ 16% of the genes are not present in all 503 lines, showing marked genetic variation[83]. The bread wheat we consume today (Triticum aestivum) is encoded by three sets of chromosomes (thus is an evolutionary hexaploid) of relatively primitive grass species so that most proteins in wheat are encoded by three sets of divergent genes that are nearly identical in some cases, or very different. In addition, the replication of genomes through sexual reproduction allows gain or loss of function and extension of the capacity of the plant to grow in different environments or have multiple options for nutrients (or anti-nutrients). Bread wheat and pasta wheat (Triticum durum or Triticum turgidum subsp. durum, an evolutionary tetraploid) are both nutritious and used extensively in human food. But both cause celiac disease in about 1% of the general population in North America and Europe, genetically susceptible individuals (25% of the population) and IgE mediated food allergy in a much smaller number of people (<0.4% of the public). Those are non-GM crops as there are no approved GM wheat varieties (yet). That illustrates that all foods represent some risks for some consumers and that it is necessary to have genetic variation to produce the foods we eat.

We should step back and consider why we eat certain foods like rice, wheat, soybeans and maize and other foods, but as humans we do not eat grain alone. Humans have selected certain food sources for ease of production but mostly for nutritional value, measured by average energy, amino acid composition, lipid content, carbohydrates, vitamins and minerals. Those crops were initially grown and consumed long ago and dramatically changed by breeding and cultivation without any scientific measure of specific amino acids, caloric density, vitamins or fatty acid profiles. In the past 100 years we have learned how to measure those components and also in many cases believe we know what a "healthy" and "nutritious" diet is made up of. Typically it is a mixture of different foods. Even though we have all that information today we do not make detailed measurements of the composition of every shipment of grain that goes into a box of cereal or a loaf of bread because would cost too much and we also know that on the average safety and nutrition of the cereal or bread is fine. We have learned the primary components of each major food crop and have typical measurements that are tested by agricultural nutritionists to ensure they formulate optimal diets for agriculturally important species. Each crop has specific components that are evaluated, and nutritionists have ranges that they deem acceptable for animal feed.

### 3.1 Key nutrients and anti-nutrients

Key nutrients and anti-nutrients expressed in the host plant (gene recipient) are to be measured and evaluated relative to non-GM varieties or lines intended for the same uses. There is an expectation that the key components will fall within the range the same components in non-GM events of similar genetic background[84-85]. But as with all statistical measures, statistically significantly different values are expected when measuring many components. However, statistical differences alone are not a reason to reject a product as unsafe; there should be a scientifically based rationale to suggest potential harm. In order to provide guidance on appropriate compositional traits for given food crops recent historical records for varieties of the same crop must be found or a number of commercial varieties must be planted in adjacent plots in multiple field trials.

Animal nutritionists understand the differences in compositional measurements that are important for canola, corn, cotton, potatoes, soybeans and wheat. And many possible compositional measurements are irrelevant to the typical use of these crops. However, some GMO regulators and critics expect that developers will measure every possible component of the GMO and compare it to the nearest genetic relative. If there are statistically significant differences some would argue it is due to the insertion of the DNA and that the food is unsafe. Yet we have also learned that plants from genetically identical plants grown in close proximity or 100 miles apart can differ in many components due to micro-environmental differences. The complexity of the genotype and environmental interactions that can lead to significant differences in expression of some components of agriculturally important crops has not been sufficiently evaluated in terms of biological relevance, yet some scientists are calling for increasing the use of various omics-techniques to measure variation with high precision (Doerrer et al., 2010). Fortunately, even though the compositional analysis is considered an important part of the safety assessment of a GM crop, in the US and most countries regulators have not blocked an approval of a GM food or feed crop due to minor statistical variations in composition as it is clear that non-GM products often have fairly marked differences in components without measurable effects on food or feed safety (Privalle et al., 2013). An important recent finding by two different groups is that compositional differences between GM and near-isogenic lines are primarily due to back-crosses and conventional breeding and are not caused by insertion of the gene[86-87]. Understanding the source of variation is an essential consideration as some authors are suggesting complex proteomics analysis of potential differences in endogenous allergen levels in GM plants might be due to insertion and require additional tests[88].

Thus we need further definition of the important components to measure and guidance on the variation of those components that may have biological relevance. In order to provide some references for composition, the biotechnology industry supports the International Life Sciences Institute (ILSI) Crop Composition Database[89] that contains compositional data for seed of corn, cotton and soybeans (https://www.cropcomposition.org/query/index.html). The data is limited to years 1995-2005 and specific countries of cultivation. The ILSI database is scheduled to a new version released by the end of 2014 that will include many more data-points and expand to include sweet corn, canola and rice. Additional information is available for rice and soybeans from a Japanese composition database (http://afdb.dc.affrc.go.jp/afdb/index_e.asp). The data is available for a limited set of varieties of these two crops and limited years of cultivation from Japan[90]. These databases provide some information about methods and ranges of components specific for the species. Interestingly the animal feed industry is most sensitive to changes in composition of commodity crops as slight variation in feed quality can mean profit or loss to major animal producers. Companies like Tyson (USA), with more than 4 000 poultry farms in chicken production and Perdue Farms (USA), second leading poultry producer in the US measure composition of feed based on nutritionally important ingredients that are crop-specific. In order to formulate optimum feed for growth and safety they measure proximate analysis of every delivery of commodity crop getting random representative samples from their extremely large shipments, measuring total protein, lipid, carbohydrates, moisture, ash, fiber and often the amino acid composition as well as crop specific vitamins, fatty acids and minerals. They also measure crop specific toxicants and anti-nutrients. The poultry industry is the most sensitive to nutritional quality changes. In addition, every shipment of corn grain or dry distiller's grain is checked for mycotoxin levels as corn is the most likely crop to have contamination. An example of a chicken broiler study on a GM stacked-trait event describes the feed ingredient evaluations and provides real data that would be similar to the analysis performed

by Tyson or Perdue[91]. The major components and measures essential to optimum chicken growth that are evaluated in feed preparation do not include a long list of metabolites, RNA transcripts or proteomic measures. Instead they focus on components that are known to contribute to the substantial growth rate (approximately a 35-fold increase in body weight from hatching through day 42 of the studies) for the chickens. The feed efficiency and weight gain are highly correlated to nutritional properties, more so than any other animal species. The production of feed lot size and the number of animals in commercial production units is normally quite large. Since chickens are fed defatted soybean meal, the composition of fatty acids and lipids is not as critical for soybean ingredients as it is for mammalian species, such as dairy cows. Most dairy farms, beef, pork, goat and sheep operations do not monitor every shipment of feed, except for mycotoxins in corn, but instead sample occasionally throughout the year to re-formulate diets if the typical component nutritional values are changing. In the US studies supplied to regulatory agencies include proximate and specific ingredient measures comparing the new GM line ingredients (seeds, grain or forage) and ingredients from a nearest genetic comparator of non-GM line and ingredients from three to five other commercial non-GM lines, all grown at multiple geographical sites to provide environmental diversity for plant growth. Some countries But in general a GMO developer must provide specific composition to regulators from multiple years of multiple geographical replicates of the GMO and a number of non-GM comparators to allow statistical comparison. The relevance for safety is usually not clear.

In addition to nutrients, specific anti-nutrients are also measured that are crop specific, including lectins and trypsin inhibitors, toxins such as solanine and allergens for highly allergenic crops (e.g. soybean). While there are generally accepted limits for many anti-nutrients (e.g. solanine at 200 mg per kg fresh weight, Friedman, 2006), acceptable limits of variation allergens have not be established[92].

### 3.2 Measuring potential changes in endogenous allergen levels

There is a requirement in the US and a recommendation by the EU to consider whether insertion of the transgene has increased the expression or accumulation of naturally occurring endogenous allergens if the gene recipient (host plant) is a common source of food allergy. Regulators recognized that the risk of food allergy is not equal from different allergenic sources. Labeling requirements for processed foods are meant to be truthful and to protect those at risk. In the US and in the EU food labeling regulations demand that all ingredients derived from the major allergenic sources must be labeled. That list includes the eight common allergenic food in the US: chicken eggs, cow's milk, peanut, many tree nuts, crustacean shellfish, fish, soybeans, wheat (http://www.fda.gov/food/resourcesforyou/consumers/ucm079311.htm). In addition in the US foods containing glutens from wheat, barley and rye must be labeled, unless the gluten content is less than 20 ppm on a mass basis. In the EU six more foods are added to the list of eight including: cereals containing gluten (wheat, rye, barley, oats, spelt, kamut and hybrids of those grains); celery (root), mustard seed, sesame seed, lupin and molluscs as well as sulphur dioxides) as be listed all whole, relatively unfractionated ingredients must be labeled as to source (e.g. wheat, eggs, milk). In both the US and EU ingredients derived from the commonly allergenic foods must also be labeled unless the processed ingredient is exempt (e.g. hexane refined soybean oil). Starch from wheat must be labeled as coming from wheat, but starch from corn, rice or tapioca may simply be labeled as modified starch, without indicating the source. Thus in the US and EU a developer must evaluate potential changes in endogenous allergens in GM peanuts, soybeans and wheat, but not in common beans (*Phaseolus vulgaris*), corn and rice as they are not common sources of allergy. The methods used to perform the evaluation have generally been consistent with measurements of allergens in diagnostic allergen products[93-94]. Pharmaceutical grade allergen extracts are expected to show similar qualitative binding using immunoblots as well as variation in total IgE binding between 50% and 150% of the extract standard mean serum IgE binding using pooled allergic sera to compare one batch of allergen extract to a previous batch[93,95]. The first herbicide tolerant soybean (event 40-3-2 from Monsanto) was tested for differences in IgE binding using western blots of soybean extracts separated on SDS-PAGE with sera from three individual soybean allergic subjects[96]. Sten et al.[97] performed a much more extensive, non-regulatory study of IgE binding by in vitro methods using sera from 10 soybean sensitized subjects to compare results between 10 genetic varieties of the same GM event (40-3-2) and 8 genetically similar non-GM varieties of soybean. They used RAST-inhibition and basophil histamine release and found no significant difference between the GM and non-GM soybeans although there

were marked individual subject to subject and soybean line to soybean line differences. My laboratory has also performed serum IgE binding studies on five different soybean events in total from three different commercial developers. The methods used included direct IgE binding, ELISA inhibition with pooled soybean allergic sera or direct ELISA with individual sera and found no significant differences in binding except between one or more of the non-GM lines[92,94]. Some differences were found in gain or loss of an IgE binding band in the qualitative IgE immunoblots in some non-GM soybeans. In addition to those standard methods for evaluating potential changes in allergen abundance, two-dimensional (2D) immunoblots were performed using individual sera to compare each GM to three non-GM soybean lines due to new regulatory demands by the EFSA[51] and EC regulations[52]. Some individual serum IgE binding spot differences were noted, but not showing specific changes for the GM lines[92,94]. Clearly the population of allergic subjects included in such studies will influence the outcome. It is impractical to include more than a few (10?) specifically allergic subjects in a study unless multiple large allergy centers are included. There will always be some uncertainties regarding which proteins and isoforms might bind IgE from individual allergic subjects. However, the suggestion by the EFSA to use proteomics (LC-MSMS) to evaluate the abundance of individual "allergens" in soybeans and other commonly allergenic food crops is not as valid as serum testing because the list of "allergens" that EFSA wants to use [e.g. allergenic proteins in the OECD composition list for soybeans, includes proteins with little or no evidence of allergenicity (Gly m 1, Gly m 2, Gly m 3 (profilin), P34 Gly m Bd 30 K, Unknown Asn-linked glycoprotein, lectin, lipoxygenase, Kunitze trypsin inhibitor, unknown 39 and 50 kD proteins and[22-25]. The important allergens in soybean that have been identified include Gly m 5 (β-conglycinins alpha-, alpha'- and beta-) and Gly m 6 (5-glycinins) and possibly Gly m 4, also known as SAM22. Thus the EFSA recommendation is not based on evidence of risk since there is no gradation of risk in the proteins chosen and in fact some have no published evidence of allergy, or the protein sequence was not determined. In addition, LC-MSMS does not provide 100% coverage of any protein and it is therefore unlikely to identify isoforms, some of which may not bind IgE. Serum IgE binding tests at least compares a biological measurement between the GM and other non-GM varieties using sera from allergic subjects.

However, it is important to consider whether there is relevance for safety to these measurements. Is there an increased risk of allergy if there is a difference? People allergic to soybean should avoid eating any soybean. People who are not allergic can eat as much as they desire. In processed foods the amount of total soybean protein can vary markedly from product to product and the food companies are not choosing lots of soybean based on specific varieties. Instead they buy in bulk with the soybeans typically mixed at the silo, during shipment, in milling and processing and during food manufacture.

An important question that has not been answered by any scientific study or any regulatory body is what difference would be required in endogenous allergen accumulation to have an adverse impact on human health for the specifically food allergic subjects who are the sensitive, at-risk population? An informative estimate might be made based on the dose-increase interval highly trained clinical food allergists use in performing double-blind placebo-controlled food challenges (DBPCFC). There are a few publications describing protocols for testing high risk patients with the intent of establishing thresholds of doses for various allergens. A review of studies by Crevel et al.[98] reported protocols with increasing challenge doses between 3-fold and 10-fold for peanuts with peanut allergic subjects. The experimental design for DBPCFC in the EuroPrevall studies began at three micrograms of protein from allergenic sources and used ten-fold increasing doses to 30 mg of protein, then reducing the step increase to three-fold above 30 mg as the risk of serious reactions were felt to increase above that dose[99]. Therefore it seems logical to conclude that at least a three-fold increase might of concern.

### 3.3 Assessing potential new, unintended proteins

During characterization of each new GM event the insertion of DNA is to be analyzed to confirm the sequence of the insert as well as the immediate surrounding DNA. Typically a few hundred bases to a thousand bases are provided beyond the insert. The sequence of the insert is to ensure that the protein(s) intended to be expressed (if any) are correct. If an unexpected change has occurred, that should be evaluated in terms of the function of the new protein as well as possible risks for allergy and toxicity using bioinformatics. The flanking DNA is considered to determine if there is a possibility a new fusion protein might be expressed in the plant. All six potential reading frames in the DNA sequence are evaluated using computer algorithms to identify potential open

reading frames (ORF). Some regulators are satisfied with start (methionine) to stop codons to define a potential ORF. Others want all hypothetical ORFs meaning stop to stop. The potential ORFs are then evaluated using bioinformatics to search for matches to allergens and toxins. The critical segment is the fusion site. The plant DNA on each side of the insert was already there and if it encoded an allergen or toxin, those would have been endogenous hazards. The safety assessment is focused on new potential hazards and risks. If there were matches to an allergen or a toxin, further analysis may be performed to evaluate whether and what tissues would transcribe RNA from that region of the DNA. If the specific RNA is present, measurements could be made to determine if there is translation product (protein) using either LC-MSMS or antibodies generated against a synthetic peptide "encoded" by the ORF in assays. If there is a negligible level of protein, then the risk is minimal.

Some regulators ask for flanking sequence until it is clear that the transgene has not interrupted an endogenous plant gene in the coding or intervening sequences (introns). However evaluation of agronomic traits of the plants in field trials with geographical replicates will help identify any biologically significant differences of the GM vs non-GM varieties. That type of evaluation is about performance of the plant, not safety. The US regulators very interested in obtaining information relevant to safety of the food and feed products. The GM developer and associated seed companies must show data to farmers to convince them that the GM plants produce adequately in terms of yield and overall composition. Otherwise farmers will not purchase the seeds.

### 3.4 Assessing unintended effects conclusions

The conclusion of the compositional analysis is generally whether the total nutrients and anti-nutrients for the specific crop are substantially equivalent to non-GM comparators or not. These analyses are performed using field-trial grown material of the GM and non-GM varieties in geographical replicates. Certainly there can be some statistically significant differences of measuring a number of components in many samples over different geographies will often result in a few statistically significant differences. Most of the variation is due to actual genetic differences that are associated with the whole plant genomes and back-crossing and breeding programs and have nothing to do with transgene insertion[82-83]. In addition, recent discoveries that DNA methylation patterns can be inherited and alter gene expression without any change in DNA help us realize that we cannot expect to control or understand every measureable difference based on DNA sequence information[100]. And it is extremely important that we realize that every measurable difference does not constitute a risk for consumers, in fact very few do. Humans selected and have improved most of the domesticated crops hundreds to thousands of years ago. We know that genetic variation is needed to be able to grow the same species in a wide variety of environmental conditions in order to produce food and feed.

In the US the values from individual measurements are compared between the GM event and the near genetic relative (near isoline or parental variety) and also compared to either a number of commercial lines grown in the same field trials or recent historical data from real production samples. If the measures from the GM crop fall within the typical range of variation as a benchmark for potentially relevant biological differences, a difference between the GM and near genetic relative is considered acceptable. The GM plant is therefore deemed "substantially equivalent" to other varieties of the crop. Similar inferences are made from data obtained by measuring animal responses in feeding studies such as the 90-day rat feeding trial that some regulators require; 42 day broiler studies or large animal feeding trials that are generally used as industry acceptance studies in many countries, but are required in some (e.g. India).

### 3.5 Current status of GMO approvals

How many GM events have been developed and gotten regulatory approvals for growing, of use as food and feed? It is hard to find accurate information. The Center for Environmental Risk Assessment (CERA) GM crop database www.cera-gmc.org/GmCropDatabase lists 153 total crop-events. Not all of those were developed through GM technology as some were developed by mutagenesis or traditional breeding. In addition, not all of those are approved anywhere and some are approved but not used. The International Service for the Acquisition of Agri-Biotech Applications (ISAAA) also maintains a GM crop database that lists 353 events (www.isaaa.org/gmapprovaldatabase). By quick examination it seems ISAAA shows some crop types not listed by CERA including beans (*Phaseolus vulgaris*), eggplant or brinjal (*Solanum melongea*), poplar trees (*Populus* sp.), sugar cane (*Saccharum* sp.) and pepper (*Capsicum annuum*) that have not been submitted to U.S. or Canadian regulators. It is likely that each of these databases misses a few events, but unlikely that either

of them miss globally traded GM crops. In addition the three US regulatory bodies each have a separate database that presents their actions on individual GM events. The USDA website is: http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml. The FDA website is: http://www.accessdata.fda.gov/scripts/fdcc/?set=Biocon. The EPA website is: http://www.epa.gov/oppbppd1/biopesticides/pips/pip_list.htm.

Even though many events with different properties and in different plants are approved for use, the bulk of the GM events are in a few commodity crops (canola, cotton, maize, and soybean). The rate of adoption as measured in percent of hectares planted in GM crops in the U.S. has been extremely rapid, going from zero in 1994 to more than 90% of our soybeans and corn (maize) in 2014. A significant fraction of the cotton production in the U.S. is from GM events while 95% of cotton in India and 90% of cotton in China is GM cotton. There are now multiple events from different developers having similar functions (herbicide tolerance or specific insect resistance). At the same time a number of previously approved GM crops (post-1994) have disappeared from the market. Some products were dropped due to consumer or company pressures including the viral resistant, Colorado potato beetle resistant potatoes developed by Monsanto as major potato markets are dominated by French fry and fast food restaurants that are very sensitive to perceived consumer preferences. Those products that dramatically reduced insecticide use on potatoes were withdrawn in about 2002 due to pressure from the fast food industry. Herbicide tolerant wheat was submitted by Monsanto to Canada and the US, but was withdrawn before approval due to pressure from the Canadian Wheat Board because of fears export markets to Asia would block trade. Delayed ripening tomatoes were dropped as they were not commercially viable (four companies including Zeneca and Monsanto had approved GM events) because fresh food qualities were not as good as non-GM varieties. The viral resistant squash that was developed by Asgrow is still on the market, though now owned by Seminis. Viral resistant papaya was developed by researchers at Cornell University and was approved for use in the US because the Hawaiian trees were being decimated by ring spot virus. The GM construct blocked replication of the virus and the introduction of this trait saved the industry in Hawaii.

## 4 Summary

Some experts predict an eminent global food crisis while others suggest continuation of more regionalized crises that may be caused by local draught, disease or have artificial political or economic causes[101-102] (Butler, 2009a; Butler, 2009b). Some solutions for improving the global sustainable agriculture are and can be contributed through biotechnology, with current and future GM crops. Yet progress is being stifled by a very focused, well financed vocal minority of NGOs and by celebrities who are stirring public uncertainty even though they clearly do not have a good understanding of agriculture, food production and costs. Can we find common ground in this debate? Few who are students of food production, agriculture and human health would deny that at some point the world's growing global population will outstrip the capacity to maintain food production in in the long-run even though the efficiency of production has increased markedly in the past century[103]. Yet our ability to increase production currently comes through the use of adding mined minerals, increased use of fossil fuels for fertilizer and tillage and the use of machines to replace human labor and draft animals in intensive agricultural practices. Can we maintain our current rate of expansion? Experiences in the US agricultural system may provide useful examples for the potential benefit of GM crops in China and other Asian countries and in setting a standard for food safety of newly developed products.

In considering the experiences in the US regarding the safety evaluation process and regulations of genetically modified (GM) crops, it is necessary to look also at the global nature of food supplies, the concerns of various food safety regulatory bodies as well as consideration of the long history of various food crops. No country is self-sufficient and most foods consumed in any one country originally came from, or is dependent to some extent on inputs from other countries. The adaptation of wheat, rice, potatoes, tomatoes, peppers, various legumes and the specific animals we consume today were developed from naturally occurring ancestral organisms from very different geographical locations than those used for production today. They were selected and improved through breeding processes that took hundreds or thousands of years. They were chosen by experience, but based on food utility (nutritional and anti-nutritional) characteristics, ease of production and food safety. Yes there are real risks of foods for those with allergies and celiac disease. There are risks for those who do not prepare or store food properly to suppress microbes and spoilage and to inactivate anti-

nutrients. The primary potential risks of new proteins are relatively easy to prevent through the current assessment scheme.

There are a few uncertainties that US regulators are still finding perplexing. Primarily if the protein is stable in the pepsin assay, they are concerned that it might sensitize and become an allergen. Low abundance stable proteins have little risk, and they should find an acceptance level. We also need to continue working on a better way of predicting sensitization. The current suggestions of computer programs to predict antigenicity are far from perfect and over-predict risk. Animal models so far have failed to provide sufficiently accurate predictions to be useful. Cell based assays using human antigen presenting cells, T cells and B cells have not been validated to demonstrate accurate predictions. Therefore additional research is needed for difficult proteins where the current Codex guideline[1] and US evaluation process do not show results leading to a conclusion of unlikely harm. But most GM products today are easily cleared with bioinformatics for allergenicity, celiac disease and toxicity. In a few cases serum IgE tests are needed and simple, predictive toxicity tests are needed.

Labeling of foods is a major obstacle around the world. Some countries like China have rules demanding labeling at least some foods if they contain GMOs. In the US a few states have passed laws that may take effect in the near future and a few states will vote on labeling in November, 2014. Major economic and practical food production hurdles make this approach untenable. Crops are grown and traded across state lines and national boundaries. Food companies often make products for all 50 states and for export. There are many individual ingredients that might contain a GMO, but that is not consistent from lot to lot. As an example, figure 2 shows the labeled ingredients in a black vegetarian bean burger produced in the US. Each component derived from soybeans, corn (maize), canola or cotton may be from a GMO. The CERA GM crop database (http://cera-gmc.org/index.php/GMCropDatabase) lists 12 approved GM soybeans representing 8 GM proteins and 57 approved maize lines representing at least 15 different proteins. Suppliers of commodities, ingredients and final food products would have to control and test for all of those ingredients if they do not want to list "GMO" on the label if these laws pass. There will be added expense,

**Ingredients: Black Bean Veggie Burgers**

**(frozen meat-substitute meal, by a US company)**

WATER, COOKED BLACK BEANS (BLACK BEANS, WATER), COOKED BROWN RICE (WATER, BROWN RICE), ONION, **WHOLE KERNEL CORN**, **CORN OIL**, **SOY PROTEIN CONCENTRATE**, WHEAT GLUTEN, EGG WHITES, DICED TOMATOES, BULGUR WHEAT, GREEN CHILES, CALCIUM CASEINATE, **CORNSTARCH**, CONTAINS TWO PERCENT OR LESS OF ONION POWDER, SPICES, TOMATO JUICE, YEAST EXTRACT, TOMATO POWDER, DEXTROSE, SALT, GARLIC POWDER, HYDROLYZED VEGETABLE PROTEIN (**CORN GLUTEN**, WHEAT GLUTEN, **SOY PROTEIN**), **SOY SAUCE** (***SOYBEANS***, WHEAT, SALT), NATURAL AND ARTIFICIAL FLAVORS, PAPRIKA, JALAPENO PEPPER, CITRIC ACID, XANTHAN GUM, DISODIUM INOSINATE, , CARAMEL COLOR, LACTIC ACID.

**Allergen Information:**

CONTAINS: **SOY**, WHEAT, EGG AND MILK INGREDIENTS.

**Fig. 2** Ingredient label of a commercial black bean burger produced in the US in 2014. Ingredients that may contain a currently approved GMO are listed in bold and underlined. Those ingredients may be subject to GMO labeling laws if mandatory labeling laws are passed. The Allergen information is a safety label as it shows major allergenic ingredients that have to be avoided by some consumers with specific food allergies so they would know to avoid this product for safety reasons if they are allergic to soybean, wheat, eggs or milk.

and no safety benefit. For foods that are already cluttered with labeled information, critical safety information such as allergen content gets lost.

The foods humans consume are tremendously diverse in composition, nutritional qualities and to some extent, risks. We are omnivores and our ancestors adapted to many different climates and conditions as they spread across continents and changed from migratory hunter-gatherers to migratory pastoralists and then to relatively sedentary agriculturalists[104,106]. The adaptations seem to have been possible because of the ability of humans to cooperate and accept added costs of helping to ensure survival of others rather than protection of the immediate family, an adaptation that was not always beneficial to the immediate relatives, but was beneficial for the society[106]. In the post-industrial era humans have become highly mobile individually. However, within each society the basic food production infrastructure needed to maintain the population is slow to change for many reasons including the large investment in equipment, complexity of the commodity and food processing facilities and the relatively restricted genetic pool of plants and animals that are used for production. But adaptation occurs and the efficiency of production has increased, especially during the last century. Increased have occurred even as land is available for farming as the population concentrated in cities away from the production of food crops[107]. Since the world population is now estimated to be over 7.25 billion people, and with a total biomass exceeding the combined total of all other terrestrial vertebrates we need to think hard about how to improve food and feed production. It took hundreds or thousands of years to learn how to manage and accept many new methods of food production. In the past 100 years food production has shifted markedly to more industrialized methods to meet food demands. Some people would seek to stop the technology, restrict the tools of introducing new improvements into food crops because of claims they produce unsafe foods. But as I search for evidence of harm from GM crops, it is not there.

It is helpful to consider that none of the plant foods that we grow and consume today are completely natural. Although they are genetically fairly similar to some native plants, the grains (wheat, barley, rye, rice, maize, sorghum) have been bred and selected for hundreds of years. Many varieties of tomatoes, potatoes, eggplant and peppers are quite safe for consumption after many forms of cooking and processing. But they are closely related, in the same plant family (Solanaceae) as toxic nightshade, which along with tobacco and petunias are really not edible. The edible solanaceous plants have wild relatives in the same species that produce sufficient levels of glycoalkaloids (solanine, tomatine and others) and lectins that are quite harmful to us and to many domestic animals if consumed. We can only consume the current varieties of these crops because our ancestors went through a process of breeding and selecting varieties with low levels of these toxins and anti-nutrients in the edible plant parts. They did that without the complex scientific tests and instruments we use today to detect specific substances that cause harm. They did that without having standardized animal feeding trials. Even though we are omnivores and can consume many different plants and animals, we have had to learn the limits of what we can consume. And even though the potatoes that we eat today are safe, we have learned that some wild relatives produce sufficiently high concentrations of a solanine, tomatine and other glycoalkaloids to cause harm or even death.

Beyond a historical perspective, it is also important to remember that we live in an age of increasing information distribution with frequent unintended impacts of miss-information. There are many claims of real or potential harm from various foods that would never have been noticed centuries or event decades ago, but often the communicated fears are hypothetical risks. However, instant messaging and the internet compress years to seconds. When European explorers brought tomatoes and potatoes from South America to Italy and the United Kingdom in the 1500's they were introducing crops that had been grown and consumed safely for over a thousand years. But in Europe people did not have full knowledge of how to grow and use the plants. Some who became ill due to improper food preparation or eating the green part of the plants and after falling ill people suggested that the entire plants were poisonous including the fruits and tubers. Natives of South American knew to avoid consuming the green plant material. The rare cases of harm in Europe lead to wide spread fear that stifled the introduction of these now staple foods into the European diets. Now false claims about GMOs are common and effects long lasting. Recent claims by Dr. Oz, Jeffrey Smith, Oprah Winfrey or Cui Yongyuan claim that GM crops are unsafe or untested have caused consumers to become skeptical of claims by biotechnology companies and governments that they are safe. Those media personalities however have not read the dossiers or performed safety studies that have convinced US regulators the products like European Corn Borer

resistant MON810 is safe. How do we present the truth to consumers when there are "trusted" personalities telling consumers that the government is corrupt and that big biotech companies like Monsanto did not do an adequate job of testing and evaluating safety?

## 5 Conclusions

The US regulatory system for evaluating the safety of GM crops involves three federal agencies, the USDA, the FDA and EPA. The process for evaluation was initiated in the late 1980s and early 1990s through consultations that included academics, industry scientists and governmental regulatory scientists and policy makers. The assessment was refined in the late 1990s through 2003 and aligned with the Codex Alimentarius Commission Guidelines for the safety assessment of GM crops. Potential risks of allergenicity of foods produced from the GM crops must be evaluated using scientifically acceptable methods. The process is efficient for identifying proteins that are likely to present a significant risk of food allergy, which would be the transfer of a known allergen or a likely cross-reactive protein. There is a bit less certainty trying to predict whether a new protein with no obvious risks factors might sensitize de novo, but risks are clearly low in those cases where the protein is rapidly digested by pepsin in a test-tube assay and/or low in abundance in the food component. The potential that a transgenic has significantly higher expression of endogenous allergens is quite low compared to non-GM varieties, but in addition the risk is for those consumers who should be avoiding consumption of food from the host plant anyway. Thus there is no practical increase in risk even if the content of endogenous allergens was increased. Potential food toxicity is also evaluated based on criteria established for non-GMOs. Few proteins are toxic and the comparison of the sequence of the GM protein to those of known toxins along with evaluation of the gene source and the mechanism of action of the protein will identify high risk proteins. The US has evaluated and approved the commercialization of approximately 100 new events or varieties of GMO. There are no documented cases where an approved GM crop has caused harm to humans or animals who have consumed edible parts of the plants. However, the regulatory process is expensive and time consuming. Since most food crops are traded on an international market, it is unfortunate that there isn't a single safety evaluation process that is standardized and accepted across all countries to avoid duplication of studies.

**Acknowledgements:** The National Academy of Science (USA) and the Chinese Academy of Science were kind in providing travel funds for me to attend a joint meeting in Wuhan, China to discuss the safety assessment. My laboratory has been fortunate in obtaining funding from the US EPA for three studies to evaluate possible improvements in the allergenicity assessment of GM crops. The USDA FAS Borlaug program as funded my laboratory to provide training for a number of international scholars on the allergenicity assessment of GM crops, as has the Gates Foundation through the Danforth Plant Sciences Center. I have had the privilege of trying to help a number of scientists in Africa, Australia, India and the US evaluate the potential allergenicity and potential toxicity of a number of important GM products as well as funding from eight agricultural biotechnology companies to maintain the Allergen Online database and to perform studies on potential changes in endogenous allergens in GM soybeans. Finally I have had wonderful experiences working with a number of talented, hardworking graduate students, technicians and colleagues who are allergy/allergen experts and have consulted on the database and other allergen challenges.

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