Genetically Engineered Plants and Foods: A Scientist's Analysis of the Issues (Part I)

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Abstract

Through the use of the new tools of genetic engineering, genes can be introduced into the same plant or animal species or into plants or animals that are not sexually compatible—the latter is a distinction with classical breeding. This technology has led to the commercial production of genetically engineered (GE) crops on approximately 250 million acres worldwide. These crops generally are herbicide and pest tolerant, but other GE crops in the pipeline focus on other traits. For some farmers and consumers, planting and eating foods from these crops are acceptable; for others they raise issues related to safety of the foods and the environment. In Part I of this review some general and food issues raised regarding GE crops and foods will be addressed. Responses to these issues, where possible, cite peer-reviewed scientific literature. In Part II to appear in 2009, issues related to environmental and socioeconomic aspects of GE crops and foods will be covered.

Contents

1. INTRODUCTION

"Be very, very careful what you put into that head, because you will never, ever get it out," said Thomas Cardinal Wolsey (1471–1530). Although spoken centuries ago, this admonition rings true today when it comes to the impact of information people receive, particularly in the popular press, about genetically engineered (GE) crops and foods. Genetic engineering enables the introduction of genes, using the modern tools of recombinant DNA (rDNA), from the same species into an organism; of more concern to some, genes from organisms in other kingdoms can be introduced. Although much relating to GE crops and foods has been written, both pro and con, this review attempts, where possible, to address issues by linking responses to peer-reviewed literature. The intent is to present as accurate a scientific picture as possible, although this does not imply that people possessing the same scientific understanding will necessarily make the same choices about the advisability of GE crops for consumption, because different people have different values.

GE crops, and products made from them, must be evaluated on a case-by-case basis and, although scientific information should be a part of the considerations for using and consuming these crops, issues beyond the technical, science-based facts also need to be considered. Here, aspects of a number of issues related to GE crops and foods are reviewed from a detailed scientific viewpoint. Not all issues raised are discussed and not all aspects of the issues raised are addressed.

GE: genetically engineered

Recombinant DNA (rDNA): DNA that is manipulated in the laboratory using recombinant DNA technologies

GMO: genetically modified organism

2. GENERAL ISSUES

2.1. Terminology

Biotechnology literally means the use of a living organism (hence, "bio") to perform a task or function. Historically the term was used to describe processes like cheese, yogurt, wine, or beer production. In modern parlance, however, biotechnology is commonly used to refer to the newer methods of genetic engineering of organisms through the use of recombinant DNA or rDNA. People use the term GMO today to refer to a genetically modified organism, one that has been engineered using rDNA. Others refer to foods created in this manner as genetically engineered or GE foods. So, a GE or GMO food is a food modified using rDNA methods or one that contains a GE ingredient. The term LMO, for living modified organism, refers to a GE organism that is alive, such as a fresh fruit, vegetable, or seed that was created using rDNA. A seed is an LMO, whereas flour made from seeds or grain would not be an LMO. Use of the terms GMO and LMO can be confusing, especially to geneticists, given that all foods eaten today have been altered or modified genetically through natural or human-imposed mutations or crossing. Frankenfoods, or Frankenstein foods, is a term first coined by Paul Lewis in his 1992 letter to the Editor of the *New York Times* (139), arguing against GE tomatoes and calling for action against Frankenfoods. The term gained popularity after 1998 when nongovernmental organizations (NGOs) started using the term to call consumers to action against GE foods (102).

GRAS: generally recognized as safe

Classical breeding: methods used by humans to facilitate genetic exchange between one organism and another

2.2. Besides Genetically Engineered Crops, Does Genetic Engineering Play a Role in Producing Food?

Much processed food is produced using enzymes, or whole organisms with enzymes, that are responsible for altering the nature of the food—such as bacteria (e.g., yogurt), yeast (e.g., beer, wine), and multicellular fungi (e.g., blue cheese). These enzymes were modified genetically through traditional methods and in some cases rDNA methods as well. One example of the latter is the modification of an enzyme used in making cheese, rennin. This enzyme is present in rennet, historically isolated from the stomachs of slaughtered calves where it was needed to clot mother's milk to slow its digestion. In cheese-making, rennet is used to coagulate milk to separate the curds (solids) and whey (liquid). Rennin or chymosin was the first protein produced through rDNA means to be used in food (95). The chymosin gene from a cow was cloned into yeast and *Escherichia coli*, from which rennin can be made in larger quantities and with more consistent quality than from calves' stomachs. Engineered chymosin is currently used in approximately 60% of U.S. hard cheese products (27).

Other products produced by rDNA methods include food supplements, such as vitamin B2 (riboflavin) (181), α-amylase (used to produce high-fructose corn syrup and dry beer), and lactase (added to milk to reduce the lactose content for persons with lactose intolerance). The Food and Drug Administration (FDA) granted GRAS (generally recognized as safe) (See section 3.4) status for these enzymes produced by GE microorganisms prior to their use in food (125).

2.3. How Does the Creation of a Genetically Engineered Crop Differ from That of a Classically Bred Crop?

Similarities and differences exist between the modifications made in organisms by classical breeding versus those made via rDNA methods. Both approaches can involve changes in the sequence, order, and regulation of genes in an organism and can utilize many of the same enzymes. However, with the rDNA approach the amount of genetic information modified is small, one or a few genes, compared with the classical breeding approach where all the tens of thousands of genes in the organism are involved, potentially exchanging positions. Another difference is that with the rDNA approach when and where a gene product is made can be controlled precisely. Thus, if a change in seed characteristics is desired, a gene can be linked to regulatory signals that result in expression only in the seed or even in a specific compartment of the seed (43)—an outcome difficult to achieve with classical breeding.

With classical breeding approaches, crosses can only be accomplished between closely related species or genera. For example, a wild *Lycopersicon* variety can be crossed with cultivated tomato (*Lycopersicon esculentum*) varieties (42), and wheat (*Triticum aestivum*) can be crossed with rye (*Secale cereale*) to yield triticale (218). But in many crosses, wild relatives and different genera or species are not compatible or crosses can be made but the resulting embryos must be rescued by in vitro culture to obtain a plant (204). In contrast, rDNA approaches can utilize genetic material from any living organism, which permits DNA from bacterial or animal sources to be introduced into plants. Therefore, rDNA can result in gene combinations not previously seen.

2.4. Can Marker-Assisted Selection Be Used Instead of Genetic Engineering to Improve Crops?

When it is determined what genic sequences are responsible for certain traits, that information can be used to develop breeding aids. The ability to select desirable alleles and eliminate deleterious ones in a fast, reliable manner is critical to the development of

improved germplasm through breeding. Genetic markers can speed identification of plants with desired (or deleterious) alleles in large populations via a process termed marker-assisted selection (MAS). Markers closely linked to desired traits are used to select indirectly for the trait (58). With the whole and partial genome sequences and other molecular tools now available, markers can now be identified in the responsible gene, rather than linked to it, which makes MAS even more valuable. Thus, a marker was found in *Xa5*, a rice (*Oryza sativa*) bacterial blight disease resistance gene, and its use prevented separation of the marker from the trait by recombination (115).

MAS is particularly valuable for traits when *a*) phenotypic screens in the field are difficult or costly, e.g., drought/frost tolerance or resistance to exotic diseases or pests; *b*) multiple alleles exist; and *c*) recessive or low heritability traits exist that require progeny testing. MAS is used for some important traits, e.g., leaf rust resistance in wheat (165), erect panicle in rice (126), soybean (*Glycine max*) rust resistance (108), drought adaptation in maize (190), and root-knot nematode resistance in cotton (*Gossypium hirsutum*) (255).

Utilization of GE crop varieties is not permitted for organic growers. National Organic Standards specify that these varieties cannot be intentionally grown by organic farmers and labeled "organic" (161) (See section 3.16); however, MAS can be used to introduce desired traits from wild or related species and these species are acceptable to organic growers. However, this approach is limited to diversity extant in sexually compatible species, which is often not sufficiently broad to provide needed traits. Utilizing MAS to create food crops has led to the term, "Super Organics" (144), crops that are grown under certified organic conditions and were created through classical breeding using genomic information and MAS, not through rDNA methods.

2.5. Does the Use of rDNA Always Involve Moving Genes from One Organism to Another?

MAS: marker-assisted selection

Substantial equivalence: an assessment of a new food must demonstrate it is as safe as its conventional counterpart

Using rDNA methods, a gene from any living organism can be inserted into a plant and, given appropriate regulatory signals and codon usage, can be expressed efficiently in a plant cell. To introduce a gene from the same or a heterologous source, the gene must be identified in a donor organism and cloned into a bacterium to obtain sufficient DNA to build plant transformation vectors (210) and perform the transformation. Given the intermediate bacterial step, even if structural genes and regulatory sequences are from the plant, plant transformation would involve moving DNA from one organism to another.

Sources of genes to engineer plants can be derived from the same plant, a different plant, a related wild species, or from bacteria, fungi, viruses, and mammals. The capacity to introduce genes from different living organisms raises the issue of whether all engineered plants, regardless of the source of the genetic material, should be considered as a homogeneous group. Alternatively, it has been suggested that GE plants be placed in three classes: *i*) wide transfer, referring to gene movement from organisms of other kingdoms into plants; *ii*) close transfer, referring to movement between species of plants; and *iii*) tweaking, referring to the manipulation of levels or patterns of expression of genes already present in the plant (232).

Using such a classification scheme would provide some clarity to several issues relating to regulation and public perception of GE crops. A determination of substantial equivalence (See section 3.4) could then be carried out at different levels of scrutiny, depending on classification level. A GE plant created by tweaking or close transfer would result in changes unlikely to be dramatically different from those created by processes used by traditional breeders. Conversely, introduction

via a wide transfer would likely require more thorough testing to establish substantial equivalence.

EPA: Environmental Protection Agency

USDA: United States Department of Agriculture

Pesticide: any substance or mixture of substances that prevents, destroys, repels, or mitigates any pest, including insects, weeds, fungi, bacteria, viruses, mice, and other animals

Bt: *Bacillus thuringiensis*

APHIS: Animal and Plant Health Inspection Service

EIS: environmental impact statement

2.6. Which U.S. Agencies Have Regulatory Authority Over Genetically Engineered and Classically Bred Crops?

In the early 1980s, the U.S. established a formal regulatory structure for GE organisms by expanding existing legislation to accommodate products created by rDNA. This approach was outlined in an Office of Science and Technology Policy document entitled *Coordinated Framework for Regulation of Biotechnology* (169), which established the concept that GE foods would be regulated on the basis of product, not process, and on a case-by-case basis.

GE foods and products made from them are under regulatory control of three federal agencies: the FDA, the Environmental Protection Agency (EPA), and the U.S. Department of Agriculture (USDA) (see 150 for a review). The FDA is responsible for the safety and labeling of foods and animal feeds from all crops, including those that are GE. The FDA requires full evaluations of GE foods containing uncharacterized DNA sequences, significantly altered nutrient levels, different composition relative to existing foods, potentially allergenic or toxic proteins, and/or new selection marker genes. The EPA evaluates food safety and environmental issues associated with new pesticides and pesticidal products. Bt corn (*Bacillus thuringiensis; Zea mays*) and the pesticidal Bt product it contains, used to control the European corn borer, for example, fall under its jurisdiction. The EPA's control also encompasses GE plants in which a small part of a pest, such as a viral regulatory sequence (e.g., 35S promoter), is used to develop the GE crop. A division of the USDA, the Animal and Plant Health Inspection Service (APHIS), oversees environmental consequences and safety of planting and field-testing GE plants; their role is to ensure that field tests of GE crops are conducted under controlled conditions and that any unusual occurrences are reported. Every GE crop will not be overseen by all three agencies; however, all three agencies have the legal power to ask for immediate removal from the market of any product, if valid scientific data show a safety concern for consumers or the environment.

The federal government considers each GE plant with a specific DNA segment introduced via rDNA methods to be a "regulated article" and each gene transfer is defined as an event. Creating a second transformed plant with an identical DNA construct inserted in a different location is considered to be a separate event, a regulated article requiring oversight, even if the first event received regulatory approval and attained nonregulated status. As of October 2007, 113 petitions have been received at APHIS; 90 petitions received nonregulation status and no longer require APHIS review for movement or release in the U.S. (113).

In 2005, an audit by the USDA Inspector General (110) indicated the USDA lacked basic information about where GE crops were grown and their fate after harvest, raising concerns particularly about the fate of crops engineered to produce pharmaceuticals (See section 3.14). Two additional concerns were raised in 2007. First, a U.S. federal court ordered the USDA to conduct more detailed reviews of applications for experimental plots of GE bent grass when pollen was found to have spread 13 miles from the original cultivation site (61). Second, in early 2007 questions were raised about the approval of deregulation status for Roundup Ready® alfalfa when a U.S. District Court Judge ruled that the USDA had erred in approving deregulation without a proper environmental impact statement (EIS). Roundup Ready® alfalfa was returned to regulated status, pending submission and review of an appropriate EIS (11).

2.7. Which Genetically Engineered Crops Are Grown Commercially?

The first GE plant was tobacco, reported in 1983 (23), but no plants were commercially grown until the FlavrSavr™ tomato was commercialized in 1994 (146). Although the FlavrSavr™ tomato was ultimately taken off the market, other commercial crops entered the market—most notably large acreage crops, such as canola (*Brassica napus*), corn, cotton, soybean, and most recently, alfalfa (*Medicago sativa*) (See section 3.20). If success is measured by increases in global acreage or farmer acceptance, certainly these GE crops have been successful. In 2005, the billionth acre of a GE crop was planted (116). In 2006 the worldwide acreage of GE crops was 252 million acres grown by 10.3 million farmers in 22 countries (117); the majority of the farmers are in the U.S. and almost none are in Europe. In the U.S. the adoption of herbicide-tolerant (HT) soybeans represented 87% of total U.S. soybean acreage in 2006. HT cotton represented 60% of total cotton acreage (71); pest-resistant (Bt) cotton was 52%, whereas Bt corn was 35% of total corn acreage.

Despite sizeable GE crop acreage, the diversity of crop types and traits in commercial production is limited. Few minor acreage GE crops are at present commercially successful, i.e., papaya (*Carica papaya*), certain types of squash (*Cucurbita* sp.), and sweet corn (117). Nearly all major-acreage, commercial releases of GE crops are based on pest protection via genes from Bt or HT, predominantly resulting in tolerance to Monsanto's RoundUp® herbicide, although some result in tolerance to Bayer's Liberty® herbicide. More recently, stacked versions of these traits were released—e.g., maize engineered for rootworm and European corn borer resistance (both Bt-based) and tolerance to RoundUp®. Except GE papaya, all commercial varieties in 2007 are from the private, not the public, sector.

2.8. How Many Foods Are Genetically Engineered?

HT: herbicide tolerant

Estimates suggest that as much as 80% of U.S. processed food may contain an ingredient from a GE crop, such as corn starch, high-fructose corn syrup, corn oil, canola oil, soybean oil, soy flour, soy lecithin, or cottonseed oil (98). Despite this percentage in processed foods, there are very few commercially available whole GE foods. The first commercial GE whole food was the FlavrSavr™ tomato, engineered to have a longer shelf life (129) so tomatoes could be kept on the vine longer to ripen, develop more flavor, and allow later shipments to stores. Although grown in California, the tomatoes were made into tomato paste, clearly labeled, and sold in the U.K. The paste gained an estimated 60% share of the canned tomato market by 1999, but left the market shortly thereafter owing to market concerns (146). Endless Summer™ tomatoes, also engineered to control ripening and introduced at approximately the same time, were commercially available for only a short time.

GE papaya is the only engineered fruit commercially available in the U.S. today. This occurred because in Hawaii, where most papayas for the U.S. are grown, production fell owing to losses to papaya ringspot virus, PRSV (93). PRSV, discovered in Hawaii in the 1940s, virtually eliminated large-scale production on Oahu in the 1950s, forcing the industry to relocate in the early 1960s to the island of Hawaii. There it thrived, which led to 95% of Hawaii's papaya being produced there by the 1980s. Delay in spread of the disease gave researchers time to look at possibilities to protect against the virus. Infecting papaya with milder virus strains (205) met with limited success owing to a more aggressive PRSV, but a GE papaya containing a viral coat protein gene was successful (92, 141). In 2006 the GE varieties "Rainbow" and "SunUp" accounted for >50% of papaya production in Hawaii, although much of the

papaya consumed in the U.S. is from Brazil, Mexico, and the Caribbean, where PRSV is not a serious problem.

Another commercial whole food available in the U.S. is GE squash (yellow crookneck, straightneck, and zucchini). The first variety of GE yellow squash, termed Freedom II, was the second GE crop to be cleared by U.S. regulators. Freedom II was engineered with viral coat protein genes to be resistant to two viruses—Watermelon Mosaic Virus 2 (WMV2) and Zucchini Yellow Mosaic Virus (ZYMV) (238). Freedom II reached the market in 1995 but was not labeled like the FlavrSavr^{TM} tomato. Viral resistance was transferred to zucchini by breeding and, because squash is usually infected with a third virus, Cucumber Mosaic Virus (CMV), a GE squash resistant to all three viruses was developed. Six varieties of GE yellow squash and zucchini, bearing various names, e.g., Independence II, Liberator III, Freedom III, and Destiny III, are currently being sold. U.S. acreage is limited in part because of the negative effects of other viruses against which the GE varieties are not protected, but resistance to the original three viruses remains strong (88).

The last whole GE food available in the U.S. is GE sweet corn, engineered with a Bt gene to protect against earworms (*Helicoverpa zea*), one of the most costly crop pests in North America. Earworm damage results in subsequent fungal and bacterial attack and quality loss (107). Expressing Bt in corn results in reductions in insect attack. By reducing insect damage, mycotoxigenic fungi numbers are decreased and this results in lower levels of mycotoxins, such as fumonisins, which have toxic effects on humans such as elevated rates of liver and/or esophageal cancer (243). Comparing fumonisin levels in corn from Bt hybrids versus control hybrids, Bt hybrids give higher percentages of grain suitable for human and animal use (99).

2.9. What Is in the Crop Biotechnology Pipeline?

Although commercialized GE crops are limited in trait diversity, proof-of-concept for many other traits has been reported in laboratory experiments and small-scale field trials. These traits fit into several categories: pest resistance, agronomic performance, abiotic stress tolerance, medical applications, biofuels, and improved food, feed, and environment.

Pest resistance traits are aimed at improving crop performance by protecting against pests. For example, researchers found a gene in the genome of a wild Mexican potato (*Solanum tuberosum*) variety that was subsequently engineered into cultivated potato, allowing the GE potato to survive exposure to the many races of *Phytophthora infestans*, the fungus responsible for the Irish potato famine (215). A native gene, *Mi*, from tomato was upregulated to protect the roots against root knot nematode (196). Although Europe has been reluctant to embrace engineered crops, the first field trial of GE grapes (*Vitis vinifera*) took place in the northern Alsace region of France in 2005. A coat protein gene from fanleaf virus was inserted into the grape rootstock (29), but not in the scion, the portion of the plant that bears fruit.

Some traits aimed at improving field performance of crops for farmers could, given responsible usage, also positively impact the environment. One key aspect of crop performance is yield. In 2001, transgenic rice plants expressing the maize proteins pyruvate orthophosphate dikinase (PPDK) and phosphoenolpyruvate carboxylase (PEPC) exhibited a higher photosynthetic capacity ($\geq$35%) compared with untransformed plants (130). Another agronomic improvement focuses on nitrogen use efficiency, aimed at reducing fertilizer usage and increasing sustainability. The plant-specific transcription factor Dof1, when introduced into the model plant species *Arabidopsis*, increased nitrogen content by ~30%,

improving growth under low-nitrogen conditions (253).

Another focus is on improving abiotic stress tolerance, e.g., high salt, high and low water availability, and temperature extremes. Constitutive expression of *CBF* genes from the cold response pathway in GE *Arabidopsis* induces expression of target *COR* (cold-regulated) genes and enhances freezing tolerance in nonacclimated plants (140). Transgenic tomato plants overexpressing a vacuolar Na^+/H^+ antiport produce fruit when grown in 200 mM sodium chloride, ~40% of sea water concentration (257), and the tomato fruits display very low sodium content. The first use of GE to alter nutritional quality was the introduction of three genes into rice to create the much publicized Golden Rice variety, enriched in provitamin A (254) (See section 3.21). Efforts have also been successful in increasing calcium levels threefold in potato (174), as well as increasing folate levels in tomato (54).

Approaches utilizing GE plants have also focused on combating human diseases and include the development of a subunit vaccine against pneumonic and bubonic plague that is immunogenic in mice (6); a potato-based vaccine for hepatitis B, shown to raise immunological responses in humans (233); a GE pollen vaccine that reduces allergy symptoms (164); and an edible rice-based vaccine targeted at alleviating allergic diseases such as asthma, seasonal allergies, and atopic dermatitis (225) (See section 3.14).

The utilization of plants to produce alternative energy sources is a present focus of attention, given the global rise in nonrenewable energy usage and greenhouse gas emissions. One approach involves engineering the green alga, *Chlamydomonas reinhardtii*, to produce hydrogen gas, a clean, renewable fuel source (151). Paper waste, particularly from newspapers, is a major environmental pollutant that because of compaction remains in landfills for decades without decomposition. GE bacteria engineered with trifunctional designer cellulosomes or bifunctional systems can degrade microcrystalline cellulose and straw (72). Efforts are also aimed at improving the ability of engineered plants and microbes to process cellulosic biomass into usable biofuels (For reviews, see 221 and 237).

3. FOOD ISSUES

The topics addressed in this section represent some major issues that have been raised regarding GE foods. These include food safety of GE plants and animals, pharma crops, labeling, allergenicity, nutritional composition, organic foods, and food safety testing.

Allergenicity: reaction to a substance that is foreign to the body and can cause a hypersensitive or allergic reaction in certain people

Nutritional composition: includes protein, carbohydrate, fat, vitamins, minerals, fiber, moisture, and phytochemical levels

3.1. Did People Die After Consuming Tryptophan Made By Genetically Engineered Bacteria?

In 1989 claims surfaced that a nutritional supplement, L-tryptophan, used to treat insomnia, premenstrual syndrome, and depression, caused an epidemic of eosinophilia-myalgia syndrome (EMS) in the U.S.; the number affected was reported to be "between 5000 and 10,000 people and the number of deaths near 40" (213). All affected people had consumed tryptophan made by one Japanese company (197) that had produced L-tryptophan using GE bacteria without incident prior to 1989. However, in 1989 the company changed GE bacterial strains and manufacturing processes, eliminating some filtration steps and reducing by half the amount of active carbon used for purification. Although the final product was 99.6% pure, it still contained 60 different impurities (148), any one of which could have caused the illness, although the cause of the problems was never conclusively linked to the organism or the manufacturing process. But reconstruction experiments (148) make it likely that the presence of the causative impurity was not due to the GE bacterium, but to the changes in processing. In a legal summation, it was stated that "the fermentation and later cooking of industrial sized lots of L-tryptophan generated the contaminant" that was legally responsible for the

GM: genetically modified

Toxicity: adverse physiological effects following exposure to a substance

autoimmune EMS disease (234). Procedures should have been conducted to assess safety after changes were made in the strains and production methods.

3.2. Were Potatoes Genetically Engineered with a Lectin Protein Unsafe to Eat?

In the late 1990s, Ewen & Pusztai (69) conducted studies on rats fed potatoes engineered to express an introduced lectin gene from a snowdrop plant (*Galanthus nivalis*), intended to reduce insect damage. After feeding, they observed stomach lesions in the rats and concluded that "the damage to the rats did not come from the lectin, but apparently from the same process of genetic engineering that is used to create the GM foods everyone was already eating" (211). This study and its conclusions were strongly criticized by the scientific community (186), because the study was conducted with too few animals and inadequate controls. Following the initial announcement of the findings to the popular press, the original study was published in the *Lancet* to provide researchers an opportunity to view the data. But the data in the paper left researchers unable to draw firm conclusions (134) or confirm or deny results. The U.K.'s Royal Society criticized the study for lack of proper controls. In the same issue of *Lancet* in which the paper was published, Dutch scientists concluded the observed toxic effects might be due to nutritional differences between control and GE potatoes, not from the GE process (133). To reach firm conclusions, experiments should be repeated on larger numbers of animals with proper controls. Notably, this product was not marketed and the results do not extend to safety analyses of other GE crops. (See section 3.4)

3.3. Were Fish Genes Introduced into Strawberries?

An antifreeze gene from Artic flounder was introduced into tobacco and tomato (103) and field-tested in tobacco (*Nicotiana tobacum*) (137) and tomato (114); it was not introduced into other crops, like strawberries (*Fragaria* × *ananassa*), nor was it commercialized. The gene used, *afa3*, encoded an antifreeze protein, which in the blood of polar fish was found to inhibit ice recrystallization; however, despite high mRNA levels in the leaves of transformed tobacco, no inhibition of ice recrystallization was detected. If the approach had been pursued, additional environmental and food safety tests would have been conducted to study the impacts of this gene on the plant, the environment, and consumers. Although humans consume flounder and this protein, substantial equivalence and allergenicity and toxicity tests (See section 3.4) would have been done to assure the safety of the gene product in new foods.

However, issues with foods such as those engineered with a fish gene go beyond scientific risk, they raise questions of whether exchanges between certain organisms should be carried out. Cross-kingdom transfer of animal genes to plants is not popular with consumers worldwide (143), who are more comfortable with gene transfer among plants or between plants and bacteria (118). In fact, in a 2001 poll, 33% of U.S. respondents believed that it was not possible to transfer animal genes into plants and 16% weren't sure (199). To date, no human or animal genes have been introduced into any commercialized GE crops in the U.S., but rice engineered with human lysostaphin and lysozyme to combat childhood diarrhea (256) has been grown in the field (87).

3.4. Are Food Safety Studies Conducted on GE Foods?

GE foods and products made from GE crops that are used in foods today have undergone safety testing by the companies or institutions that developed them (See sections 3.6 and 3.7). The data were then reviewed by federal regulatory agencies. Frequently GE foods and products made from GE crops are also tested by outside groups and the results published in

peer-reviewed journals. This process is comparable to safety assessments done for pharmaceutical drugs and biomarkers; pharmaceutical companies provide safety data that are subsequently reviewed by FDA scientists (82). Consultation with and submission to regulatory agencies of certain safety data for GE foods is voluntary, as are some data for pharmaceutical products (82); however, the legal requirements that foods (and pharmaceuticals) have to meet are not voluntary. Although GE foods can be marketed without certain regulatory approvals, to date all products in the marketplace have undergone full review by regulatory agencies regarding safety and content relative to unmodified forms (searchable data on specific events available at 84). Submitting the safety data is in the developer's best interests, however, given the legal liabilities incurred should a problem with the food arise following market introduction (See section 3.14).

The EPA focuses on environmental and human health impacts of pesticides and therefore evaluates GE plants with altered pesticide traits. The EPA's regulatory oversight of Bt crops is based on the presence in the plant of Cry proteins from *B. thuringiensis* (See section 3.7), which are termed plant-incorporated protectants (PIPs), substances that alter the crop's pesticidal properties (65).

Health safety assessments of GE foods are based in part on the concept of substantial equivalence (132). If the food and/or its new ingredient(s) is substantially equivalent to existing foods or food ingredients, it is treated like conventional foods with respect to certain aspects of its safety (124). Food or food ingredients used safely for long periods or foods substantially equivalent to these foods in nutritional characteristics do not require additional extensive safety testing. Substances that result in scientifically based safety issues require additional testing in the laboratory or in animal models.

A determination of substantial equivalence requires analysis of GE foods relative to comparable existing foods in terms of protein, fat, starch, amino acid, vitamin, mineral, and phytonutrient composition (20, 209, 229). GE foods can be designated substantially equivalent to their existing counterparts, substantially equivalent except for certain defined differences (on which safety assessments are then needed), or not substantially equivalent, meaning more safety testing and further review are necessary. When making such comparisons, it is important to note that the composition of components varies across a range—whether conventional, organic, or GE. For example, when polyphenol profiles of fresh apple juices from various apple (*Malus domestica*) cultivars and commercially available apple juices were compared, significant differences were found in total polyphenol content, as well as in profiles of individual polyphenols, as analyzed by high-performance liquid chromatography (HPLC)-photodiode array detection and HPLC-electrospray ionization-tandem mass spectrometry (123).

Cry: crystal protein

Large numbers of animal tests on GE foods and GE ingredients have been conducted and published in the literature (See 40, 76, 127, 185, and 244 for reviews). In the studies reported in these reviews, both chemical analyses and studies in a variety of animals (e.g., dairy cows, beef cattle, pigs, laying hens, broilers, fish, and rabbits) revealed no significant, unintended differences between GE and conventional varieties in composition, digestibility, or animal health and performance. The lack of significant differences between GE food and feed and isogenic counterparts in these tests strongly supports their substantial equivalence.

Food safety testing in animals is used to determine toxicity and allergenicity of the GE food or ingredient; however, such testing of whole GE foods and feeds is difficult or impossible owing to the need for animals to consume large amounts of food to obtain sufficient quantities of the GE ingredient. Compositional analyses and toxicity testing of individual components are actually more sensitive and accurate in assessing safety (40). Therefore, in addition to whole foods, safety

Transgene: a gene that is manipulated using recombinant DNA technologies and reintroduced into a host organism

tests are conducted on individual products of introduced genes, both target and selectable marker genes, on the basis of the food additive provision (Section 409) of the 1992 Federal Food, Drug, and Cosmetic Act (83). This act states that substances intentionally added to food are food additives, unless they are GRAS or are exempt, as with a pesticide, and are then the responsibility of the EPA. GRAS status is established by a long history of food use or when the nature of the substance does not raise significant, scientifically based safety issues (77). For example, the FlavrSavr™ tomato (See section 2.8) was created using a kanamycin resistance selectable marker gene; data on the selection gene and its product were submitted by the company and, following review, the gene and its product were granted GRAS status (188).

3.5. What Happens to the DNA in Foods When They Are Eaten?

The daily human intake of DNA in food is estimated at 0.1–1 g (75). Estimates of the total daily transgene DNA intake can be calculated, assuming 50% of the diet is from GE foods and transgenes represent an estimated 0.0005% of total DNA in food, as 0.5–5 μg/day. DNA is chemically identical regardless of its source and is mostly degraded during industrial processing and in the digestive tract. Small fragments can be detected in certain body tissues, such as leukocytes, liver, and spleen. For example, fragments of orally administered phage M13 and plant DNA were taken up by phagocytes as a part of their normal function as immune system cells (200, 201). In rare instances fragments could pass into other organs, including the fetus, but were never demonstrated to be intact. Others reviewing the published data in these papers argued that the rare events observed more likely resulted from contamination (18, 91, 120).

In July 2007, the European Food Safety Authority released statements on the fate of genes and proteins in food and feed: "After ingestion, a rapid degradation into short DNA or peptide fragments is observed in the gastrointestinal tract of animals and humans" and "To date a large number of experimental studies with livestock have shown that rDNA fragments or proteins derived from GM plants have not been detected in tissues, fluids or edible products of farm animals" (68).

No reproducible data exist to show that transgene DNA in commercialized GE crops has unique behavior relative to native plant DNA. However, in late 2005 Dr. Irina Ermakova (184) of the Russian Academy of Sciences publicly announced her study, describing stunted development and higher infant mortality in rats fed diets containing Roundup Ready® soybeans (55.6% mortality) compared with rats fed conventional soybeans (9% mortality). Among the possibilities, she claimed that animals died of mutations induced solely by the transgene DNA—on the basis of earlier claims that DNA insertion in the plant genome is highly mutagenic (136). Her results were not published in a peer-reviewed scientific journal, but were presented at international symposia (184) and during parliamentary debate in New South Wales, where the data were used to push for a ban on GE crop cultivation in the European Union (E.U.) (175).

The results of the Ermakova study contradict the results from a number of other, sometimes multigenerational, studies on rats and mice fed Roundup Ready® soybeans that revealed no adverse effects on litter size, histological appearance of tissues, or numbers of deaths of progeny (30, 231, 259; For review, see 217). Differences between these studies and those of Ermakova likely relate to aspects of her experimental procedure: *i*) the conventional diet was from an uncharacterized soy variety, *ii*) the number of pups in the litters was small, and *iii*) reproductive rates in rats fed conventional soy were low. In an attempt to understand her studies, the editor of *Nature Biotechnology* invited Dr. Ermakova to provide a detailed account of her work (145). In this dialogue, Ermakova admits to having

questions about her own results, making the need for peer-review and controlled repetition of her studies using proper controls essential.

3.6. Do Genetically Engineered Foods Have Changes in Nutritional Content?

Preventing adverse health effects of foods requires the application of appropriate scientific methods to predict and identify unintended compositional changes resulting from genetic modification of plants, animals, and microbes—whether by classical or rDNA methods. It is the final product, rather than the means by which it is modified, that is more likely to result in unintended effects (50). Nonetheless, the nutritional composition of GE foods, including levels of protein, carbohydrate, fat, vitamin, mineral, fiber, moisture, and phytochemicals, is analyzed for substantial equivalence, and levels of individual nutrients and antinutrients in GE foods are compared with levels in conventional counterparts (See section 3.4).

When considering substantial equivalence, it is important to note that a range of natural variation is observed in conventionally bred cultivars when grown under similar conditions (208). Therefore, comparisons of nutritional content of GE foods must be measured against variation in conventional foods grown under comparable conditions. For example, nutrient composition of GE potato tubers was compared with control wild-type and tissue culture–derived non-GE potato tubers of two cultivars, cv. Record and cv. Desiree, grown under the same conditions. Data were analyzed using targeted compositional analyses (207). An analysis of variance (ANOVA) for the major consensus nutrient compounds, recommended by the Organization of Economic Cooperation and Development (171) as being appropriate for safety assessment of novel foods, was conducted and no consistent differences, outside normal variation, were found among the tubers.

Extensive nutritional equivalence studies of Roundup Ready® soybeans have been conducted. These studies include analyses of protein, oil, fiber, carbohydrate, ash, and moisture content and the amino acid and fatty acid composition in both seeds and toasted soybean meal; the values were compared with those from conventional soybeans. Special attention was given to levels of antinutrients and phytonutrients typical for soybeans, e.g., trypsin inhibitors, lectins, and isoflavones (172). One significant difference was detected in defatted, nontoasted soybean meal, the starting material for the production of commercially utilized soybean protein. The variation was in trypsin inhibitor levels, which were 11%–26% higher in GE soybeans than in wild-type. However, levels in seeds and defatted, toasted soybean meal, the form used in foods, were similar for all lines. The results demonstrated that the composition of these GE lines is equivalent to that of conventional soybean cultivars in the form consumed by humans. Equivalence of the feeding value of this GE soy was also demonstrated by feeding it to rats, chicken, catfish, and dairy cattle (100). A broader study using Bt corn and Roundup Ready® corn and soybean to look at composition, digestibility, and feeding value for sheep, chickens, and beef and dairy cattle concluded that seeds of the GE varieties were substantially equivalent to seeds from isolines of non-GE varieties (46).

A 1999 study of nutritional equivalence by Lappé and others (135), often cited by those concerned about GE crops, showed that Roundup Ready® soybeans had reduced levels of isoflavones, notably genistin and daidzin, and thus had significant implications for human health given the potential positive health benefits of the two compounds. The American Soybean Association published a response to this study indicating the variation in phytoestrogen levels was within the limits of variability for conventional soybean varieties (1). In fact, not all comparisons in the Lappé study of the two compounds in conventional versus transgenic varieties showed

reduced levels; some showed significant increases (128, table 1). Another phytoestrogen, glycitin, showed significant decreases in only two of seven samples. These results underscore the variability of phytoestrogen levels from sample to sample. A premise of the Lappé study (128) was that other studies on Roundup Ready® soybean used seeds from non-herbicide-treated plants and this raised concerns on the basis of preliminary data from *Phaseolus* that herbicide treatment might generate increased levels of phytoestrogens (198). However, the original 1999 study on Roundup Ready® soybean safety was performed on seed from herbicide-treated plants and no differences in phytoestrogen levels were observed (229).

It is important to note that genetic engineering can purposefully be used to change the nutritional profiles of foods. In these cases studies similar to those described above would be conducted; the mandate for substantial equivalence would apply only to compounds unrelated to the introduced trait. Examples of such foods include those with increased β-carotene (173, 254), flavinoids (53, 189), calcium (174), folate (54), and iron availability (57) (See section 2.9). According to FDA policy, GE foods with altered nutritional traits must be labeled to indicate nutritional differences; one example is Vistive™, a low-linoleic oil from GE soybeans that can be used instead of trans fat–containing oils (157).

3.7. Is the Bt Protein Safe for Human Consumption?

Bt proteins, naturally occurring insecticides produced by the soil bacterium, *B. thuringiensis*, have been used to control crop pests since the 1920s (89), generally as microbial products. Many strains of *B. thuringiensis* exist that produce different Bt proteins varying in the insects they target, e.g., larvae of butterflies and moths, beetles, and mosquitoes. The insecticidal Bt proteins form crystalline protein bodies inside the bacterium, hence the name Cry proteins. Full-sized Cry proteins are inactive until eaten by target insect larva, and inside the midgut they are cleaved and become active. The smaller, active peptides bind to specialized receptors, creating holes in the gut membrane that cause contents to leak and kill the larvae. The precision of different Bt proteins for their targets resides in the specificity of their tight binding to companion receptors in the insect gut (70).

Bt microbial products have a long history of safe use (~40 years) with only two reports prior to 1995 of possible adverse human effects, neither of which was due to exposure to Cry proteins (149). In a 1991 study that focused on exposure via inhalation of Bt sprays, results showed immune responses and skin sensitization to Bt in 2 of 123 farm workers (21). In a 2006 article, the Organic Consumers Association linked this observation to possible impacts of Bt in GE foods, warning that "Bt crops threaten public health" (38). But the respiratory sensitization observed in the farm workers does not provide validation that oral exposure to Bt would result in allergic responses.

In recent years a variety of safety studies were conducted specifically on native Bt proteins to show that they do not have characteristics of food allergens or toxins (See 64, 70, and 152 for reviews). In its review of Bt proteins, the EPA stated that, "several types of data are required for Bt plant pesticides to provide a reasonable certainty that no harm will result from the aggregate exposure of these proteins." The data must show that Bt proteins "behave as would be expected of a dietary protein, are not structurally related to any known food allergen or protein toxin, and do not display any oral toxicity when administered at high doses" (64). The EPA does not require long-term studies because the protein's instability in digestive fluids makes such studies meaningless in terms of consumer health (206). In vitro digestion assays were used to confirm degradation characteristics of Bt proteins, whereas murine feeding studies were used to assess acute oral

toxicity (22, 64). Data on *Cry1Ab* in maize and cotton and *Cry1Ac* in tomato, maize, and cotton have been carefully reviewed by regulatory agencies in numerous countries, including the U.S., Canada, Japan, U.K., E.U., Russia, and South Africa (4).

The possibility for allergenic effects of four maize Bt varieties was specifically investigated in potentially sensitive populations (16). Skin prick tests were performed with protein extracts from MON810, Bt11, T25, and Bt176 and from nontransgenic control samples in two sensitive groups: children with food and inhalant allergies and individuals with asthma-rhinitis. Immunoglobulin E immunoblot reactivity of sera from patients with food allergies was tested versus Bt maize and pure Cry1Ab protein. No individual reacted differently to transgenic and nontransgenic samples; none had detectable IgE antibodies against pure transgenic proteins.

A truncated version of the full-length 131-kDa Bt protein, containing only the insect-toxic fragment, is used to engineer some crops. For example, Mon810 maize contains a truncated *cry1Ab* gene that codes for a 91-kDa protein. The potential for mammalian toxicity of the truncated protein was assessed by administering purified, truncated Cry1Ab protein from *E. coli* to groups of ten male and female CD-1 mice at $\leq$4000 mg/kg body weight (2). These doses represented a 200–1000-fold excess over the exposure level predicted on the basis of human consumption of MON810 grain. Mice were observed up to 9 days after dosing; no treatment-related effects on body weight, food consumption, survival, or gross pathology upon necropsy were observed for mice administered Cry1Ab truncated protein.

Despite extensive evaluations of Bt food safety, in June 2005 a Greenpeace press release, published in the *New York Times* and other international newspapers, stated, "There are strong warning signs that this GE Bt rice could cause allergenic reactions, as it did when tested on mice based on a study (158) and references therein". However, in the Moreno-Fierros study (158) referred to in the press release, *Cry1Ac* was being tested as an oral adjuvant to boost vaccine titers. As such, the protein was used in large amounts and the stomach pH was raised to prevent degradation of Cry1Ac. It had been chosen as an adjuvant precisely because it is nontoxic to vertebrates (193).

The native Cry9c, a protein effective against lepidopteran insects, was engineered into a variety of corn called Starlink^{TM}. Researchers knew the Cry9C protein did not originate from an allergenic source and had no amino acid homology with known toxins or allergens in available protein databases. However, when Starlink^{TM} corn was created, the Cry9C protein had no history of human dietary exposure, and in addition it was not readily digestible and was stable at 90°C (62), both hallmarks of certain allergens (See section 3.9); Cry9C also had biochemical characteristics that differentiated it from other previously reviewed Cry proteins (63). To determine with reasonable certainty that no harm would result from human exposure to this protein, it was necessary for the EPA to determine if proteins with these biochemical characteristics were likely to affect the safety of a food. Because it was slow to digest, it provided longer lasting protection against insect damage, but the altered digestibility characteristics in humans and its relative stability to heat caused regulators to delay approval of the crop for human consumption (although it was approved for animals) so that they could reexamine its potential as a human allergen (See section 3.9).

A positive aspect of safety regarding Bt corn is the lower levels of mycotoxins compared with non-Bt corn. Mycotoxins are toxic and carcinogenic chemicals produced as secondary metabolites of fungal colonization (252) that occur as a result of insects such as the corn earworm carrying the mycotoxin-containing fungi that infest the kernels following wounding. In some cases, the reduction of mycotoxins in Bt corn results in a positive economic impact on U.S. domestic

and international markets. More importantly, in less-developed countries certain mycotoxins are significant contaminants of food and their reduction in Bt corn could improve human and animal health.

In 2002, APHIS announced the deregulation of a corn variety, Mon 863, with increased rootworm (*Diabrotica* spp.) resistance. Food safety assessments by the company used 90-day mouse feeding trials to demonstrate safety (156); independent assessments also demonstrated the safety of Mon 863 (94, 109, 228). Mon 863 contains a variant Cry3Bb1 with seven amino acid differences from wild-type Cry3Bb1 to enhance plant expression and insecticidal activity against corn rootworm (3). A 2007 paper (203) contained a statistical reanalysis of the original data that was different from the earlier risk assessment analyses, which caused the authors to conclude that "with the present data it cannot be concluded that GM corn MON 863 is a safe product." After the 2007 peer-reviewed publication, the European Commission requested the European Food Safety Authority (EFSA) to determine what impact the reanalysis had on their earlier decision. The EFSA concluded that the reanalysis did not raise new safety concerns (67).

3.8. Have Allergens Been Introduced into Foods Through Genetic Engineering?

The use of genetic engineering to introduce genes into an organism raises the possibility of the introduction of allergens. Under the FDA's biotechnology food policy, GE foods must be labeled if the source of the gene is one of the common allergy-causing foods [e.g., cow's milk, eggs, fish and shellfish, tree nuts, wheat, soybeans, and especially peanuts (47)], unless the gene product is proven not to be allergenic through additional safety testing. Although not mandatory, to date all companies marketing new GE foods have consulted with the FDA and performed recommended analyses to determine if introduced proteins have properties that indicate possible allergenicity, i.e., similarities to known allergens, small size, slow digestibility, and/or high heat stability (230). Although there are exceptions in each category, these characteristics indicate the protein might be allergenic and therefore merits further study.

One example of an introduced allergen that was forestalled by this process was the attempt to engineer soybean with a Brazil nut protein, the methionine-rich 2S albumin, to improve soy protein's deficiency in the essential amino acid, methionine. Attempts to manipulate this nutrient through traditional breeding had failed because of lower yields or grain quality. In the development of the GE soybean researchers recognized that allergies to nuts are among the most common types of allergies and allergies specific to Brazil nut had been documented (14). Therefore, testing of the new soybeans for allergenicity was conducted in university and industrial labs during product development. Sera from people allergic to Brazil nut reacted with the new soybean (166), so development of the new soybean was halted and it was never marketed.

Foods can also be engineered to remove offending allergens to create, for example, more hypoallergenic foods (See section 3.11).

3.9. Were Foods Made From Bt Corn Removed from the Market Because of Allergenicity Concerns?

An example of a commercialized GE crop that was recalled owing to concerns about allergenicity is Starlink^{TM} corn, a variety engineered to express the Bt *Cry9C* protein (See section 3.7). The EPA did not approve use of StarLink^{TM} corn for for human consumption; animal consumption was approved because farm animals do not have food allergies. The concern was that the Cry9c protein shared several molecular properties with proteins that are known food allergens (39)—namely, increased heat stability and slower digestibility characteristics. While additional

testing was being conducted to determine human safety, Starlink™ entered the human food supply because of problems encountered with segregating feed and food corn. As a result, the FDA issued a recall of numerous food products containing Starlink™ corn.

In October 2000 the FDA asked the Centers for Disease Control and Prevention (CDCP) to investigate 51 reports of human illness that individuals claimed were related to consumption of products containing Starlink™ corn. Of the 51 reports, 28 described symptoms consistent with a possible allergic reaction to corn products. Blood serum samples from 17 patients were tested using an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to the Bt protein. The CDCP study (39) concluded that Starlink™-specific antibodies were not detected in those human sera; however, the study was not conclusive for two reasons. First, food allergies can occur in individuals even if they have no detectable allergy-specific antibodies that bind to the allergen (170). Second, the source of the protein to make antibodies was of bacterial origin, not plant, and this could have changed the conformational shape of the protein, compromising the ability of the antibodies to recognize the plant-made protein. However, researchers analyzed the corn-containing foods consumed by 10 of the 17 test subjects who reported allergic reactions. Detection of Bt protein was negative in 9 of 10 samples; the tenth was inconclusive (74)

Taken together, these results suggest that the Bt protein in Starlink™ was not involved in the allergic reactions of the 17 individuals tested. But uncertainty still exists because blood and food samples were not received from all 28 individuals who experienced a true allergic reaction. In separate studies, an EPA scientific advisory panel concluded that the Bt in Starlink™ had a moderate chance to cause allergies, on the basis of its biochemical nature. But the level of its presence in food at that time was low; Starlink corn represented between 0.4–0.5% of U.S. corn production (202) and levels of protein also influence its potential for allergenicity (73). Starlink™ corn was removed from the market in 2000 and, on the basis of USDA monitoring, the food supply is now 99.99% Starlink-free (242) and Starlink™ corn therefore is not currently likely to cause allergy-related problems.

3.10. Do Only Genetically Engineered Foods Cause Food Allergies?

Allergies are present in conventional foods such as milk, eggs, fish, shellfish, tree nuts, soybeans, wheat, and peanuts, termed the "big eight"—the foods that are the major allergen sources for adults and children in the U.S. Another example of a conventionally bred food, not considered to be allergenic when introduced in the U.S. in the 1960s but now known to cause allergenic responses, is the kiwi (*Actinidia arguta*). No allergenicity testing or screening was conducted on the fruit when introduced; however, today kiwi is known to cause allergic reactions (222), some of them lethal due to cross allergies with latex (245). This raises the question of how much testing introduced foods, GE or classically bred, should undergo in the U.S. before being offered to consumers.

Given that food safety testing conducted on GE foods focuses on the introduced gene and its protein product (See section 3.4), it seems unlikely that allergenicity issues related to a commercialized GE food that has undergone FDA scrutiny will be greater than that of conventional foods, created by classical breeding and mutation, that have not undergone such scrutiny (50). Does this mean GE foods are 100% safe? No, a statement that a food is 100% safe cannot be made about any food—be it conventional, GE, or organic. For example, a peanut—whether grown conventionally or organically, whether GE or non-GE—can cause severe allergies in sensitive individuals (178).

CaMV: cauliflower mosaic virus

3.11. Can Genetically Engineered Foods Have Fewer Allergens than Non-GE Foods?

On the basis of data from the third National Health and Nutrition Examination Survey (160), 54.3% of individuals aged 6–59 had a positive skin test to at least one of the ten allergens tested (12). The highest prevalence was for dust mite, rye, ragweed, and cockroach; approximately 25% of the population tested positive to each allergen. Peanut allergy was the least common—only 9% of the population—but it is one of the most severe and durable allergies. Other food allergies include those to milk, eggs, fish, shellfish, tree nuts, soybeans, and wheat (See section 3.10). The nature of the proteins causing these allergic reactions is well characterized in certain cases, thus making it is possible to engineer the organism to make lower levels of the proteins responsible for the allergies or change their conformation to reduce allergic responses (33). Reported successful examples of engineering approaches that reduce allergenicity include those aimed at grass pollen (24, 25) and foods such as wheat (34, 35), rice (224), and peanuts (*Arachis hypogaea*) (219).

3.12. Do Viral Sequences Used in Plant Genetic Engineering Create a Human Health Risk?

Introduced transgenes are regulated by promoter sequences that determine how much, where, and when the encoded protein is expressed. The 35S promoter from the cauliflower mosaic virus (CaMV) (168) was used in some commercial GE crops, e.g., Bt11, Bt176, Mon810 maize, and Roundup Ready® soybean (4). This promoter was used to obtain strong expression of the linked gene throughout the plant (19). In other GE crops such as high laurate canola, a native promoter (*Brassica napin* storage protein promoter) led to expression of the California laurel (*Umbellularia californica*) thioesterase in embryos, but not in leaves or pollen (187).

It has been claimed that the 35S promoter may be unstable and prone to transfer and insertion into DNA of other cells, on the basis of a recombinational hotspot in the promoter (104). This theory led to claims that use of the 35S and other viral promoters in GE crops might increase human cancer rates by activating nonviral genes in the species into which it was transferred (humans) by horizontal transfer. Although not based on direct scientific experimentation, Stanley Ewen, who collaborated with A. Pusztai on the snowdrop lectin studies in potato (69), speculated that the CaMV promoter "could affect stomach and colonic lining by causing a growth factor effect with the unproven possibility of hastening cancer formation in those organs" (212).

These speculations have been extensively rebutted by the scientific community, as summarized in 105. One major thrust of the rebuttals is that the 35S promoter is ubiquitous in nature. In the U.K. an estimated ~14–25% of oilseed rape in the field is infected with CaMV (101); similar numbers have been estimated for cauliflower and cabbage. Because of its prevalence in foods, humans have consumed CaMV and its promoter at high levels for decades with no observable effects. The presence of the CaMV promoter in GE plants does not in principle present a different situation. Additionally, DNA in food is rapidly broken down during digestion, giving it little time to interact with the stomach and colonic linings (See section 3.5).

A documented issue with this promoter in the laboratory is that it can become inactivated if CaMV infects the GE plant with a CaMV-driven transgene. This inactivation was demonstrated when CaMV-driven herbicide resistance in oilseed rape was compromised, causing the virally infected plants to become susceptible to the herbicide (5). Although not related to human safety, this situation should be carefully monitored in the field to avoid unexpected situations. At present other promoters that are not derived from plant viruses are being used in GE plants (45, 183, 247).

3.13. Can Genetically Engineered Foods Increase Antibiotic Resistance in Human and Animal Intestinal Flora?

The frequency of resistance to antibiotics in bacteria and the numbers of drugs to which they are resistant is increasing. Several factors have been suggested as exacerbating this problem (163). One potential causative factor is the widespread use of antibiotics in human therapy (90, 119). Another potential causative factor is the subtherapeutic use of antibiotics for growth promotion in farm animals (41, 138, 216). In a 2007 report on levels of antibiotics in the manure of animals fed antibiotics, data were presented on the passage of antibiotics to foods, especially root crops, when manure was used as fertilizer (56). This is of potential importance to all farmers who utilize animal manure as a primary source of fertilizer.

Antibiotic-resistance genes—sometimes used as markers to identify GE plant cells that receive transgenes—might add to the problem of antibiotic-resistant bacteria. For marker genes in GE foods to increase antibiotic resistance in humans or animals, they must be transferred to bacteria in the respective digestive tracts. Functional transfer of plant DNA into microorganisms is directly impacted by intactness of DNA. Complete transfer of the antibiotic resistance gene, and possibly its controlling elements, and its integration in the bacterial chromosome must occur to make a bacterium antibiotic-resistant.

During chewing, cells in food are broken down. As cells are destroyed, DNA is released and highly active enzymes in saliva and in the plant start degrading DNA (153)—a process that continues in the digestive tract, where other enzymes further break down DNA and proteins (See section 3.5). In mouse studies, fragments but not intact pieces of M13mp18 DNA were found in 0.1% of white blood cells and spleen or liver cells at 2–24 h after feeding, but not later (201). In humans, foods remain in the stomach for ~2 h, where the remaining DNA is fragmented into small pieces. To demonstrate the fate of transgene DNA in humans, the antibiotic resistance gene from GE maize was shown not to transfer to gut bacteria in chickens fed GE maize (48).

Although GE crops are not likely to be significant factors in increasing the incidence of antibiotic-resistant bacteria, new selection strategies for identifying engineered plants were developed, in part as a response to public concerns, and these offer alternatives to the use of antibiotic resistance genes as selectable markers. These approaches include genes such as phosphomannose and xylose isomerase that facilitate selection by giving transgenic cells a metabolic advantage over nontransgenic cells (180). Also, means exist to segregate marker genes so they do not remain in the commercial product (128, 258). An *Agrobacterium*-mediated method is available that uses plant-derived transfer DNA and a novel transient selection system that can result in only native DNA in GE plants (194).

3.14. Can Genetically Engineered Food Crops Be Used to Make Pharmaceuticals? Could They Contaminate the Food Supply?

In the early 1990s, efforts were made to evaluate the effectiveness of plants and foods to deliver pharmaceuticals, particularly vaccines. These efforts involved using tobacco to express a bacterial surface protein to prevent dental caries and to express the hepatitis B surface antigen (52, 147). Since then, maize, potato, rice, soybean, and tomato have been used to produce vaccines for both humans and animals (177). These include subunit vaccines against pneumonic and bubonic plague, shown to be immunogenic in mice (6); a potato-based vaccine for hepatitis B that raises an immunological response in humans (233); a GE pollen vaccine that reduces symptoms in allergy sufferers (164); and an edible rice-based vaccine targeted to allergic diseases such as asthma, seasonal allergies, and atopic dermatitis (225) (See section 2.9).

Plant vaccines have the advantage of being readily consumed with limited or no processing and of obviating the need for cold storage, clear advantages in developing countries. However, with this ease of delivery comes the possibility that such products could enter the food supply if food crops are engineered. Under U.S. regulations, GE plants containing pharmaceutical or industrial products are not permitted to enter the food supply. The FDA prohibits "adulterated" foods in the supply chain, including foods from GE crops that might contain potentially harmful proteins (81). APHIS, which regulates the movement and field testing of GE plants (See section 3.6), requires special steps to prevent plants that produce drugs or industrial enzymes from contaminating food crops: *i*) labeling, packaging, and segregating regulated plant materials; *ii*) reproductive isolation to prevent GE pollen from fertilizing conventional plants; *iii*) postharvest monitoring to remove volunteer plants; and (*iv*) proper disposal of the transgenic material.

In 2005 these rules were tightened to include the following: *i*) exclude field growth without a permit; *ii*) include crop inspections seven times/year, twice after harvest; *iii*) increase field isolation distances; and *iv*) use dedicated farm equipment (9). This tightening resulted from early violations of field-testing permits. For example, in two cases regulators found volunteer engineered corn plants producing a pharmaceutical protein (8) that had tassled in a soybean field.

Cases like these demonstrate that "pharming" in food plants can result in mixing with food. The Grocery Manufacturers of America urged the USDA to restrict plant-made pharmaceutical production to nonfood crops (96). The National Corn Growers Association countered by proposing safeguards such as *i*) using plants that are male-sterile or that produce non-GE pollen, *ii*) dedicated production systems that isolate pharma crops, *iii*) third-party verification, and *iv*) grower training programs (159). In September 2002, the FDA released a guidance document that recommends multiple strategies to prevent pharma crops from contaminating human or animal feed (79). This document suggests that those who are growing drug-producing plants that cross pollinate, such as corn and canola, strengthen containment procedures by growing plants in geographical regions where little or none of that crop is grown for food. Following this strategy, Ventria, a company that developed self-pollinating rice engineered to produce human lysostaphin and lysozyme to shorten the duration of childhood diarrhea, relocated their fields from their home rice-growing state, California, to Kansas, where commercial rice is not grown (87).

3.15. Why Doesn't the FDA Require Labeling of Genetically Engineered Foods?

The FDA's labeling policy for GE foods is the same as for conventional foods and it assures that consumers are given information about nutritional, health safety, or food quality changes in the end product. FDA-mandated labels are not used to provide information about the process by which the food is made. If a GE food is significantly different from its conventional counterpart, the food must be labeled to indicate the difference. Instances where the nutritional profile changes are included, for example if the GE food is created using genetic information from a previously recognized allergenic source, such as peanut, soy, or wheat, or if the new protein has characteristics of known allergens. For example, oils made from GE soybean and canola varieties with changes in fatty acid composition must be labeled; foods containing those oils must be labeled and companies producing that oil must use a new name. For example, Monsanto is using the name Vistive™ to market its low–linoleic acid product from GE soybean oils (157). If a food contains a new, potentially allergy-causing introduced protein, the label must state that the product contains the allergen and name its source.

3.16. Are Organic Foods Healthier or Safer?

Organic farming is a method of agricultural production that does not allow the use of synthetic pesticides, fertilizers, or growth enhancers. Foods grown under organic certification differ from conventionally produced food by the manner in which they are grown, handled, and processed, but an "organic" label does not guarantee the nature of the product, the food, or ingredient, only its production method. The important factors for many people who consume organic foods relate to the perceptions that they are healthier, taste better, are better for the environment, have lower pesticide levels and fewer food additives, and are better for animal welfare (214). However, organic certification does not imply that foods produced using organic methods are more nutritious or safer than those produced without organic methods (195).

A 2007 review by the British Nutrition Foundation stated, "There appears to be a perception among many consumers that organic foods are more nutritious and therefore healthier than conventionally produced foods. However, to date there are limited data to support this view" (248). This perception has led in part to increases in the world market for certified organic foods to ~$34 billion in 2005 (111). A 2007 poll showed that 57% of polled consumers strongly believed that science had proven that organic food was healthier than conventional (182, figure 17). Because of the paucity of scientific data, the UK Food Standards Agency decided in October 2007 to seek a contractor who will evaluate relevant studies and compare the nutrient and non-nutrient content of organic and conventional foods to determine if any compositional differences have nutritional or other health effects in the context of the complete diet (86).

In general, only a small number of peer-reviewed studies exist that analyze nutritional differences between foods produced conventionally and organically. Although statistically significant differences have been observed for a limited number of metabolites for a few foods grown under differing environmental conditions using conventional and organic production systems, more research is required to determine if any of these differences have actual health-promoting effects. Some examples of such studies follow.

i. Zörb and colleagues (260) looked at the profiles of 44 metabolites in wheat grown under comparable organic and conventional conditions as a part of a long-term biodynamic, bioorganic, and conventional farming system in Switzerland. Statistical analyses of data, obtained with high-throughput gas chromatography-mass spectrometry, showed that metabolite status of wheat grain from organic and conventional farming did not differ in the levels of 44 metabolites, which indicates low or no impact of farming systems on wheat metabolite composition.

ii. Another study found increases in vitamin C in organically grown kiwifruit compared with conventionally grown fruits, both before and after storage. Postharvest performance was measured for both types of kiwifruit, grown on the same farm and harvested at the same maturity stage (7). Total phenolics and antioxidant activity were also higher in organic fruit.

iii. In tomatoes, levels of the flavonoids quercitin and kaempferol aglycones in archived samples of organically produced tomatoes, grown from 1994–2004 in the Long-Term Research on Agricultural Systems project at University of California, Davis, were at statistically higher levels than those grown in the same tract using conventional production practices (155). Flavonoid levels increased over time in the tomatoes grown organically, but not in those grown conventionally.

iv. Increases in total antioxidant activity were also found in a 2005 study of red

oranges (*Citrus aurantium*). Organic oranges had significantly higher total phenolics, total anthocyanins and ascorbic acid levels, and total antioxidant activity versus corresponding nonorganic oranges (227). Four lots of fruits, purchased from certified producers grown under statutory European Community regulations at the same time of year, were analyzed; however, no assurances were given that the two sources of oranges were grown under comparable environmental conditions. Also, no indications of the natural variation in these phytonutrients were given for comparison.

v. There are "moderately strong and consistent data showing that organic potatoes are richer sources of vitamin C than their conventionally grown counterparts"; no studies have shown lower levels of vitamin C in organic potatoes (248).

Several studies of nutritional differences between organically and conventionally produced dairy products have been reported.

i. Several small-scale studies reported different conclusions when comparing the effects of farming systems on the content in milk of conjugated linoleic acid content, known for its health-promoting effects (cited in 60).

ii. In one large-scale study a higher proportion of polyunsaturated fatty acids and n-3 fatty acids relative to monounsaturated fatty acids was observed in milk from cows raised under organic production methods, compared with those that were conventionally raised (60). No differences were seen in the proportion of conjugated linoleic acid or vaccenic acid, but factors other than farming systems, e.g., time of year, breed, type of feed, and access to fresh grazing, are known to affect the fatty acid content of milk.

iii. A 2007 study conducted on 312 breastfeeding mothers demonstrated that mothers' milk from women eating a diet that consisted of 89% or more of organic dairy and meat products was measurably higher in conjugated linoleic acid (192).

iv. Kuhnert and coworkers (131) looked at the incidence of *E. coli*, particularly Shiga toxigenic and 0157:H7 strains, in milk. Although levels were relatively high in cattle feces, no differences in prevalence of the two types of organisms in milk were found between those raised using organic practices versus conventional farming systems.

Differences reported in nutrient composition between organically and conventionally produced foods are interesting but, as seen in the examples given, it is very difficult to control all variables that might affect nutritional quality and ensure that the observed variations are significant and reproducible. In addition, there are many important nutrients for which no significant differences have been found. For example, in milk no significant differences have been reported in other major nutrients such as calcium, zinc, vitamin B2, or vitamin B12 (248). Much more research is needed to determine whether the nutritional differences observed between organic and conventional food products are reproducible and have a significant impact on human health.

One notable difference between conventional and organic production methods, which may be perceived by consumers as healthier, is the ban on the use of synthetic pesticides in organic agriculture. Synthetic pesticides can only be used in organic farming when an efficacious, natural version is not available and no organic substitutes exist. Lists of chemicals approved for organic agriculture are available (162). With regard to this aspect of food safety of organics, very little peer-reviewed research has been conducted. A small-scale study looked at levels of certain pesticides in children's urine following

consumption of conventional and organic foods. Researchers looked at contributions of daily dietary pesticide intake on overall pesticide exposure during a 15-day period in 23 children, aged 3–11 (142). Children ate conventional foods on days 1–3 and 9–15 and organic foods on days 4–8; attempts were made to substitute comparable food items so as not to change their diets. Analysis of urine specimens collected twice daily showed that concentrations of the organophosphate pesticides malathion and chlorpyrifos decreased to undetectable levels immediately after organic diets were consumed and remained undetectable until conventional diets resumed. However, no direct determinations of levels of organophosphates in the foods were carried out and it was not stated whether the already low levels of organophosphates in the conventional foods would have adverse impacts on health.

Strictly from a nutritional perspective not enough data exist at present to show nutritional benefits from conventionally or organically produced foods that favors consuming either for health benefits. However, if the goal is to promote healthy eating, it is more important for consumers to focus on eating a healthy, balanced diet, rich in fruits and vegetables, than focusing on foods that are produced by particular methods. Convincing epidemiological evidence shows that diets rich in fresh fruits and vegetables, regardless of the methods used to produce them, improve health and are associated with reduced frequency and severity of a number of health conditions (191).

3.17. Should Genetically Engineered Crops and Foods Be Banned Until They Are Proven to Be 100% Safe?

Acceptance of the new GE foods depends on several factors, including perception of risk and benefit, assurance of safety, and one's own values. Nearly everything in our technologically complex world comes with risks. The introduction of the automobile, hybrid crops, margarine, pasteurized milk, and vaccines all came with attendant risks. Only after individuals gained experience with these new products did they become comfortable with choosing those products for which the benefits for them outweighed the risks.

The first GE crops to be released commercially benefited farmers, the companies that produced them, and in some cases the environment, but consumers saw little benefit. In the development pipeline are GE crops and foods that might be attractive to consumers and have greater benefit for the environment (See section 2.9), but benefits realized depend on which products are developed, how they are deployed, and how different individuals value them. Potential advantages from GE crops and foods could be substantial in terms of the environment and human health. In fact, continuing to deplete our resources as we do now is likely to be more harmful than making the best possible use of all available technologies (31).

The second factor relating to acceptance of GE food has to do with assurance of safety. GE foods that make it to the market go through extensive safety testing, the data from which are reviewed by the USDA, FDA, and/or EPA (See sections 2.6 and 3.4). GE foods cannot be guaranteed to be 100% safe, just as foods created by conventional breeding or grown using conventional or organic practices cannot be guaranteed to be completely safe (15). Given that safety testing of GE foods focuses on the introduced gene and its product and a determination of substantial equivalence, food safety issues with a commercialized GE food that are greater than those experienced with conventionally modified foods are unlikely to arise (50).

The third factor relating to acceptance of GE foods has to do with individual values. This aspect cannot be addressed with scientific data.

bGH: bovine growth hormone

IGF-I: insulin-like growth factor I

rbGH: recombinant bovine growth hormone

3.18. Are Milk and Meat from Cloned Cows Safe to Eat?

A clone of an organism is genetically identical to a single common ancestor. Cloning of animals can be achieved by splitting an early-stage multicellular embryo to create twins; the first split-embryo calves were produced in 1981. Clones are also produced by nuclear transfer, in which DNA from the nucleus of one cell is introduced into a recipient unfertilized egg from which the nucleus was removed (223). Nuclear transfer has been performed successfully since the mid-1980s, but Dolly the sheep was different—she represented the first successful nuclear transfer to an adult cell (249). Since then, several adult tissues have been used to produce clones of cattle, pigs, horses, cats, rabbits, goats, and fish (59). Cloning animals is one of many methods used to assist animal reproduction (154).

One of the food safety issues raised regarding consumption of food from cloned animals is whether the process causes changes in the composition of food derived from the animal. The Center of Veterinary Medicine in the FDA has the responsibility to evaluate food safety and animal health issues. In their draft risk assessment of the safety of food from cloned animals, they state it is "highly unlikely that 'silent' pathways producing intrinsic toxicants exist in food animals" and that the only hazards that could arise "would be from incomplete or inappropriate reprogramming of the genetic information from the donor somatic nucleus (i.e., epigenetic effects)" (80). With regard to compositional differences in meat from cloned cows, numerous studies found no obvious differences in milk or meat (167, 226, 235, 236, 246). The FDA draft risk assessment on livestock cloning states, "the current weight of evidence suggests that there are no biological reasons, either based on underlying scientific assumptions or empirical studies, to indicate that consumption of edible products from clones of cattle, pigs, sheep or goats poses a greater risk than consumption of those products from their non-clone counterparts" (80).

3.19. Is Milk from rbGH-Injected Cows Safe? Why Isn't It Labeled?

Bovine growth hormone (bGH), also called bovine somatotropin (bST), is unrelated to steroid hormones. bGH, produced in the pituitary glands of dairy cows, is a naturally occurring protein hormone in milk, which stimulates the liver to produce insulin-like growth factor-I (IGF-I). The structure of human somatotropin differs from bGH, and the latter is not biologically active in humans (176). Upon pasteurization, 90% of bGH is destroyed; digestive enzymes degrade the remainder. Other growth factors in milk (e.g., the cytokines IL-1 and IL-2), though sometimes slightly elevated in milk from bGH-injected cows, are inactive in other mammals (122).

Since the late 1920s it was known that lactating mammals produce more milk when treated with extracts of the pituitary hormone bGH, but because that hormone could only be isolated from the pituitary glands of slaughtered cattle, bGH was not available in sufficient quantities for commercial use in the dairy industry (37). Sufficient quantities were made available when a synthetic gene for bGH was inserted into a bacterium to produce recombinant bGH (rbGH or rbST), which is chemically identical to bGH. When rbGH is injected into cows, the efficiency of conversion of feed to milk is increased and milk yields can be increased by 15% to 20% (17, 55). Trace amounts of bGH is found in all milk; cows given rbGH contain no more bGH than unsupplemented cows (122). Published data indicate that the use of rbGH to increase milk production does not impact its nutritional quality.

Extensive studies of rbGH safety have been conducted worldwide and reviewed by the FDA, after which both milk and meat from

rbGH-injected cows were deemed safe (78). Separate reviews of the data by the National Institutes of Health, the World Health Organization, the Office of the Inspector General of the Department of Health and Human Services, and reviews by the Journal of the American Medical Association and the Journal of the American Dietetic Association all independently concluded that milk from rbGH-injected cows is safe.

Despite these safety assurances, claims were made as recently as 2001 (66) that milk from rbGH-treated cows contains elevated levels of IGF-I, a protein hormone normally present in milk (44). An elevated content of IGF-I has been suggested to have adverse implications for human health and cancer frequency. Comparisons of marketed milk indicate that there are no differences in IGF-I concentrations between milk derived from cows treated or not with rbGH (240), and levels are within the limits of natural variation (for review, see 85). In fact, IGF-I levels in human breast milk and saliva are higher than in cow's milk. Additionally, IGF-I is digested as other food proteins are and is inactive when consumed (for review, see 85). IGF-I content of milk from rbGH-treated cows has been extensively reviewed and its safety confirmed (78, 97, 240).

The FDA concluded that the use of rbGH in dairy cattle presents no confirmed health risks to consumers and the milk is substantially equivalent to milk from cows not treated with rbGH (See section 3.4). However, aside from safety issues, some consumers view the use of rbGH to increase milk production as "unnatural" and this has been promoted as a reason to oppose milk from cows injected with rbGH (37). This perspective led some dairies to voluntarily, although not legally, label milk as being from cows not injected with rbGH, even though FDA labeling policy for foods produced from GE ingredients (which is the same as for all other foods and food ingredients) specifies no label is needed if the food is substantially equivalent to non-GE foods in safety, composition, and nutrition. Of note, in January 2008, the Pennsylvania Department of Agriculture issued a new labeling standard indicating that milk could be labeled as coming from cows not treated with rbGH as long as the labeling was uniform (179).

Outside the U.S., countries that are signatories to the World Trade Organization cannot bar milk from cows injected with rbGH based solely on its production method, unless there is scientific evidence that it affects human health or safety (37). But the E.U. has been staunch in its opposition to such milk in part due to consumer concerns that arose in the 1990s as a result of certain food safety outbreaks, such as bovine spongiform encephalopathy (32), that were not effectively handled by existing regulatory systems. In 1999 the E.U. decided not to approve sales of milk from rbGH-treated cows in E.U. member countries, based not on human health concerns but on animal welfare issues (51). Today milk and milk products from rbGH-treated cows are recognized as safe in the E.U. and can be marketed in E.U. countries (49, 97), but the use of rbST in their dairy herds is not approved.

With regard to animal health, some studies have reported an increased frequency of mastitis in groups of rbGH-treated cows. This increase has been attributed mainly to increased milk volume in the mammary glands of treated cows and no convincing data are available that show a decrease in secretion of mammary gland immune factors as a result of growth hormone treatments (36). A 1999 study (106) indicated the rbGH can actually provide a protective effect against *Streptococcus uberis* mastitis following experimental infection.

3.20. Can the USDA Stop the Planting of Genetically Engineered Crops that Pose Health or Environmental Risks?

After the commercialization process for a GE crop is complete, including deregulation, all federal regulatory agencies (FDA, EPA, and

USDA) have the legal authority to demand the immediate removal of any product from the marketplace. Removal can be demanded if new, science-based evidence raises questions about consumer or environmental safety (10). A case in point is the rescinding of deregulatory status of Roundup Ready® alfalfa by a U.S. District Court Judge in 2007, on the basis of the lack of a full EIS (112). A concern was that cultivation of GE alfalfa would result in the spread of the Roundup Ready® gene to "natural alfalfa" causing a "significant environmental impact" (121). After a specified date in 2007, farmers were not able to plant Roundup Ready® alfalfa and will have to await more evaluation and approval of the EIS.

Bioavailability: degree to or rate at which substance is absorbed and becomes available for physiological activity

Recommended daily allowance (RDA): amounts of vitamins and minerals to be consumed to maintain good health, specified by the Food and Nutrition Board of the National Research Council

3.21. Is Golden Rice the Only Way to Provide Vitamin A to People in Developing Countries?

Vitamin A deficiency, along with iron and zinc deficiencies, pose the greatest public health consequences of all micronutrient deficiencies. Vitamin A deficiency is most common in young children and pregnant women and can lead to blindness, susceptibility to infectious diseases, and death (251). The Food and Agricultural Organization and the United Nations have developed different strategies to overcome deficiency of vitamin A, including dietary diversification, food fortification, and vitamin supplementation. When applied, there has been varying success in different regions of the developing world with the various approaches, e.g., distribution of vitamin A pills in Nepal (241), the fortification of sugar with vitamin A in Guatemala (13), and gardening projects in Bangladesh and Thailand (239). All these efforts required continuous public education and financial support from the public and private sector. For example, vitamin A fortification of sugar was temporarily suspended owing to an economic downturn that increased vitamin A prices and at that point vitamin A deficiency reappeared (239).

Despite these various efforts, ~250,000 to 500,000 children deficient in vitamin A become blind each year; half of them die within twelve months (250). Recent studies indicate that biofortification, i.e., incorporating micronutrients into food, has the potential to control deficiencies and is cost-effective and efficient compared with alternative public health and agricultural measures when coupled with other micronutrient interventions (220). To develop a biofortification strategy to address vitamin A deficiency, researchers developed the first variety of Golden Rice (GR1), a GE variety with increased levels of β-carotene, a precursor to vitamin A, compared with non-GE rice (254). The rice contained three new genes, two from daffodil (*Narcissus pseudonarcissus*) and one from a bacterium (*Erwinia uredovora*). In 2005 the development of a new Golden Rice variety, GR2, was published; in GR2 a maize gene is substituted for the daffodil genes, boosting β-carotene levels to 37 μg/g—estimated to provide 50% of a child's RDA of vitamin A in 72 g of dry GR2 rice (173). However, the actual impact of this rice also depends on several other variables, e.g., uptake and conversion to vitamin A, amount consumed, bioavailability, effects of cooking, and consumer acceptance (28).

The GR1 and GR2 rice varieties are in use in breeding programs in the Philippines, India, Bangladesh, China, and Vietnam; the use of Golden Rice is being governed by the Golden Rice Humanitarian Board and is based on full regulatory compliance (G. Berry, personal communication). Although perhaps not legally needed, because often no intellectual property restrictions exist in these countries on commonly employed genes [e.g., 35S promoter, hygromycin resistance gene (26)], all companies with patents applying to Golden Rice licensed them at no charge for use in resource-poor countries.

Golden Rice might increase vitamin A sufficiency for people in areas difficult to reach with other vitamin A distribution efforts or for people with limited opportunities to grow or purchase sufficient amounts of fresh vegetables or fruits. Golden Rice will not be the single solution to vitamin A deficiency

worldwide, but it is another tool that can be used in public health programs to combat vitamin A deficiency.

4. CONCLUDING REMARKS

Researchers using rDNA methods now have the technology to transfer genes, not only within a species, but also from one kingdom to another. This technology opens the door to changing agricultural crops in ways not previously possible. These changes can result in plants that are better able to survive pest attack and abiotic stresses, can be enhanced nutritionally, or can be used to immunize humans and animals. But, with this capacity to change comes the responsibility to proceed with caution, investigating possible outcomes carefully. Conversely, there is also a responsibility to utilize the technology where it can provide improvements to human health and the environment and make farmers' efforts more productive.

On the basis of the intensive look at the data and the peer-reviewed research in this review, the development of GE crops to date seems to have been responsible and regulatory agencies have, in general, proceeded with caution in releasing GE varieties. Although no human activity can be guaranteed 100% safe, the commercial GE crops and products available today are at least as safe in terms of food safety as those produced by conventional methods. This does not mean we should relax our vigilance in investigating products resulting from this new technology as well as the time-honored methods. But, we should not hold the new GE products to standards not required for food and feed products produced by other technologies and methods.

With the proper balance of caution and scrutiny, we can take advantage of the power of this technology without compromising the health of humans, animals, or the environment. To achieve that proper balance it is important to know the facts about the technology and its products. This is the information that I have attempted to provide in Part I of this review on general and food issues. In Part II, I will cover environmental and socioeconomic issues. In this way, paraphrasing Cardinal Wolsey, I hope that this will help us to be "very, very careful what we put into our heads"!

SUMMARY POINTS

1. Foods consumed today are derived from plants and animals whose genetic makeup has been modified by sexual crosses and mutation. Recombinant DNA provides a new tool to make genetic modifications, and this technology is termed genetic engineering or biotechnology.
2. Technically, researchers are now able to transfer genes using recombinant DNA methods, not only within a species, but also from one kingdom to another, which can lead to significant changes in various attributes of agricultural crops.
3. The safety of genetically engineered crops and foods, just as those created by classical breeding and mutation and grown conventionally or organically, needs to be evaluated on a case-by-case basis so that informed decisions can be made about their utility, safety, and appropriateness.
4. Data and information from peer-reviewed science on the safety of these products should be a part of the information considered when growing and consuming foods from these crops.
5. Factors beyond the technical, science-based facts should also be considered during the decision-making process.

6. Although scientific testing and governmental regulation can reduce the safety risks of conventionally and organically produced and genetically engineered crops and food, 100% safety is not achievable.
7. To date, no scientifically valid demonstrations have shown that food safety issues of foods containing genetically engineered (GE) ingredients are greater than those from conventionally or organically produced foods.
8. In commercial fields only a few crops have been modified using rDNA technologies (i.e., canola, corn, cotton, papaya, squash, and soy), but many others are in development.

FUTURE ISSUES

1. The introduction of pharmaceutical and industrial proteins into edible genetically engineered crops raises issues that require additional safety and regulatory scrutiny.
2. Measures that permit farmers to use their production techniques of choice, while respecting their neighbors' rights to do the same, must be pursued to achieve economic coexistence.
3. Interest in and funding for independent peer-reviewed studies on the food safety of conventional, organic, and GE foods must be encouraged.
4. Rigorous, fact-based governmental regulatory policy should be in place to allow public- and private-sector scientists to play a role in the creation and evaluation of genetically engineered crops.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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