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Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology

Prepared by a Task Force of the ILSI International Food Biotechnology Committee

ILSI
International Life Sciences INSTITUTE

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PREPARED BY A TASK FORCE OF THE ILSI INTERNATIONAL FOOD BIOTECHNOLOGY COMMITTEE

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Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology: *An Executive Summary*

A Task Force Report by the International Life Sciences Institute, Washington, D.C.

The global demand for food is increasing because of the growing world population. At the same time, availability of arable land is shrinking. Traditional plant breeding methods have made and will continue to make important contributions toward meeting the need for more food. In many areas of the world, however, the problem is food quality. There may be enough energy available from food, but the staple foods lack certain essential nutrients. In the developed world, demand for "functional foods" (that is, foods that provide health benefits beyond basic nutrition) is increasing. Nutritional improvements in foods could help to meet both of these demands for improved food quality. Modern agricultural biotechnology, which involves the application of cellular and molecular techniques to transfer DNA that encodes a desired trait to food and feed crops, is proving to be a powerful complement to traditional methods to meet global food requirements. An important aspect of biotechnology is that it provides access to a broad array of traits that can help meet this need for nutritionally improved cultivars. The new varieties developed through modern biotechnology have been identified by a number of terms, including genetically modified (GM or GMO), genetically engineered (GE or GEO), transgenic, biotech, recombinant, and plants with novel traits (PNTs). For the present discussion, the term "GM" will be used because of its simplicity and broad public recognition.

Foreword

Most of the initial crops derived from modern biotechnology (also known as genetically modified or GM crops) consist of varieties of maize, soybeans, potato, and cotton that have been modified through the introduction of one or more genes coding for insect or disease resistance, herbicide tolerance, or combinations of these traits. It is well recognized that absolute safety is not an achievable goal in any field of human endeavor, and this is particularly relevant with respect to ingestion of complex substances like food and feed. The safety of foods and feeds derived from such crops, therefore, was established using the internationally accepted concept of "substantial equivalence." A key element of this comparative safety assessment is that a food or feed derived from a GM crop is shown to be as safe as its conventionally bred counterpart. Application of the principle of substantial equivalence involves identifying the similarities and any differences between a product and its closest traditional counterpart and subjecting the differences to a rigorous safety assessment.

Today, GM crops include plants with "quality traits" that are intended to improve human or animal nutrition and health. These crops (for example, rice with provitamin A, maize and soybeans with altered amino acid or fatty acid contents) are typically improved by modifying the plant's metabolism and composition. In some cases, these modifications result in a product with complex qualitative and quantitative changes. Experts convened by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Organization for Economic Cooperation and Development (OECD) have agreed that the concept of substantial equivalence is a powerful tool for assessing the safety of food and feed derived from GM crops. This conclusion was based on the recognition that whole foods and feeds do not lend themselves to the standard safety assessment principles used for additives and other chemicals and that quantitative assessment of risk of individual whole foods from any source cannot be achieved (1996 Report of the Joint FAO/WHO Expert Consultation on biotechnology and food safety: review of existing safety assessment strategies and guidelines, Rome, Italy).

Substantial equivalence is not a conclusion drawn from a safety assessment. It is a process to identify differences that warrant safety assessments before commercialization. Therefore, an essential element in the application of the concept of substantial equivalence to nutritionally improved products is the availability of appropriate methods and technologies to identify biologically and/or toxicologically significant differences that require a safety assessment. Profiling methods (for example, metabolomics) that allow the simultaneous screening of many components without prior identification of each component can contribute to this purpose. Such methods have the potential to provide insight into metabolic pathways and interactions that may be influenced by both traditional breeding and modern biotechnology. A major challenge in the use of profiling techniques is to determine whether observed differences are distinguishable from natural variation associated with varietal, developmental, and/or environmental factors. Profiling techniques must, therefore, be validated and the baseline range of natural variations must be clearly established before they can be used in a regulatory framework. For now, these profiling methods may be useful primarily as prescreens for nutritionally improved products to aid in the identification of compounds that need to be evaluated.

In 2001, the ILSI International Food Biotechnology Committee convened a task force and an expert working group to develop a framework for the scientific underpinnings of the safety and nutritional assessment of nutritionally improved GM products. This working group consisted of individuals from leading scientific institutions with expertise in the areas of human and animal nutrition, food composition, agricultural biotechnology, food and animal feed safety assessment, and global regulations pertaining to novel foods and feeds. In addition, the document was reviewed by 23 experts worldwide, and an international workshop was convened to facilitate broader involvement of global stakeholders in developing and refining a safety and nutritional assessment framework for nutritionally improved products. Reviewers and workshop participants included food scientists; plant biotechnologists; scientists from regulatory agencies with responsibilities for

food, feed, and environmental safety; human food and animal feed nutritionists; food toxicologists; representatives from the food, feed, livestock, and biotechnology industries; and public interest sector scientists.

The resulting document provides the scientific underpinnings and recommendations for assessing the safety and nutritional effects of crops with improved nutritional qualities. It includes terms and definitions for describing such products, identifies the key safety and nutritional challenges, and introduces potential approaches and methods to address those challenges. To keep this document to a manageable size, its scope was intentionally limited. The document does not discuss the safety or nutritional assessment processes for functional foods (that is, foods that offer potential health benefits that go beyond satisfying basic nutritional needs), food or feed traits that are principally targeting a health or pharmacologic benefit, or crops that combine (that is, stack) several improved nutrition traits into a single crop.

The document also discusses the extensive experience available from the commercialization of GM crops to date and focuses on the unique questions and challenges associated with nutritionally improved products. This is a forward looking document that attempts to incorporate the current scientific principles and acknowledges the concerns raised to date, but it has not been used as an opportunity to directly revisit specific arguments, nor does it address the scientific principles and rationale for assessing the environmental safety of improved nutrition crops.

Chapter 1 of this document presents a synopsis of modern agricultural biotechnology. Chapter 2 discusses examples of nutritionally improved crops under development and/or consideration. The safety assessment process for nutritionally improved foods and feeds is presented in Chapter 3. This assessment builds on principles and processes that have been successfully employed for GM crops with improved agronomic traits that are currently on the market. Chapter 4 focuses on the nutritional assessment process for nutritionally improved food crops, and Chapter 5 focuses on nutritionally improved animal feeds. An overview of analytical methods both in place and in development to identify unanticipated or unintended changes in nutritionally improved crops is provided in Chapter 6. Lastly, an analysis of possible postmarket monitoring strategies for nutritionally improved GM crops is presented in Chapter 7.

It is our intention that this document will serve as a key reference for scientific and regulatory considerations on both general and technical issues.

Background

The first GM crops to be planted on a widespread basis consisted primarily of varieties with improved agronomic characteristics. These have been widely adopted and safely grown and used on a large scale in an increasing number of countries. A newly emerging class of GM crops is being developed with a focus on improved human or animal nutrition. A number of these crops have reached the field trial stage and/or are advancing through regulatory approval processes toward commercialization. These nutritionally improved crops have the potential to help offset nutrient deficiencies; improve the nutritional value of foods and feeds; promote well-being through elevated levels of beneficial compounds; lower levels of natural toxins, toxic metabolites, or allergens; improve processing; and/or enhance taste. To keep this document to a manageable size, its scope was intentionally limited. The document does not discuss the safety or nutritional assessment processes for functional foods (that is, foods that offer potential health benefits that go beyond satisfying basic nutritional needs), food or feed traits that are principally targeting a health or pharmacologic benefit, or crops that combine (that is, stack) several improved nutrition traits into a single crop.

As long ago as 1263, the English Parliament decreed that nothing could be added to staple foods that were "not wholesome for a man's body." Consequently, a well established history and process for assessing the safety of foods introduced into the marketplace exists that long precedes the introduction of GM crops. The assessment of crops with improved nutritional properties, regardless of how those crops are developed, can follow these same well-established principles and processes to assure that the intakes of essential nutrients in animal and/or human diets are not compromised. A key purpose of the assessment is to determine if adverse effects on health are likely to result from the intended compositional change. This kind of analysis has already been applied in several countries to crops with altered composition, and the principles of the evaluation are applicable to all novel foods. The scientific procedures for this kind of analysis require an integrated multidisciplinary approach, incorporating molecular biology, protein biochemistry, agronomy, plant breeding, food chemistry, nutritional sciences, immunology, and toxicology.

It is well recognized that absolute safety is not an achievable goal in any field of human endeavor, and this is particularly relevant with respect to ingestion of complex substances like foods and feeds. The safe use of a given food or feed has typically been established either through experience based on common use of the food or by experts who determine its safety based on established scientific procedures. Starting in the 1990s, the standard applied to novel, especially GM, food and feed crops has been that they should be as safe as an appropriate counterpart that has a history of safe use. This comparative assessment process (also referred to as the concept of substantial equivalence) is a method of identifying similarities and differences between the newly developed food or feed crop and a conventional counterpart that has a history of safe use. The analysis assesses: (1) the agronomic/morphological characteristics of the plant, (2) macro- and micronutrient composition and content of important antinutrients and toxicants, (3) molecular characteristics and expression and safety of any proteins new to the crop, and (4) the toxicological and nutritional characteristics of the novel product compared to its conventional counterpart in appropriate animal models. The similarities noted between the new and traditional crops are not subject to further assessment since this provides evidence that those aspects of the newly developed crop are as safe as crops with a history of safe consumption. The identified differences are subjected to further scientific procedures, as needed, to clarify whether any safety issues or concerns exist. By following this process, the safety assessment strategies for GM crops have proved, over the past 10 years, to be scientifically robust, providing a level of safety assurance that is comparable to, or in some cases higher than, that available for conventional crops. Approximately 30000 field trials have been conducted with more than 50 GM crops in 45 countries. As an endorsement to the robust nature of the comparative safety assessment process, more than 300 million cumulative hectares of GM crops have been grown commercially over the past decade with no documented adverse effects to humans or animals.

Numerous independent evaluations of GM crop assessment strategies by scientific organizations (for example, WHO, FAO, OECD, EU Commission, French Medical Association, U.S. National Academy of Sciences, Society of Toxicology) have concluded that current safety assessment processes for today's GM crops are adequate to determine whether significant risks to human or animal health exist. Indeed, a number of these reports suggest that the use of more precise technology for GM crops may provide a higher level of safety assurance for these crops than for conventionally bred plants, which are usually untested. For example, the

2001 European Commission Report (EC-sponsored Research on Safety of Genetically Modified Organisms; Fifth Framework Program—External Advisory Groups, "GMO research in perspective," report of a workshop held by External Advisory Groups of the "Quality of Life and Management of Living Resources" Programme) summarized biosafety research of 400 scientific teams from all parts of Europe over 15 y. This study stated that research on GM plants and their products following usual risk assessment procedures has not shown any new risks to human health or the environment beyond the usual uncertainties of conventional plant breeding. Another example is a 2002 position paper by the Society of Toxicology, *The Safety of Genetically Modified Foods Produced through Biotechnology*, which corroborated this finding. It is, therefore, important to recognize that it is the food product itself, rather than the process through which it is made, that should be the focus of attention in assessing safety. This paper goes on to state that the Society of Toxicology supports the use of the substantial equivalence or comparative assessment concept as part of the safety assessment of foods derived from GM crops.

The assessment process

The methods presently used to assess the safety of foods and feeds from GM crops with improved agronomic traits are directly applicable to nutritionally improved crops. Molecular characterization studies that assess the sequence and stability of the introduced DNA and studies that assess the potential toxicity and allergenicity of any new proteins produced from the inserted DNA are as applicable to nutritionally improved crops as to other GM products. Compositional analyses that quantify expected and unexpected changes in more than 50 key components (for example, proximates, amino acids, fatty acids, vitamins, minerals, antinutrients) for agronomically improved GM crops are also appropriate for nutritionally improved GM crops. In 2001/2002, the OECD published lists of analytes for the compositional evaluation of specific crops, with the understanding that the need for analysis of specific compounds should be determined on a case-by-case basis. The compositional analyses provide information on the concentrations of macronutrients, micronutrients, antinutritive factors, and naturally occurring toxins. A database that contains detailed information on the composition of conventionally bred crops has been developed and made available by the International Life Science Institute (ILSI) at www.cropcomposition.org.

Any single safety assessment study has strengths and weaknesses, which leads to the conclusion that it is unlikely that any single study is sufficient to assess the safety of a food product whether developed through biotechnology or any other method. Therefore, consideration of the sum total of studies that comprise the safety and nutritional assessment of the crop is necessary to reach a conclusion that the food or feed products derived from a new GM crop are as safe as the food or feed derived from the conventionally bred counterpart. The strength of the risk assessment depends not only on the sensitivity of any single method, but also on the aggregate sensitivity and robustness of the evidence provided by all methods combined.

Analysis of composition

The fundamental concepts used in food/feed assessments have been refined through extensive international dialogue and consensus building. The key concept is the need to determine whether changes other than the intended new trait have occurred in the new crop. It is recognized that statistically significant differences between the modified crop and its counterpart do not necessarily imply an outcome that might have an effect on human or animal health (that is, the differences may not be biologically meaningful), but may indicate the need for follow-up assessment on a case-by-case basis. Also, the occurrence of unintended effects is not restricted to modifications introduced via biotechnology; unintended effects also occur frequently during conventional breeding. Therefore, the impact of the insertion of DNA into the plant genome as well as the potential of the introduced trait to alter plant metabolism in an unexpected manner must be evaluated in the context of natural variation present in conventionally bred plants.

A detailed agronomic assessment is one important way to help identify unintended effects. The agronomic assessment evaluates unintended effects at the whole-plant level (that is, the morphological phenotype and agronomic performance data such as yield). Targeted analysis of composition focused on possible changes at the metabolic level (that is, the biochemical phenotype) is also an important tool to evaluate unintended effects. Where crops have been modified with the specific intent to change nutritional characteristics, the analysis should include examination of metabolites relevant to the modified anabolic and/or catabolic pathways and the impact of such modifications on the metabolites in related pathways. In the case of nutritional improvements that do not directly modify specific metabolic pathways, special attention to the mechanism of action of the desired trait should be considered. Examples of such traits are crops expressing a protein with an amino acid composition that results in higher levels of specific essential amino acids or crops with other desirable functional or organoleptic properties.

Since the types of nutritionally improved crops anticipated are diverse, the safety and nutritional assessment of each new product should be approached on a case-by-case basis, building on the comparative assessment principles and methods applicable to any new food or feed. A significant change in the dietary intake of a nutrient is defined here as a change that meaningfully affects health, growth, or development. In addition, the safety assessment of foods and feeds containing improved levels of nutrients will take into account the frequency and quantities in which the food or feed is consumed in by humans or animals, as well as the existing knowledge concerning the safety of the nutrient in question. Conventional crops vary widely in composition, as indicated in the 2001/2002 OECD consensus documents and in the ILSI crop composition database (www.cropcomposition.org). Determining the most appropriate conventional comparator for a nutritionally improved crop needs careful consideration. In some cases, it may be appropriate to use the closest genetically related or near isogenic variety, considering simply the nutritional impact of the altered component when the modified crop is used as a direct replacement of the comparator. In other cases, where the nutrient composition is altered to an extent that no suitable comparator can be identified within the same crop, the comparator may be a specific food component derived from another food (for example, a specific fatty acid profile). In these circumstances, the assessment should focus on the safety of the changed levels of the nutrient in the context of the proposed use and intake of the food or feed as well as the safety of the altered crop. It should also be noted that in cases where one part of the plant is eaten by humans (for example, grain) and other parts are eaten by animals (for example, forage) compositional analysis of both will need to be examined separately (for example, seeds vs. seeds and forage vs. forage) and may lead to different results. Targeted compositional analyses using validated quantitative methods will continue to be the core method to assess whether unintended changes have occurred.

Nontargeted methods

Nontargeted "profiling" methods may supplement targeted analytical methods in the future for the detection of unintended effects in GM crops. Examples of profiling methods include functional genomics, proteomics, and metabolomics for analysis of gene expression (for example, mRNA), proteins, and metabolites, respectively. These methods provide a broad view of complex metabolic net-

works without the need for specific prior knowledge of changes in individual plant constituents or pathways. These techniques have the potential to provide insight into metabolic pathways and interactions that may be influenced by both traditional breeding and modern biotechnology. A major challenge in the use of profiling methods for the detection of unintended effects is determining whether any observed differences are distinguishable from natural qualitative and quantitative variation due to varietal, developmental, soil, and/or environmental factors. In other words, it must be assessed whether the identified differences are biologically meaningful. Nontargeted profiling methods may thus provide additional opportunities to identify unintended effects, but they must be validated for the purpose, and the baseline range of natural variations must be clearly established and verified before they can be used in a regulatory framework. Profiling methods could, however, target specific metabolic pathways and identify expressed genes, proteins, or metabolites for which specific quantitative analytical methods could then be validated for the regulatory studies. These methods could also be used to assess whether there were changes in associated metabolic pathways. Hence, these methods may be useful during the developmental phase of a product because they can help to focus the safety assessment process by identifying the exact compounds that need to be measured in a specific nutritionally improved product.

The role of animal studies

Feeding studies in laboratory animals and targeted livestock species may be useful to assess the nutritional impact of the intended changes (for example, the nutritional value of the introduced trait). Studies in laboratory animals may also serve a useful role in confirming observations from other components of the safety assessment, thereby providing added safety assurance.

The safety of the intended changes to a crop are normally tested using a tiered approach consisting of bioinformatic structure–activity relationship investigations for sequence homology with allergens and toxins, followed by in vitro determinations of the digestibility of newly expressed proteins and in vivo studies with appropriate animal species. The types of changes assessed in this manner include the newly expressed proteins, any new metabolites present in the improved nutritional quality of the crop, and substantially altered levels of metabolites preexisting in the crop. Because the type of modification to each new crop is unique, the specific scientific procedures for an assessment should be determined on a case-by-case basis. For this purpose, existing OECD toxicology test protocols may be applicable. In some cases, appropriately designed animal toxicity studies can provide an additional measure of safety assurance. In general, however, such studies in laboratory animals and targeted livestock species are unlikely to reveal unintended minor compositional changes that have gone undetected by targeted analysis because they lack adequate sensitivity.

Numerous animal feeding studies have been conducted with approved and commercialized GM crops with improved agronomic traits. All published animal feeding studies have shown that performance of animals fed ingredients from GM crops was comparable to that of animals fed the conventional counterpart. Thus, it has been concluded that routine feeding studies with multiple species generally add little to the nutritional and safety assessment of GM crops that have no intended compositional changes.

Although animal feeding studies with crops (for example, maize, soybeans, wheat) that are normal components of animal diets can be relevant and meaningful, animal testing of some food products (for example, vegetables, fruits) presents additional challenges because animals may not normally consume these products (for example, macadamia nuts can be eaten by humans with impunity, but cause transient paralysis when fed to dogs). In addition, some nutritionally improved crops create special challenges when choosing a comparator. Examples of these challenges include crops with increased nutrient content that enhances animal performance and crops from which an edible coproduct may remain after the desired nutritional ingredient has been extracted for other purposes. It is noteworthy that the most appropriate comparator may, in some cases, be a formulated diet that allows for comparison of the nutritionally improved crop to the conventional crop supplemented with a purified source of the enhanced nutrient (for example, amino acid or fatty acid).

Animal studies also may play a role in testing the nutritional value of the introduced trait in a nutritionally improved crop. Analyses of nutrient composition provide a solid foundation for assessing the nutritional value of foods and feeds; however, they do not provide information on nutrient availability. Therefore, depending on the specific nutritional modification being introduced, it may be important to assess nutrient bioavailability in relevant animal studies. The intended changes in each nutritionally improved crop will determine which animal studies are most appropriate. Attention is drawn to guidelines being developed by an ILSI Task Force for animal study designs appropriate for nutritionally improved crops developed through biotechnology.

Postmarket monitoring

The premarket safety assessment of GM foods and feeds provides a scientific basis for ensuring the safety of the food and generally eliminates the need for postmarket monitoring. The premarket safety assessment principles applied to foods derived from GM crops are the same as those applied to other novel foods improved through other processes or methods. These scientific procedures and principles provide the basis for concluding that foods from GM crops are as safe as foods with a history of safe use and consumption. Postmarket monitoring has not been a routine requirement in supporting the safety or regulatory approval of food products, except in a few unique instances where there has been a need to confirm premarket dietary intake estimates to ensure safety and/or nutritional impact. For example, in some cases regulators have used active postmarket monitoring for novel (albeit non-GM) foods where such issues were identified in the premarket assessment of food ingredients (for example, potential for digestive tract side effects of olestra or confirmation of consumer intake levels of aspartame and yellow fat spreads enriched with phytosterols).

Postmarket monitoring may be appropriate when there is a need to corroborate estimates of dietary intakes of a nutritionally improved food with expected beneficial effects on human health. Postmarket monitoring must be based on scientifically driven hypotheses relative to endpoints that potentially affect human safety or health. The investigation of adverse events or the potential for chronic health effects, the confirmation of premarket exposure estimates, or the identification of changes in dietary intake patterns represent examples where, in very specific instances, hypotheses may be appropriately tested through postmarket monitoring programs. In the absence of a valid hypothesis, postmarket monitoring for undefined hypothetical adverse effects from foods from a GM (or non-GM) crop is not feasible and adds nothing to the premarket testing results, while potentially undermining confidence in the overall safety assessment process.

The success of any postmarket monitoring strategy is dependent on the accurate estimation of exposure in targeted or affected population groups and the ability to measure a specific outcome of interest and associate it with exposure. There must be traceability from field to consumer and the ability to control confounding factors. Adequate data must be available, therefore, to assess the use, distribution, and destination of the product or commodity

within the food supply. The safety and nutritional quality of nutritionally improved products can only be fully assessed in the context of their proposed uses and consequent human and animal exposure/intake. For example, exposure to enhanced levels of dietary components, such as fatty acids, in particular foods needs to be assessed in the context of total dietary exposure, which may be derived from multiple sources. Although this must be performed on a case-by-case basis, the analysis itself need not be complex. Methodologies for assessing human intake of nutrients and other dietary constituents range from per capita methods to methods that use available food consumption databases or direct consumer food consumption surveys. The analysis does not differ, in principle, from that applied to new food ingredients and food and feed additives. Another factor that may complicate the evaluation of nutritional exposure is the variability of the human diet and the global difference in diets and dietary consumption and, as a consequence, the resulting broad distribution of individual nutritional states. Unfortunately, reliable comprehensive dietary intake data are only available for a few countries.

Conclusions and Recommendations

The crops being developed with a focus on improved human or animal nutrition hold great promise in helping to address global food security. The existing comprehensive safety and nutritional assessment processes used to assess the safety of GM foods and feeds already introduced into the marketplace are fitting for nutritionally improved crops, although some additional studies may be needed to assess potential human health effects resulting from changed levels of the improved nutritional factor(s). The comparative assessment process provides a method of identifying similarities and differences between the new food or feed crop and a conventional counterpart with a history of safe exposure. The similarities noted through this process are not subject to further assessment since this provides evidence that those aspects of the new crop are as safe as crops with a history of safe consumption. The identified differences then become the focus of additional scientific studies and evaluation. The types of nutritionally improved products anticipated are diverse; therefore, the safety and nutritional assessment of each new product should be approached on a case-by-case basis. Many nutritionally improved crops have modified biosynthetic and/or catabolic pathways, and the impact of such modifications on metabolites in those and related pathways should be specifically and carefully examined. The use of profiling techniques to detect unintended effects is still limited by the difficulties in distinguishing possible product-specific changes from natural variation due to varietal, developmental, and/or environmental factors, and therefore, building databases containing information on natural variation is of high priority. These profiling methods may be useful as prescreens to help focus the safety assessment process by identifying the specific compounds that need to be measured in a particular nutritionally improved product. Depending on the nutritional modification being introduced, it may be important to assess nutrient bioavailability in relevant animal studies. Animal studies can play an important role in assessing the nutritional impact of the intended changes (for example, the nutritional value of the introduced trait) and in confirming observations from other components of the safety assessment, thereby providing added safety assurance. Any postmarket monitoring that is deemed necessary must be based on scientifically driven hypotheses relative to endpoints that potentially affect human and animal safety or health. In the absence of an identified risk, postmarket monitoring for undefined adverse effects for foods from nutritionally improved (or any other) crop is virtually impossible to carry out, is unnecessary, and is inconsistent with, and may undermine confidence in, the premarket safety assessment process.

Recommendation 1. All nutritionally improved foods and feeds should be evaluated for their potential impact on human and animal nutrition and health regardless of the technology used to develop these foods and feeds.

Recommendation 2. The safety assessment of a nutritionally improved food or feed should begin with a comparative assessment of the new food or feed with an appropriate comparator that has a history of safe use.

Recommendation 3. The safety and nutritional assessment of any new nutritionally improved crop varieties should include compositional analysis. In cases where the nutrient composition is altered to an extent that no suitable comparator can be identified, the assessment should focus on the safety of the changed levels of nutrients in the context of the proposed use and intake of the food or feed.

Recommendation 4. To evaluate the safety and nutritional impact of nutritionally improved foods and feeds, it is necessary to develop data on a case-by-case basis in the context of the proposed use of the product in the diet and consequent dietary exposure.

Recommendation 5. Current approaches of targeted compositional analysis are recommended for the detection of alterations in the composition of the nutritionally improved crop. New profiling techniques might be applied to characterize complex metabolic pathways and their interconnectivities. These profiling techniques can also be used in a targeted fashion to generate information on specific nutrients or other metabolites. However, before using profiling methods, baseline data need to be collected and the methods must be validated and harmonized globally.

Recommendation 6. Studies in laboratory animals may serve a useful role in confirming observations from other components of the safety assessment, thereby providing added safety assurance. However, studies in laboratory animals and targeted livestock are unlikely to reveal unintended minor compositional changes that have gone undetected by targeted analysis because they lack adequate sensitivity.

Recommendation 7. Animal feeding studies should be conducted in target species to demonstrate the nutritional properties that might be expected from the use of the modified crop, crop component, or coproduct.

Recommendation 8. The premarket assessment will identify safety and nutritional issues before product launch. It is unlikely that any new product with scientifically valid adverse health concerns will be marketed. Postmarket monitoring of nutritionally improved food products may be useful to verify premarket exposure assessments or to identify changes in dietary intake patterns. Postmarket monitoring should only be conducted when a scientifically valid testable hypothesis exists, or to verify premarket exposure assessments.

About ILSI

The International Life Sciences Institute (ILSI) is a nonprofit, worldwide foundation established in 1978 to advance the understanding of scientific issues relating to nutrition, food safety, toxicology, risk assessment, and the environment. ILSI also works to provide the science base for global harmonization in these areas.

By bringing together scientists from academia, government, industry, and the public sector, ILSI seeks a balanced approach to solving problems of common concern for the well-being of the general public.

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North Africa and Gulf Region, North America, North Andean, South Africa, South Andean, Southeast Asia Region, the Focal Point in China, and the ILSI Health and Environmental Sciences Institute. ILSI also accomplishes its work through the ILSI Research Foundation (composed of the ILSI Human Nutrition Institute and the ILSI Risk Science Institute) and the ILSI Center for Health Promotion. ILSI receives financial support from industry, government, and foundations.

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Chapter 1: An Introduction to Modern Agricultural Biotechnology

During the next decade, food and agricultural production systems will need to be significantly enhanced to respond to a number of remarkable changes, such as a growing world population; increasing international competition; globalization; shifts to increased meat consumption in developing countries; and rising consumer demands for improved food quality, safety, health enhancement, and convenience. New and innovative techniques will be required to ensure an ample supply of healthy food by improving the efficiency of the global agriculture sector. Modern biotechnology encompasses one such set of techniques. In recent years, agricultural biotechnology has come to mean the use of recombinant DNA technology. Biotechnology has proven to be a powerful complement to traditional plant breeding.

From a scientific perspective, the terms "genetically modified organism" (GMO) and "living modified organism" (LMO) apply to virtually all domesticated crops and animals, not just the products of recombinant DNA technology. Genetic manipulation by selection and conventional crossbreeding has gone on for centuries. During the last century, plant and animal breeders expanded the tools of genetic manipulation beyond traditional breeding to use a variety of other techniques. In the case of plants, these include aneuploidy, diploidy, embryo rescue, protoplast fusion, somaclonal selection, and mutagenesis with either radiation (cobalt-60) or ethyl methanesulfonate (Brock 1976). These techniques do not allow targeted modifications at the genome level; rather multiple genes are transferred or affected simultaneously and years of backcrossing are required to remove or reduce unwanted effects (Rowe and Farley 1981). In addition, traditional breeding programs are time consuming, labor intensive, and limited to transfers of genes between closely related species. With few exceptions, plants created by these conventional phenotypic selection techniques are not defined as a separate class of crops, and in most parts of the world they undergo no formal food or environmental safety assessment or review before introduction into the environment and marketplace (FDA 1992). Genetically modified, conventionally produced crops account for the majority of the current agriculture food production.

Recombinant DNA technology permits a more precise and predictable introduction of a broader array of traits than does traditional plant breeding. The class of plant products developed through modern biotechnology has been identified by a number of names, including genetically modified (GM or GMO), genetically engineered (GE or GEO), transgenic, biotech, and recombinant. For the present discussion, the term "genetically modified" (GM) will be used because of its simplicity and broad recognition. Using biotechnology, single traits can be modified much more quickly and precisely than is possible using traditional selection and breeding methods. The set of tools provided by modern biotechnology has thus introduced a new dimension to agricultural innovation.

Agricultural biotechnology has the potential to increase the efficiency and yield of food production, improve food quality and healthfulness, reduce the dependency of agriculture on synthetic chemicals, reduce biotic and abiotic stress, and lower the cost of raw materials, all in a sustainable environmentally friendly manner.

The first generation of GM crops contained traits with improved agronomic characteristics, and these crops have been in the market for more than 7 y. The next generation of GM crops will include traits with improved nutritional characteristics. A limited number of GM improved nutritional crops have also been introduced. Many others are being developed and are expected to be commercialized within 10 y. It is recognized that there have been questions and concerns about the safety assessment process and nutritional characterization of the agronomic-trait GM crops. As will be demonstrated later, these crops have been more thoroughly tested than any others in the history of crop agriculture. Many different GM crop products have now completed the regulatory process in several countries around the world including the U.S., Canada, and Argentina, with a lesser numbers in Japan, the European Union, Australia, New Zealand, India, Russia, China, and South Africa. Taking into consideration the experience gained with GM crops with improved agronomic traits, the focus of this document is on the scientific principles and methods for assessing the safety and nutritional qualities of nutritionally improved GM crops.

1.1 Progress to Date

The global acreage of GM crops increased by 15%, or 9 million ha in 2003, according to a report released by the International Service for the Acquisition of Agri-biotech Applications (ISAAA 2003; James 2003). According to the report, global adoption of GM crops reached 67.7 million ha in 2003 and over half of the world's population now lives in countries where GM crops have been officially approved by governmental agencies and grown. In addition, more than one-fifth of the global crop area of soybeans, maize, cotton, and canola contain crops produced using modern biotechnology. Nearly 7 million farmers in 18 countries grew GM crops in 2003 with more than 85% of these farmers being resource-poor farmers in developing countries. The report also projects continued near-term growth in global acreage of GM crops and in the number of farmers who use the technology (James 2003).

In 2003, six principal countries grew 99% of the global GM crops. The USA grew 42.8 million ha (63% of global total), followed by Argentina with 13.9 million ha (21%), Canada with 4.4 million ha (6%), Brazil with 3.0 million ha (4%), China with 2.8 million ha (4%), and South Africa with 0.4 million ha (1%). Globally, the principal GM crops were soybeans (41.4 million ha; 61% of global area), maize (15.5 million ha; 23%), cotton (7.2 million ha; 11%), and canola (3.6 million ha; 5%). The breakdown by crop and country from 1996 to 2003 is illustrated in Figure 1-1 and 1-2 (data from ISAAA Briefs).

During the 8 y since introduction of commodity GM crops (1996 to 2003), a cumulative total of over 300 million ha (almost 750 million acres) of GM crops were planted globally by millions of large- and small-scale farmers (James 2003). Rapid adoption and planting of GM crops by millions of farmers around the world; growing global political, institutional, and country support for GM crops; and data from independent sources confirm and support the benefits associated with GM crops (James 2003).

The most obvious benefits of GM crops with improved agronomic traits have been to farmers who have been able to increase

their production, reduce input costs, use less insecticide, increase insect and weed control in an environmentally managed way, enhance conservation tillage, and increase their economic return (Gianessi and others 2002). Consumers are largely unaware of any benefits to them from this first generation of agricultural biotechnology. For example, it is largely unknown that the level of fumonisin mycotoxin contamination of maize has been reduced by up to 93% with the reduction in insect damage, and therefore decreased fungal spore infections, realized by the introduction of European Corn Borer-resistant Bt maize (Munkvold and others 1999). This reduction in fumonisin levels has direct safety benefits to humans and animals because those mycotoxins are some of the most noxious substances on crops, resulting in ailments from liver cancer to brain damage. Most consumers are also unaware of the significant reduction in use of chemical insecticides (Gianessi and others 2002).

The next major phase for agricultural biotechnology is the introduction of traits that provide more readily apparent benefits to the consumer and traits that will confer value-added components from the perspective of the food or feed processor. Many of these traits will be ones that provide readily apparent benefits to the consumer; others will be value-added components from the perspective of the food or feed processor. Adoption of the next stage of GM crops may proceed more slowly, as the market confronts issues of how to determine price, share the value, and adjust marketing and handling to accommodate specialized end-use characteristics. Furthermore, competition from existing products will not evaporate. Challenges that have accompanied GM crops with improved agronomic traits, such as the stalled regulatory processes in Europe, will also affect adoption of nutritionally improved GM products.

1.2 Safety of GM Crops

The consensus of scientific opinion and evidence is that the application of GM technology introduces no unique food/feed safety concerns and that there is no evidence of harm from those products that have been through an approval process. This conclusion has been reached by numerous national and international organizations (for example, Food and Agriculture Organization/World Health Organization [FAO/WHO] of the United Nations, Organization for Economic Cooperation and Development, EU Commission, French Academy of Sciences, National Research Council of the U.S. National Academy of Sciences, Royal Society of London, and Society of Toxicology; Table 1-1 and 1-2).

A rigorous safety-testing paradigm has been developed and implemented for GM crops, which utilizes a systematic, stepwise, analytical, and holistic safety assessment approach (Cockburn 2002). The resultant science-based process focuses on a classical evaluation of the toxic potential of the introduced novel trait and the wholesomeness of the GM crop. In addition, detailed consideration is given to the history and safe use of the parent crop as well as that of the gene donor(s). The overall safety assessment begins with the concept known as "substantial equivalence", a model that is found in all international crop biotechnology assessment guidelines. This concept is essentially a comparative approach that seeks to identify the similarities and differences between the GM product and one or more appropriate comparators with a known history of safe use. Detailed consideration is given to the history and safe use of the parent crop, which is often the principal comparator, as well as the gene donor. This ensures that the identification of similarities with the comparator provides a solid basis for concluding that these aspects of the product are not likely to raise concerns. Consideration of the safety of the parent crop and the gene donor helps to eliminate the possibility of potentially undesirable traits being introduced from those sources or, alternatively, permit a directed search for these traits to determine to what extent they have been transferred into the modified organism. The differences from the comparator that are noted, which include the introduced novel trait, are then subjected to a classical evaluation of their potential toxic, allergenic, or nutritional impact. By building a detailed profile on each step in the transformation process (from parent to new crop) and by thoroughly evaluating the significance, from a safety perspective, of any differences that may be detected between the GM crop and its comparator, a comprehensive matrix of information is constructed. This information is used to reach a conclusion about whether food or feed derived from the GM crop is as safe as food or feed derived from its traditional counterpart or the appropriate comparator. Using this approach in the evaluation of more than 50 GM crops that have been approved worldwide, the conclusion has been reached that foods and feeds derived from GM crops are as safe and nutritious as those derived from traditional crops (Table 1-1). The lack of any proven adverse effects resulting from the production and con-

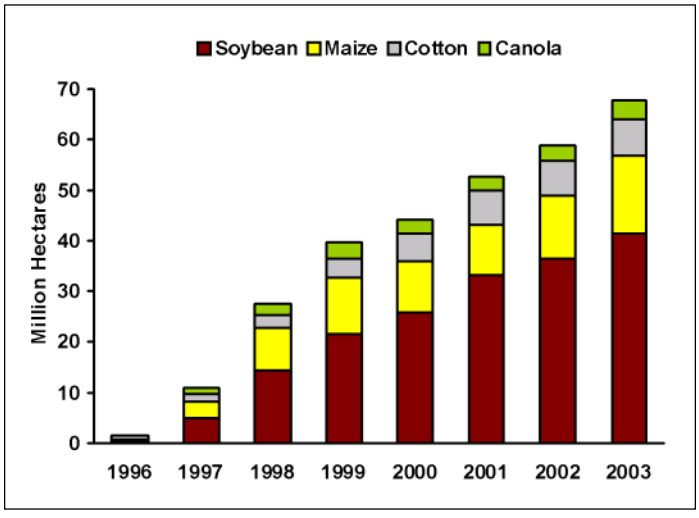

Figure 1-1—Areas planted to 4 primary GM crops. Source: ISAAA briefs.

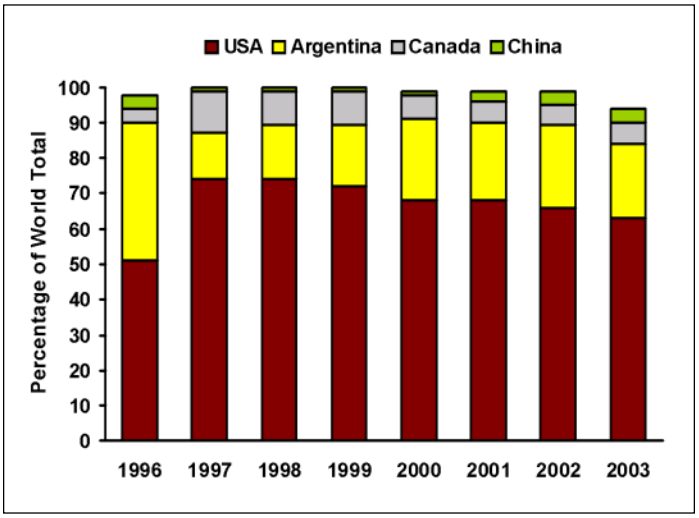

Figure 1-2—Areas planted to GM crops in 4 principle countries. Source: ISAAA briefs.

Table 1-1—Milestones in the international consensus on the safety assessment of biotechnology-derived foods

Year	Organization	Item	Reference
1990	IFBC	Guidelines on the safety assessment in general	IFBC 1990
1991	FAO/WHO	Report describing strategies for safety assessment of foods derived from modern biotechnology	
1993	OECD	Report describing principles of substantial equivalence	OECD 1993
1996	ILSI/IFBC	Decision tree for assessment of potential allergenicity	Metcalfe and others 1996
1996	FAO/WHO	Expert consultation on safety assessment in general, including the principle of substantial equivalence	FAO/WHO 1996
1997	ILSI Europe	Novel Foods Task Force. The safety assessment of novel foods.	ILSI 1997
1999–pres.	OECD	Installment of the Task Force for the Safety for Novel Foods and Feed, among others compilation of consensus documents on composition of crops as support for comparative evaluation	
2000	FAO/WHO	Expert consultation on safety assessment in general, including the principle of substantial equivalence	FAO/WHO 2000
2001	ILSI Europe	Concise monograph series genetic modification technology and food consumer health and safety	Robinson 2001
2001	EU	EU-sponsored Research on Safety of Genetically Modified Organisms. "GMO research in perspective." Report of a workshop held by External Advisory Groups of the "Quality of Life and Management of Living Resources" Program.	EU 2001
2001	NZRC	New Zealand Royal Commission on Genetic Modification	NZRC 2001
2000–2003	FAO/WHO	Guidelines for Codex alimentarius committee, developed by Task Force for Foods Derived from Biotechnology Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology, Food and Agriculture Organisation of the United Nations, Rome, Italy.	FAO/WHO 2002, 2003
2003	ILSI	Crop composition database (www.cropcomposition.org)	ILSI 2003

Table 1-2—Examples of reports on biotechnology-derived foods and/or their safety that appeared in 2001/2003

Organization/authors	Relevant conclusions/recommendations	Reference
Royal Society of the United Kingdom	Endorsement of comparative approach development of "profiling methods" for compositional analysis building of reference data sets by public-private co-operation allergy assessment should include food and inhalant allergies allergy part of post-market surveillance.	Royal Society 2002
Irish Council for Science Technology and Innovation	Biotechnology derived foods no less safe than conventional foods. Transgenic viral sequences in plants comparable to natural presence of virus genes.	ICSTI 2002
Society of Toxicology	Substantial equivalence as guidance for safety assessment of biotechnology derived foods as safe as conventional foods, presently used assessment methods adequate for current products, update of toxicological and assessment methods for future products, development of profiling methods to assess complex modifications, further identification and characterization of protein allergens.	Hollingsworth and others 2003
Canadian Biotechnology Advisory Committee	Research into hypothesis of long-term health effects and development of accessible food consumption data.	CBAC 2002
The French Academy of Science	Report. Les plantes génétiquement modifiées "Genetically Modified Plants" (Académie des sciences 2003 "The Genetically Modified Plants" called for an end to the European moratorium on biotech crops. Criticisms against GMO can be adequately addressed on strictly scientific criteria. Furthermore, any generalization on the potential risks linked to GMO is impossible since scientific rigor can only proceed from a case-by-case analysis.	ADSF 2002
Australia and New Zealand	Regulation of genetically modified foods in Australia and New Zealand	Brent and others 2003

sumption of GM crops grown on more than 235 million cumulative ha over the last 7 y supports these safety conclusions.

The U.S. National Research Council (NRC 2000) determined that no difference exists between crops modified through modern molecular techniques and those modified by conventional breeding practices. The authors of the NRC report emphasized that they were not aware of any evidence suggesting foods on the market today are unsafe to eat because of genetic modification. In fact, the scientific panel concluded that growing such crops could have environmental advantages over other crops.

The committee chair, Perry Adkisson, noted that the focus of risk assessment should be on the properties of a GM plant, not on the process by which it was produced. However, the NRC cautioned that, even given the strengths of the U.S. system governing GM plants, regulatory agencies should do a better job of coordinating their work and expanding public access to the process as the volume and mix of these types of plants on the market increase. Any new rules should be flexible so they can easily be updated to reflect improved scientific understanding.

In a 2003 position paper, the Society of Toxicology (Hollingsworth and others 2003) corroborated this finding and noted that there is no reason to suppose that the process of food production through biotechnology leads to risks of a different nature than those already familiar to toxicologists or to risks generated by conventional breeding practices for plant, animal, or microbial improvement. It is therefore important to recognize that it is the food

product itself, rather than the process through which it is made, that should be the focus of attention in assessing safety. The paper goes on to state that the Society supports the use of the substantial equivalence concept as part of the safety assessment of foods and feeds from GM crops. This process seeks to establish whether the food from a GM crop is significantly different from foods from conventionally bred crops, a source that is generally considered safe by consumers. In addition, the process is designed to assure the safety of any identified differences and to provide a critical assessment as to the nature of any increased health hazards in the new food source (Hollingsworth and others 2003).

An EU Commission Report (2001) that summarized biosafety research of 400 scientific teams from all parts of Europe conducted over 15 y stated that research on GM plants and derived products so far developed and marketed, following usual risk assessment procedures, has not shown any new risks to human health or the environment beyond the usual uncertainties of conventional plant breeding. Indeed, the use of more precise technology and the greater regulatory scrutiny probably make GM plants even safer than conventional plants and foods. If there are unforeseen environmental effects—none have appeared yet—these should be rapidly detected by existing monitoring systems. The Royal Society of the United Kingdom released 2 reports (Royal Society 2002, 2003) that support this conclusion. It does caution that the regulatory environment needs to be kept flexible to accommodate evolving data sets on risk.

The medical community has supported the introduction of GM plants. The American Medical Association (AMA 1999), states, "it is the policy of the AMA to endorse or implement programs that will convince the public and government officials that genetic manipulation is not inherently hazardous and that the health and economic benefits of recombinant DNA technology greatly exceed any risk posed to society." A French Academy of Sciences report (ADSF 2002) called for an end to the European moratorium on GM crops. The report states, "Criticisms against GMOs can be adequately addressed on strictly scientific criteria. Furthermore, any generalization on the potential risks linked to GMOs is impossible since scientific rigor can only proceed from a case-by-case analysis." Even the British Medical Association (which originally expressed concerns about GM crops) is to change its advice on the health risks of foods from GM crops. The Head of Science and Ethics, Dr. Vivienne Nathanson, said she had seen "no evidence" that it posed a threat and that there was no direct health risk to people. However, she cautioned that work needed to be done on the environmental impact of GM crops and on reassuring the public that there were "global benefits" (Ahmed 2003).

1.3 A Real World Example of Product Compared with Process

An example from work conducted at the Univ. of California (UC) Davis helps to illustrate that a similar endpoint can be reached by traditional imprecise and modern precise methods (Klann and others 1993, 1996). High-soluble solids are commercially desirable for tomato processing—the higher the solids the more paste for the cannery. The common processing variety of tomato, *Lycopersicon esculentum*, accumulates glucose and fructose and has about 5% soluble solids; it is termed a hexose accumulator. There is a wild variety of tomato, *L. chmielewskii,* that has 10% soluble solids and accumulates high levels of soluble sugar in mature fruit unlike the domesticated tomato species. However, that is the only desirable characteristic of the wild tomato variety. The other characteristics of *L. chmielewskii* are undesirable and include small size, bitter taste, low yield, and toxicity. Like the potato, the tomato is a member of the deadly nightshade family that produces glycoalkaloid toxins. Researchers at UC Davis used classical breeding over many years to transfer the higher soluble solids characteristic from the wild tomato to the domesticated tomato, while retaining all of the other desirable characteristics of the domesticated variety. Unfortunately, the new varieties were hampered by reduced fertility in addition to technical difficulties in determining how much of the toxic substances were introgressed. This illustrates that classical plant breeding does not always yield the desired array of characteristics and sometimes results in undesirable characteristics over which the breeder has little control. Genetic and biochemical analyses of progeny showed that the lack of acid invertase activity in sucrose-accumulating fruit was consistent with the absence of acid invertase mRNA although the gene encoding the protein was intact. This suggests that the *L. chmielewskii* invertase gene is transcriptionally silent in fruit and that this is the basis for sucrose accumulation in progeny derived from the interspecific cross of *L. esculentum* and *L. chmielewskii* (Klann and others 1993).

Armed with this information, a 2nd approach with the same goal was undertaken to increase the soluble solid content of the tomato (Klann and others 1996). Through use of genetic engineering the researchers switched off expression by adding a complement of the gene using a technology termed antisense, without substantially altering any other desirable traits of the fruit. Therefore, if one were to ask which fruit was more equivalent to the commercial cultivar (that is, the one produced from a traditional wide cross with introgressed genes from the toxic relative or the one produced by modern biotechnology techniques without introducing genes coding for high levels of glycoalkaloid toxins), most people would conclude that the modern biotechnology approach produced a more substantially equivalent, potentially safer fruit. Yet, the variety produced using the less-precise technology is the one commercialized because of the prohibitive cost of registering a GM product for deregulated status. So, it is important that safety assessment processes be developed and implemented that are science-based and cost-effective to encourage the development of the safest and most effective and efficient agricultural products.

1.4 Regulatory Oversight of GM Crops

Genetically modified crops and foods derived from them have been thoroughly and extensively tested during the past 15 y, both in the laboratory and in controlled natural environments under the oversight of numerous regulatory agencies For example, in the U.S., the following agencies have oversight: U.S. National Institutes of Health (NIH), U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration (FDA), Animal & Plant Health Inspection Service/U.S. Dept. of Agriculture (APHIS-USDA). For example, the USDA has approved at least 8700 field tests involving more than 35000 sites throughout the United States. The Agency has assessed the GM plants for their suitability for release in the environment. Globally, approximately 30000 field trials have been conducted on 100 organisms in 45 countries (International Field Test Sources 2002). There has not been a single report of an unexpected or unusual outcome that resulted in a reported safety concern.

Traditional foods eaten for millennia have not been rigorously regulated by national governments nor have elaborate procedures for regulatory oversight been implemented. However, there is a rigorous testing and safety assessment process for GM crops. Many crop varieties improved using much less precise methods such as crossbreeding, mutation-induced breeding, or species-wide crosses (in which tens of thousands of untested genes are combined) did not undergo the same type of scrutiny or inquiry as GM crops in most parts of the world. Foods from GM crops are thoroughly assessed for their safety prior to marketing. Several re-

cent reports and activities focus on the strategies by which this assessment is carried out (Table 1-1). In spite of national differences regarding the approval procedures, the actual safety assessment of foods from GM crops follows an internationally acknowledged consensus approach (Table 1-1). This consensus has been reached through the activities of international organizations, including FAO/WHO, OECD, ILSI, and IFBC, which have been working together with scientists, regulators, and other interested parties. Their activities date back to the years preceding the introduction of the first commercial GM crops. Since then, numerous landmark publications have appeared. These publications are summarized in Table 1-1.

The main principles of the international consensus approach, which are also discussed in more detail in the following chapters, are listed below. They illustrate the varieties of principles at the center of the discussions and they are continuously updated.

Substantial equivalence: This is the guiding principle for the safety assessment. In short, substantial equivalence is the concept of comparing of the GM product to a conventional counterpart with a history of safe use. Such a comparison commonly includes agronomic performance, phenotype, expression of transgenes, composition (macro- and micronutrients), and amounts of antinutrients and natural toxicants and identifies the similarities and differences between the GM product and the conventional counterpart. Based on the differences identified, further investigations may be carried out to assess the safety of these differences. These assessments include any protein(s) that are produced from the inserted DNA.

Potential gene transfer: Where there is a possibility that selective advantage may be given to an undesirable trait from a food safety perspective, this should be assessed. For example, the highly unlikely event that a gene coding for a plant-made pharmaceutical is transferred to commodity corn. Where there is a possibility that the introduced gene(s) may be transferred to other crops, the potential environmental impact of the introduced gene and any conferred trait must be assessed.

Potential allergenicity: Since most food allergens are proteins, the potential allergenicity of newly expressed proteins in food must be considered. A decision-tree approach introduced by ILSI/IFBC (Metcalfe and others 1996) has become internationally acknowledged and recently updated by Codex (FAO/WHO 2002). The starting point for this approach is the known allergenic properties of the source organism for the genes. Other recurrent items in this approach are structural similarities between the introduced protein and allergenic proteins, digestibility of the newly introduced protein(s), and, eventually and if needed, sera-binding tests with either the introduced protein or the biotechnology-derived product.

Potential toxicity: Some proteins are known to be toxic, such as enterotoxins from pathogenic bacteria and lectins from plants. Commonly employed tests for toxicity include bioinformatic comparisons of amino acid sequences of any newly expressed protein(s) with the amino acid sequences of known toxins, as well as rodent toxicity tests with acute administration of the proteins. In addition to purified proteins, whole grain from GM crops has been subjected to in vitro digestibility tests as well as tested in animals (for example, classic, subchronic (90-d) rodent studies).

Unintended effects: Besides the intended effects of the genetic modification, interactions of the inserted DNA sequence with the plant genome are possible sources of unintended effects. Another source might be the introduced trait unexpectedly altering plant metabolism. Unintended effects can be both predicted and unpredicted. For example, variations in intermediates and endpoints in metabolic pathways that are the subject of modification, while undesirable are predictable; whereas the turning on of unknown endogenous genes through random insertion in control regions is both unintended and unpredictable. The process of product development that selects a single commercial product from hundreds to thousands of initial transformation events eliminates the vast majority of situations that might have resulted in unintended changes. The selected commercial product candidate event undergoes additional detailed phenotypic, agronomic, morphological, and compositional analyses to further screen for such effects.

Postmarket surveillance: It is acknowledged that the premarket safety assessment should be rigorous to exclude potentially adverse effects of consumption of foods or feeds derived from GM crops. Nevertheless, some have insisted that such foods should also be monitored for long-term effects by postmarket surveillance. No international consensus exists as to whether such surveillance studies are technically possible without a testable hypothesis in order to provide meaningful information regarding safety, and a GM crop with a testable safety concern would most likely not pass regulatory review. The notion of using measurable biomarkers has been suggested, but these then need to be determined for all foods and feeds, whatever the source and whenever the question of reasonable economic burden arises.

Besides the international organizations such as FAO/WHO, OECD, ILSI, and IFBC, other organizations have also formulated their views and recommendations on safety of foods from GM crops. Table 1-2 lists recent examples of expert reports with some of their most relevant conclusions that appeared in 2001/2002.

The general conclusions of these reports are that the current safety assessment methods are considered appropriate for the GM crop products presently on the market. It is suggested that additional validated methods be developed for the safety assessment of future GM crops with more complex modifications. In addition, one report recommends hypothesis-based postmarket surveillance, while another specifically recommends allergy-oriented surveillance (Table 1-2).

Several comprehensive overviews of the food safety assessment of GM crops have been published in the scientific literature (for example, Kuiper and others 2001; Cockburn 2002). This comparative assessment concept and its application are discussed in more detail in Chapter 3.

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Chapter 2: Improved Nutritional Quality through Modern Biotechnology

2.1 Introduction

Agriculture's traditional role of providing food, feed, and fiber is being augmented by biotechnology. Biotechnology will be a critical element in the development of crops, foods, and ingredients with traits with improved nutritional properties. Developing plants with these improved traits involves overcoming a variety of technical challenges inherent in metabolic engineering programs. Both traditional plant breeding and biotechnology-based techniques are needed to produce plants with the desired quality traits. Continuing improvements in molecular and genomic technologies are contributing to the acceleration of product development. Table 2-1 presents examples of crops that have already been genetically modified with macro- and micronutrient traits that may provide benefits to consumers and domestic animals. Some of these crops have already been approved and commercialized, whereas others are still in development.

2.2 The Plasticity of Plant Metabolism

Plants are remarkable in their ability to synthesize a variety of organic compounds, such as vitamins, sugars, starches, fatty acids, and amino acids. As many as 80000 to 100000 different substances are synthesized in plants, including macronutrients (for example, proteins, carbohydrates, lipids [oils], and fiber), micronutrients (for example, vitamins and minerals), antinutrients (for example, compounds such as phytate that reduce bioavailability), allergens (for example, albumin), endogenous toxicants (for example, glycoalkaloids and cyanogenic glycosides), and other plant-specific compounds (some of which may have beneficial effects) that are significant to human and animal health (Conn 1995). This plasticity is elegantly demonstrated in the way that plants respond to environmental stimuli such as pathogen attack. Functional complexity begins with the exogenous signals perceived from the pathogen, continues with the mechanisms of signal perception and signal transduction, and results in extensive "reprogramming" of cellular metabolism, involving extensive changes in gene activity. Thus, pathogen defense entails a major shift in metabolic activity, rather than altered expression of a few unique, defense-related genes. The observed complexity serves as a paradigm of the flexibility and plasticity of plant metabolism. Many of these same metabolites have either positive or negative impacts on the nutritional characteristics of plants. For example, the shikimate pathway includes a number of phytochemicals that can have either good or bad effects. These compounds include phenylpropanoids, coumarins, stilbenes (some such as resveratrol are beneficial, while others such as kawain have negative effects), flavonoids, and tannins (Buchanan and others 2000).

2.3 The Challenge: Improved Nutritional Quality

The next generation of plants will focus on value-added output traits where valuable genes and metabolites will be identified and isolated, with some of the metabolites being produced in mass quantities for niche markets. This chapter will focus only on nutritionally-enhanced crops for food and feed and will not cover the use of plants as factories for the production of therapeutics or industrial products, even if the products are intended for use in the food or feed industry. The nutritionally improved crops in the current development pipeline will be well understood and well characterized from a compositional perspective as they undergo safety and nutritional assessment following existing regulations that are more than adequate to address any potential concerns. However, some of the more potentially beneficial modifications will require a more thorough understanding of plant metabolism and methods to achieve effective changes in the desired metabolic endpoints. Although progress in dissecting metabolic pathways and our ability to modify gene expression in GM plants has been most impressive during the past 2 decades, attempts to use these tools to engineer plant metabolism have met with more limited success.

Metabolic engineering typically involves the redirection of cellular activities by the modification of the enzymatic, transport, and regulatory functions of the cell using recombinant DNA (rDNA) and other techniques. Since the success of this approach hinges on the ability to change host metabolism, its continued development will depend critically on a far more sophisticated knowledge of plant metabolism, especially the nuances of interconnected cellular networks, than currently exists. Although the enzymological sequences and intermediates of many metabolic pathways in a small number of well-studied organisms are known with some confidence, little is known in quantitative terms about the controls and integration of these pathways. The necessary knowledge also includes conceptual and technical approaches necessary to understand the integration and control of genetic, catalytic, and transport processes. Though there are notable exceptions, most successful attempts at metabolic engineering thus far have focused on modifying (positively or negatively) the expression of single genes (or a series of individual enzymatic steps) affecting pathways. Generally, more success has been achieved when conversion or modification of an existing compound to another has been targeted than when an attempt has been made to significantly change flux through a pathway (for example, increasing the oleic acid concentration in canola oil, as will be discussed later). Attempts to modify storage proteins or secondary metabolic pathways have also been more successful than have alterations of primary and intermediary metabolism (Della Penna 1999).

Research to improve the nutritional quality of plants has historically been limited by a lack of basic knowledge of plant metabolism and the stimulating challenge of resolving complex interactions of thousands of metabolic pathways. With the tools now being harnessed through the fields of genomics and bioinformatics, there is the potential to identify genes of value across species, phyla, and kingdoms. Through advances in proteomics, it is becoming possible to quantify simultaneously the levels of many individual proteins and to follow posttranslational alterations that occur in pathways. Metabolomics allows the study of both primary and secondary metabolic pathways in an integrated fashion.

With these evolving tools, a better understanding of global effects of metabolic engineering on metabolites, enzyme activities, and fluxes is beginning to be developed. The increase in our basic knowledge of plant metabolism during the coming decades will provide the tools necessary to modify more effectively the nutritional content of crops to have a positive effect on many aspects of human and animal health.

In addition to metabolic considerations, attention needs to be given to the site of synthesis and site of activity of an enzyme. Signal sequences or transit peptides coding sequences attached to introduced genes are not always sufficient to ensure appropriate targeting. For example, charge and size of a protein may affect the efficiency of transportation into plastids. Another complexity found in biological systems is redundancy of pathways and the ability of plants to compensate as they often contain more than one enzyme capable of catalyzing a similar reaction. A potential approach to counter some of these problems in metabolic engineering of pathways involves the manipulation of transcription factors that control networks of metabolism (Kinney 1998; Bruce and others 2000). For example, expression of maize transcription factors C1 and R, which regulate production of flavonoids in maize aleurone layers, together under the control of a strong promoter resulted in a high accumulation rate of anthocyanins in Arabidopsis, presumably by activating the entire pathway (Bruce and others 2000). Such expression experiments hold promise as an effective tool for the determination of transcriptional regulatory networks for important biochemical pathways. In summary, metabolic engineers must not only understand the fundamental physiology of the process to be impacted, but also the level, timing, subcellular location, and tissue or organ specificity that will be required from a transgene to ensure successful manipulation of that physiology. Gene expression can be modulated by numerous transcriptional and post-transcriptional processes. Correctly choreographing these many variables is the element that makes metabolic engineering in plants so challenging.

In conjunction with such increases in the understanding of plant metabolism, the challenge then remains to understand how components in the diet interact with human or animal metabolism to benefit their health and well-being. This challenge is at least as complex as the task of increasing or decreasing the amount of a specific protein, fatty acid, or other component of the plant itself. It is of little use producing a plant with a supposed nutritional benefit unless that benefit actually improves the health of humans or animals.

Specific examples of work being done to improve nutritional quality at the macro- (protein, carbohydrates, lipids, fiber) and the micro- (vitamins, minerals) level and to reduce the amounts of endogenous toxicants, allergens, and antinutrients will be discussed later in this chapter, but first the technology that makes plant trait modification feasible is examined.

2.4 The Tools

Metabolic engineering is generally defined as the redirection of one or more enzymatic reactions to improve the production of existing compounds, produce new compounds, or mediate the degradation of compounds. Substrate-product relationships in plant pathways were initially elucidated through the application of radiolabel tracer studies during the 1960s and 1970s. In the 1980s, with the advent of rDNA technology, tools such as cloning, promoter analysis, protein targeting, plant transformation, and biochemical genetics were developed. The GM crops with improved agronomic traits presently being grown on more than 60 million ha around the world are a product of the application of these technologies to crop plants. These products provide benefits to the farmer and community in reducing insecticide and herbicide usage and increasing the ability of farmers to conserve soil and other resources (Gianessi and others 2002). They generally involve the relatively simple task of adding a single gene or small number of genes to plants. These genes in the main function outside of the plant's primary metabolic processes and thus have little or no effect on the composition of the plants.

The more complex task lies in engineering metabolic pathways and plant metabolites. Significant progress has been made in recent years in the molecular dissection of plant metabolic pathways and in the use of cloned genes to engineer plant metabolism in ways that are more complex. Table 2-1 presents examples of crops that have already been genetically modified with nutritionally improved traits that may provide benefits to consumers and domestic animals. This table includes many modifications that have not yet progressed, and may never progress, to commercial production. These products are being tested for applications in food, feed, and industrial markets.

In addition to these numerous success stories, some studies have yielded unanticipated results. For example, the concept of gene silencing emerged from the unexpected observation that adding a chalcone synthase gene to increase color in petunias resulted instead in the switch off of color producing white and variegated flowers (Napoli and others 1990). This initially unexpected observation has now been turned to advantage in switching off expression of an allergen in soybeans, as will be discussed later. Metabolic pathway modifications are complex, and the state of understanding of plant metabolism is sometimes insufficient to bridge the gap between the ability to clone, study, and modify individual genes and proteins and the understanding of how they are integrated into and affect the complex metabolic networks in plants. Regulatory oversight of such products has been designed to detect such unexpected outcomes and to ensure that products from GM plants are safe before they are commercialized.

Genomics-based strategies for gene discovery, coupled with high-throughput transformation processes and miniaturized automated analytical and functionality assays, have accelerated the identification of product candidates. Identifying rate-limiting steps in synthesis could provide targets for genetically engineering biochemical pathways to produce augmented amounts of compounds and new compounds. Targeted expression will be used to channel metabolic flow into new pathways, while gene-silencing tools can reduce or eliminate undesirable compounds or traits, or switch off genes to increase desirable products (Kaiser 2000, Liu and others 2002, Herman and others 2003). In addition, molecular marker-based breeding strategies have already been used to accelerate the process of introgressing trait genes into high-yielding germplasm for commercialization.

2.5 Lessons Learned from Experimental Modification of Pathways

Analysis of fluxes in metabolic pathways in response to an environmental or genetic manipulation can help identify rate-limiting steps. Traditional biochemical hallmarks of potential regulatory, or rate-controlling, enzymes are that they catalyze reactions and are regulated by appropriate effector molecules. The modification of enzymes of the carbon cycle to study their role in regulating pathway flux has provided some of the more interesting results from metabolic engineering studies in plants.

For example, when the highly regulated Calvin cycle enzymes, fructose-1, 6-bisphosphatase and phosphoribulokinase, were reduced 3- and 10-fold in activity, respectively, minor effects on the photosynthetic rate were observed (Hajirezaei and others 1994; Paul and others 1995). In contrast, a minor degree of inhibition of plastid aldolase, which catalyzes a reversible reaction and is not subject to allosteric regulation, led to significant decreases in photosynthetic rate and carbon partitioning (Haake and others 1998). Thus aldolase, an enzyme seemingly irrelevant in regulating pathway flux, was shown to have a major influence over the pathway (Haake and others 1998). Understanding of the individual kinetic properties of such key enzymes may not always be sufficient to understand their wider role in central metabolism.

Table 2-1—Examples of crops genetically modified with nutritionally improved traits intended to provide health benefits to consumers and domestic animals.

Crop/Species	Trait	Transgene	Reference
Alfalfa	+Phytase	Phytase (*Aspergillus*)	Austin-Phillips and others 1999
	+Resveratrol	Resveratrol glucoside	Hipskind and Paiva 2000
	Lignin ↑	Downregulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-methyltransferase	Guo and others 2001
Arabidopsis & tobacco	+Catechol	Salicylate hydroxylase (nahG)	Friedrich and others 1995
Beet	+Fructans	1-Sucrose:sucrose fructosyl transferase	Smeekens 1997
Canola	Vitamin E↑	γ-Tocopherol methyl transferase (*Arabidopsis*)	Shintani and DellaPenna 1998
	Lauric acid↑	Lauroyl ACP thioesterase (California bay tree)	Del Vecchio 1996
	γ-Linolenic acid↑	δ-6- and δ-12 desaturases	Liu and others 2002
	+ ω-3 Fatty acid	δ-6 Desaturase gene *(Mortierella*)	Ursin 2000, James and others 2003
	+ β-Carotene	Phytoene synthase (daffodil) Phytoene desaturase *(Erwinia*) Lycopene cyclase (daffodil)	Ye and others 2000
	8:0 and 10:0 Fatty acids Medium Chain Fatty Acids ↑	Ch FatB2, a thioesterase cDNA (*Cuphea hookeriana*)	Dehesh and others 1996
Cassava	Cynaogenic glycosides ↑	Hydroxynitril lyase	Siritunga and Sayre 2003
Cotton	Oleic acid↑	Mutant δ-12 desaturase	Chapman and others 2001
	High-oleic and high-stearic cottonseed oils	hpRNA-mediated post-transcriptional gene silencing desaturases	Liu and others 2002
Coffee	Caffeine↑	Antisense xanthosine-N-7-methyl transferase (coffee)	Moisyadi and others 1998
Lupin	Methionine↑	Seed albumin (sunflower)	White and others 2001
Maize	Methionine↑	mRNA stability by intron switiching Dzr1 target	Lai and Messing 2002
	Fumonisin↑	de-esterase+de-aminase (mbial)	Duvick 2001
	Insect resistance	Avidin (chicken)	Kramer and others 2000
	Protein with favorable amino acid profile↑	α-Lactalbumin (porcine)	Yang and others 2002
	Sulfur amino acids↑	Maize 15kDa-zein	Dinkins and others 2001
Maize	Vitamin C↑	Wheat dehydroascorbate reductase (DHAR)	Chen and others 2003
Potato	Starch↑	ADP glucose pyrophosphorylase (*Escherichia coli*)	Stark and others 1992
	Very-high-amylose starch↑	Inhibition of SBE A and B	Schwall and others 2000
	Inulin molecules↑	1-SST (sucrose:sucrose 1-fructosyltransferase) and the 1-FFT (fructan:fructan 1-fructosyltransferase) genes of globe artichoke (*Cynara scolymus*)	Hellwege and others 2000
	+Sulphur-rich protein	Nonallergenic seed albumin gene (*Amaranthus hypochondriacus*)	Chakraborty and others 2000
Potato	Solanine↓	Antisense sterol glyco transferase (Sgt) gene	McCue and others 2003
Rice	+ β-Carotene	Phytoene synthase (daffodil) Phytoene desaturase *(Erwinia*) Lycopene cyclase (daffodil)	Ye and others 2000
	Iron↑	Ferritin *(Phaseolus*) Metallothionein (rice) Phytase (mutant, *Aspergillus)*	Lucca and others 2002
	Allergenic protein↓	Antisense 16kDa allergen (rice)	Tada and others 1996
Rice	+ Puroindolinone compounds: softer rice kernels, flour yields more finer particles, less damage to starch	Wheat puroindoline genes	Krishnamurty and Giroux 2001
Sorghum	Improved digestibility of livestock feed	Mutated Brown midrib (Bmr) encodes caffeic acid O-methyltransferase (COMT), a lignin-producing enzyme	Vermerris and Bout 2003
Soybeans	Improved amino acid composition	Synthetic proteins	Rapp 2002
	Increased sulfur amino acids	Overexpressing the maize 15 kDa zein protein	Dinkins and others 2001
	Oleic acid↑	Δ-12 Desaturase (soybean, sense suppression)	Kinney and Knowlton 1998
	Oleic acid↑	Ribozyme termination of RNA transcripts down-regulate seed fatty acid	Buhr and others 2002
	Immunodominant Allergen ↓	Gene silencing of cysteine protease P34 (34kDa)	Herman 2002
Soybean/arabidopsis	Isoflavones↑ +isoflavones	Isoflavone synthase	Jung and others 2000)
Sweet Potato	Protein content↑	Artificial storage protein (ASP-1) gene	Prakash and others 2000
Tomato	Provitamin .A↑ and lycopene↑	Lycopene *cyclase (Arabidopsis*)	Rosati and others 2000
	Provitamin.A↑	Phytoene desaturase *(Erwinia*)	Fraser and others 2001
	Flavonoids↑	Chalcone isomerase (*Petunia)*	Muir and others 2001
	Lycopene ↑	Engineered polyamine accumulation	Mehta and others 2002
Wheat	Glutenins ↑	High molecular weight subunit genes	Barro and others 1997, Rooke and others 1999
	Caffeic and ferulic acids ↑	Wheat gene	UPI 2002

2.6 Functional Foods

In recent years, a new category called "functional foods" has appeared in the marketplace, and sales are growing quickly. For many, functional foods include not only those with added components that enhance their health claims but also include unsupplemented foods for which new health claims are recognized through the addition of a new product label. Functional foods are intended to appeal to consumers by offering potential health benefits that go beyond satisfying basic nutritional needs. These foods exploit the growing scientific evidence supporting the role of a diet containing certain types of foods or phytochemicals in the prevention and treatment of disease. Epidemiological research has shown a positive association between dietary intake of food components found in fruits, vegetables, grains, fish oil, and legumes and their effect on chronic disease. In 1992, a review of 200 epidemiological studies (Block and others 1992) showed that cancer risk in people consuming diets high in fruits and vegetables was only half that in those consuming low amounts of these foods. Functional food components have been associated with the prevention and/or treatment of at least 4 of the leading causes of death in the USA: cancer, diabetes, cardiovascular disease, and hypertension. The U.S. National Cancer Institute estimates that 1 in 3 cancer deaths are diet related, and that 8 of 10 cancers have a nutrition/diet component (Steinmetz and Potter 1996). Other nutrient-related correlations link dietary fat and fiber to colon cancer, folate to the prevention of neural tube defects, calcium to the prevention of osteoporosis, psyllium to the lowering of blood lipid levels, and antioxidant nutrients to the scavenging of reactive oxidant species and protection against oxidative damage of cells that may lead to chronic disease (Goldberg 1994). One group of phytochemicals, the isothiocyanates (glucosinolates, indoles, and sulforaphane), found in cruciferous vegetables such as broccoli, has been shown to trigger enzyme systems that block or suppress cellular DNA damage and that seem to reduce tumor size (Gerhauser and others 1997). The large numbers of phytochemicals that are implicated in this type of activity suggest that the potential impact of phytochemicals and functional foods on human and animal health is worth examining.

Beyond understanding of plant metabolism, the challenge then remains to better understand how components in the diet interact with human or animal metabolism to benefit their health and well-being. Although there exists extensive research and clinical support for specific nutrient effects as documented in the following sections, improving our knowledge at the fundamental level of molecular effects will better inform the decisions being made with respect to nutritional quality improvement. This challenge is at least as complex as the task of increasing or decreasing the amount of a specific protein, fatty acid, or other component of the plant itself. It is of little use producing a plant with a supposed nutritional benefit unless that benefit can be translated into positive health or nutritional impacts in humans or animals. Table 2-2 illustrates some examples of components with suggested functionality.

The application of rDNA technology to improve plant-specific components known to have benefit for human health that goes beyond meeting basic nutritional requirements is one way to introduce new functional foods into the marketplace. In addition to functional foods, rDNA technology allows the engineering of plants to address issues of animal nutrition and the impact of animal effluent on the environment. A good example of this is the addition of phytase enzymes to crops to reduce the need to add phosphorus to feed (Austin-Phillips and others 1999; Lucca and others 2002). Most of the phosphorus is added because the phosphorus in phytic acid is not bioavailable and because of the sequestering effect of phytic acid on uptake of divalent mineral ions. Chapter 5 will discuss the nutritional assessment of nutritionally improved feed ingredients derived from GM crops.

2.7 Examples of Modifications

The following sections will examine a number of areas where metabolic engineering has been carried out or may be beneficial. The examples will illustrate the types of modifications that have been carried out or are being contemplated and describe their purpose, examine the successes and failures that have been documented, and provide insight into the technology used to produce nutritional alterations in plants so that readers will have a greater understanding of the problems that could arise from metabolic engineering. Further examples can be found in the references listed in Table 2-1.

2.7.1 Proteins and amino acids

Humans, as well as poultry, swine, and other nonruminant animals, have specific dietary requirements for each of the essential amino acids. A deficiency of 1 essential amino acid limits growth and can be fatal. In animal feeds, the primary limitations of maize and soybean meal-based diets are for lysine in nonruminant mammals and methionine in avian species. Maize with increased levels of lysine and soybeans with increased levels of methionine could allow diet formulations with improved amino acid balance, without the need to add crystalline lysine and methionine.

Most plants have a poor balance of essential amino acids relative to the needs of animals and humans. The cereals (maize, wheat, rice, and so on) tend to be low in lysine, whereas legumes (soybean, peas, and so on) are often low in the sulfur-rich amino acids methionine and cysteine. Successful technical examples to date to enhance free amino acids levels include high-lysine maize (O'Quinn and others 2000) and high-lysine canola and soybeans (Falco and others 1995). Dinkins and others (2001) increased sulfur-rich amino acids in soybean plants by overexpressing the methionine-rich 15-kDa zein protein from maize.

In areas such as less-developed countries, where it is difficult to obtain access to the components necessary for a balanced diet, these types of modifications could offer a particular advantage. Consumption of foods prepared from these crops potentially can help prevent protein malnutrition in such regions, especially among children, as well as increase the availability of animal protein in developing countries by improving the quality of animal feed.

From an engineering perspective, one of the most straightforward methods to modify amino acid compositions of food and feed is by expressing proteins with high levels of the desired amino acids in the seed (the major food source). One method of modifying storage protein composition is to introduce heterologous or homologous genes that code for proteins containing elevated levels of sulfur-containing amino acids (methionine, cysteine) and lysine. These proteins can be from other natural sources or can be synthetic.

An example of the synthetic approach was published by Beauregard and others (1995). An 11-kDa synthetic protein, MB1, was created to contain the maximum number of the essential amino acids methionine, threonine, lysine, and leucine in a stable, helical conformation. The structure was also designed to resist proteases to prevent degradation in-planta. The high methionine (16%) and lysine (12%) contents make it a desirable candidate for improving soy protein quality. The MB1 protein was targeted to seed protein storage bodies using appropriate leader sequences and seed-specific promoters (Simmonds and Donaldson 2000). Using a similar approach, another artificial storage protein (ASP-1) has been used to modify sweet potatoes (Prakash and others 2000). Transgenic plants exhibited a 2- and 5-fold increase in the total protein content in leaves and roots, respectively, over that of control plants. A significant increase in the level of essential amino acids such as methionine, threonine, tryptophan, isoleucine, and lysine was also observed (Prakash and others 2000).

Table 2-2—Examples of plant components with suggested functionality[a]

Class/components	Source[b]	Potential health benefit
Carotenoids		
α-carotene	Carrots	Neutralizes free radicals that may cause damage to cells.
β-carotene	Various fruits, vegetables	Neutralizes free radicals.
Lutein	Green vegetables	Contributes to maintenance of healthy vision
Lycopene	Tomatoes and tomato products (ketchup, sauces)	May reduce risk of prostate cancer.
Zeaxanthin	Eggs, citrus, maize	Contributes to maintenance of healthy vision.
Dietary fiber		
Insoluble fiber	Wheat bran	May reduce risk of breast and/or colon cancer.
β glucan	Oats	May reduce risk of cardiovascular disease (CVD).
Soluble fiber	Psyllium	May reduce risk of CVD.
Whole Grains	Cereal grains	May reduce risk of CVD.
Collagen hydrolysate	Gelatin	May help improve some symptoms associated with osteoarthritis
Fatty acids		
Omega-3 fatty acids - DHA/EPA	Tuna; fish and marine oils	May reduce risk of CVD and improve mental, visual functions.
Conjugated linoleic acid (CLA)	Cheese, meat products	May improve body composition, may decrease risk of certain cancers.
Flavonoids		
Anthocyanidins: cyanidin	Berries	Neutralize free radicals, may reduce risk of cancer.
Hydroxycinnamates	Wheat	Antioxidant-like activities, may reduce risk of degenerative diseases.
Flavanols: catechins, tannins	Tea (green, catechins), (black, tannins)	Neutralize free radicals, may reduce risk of cancer.
Flavanones	Citrus	Neutralize free radicals, may reduce risk of cancer.
Flavones: quercetin	Fruits/vegetables	Neutralize free radicals, may reduce risk of cancer.
Glucosinolates, indoles, isothiocyanates		
Sulphoraphane	Cruciferous vegetables (broccoli, kale), horseradish	Neutralizes free radicals, may reduce risk of cancer.
Phenols		
Stilbenes – resveratrol,	Grapes	May reduce risk of degenerative diseases; heart disease; cancer.
caffeic acid, ferulic acid	Fruits, vegetables, citrus	Antioxidant-like activities; may reduce risk of degenerative diseases; heart disease, eye disease.
Plant stanols/sterols		
Stanol/sterol ester	Maize, soy, wheat, wood oils	May reduce risk of coronary heart disease (CHD) by lowering blood cholesterol levels.
Prebiotic/probiotics		
Fructans, inulins, fructo-oligosaccharides (FOS)	Jerusalem artichokes, shallots, onion powder	May improve gastrointestinal health.
Lactobacillus	Yogurt, other dairy	May improve gastrointestinal health.
Saponins	Soybeans, soy foods, soy protein-containing foods	May lower LDL cholesterol; contains anti-cancer enzymes.
Soybean protein	Soybeans and soy-based foods	25 g/day may reduce risk of heat disease.
Phytoestrogens		
Isoflavones- daidzein, genistein	Soybeans and soy-based foods	May reduce menopause symptoms, such as hot flashes, reduce osteoporosis, CVD.
Lignans	Flax, rye, vegetables	May protect against heart disease and some cancers; may lower LDL cholesterol, total cholesterol, and triglycerides.
Sulfides/thiols		
Diallyl sulfide	Onions, garlic, olives, leeks, scallions	May lower LDL cholesterol, helps to maintain healthy immune system.
Allyl methyl trisulfide, dithiolthiones	Cruciferous vegetables	May lower LDL cholesterol, helps to maintain healthy immune system.
Tannins		
Proanthocyanidins	Cranberries, cranberry products, cocoa, chocolate, black tea	May improve urinary tract health. May reduce risk of CVD, and high blood pressure

[a]Examples are not an all-inclusive list.
[b]U.S. Food and Drug Administration approved health claim established for component.
Modified from IFIC 2002.

An example of the use of proteins from natural sources is the work of Chakraborty and others (2000), who reported introducing an albumin gene for a nonallergenic protein from *Amaranthus,* rich in all essential amino acids, into potato. The resulting tuber composition corresponds well with the World Health Organization (WHO) standards for a nutritionally rich protein for optimal human nutrition (WHO 1999). In this case, there was a striking increase in the growth rate and production of tubers in transgenic populations compared to the control. There was also an increase in the total protein content, with an increase in most essential amino acids (Chakraborty and others 2000). The results of these experiments document, in addition to successful nutritional improvement of potato tubers, the feasibility of genetically modifying other non-seed food crop plants with novel protein composition. An important issue is that of ensuring that the total composition of storage proteins, for example, is not altered to the detriment of the development of the crop plant when attempting to improve amino acid ratios. Rapp (2002) reported modifying soybean storage pro-

teins in such a way that the 3-dimensional structure is maintained, and so that the modified proteins can accumulate in the seed at levels comparable to the endogenous seed proteins. A novel method of increasing essential amino acids was demonstrated by Lai and Messing (2002). Maize produces a methionine-rich protein (delta-zein) in the grain but at a low level. Lai and Messing (2002) found a protein, Dzr1, that binds an intronic region and degrades delta-zein mRNA before translation. They replaced the targeted intronic region with an intron from another maize gene. This prevented Dzr1 from degrading delta-zein RNA and maximized the production of the methionine-rich protein. Chickens fed diets containing this maize grew significantly faster than chickens fed conventional maize. This modification could potentially save animal farmers $1 billion per year in synthetic methionine supplements to maize-based feed.

Attempts to manipulate the free lysine content of seeds illustrate that one needs to consider catabolic, as well as anabolic, variables when trying to engineer a particular metabolic phenotype in plants. A key step in lysine synthesis is catalyzed by dihydrodipicolinate synthase (DHDPS), which is feedback inhibited by the pathway endproduct (lysine) and, thus, plays a key role in regulating flux through the pathway. Engineering plants to overexpress a feedback-insensitive bacterial DHDPS greatly increased flux through the lysine biosynthetic pathway. However, in most cases this did not result in greater steady-state lysine levels because the plants also responded by increasing flux through the lysine catabolic pathway through elevation of lysine-ketoglutarate reductase. Substantial increases in lysine only occurred in plants where flux increased to such a level that the first enzyme of the catabolic pathway became saturated (Brinch-Pedersen and others 1996), again illustrating the potential complexities of metabolic regulation.

2.7.2 Carbohydrates

Plants make both polymeric carbohydrates (for example, starches and fructans) and individual sugars (for example, sucrose and fructose). The biosynthesis of these compounds is sufficiently understood to allow the bioengineering of their properties and to engineer crops to produce polysaccharides not normally present.

The term prebiotic is used to describe an indigestible food ingredient, such as fructooligosaccharides (FOS), that beneficially affects the microflora by selectively stimulating the growth and/or activity of beneficial bacteria. Fructans (plant inulins) and fructooligosaccharides may be important ingredients in functional foods, because evidence suggests that they promote a healthy colon and help reduce the incidence of colon cancer. The FOS may have anticarcinogenic, antimicrobial, hypolipidemic, and hypoglycemic actions in some (Pierre and others 1997; Roberfroid and Delzenne 1998, Sahaafsma and others 1998). They may also help improve mineral absorption and balance, and may have antiosteoporotic and antiosteopenic activities (Ohta and others 1998). Inulins are only slightly digested in the small intestine. They are, however, fermented by a limited number of colonic bacteria (Wang and Gibson 1993). This could lead to changes in the colonic ecosystem in favor of some bacteria, such as *Bifidobacteria*, which may have health benefits (Bouhnik and others 1999). Oral administration to humans of fructans, such as oligofructose and inulin, has been shown to increase the number of bifidobacteria in stools (Isolauri and others 2002). *Bifidobacteria* may inhibit the growth of pathogenic bacteria, such as *Clostridium perfringens* and diarrheogenic strains of *Escherichia coli* (Bouhnik and others 1999). Inulins are considered to be bifidogenic factors. Their energy content is about half that of digestible carbohydrates or about 1 to 2 kcal/g. The possible anticarcinogenic activity might be accounted for, in part, by the possible anticarcinogenic action of butyrate (Watkins and others 1999). Butyrate, along with other short-chain fatty acids, is produced by bacterial fermentation of FOS in the colon. Some studies have shown that butyrate induces growth arrest and cell differentiation and may also upregulate apoptosis, 3 activities that could be significant for antitumor activity (Watkins and others 1999, Stringer and others 1996). The FOS may lower serum triglyceride levels in some individuals. The mechanism of this possible effect is unclear. Decreased hepatocyte triglyceride synthesis is a hypothetical possibility. The FOS may also lower total cholesterol and LDL-cholesterol levels in some people (Smith and others 1998, Watkins and German 1998). Again, the mechanism of this possible effect is unclear. Propionate, a product of FOS fermentation in the colon, may inhibit HMG-CoA reductase, the rate-limiting step in cholesterol synthesis (Watkins and German 1998).

Thus, there is interest in modifying plants to produce this polymeric carbohydrate. The main crop of interest for producing fructan is the sugar beet because the major storage component of this species is sucrose, the direct precursor for fructan biosynthesis. Sévenier and others (1998) have reported high-level fructan accumulation in a GM sugar beet without adverse effects on growth or phenotype. This work has implications both for the commercial manufacture of fructans and for the use of genetic engineering to obtain new products from existing crops. Hellwege and others (2000) produced GM potato (*Solanum tuberosum*) tubers that synthesize the full spectrum of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*) roots. A similar approach (Allen and others 2002) is being used to derive soybean varieties that contain some oligofructan components that may selectively increase the population of beneficial species of bacteria (for example, *Bifidobacteria*) in the intestines of humans and certain animals and, thus, inhibit harmful species of bacteria (for example, *E. coli* 0157:H7, *Salmonella* SE, and so on).

The soluble oligosaccharides, stachyose and raffinose, which are found in soybeans, are not digested and can cause flatulence and digestive problems (Hartwig and others 1997; Suarez and others 1999), producing discomfort in humans. These compounds in conventional soybean or soybean meal are similarly not digested by nonruminant animals, resulting in reduced feed efficiency. Researchers found that the incorporation of low-stachyose soybean meal from nonmodified sources in prestarter pig diets tended to improve growth performance (Risley and Lohrmann 1998). In addition, the increased sucrose content of low-stachyose soybean results in foods with a sweeter taste than do their traditional counterparts. Manipulating the level of this family of oligosaccharides through rDNA technology has been achieved by inhibiting galactinol synthase activity (Kerr and others 1998). This is the first committed step in the pathway and involves the synthesis of galactinol from UDP-Gal and myo-inositol. The individual members are then synthesized by distinct galactosyl transferases (for example, raffinose synthase and stachyose synthase). As raffinose and stachyose may be crucial during seed development and storage, perhaps an alternate strategy would be that suggested by Griga and others (2001), which is based on the transfer of α -galactosidase from a thermophilic bacterium (*Thermotoga neapolitana*) into legumes and inducing α-galactosidase to degrade the oligosaccharides after harvesting by changing the temperature.

Starch is an important storage polysaccharide in many plants. It is composed of densely packed α-glucans, consisting of α-1,4- and α-1,6-linked glucose residues. Engineering starch content and composition in potatoes is of interest. Plant ADP glucose pyrophosphorylase (ADPGPP) is sensitive to allosteric effectors and has been proposed to be a key regulator of starch biosynthesis. Stark and others (1992) engineered wild type and mutant allosterically insensitive *E. coli* ADPGPP for chloroplast-targeted, tuber-specific expression in potatoes. Tubers from potato plants trans-

formed with the allosterically insensitive *E. coli* ADPGPP enzyme had starch levels up to 40% higher than the wild type. The higher starch content results in far less fat absorption during frying, because the moisture lost during frying is replaced by oil. However, there are still problems of irregular granule distribution throughout the tuber to be solved. Schwall and others (2000) created a potato producing very high amylose (slowly digested) starch by inhibiting 2 enzymes that would normally make the amylopectin type of starch that is rapidly digested. This "resistant starch" is not digested in the small intestine, but is fermented in the large intestine by the microflora. Clinical studies have demonstrated that resistant starch has similar properties to fiber and has potential physiological benefits in humans (Yue and Waring 1998, Richardson and others 2000). The next section will discuss this in more detail.

2.7.3 Fiber and lignans

Fiber is a group of substances chemically similar to carbohydrates, except that nonruminant animals poorly digest fiber. Fiber provides bulk in the diet, such that foods rich in fiber are satisfying without contributing significant calories. Current controversies aside, there is ample scientific evidence to show that prolonged intake of foods high in dietary fiber has various positive health benefits in humans, especially the potential for reduced risk of cardiovascular disease and colon and other types of cancer. A study that covered nearly 30000 middle-aged Finnish men found strong evidence of an inverse association between the amount of dietary fiber in the diet and coronary heart disease. The relative risk for fatal myocardial infarction was 0.45 among men with the highest intake of fiber (median 28.9 g/d) compared with men with lowest intake of fiber (median 12.4 g/d) (Pietinen and others 1996).

Fiber type and quantity are undoubtedly under genetic control, although this topic has received little attention. The technology to modify fiber content and type by genetic engineering would be a great benefit in persuading the many individuals who, for taste or other reasons, do not include adequate amounts of fiber in their daily diet. For example, fiber content could be added to more preferred foods or the more common sources of dietary fiber could be altered for greater health benefits. Other fiber-associated compounds include lignans. The 2 lignans of primary interest in mammals, enterodiol and its oxidation product, enterolactone, are formed in the intestinal tract by bacterial action on plant lignan precursors (Rickard and Thompson 1997). Flaxseed is the richest source of mammalian lignan precursors. Because enterodiol and enterolactone are structurally similar to both naturally occurring and synthetic estrogens, and have been shown to possess weakly estrogenic and antiestrogenic activities, they may play a role in the prevention of estrogen-dependent cancers (Rickard and Thompson 1997). Genes encoding all the enzymes for the conversion of coniferyl alcohol (lignan and lignin precursor) to secoisolariciresinol, a major dietary phytoestrogen, have been cloned. Other alcohol derivatives such as plant sterols (mainly sitostanol) exhibit a dose-dependent action inhibiting cholesterol absorption while increasing cholesterol excretion and upregulating cholesterolgenesis in hamsters, resulting in lower circulating lipid levels (Wong 2001).

However, as discussed elsewhere, low-fiber feedstuffs are often favored for nonruminant animals. Nonruminant animals do not produce enzymes necessary to digest cellulose-based plant fiber. Plants low in fiber should yield more digestible and metabolizable energy and protein and less manure and methane when fed to these species (North Carolina Cooperative Extension Service 2000). US Dairy Forage Center (USDFRC) estimates that a 10% increase in fiber digestibility would result in an annual $350 million increase in milk/beef production and decreased generation of manure, USDFRC estimates that a 10% increase in fiber digestibility is equivalent to 2.8 million tons decrease in manure solids produced each year (McCaslin 2001). Improved digestibility of livestock feed is therefore highly desirable. Guo and others (2001) developed low-lignin transgenic alfalfa through knockouts of enzymes involved in lignin biosynthesis. The altered lignin content and composition resulted in increased rate and extent of rumen digestion. Vermerris and Bout (2003) identified and cloned a brown midrib (Bmr) gene, which encodes caffeic acid O-methyltransferase (COMT), a lignin-producing enzyme. They generated mutants that give rise to plants that contain significantly lower lignin in their leaves and stems, leading to softer cell walls compared to wild type. The plant-softening mutations improve the digestibility of the food, and livestock seem to prefer the taste. Such improved fiber digestibility in nonruminants should have significant beneficial effects because the efficiency of digestion of most high-fiber diets for nonruminants is far from optimized.

2.7.4 Oils/lipids

Gene technology and plant breeding are combining to provide powerful means for modifying the composition of oilseeds to improve their nutritional value and provide the functional properties required for various food oil applications. The technology also has the potential to produce industrial oils and chemicals in genetically engineered crops. Mazur and others (1999) recently reviewed this topic.

Genetic modification of oilseed crops can provide an abundant, relatively inexpensive source of dietary fatty acids with wide-ranging health benefits. Production of lipids shown to have health benefits in vegetable oil provides a convenient mechanism to deliver healthier products to consumers without the requirement for significant dietary changes. The lipid biosynthetic pathway was one of the earliest pathways to be targeted for modification, and it represents one of the better examples of metabolic engineering in plants to date. Most enzymes required for fatty acid synthesis in plants have been cloned, and various academic and industrial groups have modified their expression to manipulate oilseed fatty acid composition. Major alterations in the proportions of individual fatty acids have been achieved in a range of oilseeds using conventional selection, induced mutation, and, more recently, posttranscriptional gene silencing. Examples of such modified oils include low- and zero-saturated fat soybean and canola oils, canola oil containing medium chain fatty acids (MCFA), high-stearic acid canola oil (for trans fatty acid-free products), high-oleic acid (monounsaturated) soybean oil, and canola oil containing the polyunsaturated fatty acids (PUFA), γ-linolenic (GLA; 18:3 n-6), stearidonic acids (SDA; C18:4 n-3), and other omega-3 fatty acids (Yuan and Knauf 1997).

Altering the chain length and saturation level of the fatty acids can improve the nutritional qualities of some oils. In addition, genes from various plant species may be introduced to produce unusual fatty acids in oilseed crops. Laurical™, canola oil with high amounts of lauric acid (C12:0), was the first commercial GM food oil. In this case, lauroyl-ACP thioesterase genes from the California bay laurel were cloned and transferred to canola (low-erucic acid rapeseed) oil crops. In 1995, the FDA completed its evaluation of Laurical for use in food products (Del Vecchio 1996).

Medium chain fatty acids (MCFA) range from 6 to 10 carbons long and are only minor components of natural foods. The medium chain triglycerides (MCT) with these MCFA aid in absorption of calcium and magnesium (Fushiki and others 1995) and are rapidly oxidized as a quick source of energy. When MCT are substituted for long chain triglycerides (LCT) in the diet, animals gain less weight, store less adipose tissue, and experience an increase in metabolic rate (Baba and others 1982; Geliebter and others 1983). Mice fed diets with MCT have also been shown to possess

increased endurance in swimming tests over that of mice fed diets with LCT (Fushiki and others 1995). Medium chain triglyceride oil has been included in medical foods, ergogenic aids, and dietary supplements.

Because MCT are not readily available in high quantities in ordinary foods, they must be produced synthetically, making them of great interest to researchers. Thus, Dehesh and others (1996) have used the morilena mushroom and plants to identify enzymes involved in production of the MCT capric and caprylic acid. Expression of an acyl-ACP thioesterase cDNA from *C. hookeriana* in seeds of canola, an oilseed crop that normally does not accumulate any capric and caprylic acid, resulted in a large increase in the levels of these 2 MCT (Dehesh and others 1996). This illustrates the capacity to harness, through biotechnology, the genes contributing to phytochemical biodiversity in wild species and offers significant potential in the treatment of disease where such phytochemicals have proven health benefits.

Many types of fats are important, and the following sections will discuss different types of modifications with differing health implications. Edible oils rich in monounsaturated fatty acids provide improved oil stability, flavor, and nutrition for human and animal consumption. Oleic acid (C18:1), a monounsaturate, can provide more stability than the polyunsaturates, linoleic (C18:2) and linolenic (C18:3) acids. Higher monounsaturates are also preferred from a health perspective (Marsic and others 1992; McDonald 1995). Antisense inhibition of oleate desaturase expression in soybean resulted in oil that contained >80% oleic acid (23% is normal) and had a significant decrease in polyunsaturated fatty acids (Kinney and Knowlton 1998). Clemente (Buhr and others 2002) achieved a more stable effect using termination of transcripts with a self-cleaving ribozyme to enhance nuclear retention and serve as a tool to decrease specific plant gene expression achieving greater than 85% oleic, and saturated fatty acids levels of less than 6%. High-oleic soybean oil is naturally more resistant to degradation by heat and oxidation, and so requires little or no postrefining processing (hydrogenation), depending on the intended vegetable oil application. Liu and others (2002) produced high-stearic and high-oleic cottonseed oils by using posttranscriptional gene silencing.

While many lipids have important health implications, the long-chain polyunsaturated fatty acids (PUFA), especially the omega-3 fatty acids found in fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are present in the retina of the eye and cerebral cortex of the brain, are some of the most well documented from a clinical perspective. Docosahexaenoic acid is also the predominant structural fatty acid in the gray matter of the brain. It is believed that EPA and DHA play an important role in the regulation of inflammatory immune reactions and blood pressure, treatment of conditions such as cardiovascular disease and cystic fibrosis, brain development in utero, and, in early postnatal life, the development of cognitive function (Dry and Vincent 1991; Fortin and others 1995; Katz and others 1996; Yehuda and others 1996; Broughton and others 1997; Landmark and others 1998; Carlson 1999; Christensen and others 1999; Smuts and others 2003). They also possess anticancer properties (Anti and others 1994; Wigmore and others 1996; Gogos and others 1998; Simonsen and others 1998; Norrish and others 1999). Omega-3 fatty acids also seem to be beneficial in certain neuropsychiatric illnesses such as bipolar disorder, schizophrenia, and depression (Stoll and others 1999). Current Western diets tend to be relatively high in omega-6 fatty acids and relatively low in omega-3 fatty acids. This is due in part to our high intake of vegetable oils that are rich in omega-6 fatty acids, and our low intake of oils and foods rich in omega-3 fatty acids, such as canola oil, flaxseed oil, or fatty fish. In plants, the microsomal ω-6 desaturase-catalyzed pathway is the primary route of production of polyunsaturated lipids. Ursin (2000) introduced genes encoding fatty acid desaturase from plants and fungi (such as the Δ-6 desaturase gene from a fungus *(Mortierella*) succeeding in producing omega-3 fatty acids in canola. In a clinical study designed to determine the relative efficacy of various fatty acids, metabolism of α-linolenic acid (ALA) and SDA, to the long-chain PUFA EPA, DPA n-3 (docosapentaenoic acid), and DHA in humans was measured. Researchers observed that SDA was superior in producing EPA by a factor of 3.6 over ALA (James and others 2003). Transgenic canola oil was obtained that contains >23% SDA, with an overall n-6:n-3 ratio of 0.5. Many food quality and health considerations encourage the development of oils containing altered ratios of saturated/unsaturated fatty acids. For a more complete list, see Table 2-1 and 2-2.

2.7.5 Vitamins and minerals

For selected minerals (iron, calcium, selenium, and iodine) and a limited number of vitamins (folate; vitamins E, B_6, and A), the clinical and epidemiological evidence is clear that they play a significant role in maintenance of optimal health and are limiting in diets worldwide. In addition, there is a growing knowledge base indicating that elevated intake of specific vitamins and minerals (for example, vitamins E and C, carotenoids, and selenium) may reduce the risk of diseases such as certain cancers, cardiovascular diseases, and chronic degenerative diseases associated with aging (Kehrer and Smith 1994; Steinmetz and Potter 1996; AIFCR 1997). Because of the difficulty in separating individual nutrient effects from an overall dietary pattern that may be fundamental to achieving these health benefits, improved dietary patterns should still be encouraged. If nutrient intakes associated with optimal health benefits are not achievable by dietary modification alone, fortification of foods will be an alternative route. Genetic engineering is a potentially important route of fortification, particularly since it would seem to avoid many of the technical problems associated with food fortification such as uneven distribution of minute quantities of nutrients, unstable mixing and settling, over- or underaddition, and so on. Various groups (for example, the Consultative Group on International Agricultural Research) are using both traditional breeding and recombinant DNA approaches to develop biofortified crops that will be especially valuable in developing countries.

Rice is a staple that feeds nearly half the world's population, but milled rice does not contain β-carotene or significant amounts of its precursors. Integrating observations from prokaryotic systems into their work has enabled researchers to clone the majority of the carotenoid biosynthetic enzymes from plants during the 1990s. Ingo Potrykus and his research team at ETH-Zurich reported that immature rice endosperm is capable of synthesizing the early intermediate of β-carotene biosynthesis (Ye and others 2000). Using carotenoid pathway genes from daffodil and *Erwinia* and a *Rubisco* transit peptide, his team succeeded in producing β-carotene in the rice endosperm. This major breakthrough in the modified rice plant (cv T304) led to the development of "Golden indica Rice" (Datta and others 2003) based on the concept reported earlier, which showed that an important step in provitamin A synthesis can be engineered into a non-green plant part that normally does not contain carotenoid pigments (Ye and others 2000). Chen and others (2003) took advantage of the fact that vitamin C can be scavenged by the enzyme dehydroascorbate reductase (DHAR) by introducing the gene encoding DHAR from wheat into maize and succeeded in increasing the amount of vitamin C by up to 100-fold.

Iron is the most commonly deficient micronutrient in the human diet, and iron deficiency affects an estimated 1 to 2 billion people. Anemia, characterized by low hemoglobin, is the most widely recognized symptom of iron deficiency, but there are other serious problems such as impaired learning ability in children, in-

creased susceptibility to infection, and reduced work capacity (Moffatt and others 1994; Seshadri and Gopaldas 1989). Three research groups led by Goto (Goto and others 1999), Potrykus (Lucca and others 2002), and Datta (Vasconcelos and others 2003) employed the gene for ferritin, an iron-rich storage protein, under the control of an endosperm-specific promoter. Grain from these GM rice plants contained 3 times more iron than normal rice. To increase the iron content in the grain further, the researchers also focused on iron transport within the plant (Potrykus 1999; Lucca and others 2002; Vasconcelos and others 2003). Other examples of this kind of approach to increasing nutrient levels in foods are provided in Table 2-1, including attempts to increase vitamin E in soybean, maize, and canola and to increase folate in rice.

2.7.6 Nutraceuticals

The search for new compounds to treat human disease has led to the formation of specialized biotechnology firms searching for nutraceuticals (see the Glossary for a definition of the term nutraceutical). The recommended dietary allowances do not reflect the growing knowledge base, which indicates that elevated intakes of specific vitamins and minerals (that is, vitamins E and C, carotenoids, and selenium) significantly reduce the risk of diseases such as certain cancers, cardiovascular diseases, and chronic degenerative diseases associated with aging. To obtain such therapeutic levels in the diet, additional fortification of the food supply will be required as well as modification of dietary preferences, or direct modification of micronutrient levels in food crops. Studies by Bao and others (2001) and Bacon and others (2003) demonstrate that maximized dietary intake is not always correlated with optimized dietary benefit. Quercetin is a flavonoid that has been demonstrated in some studies to work optimally at very low concentrations in protecting against cancerous cell proliferation and the actions of the carcinogen PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) found in cooked meat (Bao and others 2001). After activation in the liver, PhIP can attack DNA to form DNA adducts. Using accelerator mass spectrometry (AMS), this group has shown that both quercetin and sulforaphane can inhibit DNA adduct formation in a dose-dependent manner. The protective mechanism of quercetin is through the inhibition of the phase I enzyme CYP 1A2, while sulforaphane acts through the induction of phase II detoxification enzymes such as glutathione transferases and UDP-glucuronosyl transferases. They further found that quercetin could ameliorate the effects of PhIP optimally at very low concentrations. As the concentration was increased, the effect was attenuated (Bacon and others 2003). Similar effects may be found for other phytochemicals. This also illustrates the importance of taking a cautious approach to any research to increase phytochemicals with putative beneficial effects under the premise of "more is better."

Unlike vitamins and minerals where mode of action is known, the primary evidence for the health-promoting roles of phytochemicals comes from epidemiological studies, and the exact chemical identity of many active compounds has yet to be determined. However, for select groups of phytochemicals, such as non-provitamin A carotenoids, glucosinolates, and phytoestrogens, the active compound or compounds have been identified and rigorously studied (Lachance 1998). Other targets include improved iron content, through the production of iron-rich storage protein, bioavailable phosphorus released from phytate, and isoflavonoids (Lucca and others 2002).

Other interesting products in the carotenoid pathway include lycopene, which may benefit the cardiovascular system by reducing the amount of oxidized low-density lipoprotein (LDL). Recent epidemiologic studies have suggested a potential benefit of this carotenoid in reducing the risk of prostate cancer, particularly the more lethal forms of this cancer. Five studies support a 30% to 40% reduction in risk associated with high tomato or lycopene consumption in the processed form in conjunction with lipid consumption, although other studies with raw tomatoes were not conclusive (Giovannucci 2002)., In an intriguing paper, Mehta and others (2002) used a GM approach to modify polyamines in tomato fruit to retard the ripening process. These modified tomatoes had longer vine lives, suggesting that polyamines have a function in delaying the ripening process. There was also an unanticipated enrichment in lycopene content of the GM tomato fruit. The lycopene levels were increased 2- to 3.5-fold compared to the conventional tomatoes. This is a substantial enrichment, exceeding that so far achieved by conventional means. This novel approach may work in other fruits and vegetables.

Stilbenes, including resveratrol (3,5,4'-trihydroxystilbene), are phenolic natural products that accumulate in a wide range of plant species, including pine, grapevine, peanut, and rhubarb (Tropf and others 1994). Grapes and related foods, such as raisins and red wine, are among the few human dietary sources of resveratrol. This compound has attracted considerable notice as a substance with possible beneficial effects on human health (Wieder and others 2001). An excellent antioxidant, resveratrol inhibits platelet aggregation and eicosanoid synthesis and is thought to contribute to improved heart function and lower blood cholesterol, based on epidemiological studies (Frankel and others 1993; Pace-Asciak and others 1995). It was shown to have "chemo-preventive" activity, preventing the formation of tumors in mouse skin bioassays, and, therefore, may help reduce cancer rates in humans (Jang and others 1997). Hipskind and Paiva (2000) have genetically engineered the constitutive accumulation of a resveratrol glucoside in alfalfa leaves and stems.

Other phytochemicals of interest include flavonoids, such as tomatoes expressing chalcone isomerase that show increased contents of the flavanols rutin and a kaempferol glycoside; glucosinolates and their related products such as indole-3 carbinol (I3C); catechin and catechol; isoflavones, such as genistein and daidzein; anthocyanins; and some phytoalexins (Table 2-2).

2.7.7 Antinutrients

Reducing phytate is an example of a biotechnology approach that solves both a nutritional and an environmental problem. Seeds store the phosphorus needed for germination in the form of phytate, a sugar alcohol molecule having 6 phosphate groups (inositol hexaphosphate). However, phytate is an antinutrient because it strongly chelates iron, calcium, zinc, and other divalent mineral ions, making them unavailable for digestive uptake. Nonruminant animals generally lack the phytase enzyme needed for digestion of phytate. Poultry and swine producers in most countries currently add mined and processed (powdered) phosphate to the diets of their animals to enable optimal growth. Excess phosphate is excreted into the environment, resulting in water pollution. When low-phytate soybean meal is utilized along with low-phytate maize for animal feeds, the phosphate excretion in swine and poultry manure is reduced by half. A series of GM rice lines (Japonica and Indica) have been developed to solve this problem (Potrykus 1999). In addition, low-phytate maize was commercialized in the USA in 1999 (Wehrspann 1998). Research indicates that the protein in low-phytate soybeans is also slightly more digestible than the protein in traditional soybeans (Austin-Phillips and others 1999). Austin-Phillips and others (1999) have genetically engineered alfalfa to produce phytase. A number of studies have shown that optimal performance and bone mineralization can result from diets without added phosphorus when phytase is included (Keshavarz 2003). Viveros and others (2002) demonstrated that phytase supplementation to low-phosphorus diets improved performance, mineral use, tibia weight, and relative liver weight in broiler chickens fed different levels of phos-

phorus. Harper and others (1997) showed similar effects in growing-finishing swine. Phytase supplementation of low-phosphorus diets improves performance, phosphorus digestibility, and bone mineralization and reduces phosphorus excretion in pigs (Harper and others 1997). Poultry grew well on the engineered alfalfa diet without any inorganic phosphorus supplement (Austin-Phillips and others 1999). Thus, phosphorus supplements may be eliminated from poultry feed to reduce costs and reduce pollution.

Other antinutrients that are being examined as possible targets for reduction are trypsin inhibitors, lectins, and several other heat-stable components found in soybeans. Consideration must be given to possible increased susceptibility to pests and diseases when natural toxicants are removed, so the base germplasm should have input traits to counter this. Reducing the amounts of trypsin inhibitors in soybeans would have a positive effect on the domestic feed industry and offer a competitive advantage for on-farm feeding of this protein source. If this can be combined with increases in the amounts of essential amino acids, very large improvements in productivity may be achieved.

2.7.8 Allergens and Substances Causing Food Intolerance

While symptoms of food intolerance are common, true food allergy is less common (Taylor and others 2000; Taylor and Hefle 2001). A food allergy is distinguished from food intolerance and other disorders by the production of antibodies (IgE) and the release of histamine and similar substances. The best-characterized true allergens include the superfamily cupins, which include globulins found in nuts and beans and albumins in nuts, and the superfamily prolamins found in cereal grains. Other common allergens are hevein (initially from rubber trees), which causes contact dermatitis from latex, and chitinases (Taylor and Hefle 2001). Foods that frequently cause malabsorption or other food intolerance syndromes other than direct IgE immune responses include wheat and other gluten-containing grains (celiac disease or gluten-sensitive enteropathy is a multifactorial disorder caused by an inappropriate T-cell-mediated response to ingested gluten, resulting in chronic intestinal inflammation characterized by villous atrophy and malabsorption; Kay 1997) and cow's milk (milk/lactose intolerance and intolerance of dairy products–other than lactoglobulins, which are allergenic). Buchanan and others (1997) have indicated that extensions of the biochemical and molecular studies have led to the use of thioredoxin to reduce allergenicity. Allergen reduction by thioredoxin changes the biochemical and physical properties of proteins. According to present evidence, thioredoxin may be used to improve foods through, among other changes, lowering allergenicity and increasing digestibility. Using dogs, researchers have shown that thioredoxin reduces disulfide bonds of allergens (converting S-S to 2 SH), and thereby alters the allergenic properties of proteins extracted from wheat flour (Buchanan and others 1997). By changing the levels of expression of the thioredoxin gene, scientists have been able to reduce the allergenic effects of the protein fractions extracted from wheat and other cereals. Thioredoxin mitigated the allergenicity associated with the major protein fractions such as the gliadins (including the alpha, beta, and gamma types) and the glutenins, but gave less consistent results with the minor fractions, the albumins and globulins (Buchanan and others 1997).

One soybean storage protein (P34) accounts for 85% of IgE responses in soybean-sensitive individuals. Sense suppression (gene silencing), driven by a seed-specific β-conglycinin promoter, was used to eliminate the accumulation of P34 in transgenic soybeans, removing the principal source of food allergenicity in soybeans (Herman 2002; Herman and others 2003). Early results from human blood serum tests indicate that P34-specific IgE antibodies could not be detected in soybean-sensitive people fed the gene-silenced beans (Helm and others 2000, Herman 2002; Herman and others 2003).

2.7.9 Toxins

Plants are not always benign and produce many phytochemicals to protect themselves from pests. Over years of breeding and selection, most of the genes involved in the production of noxious products have been eliminated from plants used as food and feed crops.

Potatoes and tomatoes are members of the deadly nightshade family and can contain toxic glycoalkaloids (for example, solanine) that have been linked to spina bifida (Friedman and others 1991). Lectins are toxic glycoproteins that have the ability to bind to carbohydrate-containing molecules on the epithelial cells of the intestinal mucosa, thus causing toxicity. They are also called hemaglutinnins, based on their ability to agglutinate red blood cells (van Heugten 2001). Kidney beans contain phytohemagglutinin and are poisonous if undercooked (Pusztai and others 1975). A number of people die each year from cyanogenic glycosides from peach and apricot seeds (Hall and Rumack 1986) and many become ill from the sodium channel binding of grayanotoxin in honey produced from the nectar of rhododendrons (Codding 1983).

It is conceivable that biotechnology approaches can be employed to downregulate or even eliminate the genes involved in the metabolic pathways for the production, accumulation, and/or activation of these toxins in plants. For example, the solanine content of potato has already been reduced substantially using an antisense approach, and efforts are underway to reduce the level of the other major potato glycoalkaloid, chaconine (McCue and others 2003). Work has also been done to reduce cynaogenic glycosides in cassava through expression of the cassava enzyme hydroxynitrile lyase (HNL) in the roots (Siritunga and Sayre 2003).

2.8 Implications for Safety Assessment

As stated previously, metabolic engineering is generally defined as the redirection of one or more enzymatic reactions to improve the production and accumulation of existing compounds, produce new compounds, or mediate the degradation of compounds. Significant progress has been made in recent years in the molecular dissection of many plant pathways and in the use of cloned genes to engineer plant metabolism. There have been numerous success stories, as well as a number of research studies that have yielded unintended results, such as attempts to modify photosynthesis. Trait modifications with the additions of 1 or 2 genes that do not act on central or intermediary metabolism produce targeted, predictable outcomes, whereas major modifications of metabolic pathways can produce unanticipated effects. It is, therefore, very encouraging that the presently available analytical technologies have been able to detect and assess the safety of these unanticipated effects. In addition, regulatory oversight of GM products has been designed to detect such unexpected outcomes in GM crops. As more metabolic modifications are introduced, we must continue to study plant metabolism and the interconnected cellular networks of plant metabolic pathways to increase the likelihood of predicting pleiotropic effects that may occur as a result of the introduced genetic modification. This topic is considered in more depth in Chapter 6.

2.9 The Future

The need for approaches to modify the amounts of essential minerals and vitamins in major crops is clear. Improvement strategies should clearly be pursued, as long as attention is paid to the upper safe level of intake for each nutrient. However, for many other health-promoting phytochemicals, clear links with health

benefits remain to be demonstrated. Such links, if established, will make it possible to identify the precise compound or compounds to target and which crops to modify to achieve the greatest nutritional impact and health benefits. Because these decisions will require an understanding of plant biochemistry, human and animal physiology, and food chemistry, strong interdisciplinary collaborations will be needed among plant scientists, nutritionists, and food scientists to ensure a safe and healthful food supply for this new century.

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Chapter 3: Safety Assessment of Nutritionally Improved Foods and Feeds Developed through the Application of Modern Biotechnology

3.1 General Principles

The safety standard that has been applied traditionally to ingredients in foods and feeds is that they should present a reasonable certainty of no harm under intended conditions of use (FAO/WHO 1996). It has long been recognized that absolute safety is not an achievable goal. This is because many foods and feeds contain inherent toxic factors (for example, glycoalkaloids in potatoes) or antinutrients (for example, phytates) and the unavoidable presence of these naturally occurring substances must be considered in assessing the safety of traditional varieties. There is a general agreement (FAO/WHO 2000; CAST 2001; Kuiper and others 2001; Cockburn 2002) that the standard of safety that should be applied to food products derived from GM crops should be equivalent to that applied to foods and feeds derived through traditional plant breeding. It is a fact, however, that, unlike most foods derived from traditional plant breeding, nearly all new foods and feeds derived from GM crops have been subjected to detailed compositional analysis and many have been assessed in toxicological and nutritional studies (Astwood and others 1996; Hammond and others 1996; Brake and Vlachos 1998; Kaniewski and Thomas 1999; Taylor and others 1999; Betz and others 2000; Edwards and others 2000; Martens 2000; Rogan and others 2000; Sidhu and others 2000; Aulrich and others 2001; Bohme and others 2001; CFSAN/FDA 2002; Cromwell and others 2002; Nair and others 2002). So, while the standard of safety may be the same in both cases, foods derived from GM crops have been subjected to more detailed scrutiny from the point of view of safety and nutrition.

In keeping with internationally recognized principles for the safety assessment of foods derived from GM crops (OECD 1993, 2002; FAO/WHO 1996, 2000; MacKenzie 2000; DEFRA 2001; EC 2003), the general approach involves comparison of the newly developed food with a suitable comparator food that has a history of safe use. This concept, referred to as substantial equivalence, includes a detailed comparison of agronomic features and composition of key nutrients, antinutrients, and natural toxicants of the new crop compared to the conventional counterpart. The purpose of this evaluation is to identify similarities and differences between the new variety and its comparators. Any differences then become the focus of the safety assessment.

Sufficient experience has been gained with the more than 50 GM crops that have been assessed by regulatory agencies, to date, to state with considerable confidence that the process of biotechnology as applied to date has not resulted in major unintended compositional changes in the food or feed. Indeed, as predicted, the application of biotechnology has resulted in minimal or no change in composition apart from the intended expression of specific traits. In addition, because the novel protein introduced is examined closely with respect to toxicity and allergenicity, it can be concluded that GM crops are as safe as their conventional counterparts.

With this experience in hand, the challenge is to develop safety assessment procedures that can be applied to nutritionally improved GM foods and feeds. The fundamental purpose here is to determine whether the composition of a nutritionally improved variety differs significantly from its traditional counterpart aside from the intended change in nutrient composition and to assess the safety of the intended change and any unintended changes.

Nutritionally improved varieties may be expected to contribute significant new sources of dietary nutrients or other bioactive phytochemicals. To assess the safety and nutritional impact of these products, it is important to have knowledge of how much of these products will be consumed in the overall human diet or in animal feeds. The safety and nutritional quality of these products can only be assessed in the context of their proposed uses and consequent intake.

3.1.1 Safety assessment concepts applied to nutritionally improved foods and feeds

A key basic principle is that both foods and feeds should meet the same safety and quality standards and should be subjected to the same safety assessment procedures. In the case of nutritionally improved foods and feeds, there is no single safety assessment approach that can be applied to all new products, although some core procedures, such as compositional analyses, that have been applied to GM crops to date are warranted. The guiding principle in approaching the safety assessment is to have clear understanding of the introduced genetic changes and how these changes affect the nature and amount of expression products and metabolites. Since the types of nutritionally improved products anticipated are diverse (see Table 2-1 and 2-2), each new product must be approached on a case-by-case basis, applying the general principles that have evolved for products derived from GM crops with improved agronomic traits.

3.1.1.1 Exposure assessment

Because nutritionally improved varieties may be expected to have major changes in the amounts of one or more nutrients, assessing human and animal exposure to these products is important, particularly if the exposures are significant. Exposure to altered levels of nutrients, such as fatty acids, from foods and feeds derived from GM crops needs to be considered in the context of total dietary exposure consumption of those same substances, which may appear in the diet from multiple sources (OECD 2002). This will require knowledge of how much of the product is consumed in the diet of humans or, in the case of livestock, the extent to which it is used in animal diets. A key consideration in the exposure assessment is the criterion that will be used to assess whether the use of a new variety will result in a significant change in dietary intake to the nutrient of interest. The word "significant" as used here refers to a change in the dietary intake of a nutrient that has the potential to materially affect health, rather than simply some defined percentage change in composition of that nutrient in the new variety. It is conceivable that a large and unintended change in content of a specific nutrient in a given food could have relatively little effect on human nutritional status with respect to that nutrient. In contrast, seemingly small decreases in content of a specific micronutrient might conceivably have serious effects on a specific at-risk subpopulation that has marginal intake of that

nutrient. The issue of what constitutes a significant change in intake of nutrients was discussed in the report of the International Food Biotechnology Council (IFBC 1990). For nutrients, it was recommended that if a food supplies less than 5% of the average daily need (intake) in an amount of the food typically consumed per day by the population in question, then the intake from that source can be regarded as nonsignificant. Similarly, it could be stated that if the intake of any inherent constituent from a food or feed derived from a GM crop were increased by 5% or less, that would not be considered a significant change. As pointed out by IFBC (1990), the distinction between a nonsignificant and a significant change is judgmental. The determination of the significance of a change in the level of a nutrient will also vary depending on the nutritional importance of the food and the availability of the nutrient in the food supply of the population. Recommended dietary intakes can be or have been set for most nutrients. Since each nutrient has a unique role and function and is present at different levels in different foods, the potential impact of changes in the dietary content of nutrients must be assessed on a case-by-case basis.

It should also be recognized that certain new varieties may be developed to achieve a particular nutritional purpose within a specific age or gender group. This will require that intake assessment be tailored to the specific demographic group who consume the greatest amount of the new product. The issue of what constitutes a significant change in dietary intake is discussed further in Chapter 5.

Methodologies for assessing intake of nutrients and other dietary constituents are widely available. These range from per capita methods to methods that use available food consumption databases or to actual food consumption surveys (Anderson 1986; Löwik 1996).

Per capita methods include food availability estimates or food disappearance data, presumably food eaten. Although per capita methods provide a representative general population mean of food consumption, they cannot provide consumption estimates for specific segments of the population. Specific segments may include populations who consume greater amounts of particular foods, either as a function of age, health status, or choice (for example, children, athletes, vegans; Lauer and Kirkpatrick 1991).

Food consumption survey methods vary in their design and collection of dietary intake data and can range from 24-h dietary recalls to multiple-day dietary records. It is well known that short-term food consumption data do not represent actual intake over a longer time period. Twenty-four-h dietary recall data have been found to overestimate consumption of specific food components, particularly for users or eaters of specific food products (Lauer and Kirkpatrick 1991). In addition, these types of surveys are generally considered to provide worst-case estimates of consumption because of the numerous conservative assumptions inherent in the methodology for estimating intake. Because of significant intraperson variability in food consumption, food consumption does not follow a normal distribution and it is difficult to determine accurately the consumption of those individuals in the 90th to 99th percentile. The greater the length of the dietary survey, the more accurate are the consumption estimates of consumers at the extremes of consumption. Detailed methods for assessing the intake of nutrients and other dietary constituents are provided by Kroes and others (2002) and the Journal of Nutrition supplement on "The Integrated CSFII-NHANES" (Madans and others 2003). Statistical and logistic issues associated with assessing intake of nutrients are discussed in Chapter 5.

3.2 Specific Evaluation Issues

The recommended approach for the safety and nutritional evaluation of foods derived through biotechnology involves a thorough knowledge of the parent or traditional crop, molecular characterization of inserted DNA, evaluation of the safety of any proteins and other products expressed from the inserted DNA, application of the concept of substantial equivalence to identify similarities and differences in composition in comparison to suitable control conventional counterparts, and the evaluation of the safety and nutritional consequences of the intended alterations in nutrient composition and any other alterations identified (OECD 1993, 2002; FAO/WHO 2000; Kuiper and others 2001; Cockburn 2002).

3.2.1 Molecular characterization

A core component of the safety assessment of foods derived from GM crops is the molecular characterization of the introduced DNA. A primary purpose of this analysis is to establish that the integrity of the vector DNA has not been modified as a result of the transformation process. The molecular characterization of GM plants is comprised of essentially 2 basic components (1) a comprehensive description of the genetic elements and constructs used for plant transformation, and (2) the description of those elements as integrated in the transgenic event of interest. Outlined below are the generic requirements for molecular characterization applied in North America. It should be noted that regulatory requirements for molecular characterization may be different for Europe (EEC 2001), Japan (Ministry of Heath and Welfare 2000), Australia and New Zealand (ANZFA 2001), Argentina, and other countries.

3.2.1.1 Transformation system and DNA.

The constructs and transformation method used to generate the GM plant must be described. This includes a detailed description of the transformation method (for example, *Agrobacterium*-mediated transformation or direct transformation by methods such as particle bombardment, electroporation, or PEG transformation of protoplasts). For *Agrobacterium*-mediated transformation, the strain designation of any *Agrobacterium* used during the transformation process and how the Ti plasmid based vector was disarmed should be described, as well as the process used to free the system of remaining *Agrobacterium* cells once transformation was complete. For direct DNA-based transformation systems, the information should include information on whether the system utilized a pathogenic organism or nucleic acid sequences from a pathogen; how such sequences, if present, were removed prior to transformation; and whether the transformation process involved the use of helper plasmids or a mixture of plasmids or carrier DNA.

A detailed physical map of the vector used for transformation, including as appropriate the location of restriction sites, should be supplied, noting those portions of the vector used as primers in PCR analysis or as probes in Southern analysis. In addition, a summary of all genetic components that comprise the vector, including coding regions and noncoding sequences of known function, should be supplied. The data on coding regions should detail the size of the individual DNA elements; the location, order, and orientation of the elements in the vector; the source of each element; and their probable function (if any) in the plant. In addition, information indicating whether any of the donor organisms or derived genetic components are known to cause disease or injury to plants or other organisms or are known toxicants, allergens, pathogenic factors, or irritants is supplied. If there is a history of safe use of the donor organism(s) or components thereof, that is also taken into account.

With regard to coding sequences (open reading frames), significant DNA sequence alterations to the native gene that resulted in a change in the amino acid sequence must be described. If the

modified amino acid sequence has not been previously published, the complete sequence (highlighting the modifications) is to be reported, while DNA sequence modifications that affect only a few amino acids can be described without providing the complete sequence. Modifications known or anticipated to result in posttranslational modifications or alterations to the structure or function of the gene product must be described.

3.2.1.2 Characterization of the DNA inserted into the plant

The complete nucleotide sequence of the DNA that is transformed into the plant is not generally required. However, sufficient data must be provided to demonstrate that the nature and order of the genetic elements as they existed in the vector DNA used in the transformation process have not been substantially altered following introduction into the plant. This may include Southern blot analysis, analysis with appropriate PCR analysis, DNA sequencing, RT-PCR data, and characterization of the protein product produced from the inserted DNA to demonstrate that the expected protein is expressed in the plant. Data describing the number of gene copies inserted into the plant, including the integration of partial gene fragments should be provided. In the case of allopolyploid plants, information identifying which parental genome the transgenic DNA has inserted into may also be required.

3.2.1.3 Inheritance, Stability, and Safety of the Introduced DNA

The pattern and stability of inheritance of the introduced DNA (and gene function) must be demonstrated for plants that are male or female fertile, or both. A variety of methods can be used to demonstrate this, such as retention of phenotype, immunoassays, PCR, or Southern hybridization. For plants that are infertile or for which it is difficult to produce seed (such as vegetatively propagated male-sterile potatoes), data must be provided to demonstrate that the transgenic trait is stably maintained and expressed during vegetative propagation over a number of generations appropriate for the crop.

DNA is an integral part of every plant cell and is rapidly degraded by normal digestive processes, leading a number of organizations to conclude that consumption of DNA, including DNA introduced into GM crops, is safe (Kessler and others 1992; OECD 1998, 2000; FAO/WHO 2000). To date, fragments of low-copy plant transgenes have not been detected in the tissues of animals that are typically consumed by humans (Jonas and others 2001; Aumaitre and others 2002).

3.2.2 Evaluation of protein safety

As with foods and feeds derived from GM crops with improved agronomic traits, the safety of any protein(s) that may be expressed from the inserted DNA in nutritionally improved products derived from GM crops as a result of any genetic change must be established. The need for studies to support safety requires consideration on a case-by-case basis and depends, in part, on available knowledge about the function and biological activity of the protein, as well as any history of prior exposure. Where appropriate, safety studies may include standard animal testing to evaluate toxicological effects or immunological studies and bioinformatic approaches necessary to assess potential allergenicity (WHO 1987; Munro and others 1996a; LSRO 1998; FAO/WHO 2000; NAS 2000b; Codex 2002). This may require the isolation of the protein from the plant or the synthesis of the protein by other means such as by *E. coli*, in which case there is a need to demonstrate biochemical, structural, and functional equivalence between this test material with that found in the plant (Codex 2002).

3.2.3 Application of the concept of substantial equivalence

In 1993, OECD formulated the concept of substantial equivalence as a starting point for the safety assessment of GM crops. A joint FAO/WHO consultation in 1996 and the Codex Alimentarius Commission of FAO/WHO in 2000 and 2002 endorsed the concept as a strong and robust starting point for the safety assessment of GM crops, and the concept has been reviewed by a number of workers including Chesson (2001), Kuiper and others (2001), Aumaitre and others (2002), and Cockburn (2002). As has been pointed out by others (OECD 1993, 2002; FAO/WHO 2000, 2001), application of the substantial equivalence concept is not a safety assessment per se, but provides a basis to identify similarities and differences between the new variety and some suitable comparator variety. Differences are then subjected to further safety assessment. Examples of the application of substantial equivalence are provided in Chapter 4.

Nutritionally improved products are expected to consist of 2 categories of products. One category will be nutritionally improved foods and feeds intended to replace traditional varieties in the human diet or in animal diets. The 2nd category of products is food or feed ingredients derived from nutritionally improved crops. Some of these will be identical chemically to ingredients currently derived from food crops, whereas others could be chemically altered products, such as cross-linked modified starches that are modified to have specific processing or health attributes. The approach to the evaluation of these 2 categories will differ and this is discussed further below.

3.2.3.1 Compositional analysis. Compositional analysis is the major factor assessed in the determination of substantial equivalence. Various grain, plant parts, and/or processed fractions are analyzed to determine the amounts of specific analytes in the matrix. These analyses range from the crude proximates (protein, fiber, fat) to very detailed analysis of the amino acid composition of the matrix. Thus a typical composition profile consists of moisture, crude protein, crude fat, ash, fiber fractions, amino acid and fatty acid profiles, vitamins, and minerals. In addition, data on antinutrients and other biologically significant compounds present in the crop, such as trypsin inhibitors, endogenous toxins, isoflavones, or phytic acid should be obtained.

3.2.3.2 Statistical issues. It is critical that data used in the assessment of composition are statistically robust. This means that the data must come from a sampling plan that has been set up to a defined protocol in order to obtain a representative and substantially robust sample. Developers have often adopted practices employed in pesticide residue trials, as required by EPA (1996) and in line with Codex (1987) recommendations. In other studies, replicate samples are collected or samples are collected from multiple plots at the same location. In some cases, the sample may be from a much larger number of plants (for example, from a bulk sample from a large plot), and in these cases care must be taken to obtain a representative sample from the bulk sample, either by employing appropriate sampling methods or by sampling multiple times while harvesting the plot.

Although many of the analytes show a normal distribution, this cannot be assumed. Thus, a statistical test that is relatively insensitive to such effects is best utilized. When comparing data, care must be taken to account for the distribution of the data.

3.2.3.3 Selection of appropriate comparator. One of the key considerations in applying the concept of substantial equivalence is the selection of an appropriate comparator. Should a new variety of maize be compared to genetically closely related (near isogenic) material or to the total population of the crop in the real world (that is, to a single variety of maize or to all maize varieties)? If a specific food or feed component is modified (for example, the fatty acid content of the oil), it may be more appropriate to compare the component to the composition of the oil from another crop or other source than to the oil from the crop that was modified. This method was used for canola with increased levels of lau-

Table 3-1—Carotenoid concentrations of canola seeds from selected lines transformed with phytoene synthase (*crtB*) gene (from Shewmaker and others 1999)

Sample ID	Generation segregation ratio & production site	Carotenoid concentration (μg gFW^{-1})					
		Lutein	Lycopene	α-Carotene	β-Carotene	Phytoene	Total
Q control	homo, GH	30	ND	ND	3	ND	33
Q3390-2	T2, 15:1, GH	50	6	372	721	192	1341
Q3390-9	T2, >63:1, GH	68	12	394	949	194	1617
Q3390-12	T2, 3:1, GH	48	10	400	739	171	1368
Q3390-15	T2, null, GH	27	ND	ND	1	ND	28
Q2290-18	T2, >63:1, GH	50	8	449	759	128	1394
Q3390-26	T2, 3:1, GH	34	9	311	584	149	1087
Q3390-37	T2, 3:1, GH	50	10	291	626	119	1096
Q3390-49	T2, 15:1, GH	28	2	346	677	146	1199
Q3390-12	T4, homo, GH	45	10	395	565	443	1458
Q3390-26	T4, homo, GH	30	9	234	672	393	1337
Q3390-26	T4, homo, field	57	14	279	379	344	1073
S control	homo, GH	31	ND	ND	5	ND	36
S3390-1	T3, homo, GH	52	2	440	669	430	1163
S3390-4	T3, homo, GH	44	17	282	637	239	1219
S3390-5	T3, hetero, GH	51	2	191	387	120	751
S3390-5	T3, homo, GH	46	4	256	633	220	1159
S3390-11	T3, homo, GH	54	10	406	556	427	1453
S3390-14	T3, homo, GH	66	13	431	674	263	1447
S3390-35	T3, hetero, GH	38	2	125	314	76	555
S3390-35	T3, homo, GH	44	5	234	504	169	956
S3390-1	T4, homo, GH	44	26	344	599	175	1188
S3390-1	T4, homo, field	72	25	225	401	332	1055

Abbreviations: FW, fresh weight; GH, greenhouse; ND, not detected. Seeds were randomly sampled in each generation.
Reprinted with permission from Shewmaker CK, Sheehy JA, Daley M, Colburn S, Yang Ke D. 1999. Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant J 20:401-12. Copyright 1999 Blackwell Publishing Ltd.

rate, in which the oil content was compared with tropical oils instead of with conventional canola oil.

Two approaches are in use. In the first approach, the package should include data from a genetically similar comparator grown alongside the GM crop as well as data on the range of composition from other varieties of that crop (data specifically generated or from the published literature). In some cases, the GM crop has also been compared to a number of commercial varieties. In practical terms, applicants wishing to register GM crops have carried out both comparisons. There are a number of limitations to this approach. The first is that, although a comparator may be considered near-isogenic, it is certainly the case that normal Mendelian genetics result in a large number of genetic loci potentially differing between the GM crop and the closest comparator. This is especially true where the comparator is not a line that has been specifically bred to be a comparator for the line being tested.

In the second approach, the data obtained from the GM crop are compared to the publicly available data. For maize, data are typically obtained from publications that have been compiled for the feed trade. These include Watson (1987), Ensminger and others (1990), various publications (for example, U.S./Canadian feed tables), and various private publications. While there is a wealth of information for maize grown in North America, the data may be limited for other geographic regions. The biggest concerns about these data are that the sources are often dated and lack association with specific analytical methods. Users therefore cannot compare their data directly with data obtained using the same quantitative methods.

To alleviate this problem, the ILSI International Food Biotechnology Committee (ILSI 2003) constructed a comprehensive up-to-date database on the composition of crops that is accessible via the internet (www.cropcomposition.org). By pooling data generated by the agricultural biotechnology industry, the scientific basis for comparison of composition data with the larger data set of each crop will be significantly improved. Public data that meet the acceptability criteria will be accepted added to the database, so that other publicly available data can be incorporated in a consistent manner from throughout the world. This robust database will further the understanding of the phenotypic diversity in composition of conventional crops and their products and will allow better evaluation of the composition of nutritionally improved GM crops and their products.

3.2.3.4 An example of comparative assessment. Considerable experience has been gained to date with the application of a comparative analysis of agronomic trait crops, and is beginning to be applied to nutritionally improved, GM crops. An example taken from a paper by Shewmaker and others (1999) provides an analysis of the fatty acid and carotenoid composition of a nutritionally improved GM variety of canola. Insertion of a bacterial phytoene synthase gene resulted in a 50-fold increase in the concentration of carotenoids and a substantial increase in oleic acid composition (Table 3-1 and 3-2).

3.2.4 Approaches to the evaluation of the safety and nutritional quality of foods and feeds

The recommended approach for the safety and nutritional evaluation of nutritionally improved foods and feeds follows concepts already successfully employed for the evaluation of products derived from GM crops with improved agronomic traits. As indicated previously, foods and feeds derived from GM crops with improved agronomic traits have not been reported to be significantly altered in terms of the concentrations of macro- and micronutrients and other inherent constituents, providing a high degree of confidence that the amount of food and feed from nutritionally improved GM crops will not present new safety issues. Hence the safety and nutritional assessment of these products can rely on

Table 3-2—Fatty acid composition of napin-*crB* lines[a] (from Shewmaker and others 1999)

Line	Location	Generation	Segregation ratio	16:0	18:0	18:1	18:2	18:3	20:0
S control	GH	N/A	N/A	5.1	1.7	59.9	17.1	12.0	0.6
S control	GH	N/A	N/A	4.7	1.6	62.7	15.3	11.3	0.6
S3390-1	GH	T2	3:1	4.6	2.7	70.1	12.4	6.7	1.0
S3390-25	GH	T2	>63:1	5.0	2.1	71.6	12.8	5.8	0.9
S3390-15	GH	T2	3:1	4.4	1.5	69.3	14.9	7.7	1.4
S3390-4	GH	T2	3:1	5.0	2.4	67.4	14.5	8.6	1.2
S3390-21	GH	T2	15:1	5.6	2.2	67.6	14.5	7.7	0.8
S3390-1	GH	T4	homo	4.0	2.1	75.9	9.8	5.0	0.9
S Control	Field	N/A	N/A	5.4	1.6	56.7	20.7	13.3	0.5
S Control	Field	N/A	N/A	5.3	1.6	56.4	20.9	13.5	0.5
S3390-1	Field	T4	homo	5.3	2.1	61.2	18.3	10.7	0.7
S3390-1	Field	T4	homo	5.1	2.6	64.3	15.8	9.9	0.7
Q Control	GH	N/A	N/A	3.8	1.8	59.8	21.2	10.1	0.7
Q3390-2	GH	T2	15:1	3.7	2.1	65.0	18.0	7.9	0.8
Q3390-12	GH	T2	3:1	4.0	2.4	62.9	18.8	8.7	0.9

[a]All values were determined on random pools of 50 seeds. Each value represents the relative fatty acid percentage (w/w) of total fatty acids.

historical practices employed to date.

The range of new nutritionally improved products derived from GM crops is potentially very diverse, including varieties with altered levels of amino acids (for example, high-lysine maize) and vitamins, reduced levels of antinutrients (for example, phytates), altered fatty acid composition, and the use of plants for the production of new ingredients that may not be native to the plant. In approaching the evaluation of the safety and nutritional value of such products, 2 key questions emerge. The first of these is how the product will be used. Is the product intended to be consumed as a whole food or feed replacing a traditional product, or is it intended that the product of the genetic modification will be separated from its plant production system and consumed as an ingredient? The approach to safety assessment will be different in these 2 cases. The second key question that emerges relates to the extent of consumption of the new nutritionally improved food or ingredient. This must be known or predictable in advance of performing a safety or nutritional evaluation.

Nutritionally improved foods or feeds derived from plants and intended for use as replacements for traditional products are best compared initially with their parental varieties. The initial approach is to apply the concept of substantial equivalence focusing on constituents other than the altered level of nutrients. Detailed analysis of major and minor constituents should be undertaken with a view to determining whether the intended genetic change has altered the concentration of inherent constituents other than the intended improvement in nutrient composition. If no significant changes are observed from compositional analysis, the safety and nutritional evaluation then focuses on the altered levels of nutrients arising from the genetic modification.

It should be established that, under the conditions of intended use of the new food or feed, there is no increased safety concern due to the altered level of nutrients compared to the traditional source. As noted above, a key dimension of this is determining the most likely exposure level for the altered nutrient(s). Safety can only be evaluated in the context of use patterns and exposure. For new crops that contain altered amounts of nutrients, the range of safe intakes can be established from the literature (NAS 2000a). For example, there are adequate data on amino acid or fatty acid toxicity to establish whether altered concentrations of these substances in a whole food/feed would present a safety concern. It can be concluded that, for the vast majority of new nutritionally improved GM varieties, the principal focus will be on enhancing nutrient composition or improving bioavailability or functionality of existing inherent constituents. Such compositional changes are unlikely to raise safety concerns because of the well-established role of nutrients in human and animal nutrition. The only residual issue of potential concern might be the presence of unintended changes in composition or metabolic pathways. Procedures for evaluating this possibility are presented in Chapter 6.

In cases where the nutrient is separated from its plant source with the intention to use it as an ingredient in foods or feeds, the use pattern and exposure again dictate the approach to the safety assessment. Information must be obtained on how the product will be used and the consumption that might be anticipated from its use. As indicated earlier, nutrients derived from nutritionally improved crops may be chemically identical to existing nutrients or they may be chemically altered to improve their functional or physiological properties. The use of these materials in food or feed will be subject to existing regulations, and chemically altered substances may require detailed safety assessment and regulatory approval prior to use.

3.2.4.1 Role of animal tests. Historically, toxicity tests in laboratory animals have played a significant role in ensuring the safety of chemicals present in foods, including food additives and contaminants that typically are consumed by humans in very small amounts. However, their value for assessing the safety of whole foods or major food constituents presents a number of difficulties, which are discussed below.

Before considering this matter, it is important to point out that, consistent with the concept of substantial equivalence, the safety assessment of foods derived from GM crops focuses on the examination of any differences between a suitable traditional variety and the new GM variety. This concept also holds in the conduct of animal tests where test groups are fed the food derived from the GM crop while the control group is fed a suitable comparator food. A key challenge for future consideration is the role of animal tests in the safety assessment of new GM varieties with significantly different nutrient composition from traditional varieties. In these cases, suitable comparator (control) varieties may not be available and existing study protocols may need revision to ensure the safety assessment is appropriate and adequate.

The difficulties encountered in assessing the safety of foods derived from GM crops in bioassays such as animal tests are well recognized (OECD 1993, 2002; LSRO 1998; FAO/WHO 2000). It has been pointed out on numerous occasions that animal feeding studies with whole foods or feeds must be designed and conducted with great care to avoid problems encountered with nutritional im-

Table 3-3—Toxicity studies performed with genetically modified food crops[a]

Crop	Trait	Species	Duration	Measurements	Reference
Cottonseed	Bt endotoxin (*Bacillus thuringiensis*)	Rat	28 d	Body weight Feed conversion Histopathology of organs Blood chemistry	Chen and others 1996
Maize	Cry9C endotoxin (*B. thuringiensis* var. tolworthi)	Human		Reactivity with sera from maize-allergic patients	EPA 2000
Maize	Cry9C endotoxin (*B. thuringiensis* var. tolworthi)	Rat, mouse	91 d	Body weight Blood chemistry Blood count Organ weights Histopathology of immune-related organs Serum IgE, IgG, and IgA levels	Teshima and others 2002
Potato	Lectin (*Galanthus nivalis*) Ewen and Pusztai 1999	Rat	10 d	Histopathology of intestines	
Potato	Cry1 endotoxin (*B. thuringiensis* var kurstaki HD1)	Mouse	14 d	Histopathology of intestines	Fares and El Sayed 1998
Potato	Glycinin (soybean [*Glycine max*])	Rat	28 d	Feed consumption Body weight Blood chemistry Blood count Organ weights Liver and kidney histopathology	Hashimoto and others 1999a,b
Rice	Glycinin (soybean [*Glycine max*])	Rat	28 d	Feed consumption Body weight Blood chemistry Blood count Organ weights Liver and kidney histopathology	Momma and others 2000
Rice[b]	Phosphinothricin acetyltransferase (*Streptomyces hygroscopicus*)	Mouse, rat	acute & 30 d	Feed consumption Body weight Median lethal dose Blood chemistry Organ weight Histopathology	Wang and others 2000
Soybean GTS 40-3-2	CP4 EPSPS (*Agrobacterium*)	Rat, mouse	105 d	Feed consumption Body weight Histopathology of intestines and immune system Serum IgE and IgE levels	Teshima and others 2000
Soybean GTS 40-3-2	CP4 EPSPS (*Agrobacterium*)	Human		Reactivity with sera from soybean-allergic patients	Burks and Fuchs 1995
Soybean GTS 40-3-2	CP4 EPSPS (*Agrobacterium*)	Rat	150 d	Blood chemistry Urine composition Hepatic enzyme activities	Tutel'yan and others 1999
Soybean	2S Albumin (Brazil nut [*Bertholetta excelsa*])	Human		Reactivity with sera from Brazil nut-allergic patients	Nordlee and others 1996
Tomato	Cry1Ab endotoxin (*B. thuringiensis* var. kurstaki)	Rat	91 d	Feed consumption Body weight Organ weights Blood chemistry Histopathology	Noteborn and others 1995
Tomato	Antisense polygalacturonase (tomato [*Lycopersicon esculentum*])	Rat	28 d	Feed consumption Body weight Organ weights Blood chemistry Histopathology	Hattan 1996

[a]Reproduced from Kuiper and others 2001; Table 4). Data from publicly available reports.
[b]Mutagenicity also tested.

balance from overfeeding a single whole food, which itself can lead to adverse effects. In undertaking such tests, a balance must be struck between feeding enough of the test material to have the possibility of detecting a true adverse effect and, on the other hand, not inducing nutritional imbalance. In any event, the multiples over anticipated human intake one would like to attain in animal tests are simply not achievable for practical reasons, and margins of safety of 1 to 3 times have to be accepted (WHO 1987; Hattan 1996; Munro and others 1996a). This limits the sensitivity of animal bioassays to detect small differences in composition, which may be more readily detected with thorough analytical characterization. These issues are discussed in more detail in Chapter 6.

Even though animal tests lack the sensitivity to detect minor changes in composition, in some instances, properly designed studies can confirm conclusions from other elements of the safety assessment and provide added assurance of safety. However, it must be recognized that the ability of rodent bioassays to detect adverse effects from an inherent constituent of a food derived from a GM crop depends upon the intrinsic toxicity of the constituent and whether it is present in the food in sufficient amounts to induce toxicity under conditions of a bioassay. In general, it is difficult to feed experimental animals more than 25 to 30% of the diet of a food product without creating nutritional imbalances, so the concentration of toxicant would have to be sufficiently high (or the toxicity so significant) in the food product portion of the rodent diet to produce toxicity. If it is not, the rodent bioassay simply will not detect the presence of the toxicant.

A review (Munro and others 1996b) of 120 rat bioassays (each of 90 d duration) of chemicals of diverse structure including food additives, pesticides, and industrial chemicals found lowest observed adverse effect levels (LOAEL) to range from 0.2 to 5000 mg/kg body weight with a median of 100 mg/kg and a 5th percentile of 2 mg/kg. To achieve the 5th percentile of exposure from a toxic constituent present in, say, a food crop in a rodent bioassay (at a food incorporation rate of 30%) the toxin would have to be present at a level of 80 ppm. To achieve the median exposure of 100 mg/kg it would have to be present at 5000 ppm. These concentrations fall well within the range of existing analytical techniques for detection of inherent toxicants in food. The concentrations should also be readily detected during compositional analysis of the known toxicants in the host organism used to generate the improved nutrition crop.

Box 3-1-Animal Feeding Studies – Nutritionally Improved Varieties

- Whole food testing: limitations (for example, dose levels, bulk of the material, palatability, and confounding factors).
- Ninety-day rodent toxicology study recommended where appropriate.
- Broiler chicken: commonly used, fast growing–useful for assessing nutritional quality, but not optimized for toxicity studies.

The broiler chicken has emerged as a useful animal model for assessing nutritional value of foods and feeds derived from GM crops. It should be noted, however, that, contrary to laboratory rodents, the rapidly growing broiler has been obtained through breeding efforts with the aim to create an efficient food-producing animal. This may, therefore, not render it optimal for toxicological testing of foods and feeds. In fact, disorders such as "sudden death syndrome" and "ascites," are considered related to metabolic disorders associated with its rapid growth (Olkowski and Classen 1995). On the other hand, broiler chickens have been optimized for growth relative to highly characterized diets such that small changes in nutrients or antinutrients in the diet are readily manifested in reduced growth. In addition, one of the first indications of an ill animal is loss of appetite or reduced growth rate. Also associated with the rapid growth of broiler chickens is the reduced fertility of overweight broilers allowed ad libitum access to feed (Robinson and others 1993). Live weight gain, efficiency of feed conversion, carcass weight, and breast muscle and fat pad weight are the traits usually measured in broiler feeding studies with feedstuffs from GM crops (Clark and Ipharraguerre 2001). Given the background of adverse symptoms related to the rapid growth of these animals, it seems that broiler chickens are not as useful for toxicological testing as are the common laboratory animals such as rats, mice, rabbits, and guinea pigs.

Examples of feeding studies with whole foods derived from GM crops with single inserted traits (improved insect protection or herbicide tolerance) are provided in Table 3-3 (updated from Kuiper and others 2001). Among the traits measured are body and organ weight, feed consumption and conversion, blood chemistry, serum IgE and IgG levels, urine composition, hepatic enzyme activities, and histopathology of organs and intestinal tissues. There are no indications from these experiments that unintended effects that affect animal health or productivity occur as a result of the genetic modification process, but one should realize that animal models have the limitations discussed above. All animal studies should be conducted according to internationally accepted protocols (for example, ILSI Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Input Traits 2003).

Whether the rat, broiler, or other species are selected as animal models, great care must be taken in formulating the diets to be administered. The key issues to be considered here are the formulation of diets with appropriate nutritional characterization and the avoidance of diets that are nutritionally unbalanced. A further issue is the selection of an appropriate control diet. Ideally, the control diet should be comprised of the foods or feeds selected for the analytical trials. Improved nutrition products derived from GM crops may differ considerably in nutrient composition from traditional varieties making direct comparisons difficult. It is also essential that the experiment is properly designed with an adequate number of replications to provide sufficient statistical power. Clearly, each new food or feed derived from a GM crop needs to be assessed on a case-by-case basis and it is not possible to formulate in advance any routine approach to animal safety testing. A summary of key issues for consideration in applying animal feeding studies to nutritionally improved varieties is presented in Box 3-1.

3.3 Conclusions

Nutritionally improved foods and feeds derived through biotechnology raise no new safety concerns. The approach to safety assessment is similar in many respects to the approach used for foods and feeds derived from GM crops with improved agronomic traits. This consists of detailed molecular characterization of genetic events and safety assessment of any expressed protein(s) or other products from the inserted DNA, coupled with extensive compositional analyses to ensure that the amounts of inherent constituents are not altered in comparison to an appropriate comparator or literature values, apart from the intended change in nutrient composition. The safety assessment of foods and feeds containing altered levels of nutrients will depend on the extent to which the food or feed is used in the human diet or in animal diets and existing knowledge concerning the safety of the nutrient in question. For many nutrients, safe upper intake levels have been established from the literature (NAS 2000a). In cases where the nutrient is separated from its plant source and used as an ingredient in foods or feeds, existing regulations would be expected to govern its safety assessment and use.

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Chapter 4: Nutritional Assessment Process for Nutritionally Improved Food Crops

4.1 Introduction

4.1.1 Background

Although the composition of plants can vary significantly from one variety to another of the same species, as a group, the great majority of new crop varieties do not differ significantly in composition from older varieties currently being cultivated. Plant breeders have focused much of their effort during the past decades on improving a variety of traits such as disease resistance and yield potential without changing composition. There are, however, a few examples of selected compositional changes that improve nutritional value, and many more are under development (Chapter 2). The recent introduction of modern molecular biotechnology, including genomics, marker assisted breeding, and recombinant DNA techniques, has enhanced the breeders' capacity to create and select for compositional changes.

Evaluation of the major compositional components of new varieties is a standard part of the breeders' evaluation process, particularly if changes in composition were an intended outcome. Potential changes in known micronutrient composition, beneficial phytochemical content, levels of antinutrients, and toxicant concentrations have been selected for and analyzed. These principles of food safety assessment developed for traditional crops have been extended to crops produced through the use of biotechnology, except that the analyses are much more extensive (Chapter 3).

The development of new crops with intentionally altered nutrient content adds one additional consideration to the premarket safety assessment described in Chapter 3. The potential impact of changes in nutrient composition on human or animal diet and health must also be evaluated to assure that the intake of essential nutrients is not compromised. The purpose of the assessment is to determine whether adverse effects on health could result from the intended compositional change. As will be discussed below, this kind of analysis has already been applied in several countries to crops with altered composition. The principles of the evaluation apply equally to all novel foods regardless of the methods used to develop them.

4.1.2 Estimating dietary intake of nutrients is complex

An important part of the safety assessment of nutritionally improved GM crops is the need to develop effective methods to evaluate the potential impact of such crops on the diet (OECD 2002). Such analyses are complex for a number of reasons, among which are: (1) the challenges associated with estimating dietary intake of specific foods, (2) the highly variable nature of the human diet, (3) the complex relationship between individual nutrients and human health, (4) the possibility of nutrient-nutrient interactions, and (5) the unique lifestyles and genetic makeup of individuals.

The nutritional impact of a few major plant-based commodities such as wheat, rice, potatoes, and maize is relatively easily assessed since they may comprise a large and relatively fixed proportion of the human diet for some populations (FAO 2002). The impact of a significant change in the content of a nutrient, for example β-carotene in "Golden Rice" or "Golden Mustard," can be reasonably simple to compute. Changes in foods that comprise a large percentage of the dietary intake also have good potential for significant nutritional impact. In many industrialized countries, the average consumer's diet can be derived from hundreds or even thousands of food products. Dietary intake patterns may also change with time. New products and new consumer preferences for these products may gradually change the overall diet. Reliable methods for establishing the current intake of nutrients of a population and estimating the range of changes in nutrient intake that might be caused by a nutritionally improved product are needed to assess its impact on nutrition and health.

4.1.3 Nutritional changes that may be introduced

A variety of distinct kinds of compositional changes have been described in Chapter 2. One or more of the following types of changes could be incorporated into nutritionally improved GM crops:

· Increases in the content of a single nutrient in a manner such that there is no significant impact on other components but there is an enhancement of nutritional value (for example, enhancing the lysine content of maize or enhancing the level of β-carotene of "Golden Rice" or "Golden Mustard").
· Increase in the content of a nutrient with a compensatory decrease in another (for example, increasing the protein content of a grain with a compensatory decrease in carbohydrate or lipid content, as occurred in Quality Protein Maize [QPM] [Vasal 1994; Córdova 2000]).
· Alteration in the composition of a nutrient with no concomitant change in overall macronutrient composition (for example, exchange of linolenic acid and linoleic acid by oleic acid to make a high-oleic acid vegetable oil).
· Changes in bioactive substances, such as an increase in an antioxidant, with expected benefits to health.
· Reductions in the content of an antinutrient, toxin, or allergen.
· Changes that affect bioavailability, absorption, or utilization of a nutrient (for example, low phytate or high phytase content in soybeans as a means to enhance phosphorus or trace mineral absorption).

This chapter is primarily focused on nutritionally improved crops in which the macro- and/or micronutrient content is altered. Changes in macro- or micronutrient content intended to improve quality traits can impact nutritional value as well. Increased starch content in potatoes (Fromm and others 1993) and elevated solids content in tomatoes (Roller and Harlander 1998) are examples of changes that improve nutritional value and impact functional qualities as well. High-oleic vegetable oils were first developed as stable frying oils, but were later recognized for their potential to influence dietary fatty acid intake (see Box 4-1). It is clear that alterations in composition that might change dietary intake of nutrients should be evaluated regardless of the intended purpose for which they were developed.

4.1.4 How the nutritionally improved food will be marketed is important

There are at least 2 primary ways in which nutritionally improved foods might be introduced into to the consumer's diet. This chapter assumes that foods and food ingredients that have enhanced nutrient content and/or bioavailability will be developed and marketed to consumers as higher value products that

identify the enhancement on the label. It would be required to state changes in nutrient composition on the label, and the developer would almost certainly want to market some perceived benefit of the product's consumption to consumers. It is important to point out that nutritionally improved food products that are described in labeling or advertising as having particular health benefits (for example, cholesterol reduction) will require the generation of data to substantiate these claims. These data are distinct from the data that are generated to demonstrate the safety and nutritional value of such products mentioned above (4.1.3), and are not the subject of this report.

The alternative pathway by which an enhanced nutrition product might reach consumers is as a food or food ingredient intended to replace a generic commodity. An example might be the introduction of higher levels of sulfur-containing amino acids into soybeans, or the increase in lysine and tryptophan concentrations in maize (see QPM below). It is conceivable that a thorough safety review would provide evidence that these enhanced commodities were in all ways as safe as their traditional counterparts, and that they provided improved essential amino acid intake. Thus, setting aside commercial and market considerations, one could conceive of approving the wholesale replacement of the traditional varieties of maize and soybeans with these new nutritionally improved varieties. Such wholesale replacement would be analogous to food fortification, and this possibility is in fact sometimes called biofortification. The same scientific logic that applies to national policies regarding nutrient fortification *could* apply to these novel commodities. From a scientific point of view, the process used to create the fortification is immaterial to the evaluation of safety and nutritional efficacy.

Box 4-1—Case study of the comparative approach applied to modified vegetable oils

High oleic acid sunflower is an example of a crop with a modified oil composition that was created through mutation breeding and which is widely cultivated. High-oleic acid sunflower oil became commercially available around 1985. Animal and human experiments with high-oleic acid sunflower oil are well documented, often in comparison with olive oil. Oleic acid levels are high in both oils: 76% in virgin olive oil and 77% in high-oleic sunflower oil (Bockisch 1998). Levels of other fatty acids, including stearic, linoleic, and linolenic acids are different between these two oils, however. Triacylglycerol substitution patterns also differ: for example, olive oil contains less triolein but more palmitoyl-dioleoyl-glycerol than high-oleic sunflower oil (Pacheco and others 2001). In addition, olive oil is a source of flavonoid antioxidants, whereas high-oleic acid sunflower oil is a source of vitamin E and sterols (Oubina and others 2001). It has been verified that the compositional equivalency of high-oleic acid sunflower oil and olive oil is a reasonably good indicator of the nutritional equivalence of the 2 oils. Differences have been noted, however, between the physiological effects of olive oil and high-oleic acid sunflower oil. These differences in general could be related to differences in the content of fatty acids other than oleic acid, differences in substitution patterns of the triacylglycerol molecules, and/or differences in the nature and levels of antioxidants (polyphenols in olive oil versus tocopherols and sterols in high-oleic acid sunflower oil).

Meta-analysis of data pooled from several clinical studies on the effect of dietary oils on the plasma level of cholesterol shows that the level of low-density lipoprotein cholesterol is higher for diets containing olive oil than for those containing high-oleic acid sunflower oil (Truswell and Choudhury 1998). Research reports of human and animal trials also indicate that olive oil and high-oleic acid sunflower oil have different effects on parameters related to, among others, cardiovascular health. Abia and others (2001), for example, observed in healthy subjects that plasma triacylglycerols increased less after meals if these meals contained olive oil instead of high-oleic acid sunflower oil. In healthy and hypertensive subjects consuming either olive oil or high-oleic acid sunflower oil, Ruiz-Gutierrez and others (1997) observed differences in composition and substitution patterns of triacylglycerols in very-low-density lipoproteins. Oubina and others (2001) found that serum levels of vitamin E, peroxides, and thromboxane were higher in normo- and hypercholesterolemic subjects who consumed high-oleic acid sunflower oil than in subjects consuming olive oil for 14 d. These findings demonstrate that 2 oils of seemingly comparable composition may give rise to differing physiological responses due to differences in their content of micronutrients.

4.2 Nutritionally Improved Foods

4.2.1 Enhancing the nutritional composition of plants

Quality Protein Maize (QPM) describes a family of maize varieties that contain higher levels of lysine and tryptophan than conventional maize (Vasal 1994). In addition, QPM has twice the protein content of conventional maize and is 10% more productive in the field relative to local varieties. Quality protein maize was developed to improve the protein nutrition of populations who consume significant quantities of maize in the diet and are at risk of protein malnutrition. Drs. Evangelina Villegas and Surinder Vasal were awarded the World Food Prize in 2000 (see www.worldfoodprize.org/Laureates/villegas.htm; www.worldfoodprize.org/Laureates/vasal.htm) for their 30-y efforts on the development of QPM. The initial development of QPM was the isolation of the high-lysine maize mutant *opaque*-2 (Mertz and others 1964; Cromwell and others 1967). High-lysine maize that would improve the essential amino acid balance of maize for use in animal feed has also been under development by plant breeders for over 40 y (Mertz and others 1964; Cromwell and others 1967).

Another compositionally altered product of conventional breeding that has found widespread commercial acceptance is canola (a rapeseed variety bred to be low in erucic acid and glucosinolates; Canola Council of Canada 2001). A variety of vegetable oils with improved nutritional and/or processing properties are described in Section 4.2.3.

4.2.2 The nutritional safety assessment of nutritionally improved crops

It is worth reiterating that the process described herein to evaluate the safety and nutritional value of nutritionally improved products derived via biotechnology is basically no different than the process used to evaluate the nutritionally improved products developed by any other technology (for example, traditional breeding, use of novel food ingredients). The analysis does not differ significantly in principle from that applied to new food ingredients. The sequence of steps in the analysis for such nutritionally modified foods is as follows:

· Estimation of magnitude of changes in nutrients (compositional analysis)
· Determination of the expected level of use in food products
· Estimation of frequency of use in products (foods in which the new variety or product is incorporated or for which it is substituted; generation of food lists)
· Estimation of anticipated distribution of dietary intakes by selected groups (intake ranges by age, gender, demographic, health status), also known as "exposure assessment"
· Assessment of the potential nutritional and health outcomes, taking into account nutrient content and bioavailability and the effects of processing.

The strategy for the evaluation of the nutritional impact for a crop variety that has altered nutrient composition can also be applied to the evaluation of changes in nonnutritive food components that are intended to provide health benefits. Specifically, plant breeders are developing crops with elevated levels of vitamin C, vitamin E, and β-carotene, nutrients with well-known safety and nutritional benefits. In addition, a number of nonnutritive

Table 4-1—Summary of data on toxicity and nutritional testing of high-oleic acid soybeans

Crop	Test	Duration	Traits	Reference
Soybeans	Composition		Crude composition, amino acids, fatty acids, vitamins, minerals, isoflavones, stachyose, raffinose, trypsin inhibitor, phytate, lectin	FSANZ 2001
Soybean meal	Pig feeding	17 d	Weight gain, feed conversion	FSANZ 2001
Soybean meal	Cow feeding		Performance, milk fat composition	CFIA 2001
Soybean meal	Poultry feeding	18 d	Weight gain, feed conversion	FSANZ 2001
Soybean extract	Reactivity toward sera from soybean allergic patients		Radio-allergo sorbent assay (RAST), inhibition-RAST, immunoblotting	FSANZ 2001
Soybeans	Estimation of impact of dietary intake		Intake of specific classes of fatty acids if shortenings and frying oil were to be completely substituted by high-oleic soybean oil	FSANZ 2001

phytochemical components, such as isoflavones, flavonoids, and saponins, are being considered for overexpression in plants. As noted in Chapter 2, there is some preliminary evidence for health protective or health beneficial roles for various phytochemicals. Products with enhanced phytochemical content are not a major focus of this report, because the consequences of increased dietary intake by humans are not fully elucidated. Thus, the evaluation of such products is complicated by the need to generate basic information about safety of high intake levels, as well as metabolism, interaction with other dietary components, and long-term physiological effects. The foregoing is not to be taken as recommending that a different or higher standard be applied to these products, but simply acknowledges that there is not as much evidence and experience with many of these products as is available for compounds for which a requirement in human nutrition has been established for some years. This issue points to the need to consider each unique example on a case-by-case basis.

4.2.3 Case study: Regulatory review of vegetable oils with altered fatty acid content

There are several examples of vegetable oils in which fatty acid compositions have been altered to produce nutritional and/or functionality improvements. It is instructive to review the safety assessment process undertaken for each product as a way to illustrate the process that could be established for other nutritionally improved products.

· High-oleic soybean oil was first developed by traditional plant breeding methods to improve the oxidative and thermal stability of soybean oil by replacement of polyunsaturated fatty acids with oleic acid (Broun and others 1999). Varieties have also been developed using biotechnology (Broun and others 1999). In these varieties, the formation of monounsaturated oleic acid is favored over the production of polyunsaturated fatty acids due to suppression of Δ-12 desaturase activity by the inserted DNA. Regulatory reviews have been completed for food use in USA, Canada, Australia-New Zealand, and Japan. High-oleic soybeans developed via biotechnology have been tested for their effects on performance of pigs, cattle, and poultry (Table 4-1). In addition, the allergenicity of the high-oleic soybeans has been compared to that of conventionally produced soybeans and shown to have a similar allergenicity profile.

· High-laurate canola has been produced by insertion of a fatty acyl thioesterase gene from California bay tree (Broun and others 1999). The thioesterase preferentially cleaves lauroyl groups, leading to an enrichment of lauric acid levels compared to conventional canola. This canola has completed regulatory review for food use in USA and Canada.

· Solin oil is a form of linseed oil isolated from a flax variety that produces reduced levels of linolenic acid, making it more suitable for certain processed food applications (Broun and others 1999). Solin oil was developed and approved in Canada and has been self-affirmed as "generally recognised as safe" (GRAS), and the United States Food and Drug Administration has not objected.

· High-oleic acid sunflower oil was commercialized in the mid 1980s(see Box 4-1).

The potential health consequences of the replacement of cooking and frying oils with high-oleic acid soybean oil were considered in the premarket safety review (Health Canada 2000; FSANZ 2001). It was noted that the oil was similar in composition to olive oil. The impact of substitution of the modified oil on overall dietary intake of fatty acids was calculated based on consumption data for British consumers (FSANZ 2001). Of particular interest was the estimation of the impact of this substitution on consumers at the highest extreme of consumption whose overall intake of fatty acids would be the most altered. It was concluded that a potential "worst case" decrease of 29% in the consumption of linoleic acid would have negligible effects on the incidence of cardiovascular disease and that the concomitant reduction in saturated fatty acid intake was likely to have a more significant beneficial effect on health. It also was concluded that the intake of monounsaturated fatty acids would increase, at the expense of saturated fatty acids and n-6 polyunsaturated fatty acids. Thus, the substitution in the diet of oil from soybeans modified to be high in oleic acid would have an effect on the diet comparable to that of substitution with olive oil. Some nutritionists have supported such a dietary change as a way to achieve the health benefits of a more "Mediterranean" diet (Simopoulos 2001).

For the high-laurate canola, no nutritional concerns arising from its replacement in the diet of other high-lauric acid oils such as coconut oil were raised. The levels of antinutrients were also similar to those found in conventional canola (Health Canada 1999).

In the GRAS affirmation of Solin oil, its dietary impact was calculated assuming it would completely substitute for sunflower oil (FDA 1998). In reviewing Solin, of which substantial amounts are consumed in the European Union, the British Advisory Committee on Novel Foods and Processes (ACNFP) expressed in its Annual Report a concern over the dietary impact of the use of modified oils with longer shelf life (ACNFP 1997):

Irrespective of whether food ingredients from this variety of linseed are considered to be novel or not, the ACNFP was concerned that the levels of α-linolenic acid had been reduced in the oil in order to improve shelf life. In reducing the levels of this fatty acid to prevent rancidity, the ratios of n-3 to n-6 fatty acids were dramatically changed in the oil. The ACNFP was concerned that altering the fatty acid ratio in this way may have long-term effects on public health. The Committee acknowledged that this problem was not unique and that there is a growing trend in altering the fatty acid composition of vegetable oils, through the use of traditional plant breeding techniques, in order to improve their shelf life but at the expense of nutritionally beneficial fatty acids. It was

agreed that the generic question of the desirability of changing the composition of fats and oils in this way should be referred to COMA (Section 4.3). [COMA is the Committee on Medical Aspects of Food and Nutrition Policy, a Food Safety Agency of the United Kingdom.]

It should be emphasized that the focus of the ACNFP was on the impact on diet and health of changes in oil composition rather than the process used in plant breeding (traditional plant breeding versus biotechnology).

It should also be noted that the Canadian authorities treat plants that have been modified through biotechnology and mutation breeding as "plants with novel traits"—they do not distinguish between the process of production of the new varieties. Conventionally bred crop varieties with modified oil compositions have, therefore, been subjected to the same approval procedure as those developed with the use of biotechnology in Canada.

Varieties of high-oleic acid canola and low-linolenic soybean produced through traditional breeding have been approved for food use in Canada. In these cases, toxicity testing was not considered necessary, because of the similarity of these oils to the oils from other conventional foods and the removal from the oil of proteins that might have caused concerns about their allergenicity (Health Canada 2000; 2001).

The foregoing example demonstrates the limitations of comparing clinical trials and animal experiments to real diets. In everyday life, it is not likely that sunflower oil will substitute 100% for olive oil, and a number of sources will contribute to total oil intake, thus such differences would likely not be observed. The comparison of olive oil and high-oleic sunflower oil presented in Box 4-1 demonstrates that an adequate nutritional assessment cannot focus solely on the change in composition of a single macrocomponent of a food. In the example cited, while the 2 oils compared are similar in oleic acid content, significant differences in fatty acid content, triglyceride structure, and the content of other constituents gave rise to differences in the physiological effect of these 2 oils in animal and human studies. In general, nutritional assessment should consider changes in content of all components of a food that are known to play a significant role in diet or health through consumption of that food. Which nutrients should be analyzed will depend on whether that food is a significant dietary source of that nutrient, thus the assessment must be made on a case-by-case basis.

4.3 Issues in Assessing the Impact of Changes in Nutritional Composition

4.3.1 Compositional analysis

Changes in nutritional content can be quantified by compositional analysis (see Chapters 3 and 6 for details). When a plant variety has been purposefully developed to contain altered levels of a specific nutrient or nutrients, the intended changes in those specific nutrients should be well documented. It is possible, however, that a significant compositional change in one nutrient might result in additional changes in composition. Therefore, an analysis of macro- and micronutrient composition should be performed either to document that no ancillary changes in nutrient content have occurred or to identify those changes. A list of analytes for a detailed nutrient evaluation is given below (OECD 2002):

· Proximate analysis (protein, lipid, carbohydrate, fiber, ash, moisture)
· Amino acids
· Fatty acids
· Fat-soluble vitamins
· Water-soluble vitamins
· Minerals for which a need in human nutrition has been established
· Known beneficial nonnutritive substances
· Known antinutrients
· Toxicants

4.3.2 Determining the nutritional significance of a change in nutrient composition

The analysis of the impact on dietary intake should include all nutrients for which a "significant" change in content has occurred. It will, therefore, be necessary to develop a consensus definition of "significant" in this context. As first defined in Chapter 3, the word "significant" as used here refers to a change in the dietary intake of a nutrient that meaningfully affects health, growth, or development, rather than simply some defined percentage change in composition of that nutrient in the new variety.

It has been suggested that if the introduction of a newly developed food or food ingredient has an effect on the total dietary intake of a specific nutrient that exceeds 15% of the recommended daily allowance (RDA) for that nutrient (IOM 1989), the health consequences should be evaluated (ILSI 1995). While it is tempt-

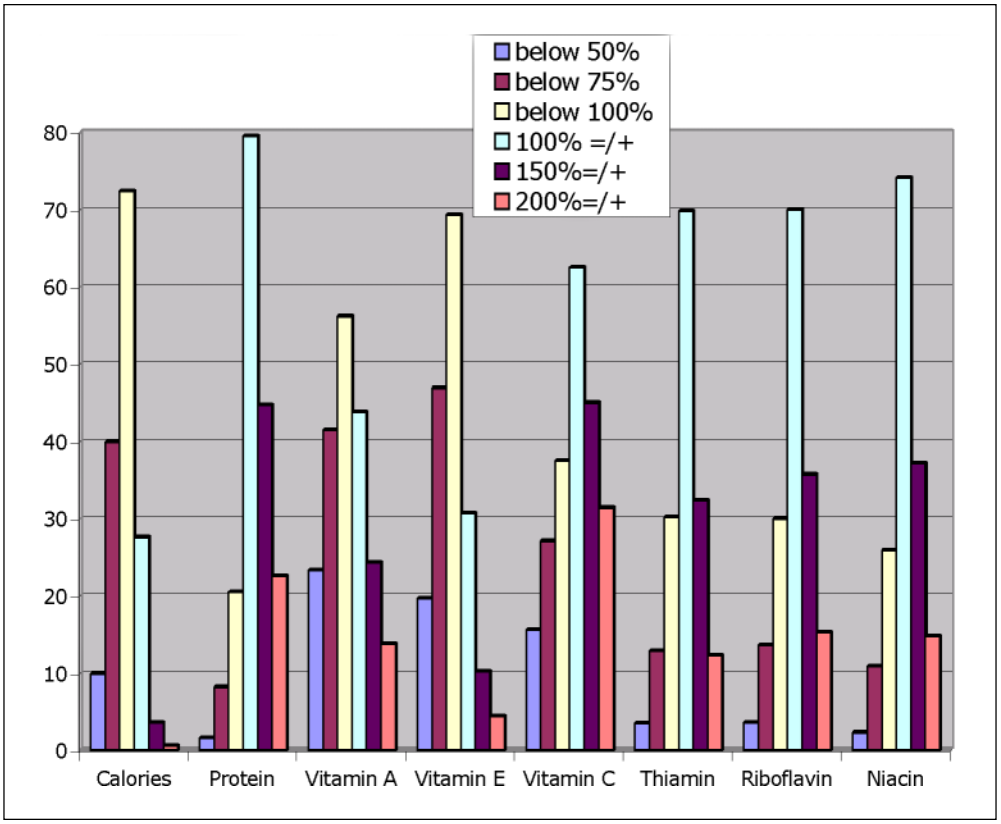

Figure 4-1—Distribution of calorie and nutrient intakes in the United States 1994–1996 (Data adapted from Table 14, USDA 2002b. Values represent percentage of the population who consume < 50% of the US RDA, < 75% of the US RDA, and so on)

Table 4-2—Examples of the composition of Bt-maize compared to literature and other database values

Nutrient	Bt-Maize % dry weight	Literature % dry weight	ILSI crop composition database % dry weight	USDA database converted to % dry weight	OECD consensus document	"Commercial" % dry weight	"Range" % dry weight
Protein	13.1	6.0–12.0	6.15–17.53	(10.34)	10.5	9.6–12.7	6–12.7
Fat	3.0	3.1–5.1	2.70–4.87	(3.74)	5.3	3.6–5.3	3.1–5.8
Fiber	2.6	2.0–5.5	1.82–11.34	(3.85)	N/A	3.7	3.0–4.3
Ash	1.6	1.1–3.9	0.62–6.28	(1.45)	1.34	1.28–1.5	1.1–3.9

Bt-Maize and literature values from Astwood and Fuchs (2001), data from the ILSI crop composition database (range and mean given), the USDA Nutrient Database SR-15 (n = 4–7), and the OECD consensus document (OECD 2002) data for commercial and literature (range) values. N/A indicates not available.

ing to set specific thresholds for action, it will be necessary to consider the acceptable limits of change in nutrient composition in each specific case. A simple rule of thumb such as 15% will probably not suffice because the concentration of any given nutrient varies between samples and dietary intakes vary significantly among individuals. In particular, dietary intakes vary widely across a population. A decrease in dietary content of a nutrient by 15% of the RDA may be inconsequential to well-nourished members of the population but would be detrimental to those whose diet is already borderline or low in that nutrient (see Figure 4-1 and 4-2). The impact of a change in composition is, therefore, a function of both consumption and nutritional status. It is conceivable that a threshold for action of 15% could be either too low or too high. It would be more appropriate to judge the impact of a change in nutrient concentration on a case-by-case level without setting some a priori action level such as 15%.

4.3.3 Determining the significance of a change in nutrient content of a food

It is challenging to determine a mean concentration value for a specific nutrient in a given food crop such as maize, wheat, or soybeans. Large variations in content of specific nutrients are commonly encountered in different samples of the same food (USDA 2002a; ILSI 2003). Different varieties of the same crop plant have been observed to have significant differences in composition. Representative compositional analyses of the same variety will also vary because of environmental effects such as geography, soil, climate, harvesting, and postharvest handling. In fact, several-fold variations in content of some less stable nutrients such as vitamin C are commonly encountered. The data in Table 4-2 present the composition of a specific variety of insect-protected maize and comparisons with the literature values for composition. The values for protein, fat, ash, and fiber varied over a more than 2-fold range.

Box 4-2—Average Nutrient Intakes in the United States

The mean intakes of essential nutrients in the United States as measured in the NHANES and CFS surveys appear to be on the whole quite adequate, with the consumption of most nutrients being above the recommended daily allowance (RDA, Table 4-3). Mean consumption numbers, however, do not reflect variability of individual consumption patterns. The data in Figure 4-1 and 4-2 demonstrate that more than 22% of the population consumes less than 50% of the RDA for vitamin A, and 20% consumes less than 50% of the RDA for vitamin E and calcium. In general, 10 to 20% of the population consumes less than 50% of the RDA for a given nutrient while 25 to 50% will consume greater than 200%. Five- to 10-fold differences in consumption are not uncommon.

4.3.4 Variability in the human diet

The highly variable nature of the human diet and the resulting variability in nutritional states must be considered in order to fully understand the nutritional adequacy of a food. Data on human dietary intake are usually gathered through carefully designed food intake surveys. Intake studies present a number of methodological challenges, not the least of which is the need to rely on self-reporting of intakes by subjects. The most useful studies survey large, demographically representative populations repeatedly over a number of years, while simultaneously tracking an array of environmental, social, and health parameters. Protocols have improved markedly in recent years. Nonetheless, reliable comprehensive dietary intake data are only available for a few countries such as the United States and the United Kingdom (WHO 2002).

The United Nations recently has placed emphasis on defining nutritional status and health around the globe, with particular attention to food insecurity and the definition of "at-risk" populations. The most critical nutrient deficiencies in regions and subpopulations of most countries are now fairly well documented. Insufficient energy intake is often accompanied by a shortfall in specific nutrients as well; in some cases, energy intake is adequate while undernutrition of specific nutrient(s) is prevalent. Worldwide, vitamin A, iron, iodine, and protein deficiencies dominate the list of nutrients for which numerous subpopulations are at risk for undernutrition (FAO 2002; WHO 2002).

4.3.5 Dietary intake surveys

In the United States, human dietary intake data are reported in the National Health and Nutrition Examination Survey (NHANES; CDC 2002) and Continuing Survey of Food Intakes by Individuals (CFSII; USDA 2002b) databases (Box 4-2). The National Nutrition Monitoring and Related Research Act of 1990 called for merging the 2 U.S. surveys into a single comprehensive food intake reporting system. The data reported in these surveys allow the estimation of intakes of thousands of individual foods, major food groups, and specific macro- and micronutrients. The range of intakes for a nutrient, food, or food group can be sorted by a variety of criteria including age, gender, health status, socio-economic status, geography, and ethnicity, in order to reveal group-related variations in estimated daily intakes. Analysis of the data allows the identification of populations who are at risk for undernutrition of specific nutrients (that is, folate in pregnancy, vitamin B_{12} in the elderly, or iron in vegetarians).

A major challenge to prediction of the potential health impact of a change in nutrient composition of a food is that consumption by the most critical groups—those who are at risk and/or who represent extremes of consumption—is often the most difficult to project accurately. Recent research has focused on the development of statistical modeling methods that will allow intakes by specific subpopulations to be projected accurately. The Monte Carlo simulation technique (in which values for uncertain vari-

ables are randomly generated over and over) is one such example. These models may be particularly useful in identifying extreme consumption patterns when coupled with behavioral characteristics. It is also becoming more clearly established that genetic and biochemical differences lead to variations in human response to diet. In the future, behavior and genetic typing as well as biomarker analysis may be used to predict both intakes and health outcomes. It may even be possible at some point to develop a recommended optimal list of nutrient intakes for each individual, as an aid to preventative medicine.

4.3.6 Single trait compared with total diet comparison

Assessment of the potential health impact of a change in a single nutrient is a prerequisite for evaluating the potential impact of the new variety on the adequacy of the diet, but it is not sufficient information. The role of folate in masking vitamin B_{12} deficiency is a classic example that illustrates the need to evaluate the diet rather than the intake of an individual nutrient. As noted previously, the health impact of all the changes in nutrient composition in an individual new variety should be assessed—if these changes are deemed "significant." A comprehensive analysis should include the change in composition multiplied by the percent each food containing the novel variety represents in the diet. Each of these contributions is then summed to determine the overall dietary intake. This process needs to be repeated for all nutrients so the potential health impact of a change in composition of a single nutrient can be evaluated in the context of intake of all nutrients.

Some novel foods will be altered with respect to specific components such as fatty acids, amino acids, or specific vitamins. If no collateral changes in nutrient composition are present in the variety, such changes can be viewed as a means to augment the intake of specific nutrients. The substitution of a monounsaturated fatty acid for a saturated fatty acid or an essential amino acid for a nonessential one might improve nutritional quality.

If it is proposed to substitute a nutritionally enhanced crop for a conventional crop as one means of biofortification for a specific nutrient, it should be shown that the change in composition results in an improvement in nutritional status of the population for that nutrient. As with other fortified foods, on a case-by-case basis, digestibility and absorption studies may be appropriate to demonstrate that the enhanced nutrient is bioavailable. Any potential antagonisms, for example, competition in amino acid uptake, should also be assessed. Finally, it should be demonstrated that the increased intake of the novel food does not simply replace another source of the same nutrient or partially displace another nutrient from the diet.

Table 4-3—Mean intake of calories and nutrients in the United States 1994–1996 (CDC 2002)

Nutrient	Mean intake as % of RDA
Calories	88
Protein	161
Vitamin A	121
Vitamin E	94
Vitamin C	179
Thiamin	137
Riboflavin	143
Niacin	144
Vitamin B_6	106
Folic acid	167
Vitamin B_{12}	276
Calcium	92
Phosphorus	140
Magnesium	101
Iron	137
Zinc	86

Recommended dietary allowances and dietary reference intakes from IOM (1989; 2002).

4.4 Hypothetical Case Study: Soybean Oil with Enhanced Levels of α-Tocopherol

It is possible to genetically modify plants to express elevated concentrations of α-tocopherol, the nutritionally relevant form of vitamin E (DellaPenna 2001). As a hypothetical case study, assume that such a modification could be made in soybeans such that the α-tocopherol content of 1 tablespoon (14 g) of the oil increased by 2 mg. The questions that need to be answered regarding the safety and nutritional value of such a product are as follows:

- Scope of compositional change
  - · What is the composition of the new soybean oil?
  - · How does this compare to regular soybean oil?
  - · Has the genetic transformation caused any increases or decreases in components other than α-tocopherol?
  - · Have any new components, such as intermediates in the α-tocopherol synthetic pathway, appeared, decreased, or increased in the oil?
  - · Have any usual components of the oil decreased?
  - · How does the new level compare to levels of addition to oils permitted for vitamin E either as nutrients or as antioxidants?
- Estimation of human dietary intake

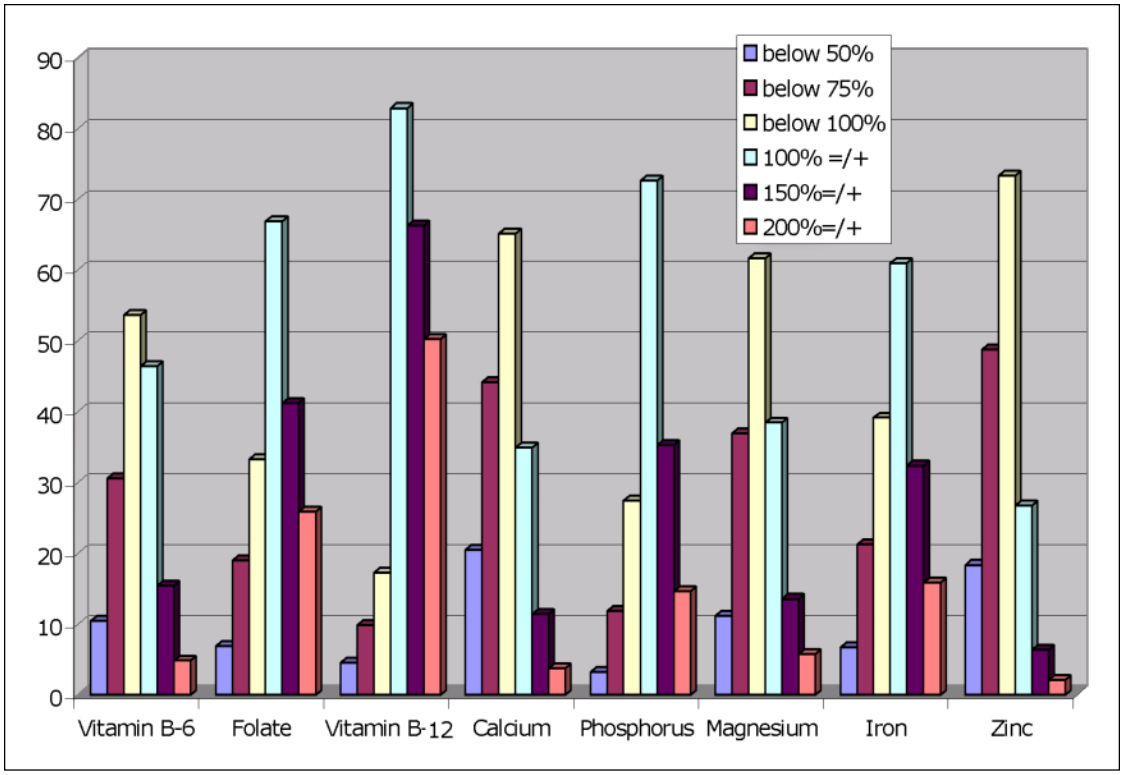

Figure 4-2—Distribution of vitamin and mineral intakes in the United States 1994–1996 (Data adapted from Table 14, USDA 2002b. Values represent percentage of the population who consume < 50% of the US RDA, < 75% of the US RDA, and so on)

· What would be the expected dietary intake of the new oil for various subgroups of the population?
· What impact would substitution of high α-tocopherol soybean oil for traditional soybean oil have on the α-tocopherol content of the diet?
· If the transformation caused increases or decreases in other nutrients or components of the oil, what effect would these changes have on the nutritional content of the diet?
· How does this increase in α-tocopherol level in a reasonable daily intake of oil compare to recommended intakes of vitamin E?
• Impact on human nutrition and health
· Do any of these changes raise questions of safety?
· Is the increased nutrient bioavailable and absorbed?
· Are there any potential antagonisms to absorption of other nutrients?
· Would there be there any significant decreases in dietary adequacy for any nutrient because of this change, especially for special sensitive population groups, such as infants, children, the elderly, and pregnant and nursing women?
· If the novel food were proposed as a means of biofortification, would there be a significant increase in dietary adequacy for the population (or for at-risk subpopulations)?

4.5 Conclusions and Recommendations

Nutritionally improved GM foods have already been introduced into the marketplace after completion of premarket safety assessments in a number of countries. The paradigm previously applied to evaluation of novel foods produced through conventional breeding appears applicable to those produced through the application of biotechnology. The availability of comprehensive and robust composition and dietary intake databases greatly facilitates evaluation of nutritionally improved foods. In the case of nutritionally improved foods or ingredients introduced into the marketplace as novel products, it is assumed that regulations will require changes in nutritional content to be identified on the food product label. Historically, nutritionally improved foods have been subjected to premarket safety assessment but have not been required to demonstrate efficacy beyond a statement of the nature of the compositional change. No safety or health-based rationale for changing this paradigm was identified. Developers wishing to make marketing claims regarding the potential health benefits of a nutritionally improved product may wish to develop additional scientific support for the claim; in some jurisdictions such evidence may be required to support advertising claims and to substantiate bioavailability.

Recommendation 4-1. All nutritionally improved novel foods should be evaluated for the potential impact on nutrition and health regardless of the technology used to develop them. It is important to shift the primary focus from the composition of the individual foods or ingredients to the composition of the diets of individuals.

Recommendation 4-2. Alterations in composition that change dietary intake should be evaluated regardless of the intended purpose for which the food or food ingredient was developed.

Recommendation 4-3. Premarket assessment should be required to demonstrate that the introduction of a novel food will not significantly and adversely change nutrient intake for a large cross-section of consumers.

Recommendation 4-4. Human dietary intake data and dietary intake forecast models should be developed for all target and at risk populations.

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Chapter 5: Nutritional Assessment of Animal Feeds Developed through the Application of Modern Biotechnology

5.1 Scope

This chapter starts by outlining the main feed resources used in animal production systems and the extent to which feed ingredients from GM crops modified for agronomic traits have become an integral part of both nonruminant and ruminant livestock production systems. It continues by highlighting the development of nutritionally improved feed ingredients derived from modern biotechnology and describes the role of compositional analyses in the nutritional assessment of these ingredients. The role of livestock feeding studies in establishing nutritional equivalence for GM crops with an improved agronomic trait is described, and the need for livestock feeding studies to evaluate GM crops with improved nutritional characteristics is addressed. Attention is drawn to the recent OECD (2003) publication on the safety assessment for animal feedstuffs derived from genetically modified plants and the ILSI publication, Best Practices for the Conduct of Animal Studies to Evaluate Crops Genetically Modified for Input Traits (2003).

5.2 Feed Sources Used in Animal Production Systems

5.2.1 Background

In most nonruminant (for example, poultry and pigs) and ruminant (for example, beef and dairy) diets, the main dietary energy and protein components are usually cereal grains, such as maize and wheat, and oilseed meals, such as soybean, canola, and cottonseed meal. While cereal grain is a specific plant component, oilseed meal is a coproduct left after oil extraction. Both cereal grains and oil seed meals are processed before being incorporated into livestock diets. Cereal grains are usually subjected to a physical process that cracks or grinds the grain to enhance nutrient digestibility. Oilseeds are subjected to either a physical or chemical process that involves heat and pressure to extract oils from the seed, which leaves a protein rich coproduct. The remaining residue is also treated with heat/water vapor to destroy antinutrients such as trypsin inhibitors.

In addition, ruminant diets contain varying quantities of either fresh (for example, grazed grass) or conserved (for example, hay or silage) forage. Whereas hay is preserved through air-drying, silage is the result of the anaerobic fermentation of fresh forage in which organic acids are produced giving rise to a conserved feed with a low pH.

The proportion of forage in ruminant diets will depend on a number of factors including class of livestock (beef or dairy) and system of production (extensive or intensive) and can range from 50 to 100% of total dietary dry matter (DM). For example, in the case of high-yielding dairy cows, 50% of their diet may consist of forage, with the remainder containing feed ingredients such as, but not exclusively, cereals and oilseed meals.

Box 5-1-Use of GM crops with agronomic traits in livestock production systems
The largest use made of these crops in livestock production is from oilseed meals. It is estimated that over 150 million t of soybeans were produced in 2002 and that approximately 50% of the global area was planted to GM varieties, therefore, approximately 35 million t of GM soybean meal was used by the livestock industry (Soy Stats 2001). In addition, very significant quantities of maize grain, canola meal, cottonseed meal, and maize silage have been incorporated into livestock diets (FAO 2002)

5.2.2 Conventional crops

While many different crops and coproducts are used in livestock production systems, only a limited number of crops provide the main feed resources. Although on a global basis grass is the most widely used forage resource, maize silage is used extensively and is considered one of the best high-energy feed resources available for ruminants. In developing countries, crop residues such as maize stover (leaf and stem) and rice straw are important feed for ruminant livestock.

Of the 600 million tonnes (t) of maize grain produced/year (FAO 2002) about 450 million t are used in livestock feed and more than 60 million t are traded globally. Maize grain is often the preferred energy source for livestock production systems, with approximately 75% of total production being used in nonruminant diets and the remainder for ruminants. Soybean dominates world oilseed production (180 million t/year) and is often the preferred protein supplement for livestock production with 97% of the meal being used as feed for both nonruminants and ruminants (FAO 2002). There is limited consumption of unprocessed beans due to the presence of antinutritional factors such as trypsin inhibitors and lectins (OECD 2001b). Thus 2 crops, maize and soybean, both of which have been genetically modified to have improved agronomic traits, are used extensively as feed in nonruminant and ruminant diets.

After oil extraction from other crops such as canola and cotton, their residues also provide valuable protein supplements for inclusion in livestock diets. Approximately 20 million t of canola meal are used and 12 million t of cottonseed meal are fed annually to ruminants (FAO 2002).

5.2.3 Crops genetically modified for agronomic input traits

The principal GM crops are soybean (41.4 million ha), maize (15.5 million ha), cotton (7.2 million ha), and canola (3.6 million ha) (James 2003). With few exceptions, these crops have been modified for herbicide tolerance and/or insect protection. These crops are all used in livestock production diets as either energy and/or protein feed resources (see Box 5-1). They are included either in the form of whole crop (for example, maize silage), as a specific crop component (for example, maize grain), or as coproducts (for example, oilseed meals or maize stover).

5.3 The Development of GM Crops with Improved Nutritional Characteristics

5.3.1 Background

Several crops with genetic modifications aimed at improving nutritional characteristics have been produced and are currently in trials (see Chapter 2). While GM crops with agronomic traits are generally the result of the insertion of a single gene, GM crops

with improved nutritional characteristics may require the insertion of more than one gene to achieve increasingly complex modifications to plant metabolic and physiological pathways. An example of this increasing complexity is the case of "Golden Rice" that requires the insertion of three genes and is described in detail in Chapter 2.

5.3.2 Some potential modifications

Chapter 2 and the review by Kleter and others (2001) describe the wide range of nutritionally improved GM crops that are under development. The review noted that vegetable oil accounts for 15 to 20% of total energy intake in industrialized countries and, as knowledge improves as to their related health effects, more emphasis is being placed on the quality of oils and fats in the human diet. Thus, the degree of saturation of fats, fatty acid composition, and geometric configuration of individual fatty acids are all targets for genetic modification. These modifications are being implemented in a number of crops including, but not limited to, soybean, maize, canola, cotton, and sunflower (Kleter and others 2001).

Although the oils extracted from these crops are generally destined for human consumption, the coproducts will be available for use in animal production systems (see Box 5-2). These coproducts will need to be subjected to a nutritional assessment as a livestock feed. In addition, coproducts may also arise from crops modified to produce specific products for industrial processes. These coproducts may require additional tests, but this topic is outside the scope of this document.

Box 5-2-Examples of nutritionally improved GM crops that may be used as animal feed
Cereal grains in which the fatty acid and/or amino acid profiles have been improved.
·Legume seeds from crops modified for improved protein and/or amino acid profile.
·Crops modified for improved enzyme, mineral, and vitamin composition.
·Coproducts available after oil extraction from crops modified for improved oil profile either for human consumption or industrial use.
·Coproducts available after oil extraction from crops modified for improved protein content and/or amino acid profile.

Plants are an important protein source for both humans and animals. While cereal crops such as maize, rice, and wheat contain between 7 and 14% protein, legume crops can contain up to 50% protein. However, most plant proteins are deficient in at least one essential amino acid, with cereal proteins being generally low in lysine content and legumes being low in sulfur-containing amino acids such as methionine and cysteine. Supplementation of specific amino acids is often needed to avoid deficiencies in nonruminant diets. Modern biotechnology is aiming to improve not only protein content but also the amino acid profile of a range of crops.

Phosphorus is a major pollutant in animal feces, much of it resulting from the fact that phosphate is stored by plants as phytate, a stable sugar-phosphate that is not readily digested by nonruminant animals. While phytase enzyme can be incorporated into diets to enhance phytate digestion and phosphorus availability, crops have been genetically modified either for increased phytase activity or for decreased phytate production. Both approaches should improve phosphorus utilization in the diet, reduce the need for phosphorus supplementation, decrease phosphorus excretion, and reduce the burden of phosphorus pollution.

Although forages can provide more than 50% of ruminant diets, only limited work has been conducted to date to produce GM forages with improved nutritional characteristics. Attention should be drawn to the large scope for improving fiber digestion and also the huge impact that this would have on ruminant production systems in the developing world where forage quality is very low, and, as a consequence, animal performance is also low. The agronomic evaluation of such crops is critical because reduced fiber content may lead to lodging and reduced crop yield. It would seem likely that, as in the case of GM crops with agronomic traits, those with improved nutritional characteristics or their coproducts will be used extensively in animal production systems and as such will need to be evaluated in terms of both a food and feed resource.

5.4 The Role of Compositional Analyses in the Nutritional Assessment of Animal Feeds

5.4.1 Background

For at least 50 y, plant breeders and geneticists have used crop yield and nutritional composition as 2 of their main selection criteria when developing new varieties. While significant increases in crop yield have been achieved, there are few examples of significant nutritional composition changes effected through conventional breeding. Indeed, the search for higher crop yields has, in some circumstances, resulted in a reduction in nutritional composition. For example, increased grain yield is usually achieved through increased starch content, which is inversely related to protein content. Thus, increased grain yield often occurs at the expense of protein content (Bletsos and Goulas 1999). Conversely, the search for improved crop quality through conventional breeding programs has often led to a reduced crop yield. For example, Miševic and Alexander (1989) noted that, while selection was effective in increasing oil concentration in maize grain, it was associated with a significant reduction in grain yield. It is important to note that modern biotechnology may break this inverse relationship between yield and nutritional quality.

Nevertheless, compositional analysis of feeds has played an important role in the nutritional assessment of conventionally bred varieties. It should be noted that there are significant differences in composition of conventionally bred varieties within crops, and therefore the compositional analysis of GM crops must be assessed against the background of natural variability in the conventional counterpart. Attention is drawn to the ILSI (2003) crop composition database (www.cropcomposition.org) as a key source for such data. This issue is discussed in more detail in Chapter 3.

The compositional analyses conducted should provide information on macronutrients, micronutrients, antinutritive factors, and naturally occurring toxins in the feed under evaluation. Macronutrients consist of starch, crude protein, fatty acids, amino acids, ash, and structural carbohydrate components, such as neutral-detergent fiber and acid-detergent fiber. The micronutrients assessed in most compositional analyses consist primarily of key minerals and vitamins. Examples of antinutritive factors and naturally occurring toxins are trypsin inhibitors and gossypol, which are present in soybeans/soybean meal and cottonseeds/cottonseed meal, respectively.

The data produced from such analyses form the basis of the compositional feed tables that have played a major role in the formulation of diets used for both ruminants and nonruminants. In addition, these data have been used to predict the energy value of the feed, an important nutritional assessment because it is a key element in diet formulation. Clearly, the more complete the analyses, the greater the chance of providing a balanced diet for the animals being fed. For example, although the crude protein content may appear to be adequate, knowledge of the amino acid composition is necessary to predict the adequacy of essential amino acids.

However, compositional analyses provide only a guideline to

the nutritional value of feeds; they cannot provide information on nutrient availability. In many cases, this is an important issue and in vivo studies are required to determine the bioavailability of nutrients. In addition, livestock feeding studies with target species are sometimes conducted to establish the effect of the new feed resource on animal performance with endpoint measurements such as feed intake, level of animal performance, feed conversion efficiency, animal health and welfare, efficacy, and acceptability of the new feed ingredient. The extent and type of livestock feeding studies conducted will depend on the type of feed resource developed, and their need should be determined on a case-by-case basis. This topic will be discussed in detail later in this chapter.

5.4.2 The concept of substantial equivalence

The concept of substantial equivalence has been reviewed by a number of workers including Aumaitre and others (2002), Chesson (2001), Kuiper and others (2001), and Cockburn (2002) and has been discussed in Chapter 3.

The concept is applicable to both food and feed ingredients and is based on the principle that existing crops can form the basis for comparing the properties of a GM crop with an appropriate counterpart considered safe, as shown by a long history of safe use. Application of this concept is not a safety assessment per se but helps to identify similarities and differences between conventional and GM crops, with any major differences warranting follow-up safety assessment (see Chapter 3). Agronomic and phenotypic characteristics and compositional analysis of key nutritional components are critical elements in the application of substantial equivalence to a new GM crop in order to identify differences that are then subject to further investigation. The concept of substantial equivalence is discussed further in Chapter 3.

5.4.3 Crops modified for agronomic traits

Currently, more than 50 crops, modified mainly with agronomic traits, have been assessed. Agronomic, phenotypic, and compositional analyses have typically been conducted on both GM crops and their conventional counterpart grown under the same field conditions in the same year (see an example in Box 5-3).

If differences are noted during these analyses, then further assessments are needed to assess the safety and nutritional impact of significant, biologically meaningful differences. Follow-up studies may also be needed and could include either further analytical procedures, such as described in Chapter 6, and/or livestock feeding studies, which will be discussed later in this chapter. The need for this additional work should be assessed on a case-by-case basis.

However, even when statistically significant differences in compositional analyses are noted between the GM crop and its closest conventional comparator crop, these differences should be assessed carefully, because on their own they may not indicate the presence of an unintended effect. For example, the differences may fall within the natural and often wide variation that exists between currently available commercial varieties. This emphasizes the importance of comparing the GM crop to both its near isogenic parental line and also to a number of commercially relevant and diverse varieties. The range in composition within crop varieties is well illustrated in the OECD consensus documents (OECD 2001a,b; 2002a,b,c) and the ILSI (2003) crop composition database (www.cropcomposition.org).

Box 5-3-Example of data produced comparing a GM crop with a conventional crop

Sidhu and others (2000) and Ridley and others (2002) provide an excellent example of the compositional analyses conducted when comparing the grain and forage component of maize modified for an agronomic trait with its near isogenic counterpart and a number of commercially grown varieties. Compositional equivalence was clearly demonstrated. Once compositional equivalence, which is a cornerstone in nutritional assessment, has been demonstrated, work then focuses, if necessary, on livestock feeding studies to confirm nutritional equivalence (see Appendix 5-1) and on assessing the safety of any newly expressed components (proteins or nutrients).

5.4.4 Crops modified for improved nutrition

An OECD (2002d) workshop on the nutritional assessment of novel foods and feeds, concluded that the comparative safety assessment process known as the concept of substantial equivalence, which includes testing for agronomic, phenotypic, and compositional assessment, provides at present the most appropriate scientific strategy for assessing the safety and nutritional qualities of foods and feeds derived from both agronomic and improved nutrition GM crops. The workshop emphasized that substantial equivalence identifies similarities and differences between the GM crops and an appropriate counterpart and that the safety and nutritional impact of any meaningful differences should be rigorously explored. This is particularly relevant for GM crops modified for improved nutrition in which the metabolic and physiological pathways are altered, and the alterations may have resulted in unexpected effects on plant composition.

The OECD has taken the lead in producing consensus documents in which compositional analyses are proposed for new varieties of a range of crops (OECD 2001a,b; 2002a,b,c). The Royal Society (2002) welcomed this initiative so that substantial equivalence could be applied uniformly, but emphasized that there was no evidence to suggest that the GM crops currently commercialized are unsafe.

The OECD consensus documents, although not specifically produced for improved nutrition GM crops, state that data from a new event should be compared to those obtained from the parental variety, but the documents state that a developer can also compare values obtained from new varieties with the literature values presented in the consensus documents.

The primary characteristic of GM crops with improved nutritional qualities is that there are intentional differences between them and the nearest genetic counterpart. However, unintended changes may also have occurred and would need to be assessed. The role of livestock feeding studies in the nutritional assessment of improved nutrition feeds derived from GM crops is described in a subsequent section of this chapter.

The work reported by Flachowsky and Aulrich (2001) and the consensus documents prepared by OECD (2001b; 2002c) on the compositional analyses proposed for new varieties of soybean and maize provide excellent guidance for the analyses needed as part of the nutritional assessment of GM crops modified for agronomic traits and improved nutritional characteristics.

A number of recommendations for the use of compositional analysis in the nutritional assessment of feed sources, either derived from conventional breeding techniques or from agronomic or improved nutrition GM crops, are as follows:

- Proximate analyses should be conducted on whole crop, crop components, or coproducts of all new varieties, taking into account their intended use. The specific analyses should be determined on a case-by-case basis.
- When a crop, crop component, or coproduct is a primary source of key macronutrients (for example, amino acids and/or fatty acids), it should be analyzed for those nutrients.
- The analyses of key micronutrients should be decided on a case-by-case basis. Analyses of major minerals are recommended, especially in crops that are known to have an inherently low mineral content. Relevant enzyme (for example, phytase) and vita-

min profiles need only be determined in crops that have been modified for this trait.
• All new varieties should be screened on a case-by-case basis for key antinutritional factors and key secondary metabolites associated with that crop.

5.5 The Role of Livestock Feeding Studies in the Nutritional Assessment of Feed Resources

5.5.1 Background

While compositional analyses of new varieties provide an excellent starting point in their nutritional assessment, they should be considered only as part of the overall nutritional assessment, as they provide limited information on the bioavailability of nutrients. Such information is obtained from small-scale in vivo studies carried out using target livestock species, because nutrient digestion can vary markedly between livestock species. An excellent example of this progression in the nutritional assessment of a feed resource is the development by conventional breeding of Brown Midrib mutant varieties of maize (Oba and Allen 2000). The compositional analyses indicated that fiber content and composition of Brown Midrib varieties were changed when compared with conventional varieties, which suggested that fiber digestibility might be improved. However, it required in vivo digestion studies with ruminant livestock to establish that fiber digestibility in these new varieties had, in fact, been significantly improved. Subsequently, large-scale and longer-term livestock feeding studies with dairy cows established the benefit of improved fiber digestion, which was associated with increased rate of passage and/or increased feed intake, both of which led to increased nutrient intake and resulted in enhanced milk production (Oba and Allen 2000).

5.5.2 Crops modified for agronomic traits

Numerous livestock feeding studies, which varied in length depending on the target species, were conducted to confirm nutritional equivalence of GM crops modified for agronomic traits. The crops were fed directly to target species and formed a very significant part of the total dietary intake. Measurement of feed intake; nutrient digestion; the level of production of milk, meat, and eggs; and the health and welfare of the animal were recorded and compared with measurements obtained from diets based on conventional feed ingredients.

An earlier section of this chapter noted that the compositional equivalence and bioavailability of nutrients of agronomic trait GM crops were within the range of conventional varieties. Thus, it is perhaps not surprising that, in the numerous feeding studies conducted, there has been no evidence to suggest that the performance of animals fed GM feed differed in any respect from those fed their conventional counterpart (Clark and Ipharraguerre 2001; Flachowsky and Aulrich 2001). These researchers concluded that routine longer-term livestock feeding studies with target species generally added little to a nutritional assessment, and they suggested that if compositional equivalence was established, nutritional equivalence for target species can be assumed. These studies generally spanned either the finishing period to slaughter for chickens, pigs, and beef cattle or a major part of a lactation cycle for dairy cattle.

5.5.3 Crops modified for improved nutrition

The need for short-term (42 d) growth studies with young rapidly growing livestock, such as broiler chicks, to provide further meaningful information on the possible presence of unintended effects in nutritionally improved GM crops is discussed in detail in Chapter 3. It is concluded that they are not an effective means of detecting unintended effects, as they lacked adequate sensitivity to detect minor changes in composition.

While the use of such fast growing, nutritionally sensitive livestock has also been proposed as a useful model for the initial screening of expected nutritional benefits, it is suggested that studies to demonstrate nutritional properties that might be expected from the modified crop, crop component, or coproduct, livestock feeding studies should be conducted with the relevant target livestock species. The need for such studies should be determined on a case-by-case basis and should be conducted according to internationally accepted protocols.

The exact experimental and statistical design will depend on a number of factors and will include animal species used in the study, the trait(s) being assessed, and the size of expected effect, which will in turn affect, for example, the number of animals per treatment group. Endpoint measurements would include feed intake, animal performance, bioavailability of nutrients, environmental impact, and animal health and welfare.

When studies are conducted, the following guidelines are proposed:
• In the case of crops that have been modified for improved bioavailability of nutrients (for example, low-phytate maize with improved phosphorus availability or increased nitrogen digestibility), studies to determine the bioavailability of individual nutrients in the GM crop and a range of conventional varieties should be conducted according to standard methodology.
• In the case of GM crops specifically modified with traits to enhance animal performance through increased nutrient density (for example, increased oil content or fiber digestibility) or an enhanced level of a specific macronutrient (for example, lysine, methionine, cysteine), an appropriate control diet using its nearest genetic counterpart should be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM crop. It is also suggested that a number of other commercially relevant varieties should be included in the study.
• In the case of coproducts (for example, oilseeds meals) from which the modified ingredient has been extracted, these can be compared with those derived from an appropriate counterpart and other commercial varieties on the basis that they are essentially free from the modified component.

5.6 Conclusions and Recommendations

The development of GM crops with improved nutritional characteristics is in progress. Some of these crops that are designed specifically for use in livestock feeding systems are enhanced for macro- or micronutrient content or improved bioavailability of nutrients, whilst others are modified to produce food ingredients for human consumption. The latter modifications, from which the modified ingredient is extracted, result in coproducts being available as a feed resource.

The consensus documents prepared by OECD (2001a,b; 2002a,b,c) on the compositional analyses required for new varieties of a range of crops provide excellent guidance for the analyses needed as the initial part of the nutritional assessment.

Recommendation 5-1. Compositional analysis is the starting point and cornerstone for the nutritional assessment of any new nutritionally improved crop variety. The analyses required should be determined on a case-by-case basis and may vary depending on the introduced trait.

Recommendation 5-2. Feeding studies in laboratory animals and livestock species are unlikely to contribute to the detection of unintended effects in a new crop as they lack adequate sensitivity.

Recommendation 5-3. In the case of GM crops with improved nutritional characteristics, livestock feeding studies with target species should be conducted on a case-by-case basis to establish the nutritional benefits that might be expected. These studies

should span either the finishing period to slaughter for chickens, pigs, and beef cattle or a major part of a lactation cycle, lasting at least 100 d, for dairy cattle and should be conducted according to internationally agreed standard protocols.

Recommendation 5-4. In the case where nutritional components are to be deposited in the consumed tissue of the animal, specific tests for content should be conducted.

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Appendix 5-1

Results of a substantial equivalence compositional analysis for glyphosate-tolerant maize (Roundup Ready-Line GA21).

Table 1—Fiber, mineral, and proximate composition of gratin from Roundup Ready Corn Line GA21

	1996[a]		1997[b]				
Component[c]	GA21 mean (range)[h]	Control[d] mean (range)[h]	GA21 mean (range)[h]	Control[e] mean (range)[h]	Comm[f] lines mean (range)[h]	Literature (range)[h]	Historical[g] (range)[h]
Protein	10.05 (9.39-11.00)	10.05 (9.17-11.19)	11.05 (9.48-14.06)	10.54 (9.70-12.92)	10.87 (7.8-14.20)	(6.0-12.0)[k] (9.7-16.1)[l]	(9.0-13.6)
Total fat	3.5 1 (2.94-3.72)	3.55 (2.76-3.93)	3.90 (3.04-4.63)	3.98 (3.30-4.81)	3.69 (2.48-4.81)	(3.1-5.7)[k] (2.9-6.1)[l]	(2.4-4.2)
Ash	1.27 (1.06-1.45)	1.27 (1.21-1.40)	1.38 (1.06-1.80)	1.56 (1.07-3.09)	1.79 (0.89-6.28)	(1.1-3.9)[k]	(1.2-1.8)
ADF[i]	3.73 (3.35-3.99)	3.72 (3.52-4.05)	6.35 (2.73-9.47)	6.35 (3.00-9.33)	6.06 (2.75-11.34)	(3.3-4.3)[k]	(3.1-5.3)
NDF[i]	10.82 (10.06-11.88)	11.70 (9.40-13.58)	9 33 (7.51-11.57)	9.8 (8.03- 11.58)	10.12 (7.58- 15.91)	(8.3-11.9)[k]	(9.6- 15.3)
Carbohydrates	85.15 (84.00-86.11)	85.15 (83.71-86.14)	83.66 (80.57-84.97)	83.79 (81.69-85.26)	83.68 (77.41-87.16)	not reported in this form	(81.7-86.3)
Calcium	0.0026 (0.0020-0.0031)	0.0027 (0.024-0.0033)	0.0039[j] (0.0027-0.0056)	0.0043 (0.0033-0.0058)	0.0040 (0.0022-0.0208)	(0.01-0.1)[k]	(0.0029-0.006)
Phosphorus	0.299 (0.28-0.32)	0.299 (0.28-0.31)	0.326 (0.303-0.350)	0.326 (0.292-0.349)	0.330 (0.208-0.411)	(0.26-0.75)[k]	(0.288-0.363)
Moisture	14.15 (7.44-22.60)	14.40 (7.24-23.00)	16.86 (9.57-23.10)	16.21 (8.67-24.70)	16.30 (8.18-26.20)	(7-23)[k]	(9.4-15.8)

[a]Data from 5 U.S. sites: CA21 grain harvested from plants not treated with Roundup herbicide. [b]Comblned data from 4 nonreplicated E.U. sites, 6 U.S. nonreplicated sites, and 1 U.S. replicated site; GA21 grain harvested from plants treated wlth Roundup herbicide. [c]Percent dry weight of sample, except for molsture. [d]Nontransgenic negative segregant. [e]Parental control line. [f]Commercial lines; local hybrids planted at each site. [g]Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h]Range denotes the lowest and highest individual values across sites for each line. [i]ADF, acid detergent fiber; NDF, neutral detergent fiber. [j]Statistically significantly different from the control at the 5% level ($p < 0.05$). [k]Watson (1987). [l]Jugenheimer (1976). Reprinted with permission from Sidhu and others (2000). Copyright 2000 American Chemical Society.

Table 2—Fiber, mineral, and proximate composition of forage from Roundup Ready Corn Line GA21

	1996[a]		1997[b]				
Component[c]	GA21 mean (range)[h]	Control[d] mean (range)[h]	GA21 mean (range)[h]	Control[e] mean (range)[h]	Comm[f] lines mean (range)[h]	Historical[g] (range)[h]	
Protein	7.91 (5.70-10.37)	7.58 (6.11-8.61)	7.49 (6.40-8.67)	7.45 (5.88-8.76)	7.20 (5.11-10.27)	(4.8-8.4)	
Ash	4.22 (3.20-4.67)	3.85 (2.64-5.28)	4.29 (2.12-5.29)	4.26 (2.94-5.91)	4.19 (2.00-6.60)	(2.9-5.1)	
ADF[i]	25.04 (23.06-27.96)	25.89 (22.72-28.62)	23.85 (20.08-30.21)	25.55 (21.13-34.20)	25.56 (18.32-40.99)	(21.4-29.2)	
NDF[i]	39.47 (35.94-44.48)	40.85 (36.97-44.31)	37.91 (31.47-46.29)	38.92 (33.99-49.28)	39.54 (26.37-54.45)	(39.9-46.6)	
Total fat	1.73 (1.27-2.30)	1.50 (1.24-1.93)	1.88 (0.71-2.98)	2.21 (1.16-3.22)	2.04 (0.35-3.62)	(1.4-2.1)	
Carbohydrates	86.14 (82.94-89.57)	87.04 (84.83-89.88)	86.35 (85.06-89.96)	86.06 (83.58-87.85)	86.62 (83.16-91.55)	(84.6-89.1)	
Calcium	0.1934 (0.0965-0.2488)	0.1766 (0.0866-0.2172)	0.2304 (0.1420-0.3173)	0.2177 (0.1515-0.2754)	0.1948 (0.0969-0.3184)	(not available)	
Phosphorus	0.2288 (0.1822-0.2622)	0.2124 (0.2016-0.2365)	0.2178 (0.1419-0.3475)	0..2179 (0.1602-0.2914)	0.1992 (0.1367-0.2914)	(not available)	
Moisture	72.30 (69.5-77.0)	65.52 (42.0-75.3)	68.83 (62.20-74.10)	68.73 (64.60-73.80)	68.31 (55.30-75.30)	(68.7-73.5)	

[a]Data from 5 U.S. sites: CA21 grain harvested from plants not treated with Roundup herbicide. [b]Comblned data from 4 nonreplicated E.U. sites, 6 U.S. nonreplicated sites, and 1 U.S. replicated site; GA21 grain harvested from plants treated wlth Roundup herbicide. [c]Percent dry weight of sample, except for molsture. [d]Nontransgenic negative segregant. [e]Parental control line. [f]Commercial lines; local hybrids planted at each site. [g]Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995. [h]Range denotes the lowest and highest individual values across sites for each line. [i]ADF, acid detergent fiber; NDF, neutral detergent fiber. Reprinted with permission from Sidhu and others (2000). Copyright 2000 American Chemical Society.

Table 3—Amino acid composition of corn grain from Roundup Ready Corn Line GA21.

	1996[b]		1997[c]				
Amino acid[a]	GA21 mean (range)[i]	Control[d] mean (range)[i]	GA21 mean (range)[i]	Control[e] mean (range)[i]	Comm[f] lines mean (range)[i]	Literature[g] (range)[i]	Historical[h] (range)[i]
Alanine	7.62 (7.34-7.81)	7.64 (7.45-7.84)	7.64 (7.49-7.86)	7.62 (7.50-7.97)	7.78 (7.44-8.98)	(6.4-9.9)	(7.2-8.8)
Arginine	4.13 (3.72-4.34)	4.30 (4.05-4.51)	4.48 (3.74-4.93)	4.51 (4.11-4.90)	4.36 (3.67-5.34)	(2.9-5.9)	(3.5-5.0)
Aspartic acid	6.71 (6.46-6.87)	6.78 (6.35-6.83)	6.63 (6.17-7.05)	6.65 (6.22-7.08)	6.57 (6.14-7.35)	(5.8-7.2)	(6.3-7.5)
Cystine	2.10 (1.85-2.36)	2.11 (1.91-2.24)	2.22 (1.73-2.49)	2.28 (2.06-2.57)	2.19 (1.63-2.62)	(1.2-1.6)	(1.8-2.7)
Glutamic acid	19.27 (18.70-19.71)	19.06 (18.61-19.64)	18.78 (18.12-19.45)	18.70 (18.04-19.43)	19.17 (17.83-20.53)	(12.4-19.6)	(18.6-22.8)
Glycine	3.72 (3.44-3.95)	3.78 (3.48-3.96)	3.83 (3.44-4.27)	3.89 (3.52-4.14)	3.71 (3.05-4.29)	(2.6-4.7)	(3.2-4.2)
Histidine	2.81 (2.72-2.99)	2.84 (2.75-2.93)	2.67 (2.36-2.87)	2.74 (2.46-2.86)	2.80 (2.36-3.20)	(2.0-2.8)	(2.8-3.4)
Isoleucine	3.60 (3.48-3.66)	3.58 (3.44-3.70)	3.53 (3.06-3.85)	3.57 (3.13-3.92)	3.75 (3.13-4.14)	(2.6-4.0)	(3.2-4.3)
Leucine	13.11 (12.32-13.71)	12.90 (12.37-13.49)	12.98 (12.33-13.96)	12.87 (12.26-13.69)	13.32 (11.99-15.19)	(7.8-15.2)	(12.0-15.8)
Lysine	3.02 (2.68-3.30)	3.09 (2.69-3.27)	3.11 (2.59-4.04)	3.02 (2.66-3.33)	2.96 (2.20-3.50)	(2.0-3.8)	(2.6-3.5)
Methionine	1.98 (1.78-2.24)	2.03 (1.85-2.28)	2.16 (1.80-2.34)	2.17 (1.67-2.44)	2.02 (1.53-2.44)	(1.0-2.1)	(1.3-2.6)
Phenylalanine	5.15 (4.88-5.31)	5.17 (4.98-5.30)	5.31 (5.03-5.63)	5.33 (4.96-5.76)	5.36 (4.88-6.10)	(2.9-5.7)	(4.9-6.1)
Proline	8.69 (8.41-8.92)	8.69 (8.49-9.10)	8.98 (8.22-9.38)	9.00 (8.62-9.23)	9.16 (8.08-9.94)	(6.6-10.3)	(8.7-10.1)
Serine	5.33[j] (5.25-5.49)	5.27 (5.17-5.43)	5.17 (4.43-5.60)	5.03 (3.82-5.63)	4.64 (2.87-5.63)	(4.2-5.5)	(4.9-6.0)
Threonine	3.77 (3.64-3.88)	3.73 (3.58-3.85)	3.59 (3.33-3.74)	3.54 (3.08-3.71)	3.43 (2.61-3.89)	(2.9-3.9)	(3.3-4.2)
Tryptophan	0.62 (0.55-0.66)	0.57 (0.53-0.61)	0.61 (0.52-0.75)	0.61 (0.43-1.04)	0.59 (0.41-1.04)	(0.5-1.2)	(0.4-1.0)
Tyrosine	3.81[j] (3.68-3.99)	3.95 (3.88-4.10)	3.73 (3.06-4.20)	3.77 (2.78-4.32)	3.48 (2.37-4.32)	(2.9-4.7)	(3.7-4.3)
Valine	4.58 (4.40-4.74)	4.64 (4.45-4.73)	4.57 (4.15-5.18)	4.62 (4.00-5.00)	4.79 (3.93-5.40)	(2.1-5.2)	(4.2-5.3)

[a]Values expressed as percent of totatl amino acids for statistical comparaisons. These values are slightly higher when expressed as percent of total protein, for example, alanine = 7.8% for GA21 (1996). [b]Data from 5 U.S. sites: CA21 grain harvested from plants not treated with Roundup herbicide. [c]Comblned data from 4 nonreplicated E.U. sites, 6 U.S. nonreplicated sites, and 1 U.S. replicated site; GA21 grain harvested from plants treated wlth Roundup herbicide. [d]Nontransgenic negative segregant. [e]Parental control line. [f]Commercial lines; local hybrids planted at each site. [g]Watson (1982). Values are percent of total protein [10.1% total protein (N × 6.25)]. [h]Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are percent of total protein. [i]Range denotes the lowest and highest individual values across sites. [j]Values statistically significantly different from the control at the 5% level ($p < 0.05$). Reprinted with permission from Sidhu and others (2000). Copyright 2000 American Chemical Society.

Table4—Fatty acid composition of corn grain from Roundup Ready Corn Line GA21

	1996[b]		1997[c]				
Fatty acid[a]	GA21 mean (range)[i]	Control[d] mean (range)[i]	GA21 mean (range)[i]	Control[e] mean (range)[i]	Comm[f] lines mean (range)[i]	Literature[g] (range)[i]	Historical[h] (range)[i]
Arachidic (20:0)	0.40 (0.36-0.48)	0.41 (0.39-0.46)	0.37 (0.32-0.44)	0.36 (0.33-0.41)	0.40 (0.31-0.57)	(0.1-2)	(0.3-0.5)
Behenic (22:0)	0.16 (0.14-0.18)	0.17 (0.16-0.18)	0.16 (0.12-0.24)	0.15 (0.13-0.16)	0.18 (0.13-0.24)	(not reported)	(0.1-0.3)
Eicosenoic (20:1)	0.28 (0.27-0.31)	0.29 (0.28-0.30)	0.30 (0.28-0.34)	0.30 (0.28-0.36)	0.30 (0.19-0.45)	(not reported)	(0.2-0.3)
Linoleic (18:2)	58.56 (54.20-64.70)	58.72 (53.40-65.60)	61.40 (58.2-63.4)	61.51 (59.7-63.0)	59.18 (46.9-64.3)	(35-70)	(55.9-66.1)
Linolenic (18:3)	1.10 (1.07-1.13)	1.08 (0.98-1.16)	1.14 (0.92-1.24)	1.14 (1.04-1.20)	1.11 (0.77-1.55)	(0.8-2)	(0.8-1.1)
Oleic (18:1)	27.5 (22.1-31.3)	27.4 (21.4-32.4)	24.2 (22.4-26.0)	24.1 (22.9-26.0)	26.2 (21.3-39.2)	(20-46)	(20.6-27.5)
Palmitic (16:0)	9.94 (9.59-10.40)	9.92 (9.60-10.40)	10.70 (10.30-11.40)	10.72 (10.40-11.40)	10.58 (8.75-13.30)	(7-19)	(9.9-12.0)
Stearic (18:0)	1.87 (1.52-2.11)	1.86 (1.46-2.11)	1.68 (1.44-2.04)	1.67 (1.59-1.86)	1.88 (1.36-2.65)	(1-3)	(1.4-2.2)

[a]Values expressed as percent of totatl fatty acid. The method included the analysts of the following fatty acids, which were not detected in the majority of samples analyzed; caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15:0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), hetadecenoic acid (17:1), gamma linolenic (18:3), elcosadienoic acid (20:2), elcosatrienoic acid (20:3), and arachidonic acid (20:4). Palmitoleic acid (16:1) was observed at levels of ~0.17% of total fatty acids in grain samples collected in 1996 but was not detected in the majority of grain samples collected in 1997. [b]Data from 5 U.S. sites: CA21 grain harvested from plants not treated with Roundup herbicide. [c]Comblned data from 4 nonreplicated E.U. sites, 6 U.S. nonreplicated sites, and 1 U.S. replicated site; GA21 grain harvested from plants treated wlth Roundup herbicide. [d]Nontransgenic negative segregant. [e]Parental control line. [f]Commercial lines; local hybrids planted at each site. [g]Watson (1982). Values expressed as percent of total fat except for palmitic acid (16:1), which is expressed as percent of triglyceride fatty acids. [h]Range for control lines planted in Monsanto Co. field trials conducted between 1993 and 1995; values are percent of total fatty acids. [i]Range denotes the lowest and highest individual values across sites. Reprinted with permission from Sidhu and others (2000). Copyright 2000 American Chemical Society.

Chapter 6: The Role of Analytical Techniques in Identifying Unintended Effects in Crops Developed through the Application of Modern Biotechnology

6.1 Introduction

Biotechnology-derived food plants with improved nutritional properties are under development and, in a number of cases, have reached the field trial phase and reached commercialization (for example, high-oleic soybeans; Kinney and Knowlton 1998). This new generation of food/feed plants has the potential to (1) combat nutrient deficiencies in the population through the introduction of essential nutrients, (2) improve the nutritional value of foods/feeds by enhancing essential nutrients or by improving their bioavailability, (3) promote human health through elevated levels of health protecting/promoting compounds, and (4) improve the safety of products by lowering levels of natural toxins, toxic metabolites, or allergens. Examples of products under development with improved nutritional properties are described in Chapter 2. In addition to human nutrition, functional characteristics are being developed that are targeted at animal feed and food processing.

Multiple gene inserts coding for complex components of metabolic pathways have been engineered in some of these plants. A key question to be addressed is whether these types of food and feed products require additional safety testing compared to the "first generation" of GM plants (those with improved agronomic properties such as insect protection, herbicide tolerance, or a combination of these traits) because of a greater chance of unintended changes in composition.

6.2 General Principles

The safety of conventional crops is generally based on a history of safe use. Formal safety assessment is not conducted. For new varieties, analysis of agronomic performance and other phenotypic characteristics, as well as of selected macro- and micronutrients, antinutrients and toxicants, is performed. This has given us a wealth of data on the presence of nutritionally beneficial compounds as well as antinutrients and toxicants, whose levels have been either increased and/or diminished by the selective procedures. Plant breeders have discarded from the breeding programs products with unusual agronomic performance, unpleasant taste, or harmful levels of a specific compound. These approaches toward selection of conventionally bred crops have proven their efficacy and have provided the consumer with safe and nutritious foods. Similar, but much more extensive, practices are applied to foods derived from GM crops to assess their safety and nutritional equivalence to conventional crop varieties.

New analytical technologies, based on genome research and modern chemical–analytical developments, have emerged over the last decade. These technologies may provide interesting information about the structure and function of the genome of plants. These methods also give more insight into the physiology and metabolic pathways in plants, including the regulation of levels of beneficial and toxic constituents. This chapter includes a review of the potentials of these new analytical tools with respect to their use for safety assessment of foods derived from GM crops. These techniques should not be seen as alternatives, but instead as complementary methods, once validated.

The potential occurrence of unintended changes in foods produced through the application of recombinant DNA (rDNA) technology is a topic that has attracted broad interest from scientists, regulators, and consumer groups. Insertion of DNA sequences into the plant genome may lead to modification of gene function, possibly resulting in shifts in metabolic pathways, upstream and downstream effects, changed metabolic pools, and formation of new metabolites or changes in levels of existing metabolites. Identification of unintended effects in GM crops and their assessment with respect to human and animal safety has been one of the topics of the European Network Safety Assessment of Genetically Modified Food Crops (ENTRANSFOOD), sponsored by the European Commission (www.entransfood.com), and has recently been reviewed (Kuiper and others 2001; Cellini and others 2004). Some unintended effects may be predictable on the basis of what is known about the site of insertion of the introduced DNA and function of potentially disrupted gene(s) and/or the function of the inserted trait and its involvement in metabolic pathways. Other effects are less predictable because of the limited knowledge of gene regulation and gene-gene interactions.

6.2.1 Unintended effects are not unique to GM crops

The occurrence of unintended effects is not specific to genome modification through recombinant DNA technology, as unintended effects also occur frequently as a result of conventional breeding and during the use of chemical and irradiation mutagenesis to produce new phenotypes. Insertion in plant DNA must, therefore, be evaluated with an awareness of such natural DNA events, an issue that has been reviewed recently (Cellini and others 2004). Examples of unintended effects that have occurred during conventional breeding of food crops are listed in Table 6-1. As discussed below, parallels exist between natural recombination and DNA insertion in GM crops. The molecular changes that occur during conventional breeding may nevertheless be less easy to trace than changes caused by insertion, rearrangement, or mutagenesis of exogenous, distinguishable DNA originating from the same or other species.

Natural chromosomal recombination mechanisms play an important role in plant breeding, and mechanisms can be grouped into (1) homologous recombination and (2) illegitimate recombination, a nonhomologous end-joining process. Both processes are characterized by double-strand break repair mechanisms. Nonhomologous recombination is the predominant form in plants (Siebert and Puchta 2002). Although recombination events could theoretically occur at random along the length of the chromosomes, the presence of preferential sites for recombination breakpoints is well known (Schnable and others 1998).

Using modern biotechnology, exogenous DNA is integrated into plant genomic DNA through various methods including bi-

Table 6-1—Unintended effects in traditional breeding (modified from Cellini and others 2004)

Host plant/trait	Unintended effect	Reference
Barley/Powdery mildew resistance	Low yield	Thomas and others (1998)
Celery/Pest resistance	High furanocoumarins content	Beier (1990)
Maize/High lysine content	Low yield	Mertz (1992)
Potato/Pest resistance	Low yield, high glycoalkaloid content	Harvey and others (1985)
Squash, Zucchini/Pest resistance	High cucurbitacin content	Coulston and Kolbye (1990)

Table 6-2—Unintended effects in genetic engineering breeding (from Cellini and others 2004)

Host plant	Trait	Unintended effect	Reference
Canola	Overexpression of phytoene-synthase	Multiple metabolic changes (tocopherol, chlorophyll, fatty acids, phytoene)	Shewmaker and others (1999)
Potato	Expression of yeast invertase	Reduced glycoalkaloid content (–37 to 48%)	Engel and others (1998)
Potato	Expression of soybean glycinin	Increased glycoalkaloid content (+16 to 88%)	Hashimoto and others (1999a,b)
Potato	Expression of bacterial levansucrase	Adverse tuber tissue perturbations	Turk and Smeekens (1999)
		Impaired carbohydrate transport in the phloem	Dueck and others (1998)
Rice	Expression of soybean glycinin	Increased vitamin B_6 content (+50%)	Momma and others (1999)
Rice	Expression of provitamin A biosynthetic pathway	Formation of unexpected carotenoid derivatives (β-carotene, lutein, zeaxanthin)	Ye and others (2000)
Wheat	Expression of glucose oxidase	Phytotoxicity	Murray and others (1999)
Wheat	Expression of phosphatidyl serine synthase	Necrotic lesions	Delhaize and others (1999)

olistic or microprojectile bombardment, *Agrobacterium*-mediated transformation, or other methods (Datta and Datta 2002). In the case of *Agrobacterium*-mediated transformation, DNA integration from the *Agrobacterium* Ti (tumor inducing) plasmid into the plant genome occurs in the absence of any homology with plant DNA sequences, through the process of illegitimate recombination (Gheysen and others 1991). Insertion of introduced DNA into chromosomal DNA by this method can result in single or repeated copies and in multiple insertions (Grevelding and others 1993). Moreover, rearrangements of the inserted DNA and/or of the target site DNA can be observed. Sequencing the flanking regions of DNA insertions in tobacco has highlighted the presence of motifs that flank the inserted DNA, such as AT-rich sequences, microsatellite sequences, retro-elements, or tandem repeats (Iglesias and others 1997). In *Arabidopsis,* AT-rich regions have been proposed to be the preferred targets for DNA introduced by particle bombardment (Sawasaki and others 1998), suggesting that the recombination processes of DNA insertion by *Agrobacterium* and of DNA delivered by the biolistic method are controlled by similar principles. Based on the knowledge about DNA recombination mechanisms discussed above, there is no reason to suppose that integration of introduced DNA into plant chromosomes is more likely to give rise to DNA disruption than natural recombination mechanisms. Examples of unintended effects as a result of genetic modification of food crops are given in Table 6-2. Comparison of these results with those in Table 6-1 shows that some of the unintended effects in crops derived from modern biotechnology are similar to others observed in conventional breeding.

6.2.2 Approaches to identify unintended effects

6.2.2.1 Genome analysis. In the situation where genomes are well mapped and much or all the genome has been sequenced (for example, rice, corn), localization and characterization of the site(s) of insertion may be helpful to predict effects due to the DNA insertion in the recipient plant. Data on plant genome sequences flanking the inserted DNA provide information on whether the inserted DNA is within or in the proximity of a known endogenous gene. The chromosomal location of the inserted DNA can be detected by various methods such as genomic in situ hybridization (GISH; Iglesias and others 1997), fluorescence in situ hybridization (FISH; Pedersen and others 1997), and direct sequencing of flanking DNA (Thomas and others 1998; Spertini and others 1999). Knowledge of genomes in most plants is still limited, as is the reliability of annotations in genomic databases, but the understanding of the genomic code and the regulation of gene expression in relation to the networks of metabolic activity is expanding rapidly. Therefore, sequencing of the site(s) of insertion(s) may become increasingly informative with respect to possible alterations in metabolic networks by the modification of native DNA that may affect the toxicological or nutritional status of the modified product.

However, even when the genome sequence of the host crop has been sequenced, these analyses may be more complicated than initially envisioned. Plants contain pseudogenes (for example, nonfunctional genes that are highly homologous to functional genes) and large numbers of highly repetitive DNA sequences. These sequences make it very difficult to assess whether DNA has inserted into a functional gene, nonfunctional pseudogene or into highly repetitive DNA.

6.2.2.2 Compositional analysis. A comparative analysis of (1) the agronomical/morphological characteristics, (2) macro- and micronutrient composition and content of important antinutrients and toxicants, and (3) the toxicological and nutritional characteristics of the modified product and its conventional counterpart tested in appropriate animal models may help to define or assess the importance of the occurrence of unintended effects. Such an approach has been the basis for conventionally bred products with significant nutritional modification (for example, Opaque-2 maize with increased lysine content, also known as Quality Protein Maize; Villegas and others 1992; see Chapter 4) and will be explored here for its suitability for GM crops with nutritionally improved traits.

A number of different strategies can be followed. Targeted approaches are hypothesis driven and focused on the generation of information on specific known macro- and micronutrients, toxic, allergenic, or bioactive compounds present in the GM food crop and its corresponding conventional counterpart. The spectrum of compounds is based on experience gained from analysis of food crops obtained via conventional breeding. Much as in the case of human blood sample testing, analytical measurement of key substances can indicate underlying changes in health/metabolism, which can then be followed up case by case. The OECD has recently developed international consensus documents on the particular components that could be analyzed for specific crops (OECD 2003). For the analyses of these compounds, a number of validated methods are available that are well understood through their long history of use. Such approaches work well to assess the concentrations of specific predetermined compounds that, because of their selective nature, may miss other unintended changes. By selecting the nutritionally and antinutritionally relevant compounds, this provides considerable protection against unintentional changes in known nutrients and antinutrients. However, the concentrations of preselected compounds, for example, are not necessarily indicative of changes in function or phenotype of a plant as a result of the genetic modification. In addition, because only known substances are measured, any potential changes in the concentrations of unknown toxicants and antinutrients (if they exist) caused by the genetic modification would remain unevaluated by compositional analysis (but would be picked up if toxicologically significant in animal studies). However, it is likely that those of importance will have already been identified by their effect on health and nutrition during a history of use by a substantial portion of the consumer population, so the gap is expected to be very limited. The proposed lists of nutrients, antinutrients, and toxicants include those for which data suggest that changes in the concentrations could affect safety or nutritional quality. Another limitation is that sample processing (for example, extraction) before single-compound assays may be so rigorous that relevant compounds may be lost before sample analysis. These limitations may be of particular importance for food plants with limited or no history of (safe) use, and for plants that have been modified extensively through multiple gene insertions. Given the fact that the GM crops currently on the market do not fall into either of these categories, single compound analysis has been sufficiently rigorous to screen for unintended effects and thereby corroborate the safety of these crops. In the future, single compound analysis will continue to be the method of choice, using validated and quantitative methods.

Nontargeted approaches for the detection of unintended effects rely on profiling methods that may provide information on potential changes in the physiology of the modified host organism at different cellular integration levels (that is, at the level of mRNA expression and protein translation and at the level of plant metabolism). New techniques such as the DNA/RNA microarray technology, proteomics, and hyphenated (that is, coupled) analytical methods enable an integrated and simultaneous analysis of gene expression and protein and metabolite formation. These technologies have been described recently by Kuiper and others (2001, 2003) and Cellini and others (2004).

Multiple factors determine the morphologic, agronomic, and physiologic properties of a food crop, such as genetic characteristics, agronomic attributes, environmental influences, plant-microbe interactions, developmental stage, and postharvest effects. Application of the new analytical technologies during various developmental stages of the plant and under different environmental conditions may provide important information on the dynamics of gene expression and resulting metabolic consequences. Profiling techniques provide an open-ended broad view of the complex metabolic networks of the organism without knowledge of changes at the level of single cell constituents (for example, organelles). However, appropriate use of these new technologies requires validated methods with well-understood parameters and limitations, and the generation of extensive databases on conventionally bred crop varieties grown under a variety of environmental conditions in order to assess the level of natural variability. The amounts of data on baseline concentrations of natural constituents in traditional plants are, in general, limited. In addition, the massive amounts of data generated from these methods present the challenges of proper (multivariate) statistical analysis and interpretation of changes in expression patterns, including the biological significance.

6.3 Methods for Detection of Unintended Effects

6.3.1 Targeted approach

6.3.1.1 Specific compound analysis. Current comparative compositional analysis of GM crops and their counterparts is targeted at specific compounds, including toxicologically and nutritionally relevant macronutrients, micronutrients, antinutrients, toxins, allergens, and bioactive substances. Differences in compositional profiles that may be found to be due to the targeted genetic modification will need to be assessed with respect to functionality, toxicity, efficacy, bioavailability, and so on. Significant changes in other nontargeted metabolic pathways leading to greater concentrations of toxic plant substances, such as glycoalkaloids in potatoes and tomatoes, will also need to be investigated further, if the changes lead to concentrations that fall outside the natural range of variability. It should be clear that, even in those cases where differences in composition between the modified crop and its counterpart are observed that fall outside these ranges of variability, such crops do not necessarily pose a threat to human or animal health. Such differences should be assessed on a case-by-case basis as to whether additional investigations are appropriate to address further possible concerns related to the food and feed safety of the crop plant. Information on compositional analysis of GM crops in the framework of the assessment of substantial equivalence is provided by consensus documents developed by the OECD Task Force on the Safety of Novel Foods and Feed (OECD 2003).

The ILSI International Food Biotechnology Committee has developed a database on crop composition that provides a detailed assessment of the baseline of compositional variations for conventional crop varieties, beginning with maize and soybeans. This Internet-accessible tool (www.cropcomposition.org) features search options that allow the user to select, for example, specific compositional parameters, locations, seasons, and so on.

6.3.2 Nontargeted approach

Examples of profiling techniques that could be used for compositional analysis are depicted in Figure 6-1 and are discussed in further detail below.

6.3.2.1 Gene expression analysis. A powerful tool to study gene expression is DNA microarray technology. The study of gene expression using microarray technology is based on hybridization of mRNA to a high-density array of immobilized target DNA sequences, each corresponding to a specific gene. The mRNAs from samples to be analyzed are labeled by incorporation of a fluorescent dye and subsequently hybridized to the array. The fluorescence at each spot on the array is a quantitative measure corresponding to the expression level of the particular gene. The major advantage of the DNA microarray technology over conventional gene profiling techniques (for example, restricted fragment length polymorphism - polymerase chain reaction [RFLP-PCR] followed

by electrophoresis) is that it allows small-scale analysis of expression of a large number of genes at the same time in a sensitive and relative (that is, within a test) manner (Schena and others 1995, 1996). Furthermore, it allows comparison of gene expression profiles of GM crops and conventional lines under different environmental conditions. This technology and the related field of bioinformatics are still in development and further improvements can be anticipated and will be necessary for use of these methods for GM crops (Van Hal and others 2000; Kuiper and others 2003). Current limitations to this technology are the need for microarray standards that will facilitate exchangeability and comparability of gene expression profiles of food and feed crops. Databases need to be established to generate information regarding the extent of natural variability with each of the new data points. In addition, profiling generates large data sets and appropriate software/hardware and statistical methods are needed to handle these.

The potential value of transcript profiling for the safety assessment of GM food plants is currently under investigation using the tomato as a model crop (Kuiper and others 2003). To study differences in gene expression, 2 informative tomato expressed sequence tag (EST) libraries were obtained, one consisting of EST that are specific for the red stage of ripening and the other for the green unripe stage. Both EST libraries were spotted on the array, as were a number of functionally identified cDNA, selected on the basis of their published sequence. The array was subsequently hybridized with mRNA isolated from a number of different GM varieties, as well as with the parent line and control lines. Preliminary results showed that different stages of ripening could be identified based on reproducible differences in gene expression patterns. Prospects are good that this method may be used effectively to screen for altered gene expression and, at the same time, may provide information on the nature of detected alterations (that is, whether these may affect the safety or nutritional value of the food crop under investigation). Additional studies are being carried out with GM potatoes and tomatoes in the framework of the EU sponsored project GMOCARE (GMOCARE 2003). To be useful, the variability for each transcript needs to be established and knowledge gained on the relevance of each new assay point regarding safety and nutrition. Although these techniques may prove useful to identify differences among tissues or between a food component from a GM product and its conventional counterpart, the relevance to safety assessment will be challenging and has yet to be established. Therefore, these methods are not yet suitable for use in safety or nutritional assessments.

6.3.2.2 Applicability of the microarray technology to food safety assessment. A microarray customized for food safety assessment should preferably contain primarily cDNA or EST derived from metabolic pathways leading to the relevant nutrients and antinutrients, especially the natural toxins. Examples of similar-targeted studies on the expression of genes that may have been affected by insertion of another gene in transgenic plants have been published by Heinekamp and others (2002) and Moire and others 2004. Metabolic pathways for nutrients, antinutrients, and toxins (for example, glycoalkaloid biosynthesis) are poorly mapped, and, therefore, it is likely that, for the time being, the most important contribution of the microarray technology will be to help fill this gap in our knowledge of the physiology of crop plants. Once the metabolic network is more fully elucidated, it is feasible that arrays could be constructed that can provide important information about changes in metabolic pathways that may need further investigation with respect to their implications for the food safety of the crop plant. Analysis of individual constituents can then, perhaps, be reduced to a limited number of proteins or metabolites that, based on the gene expression profile, seem to have been affected by the genetic modification. An acceptable ratio of the GM versus the parent line for individuals or groups of metabolites will then have to be established based on health implications. The usefulness of this technology for the identification of unintended effects in GM crops depends largely on documented information about natural variations in gene expression levels in crop plants, which is still lacking.

6.3.2.3 Proteomics. Proteomics is an important research tool to study protein expression patterns. High-resolution, 2-dimensional gel electrophoresis can show those proteins present in a tissue which track within a given molecular weight and isoelectric point focusing range. New developments in mass spectrometry (MS) have increased the applicability of the technique (Beranova-Giorgianni 2003). Correlation between mRNA expression and protein concentrations is generally poor because rates of degradation of individual mRNA and proteins differ (Gygi and others 1999). Care must be taken in the extrapolation of these mRNA data to the proteomic and metabolomic level. Proteomics can be divided into 3 main areas: (1) identification of proteins and their post-translational modifications, (2) "differential display proteomics" for quantification of variations in content, and (3) studies of protein-protein interactions.

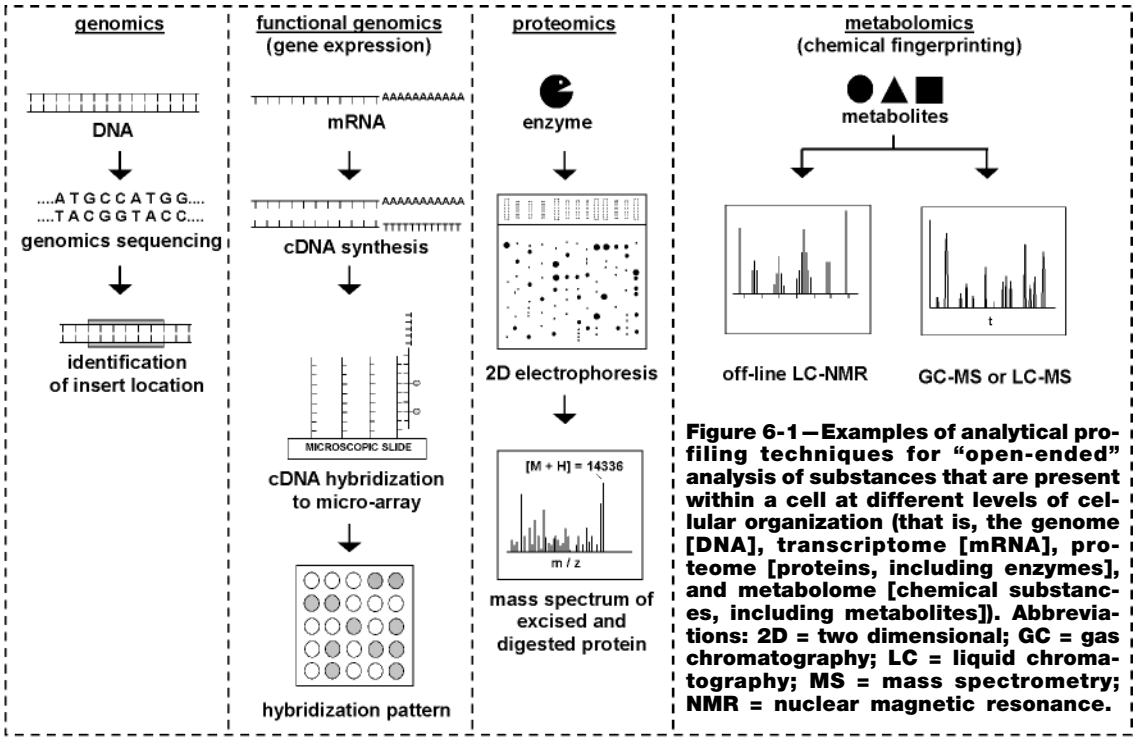

Figure 6-1—Examples of analytical profiling techniques for "open-ended" analysis of substances that are present within a cell at different levels of cellular organization (that is, the genome [DNA], transcriptome [mRNA], proteome [proteins, including enzymes], and metabolome [chemical substances, including metabolites]). Abbreviations: 2D = two dimensional; GC = gas chromatography; LC = liquid chromatography; MS = mass spectrometry; NMR = nuclear magnetic resonance.

The method most of-

ten used to analyze differences in protein patterns is 2-dimensional gel electrophoresis, followed by excision of protein spots from the gel, digestion into fragments by specific proteases, and, subsequently, analysis by MS (that is, peptide mass fingerprinting). It allows the identification of proteins by comparing the mass of the peptide fragments with data predicted by genetic or protein sequence information (see Cellini and others 2004). For crops with limited data on complete protein sequences, comparison with EST sequence databases may provide an alternative route toward elucidation of the putative function of an identified protein.

Box 6-1—Examples of profiling techniques
- Gene expression: mRNA detection with microarrays.
- Proteomics: 2-dimensional gel electrophoresis, usually followed by mass spectrometry (MALDI-TOF).
- Metabolomics: hyphenated techniques (for example, separation and universal detection, such as LC-NMR and GC-MS).

The basic methods used to identify proteins separated by gel electrophoresis are electrospray ionization and matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) MS analysis (Andersen and Mann 2000). Electrospray tandem MS allows for fragmentation of selected ion species and subsequent analysis against a database. Some newer applications eliminate the need for separation of proteins from a complex mixture by gel electrophoresis. For example, a promising technique is affinity purification by isotope-coded affinity tags for more quantitative analysis, combined with multidimensional liquid chromatography and tandem MS (Han and others 2001). Amino acid sequence data obtained from tandem MS data may also be useful for protein identification whenever no matches of single MS data with those of previously characterized proteins are found.

When searching for unintended changes by 2-dimensional polyacrylamide gel electrophoresis (PAGE), the first step is to compare proteomes obtained from extracts of leaves or seeds of the GM plant and the closest genetic counterpart. If differences in protein profiles are detected, natural variations should be further examined by analyzing the variation in levels of a number of different crop varieties grown under a number of different environmental conditions. Detection of differences specifically related to the genetic modification process by analysis of general proteomes is difficult because of the many proteins that are not involved in such changes and the many changes that may occur because of different environmental conditions. If differences fall outside natural variations, identification of the protein is carried out, and this may lead to further safety assessment studies.

Proteomics can aid in understanding changes in cellular metabolism and physiology of food plants, regardless of the technology that was used to make the change. A major limitation in the use of proteomics for the detection of unintended effects is that changes due to the genetic modifications may not be easily distinguishable from changes due to environmental factors. Therefore, restrictive conditions for protein isolation, in addition to selection of proteins involved in important metabolic pathways, may reduce the number of confounding factors and yield more informative proteomes. Further development of proteomics, combined with detection of specific proteins with antibodies or protein microarrays, may offer more effective ways to identify unexpected changes. The specificity of antibodies, for example, may allow for detection of specific proteins whose expression might have been influenced by a given genetic modification in GM plants (Carvalho and others 2003; Li and others 2001). In addition, validated and standardized protein extraction procedures have not yet been established. This is important because small variations in the many steps of sampling and extraction procedures may have a major influence on the resulting protein pattern. The pitfalls and progress with regard to proteomic analysis have been discussed by Haynes and Yates (2000). Characterization and definition of ripening stages and storage conditions and of other genetic, agronomic, and environmental factors is essential because they all have a profound influence on the proteome.

In conclusion, proteomics offers interesting possibilities for elucidation of cellular metabolic processes and their dynamics as influenced by breeding and selection processes and environmental influences. Identification of unintended effects and of toxicologically relevant proteins is one of its potential uses. Application of the technology for identification and assessment of unintended effects in GM crops is seriously hampered by the lack of information on natural variation in proteomes (for example, of commercially available lines) in addition to the abovementioned reproducibility and technical limitations. Therefore, although interesting, the use of this approach will require considerable research and development before it is used for safety assessment. Recently initiated research projects funded by the UK Food Standards Agency specifically address this issue (FSA 2003).

6.3.2.4 Metabolomics. Changes in mRNA levels or in proteins do not provide direct information about changes in biological function at the levels of metabolites in the food or feed components. A change at one level in a complex network does not necessarily lead to a particular change in function or phenotype. Therefore, these methods have limited direct value for risk assessment. Open-ended broad metabolite analysis may enable ways to define the biochemical function of plant metabolism.

A multicompositional analysis of biologically active compounds in plants (that is, nutrients, antinutritional factors, toxicants, and other compounds [the so-called metabolome]) may indicate whether intended and/or unintended effects have taken place as a result of genetic modification. The 3 most important techniques that are currently deployed for this purpose are gas chromatography (GC), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR). These methods are capable of detecting, resolving, and quantifying (in a relative sense) a wide range of compounds in a single sample. For instance, profiles of isoprenoids using an HPLC method with photodiode array detection (PDA) were recently described for a GM tomato and *Arabidopsis thaliana* (Fraser and others 2000). Approximately 42 isoprenoids could be separated on a reversed-phase C_{30} HPLC system.

The potential of GC to serve as a metabolomics tool for plants was demonstrated by Sauter and others (1991), Fiehn and others (2000), and Roessner and others (2000). Metabolomics is being developed as a tool for comparative display of gene function. It has the potential to provide insight into complex regulatory processes and to determine phenotype directly. Fiehn and others (2000) developed a GC-MS method allowing the quantification of 326 distinct compounds from *Arabidopsis thaliana* leaf extracts, with assignment of a chemical structure to approximately half of the compounds. Four genotypes were selected: 2 homozygous ecotypes and 2 single-point mutants from each genotype. Cluster analysis indicated that each genotype had a distinct metabolic profile, with the 2 ecotypes being more different than the single-point mutant compared to its parental ecotypes. Roessner and others (2000) developed a GC-MS method for simultaneous analysis of metabolites in potato tubers. Differences in profiles of soil or in vitro-grown tubers were observed. The results showed a remarkably low experimental variability (6%) compared to the much larger biological variability (20%). Differences in the content of amino acids, citric acid cycle intermediates, and compounds indicative of osmotic stress not previously seen were found in GM crop material using this open-ended approach. In

GM potato lines with increased yeast invertase or inhibited starch metabolism, differences were also observed compared to conventionally bred control lines. In the line with the invertase expressed in the apoplast, no elevated respiratory flux was observed, whereas in the line with the invertase expressed in the cytosol, appearance of phosphogluconic acid was observed, indicative of increases in components of the oxidative pentose phosphate pathway. This open-ended approach of metabolomics provides the opportunity to identify unexpected changes, and, thus, insights into metabolic networks.

Besides the metabolomic analysis of many classes of metabolites simultaneously, selective extraction, separation, and detection methods enable metabolomics of specific metabolite classes. In this way, more focused searches are possible into potential changes of (concentration/formation of) metabolites that may be caused by a particular genetic modification. One example is metabolomics of isoprenoid compounds, including carotenoids, tocopherols, quinones, and chlorophylls, from chloroform extracts of plant tissues (Fraser and others 2000). For this purpose, high-performance liquid chromatography (HPLC) was used in combination with PDA detection, and UV-spectra of the separated compounds were generated. By applying this method to tomatoes in which an additional carotenoid biosynthesis gene had been inserted, the authors could determine which carotenoids had been altered or where a new formation had occurred. In a similar fashion, Chen and others (2003) generated metabolomic profiles of phenolic compounds from methanol extracts of alfalfa tissues by HPLC followed by a combination of PDA and mass spectrometry. These authors observed, for example, that plants containing transgenes for lignin biosynthesis showed altered levels of phenols in their stem tissues but not in their leaf tissues.

In the course of the European Union sponsored SAFOTEST project, a metabolomics methodology has been developed using rice as the model crop (Frenzel and others 2002). Rice grains are characterized by a complex composition and large differences in concentrations of compounds. A method to fractionate the total rice extracts was developed enabling a GC analysis of a broad spectrum of major and minor constituents. The approach is based on consecutive extraction of lipids and polar compounds. Selective hydrolysis of silylated derivatives results in separate fractions of major (sugars) and minor (organic acids/amino acids) polar constituents. Profiles of silylated/methylated compounds are obtained by means of gas chromatography – flame ionization detection (GC-FID), and identification can be achieved by GC-MS. Further work will be carried out on GM rice varieties.

It has been shown that the use of metabolomics techniques, such as off-line LC-NMR, may provide information on possible changes in plant matrices caused by variations in environmental conditions (Lommen and others 1998). Metabolic profiles consisting of ^{1}H-NMR spectra were obtained from different water and organic solvent extracts from GM tomato varieties, such as the antisense RNA exogalactanase fruit, and from their unmodified counterpart(s) (Noteborn 1998; Noteborn and others 1998; 2000). Differences in concentration of low-molecular-weight components (MW <10 kDa) could be traced, by subtraction of the ^{1}H-NMR spectra.

To apply profiling methods on a routine basis, it is necessary to (1) standardize sample collection, preparation, and extraction procedures; (2) standardize and validate quantification; (3) generate information on profiles of plant extracts and in particular on natural variations that are relevant to food and feed production (for example, crop lines cultivated in practice); (4) further develop bioinformatic systems to treat large data sets; (5) understand the quantitative limitations of each methodology; (6) develop extensive databases to define the levels of natural variability; (7) relate changes in gene expression levels to possible changes in protein and metabolite levels; (8) evaluate the changes for their relevance to food safety; and (9) select those changes that are relevant for food safety assessment. Data analysis is of great importance, and univariate and multivariate statistics such as chemometric or pattern recognition techniques are necessary for the identification of relevant changes in metabolite patterns.

These approaches are being further explored within a European Union project, GMOCARE (GMOCARE 2003). This project includes studies of functional genomics, proteomics, and metabolite profiling. In addition, the U.K. Food Standards Agency has initiated several projects exploring the use of profiling methods for the safety assessment of foods derived from modern biotechnology (FSA 2003).

The compilation of databases on profile patterns and natural variations therein is vital to be able to evaluate the usefulness of profiles for identification of unintended effects in GM crops that are of safety and/or nutritional importance. Integrated analysis of profiles is still in its infancy. In addition, mass spectral databases are crucial to enable identification of metabolites. Therefore, these methods are not currently appropriate for use in safety assessment of either agronomically improved or nutritionally improved crops.

6.4 Discussion

6.4.1 Targeted approach for detection of unintended effects

Approaches to detect intended and unintended changes in the composition of GM food crops are based primarily on measurements of single compounds (targeted approach). This approach is hypothesis-driven and focused on the determination of levels of known specific nutritional or toxic compounds present in the food from GM crops and the food's corresponding conventional comparator. Analysis of specific compounds, while powerful, may miss some unexpected changes because of the selection of a relatively limited number of compounds to be measured. Changes in function or phenotype of a plant because of genetic modification may not be reflected in changes in these selected compounds. In addition, it is not possible to detect changes in levels of unknown toxicants and antinutrients, which may have been significantly changed through the genetic modification. These limitations are of particular importance for food plants with limited or no history of (safe) use and for plants that have been modified extensively through multiple gene insertions. Although this approach does not absolutely guarantee that unintended alterations in the plant metabolism have not occurred, experience gained with traditional plant breeding has enabled plant breeders to provide safe and nutritious crops for decades. Furthermore, much more detailed analyses are conducted for GM crops, and this provides even greater assurance of their safety and nutritional composition (Harlander 2002).

6.4.2 Nontargeted approaches

The new profiling methods offer interesting possibilities to detect secondary effects due to the genetic modification of food plants by both traditional breeding and biotechnology. Nontargeted approaches for the detection of unintended effects rely on profiling methods that may provide information on potential changes in the physiology of the modified host organism at different cellular integration levels (that is, at the level of mRNA expression and protein translation and at the level of plant metabolism). New techniques such as DNA/RNA microarray technology, proteomics, and hyphenated (that is, coupled) analytical techniques enable an integrated and simultaneous analysis of gene expression and of gene product and metabolite formation. Profiling techniques provide a broad view of the complex metabolic networks

of the organism without prior knowledge of specific changes at the level of single cell constituents. The potential of profiling techniques is, in the first place, to provide insight into metabolic pathways and their interconnectivities that may be influenced by traditional breeding and modern biotechnology. However, use of these techniques for detection and assessment of unintended effects in GM plants must be further explored, and the construction of databases containing profiles of crops obtained under different physiological and environmental conditions should be encouraged (Kuiper and others 2003). The main challenge in the use of profiling techniques for the detection of unintended effects is that any differences observed may not be easily distinguishable from natural variability due to varietal, developmental, and/or environmental factors. Another aspect one must consider while applying these nontargeted profiling techniques is that all observed differences, large or small, may not be equally significant in their impact or relevance to the overall ultimate safety and nutritional value of the food or feed product. Additionally, such profiling techniques need to be assessed for their built-in instrumental idiosyncrasies before the data are accepted to be bias-free. As discussed above, further standardization and validation of these profiling methods is needed. Of the profiling methods discussed here, the chemical fingerprinting techniques using GC/MS or HPLC-NMR are most advanced in development. These techniques also offer the best opportunities to obtain reliable metabolite profiles relevant to understanding the impact of genetic modification on the safety of the resultant improved nutrition crops. Thus, the state of the art in development of profiling methods indicates that the nontargeted methods are not yet suitable for assessment of GM crops within the safety assessment and regulatory framework. If, however, the scope of the profiling methods is narrowed to specific sets of compounds (for example, carotenoids, alkaloids), a valuable contribution can be made to the "targeted approach," perhaps in a shorter timeframe. Given the current status of profiling methods, the most valuable use of these methods from a safety assessment perspective is their use in early steps of product development or safety assessment, especially for nutritionally modified crops, to analyze for any changes in metabolites within specified biosynthetic pathways or in degradative/catabolic pathways of interest. This information can then be used to develop and validate metabolite specific analytical methods to assess these levels as part of the compositional analysis portion of the safety assessment process.

6.5 Conclusions and Recommendations

The current approach of single compound analysis for the detection of alterations in the composition because of plant breeding is the leading principle to assess the safety of food plants. This approach has shown to be adequate also for GM crops with minor modifications, and has provided us with both safe and nutritious food crops. It is expected that new profiling techniques may be a useful extension (but not a substitute) to this approach in assessing future GM crops with complex genetic modifications, thereby providing additional assurance of their safety once these methods are validated and the extent of natural variability is clearly established. With regard to the extensiveness of the data that may be generated by profiling techniques, the outcomes of the profiling analysis should be limited to those that are relevant to the safety of the pertinent food or feed. In addition, profiling techniques can be used in a more targeted fashion. For example, they might be aimed at specific groups of genes, proteins, or metabolites that are most likely to have been affected by the genetic modification. Because a targeted approach aims at effects that are predictable, some of the effects that are unpredictable may remain unnoticed. If there are remaining uncertainties in the risk assessment, supplementary animal feeding trials may be considered to assess unintended effects that are toxicologically significant.

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Chapter 7: Postmarket Monitoring

7.1 General Principles

The premarket safety assessment of foods derived through biotechnology provides a scientific basis for concluding reasonable certainty of no harm under anticipated conditions of use. The assessment of foods that are modified to provide specific nutritional benefits may require investigation of the intended beneficial effects on human health. The premarket evaluation is designed to identify endpoints of safety and efficacy, critical to the acceptability of a biotechnology-derived food, and generally negates the need for postmarket monitoring. However, postmarket monitoring may be appropriate under certain conditions where better estimates of dietary exposure and/or nutritional consequence of a biotechnology-derived or other novel food are required. There may also be a need, in selected instances, to correlate dietary intakes of a nutritionally improved food with expected beneficial effects on human health.

There are 2 elements that are essential to the monitoring process. First, adequate data must be available to assess the use, distribution, and fate of the product or commodity within the food supply and second, a monitoring program that provides requisite information on the consumption of relevant foods must be established. The feasibility of identifying any positive or negative effects on human health must be based on a hypothesis with identified biomarkers for measurement and is dependent on the availability of accurate consumption data. While current exposure assessment principles can be applied to the postmarket monitoring of biotechnology-derived foods to obtain appropriate consumption estimates and confirm premarket predictions, it should be recognized that such assessments may require traceability from field to consumer, which can be very challenging with commodity crops.

Exposure assessment methods include both deterministic and probabilistic estimates of intake, using food supply data, individual dietary surveys, household surveys, or total diet studies. If accurate exposure assessments are needed, probabilistic modeling provides a valuable but costly refinement to the coarse evaluation obtained via deterministic approaches. Habitual intake estimates may also be necessary when considering the long-term health effects of biotechnology-derived foods. The postmarket monitoring method used to estimate consumption should be evaluated a priori on a case-by-case basis to determine the most appropriate monitoring tool for establishing a specific relationship between a biotechnology-derived trait and its potential effects on human health.

7.1.1 The role of premarket assessment in ensuring the safety of biotechnology-derived foods

The premarket assessment of foods derived from biotechnology is a scientific, risk-based process that ensures the safety of the food to the consuming public. As outlined in Chapter 3, the premarket assessment of foods derived from biotechnology is a step-wise process, with specific safety determinants that vary on a case-by-case basis depending on the nature of the modification and intended use of the food. In the absence of major compositional changes in the biotechnology-derived food, the premarket assessment strategy focuses on the safety of the identified difference(s). Studies may include in vitro and in vivo evaluations of toxicological effects, potential allergenicity, and nutritional value following recommended standard methods (WHO 1987; FDA 1992; Munro and others 1996; LSRO 1998; FAO/WHO 2000; NRC 2000).

Nutritionally improved foods derived from biotechnology may be intended to have either an enhanced amount or specific composition of major inherent constituents. Consequently, those biotechnology-derived foods with major compositional changes will require consideration of these changes in the context of existing and expected dietary exposure. During the premarket safety assessment of any new food, biotechnology-derived or otherwise, it must be established that under the conditions of intended use, there would be no increased safety concern compared to that of traditional or existing foods. Safety can only be evaluated when conditions of use and exposure are known. Methods of estimating dietary exposure to biotechnology-derived foods are referred to in Chapter 3. The resultant exposure estimates are evaluated in the light of data from toxicological and nutritional studies to ensure safety and efficacy (WHO 1987; FDA 1992; Munro and others. 1996; LSRO 1998; FAO/WHO 2000; NRC 2000).

7.1.2 Is there a need for postmarket monitoring?

Postmarket monitoring has not been routine in supporting the safety or regulatory approval of food products, except in unique instances where there has been a need to confirm premarket dietary intake estimates and ensure safety. The call for postmarket monitoring of biotechnology-derived foods with respect to adverse consequences to human health is apparently driven by nebulous concerns for unexpected long-term effects. Ironically, the notion of required postmarket monitoring for adverse effects from any biotechnology-derived food contradicts the intent of any premarket safety assessment and undermines confidence in any conclusion of safety derived from that assessment (Chesson 2001). In reality, the identification of a potential food safety risk during the premarket safety assessment of a food from a biotechnology-derived crop would effectively end any endeavor to commercialize that crop. In light of the knowledge of the safety of those foods derived from biotechnology currently on the market, postmarket monitoring requires definition and a strong scientific basis to be a worthwhile endeavor that complements the premarket assessment. Moreover, there is no basis to conclude that postmarket monitoring should be applied only to biotechnology-derived foods. It is not evident that inherent differences exist between conventionally derived foods and those derived from biotechnology that would necessitate the use of postmarket monitoring specifically in one context but not the other.

7.1.3 Postmarket monitoring of foods and food ingredients

Postmarket monitoring is not standard practice in supporting the safety of a food product. However, the regulatory approval of certain novel foods and food additives has been contingent upon the conduct of monitoring programs. It is notable that these studies were conducted in the absence of regulatory guidelines on the design or conduct of postmarket monitoring for foods. For example, the United States Food and Drug Administration (FDA) made the approval of the sweetener aspartame (Butchko and others 1994) and the fat substitute olestra (Thornquist and others 2000; Allgood and others 2001; Slough and others 2001) conditional on postmarket monitoring studies. Similarly, the European Union (EU) Scientific Committee on Food (SCF) advised that required postmarket monitoring accompany the marketing of "yellow fat spreads with added phytosterol esters". Postmarket monitoring for these substances under regulatory mandate in the EU (SCF 2002) included active and passive components designed to address

specific hypotheses, with program priorities identified relative to the particular case-by-case needs of each situation. Borzelleca (1995) defined active surveillance as "…a carefully conceived and structured plan for obtaining information from consumers. It is initiated by the manufacturer who contacts consumers directly…" and passive surveillance as "…the collection of voluntary responses by consumers without input from the producer, distributor, or regulator." Passive surveillance or monitoring may be useful in cases where immediate idiosyncratic adverse effects, such as allergenicity, are of concern. However, the scope of the hypotheses that can be answered via passive monitoring and, therefore, its utility without further follow-up is limited. The confirmation of any health effect attributed to the consumption of a food requires the use of appropriately validated diagnostic tools. Active monitoring may be appropriate when confirmation of premarket consumption projections is required, when effects identified through passive monitoring require targeted investigation, or when long-term monitoring for potential chronic health effects is of interest. In the latter case, it is arguable that postmarket monitoring no longer constitutes monitoring per se, but falls instead into the realm of epidemiology, and that appropriate epidemiological techniques should therefore be applied.

Recent mandates within the EU have made environmental postmarket monitoring of GM organisms obligatory (EC 2001a), and a preliminary guidance document for notifiers and risk assessors has been published (EC 2002). The directive requires that manufacturers design and implement an environmental postmarket monitoring plan with the aim of gathering information on the immediate and cumulative long-term effects of exposure to GM organisms. The mandate includes both general monitoring for unanticipated adverse effects and, where necessary, the hypothesis-driven confirmation of potential effects identified through the premarket environmental risk assessment process.

In addition to the premarket safety assessment required by the European Novel Food Regulation (EC 1997a), postmarket monitoring of GM foods and feeds and products derived from GM food ingredients has also been proposed (EC 2001b). By definition, the proposed directive requires the implementation of both passive and active measures, with specific initiatives targeted on a case-by-case basis as a result of the risk assessment process. The EU (EC 1997b) and the Joint FAO/WHO Expert Consultation on foods derived from biotechnology (FAO/WHO 2000) have also advocated the use of monitoring programs in a nutritional context to assess the impact of the introduction of a novel food on dietary consumption patterns and to evaluate the potential effects these changes may have on the nutritional and health status of specific populations. Regulatory initiatives analogous to the recent EU directives have not been formally proposed in North America, Australia, New Zealand, or Japan.

Any postmarket monitoring program will be of limited value unless precise estimates of exposure to a particular product are available. Consequently, the collection of adequate consumption data and methods for ensuring the traceability of the biotechnology-derived food of interest are crucial elements in this process.

7.1.4 What constitutes a testable hypothesis for postmarket monitoring?

The premarket safety assessment of biotechnology-derived foods is designed to identify areas of safety or nutritional concern before the introduction of these foods for general consumption. Therefore, postmarket monitoring programs may be appropriate when specific hypotheses requiring directed investigation have been identified as a result of the premarket assessment and the data generated from premarket studies are unable to address the particular hypothesis in question. Ill-defined use of postmarket monitoring is unwarranted, and could be considered misguided, as it implies that postmarket monitoring is an appropriate means of hazard identification, as opposed to a control option for risk management possibly undermining the reliability of or the confidence in traditional approaches for determining food safety. Due to the resource intensive nature of postmarket monitoring, definite goals for a monitoring program should be established a priori, particularly when attempting to determine whether postmarket monitoring is necessary and/or appropriate.

7.2 Potential Applications of Postmarket Monitoring

The investigation of idiosyncratic adverse events or chronic health effects, the confirmation of premarket exposure estimates, or the identification of changes in dietary intake patterns, represent examples, where in certain instances, hypotheses may be appropriately tested through postmarket monitoring programs. As indicated previously, the success of any postmarket monitoring strategy is dependent on the accurate estimation of exposure in targeted or affected population groups; the ability to measure a specific outcome of interest, either directly or through appropriate biomarkers; and the control of confounding factors.

7.2.1 Idiosyncratic adverse events

The potential allergenicity of foods derived from biotechnology has been recognized as relevant for particular consideration because some of the modifications in these foods may include the expression of proteins not otherwise present in that food (Taylor and Hefle 2002). The presence of proteins per se does not merit attention as a concern regarding the safety of foods derived from biotechnology. However, the current definition of food allergens (for example, proteins eliciting an IgE-mediated hypersensitivity response in sensitive individuals following consumption) dictates that an assessment for allergenic potential is in order when the biotechnology-derived food or food ingredient contains a novel protein that would otherwise not be present in food. A premarket decision tree strategy for assessing the allergenicity of GM foods has been recommended by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO 2000; 2001) and has proceeded to Step 8 of the Codex Alimentarius Commission's assessment of the safety of foods derived through biotechnology (Codex 2002). The lack of a single definitive assay predictive of the allergenic potential of a food protein limits this strategy as a positive predictor of allergenicity. However, an absence of positive results in the overall outcome of the decision tree provides reasonable assurance that the likelihood of allergenicity is low. Therefore, based on the scientific assessment applied prior to the commercialization of a biotechnology-derived food as described in Chapter 3, a postmarket monitoring program implemented strictly to address allergenicity should not be necessary.

Food allergies represent a unique challenge in ensuring the safety of any food introduced into the population, biotechnology-derived or otherwise. Implementation of a postmarket monitoring program for allergenicity strictly to complement premarket assessment data, or appease acceptance of scientific uncertainty, should be tempered by the knowledge that allergic reactions to food represent individualistic responses in that the majority of the population can consume the offending food without adverse reaction. It would be reasonable to expect that the entire population would not be susceptible to allergic reaction following consumption of a biotechnology-derived food. Caution must be exercised in the monitoring approach taken and efforts should be made to distinguish those populations that may be at particular risk, as defined by the specific conditions under which postmarket monitoring is attempted. Guidance toward susceptible populations of individuals should be available from the multiple assessment tests conducted in the premarket evaluation.

A postmarket monitoring program to assess the potential allergenicity of a food derived from biotechnology would represent a passive reporting program for spontaneous adverse events occurring under conditions of acute postmarket exposure. In addition, the program must contend with elements related to exposure assessment (for example, accurate estimates of consumption, traceability, and so on) in order to provide monitoring data of use. Furthermore, such a program must be aware of further challenges that exist due to limitations in the extent of current understanding regarding food allergens. While it is reported that greater than 90% of all food allergies worldwide are caused by proteins associated with the consumption of cow's milk, crustaceans, eggs, fish, peanuts, soybeans, tree nuts, and wheat, or ingredients derived from these foods, the properties of a protein that make it allergenic, as well as the biological variables governing a person's susceptibility to developing an allergy, remain to be determined (Taylor and Hefle 2002). Allergic reactions to food clearly represent a special case of food safety. Research is ongoing; however, the minimum amount of allergenic protein and duration of exposure necessary to induce sensitization are not yet known. Additionally, once an individual is sensitized, the level of subsequent exposure to the allergenic protein needed to trigger an allergic response requires delineation (Taylor and others 2002).

7.2.2 Chronic health effects

The association between diet and health is well recognized. Notwithstanding this fact, it is difficult to establish a causal relationship between a particular dietary component and a specific health endpoint, even when an extensive amount of research has been conducted. For example, more than 50 y of evidence has been required to support the currently accepted relationship between dietary modification and the risk of coronary heart disease (Schaefer 2002).

The use of a postmarket monitoring program to identify chronic adverse health effects not otherwise indicated by the premarket safety assessment would be highly impractical, if not scientifically impossible. This would require the prospective long-term monitoring of a specific population in the absence of a hypothetical outcome, or alternatively, the retrospective identification of potential exposure to a particular biotechnology-derived food in a group of individuals known to have a specific disease or health condition. Without proper study design and the identification of appropriate hypotheses, confounding factors would almost certainly plague any observational investigation, as it is difficult to control for these factors even in well-defined dietary surveys with targeted outcomes. Apart from the special case of allergenicity, which could be considered an acute adverse event, the potential for adverse effects based on the chronic consumption of biotechnology-derived foods would be no different than those associated with the chronic consumption of other foods, and by definition should be minimal, because the approval of a food is dependent on its premarket assessment of safety.

A food with a potentially beneficial effect on human health, as indicated in premarket assessment studies, may require confirmation of its purported benefits through postmarket monitoring or conclusive demonstration of a positive diet-health relationship in clinical trials. With the advent of biotechnology-derived foods designed to provide specific nutritional benefits or improved functional characteristics, the use of postmarket monitoring to detect physiologically relevant beneficial health effects may be more appropriate and more readily applicable than the situation depicted for adverse effects. In this case, postmarket monitoring would constitute a traditional targeted epidemiological study specifically designed to monitor the long-term benefits of a particular biotechnology-derived food. Difficulties associated with implementing this type of investigation would be similar to the difficulties associated with any long-term dietary study, particularly in terms of compliance, mitigating or confounding factors, contributing risk factors, and the inherent heterogeneity of the study population in terms of predisposition to effects (van den Brandt and others 2002). Continual changes in both food availability and individual dietary habits also make it difficult to establish a direct causal relationship between any observed health benefits and the long-term consumption of foods derived from biotechnology.

7.2.3 Confirming premarket exposure estimates

A key step in the premarket safety assessment process is the generation of dietary intake estimates and their comparison to published guidelines, such as acceptable daily intakes for food additives, tolerable upper intake levels for nutrients, or provisional tolerable weekly intakes for contaminants. A tiered approach to exposure assessment is generally advocated, whereby crude screening tools are used to dictate the need for more highly refined consumption estimates (Kroes and others 2002). The premarket exposure assessment may provide an initial screening of consumption, as it is often based on several conservative or "worst-case" assumptions that result in overestimates of intake. When no toxicologically significant exposure is indicated as a result of this assessment, further refinement would not be warranted.

In certain situations, postmarket monitoring may provide valuable confirmation of premarket consumption projections. For instance, the premarket exposure assessment may not capture real-world exposure scenarios, or a more accurate estimate of intake may be desired for comparison with upper limits of safety. As well, premarket exposure estimates may not adequately characterize the consumption of specific population groups or certain age groups (Kroes and others 2002). In these situations, postmarket assessment provides an additional measure of safety, through the validation of premarket assumptions regarding anticipated intake levels and additional sampling to determine patterns of exposure in specific subgroups of the population, particularly those individuals at potentially greater risk.

7.2.4 Identifying changes in dietary intake patterns

With the advent of foods derived from GM crops with improved nutritional characteristics or improved functionality, consumers may actively choose products based on nutritional or physiological benefits. Consequently, actual levels of exposure and any subsequent impact on dietary consumption patterns, and therefore nutritional status, may not be evident until after the market introduction of the biotechnology-derived food product. Alterations in the micro- or macronutrient profile of a biotechnology-derived food could significantly affect dietary intakes, causing nutrient deficits or, alternatively, causing intakes to approach or exceed the upper level (FAO/WHO 2000). Similarly, nutritional status may be affected if the biotechnology-derived food product alters consumption patterns via the displacement of other core foods or food components (EC 1997b). In specific cases, these considerations may necessitate the use of postmarket monitoring. However, the presence of confounding factors makes the long-term monitoring of consumption patterns difficult and minimizes any observed associations between diet and health, because those individuals who make a nutritionally significant or healthy food-choice are likely to also have significantly different dietary patterns from individuals who do not make similar choices.

7.3 Methodological Considerations

7.3.1 Measuring population exposure to foods and food ingredients

7.3.1.1 Tracking the disappearance of biotechnology-derived

foods into the food supply. The implementation of a postmarket monitoring strategy designed to identify positive or negative effects on human health is dependent upon the availability of accurate consumption data. Exposure assessment methods that have traditionally been used to quantify the intakes of low-molecular weight chemicals, micro- and macronutrients, and whole foods, could theoretically be applied to the postmarket monitoring of biotechnology-derived foods to obtain appropriate consumption estimates and to confirm premarket predictions.

Exposure to a particular substance can be quantified if the food chemical concentration and consumption of a particular food are known or can be estimated with a certain degree of reliability. There are 2 elements in this process. Adequate data must be available to assess the fate of the product or commodity within the food supply. Secondly, a monitoring program that provides requisite information on the consumption of relevant foods must be established. The hypotheses of the monitoring program dictate the level of accuracy required in the generation of intake estimates via an exposure assessment, which in turn are dependent on the precision of the supporting data. Therefore, the contribution of exposure assessment to the overall postmarket monitoring process is directly related to the quality of information underlying the evaluation. This requirement emphasizes the need for setting study objectives a priori, to ensure that adequate and appropriate data are collected for subsequent analyses and that potential future uses of the data are anticipated. In the event that existing data are used to estimate the intake of novel foods or food components, such as biotechnology-derived foods or foods containing biotechnology-derived ingredients, this also highlights the need for standardized methods of data collection in terms of both food consumption and food composition, to enable meaningful comparisons of results among different countries.

To appropriately quantify the concentration of a biotechnology-derived food or ingredient therefrom at the level of the processed food material or brand, processes for ensuring the traceability of the commodity or food ingredient must be readily available to document the movement of such material through the feed and food chains (Stave 2002). If particular food products containing biotechnology-derived ingredients could not be identified with a high level of accuracy throughout all levels of food production, processing, and distribution, the monitoring of latent health effects from biotechnology-derived foods would be very difficult, if not impossible. For some, there is a tendency to consider postmarket monitoring not on a case-by-case basis, but as the complete representative monitoring of adverse health effects from exposure to foods derived from biotechnology. For this scenario to generate useful data, a central repository for the biotechnology content of specific foods or primary agricultural commodities would need to be available and current food composition databases would need to be expanded to incorporate industry-specific information. In addition, if the aim of a postmarket monitoring program was to monitor potential long-term effects on human health, temporal information on food composition would need to be included in these databases. Historical, current, and future levels of biotechnology-derived food components in the food supply would need to be accurately measured and predicted in order to limit discontinuity in food composition information. Clearly, this would be a time-intensive and not particularly cost-effective effort, especially in view of the low risk already assured by the premarket assessment process.

7.3.1.2 Integrating disappearance data with food consumption databases. The accuracy of the exposure assessment for foods derived from biotechnology or ingredients therefrom is dependent on the model used and the validity of the underlying parameters (Kuiper and others 2001). To assess the intake of specific food components, mathematical methods for integrating food composition and consumption data include, in ascending order of refinement, point estimates, simple distributions, and probabilistic modeling or Monte Carlo analysis. Point estimates combine a single estimate of food chemical concentration with a corresponding fixed, generally "worst-case," estimate of food consumption. Alternatively, simple distributions combine the distribution of exposures with a fixed value for chemical concentration. Lastly, probabilistic analyses include a measure of the variability associated with both food consumption and food composition data. By utilizing data from these respective distributions, an estimate of the probability of various exposure outcomes can be calculated through iterative mathematical modeling. Probabilistic modeling is especially relevant when considering the introduction of nutritionally improved foods derived from biotechnology, where several different products may be modified for a similar nutritional benefit or physiological health effect. Monte Carlo assessment could determine the probability of aggregate exposure to several different biotechnology-derived food sources with the same functional characteristics and therefore compare intended food uses with consequent exposure in particular target groups, susceptible subgroups, or the population in general. Consequently, depending on the data collected, the specific endpoint being investigated, and the precision required from the exposure assessment, an appropriate method of analysis can be selected.

When considering the long-term health effects of foods derived from biotechnology, habitual or lifetime intake estimates may be necessary. However, the overestimation of either food consumption or the biotechnology-derived content of relevant foods in a long-term epidemiologic study would lead to the overestimation of exposure. Therefore, the risks or benefits associated with the biotechnology-derived food would subsequently be underestimated. Similarly, if an acute endpoint such as allergenicity is of interest, in which a single critical exposure may precipitate an adverse event, reliable data must be available regarding the consumption of relevant foods, because knowledge of the intake required to induce or elicit a response is limited. If accurate exposure assessments are needed, probabilistic modeling provides a valuable refinement to the coarse evaluation obtained via deterministic approaches. However, the cost and resources needed to implement these methods vary substantially, and the potential to determine causation may be limited. The postmarket monitoring method used to estimate consumption should be evaluated a priori on a case-by-case basis to determine the most appropriate monitoring tool for establishing a specific relationship between the nutritional enhancement or characteristic trait of the food derived from biotechnology and its potential effects on human health.

7.3.2 Demonstration of causality

One intended outcome of a postmarket monitoring strategy for a food with some purported effect on human health may be the demonstration of causality. The consideration of a valid scientific hypothesis supported by measurable parameters is a required element of a postmarket monitoring program intended to distinguish a cause-effect relationship between the consumption of food and a specific health effect to the consumer. In the absence of defined hypotheses, data mining to investigate health-related effects is likely to identify random correlations not limited to the consumption of foods derived from biotechnology.

While the safety of foods derived from biotechnology is established in the premarket assessment, and efficacy may be demonstrated in pre- or postmarket studies, the ability to discern unpredicted adverse effects, or intended beneficial effects on health is a challenge given the myriad of confounding variables encountered under real-world conditions. Correlations that have not been adjusted for confounding factors may be spurious and not causally

related to dietary exposure to a food derived from biotechnology. Certain confounding or contributing risk factors should be anticipated before implementing a postmarket monitoring program, and these risk factors should be minimized through proper statistical design and analyses. Furthermore, the ability of any data collected in a postmarket monitoring program to demonstrate causality may be limited by the characteristics of the population included in the study program. In a postmarket monitoring program, establishing that reported effects on human health are the result of exposure to a consumed food cannot rely on the notion of definitive proof, but rather on the weight of evidence to establish a scientific basis of association. Hill (1965) put forth a list of criteria to consider before inferring causality from an observed association between a factor and an effect. Substituting the consumption of a food derived from biotechnology as a factor for causality in these criteria provides a basis for consideration of any purported health-related effects from these foods. However, inherent to Hill's criteria are clear expectations of adequate quantification of exposure to the biotechnology-derived food, incorporating accurate estimates of consumption and use, and the control of confounding factors, which may otherwise infer associations that are not valid. It is not expected that all criteria be met in order to conclude causality. However, supportive evidence within these items would contribute toward the overall strength of a cause-effect relationship.

7.4 Conclusions and Recommendations

The same premarket safety assessment principles apply to both traditional foods and foods derived from biotechnology. However, experience indicates that foods derived from biotechnology have been evaluated more rigorously than foods derived through traditional means. The use of postmarket monitoring in the absence of a specific, testable hypothesis to establish a causal relationship between the consumption of foods derived from biotechnology and potential adverse or beneficial effects on human health would be difficult, if even technically feasible.

When evaluating the feasibility of postmarket monitoring several points bear consideration:

Recommendation 7-1. Postmarket monitoring should be based on scientifically driven hypotheses relative to endpoints that potentially affect human health.

Recommendation 7-2. Monitoring should not be dependent on the technology used to develop the food, but should be applied similarly to all food products, based on a well-defined strategy for determining on a case-by-case basis whether monitoring is necessary and/or appropriate.

Recommendation 7-3. The premarket assessment will identify safety and nutritional concerns. It is unlikely that any new product with scientifically valid adverse health concerns will be marketed. Postmarket monitoring of nutritionally improved food products may be useful to verify premarket dietary exposure assessments or to identify changes in dietary intake patterns. Postmarket monitoring should only be conducted when a scientifically valid testable hypothesis exists, or to verify premarket exposure estimates.

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Glossary

Abiotic Stress: Nonliving (outside) factors that can cause harmful effects to plants, such as soil conditions, drought, extreme temperatures.

Agrobacterium tumefaciens: Microorganism (bacterium) that produces crown gall disease; most likely by introducing a part of its genetic material into the plant. The ability to transfer the DNA, called T-DNA, is carried primarily on one or more large Ti plasmids. This ability to transfer T-DNA has been used for introducing new genetic information into plant cells.

Agronomic Performance/Input Trait: Pertaining to practices of agricultural production and its costs and the management of cropland. Examples include yield, insect protection, herbicide resistance and stress tolerance.

Aldolase: An enzyme, not subject to allosteric regulation, that catalyzes in a reversible reaction the cleavage of fructose 1,6-biphosphate to form dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. This enzyme catalyzes the 4th reaction in the glycolytic pathway, which splits a monosaccharide into 2 3-carbon units.

Allopolyploid Plants: Plants having more than 2 sets of chromosomes inherited from different species.

Allosteric Regulation: Regulation of an enzyme's activity by binding of a small molecule at a site that does not overlap the active site region.

Amino Acid: The constituent subunit of proteins. Amino acids polymerize to form linear chains linked by peptide bonds; such chains are termed polypeptides (or proteins if large enough). Most proteins are composed of twenty commonly occurring amino acids.

Anabolic: That part of metabolism that is concerned with synthetic reactions.

Aneuploid: Having a chromosome number that is not an exact multiple of the haploid number, caused by one or more chromosome sets being either incomplete or present in extra numbers.

Antibody: A protein produced by the immune system in response to an antigen (a molecule that is perceived to be foreign). Antibodies bind specifically to their target antigen to help the immune system render the foreign entity harmless.

Antinutrients: Substances that act in direct competition with or otherwise inhibit or interfere with the use or absorption of a nutrient.

Antisense RNA: RNA transcribed from the noncoding DNA strand of a gene, which is expected to form a complex with the RNA transcribed from the sense (coding) strand, in many cases inhibiting translation of the target mRNA.

***Bacillus thuringiensis* (Bt):** A naturally occurring microorganism that produces a protein or proteins that only kill specific groups of organisms with alkaline stomachs, such as insect larvae. When delivered as a part of the whole organism, these proteins have been used for biological control for decades. The genetic information that encodes the proteins was identified and moved into plants to make them insect tolerant.

Bacteriophage: a virus that lives in, and for certain species, kills bacteria.

Bioinformatics: The discipline encompassing the development and utilization of computational facilities to store, analyze and interpret biological data.

Biosynthesis: Formation of a chemical compound by a living organism.

Biotechnology: the integration of natural sciences and engineering sciences, particularly recombinant DNA technology and genetic engineering, in order to achieve the application of organisms, cells, parts thereof and molecular analogs for products and services. (Modified from: European Federation of Biotechnology, as endorsed by the Joint IUFOST/IUNS Committee on Food, Nutrition and Biotechnology, 1989).

Biotic Stress: Living organisms that can harm plants, such as viruses, fungi, bacteria, harmful insects, and nematodes.

Calvin Cycle: A series of enzymatic reactions, occurring during photosynthesis, in which glucose is synthesized from carbon dioxide.

Catabolic: That part of metabolism that is concerned with degradative reactions.

Cell Cycle: The term given to the series of tightly regulated steps that a cell goes through between its creation and its division to form 2 daughter cells.

Chloroplast: A chlorophyll-containing photosynthetic organelle found in plant cells that can harness light energy to synthesize organic compounds.

Chromosome: Subcellular structures which convey the genetic material of an organism.

Comparative Genomics: The comparison of genome structure and function across different species to understand biological mechanisms and evolutionary processes.

Complementary DNA (cDNA): DNA generated from an expressed messenger RNA through a process known as reverse transcription.

Composition Analysis: The determination of the concentration of compounds in a plant or animal tissue. Compounds that are commonly quantified are proteins, fats, carbohydrates, minerals, vitamins, amino acids, fatty acids and antinutrients.

Conventional Breeding: Breeding of plants carried out by controlled transfer of pollen from one plant to another followed by selection of progeny through multiple generations for a desired phenotype. This method has included radiation or chemical mutation of plants or seeds to induce extra variation in the donor material.

Coproduct: The plant material remaining after a particular component (usually a valuable component) has been extracted. Typical examples are the meal that remains after extraction of oil from soybean or cottonseeds.

Coumarins: White vanilla-scented crystalline esters used in perfumes and flavorings and as an anticoagulant. Formula: $C_9H_6O_2$.

Crossbreeding: Interbreeding (animals or plants) between parents of different races, varieties, breeds, etc.

Diet: A specific allowance or selection of food or feed that a person or animal regularly consumes.

Diploidy: An organism that contains 2 copies of each chromosome (2N).

DNA (deoxyribonucleic acid): The chemical that comprises the genetic material of all cellular organisms. DNA consists of 2 helical complementary strands of nucleic acid made up of 4 nucleotide subunits (A, C, G, and T).

DNA Microarray: A microarray composed of nucleic acid molecules of known composition linked to a solid substrate, which can be probed with total messenger RNA from a cell or tissue

to reveal changes in gene expression relative to a control sample. This form of microarray technology allows the expression of many thousands of genes to be assessed in a single experiment.

DNA Sequencing: Technologies through which the order of base pairs in a DNA molecule can be determined.

Dose-Response Assessment: The determination of the relationship between the magnitude of exposure (dose) of a chemical, biological or physical agent to the severity and/or frequency of an associated health effect (response).

Electroporation: A method for transferring DNA, especially useful for plant protoplasts, in which high voltage pulses of electricity are used to open pores in cell membranes through which molecules (such as DNA or RNA) can pass.

Enterotoxins: Toxin affecting the cells of the intestinal mucosa.

Enzyme: A biological catalyst: a protein that controls the rate of a biochemical reaction.

Eukaryote: An organism whose cell(s) show internal compartmentalization in the form of membrane-bound organelles (includes animals, plants, fungi and algae).

Event: A term used to describe a plant and its offspring that contain a specific insertion of DNA. Events are distinguishable from each other by their unique site of integration of the introduced DNA.

Exon: The coding regions within eukaryotic genes that are separated by introns (non-coding regions). Exons are spliced together to form the messenger RNA molecule created from a gene after transcription, prior to translation (protein synthesis).

Exposure Assessment: The qualitative and/or quantitative evaluation of the likely exposure to biological, chemical and/or physical agents via different routes.

Expressed Sequence Tag (EST): Partial or full complementary DNA sequences which can serve as markers for regions of the genome that encode expressed products.

Flavonoids: Any of a group of organic compounds that occur as pigments in fruit and flowers.

Food Additive: Any substance not normally consumed as a food by itself and not normally used as a typical ingredient of food, whether or not it has nutritive value. The intentional addition of which to a food is for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results, or may be expected to result (directly or indirectly), in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods.

Food: Any substance, whether processed, semi-processed or raw, which is intended for human consumption, including drink, chewing gum and any substance that has been used in the manufacture, preparation or treatment of "food"; does not include cosmetics or tobacco or substances used only as drugs.

Fructan: A type of polymer of fructose, present in certain fruits.

Functional Foods: The Institute of Medicine's Food and Nutrition Board defines functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains."

Functional Genomics: The development and implementation of technologies to characterize the mechanisms through which genes and their products function and interact with each other and with the environment. This is usually applied to studies of the expression of large numbers of genes simultaneously.

Gas Chromatography: Analytical technique in which compounds are separated based on their differential movement in a stream of gas through a (coated) capillary at elevated temperature. This technique is suitable for the analysis of volatile compounds or compounds that can be made volatile by derivatization reactions and that are also stable at higher temperatures.

Gel Electrophoresis (1-dimensional): Analytical technique by which usually large biomolecules (proteins, DNA) are separated through a gel by application of an electric current. Separation may depend on, for example, charge and size of the molecules. Separated biomolecules may be visualized as individual bands at different positions within each lane of the gel.

Gene Expression: The process through which a gene is activated at a particular time and place so that its functional product is produced.

Gene Silencing: The effect of the expression in a cell of an mRNA complementary or identical in nucleotide sequence to an expressed mRNA from either an endogenous or introduced gene, thereby reducing the level or translation of the mRNA from the target gene.

Gene Transfer: The transfer of genes to an organism. Usually used in terms of transfer of a gene to an organism other that the original organism, through the tools of biotechnology.

Gene: The fundamental unit of heredity. In molecular terms, a gene comprises a length of DNA that encodes a functional product, which may be a polypeptide (a whole or constituent part of a protein) or a ribonucleic acid.

Genetic Code: The relationship between the order of nucleotide bases in the coding region of a gene and the order of amino acids in the polypeptide product. It is a universal, triplet, non-overlapping code such that each set of 3 bases (termed a codon) specifies which of the 20 amino acids is present in the polypeptide chain product at a particular position or stopping of translation.

Genetic Map: A diagram showing the positions of genetic markers along the length of a chromosome relative to each other (genetic map) or in absolute distances from each other (physical map).

Genetically Engineered or Genetically Modified (GE or GM): The product of the manipulation of an organism's genetic endowment by introducing or eliminating specific genes through modern molecular biology techniques. A broad definition of genetic engineering also includes selective breeding and other means of artificial selection.

Genetics: The study of heredity.

Genome: The sum total of the genetic material present in the chromosomes of a particular organism. This includes both the DNA present in the chromosomes and that in subcellular organelles (that is, mitochondrial and chloroplasts).

Genomics: Science that studies the genomes (that is, the complete genetic information) of living organisms. This commonly entails the analysis of DNA sequence data and the identification of genes.

Genotype: The total genetic constitution of an organism.

Glycoalkaloid Toxins: Steroid-like compounds produced by plant members of the botanical family *Solanaceae*, most notably "solanine" present in potato tubers.

Golden Rice: Genetically engineered rice that produces β-carotene, a substance that the body converts to Vitamin A. This improved-nutrient rice was developed to treat individuals suffering from vitamin A deficiency, a condition that afflicts millions of people in developing countries, especially children and pregnant women.

Hazard: A biological, chemical, or physical agent, or condition, with the potential to cause an adverse health or environmental effect.

Hazard Characterization: The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents. For chemical agents, a dose-response assessment should be performed if the data are obtainable.

Hazard Identification: The identification of biological, chemical,

and physical agents capable of causing adverse health or environmental effects.

Heterozygote: With respect to a particular gene at a defined chromosomal locus, a heterozygote has a different allelic form of the gene on each of the 2 homologous chromosomes.

Homozygote: With respect to a particular gene at a defined chromosomal locus, a homozygote has the same allelic form of the gene on each of the 2 homologous chromosomes.

Hormone: A molecule secreted by a cell or tissue in an organism, which has a functional consequence in other cells located remotely.

Hybrid: (1) Plant Breeding: The offspring of 2 parents differing in at least one genetic characteristic (trait). Here referring to the offspring of plant that have been bred to have multiple differences which complement in the hybrid to give the plant improved agronomic characteristics. (2) Molecular Biology: A heteroduplex DNA or DNA-RNA molecule.

Inbred: Progeny produced as a result of breeding between genetically similar parents.

Inserted DNA: The segment of DNA that is introduced into the chromosome, plasmid or other vector using recombinant DNA techniques.

Introgressed: Backcrossing of hybrids of 2 plant populations to introduce new genes into a wild population.

Intron: A non-coding sequence within eukaryotic genes that separates the exons (coding regions). Introns are spliced out of the messenger RNA molecule created from a gene after transcription, prior to translation (protein synthesis).

Inulins: a fructose polysaccharide present in the tubers and rhizomes of some plants. Formula: $(C_6H_{10}O_5)n$.

Invertase Activity: Enzyme activity occurring in the intestinal juice of animals and in yeasts, that hydrolyses sucrose to glucose and fructose.

Isoflavones: Water-soluble chemicals, also known as phytoestrogens, found in many plants and so named because they cause effects in the mammalian body somewhat similar to those of estrogen. The most investigated natural isoflavones, genistein and daidzen, are found in soy products and the herb red clover.

Knock-out: A technique used primarily in mouse genetics to inactivate a particular gene in order to define its function by insertion of an introduced DNA fragment into the gene or its controlling elements.

Lectins: Agglutinating proteins usually extracted from plants.

Library: A collection of genomic or complementary DNA sequences from a particular organism that have been cloned in a vector and grown in an appropriate host organism (for example, bacteria, yeast).

Linkage: The phenomenon whereby pairs of genes that are located in close proximity on the same chromosome tend to be coinherited.

Liquid Chromatography: Analytical technique in which substances are separated based on their differential movement within a liquid stream. A common form is column chromatography in which the dissolved substances may bind differentially to a column of solid material and be carried at different speeds by the liquid through the column, thus creating a basis for separation.

Locus: The specific site on a chromosome at which a particular gene or other DNA landmark is located.

Macronutrient: In humans and animals, a substance that is required in relatively large amounts for healthy growth and development, and belongs to one of 3 groups: carbohydrates, fats, and proteins.

Mass Spectrometry: An analytical technique by which compounds are ionized and the resulting ions are separated by mass under vacuum in route to a detector.

Metabolite: A substance produced during or taking part in metabolism.

Metabolomics: "Open-ended" analytical techniques that generate profiles of the metabolites, that is, chemical substances within a biological sample. Differences between profiles of different (groups of) samples are determined and the identity of the associated metabolites elucidated. Contrary to targeted analysis, these techniques are indiscriminate in that they do not require prior knowledge of every substance that is present.

Microarray: A microscopic, ordered array of nucleic acids, proteins, small molecules, cells or other substances that enables parallel analysis of complex biochemical samples. The 2 most common are cDNA arrays and genomic arrays.

Micronutrient: In humans and animals, a substance, such as a vitamin or trace element, essential for healthy growth and development but required only in minute amounts.

Mitochondria: Cellular organelles present in eukaryotic organisms that enable aerobic respiration, which generates the energy for cellular processes. Each mitochondrion contains a circular DNA encoding a small number of genes (approximately 50).

Modern Biotechnology: See biotechnology.

Molecular Biology: The study of biological processes at the molecular level, typically referring to DNA and/or RNA.

Mutation Breeding: Genetic change caused by natural phenomena, radiation or the use of mutagens. Stable (inheritable) mutations in genes are passed on to offspring.

Mutation: A structural change in a DNA sequence resulting from uncorrected errors during DNA replication.

Nuclear Magnetic Resonance: An analytical technique by which compounds are exposed to a magnetic field that induces magnetic dipoles within the nucleus of particular atoms inside these compounds. The magnetic energy conveyed to these atoms is subsequently released as radiofrequency waves, whose frequency spectrum provides information on the structure of the compounds.

Nucleotide (Nucleotide Base): Nucleotides are the fundamental subunits from which DNA and RNA molecules are assembled. A nucleotide is a base molecule (that is, adenine, cytosine, guanine and thymine in the case of DNA), linked to a sugar molecule and ribose or deoxyribose phosphate group for RNA or DNA, respectively.

Nucleus: In eukaryotic cells, the centrally located organelle that encloses the chromosomes containing the genomic DNA. Minor amounts of non-genomic DNA are also found in the mitochondria and chloroplasts.

Nutritionally Improved *or* Quality Trait Crops: Food or feed crops in which the quantity, ratio and/or bioavailability is enhanced for either essential macro- and/or micronutrients or other compounds for which the evidence indicates that they play a significant role in maintenance of optimal health, growth, and development.

Nutraceutical: The term was coined by the Foundation for Innovation in Medicine in 1991 and is defined as "any substance that may be considered a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease."

Organoleptic: Able to perceive a sensory stimulus such as taste.

Pesticide: Any substance intended for preventing, destroying, attracting, repelling or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities, or animal feeds, or which may be administered to animals for the control of ectoparasites. The term normally excludes fertilizers, plant and animal nutrients food additives

and animal drugs.

Phage: See bacteriophage.

Pharmacogenomics: The identification of genes that influence individual variation in the efficacy or toxicity of therapeutic agents, and the application of this information in clinical practice.

Phenotype: The observable characteristics of an organism.

Phenylpropanoids: Especially the derivatives of the cinnamyl alcohols and of cinnamic acids, isolated from medicinal plants.

Phytate (Phytic Acid): A phosphorus-containing compound in the outer husks of cereal grains that, in addition to limiting the bioavailability of phosphorous itself, binds with minerals and inhibits their absorption.

Phytochemicals: Small molecule chemicals unique to plants and plant products.

Plasmid: Circular extra-chromosomal DNA molecules present in bacteria and yeast. Plasmids replicate autonomously each time the organism divides and are transmitted to the daughter cells. DNA segments are commonly cloned using plasmid vectors.

Plasticity: The quality of being plastic or able to be molded, changed.

Plastid: Any of various small particles in the cytoplasm of the cells of plants and some animals that contain pigments (also called chromoplasts), starch, oil or protein. Here also referring to chloroplast-related components.

Polymerase Chain Reaction (PCR): A molecular biology technique through which specific DNA segments are amplified selectively. The process mimics in vitro the natural process of DNA replication occurring in all cellular organisms, where the DNA molecules of a cell are duplicated prior to cell division. The original DNA molecules serve as templates to build daughter molecules of identical sequence.

Post-transcriptional Modification: A process through which protein molecules are biochemically modified within a cell following their synthesis. A protein may undergo a complex series of modifications in different cellular compartments before its final functional form is produced.

Profiling: Creation of patterns of the substances within a sample with the aid of analytical techniques, such as functional genomics, proteomics, or metabolomics. The identity of the compounds detectable within the pattern need not be previously recognized.

Prokaryote: An organism or cell lacking a nucleus and other membrane bounded organelles. Bacteria are prokaryotic organisms.

Promoter: A DNA sequence that is located in front of a gene that controls mRNA transcription.

Protein: Biological effector molecules that consist of one or more polypeptide chains of amino acid subunits encoded by an organism's genome. The function of a protein largely depends on its 3 dimensional structure, which is determined by its amino acid composition and any post-translational modifications.

Proteomics: Techniques used to investigate the protein products of the genome and how they interact to determine biological functions. This is an "open ended" analytical technique that is used to find differences between samples and determine the identity of the associated proteins. Contrary to targeted analysis, this technique is indiscriminate in that it does not require prior knowledge of every protein.

Protoplast: A plant cell from which the cell wall has been removed by mechanical or enzymatic means. Protoplasts can be prepared from tissues of most plant organs as well as from cultured plant cells.

Protoplast Fusion: The fusion of 2 plant protoplasts.

Quality Protein Maize (QPM): A variety of maize (corn) that contains 70 to 100% more of 2 essential amino acids—lysine and tryptophan—in the grain than most varieties of maize. QPM was developed at the Mexico-based International Maize and Wheat Improvement Center (CIMMYT).

Quantitative Trait Loci: The locations of genes that together govern a multigenic trait, such as yield or fruit mass.

Ration: A fixed allowance of food or feed sufficient or adequate in amount.

Recombinant DNA Technology: The term given to some techniques of molecular biology and genetic engineering which were developed in the early 1970s. In particular, the use of restriction enzymes, which cleave DNA at specific sites, to modify sections of DNA molecules to be inserted into plasmid or other vectors and cloned into an appropriate host organism (for example a bacterial or yeast cell).

Recombinant DNA: A DNA molecule formed by joining DNA segments from different sources (not necessarily different organisms). This may also include DNA synthesized in the laboratory.

Regulatory Sequence: A DNA sequence to which specific proteins bind to activate or repress the expression of a gene.

Regulon: A protein, such as a heat-shock protein, that exerts an influence over growth and/or differentiation.

Reproductive Cloning: Techniques carried out at the cellular level aimed at the generation of an organism with an identical genome to an existing organism.

Restriction Enzyme: An enzyme derived from bacteria that cuts DNA at a site determined by a specific sequences of nucleotide bases at or near the cleavage site.

Restriction Fragment Length Polymorphism: The variation that occurs in the pattern of fragments obtained by cleaving DNA with restriction enzymes, because of differences in lengths between specific nucleic acid sequences in the DNA of individuals of a population.

Ribosome: Subcellular protein and RNA complexes that form the catalytic site for protein synthesis and at which amino acid chains are constructed as directed by the sequence of nucleotides in messenger RNA molecules.

Risk: A function of the probability of an adverse health effect and the severity of that effect, which is consequential to a hazard(s).

Risk Analysis: A process consisting of 3 components: risk assessment, risk management and risk communication.

Risk Assessment: A scientific based process consisting of the following steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization.

Risk Characterization: The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment.

Risk Communication: The interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions, among risk assessors, risk managers, population, industry, the academic community and other parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk Management: The process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of population and for the promotion of fair practices, and if needed, selecting appropriate prevention and control options.

RNA (Ribonucleic Acid): A single stranded nucleic acid molecule comprising a linear chain made up from 4 nucleotide subunits (A, C, G, and U).

Secondary Metabolites: Substances within an organism that are

not central to primary cellular functions. Examples of secondary metabolites are terpenes (such as menthol), carotenoids and steroids and alkaloids (such as solanine).

Sequence Homology: The degree of identity or similarity between 2 selected nucleotide or amino acid sequences.

Sera-binding Tests: Immunological assays that test for the presence of antigen-specific immunoglobulins (for example, IgE) in blood serum, for example serumobtained from individuals allergic to food, pollen, or other environmental antigens. Sera-binding tests include assays such as western blotting, Enzyme Linked Immunosorbent Assays (ELISA), ELISA-inhibition, RadioAllergoSorbent Test (RAST) and RAST-inhibition techniques.

Shikimate Pathway: Pathway in microorganisms and plants involved in the biosynthesis of the aromatic amino acids (phenylalanine, tyrosine, tryptophan) with a requirement for chorismate as well as shikimate. Secondary metabolites such as lignin, pigments, UV light protectants, phenolic redox molecules and other aromatic compounds such as folic acid and ubiquinone are postscript products of the shikimate pathway.

Signal Transduction: The mechanism through which a cell senses and responds to changes in its environment and changes its gene expression patterns in response.

Single Nucleotide Polymorphism (SNP): A locus at which a single base variation exists stably within populations (typically defined as each variant form being present in at least 1 to 2% of individuals).

Somaclonal Selection: Epigenetic or genetic changes, sometimes expressed as a new trait, resulting from in vitro culture of plant cells. This process occasionally generates plants that are significantly different, epigenetically and/or genetically, from the parent in a stable fashion and may provide a useful source of variation.

Southern Analysis/Hybridization (Southern Blotting): A procedure (named after its inventor E. Southern) in which DNA is transferred from an agarose gel to a nitrocellulose filter, where the DNA is denatured and then hybridized to a radioactive probe.

Splicing: The process through which exons are joined after the introns are removed from a messenger RNA.

Stem Cell: A cell that has the potential to differentiate into a variety of different cell types depending on environmental and/or developmental stimuli.

Stilbenes: A colorless or slightly yellow crystalline water-insoluble unsaturated hydrocarbon used in the manufacture of dyes; trans-1,2-diphenylethene. Formula: $C_6H_5CH{:}CHC_6H_5$. It forms the backbone structure of several compounds with estrogenic activity.

Synteny: A term describing genes that reside on the same chromosome.

Tannins: any of a class of yellowish or brownish solid compounds found in many plants and used as tanning agents, mordants, medical astringents, and so on. Tannins are derivatives of gallic acid with the approximate formula $C_{76}H_{52}O_{46}$.

T-DNA: The segment of the Ti plasmid of *A. tumefaciens* that is transferred to the plant genome.

Ti Plasmid: A plasmid containing the gene(s) responsible for transfer of DNA from *A. tumefaciens* to plant cells and the T-DNA which contains the sequences to be transferred.

Transcription: The process through which a gene generates the complementary messenger RNA molecule.

Transcriptome: The total collection of messenger RNA molecules expressed in a cell or tissue at a given point in time.

Transgene: A gene from one source that has been incorporated into the genome of another organism.

Transgenic Plant: A plant that carries an introduced gene(s) in its germ-line.

Translation: The process through which a polypeptide chain of amino acid molecules is generated as directed by the sequence of a particular messenger RNA sequence.

Transposon (Mobile Element): A segment of DNA that can move or be replicated from one location on the chromosome of an organism to another. Insertion or moving of transposons is often associated with rearrangement of the DNA at that site.

Trypsin Inhibitors: Antinutrient proteins present in plants such as soybeans that inhibit the digestive enzyme, trypsin if not inactivated by heating or other processing methods.

Unintended Effect: An effect that was not the purpose of the genetic modification or mutation.