EDITORIAL

The Editors are pleased to publish this thoughtfully prepared volume. It is the first comprehensive science-based proposal for assuring the safety of foods and food ingredients developed through the application of genetic modification. This volume furthers the commitment to have this Journal provide the type of information on which sound safety evaluation decisions can be based. Both the content of the report and the process used in its development are noteworthy.

Responsibility for the safety of our food supply lies with both government and industry. Their roles are distinct, but overlapping. Assurance of safety requires clear mutual understanding not only of the roles but of the criteria and procedures by which safety is to be judged.

To prepare this report, the International Food Biotechnology Council (IFBC) assembled a multidisciplinary team of scientific experts from universities and from food processing and biotechnology companies. Furthermore, the process used by the IFBC to develop the report deliberately included opportunities for broad-based peer review and critique by individuals around the world. Early drafts were sent to large numbers of outside reviewers and an open symposium was held to discuss those issues eliciting greatest comment.

We believe that this publication, with its extensive literature citations and glossary of terms, will contribute significantly to continuing discussions of safety evaluation criteria by scientists in government, industry, and academia. With more than 100 companies estimated to be engaged currently in biotechnology research and development of food or food ingredients, the appearance of this volume is particularly timely. Several national and international bodies are currently considering issues related to the use of a variety of traditional and recently developed techniques for genetic modification. This report should go far to stimulate and contribute to the dialogue necessary to build consensus in the scientific, industrial, and regulatory communities.

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Preface

The International Food Biotechnology Council (IFBC) was formed in February 1988, with the objective of identifying issues and assembling a set of scientific criteria to evaluate and ensure the safety of food and food ingredients derived from plants and microorganisms resulting from the application of biotechnology. The membership of the Council comprises approximately 30 companies which are almost equally divided between food biotechnology companies and food processing companies.

The scope of the IFBC effort has been limited to those new biotechnologies that lead to genetic changes in the microbe or plant used as food or in food processing. The report intentionally does not address biotechnology applied to animals used as food sources. Because its focus is food safety, it treats environmental considerations only insofar as they arise in arriving at decisions on acceptability for use in the food supply. This narrowing of the scope has allowed a focus on the issues of greatest immediate concern.

The need for the IFBC initiative is founded on the recognition that it is preferable to build a consensus on appropriate safety evaluation criteria before the widespread development of new products that may require such evaluation prior to their commercialization.

A Scientific Committee appointed from among Council members moved rapidly to enlist a number of outside academic and professional scientific experts in developing the report. The complete list of contributors follows this Preface. In addition, a Legal/Regulatory Committee defined the legal/regulatory requirements affecting food products. A Public Policy/Public Relations Committee dealt with bringing the report to the attention of its intended audiences.

Early drafts of the report were sent for peer review to approximately 150 experts in industry, government, and academia in 13 countries. More than 40 sets of detailed substantive comments were received, studied, and, in great measure, incorporated. Major issues in these drafts were discussed in an open symposium attended by over 120 experts in relevant fields.

The Council hopes that the result of the process is a report that will be accepted by, and useful to, government regulatory agencies, the food industry, and the public.

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DNA Plant Technology Corp.
E. I. du Pont de Nemours & Co.
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Takasago International

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The International Food Biotechnology Council (IFBC) expresses its deep gratitude to all the individuals who helped with the development of this report. The IFBC is indebted to each of the contributors and a complete list appears elsewhere in the report. Special mention, however, is made of the efforts of Dr. Stephen Rogers, who chaired the document drafting group, and Dr. Julianne Lindemann, who served as editor. Timely completion of this report would not have been possible without the support and guidance of the IFBC members, Board of Directors, and Executive Committee. The staff work of the IFBC has been carried out through cooperative arrangements with the International Life Sciences Institute (ILSI) and the Industrial Biotechnology Association (IBA). The complex logistics required for the development and preparation of this report were managed by Ms. Sharon Senzik, ILSI Liaison, who translated the objectives of the Board into a practical operational plan. Ms. Frances DeLuca, ILSI Executive Assistant, was responsible for the assembly of the numerous drafts and final manuscript. Other people who worked directly on important details of this endeavor include Ms. Nalini Ramalingam and Ms. Sally Treadwell. The IFBC also gratefully acknowledges the interest and assistance of experts who provided information and constructive suggestions to the authors, including Dr. Lloyd Bullerman (Department of Food Science and Technology, University of Nebraska) and Professor Cecil B. Still (Department of Biochemistry and Microbiology, Cook College, Rutgers University).

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Executive Summary

INTRODUCTION

The International Food Biotechnology Council (IFBC) was organized in 1988 to develop criteria and procedures to evaluate the safety of foods produced through genetic modification. The specific objective was to provide a comprehensive, scientifically based report, with extensive literature references and a glossary of terms for this new field, for safety criteria of foods and food ingredients derived from genetically modified plants and microorganisms. However, the immense scope of the subject required restriction to three general categories of food products: foods and food ingredients derived from microorganisms, single chemicals and simple mixtures, and whole foods and other complex mixtures. Not considered were foods derived from genetically modified animals, environmental aspects of the use or cultivation of genetically modified organisms, and whatever social and ethical issues genetic modification may be thought to raise.

A decision tree was prepared for each of the three categories of food products that embodies a series of detailed questions concerning the genetic origin, composition and safety of the food or food ingredient, and that culminates in a decision to accept, reject, or subject the test material to further study.

The report has been completed by a group of experts from both academia and industry, including those authors and other contributors named under Contributors. Drafts of the report have been reviewed by approximately 150 representatives of government agencies in 13 countries, industrial scientific organizations, professional societies, congressional-legislative staffs, public interest-consumerism groups, and academicians. In most cases their critical evaluations and extensive, written comments lent universality and accuracy to the final report.

The IFBC document drafting group who were responsible for the final report took into account the more than 40 sets of detailed, substantive, written comments from the numerous reviews, but they also had the valuable opinions derived from a 2-day symposium convened to provocatively analyze the general subject of the safety of foods produced by biotechnology.

The principal audiences for the report include regulatory agencies, the biotechnology industry, the food industry, the general public, and officials at all levels of government. Within this diverse audience, the food industry, their suppliers in the biotechnology industry, and food regulatory agencies are responsible for ensuring that the products of biotechnology will be safe for consumption. This summary describes the principal issues and the conclusions and recommendations in the report, including the specific decision criteria for the acceptance of modified foods and ingredients.

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The report recommends that no additional regulatory measures are needed for products of traditional plant breeding practices and microbial mutagenesis and selection. New products should be regulated as would their traditional counterparts. The criteria proposed ensure that food safety can be maintained and enhanced with the introduction of nontraditional genetic modification techniques.

TRADITIONAL FOODS AND TRADITIONAL METHODS OF GENETIC MODIFICATION DEFINE OUR CURRENT STANDARD OF FOOD SAFETY

In the development of criteria and procedures for the safety evaluation of genetically modified foods IFBC relied heavily on accumulated knowledge and experience regarding safe practices in plant breeding, food processing, and the use of microorganisms and microbial products in food. There can be no serious doubt that during this century our food supply has steadily improved in quality, variety, nutritional value, safety, and economy. This improvement has been an important contributor to the rapid advances we have experienced in the public health during this same period. Traditional methods of genetic modification, such as plant breeding practices, have contributed heavily to these improvements in the food supply. They have increased agricultural productivity and the availability of food with consequent reductions in real cost. They have lengthened growing seasons, increased the variety of foods available, and improved disease and insect pest resistance. In a number of instances, plant breeding has been used to remove or reduce the levels of naturally occurring toxic substances, e. g., cyanide in lima beans and cassava, gossypol in cotton seed, and solanine in potatoes (Conn, 1981; Okeke and Oti, 1988). Similarly, microorganisms and their products have been used in foods for thousands of years and are, in fact, essential in familiar foods such as bread, cheese, and yogurt. The use of microorganisms in food and for the production of food ingredients has provided numerous benefits for food preservation and processing that maintain or improve food quality. These traditional practices have a long and impressive record of safe implementation, and logically they must serve as the basis for comparison with newer techniques of genetic modification.

The Council's report starts with the assumption that we must be knowledgeable concerning the composition of traditional foods that are considered safe in order to be able to determine the health significance, if any, of compositional changes brought about through genetic modification. Our knowledge of food composition is very detailed for certain classes of components, but incomplete for others. Still, it is clear that there is considerable variability, commonly two- to threefold, and not infrequently tenfold or greater, in the concentrations of many of the normal nutrient and toxicant constituents of traditional plant foods as they enter the marketplace (Souci *et al.,* 1981). Sufficient knowledge of this variability is available to aid the developer of a food in determining the types of nutrient and toxicant constituents that should be measured when evaluating a product for potential, significant compositional changes. Natural toxicants are important because they have been, and are, occasional significant sources of human hazard, and because they will be first priority targets of any safety evaluation of a product of genetic modification (Cheeke, 1989; Liener, 1980; National Academy of Sciences, 1973).

The report concludes that the screening and testing that are a part of traditional genetic improvement of plants and microorganisms must continue if the food supply is to retain its current level of safety. However, given the long and overwhelmingly successful history of these practices, IFBC also concludes that no new regulatory measures are needed for food and food ingredient products from sources that are genetically modified using traditional procedures. Awareness of any reasonable potential for toxicant production and good judgment in monitoring when appropriate are the proper responsibilities of those who bring conventionally modified food ingredients or new plant varieties to market.

UNDERSTANDING THE METHODS AND RESULTS OF TRADITIONAL AND NONTRADITIONAL GENETIC MODIFICATION

IFBC affirms that an understanding of the methods of genetic modification allows one to put into perspective the safety and regulatory issues associated with foods and food ingredients derived from genetically modified sources. Genetic variation per se does not raise specific safety issues. IFBC devotes an entire chapter of the report to a discussion of the methods of genetic modification and their use. IFBC discusses how inherited genetic material influences food composition; the extent and mechanisms of natural genetic variability; and methods of genetic modification including traditional methods, such as plant breeding and microbial strain selection and mutagenesis, more recently developed methods such as tissue culture, and the newest nontraditional methods, such as recombinant DNA and protein engineering. Recombinant DNA techniques involving genes and vectors of known properties give us greater confidence in efficiently achieving a desired outcome than do traditional breeding, mutagenesis, and protoplast fusion.

It is significant to note that variation in composition in wild and domesticated plants and microorganisms is normal, and results from environmental and genetic influences. Selective forces, either of natural or human making, result in shifts in the genetic composition of plant and microbial populations. We depend on variability to derive improved crop varieties and microbial strains. Traditional methods of introducing genetic variability in plants, although successful in the past, are limited by crossing barriers, inability to induce directed genetic changes by mutagenesis, and inefficient selection procedures. Recombinant DNA methods of introducing additional genetic variability from diverse organisms offer unique opportunities for crop plant improvement (Gasser and Fraley, 1989; Goodman *et al.*, 1987; Kehr, 1974).

While the primary objective of plant breeders has been improved yield and pest resistance, plant breeders through selection of breeding materials, roguing of test plots, and monitoring of the ultimate commercial product have ably served nutritional quality and safety (Day *et al.*, 1985; Reitz and Caldwell, 1974; Simon, 1988). These effective and valuable practices will continue to be applied when products of nontraditional methods of genetic modification are brought into widespread, commercial use.

New genetic techniques offer more specific, precise, and frequently quicker ways of modifying plants and microorganisms to produce the desired effects in our food. Still, until we have experience with foods that have been modified using these techniques, some caution is advised, and indeed, has been incorporated into this report.

Nontraditional methods of genetic modification, particularly recombinant DNA, offer unprecedented opportunities to better understand and control the genetic constitution and nutrient composition of our food. These newer techniques also retain a potential for undirected and undesired genetic and compositional change, that is, secondary effects, as do traditional methods of genetic modification. IFBC concludes that the potential health risks associated with these undirected genetic changes have been successfully managed in the past and remain manageable. Many of the safety evaluation procedures recommended in the IFBC report are designed specifically to intercept and minimize this kind of potential risk. Once there are sufficient data on the effectiveness of these different safety evaluation procedures, some of them may be deemed overly strict or even unnecessary. IFBC intends that these procedures enhance or at the very least maintain our current standard of safety. To ensure that information concerning the safety and uniformity of foods derived using nontraditional processes is widely disseminated, IFBC recommends that academic, governmental, and industrial scientists working on nontraditional genetic modification be encouraged to publish their results in refereed journals.

GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR THE SAFETY EVALUATION OF FOODS PRODUCED USING NONTRADITIONAL TECHNIQUES OF GENETIC MODIFICATION

The report proposes a flexible, tiered-approach system of safety evaluation that is guided by decision trees. The system relies on a mix of three sources of relevant information and confidence: (1) knowledge of and confidence in the genetic background and the procedures of genetic modification; (2) knowledge, only to the necessary level of detail, of the composition of the food product including potential toxicants and significant nutrients; and (3) relevant toxicological data. Rarely, if ever, would it be necessary to pursue all three exhaustively. There must be a threshold for regulation, or even for concern, below which further evaluation on a genetically modified food product or its individual components need not be conducted. The emphasis throughout is on the safety of the food product. The process is relevant only as needed to ask the proper questions about the product.

For the products of traditional genetic modification, confidence in the parent genetic material and the procedures involved is almost always enough to ensure safety; however, some analytical monitoring for essential nutrients or potentially toxic constituents may be appropriate. Toxicological testing is seldom, if ever, even useful, much less necessary.

Separate decision trees are proposed for each of three distinct product categories: microorganisms and their products; single chemical substances and simple mixtures; complex mixtures and whole foods. In answering the questions in each tree, the evaluation will require an appropriate mix of genetic, compositional, and toxicological information that is dependent on the circumstances.

SAFETY EVALUATION OF FOOD INGREDIENTS PRODUCED BY MICROORGANISMS

Safety evaluation for foods and ingredients derived from genetically modified microorganisms should focus on the source organisms (microbial host, vector, and DNA

insert). Issues relevant to safety evaluation include five questions: (1) Does the microbe end up in the food? (2) Is the microbe free of transmissible antibiotic resistance markers? (3) Are the vectors characterized and free of attributes that would render them unsafe for use in food? (4) Does the DNA insert code for a substance that is safe for use in food? (5) Is the microbe free of intermediate host DNA that could code for a toxic product? In addition, it should be shown that the food or ingredient is free of antibiotics as well as toxins known to be produced by related microbial strains (Pariza and Foster, 1983). Finally, for foods and major ingredients (excluding incidental additives and processing aids such as enzymes), the criteria described in the decision tree for whole foods and other complex mixtures should be considered.

IFBC affirms that there are a number of microbes whose products have been consumed for a long enough time to consider the microbes and their products safe for food use. The safety of these microbes would not change on acquisition of new, characterized genes that do not result in toxin production. Similarly, there are a number of plasmid vectors that have been characterized to confirm that they do not direct toxin production. IFBC concludes that use of these plasmids should exempt them from safety testing of any plasmid-specific products.

The safety of the expression product of a new gene should be the focus of concern and evaluation when a safe microbial host is used with a safe plasmid to express a new gene. If the expression product is already part of the food chain, very little additional safety testing may be needed to supplement existing safety information on the product. In fact, transfer of a characterized gene from a complex, uncharacterized genome into a defined system such as that described above may actually lead to an increase in the safety of the final food product.

IFBC recognizes that concerns over the use of antibiotic resistance markers in microorganisms can be addressed by proper choice of marker, careful vector construction, and appropriate containment of the organism. Most importantly, the potential for movement of antibiotic resistance genes on cloning vectors from the host organism to a pathogen can be limited by design of the vector.

For most food microorganisms with a history of safe use, there is essentially no risk of toxin production after introduction of a novel gene that does not code for toxin production. If, however, the source of a DNA insert is a potential pathogenic or toxicogenic organism, then safety of the insert must be ensured by testing for toxins by looking for the presence of the gene or the expression product.

SAFETY EVALUATION OF SINGLE CHEMICALS AND SIMPLE CHEMICAL MIXTURES

Single chemicals and simple chemical mixtures warrant no new or unique safety evaluation procedures since most can be purified to discrete chemically identifiable ingredients which, for the most part, are unlikely to contain unacceptable levels of undesirable components or impurities. In these respects they are very different from whole foods and complex mixtures. Moreover, single chemicals and simple mixtures are typically consumed at low levels compared with whole foods. Thus, single chemicals and simple mixtures are treated separately using well-established criteria and procedures for the safety evaluation of food additives, micronutrients, residues, and contaminants (Food and Drug Administration, 1982; World Health Organization,

1987). These procedures need no special additions for the products of genetic modification. Currently approved single chemicals and simple chemically defined mixtures produced through genetic modification will need little or no additional safety testing if they meet specifications adequate to ensure the absence or control of toxic constituents.

SAFETY EVALUATION OF WHOLE FOODS AND OTHER COMPLEX MIXTURES

Safety evaluation of new, genetically modified plant products, microorganisms, and macroingredients derived therefrom should be based on a comparison with the traditional counterpart in regard to nutrient composition (Souci *et al.*, 1981; U.S. Department of Agriculture, 1976–1984, 1984), other desired expression products, and toxic constituents (Ames *et al.*, 1990; Cheeke, 1989; Liener, 1980; National Academy of Sciences, 1973). This, coupled with documentation on the nature of the genetic change induced and an exposure assessment (Modderman, 1986), provides the basis for a rigorous safety evaluation. In addition, as is presently the practice with traditionally bred cultivars, introduction of new foods into the marketplace should include monitored preintroduction use by human volunteers evaluating the food for acceptability and quality attributes.

The decision tree for whole foods and complex mixtures is inevitably the most complicated, because of the wide range of products that will require evaluation. It requires both common sense and expert judgment in its application. It asks a series of questions that focus first on the source of the genetic material and then on any experience-based confidence in the safety of its use. The use of genetic material from a traditional food will almost always provide greater confidence in the safety of the new food than will the use of genetic material with which we have no dietary experience. The approach anticipates an ever-expanding list of acceptable genetic elements as accumulating experience and knowledge permit.

The decision tree next addresses the composition of the food, pursuing this to a level of detail appropriate to the degree of putative risk. Once again, this requires expert judgment. The composition of traditional foods varies widely as a result of genetic, environmental, and other factors (Salunkhe and Desai, 1988; Senti, 1974). IFBC recommends that compositional screening normally be limited to any constituents intentionally introducted or modified, any constituents of nutritional or safety significance likely to vary in concentration as a result of the genetic modification, and other "inherent constituents."

In this report, "inherent constituents" of food include any identified or unidentified components naturally present in that food plant or in closely related species of food plants, including the normally edible as well as inedible portions. The term is intended to focus on essential nutrients and nonnutrient components such as naturally occurring toxic factors.

The standard for compositional comparison for safety must be the range that is "normal" in any closely related traditional foods. That information will often not be available for all, or even many, constituents of interest. The technology to generate such information is available but will require adaptation to the particular product of interest. The decision tree also considers nutrient levels and anticipated levels of human consumption of the product.

When genetic and compositional data, coupled with available toxicological information, do not suffice to establish the safety of the food, IFBC recommends limited feeding studies in animals. If the new food contains sufficient quantities of some constituent(s) with no dietary history of safe use, toxicological testing of such constituent(s) may be necessary if existing data are inadequate to ensure safety (World Health Organization, 1987). When it is not technically feasible or possible to isolate the new constituent from the food in sufficient amounts to study safety in animal tests, it may be necesary to study the safety of the whole food. Such studies normally would be confined to short-duration screens for acute or subchronic effects to detect the presence of unexpected toxicants that have escaped detection by other means. Longer-term toxicological studies on whole foods are typically insensitive and beset with confounding factors. They are rarely to be recommended, and when unavoidable, should be undertaken only with the most careful design and precautions.

Beyond these laboratory procedures, all of the field testing and screening of conventional breeding programs will still apply. One of the attractions of biotechnology is its ability to shorten and compress the development period of a new plant variety. However, opportunities for extended observation and experience prior to market introduction will remain an important part of the development cycle. For the purposes of safety evaluation, these procedures will maintain at least the present standards of safety.

LEGAL AND REGULATORY CONSIDERATIONS FOR THE SAFETY EVALUATION OF FOODS DERIVED USING GENETIC MODIFICATION

The report provides a detailed discussion of the current legal and regulatory framework for ensuring the safety of food in the United States (21 U.S.C.),[1] and parallel but briefer discussions on the regulatory systems followed by Canada, the European Community, Japan, and the Codex Alimentarius Commission. Existing U.S. food safety laws provide a comprehensive, flexible set of tools for regulating the safety of every component of the food supply and have worked well to ensure that appropriate standards of safety are met.

The IFBC proposes that the regulation of genetically modified food plants and microorganisms be patterned directly on existing law and practice. For example, if the modification results in an organism or expression product that, if produced by traditional means, would be regulated under U.S. law as a food additive or GRAS substance, the organism or expression product produced by nontraditional genetic modification should also be regulated as a food additive or GRAS substance. Examples of these include genetically modified food processing microorganisms and expression products in modified plants that perform traditional food additive functions, such as sweeteners and preservatives.

On the other hand, expression products in modified plants that affect agronomic or processing attributes, such as pest resistance or milling quality of grain, would typically not be regulated as food additives or GRAS substances but, like their tradi-

[1] The basic statutory provisions are sections 201(s), 402, 406, 408, and 409 of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §§ 321(s), 342, 346, 346a, and 348. These laws are administered primarily by the U.S. Food and Drug Administration (FDA).

tionally derived counterparts, would be required to satisfy the safety standard imposed by the food law's food adulteration provisions.

RECOMMENDATIONS FOR THE FUTURE

IFBC encourages the U.S. Food and Drug Administration (FDA) to consider some flexible, voluntary procedures for informing the agency about applications of biotechnology that might not require formal FDA review. This would help keep FDA informed about new technologies and products and contribute to public and market confidence in the food products of biotechnology. In addition, IFBC suggests that FDA affirm the practice of making independent GRAS determinations with respect to specified types of biotechnology-derived food products and that FDA also establish an informal procedure by which industry can inform FDA of these independent GRAS determinations.

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Chapter 1: Introduction

1. THE OBJECTIVE OF THIS REPORT

The objective of this report is to develop criteria and procedures for evaluating the safety-in-use of food products produced through biotechnology, in general, and through genetic modification, in particular.

There are a number of reasons for a special effort to develop criteria and procedures:

1. Several issues affecting human health and environmental safety should, if possible, be addressed without the immediate pressures of market interests, arguments between regulatory agencies and self-appointed advocates.

2. When examined in detail, some issues and situations are so closely related to past examples that established concepts can be invoked or, if they have been unproductive or misleading, modified or avoided. Beyond those close analogies is a spectrum of cases and issues that involve varying degrees of novelty and for which the science and criteria so far employed in safety evaluation are, to some degree, inadequate. Some modified or new concepts and technologies—indeed, some new science—will be needed.

3. Regulatory agencies in the United States and abroad have the continuing problem of trying to stay abreast of science they did not develop but that underlies the products they regulate and should underlie the regulatory decisions they make. When the science is moving very rapidly, this is, at best, difficult and, without outside help, often impossible. Regulatory agencies meet this need in several ways, including the use of expert advisory committees. This report is intended to aid that process. To be useful, it must have essential input from industry on actual practices but without dominance by industry, much less by suppliers or users of biotechnology and even less by any firm, organization, or individual.

4. The industry needs guidance in preparing for the degree or kind of safety evaluation that very different situations will require. There are broad categories of safety decisions that firms have always made wholly on their own or with varying degrees of regulatory guidance or control and it is desirable to extend that approach to the products of biotechnology. Explicit case-by-case regulatory approval is seldom a quick or efficient process. Biotechnology is the national monopoly of no country and we live in a very competitive world.

5. Finally, the interested public is cautiously eager for benefits but is very concerned that any possible issues of risk to health or the environment be addressed effectively. Most members of the public are not likely to maintain a close and continuing interest in a cautious, decades-long approach to regulatory issues that never gets

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away from detailed case-by-case analysis. A more productive approach should be to apply all of the knowledge and insight that can be assembled to construct a set of criteria and procedures for safety evaluation. This is the basis of this report. These criteria and procedures will need to meet at least the following demanding requirements:

- Be acceptable in detail to regulatory agencies in this country and, at least in principle, to those abroad
- Permit reasonable and predictable prospects of technical and commercial progress
- Come, and be seen to come, from a solid consensus of acknowledged experts outside the industry as well as those within the industry
- Be able to withstand, or respond to, careful, searching, peer review
- Be conducted in an open forum
- Be acceptable to informed public opinion
- Provide for update and modification as science and technology advance.

It is critically important to develop and apply procedures that clearly ensure safety, but that avoid an unnecessary burden that would discourage product development. This could be done by trial and error, but that would be tedious and unlikely to be productive.

To accomplish this in reasonable time requires forseeing the issues to be addressed and the risks that must be reduced or avoided. This is possible only through a broad perspective and a carefully balanced approach, avoiding extremes. One extreme would be to assume that everything is safe and to proceed without caution. That is simply accepting whatever unknown risks there may be. They will remain unknown until stumbled over—an unacceptable course of action. The other extreme is a blind conservatism, seeking safety so absolute it can be achieved only by restrictions so severe they prohibit all development. That extreme is out of touch with reality. We do not now have that level of safety in any traditional foods or in anything else we do. Furthermore, we live in a competitive world of international markets. What we forego will, if attractive, be done by others. We will have the consequences thrust upon us, quite possibly without our knowledge. Thus, it is imperative to become devoted observers of the "principle of commensurate effort." Efforts applied to problem analysis and regulation should be proportionate to the actual risks that appear to be involved. This requires a broad perspective. With a broad and organized view, new data gathered from experience will permit an increasingly effective and efficient evaluation process.

Case-by-case analysis is inevitable and desirable in the beginning, but it must be guided and disciplined by a broad and coherent view. Without that broad perspective, each case becomes a substitute for all the learning and analysis that should have preceded it. That lays on each case a crushing burden of proof that is appropriate only for a few, and insurmountable for most.

The probability of different national approaches to evaluating the safety of foods derived by newer methods of genetic modification, the certainty that nations will adopt these techniques at different rates, and the reality of world markets suggest that this is an appropriate field for international action. This point is mentioned again, in Chapter 7.

We begin this report with a brief history, some of it necessarily speculative, of the development of our food supply. There are some useful lessons in this history.

Chapter 2 is a discussion of the compositional variability that exists in traditional foods. The chapter concentrates on the two categories of naturally occurring constituents that are properly of the greatest public health concern: nutrients and toxicants. The importance of nutrients is obvious. Ensuring that the levels of naturally occurring toxicants are not significantly increased or, where necessary or feasible, reduced is the primary safety concern in any method of genetic modification, new or old. This is essential background for developing a valid perspective on whatever changes in composition may result from genetic modification.

Chapter 3 deals with the techniques of genetic modification and their industrial applications.

Chapters 4, 5, and 6 cover microorganisms and their products, single chemical substances and simple mixtures, and complex mixtures and whole foods, respectively. Each chapter proposes and discusses criteria and procedures for safety evaluation. These are intended as reasonably detailed general guidelines, not as specific checklists. They should be considered and applied by an interdisciplinary team possessing the appropriate backgrounds. These will include, in almost all cases, genetics and natural product chemistry and, in many cases, analytical chemistry, toxicology, and safety evaluation. The depth will depend on the need.

Then follows, in Chapter 7, a discussion of the legal and regulatory provisions that govern these applications. Because national legal systems vary too much for comprehensive discussion in this report, Chapter 7 treats only the U.S. structure in detail.

Traditional and new methods of genetic modification will not solve humankind's major problems, but they still offer great promise to an overcrowded, chronically hungry, and often polluted world. Capturing that promise will save money, misery, and lives. But we must do it prudently, in ways that avoid later regret. That is the central objective of this effort.

2. THE ORIGINS OF TRADITIONAL FOODS

The development and genetic lineage of most of our present food supply are lost in antiquity. There are hints and scattered bits of evidence in the archaeological record. The new techniques of gene mapping and DNA sequencing are permitting more inferences—and more arguments—about the origins of food plants and animals.

Humans and their immediate ancestors probably have existed on this planet for more than one million years. For all but a tiny fraction of this time they have been hunters of animals and gatherers of roots, berries, wild grasses, and other plant foods (Edlin, 1967). During this long period, avoidance of plants and animals that were harmful to health required acute skills of observation, particularly of the feeding habits of animals, birds, and other humans, judicious trial and error approaches to the selection of new foods, and transmission of information from one person to another.

Agricultural practices, involving the herding of animals and the cultivation of plants, were exceedingly difficult for early humans. Thus, these practices evolved gradually and well after the development of tool-making skills, the ability to control fire, the construction of primitive shelters, and the making of clothes from the skins of animals (Baker, 1978). The beginning of agriculture apparently occurred several times at different places over many thousands of years. Estimates of the earliest inception range from 9000 to 16,000 years ago (Edlin, 1967; Baker, 1978; Richardson and Stubbs, 1978; Janick *et al.*, 1970; Braidwood, 1970; Macneish, 1970). Plant do-

mestication appears to have begun independently in eastern North America between 2000 and 1000 BC (Smith, 1989). Cultivation of plants seems to have preceded domestication of animals, although exceptions to this pattern appear to have occurred, most notably with the dog (Zeuner, 1963).

Cultivation of plants was probably discovered accidentally, perhaps by observing that seeds of wild grains, when spilled around the living site, sprouted and matured to grasses and new seeds identical to those that were spilled (Schwanitz, 1966; Baker, 1978). Obvious, but seldom considered, is that the first cultivated plants were in fact derived from wild seed and were, therefore, identical to their wild counterparts. With passage of time, however, the characteristics of cultivated plants deviated increasingly and substantially, sometimes astonishingly, from those of their wild ancestors (Hyams, 1971). This change in characteristics resulted at first from selection and propagation of the most desirable plants and later from a combination of selection and breeding. Over the course of thousands of years, this manipulation—sometimes deliberate, sometimes accidental—of wild plants by humans has resulted in cultivars that are, in the context of wild nature, unfit. Many, within a few decades or less, would become extinct if untended by humans. As an example, plant mutants, instead of dying because of unfitness in the wild, might, if useful to humans, have been noted, protected, and propagated.

Domestication of animals conformed to the following sequence of events: limited constraints and free breeding; confinement with breeding in captivity; selective breeding, directed by humans and sometimes involving crossing with wild forms, to obtain specific characteristics; planned development of breeds with highly specialized attributes; and persecution or extermination of wild ancestors (Zeuner, 1963). Dogs, reindeer, goats and sheep were domesticated in the preagricultural period and cattle, buffalo, gaur, banteng, yaks, and pigs were domesticated in the early agricultural period (Zeuner, 1963). Candidates for domestication were selected on the basis of their hardiness, compatibility with humans, adaptability to herding, usefulness, propensity to breed in captivity, and ease of tending (Edlin, 1967).

This Neolithic Revolution had enormous consequences, then and now. It led to a large increase in food production, thus permitting and supporting a larger population. It allowed the appearance of specialized crafts not directly involved in food production. It led to a more stationary population and the emergence of cities. Hunters and gatherers were, of necessity, mobile, moving with the seasons and the food supply. A stationary population does not just encourage, but it requires the storage of food, and this promoted primitive but systematic processing and preservation. Most of these consequences were highly beneficial, and nothing that distinguishes civilization could have arisen without them. Indeed, many view the advent of successful agricultural practices as the single most important event in the development of civilization (Edlin, 1967; Schwanitz, 1966; Baker, 1978; Hyams, 1971; Macneish, 1970). This momentous train of events, however, brought new hazards that remain with us today—hazards such as obesity, alcoholism, dental caries, the "Dust Bowl" of the 1930s, the misuse of some pesticides, and the risks of plant monoculture (Garn and Leonard, 1989). There is a lesson for us. Technology offers enormous benefits but some accompanying risks. Useful progress results not from foregoing the benefits, but from controlling and reducing the risks.

Hereditary differences between plants that have been cultivated for thousands of years and their wild ancestors are worthy of further attention. Cultivated plants ex-

hibit one or more of the following traits (Schwanitz, 1966): gigantism, decreased fruit-producing ability, lessened ability to disseminate seeds and provide physical protection to fruits and seeds, reduced concentrations of bitter and toxic substances, loss of the delayed germination attribute, ability to ripen uniformly, reduced life span, altered shape of roots, altered flowers of ornamental plants, and increased diversity (color, structure, and performance of organs). Today, there is little discussion of gigantism; the focus is on the harvest index, the proportion of useful to nonuseful plant material. That and reduction in bitter and toxic substances are most pertinent to this discussion.

Increased harvest index is a factor of major importance because cultivated plants always exhibit this attribute and because this attribute renders the plant more useful to humans through an increased yield of useful parts. Such improvement may result from polyploidy (a doubling or even higher multiplication of the total number of chromosomes). Other compositional changes are often associated with increased harvest index, including concentration of nutrients. Illustrative of the importance of harvest index is the fact that a cultivated cereal plant yields at least 100-fold more grain than does its wild ancestor (Hyams, 1971).

The primitive ancestor of corn, or maize, was probably teosinte, a wild grass that can still be found in some remote areas of Mexico and Central America. Its seeds were borne on a thickened stem, 6 or 7 mm in diameter and about 2 cm long. It was unsheathed and resembled the seedheads of some wild grasses of today. By the time the first Europeans arrived, selection and cultivation had changed teosinte into Indian corn—maize. The cob was 10 cm or more in length and 3 cm or more in diameter. The individual grains are ten times heavier than those of teosinte, an example of the gigantism just discussed. It was heavily sheathed in husks that had to be removed manually to get at the useful grain. The grains of Indian corn cannot be scattered to reseed by natural forces. Thus, without human intervention, the species would survive only a few years. It is reasonable to assume that along with these extensive changes in form, there were also large changes in nutrient composition and environmental tolerance.

Turning next to bitter and toxic substances, it is interesting to note that of the approximately 51 wild plants gathered for food by the aborigines of Australia, only 36 can be eaten raw and none is pleasant tasting or highly nutritious (Schwanitz, 1966). This is not to suggest that all wild plants are unhealthful, but it should be recognized that many are and that domestication of plants has generally helped to lessen the concentrations and prevalence of substances considered undesirable in foods (see the discussion of plant toxicants in Chapter 2). Examples include reducing the toxic or antinutritional substances in soybeans, lima beans and cassava and decreasing the bitterness of crabapples, garden lettuce, and grapefruit (Schwanitz, 1966).

Hereditary changes in domesticated animals have affected body size, color, skeletal structure, and the composition, character, and location of soft parts (Zeuner, 1963). In general, domesticated animals are smaller (e.g., dogs, cats, cattle, sheep, goats, and pigs) and more variable in size than their wild counterparts; however, many exceptions exist. For example, domesticated rabbits, horses, and birds are generally larger than their wild counterparts. Differences in the soft parts of domesticated and wild animals are of particular interest here. Substantial differences exist between wild and domesticated animals with regard to the location and amounts of fat, muscle as a

fraction of body weight, blood composition, and length of the digestive tract (Zeuner, 1963). With few exceptions, domestication of animals has involved the augmentation of existing traits rather than the development of new ones.

The successes and failures of domestication have narrowed our present diet to less than 1% of the millions of available plant and animal species.

The systematic development of varieties of crop plants had to await an understanding of their breeding systems. Some species must be cross-bred; others are self-pollinating. Some pollens are spread by wind or rain, others by insects. Species that are generally cross-pollinated, by wind or by insects, do not breed true. To breed forms that are true to type, seeds must be produced by self-pollination away from other pollen sources. For some species, such as wheat, barley, and tomato, self-pollination is the rule and the isolation distance (from other crops or stands of the same species) for seed production fields is small. On the other hand, for crops such as corn, sugar beet, and rye, which are wind pollinated, or alfalfa and oilseed rape, which are bee pollinated, the isolation distances are larger. In practice, the level of homogeneity and uniformity among the plants of a cultivar varies with the breeding system of the species. Inbreeding species tend to be more uniform than outbreeding types. However, hybrid corn and other modern seed products made by controlled pollination of one inbred line by another generally have a high degree of uniformity but do not breed true.

Genetic uniformity in crop cultivars has three advantages. It ensures that the consumer gets what he or she wants rather than something else or a mixture. It allows farmers to employ precise management practices. It enables the plant breeder to benefit from measures that protect plant variety rights; these require cultivars to be not only uniform but distinctive and stable.

In their efforts to improve the cultivars available, breeders deliberately introduce new variation. Broadly speaking this is done at two levels. First, highly adapted cultivars that may differ in relatively few but nevertheless important ways are intercrossed to select, among their progeny, new forms which combine the desirable features of the parents. Because the parents were highly adapted to cultivation, their progeny tend to be adapted also. The chances are therefore good that individuals with the desired characteristics are not defective in other ways. If, however, an important character is not available in highly adapted varieties the breeder will explore crosses between adapted and unadapted forms. The latter may be cultivars from other regions, primitive varieties, and wild species, some of which may hybridize with the cultivated form only with great difficulty because they are genetically distantly related.

Clearly, the more distantly related the source material the greater the likelihood that new and unknown genetic information will be introduced. In the breeder's plots most of these forms are eliminated during succeeding generations of selection. Many are unthrifty, forming stunted, slow growing plants. Others do not flower, or are sterile, and still others have poor quality or low yield. In some examples, genes coding for toxic substances have been inadvertently introduced or their expression products increased, although examples of human health significance are uncommon. There is considerable knowledge of the toxic constituents naturally present in most foodstuffs and this is discussed further in Chapter 2 of this report (Committee on Food Protection, 1973; Liener, 1980; Cheeke, 1989). The skills of the plant breeder have usually been concentrated on yield and pest or disease resistance. Even so, they appear also to have served the ends of nutritional value and safety. Beyond that, awareness of

potential problems and some limited monitoring for their appearance by industry and regulatory agencies have been effective but not infallible protective measures.

3. THE ORIGINS OF FOOD PROCESSING AND HANDLING TECHNIQUES

Many of our current methods of food processing and preservation date back to prehistoric times (Stewart and Amerine, 1973). Peking man, perhaps 250,000 years ago, used fire for cooking and also learned very early that cooked meats were less prone to spoil than raw meats. Evidence also indicates that well before 15,000 BC, humans used drying as a method of preservation. About 15,000 BC, fish were dried, foods were smoked and meat was boiled in recently developed ceramic pots. During the period 9000 to 4000 BC such practices as alcoholic fermentation, acidification, salting, bread making and baking, sieving, pressing and seasoning came into being. The period 3500 to 1500 BC gave rise to filtration, lactic acid fermentation of vegetables, more types of seasoning, leavened bread, sausage making, freezing, clarification (beers and wine), flotation (to separate olive oil), and moderately sophisticated pressing.

Commercialization of the various methods of food processing and handling that are important today, with the exception of fermentation, occurred much more recently. Artificial drying of food began in the late 18th century (Van Arsdel *et al.*, 1973). The commercial canning industry was fathered by the work of Nicolas Appert in 1809, and advanced greatly by the discovery by Louis Pasteur in the 1860s that microorganisms are major causative agents of food spoilage. It was further advanced in the 1890s by Harry Russell from the University of Wisconsin and Samuel Prescott and William Underwood from the Massachusetts Institute of Technology when they established relationships between severity of thermal processes and inactivation of key microorganisms. The advent of commercial freezing occurred in the latter half of the 19th century when fish, meat and poultry were frozen naturally or with ice and salt. The development in the 1870s of mechanical refrigeration equipment was a key prerequisite for subsequent growth of the commercial frozen food industry. A final milestone in commercialization of frozen foods occurred in the 1920s when Clarence Birdseye developed quick freezing processes and equipment and packaging for frozen foods.

The use of ionizing radiation to preserve food is truly a latecomer to the scene. Studies to develop this technique did not begin in earnest until after World War II. No other processing method's safety has been investigated with the intensity devoted to this one. Moreover, radiation preservation of food is the only example in which substantial research on the technology and safety of the process and its products preceded commercial application of the technique.

4. USE OF MICROORGANISMS IN FOOD

Humans have used microorganisms for centuries to produce changes in natural foods and to obtain tasty "fermented" products. At first they simply allowed the natural microflora already present on or in the food to develop and to produce pickles, olives, sauerkraut, sour milk or clabber, cheese, beer, wine, bread, sausages, cured

meats, and various other fermented foods and beverages. Sometimes the wine changed to vinegar, which was relished as a condiment and preservative.

The fermentation did not always proceed as expected, so it was necessary to discover ways to control it. Our ancestors learned they could prevent growth of undesirable microorganisms by adding salt to pickles, olives, and sauerkraut without inhibiting the desirable and more salt tolerant lactic acid bacteria (an example of selective inhibition). They learned they could prevent growth of undesirable microorganisms in wine and cheese by mildly heating (pasteurizing) the grape juice and milk; then they could add desirable strains of yeasts and bacteria to make these foods more reliably and of better quality. Similar improvement in the quality of wine could be achieved by treating the must with sulfite rather than heat.

Thus, starter cultures were discovered. Their use now is universal in the commercial production of bread, wine, beer, cheese, yogurt, cultured buttermilk, dry sausage, vinegar, brewed soy sauce, and various other fermented foods. In most of these the cells of the starter organisms become part of the food and are consumed intact. In others, such as wine, beer, vinegar, and soy sauce, the cells are removed by filtration or centrifugation to eliminate turbidity.

Microorganisms also have long been the source of substances that are used as food ingredients. Prominent among these are citric acid, lactic acid, ethyl alcohol, and a wide variety of enzymes. At least one bacteriocin (nisin) is now in commercial production and approved for use as a preservative in certain foods.

Much inventive effort and, in the last century, an increasing amount of basic science lie behind this brief description of the development of our foods. The result is a food supply that is more nutritious, varied, and safer, and in terms of real income, less costly than ever before. These improvements must continue.

5. HEALTH CONSEQUENCES OF DEVELOPMENTS IN THE FOOD SUPPLY

The developments outlined here have had important consequences for human health, particularly in the past century. Between 1900 and 1986 average life expectancy in the United States rose from 51 to 75 years, and it continues to rise. Similar and in some instances slightly greater increases have occurred in others of the more developed nations. Nutrient deficiency diseases have nearly disappeared. The impact of naturally occurring toxicants has been greatly reduced. The growth of our modern food supply has paralleled and contributed to that increase in life expectancy.

Yet the current scene is hardly one of unbroken success. We have repeatedly become aware of microorganisms that we had not previously realized were causes of foodborne disease. Overnutrition and other poor dietary patterns are far too common. Yet, compared with these significant hazards that are largely within our individual control, there is often excessive public concern over the far more remote risks in the food supply.

Government agencies are necessarily responsible for regulation and for enforcement of our food laws. They also have monitoring and surveillance responsibilities over a food supply that is increasingly complex and international and that depends on a technical knowledge base that grows geometrically. Their resources are less than adequate now, and are not keeping pace.

Conquest of the major infectious diseases and increased longevity have permitted the rise of the chronic diseases—those one must live long enough to get. It is becoming increasingly apparent that diet has multiple roles, favorable and unfavorable, in the onset and development of several of the major chronic diseases including coronary heart disease and cancer. Most of the interactions of diet and disease are only partially understood. To the extent that linkage grows and leads to changes in lifestyles, food composition, dietary patterns, and methods of food preparation, those changes will apply equally to foods produced by traditional and by newer methods of genetic modification. Indeed, new methods may well ease the task of modifying diets to meet the newer guidelines, and research toward those ends is already under way.

The progress we have seen depends on a complex web of mutually supportive protective measures, explored in more detail in subsequent chapters. These include:

- the selection, screening and field testing practices of traditional breeding;
- a large and growing—though very incomplete—base of data on the composition of food plants and related species;
- consequent awareness of the sources of potential problems;
- monitoring by industry and governmental agencies of food raw material supplies;
- food standards for fortification that find the safe middle ground between nutrient deficiency and toxic excess;
- regulatory surveillance; and
- epidemiological monitoring programs.

IFBC affirms that overall these protective measures have well served the public health. Yet the major hazards—from nutritional and microbiological causes—remain. New problems continue to arise with embarrasing frequency. The resources of the major regulatory agencies continue to diminish relative to their increasing responsibilities, including those concerned with biotechnology.

IFBC recommends that these protective measures should be kept in place, strengthened with the progress of science, and adjusted as needed in their application to meet the spectrum of situations posed by new methods of genetic modification.

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Chapter 2: Variability in the Composition of Traditional Foods: Nutrients, Microorganisms, and Toxicants

1. INTRODUCTION

This chapter deals with the complexity and extensive variability characteristic of the composition of traditional foods. This wide variability is true of all categories of components including three that are of major health significance: (1) nutrients, (2) microbiological contaminants, and (3) naturally occurring toxicants. Knowledge of these components and the sources and extent of their variability is essential in evaluating their impact on health. That knowledge, moreover, is the only available standard of comparison when evaluating the safety of foods produced by genetic modification.

This chapter presents that information in a summary form not readily available elsewhere. These data are intended to be reasonably comprehensive and representative within the limits of relevance to the subject of this report. The chapter provides several examples of how to view and organize these data in preparation for the evaluation processes discussed in Chapters 4, 5, and 6.

2. COMPOSITION DATA

With the exception of a few highly refined major ingredients (sugar, salt) most individual foods are exceedingly complex mixtures that vary widely in composition. This is particularly true for foods from plants. The classes of constituents include the following:

- Carbohydrates (monosaccharides, disaccharides, oligosaccharides, and polysaccharides including gums, starches, and celluloses)
- Fats (mostly triglycerides containing fatty acids of varying chain lengths and degrees of unsaturation)
- Proteins and peptides
- Enzymes
- Minerals
- DNA and other genetic constituents
- Essential (volatile) oils, many of which contribute flavor
- Waxes
- Vitamins
- Plant pigments
- Alkaloids

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Within these substance classes, a vast array of subtypes can exist. For example, proteins can differ in molecular weight, in structure, and in their content, sequence and ratio of amino acids. Furthermore, the number of individual constituents (single chemical entities) in a single food can range from a few to many thousands. In some cases (wheat flour) processing simplifies this mixture; in other cases (coffee) it substantially increases it. Heating almost inevitably complicates the composition of food; thus, the required sterilization of a glucose solution for intravenous injection creates more than 100 new detectable compounds. More than 120 individual chemical substances have so far been isolated and identified in orange oil; the total number of volatile constituents is at least in the hundreds—most present in traces too small to identify as yet. Without any question, the total number of individual chemical substances of natural origin in the food we eat is in the hundreds of thousands.

Knowledge of food composition exists in considerable detail, though sometimes of doubtful reliability, for most macronutrients and micronutrients. There is extensive qualitative knowledge of the toxicants occurring naturally in food, and the more recent data on these are quantitative as well. Because of their real or potential importance as flavors, or occasionally as pharmaceuticals, information on essential oils and alkaloids is also abundant. Beyond these categories of constituents, knowledge of the composition of foods is very sketchy indeed.

Food is not only chemically complex; it varies widely in composition for reasons outlined in the following sections. Knowledge of this variation forms an indispensable background for assessing the significance of any compositional changes resulting from genetic modification, cultural practices, or processing (see discussion in Chapter 6, Sections 2.2 and 3.1).

2.1. Nutrients

Commercial foods derived from plants and animals exhibit considerable variability in composition. This is true for major constituents, such as fat, protein, and carbohydrate, and for minor constituents, such as vitamins. For certain trace elements and nonnutrient constituents the variation becomes even wider.

The quantity of an individual minor constituent may range over more than an order of magnitude, and may even be apparently undetectable—all in plants that are "normal."

2.1.1 Cause of Variation

The cause of this variability is chiefly genetic for both plant and animal foods (Salunkhe and Desai, 1988; Lawrie, 1985). However, environmental factors such as soil type, hours of sunlight per day, rainfall, altitude, and mean temperature and agriculture practices such as crop rotation, tillage methods, use of fertilizers and pesticides, irrigation, planting date, degree of crop maturity at harvest and storage conditions following harvest, can have major influences on the composition of plant foods. For foods from mammalian animals, nature of the basic diet, age at slaughter, degree of exercise, use of growth promoters, preslaughter procedures, and environmental conditions during and immediately after slaughter will, in addition to genetic factors, influence tissue composition (Lawrie, 1985). For fish, feeding and spawning patterns,

TABLE 1

TYPICAL VARIATION IN THE COMPOSITION OF SEVEN COMMON COMMERCIAL VEGETABLES[a]

CONSTITUENT	RANGE FACTOR[b]							
	CARROTS	POTATO	CAULI-FLOWER	SWEET CORN	TOMATO	PEAS, GREEN	BEANS, GREEN	MEAN
Protein	1.7	1.7	1.3	1.3	1.4	1.3(low)	1.5	1.5
Fat	3	4	1.6	---	1.5	1.7	2	2.3
Carbohydrate	1.5	1.4	1.5	1.1(low)	2.1	---	2.2	1.6
Crude Fiber	2.2	3	---	---	1.4	1.7	2.3	2.1
Na	2.6	6.5(high)	2.4	3.3	3.8	8(high)	3.7	4.3
K	1.7	1.8	1.1(low)	1.3	1.2(low)	1.7	1.5	1.5
Ca	2.1	2	1.3	4.5(high)	2	2.3	2	2.3
Mn	3	2.5	---	1.7	---	2.4	2	2.3
Fe	1.9	3.4	3.7	2	1.5	1.3(low)	2.2	2.3
Cu	4	4	---	1.6	---	2.9	1.8	2.9
Zinc	3	4	---	---	---	3.2	2.6	3.2
P	1.5	2	2.4	1.4	1.6	1.9	1.9	1.8
Mg	1.6	2.0	---	1.4	---	2.3	2	1.9
Carotene	3.5	---	2.5	---	15.3(high)	3.1	2.5	5.4
Vitamin K	---	4	---	---	2	5.1	5	4.0
Vitamin B_1	2	2	3	---	4	2.3	2	2.6
Vitamin B_2	2.7	2.7	2	---	2.5	1.6	2	2.2
Nicotinic acid	2.5	2	1.4	---	2.8	1.4	1.7	2.0
Pantothenic acid	5	1.7	1.5	---	1.2(low)	1.3(low)	4	2.4
Vitamin B_6	1.7	2	2	---	2	1.4	5.5	2.4
Vitamin C	2	2.5	1.1(low)	---	1.4	3	2.7	2.1
Lysine[c]	1.4(low)	1.3(low)	1.2	---	2	1.3(low)	1.2(low)	1.4(low)
Methionine[c]	2.5	1.3(low)	7	---	4.5	1.3(low)	1.4	3.0
Glucose	2	3	3.5	---	---	2	4	2.9
Sucrose	8.8(high)	1.4	10.0(high)	---	---	3.3	12.6(high)	7.2(high)
							Mean of Means	2.7

[a] Data from Souci *et al.* (1981). Foods were purchased in the marketplace so the effects of environmental conditions and agricultural practices preharvest and handling procedures postharvest are present.

[b] Range factor is the high value divided by the low value. Dashes indicate no data. High and low values apply to individual columns.

[c] Total bound and free acids.

season of harvest, location, and method of harvest can, in addition to genetic factors, cause variation in composition (Connell, 1980).

2.1.2. Extent of Variation

Data providing a quantitative overview of the range of variability encountered in plant and animal food appear in Tables 1–4.

Shown in Table 1 are range factors (high value divided by low value) depicting typical variation in desirable macro- and microconstituents of seven common commercial vegetables. These range factors were calculated from the "variation" values presented by Souci *et al.* (1981). The variation value, according to the authors, was calculated on the basis of the average variation from the mean value. Whenever calculation of the average variation was deemed, by the authors, not to be meaningful (insufficient data), the highest and lowest values known to the authors were used. For data relating to a given product, no indication was provided as to which approach was used. Averaged over the seven vegetables, range factors for the 25 constituents vary from a low of 1.4 for lysine to a high of 7.2 for sucrose, with the mean of means being 2.7.

TABLE 2

TYPICAL VARIATION IN THE COMPOSITION OF SEVEN COMMON COMMERCIAL FRUITS AND PEANUTS[a]

CONSTITUENT	RANGE FACTOR[b]							
	APPLE	PEAR	PEACH	STRAW-BERRIES	ORANGE	BANANA	PEANUT	MEAN
Protein	2.2	2.6	2.3	5.1	1.6	1.2(low)	1.1(low)	2.3
Fat	3.2	4	5.6	2.5	3.7	3.8	1.1(low)	3.4
Crude Fiber	1.7	1.8	2	3.8	1.8	2.3	1.9	2.2
Na	2.7	3	5.4	10(high)	10	6.7	1.6	5.6
K	1.7	1.5	1.6	1.6	1.4	1.5	1.2	1.5(low)
Mg	3.2	2	1.5	2	1.6	1.3	1.7	1.9
Ca	3	2	2	2	1.8	2	1.5	2.0
Mn	2.5	2	---	2	2	4	2.3	2.5
Fe	3.3	1.6	2	1.6	2.7	1.7	1.3	2.0
Cu	3	2	1.5	2	2.5	3	3.1(high)	2.4
Zn	5	2	---	1.6	2	1.9	1.1(low)	2.3
P	2.4	2	2	1.6	1.3	1.4	1.5	1.7
Se	20	13 (high)	---	---	24 (high)	17(high)	---	18.5(high)
Carotene	2.3	10	4	1.5	2	4	---	4.0
Vitamin E	4.3	1.4	---	---	---	1.2(low)	2.1	2.2
Vitamin B_1	3	7	2	2	1.4	1.7	1.4	2.6
Vitamin B_2	2.5	3	2.3	2.3	3.5	1.6	2.8	2.6
Nicotinic acid	5	3	2	5.8	2.5	1.2(low)	2	3.1
Pantothenic acid	1.4(low)	1.4	1.2(low)	1.1(low)	1.5	1.5	1.1(low)	1.5(low)
Vitamin B_6	1.5	3	1.5	1.8	2.7	1.7	---	2.0
Biotin	8	---	1.2(low)	---	1.4	2	---	3.1
Folates	1.6	---	2.2	---	4	3	1.7	2.5
Vitamin C	8.3	5	5.8(high)	2.1	1.7	3	---	4.3
Glucose	25.0(high)	1.2(low)	1.7	1.7	1.2(low)	2.4	---	5.5
Sucrose	3.1	1.3	1.6	7.9	1.3	2	---	2.9
							Mean of Means	3.4

[a] Data from Souci *et al.* (1981). Foods were purchased in the marketplace so the effects of environmental conditions and agricultural practices preharvest and handling procedures postharvest are present.

[b] Range factor is the high value divided by the low value. Dashes indicate no data. High and low values apply to individual columns.

Shown in Table 2 are range factors depicting typical variation in desirable macro- and microconstituents of seven common commercial fruits. Averaged over the seven fruits, range factors for the 25 constituents vary from a low of 1.5 for potassium and pantothenic acid to a high of 18.5 for selenium, with the mean of means being 3.4. Shown in Table 3 are range factors depicting typical variation in desirable macro- and microconstituents of five common commercial animal products. Averaged over the five products, range factors for the 13 constituents vary from a low of 1.2 for protein and sodium to a high of 3.7 for manganese, with the mean of means being 1.8. From these data it can be concluded that many of the normal constituents of plant and animal foods entering the marketplace exhibit a variance in concentration of two- to threefold.

Data in Table 4 illustrate more specifically the impact of environmental conditions, agricultural practices, and genetic composition on the range in concentration of various constituents in foods. Range factors in these specific instances generally exceed, by a substantial margin, those in the previous tables, and clearly provide a more accurate indication of the magnitude of compositional variability that environmental conditions and agricultural practices, including traditional breeding and selection, can have. Four of the ten examples in Table 4 involve range factors greater than 20.

2.1.3. Effect of Nutrient Content on Fulfillment of Nutritional Needs

The significance of these variations in nutrient content lies in the effect of the variations on the value of each food in meeting the nutritional needs of consumers. These

TABLE 3

TYPICAL VARIATION IN THE COMPOSITION OF FIVE COMMON COMMERCIAL ANIMAL FOODS[a]

	Range factor[b]					
Constituent	Cod	Salmon	Shrimp	Beef sirloin	Pork leg	Mean
Protein	1.2 (low)	1.2	1.4	1.2	1.2	1.2 (low)
Fat	4.3 (high)	1.4	2.9	4.3 (high)	1.4	2.9
Na	1.5	1.1 (low)	1.1 (low)	1.3	1.1 (low)	1.2 (low)
K	1.5	1.2	1.4	1.2	1.2	1.3
Mg	1.3	—	2.2	1.2	2.3	1.7
Ca	1.5	—	1.8	1.5	1.6	1.6
Mn	3	5 (high)	—	—	3 (high)	3.7 (high)
Fe	1.3	1.5	1.6	1.1 (low)	2.6	1.6
P	1.4	1.2	1.8	1.4	2.2	1.6
Vitamin B_1	2	1.9	3.5 (high)	1.2	1.3	2.0
Vitamin B_2	2.3	2.1	1.7	1.5	1.6	1.8
Nicotinic acid	1.5	1.8	2	1.2	1.6	1.6
Vitmin B_6	2.3	—	1.5	—	1.3	1.7
						Mean of means 1.8

[a] Data from Souci *et al.* (1981). Foods were purchased in the marketplace so the effects of environmental conditions and agricultural practices preslaughter (harvest) and handling procedures postslaughter (harvest) are present.

[b] Range factor is the high value divided by the low value. Dashes indicate no data. High and low values apply to individual columns.

needs are usually expressed as the "recommended dietary allowance" (RDA) for each essential nutrient. The RDA for each nutrient is set at a level "adequate to meet the known nutrient needs of practically all healthy persons." (National Research Council, 1989). In all cases, this is well above the amount required to avoid clinically evident nutritional deficiency. The RDAs vary somewhat by age and sex, with, in most cases, higher levels for pregnant and lactating women.

This section of the chapter provides examples of how to evaluate the role of a particular food in meeting the nutritional requirements of people, and of how to evaluate the significance of current or potential variation in the nutrient composition of a food. This process permits one to make the kinds of judgments needed to answer question 6 of the decision tree in Chapter 6. An analogous process applies to the toxicants considered later in this chapter. A more detailed discussion of estimating intakes of any food constituent is contained in the Appendix to Chapter 6.

The intake of a nutrient from a specific food depends on the amount of the nutrient in the food and on the amount of the food consumed. Tables 5 through 9 summarize this information for vitamin C and folate in white potatoes and oranges, for β-carotene (provitamin A) in carrots and broccoli, and for vitamin C and β-carotene in green bell peppers.

All of the figures in Tables 5–9 are "eaters only" figures, that is, they reflect the average of those who consumed that particular food at least once during the 3-day period covered by the National Food Consumption Survey. They are, therefore, higher than a population mean that would result from considering eaters and noneaters together.

TABLE 4

INFLUENCE OF GENETIC AND ENVIRONMENTAL CONDITIONS AND AGRICULTURAL PRACTICES ON THE COMPOSITION OF FOODS

Food	Cause of variation in composition	Constituent	Range and range factor (fold)[a]	Reference
Brown rice	Growth locale	Selenium	11–182 ng/g (16.5-fold)	Yoshida and Yasumoto (1987)
Herring	Feed and spawning cycle	Fat	0.4–30% (75-fold)	Kent (1985)
Carrots	Breeding lines	Carotene	0–370 mg/100 g tissue	Senti (1974)
Sweet potatoes	Breeding lines	Carotene	5–22 mg/100 g tissue (4.4-fold)	Senti (1974)
Muskmelon	Breeding lines	Ascorbic acid	3–61 mg/100 g tissue (20-fold)	Senti (1974)
Potatoes	Cultivar	Total glycoalkaloids	3.3–11 mg/100 g (3.3-fold)	Senti (1974)
Lima beans	Cultivar	Cyanogenic glycosides (HCN-producing capacity)	10–300 mg HCN/100 g seed (30-fold)	Conn (1973)
Turnip greens	Degree of exposure to light	Ascorbic acid	2.82×10^3 to 23.5×10^3 mg/100 g (8.3-fold)	Hamner and Parks (1944)
Tomatoes	Maturity	Ascorbic acid	2.7–7.6 mg/100 g (2.8-fold)	Malewski and Markakis (1971)
Spinach	Holding time at 20°C postharvest	Ascorbic acid	33–100% retention (3-fold)	Doesburg (1955)

[a] Range factor is the high value divided by the low value.

In Table 5, at the mean consumption level, 74 g of potatoes containing the mean level of vitamin C provides 12.6 mg, or 21% of the RDA for that vitamin. At the high level of vitamin C content, the amount rises to 18.5 mg and the percentage of the RDA to 31%. Potatoes, it is clear, are a good source of vitamin C. Indeed, before citrus products were widely available, potatoes were the principal source of that vitamin for many population groups.

Potatoes are a less useful source of folate. At the mean folate content, 7 μg, the amount consumed per day is 5.2 μg, which is 2.6% of the RDA. This rises to 7% with potatoes that have high folate levels, but is only 1.5% with potatoes of low folate content.

"Heavy eaters" are generally taken to mean the 90th centile of consumers of a particular food. For major, frequently consumed foods, such as potatoes, oranges, and carrots, the 90th centile usually is approximately—and in the case of potatoes, exactly—twice the mean. Thus for the heavy eater, the amount of vitamin C or folate consumed and the percentage contribution to the RDA are double the figures pre-

TABLE 5

EFFECT OF VARIANCE IN NUTRIENT CONTENT OF WHITE POTATOES ON ATTAINMENT OF RDA

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Nutrient concentration[b]	Amount of nutrient consumed per day[c]	Contribution to RDA[d] (%)
	Vitamin C (mg/100 g edible)		
74	Mean 17	12.6 mg	21
	High 25	18.5 mg	31
	Low 10	7.4 mg	12
	Folate (μg/100 g edible)		
	Mean 7	5.2 μg	2.6
	High 19	14.1 μg	7.0
	Low 4	3.0 μg	1.5

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Souci *et al.* (1981).

[c] The daily sum of individual intakes determined as the product of nutrient concentration in the food × weight of each portion consumed.

[d] 1989 edition, males 25–50 years: vitamin C—60 mg/day, folate—200 μg/day.

sented in Table 5. With this dietary contribution in mind, the developer of a new variety of potato should make every effort to ensure that the vitamin C content stays well above the minimum.

Table 6 displays, in similar format, the role of oranges in vitamin C intake—an even more extreme example. The average eater consuming oranges of mean vitamin C content receives 74.5 mg or 124% of the RDA. If the level of vitamin C is high, the percentage of the RDA rises to 161%. Even for oranges low in vitamin C, the intake is 97% of the RDA. Oranges are also a good source of folate. At mean folate levels, folate intake is 18% of the RDA; at high levels, 30%; and at low levels, 7%. The heavy eater of oranges consumes about 1.8 times the mean, and the figures for nutrient intake and percentage of the RDA rise accordingly.

Carrots (Table 7) play a similarly important role in β-carotene (provitamin A) nutrition. At mean carotene levels, the average eater receives 3.2 mg, or 53% of the RDA. High carotene levels result in 95% of the RDA, and even low carotene levels account for a still very useful 27%. The heavy eater receives 1.9 times these quantities.

For broccoli the per capita daily consumption (total population) is a meager 2.6 g. Those who eat broccoli, however, consume 40 g/day. Clearly, most people do not often eat broccoli. For those who do (Table 8), at mean carotene concentrations, the intake of provitamin A from broccoli is 0.76 mg, or 13% of the RDA. While broccoli is a useful source of vitamin A, and an even more useful source of vitamin C (not shown here), it clearly does not play the role of either oranges or potatoes. One who is not a broccoli eater, however, needs another source of these nutrients.

Green bell peppers (Table 9) provide a final example. They are a good source of vitamin C. Even though the average eater consumes only 16.5 g/day, this contributes, at the mean vitamin level, 35% of the RDA. Green bell peppers, however, are a poor

TABLE 6

EFFECT OF VARIANCE IN NUTRIENT CONTENT OF ORANGES ON ATTAINMENT OF RDA

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Nutrient concentration[b]	Amount of nutrient consumed per day[c]	Contribution to RDA[d] (%)
	Vitamin C (mg/100 g edible)		
149	Mean 50	74.5 mg	124
	High 65	96.8 mg	161
	Low 39	58.1 mg	97
	Folate (μg/100 g edible)		
	Mean 24	35.8 μg	18
	High 40	59.6 μg	30
	Low 10	14.9 μg	7

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Souci *et al.* (1981).

[c] The daily sum of individual intakes determined as the product of nutrient concentration in the food × weight of each portion consumed.

[d] 1989 edition, males 25–50 years; vitamin C—60 mg/day, folate—200 μg/day.

source of β-carotene and, at mean carotene content, contribute only 1% of the RDA. The heavy eater increases these low levels only by a factor of 2. Thus, variation in carotene content of green bell peppers is of no nutritional significance.

These few examples contain several instances in which single foods make a major contribution of a particular nutrient (orange juice and potatoes for vitamin C, orange juice for folate, and carrots for carotene). It is important to note, however, that even in these instances, the impact of the wide variations in nutrient content is greatly

TABLE 7

EFFECT OF VARIANCE IN THE CAROTENE CONTENT OF CARROTS ON ATTAINMENT OF RDA

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Carotene concentration[b] (mg/100 g edible)	Amount of carotene consumed per day[c] (mg)	Contribution to RDA[d] (%)
27	Mean 12	3.2	53
	High 21	5.7	95
	Low 6	1.6	27

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Souci *et al.* (1981).

[c] The daily sum of individual intakes determined as the product of nutrient concentration in the food × weight of each portion consumed.

[d] 1989 edition, males 25–50 years: vitamin A—1000 μg RE, 1 retinol equivalent = 1 μg retinol or 6 μg β-carotene. Thus, RDA = 6000 μg β-carotene or 6 mg.

TABLE 8

EFFECT OF VARIANCE IN THE CAROTENE CONTENT OF BROCCOLI ON ATTAINMENT OF RDA

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Carotene concentration[b] (mg/100 g edible)	Amount of carotene consumed per day[c]	Contribution to RDA[d] (%)
40	Mean 1.9	0.76 mg	13
	High 2.4	0.96	16
	Low 0.83	0.33	5

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Souci *et al.* (1981).

[c] The daily sum of individual intakes determined as the product of nutrient concentration in the food × weight of each portion consumed.

[d] 1989 edition, males 25–50 years: vitamin A—1000 μg RE. 1 retinol equivalent = 1 μg retinol or 6 μg β-carotene. Thus, RDA = 6000 μg β-carotene or 6 mg.

moderated by a varied and balanced diet. In the typical American diet, all citrus products, of which oranges are the major contributor, account for only 28% of the vitamin C and 9.1% of the folate. Similarly, deep yellow and dark green vegetables, of which carrots are the major contributor, provide only 22% of total dietary β-carotene. At the other extreme are large numbers of foods far more rarely consumed than broccoli and green bell peppers. It will always be important to conserve their major nutrients for the few consumers who use them at all. Variation in their minor nutrients, however, is simply of no consequence.

TABLE 9

EFFECT OF VARIANCE IN THE NUTRIENT CONTENT OF GREEN PEPPERS ON ATTAINMENT OF RDA

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Nutrient concentration[b] (mg/100 g edible)	Amount of nutrient consumed per day[c] (mg)	Contribution to RDA[d] (%)
	Vitamin C		
16.5	Mean 139	22.9	38
	High 192	31.7	53
	Low 64	10.6	18
	β-Carotene		
	Mean 0.2	0.03	0.6
	High 1.0	0.17	2.8
	Low 0.06	0.01	0.2

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Souci *et al.* (1981).

[c] The daily sum of individual intakes determined as the product of nutrient concentration × weight of each portion consumed.

[d] 1989 edition, males 25–50 years: vitamin C—60 mg/day; vitamin A—1000 μg RE/day. 1 retinol equivalent = 1 μg retinol or 6 μg β-carotene. Thus, RDA = 6000 μg or 6 mg β-carotene.

This brief discussion illustrates the key role occupied by a few foods in the supply of certain nutrients. It also illustrates the procedures required to evaluate the impact of potential changes in the content of essential nutrients. As with every other aspect of ensuring food safety and quality, it is important that attention be directed to those nutrient sources that make a significant, rather than an insignificant contribution to overall nutritional status. There can be no higher priority than conserving and enhancing the nutritional quality of the food supply.

2.2. Microorganisms Occurring Naturally in Foods

Most of our food supply, although safe and wholesome to consume, is not sterile. Raw products of all kinds commonly contain hundreds to several million microorganisms per gram. The vast majority of these are nonpathogenic and harmless to eat, and most come from the natural environment of the food source (soil, water, air). Experience has taught us how to reduce or avoid exposure to pathogenic microorganisms such as *Salmonella*, *Clostridium*, *Listeria*, and so on, by pasteurization of milk, sterilization, and proper preparation of potentially affected foods. Food processing and preservation techniques such as refrigeration, drying, salting, pickling, and fermenting are used to delay microbial spoilage.

The nonpathogenic microbial content of individual foods varies widely. For example, surveys of raw vegetables when delivered to the freezing plant have shown bacterial counts ranging from 75,000 to as high as 30,000,000 per gram. Bacterial counts of flour usually lie between 100 and 1,000,000 per gram; pasta products between 1000 and 100,000; nutmeats between a few hundred and a million, and spices between a few thousand and several million per gram, unless the products are treated to reduce microorganisms (International Commission on Microbiological Specifications for Foods, 1980).

Limits for nonpathogenic microorganisms have been established for certain types of foods (Subcommittee on Microbiological Criteria, 1985). These limits are intended primarily to ensure quality and proper handling, not necessarily safety. International microbiological specifications for precooked frozen shrimp and prawns allow up to 1,000,000 microorganisms per gram, and those for dried and frozen egg white and dried milk, up to 50,000 per gram. Canadian government standards allow up to 100,000 microbes per gram of ice cream. U.S. military specifications permit up to 50,000 microbes per gram of frozen eggs and ice cream; 75,000 per gram of various cooked foods; 20,000 to 30,000 per milliliter of several dairy drinks; and 500,000 per gram of frozen shucked oysters. Pasteurized milk in the United States may be sold with up to 20,000 microorganisms per milliliter.

A clear distinction must be made between these innocuous and ubiquitous bacteria and the disease producing (pathogenic) and toxin- or toxicant-producing (toxicogenic) organisms such as the salmonellae and clostridiae mentioned earlier. Food contamination by pathogenic and toxicogenic species is the most serious hazard associated with food. Their public health importance requires that they be recognized and controlled. Because it is important to be aware of the hazards associated with them, these organisms are listed later in Table 16.

2.3. Toxicants

As noted earlier, foods are enormously complex and variable mixtures. Virtually all except the most highly refined contain at least traces of inherent constituents that

if present in sufficient quantity, would cause serious adverse effects in those who consumed them. In a general sense, these potentially or even theoretically harmful constituents are the toxicants occurring naturally in food.

A well-accepted listing identifies six categories of food hazards:

Food Hazards

Microbiological
Nutritional
*
*
Environmental contaminants
Natural toxicants
*
*
Pesticide residues
Food additives

Natural toxicants fall in the middle—well below risks from microbiological and nutritional causes and well above those from pesticide residues and food additives (Schmidt, 1975). Natural toxicants are relevant to this report for several reasons:

1. Selection and traditional breeding practices have been among the very successful methods used to reduce concentrations of natural toxicants to levels that present no significant hazard.
2. Natural toxicants will clearly be the principal point of concern in evaluating the safety of foods produced by genetic modification of sources in which these toxicants can occur.
3. It should certainly be an intent of any genetic modification to reduce, or at least not to increase, the level of any constituent that even approaches being a significant hazard.
4. Natural toxicants are an important and, within professional circles, well-recognized source of risk in food. However, below a level of practical significance, we tolerate them because we have come to value the foods in which they occur. To the extent we are aware of such risks, we judge them to be remote or insignificant, and not worth giving up the food or taking other steps to avoid. These are "risk–benefit" decisions. Knowledge of the nature and amounts of natural toxicants helps us to make these decisions in a more informed way. Moreover, at the level at which we choose to ignore them, natural toxicants form a useful benchmark—a kind of tolerable extreme upper limit—against which to compare the relevance and significance of other food risks within our control.

This does not suggest that we do, or should, accept "new" risks from changes in food caused by human activity on a par with risks from traditional natural sources. Initial caution is essential, and even then, experience will always be the final teacher. But the comparison of the risks of the new with the risks of the long-accepted can be instructive. Without that comparison, we would seldom have the opportunity to reduce existing risks.

2.3.1. Intrinsic Toxicity and Toxic Risk

Toxicity is simply chemical disruption of the normal biological processes of living organisms. In the broadest sense, all substances are toxic, that is, they possess intrinsic

ability to cause harm. Pure oxygen is toxic; so is water. There are several documented cases of coma and at least one death from voluntary—though psychotic—drinking of 10 liters or more of water. At the other extreme, the human lethal dose of the most potent toxin known, that of botulism, is approximately 2 μg. Between these two extremes of short-term (acute) toxicity lie about nine orders of magnitude. That gap between the extremes is unlikely to grow; substances less toxic than water or more toxic than botulism toxin are unlikely to be found.

Intrinsic toxicity, however, is not the sole or even the largest component of toxic risk. The conditions of exposure and the susceptibility of the organism are the other major determinants of risk, and it is risk under foreseeable conditions of use and exposure with which we are really concerned. Of these factors, the conditions of exposure, particularly the dose, are by far the most important.

The Renaissance physician Paracelsus captured this in a famous dictum that is still a basic tenet of modern toxicology: "Everything is poison. There is nothing without poison. Only the dose makes a thing not a poison" (Paracelsus, 1564).

One may pursue this point again with the example of water. Our average daily intake of water in all forms is about 1.5 liters (1.5 kg). At the other extreme, current methods of analysis routinely detect trace constituents of food at the 100 part per trillion (ppt) level. If the analyzed food forms 1% of the daily diet, that trace constituent is 1 ppt in approximately 2 kg of food and beverage, or about 2×10^{-9} g. Between these two extremes lie about 12 orders of magnitude. That gap continues to increase as improvements in methods of analysis detect even lower levels of constituents. Thus, our awareness of the importance of dose in determining risk—or rather, lack of risk—will continue to grow.

2.3.2 Definitions of "Toxicant"

It is clear from the foregoing that a useful discussion of toxicants that occur naturally in food requires a definition of "toxicant" narrower than one which includes all food constituents.

There are regulatory definitions of "toxic" and "acutely toxic" (Occupational Safety and Health Administration, 1987). Their focus on massive single doses, however, makes them of little use for constituents of food.

The term *toxicant* clearly needs to include both acute and chronic toxicity. It should include the more serious, the unusual, and the irreversible toxic effects. Because our concern is assessing risk, not just toxicity, the term must not include all theoretically harmful constituents, but only those that are consumed in sufficient quantity to present some significant degree of possible risk. Lastly, and related to dose, we will want to include those constituents that we consume with relatively narrow margins of safety in a reasonably normal diet. For intentional additives to food, a safety factor of 100 is commonly employed to derive safe human exposure from animal data. This is—appropriately—far greater than the margins of safety with which we consume many food toxicants. For those that present some significant risk, the margin may range from as low as 4 or 5 to perhaps 30.

It should be noted that several vitamins (e.g., A and D), certain trace minerals (e.g., fluorine, iodine, copper, and selenium), and other essential nutrients are consumed safely only within a fairly narrow range. Intake below that range results in deficiency

disease; intake above the range, in toxicity. Diets must stay within this "window of safety."

Probably the greatest risks from toxicants in the food supply, other than toxins from organisms causing foodborne disease, are the risks from natural contaminants. Chief among these are the mycotoxins, such as aflatoxin and the ergot alkaloids. Clearly there will always be the need to reduce or eliminate such contaminants.

Although the term *toxicant* as used in this report is intended to include all potentially toxic substances in food, the term *natural toxicant* is intended to apply only to those toxicants that are *inherent constituents* of food (see definition in Glossary). For purposes of this document, a natural toxicant must fulfill two requirements:

1. It is any substance that occurs in food as a consequence of biosynthesis in the organism (see definition of an inherent constituent), *or* absorption by the organism resulting from its natural occurrence in the environment, including the "pass-through" toxicants.
2. The toxic effects that the substance causes in humans, domestic animals, or experimental animals either are irreversible (e.g., carcinogenicity, teratogenicity, certain neurotoxicities) *or* occur with narrow margins of safety, that is, at low multiples (approximately 25 or less) of ordinary exposures.

Because contaminants are not natural toxicants as defined here, they are considered separately, and a representative list appears in Table 16.

2.3.3. Sources, Nature, and Relative Risks of Natural Toxicants

There has been much speculation and growing but still limited knowledge on the utility of toxicants to the plants that produce them. Some, for examples, phytoalexins and protease inhibitors, confer survival value by protecting the plants that contain them against insect pests or pathogens. Some may inhibit competitors for the same ecological role. Toxicants may also be metabolic "dead ends"—accumulated end products of plant metabolism. Whatever their roles in plant physiology, some of them have long been a significant source of human hazard.

Those few foods that are not known to contain at least traces of some naturally occurring toxicants doubtless have not yet been analyzed in sufficient detail. However, of the hundreds of thousands of naturally occurring substances we consume every day in our food, only a very small proportion are toxicants as we have just defined them. To document this, we have tabulated in Table 14, within the limits of the information available to us, identified toxicants relevant to genetic modification of plants. The list is intended to be reasonably comprehensive and representative, but it is inevitably incomplete, even within its intended scope. The literature is enormous and additions are frequent. The principal sources for these data were three major compendia on the subject (National Academy of Sciences, 1973; Liener, 1980; Cheeke, 1989).

Dairy products and the flesh of common domestic animals generally contain fewer and much lower levels of toxicants than do plants. The animals function as "biological filters." Rarely, the filter fails, and toxicants from forage (e.g., cicutoxin) are passed through into edible animal products.

In the overall pattern of human harm caused by toxicants occurring naturally in food, toxicants produced by certain nondomesticated animals, particularly seafood, loom at least as large as those produced by plants. Such toxicants include puffer fish poison, paralytic shellfish poison, and ciguatera poisoning. But because animal toxicants are not relevant to genetic modification of plants, they are not included in this report except as "pass through" toxicants listed in Table 16.

Though not as large a hazard as the seafood toxicants, poisonous mushrooms cause each year in the United States several dozen reported outbreaks of food poisoning and more than a few deaths. Toxic mushrooms are not food contaminants, but are consumed by mistake or in certain native American religious rites. Because they are significant sources of human hazard, they are also listed in Table 16.

While we will continue to use food plants that naturally produce toxicants as sources of genetic material for conventional breeding, we are not likely ever to use pathogenic or mycotoxin-producing organisms in less than highly specific methods of genetic modification. Where they are so used, the criteria and procedures in this document will apply to the safety evaluation of the resulting products.

The definition of natural toxicant used here involves narrow margins of safety for substances that exhibit only reversible effects that are observed in humans and domestic and laboratory animals. Substances with very wide margins of safety have generally been excluded simply because the number is very large and genetic modification is not likely to raise their concentrations to levels that pose threats to higher animals and humans. One should note in passing, however, that these omissions contain some of the growing number of substances now being recognized as naturally occurring pesticides (Ames *et al.*, 1990). These may well become the focus of efforts at genetic modification. If this results in large increases in the concentrations of such substances, the safety implications of this will require evaluation.

Some natural toxicants exist that have not yet been isolated or structurally identified. The number of these is not known, but for the reasons discussed in item 2, page S25, it is likely not to be a large number. The introduction to Appendix A provides more detail on the criteria employed to focus on those toxicants relevant to this report.

The tabulation in Table 14 shows substances that exhibit a wide range of adverse effects. These include antinutrient, cathartic, neurotoxic, cytotoxic, hormonal, hallucinogenic, carcinogenic, and fetotoxic effects, among many others. This compilation of adverse effects results from an accumulation of millennia of human experience and a century of systematic scientific study of food constituents.

In the context of this chapter, "normal diet" includes any item of food that is customary, accepted, and familiar to the locality and the culture. It does not include foods consumed only in times of unusual deprivation or foods of primarily ceremonial or religious significance. Anything that is consumed but not in a "normal" diet is classified in Table 14 as "atypical use."

The categorizations in Tables 10, 13, and 14 have a rational basis but inevitably involve some arbitrary choices and uncertainties caused by incomplete data. The quinolizidine alkaloids such as (−)-spartein, found in the lupines, illustrate these problems. Many of the data on them are fairly recent and sketchy. Many of the reported adverse effects were observed not by feeding to test animals single substances of known identity, but by feeding the plants or mixed alkaloids. The lupines are range crops, but are also cultivated intentionally for feed and have some limited use in

TABLE 10

ANALYSIS OF NATURAL TOXICANTS IN TABLE 14

	Number	% of total
Total number of toxicants	209	100
Documented as causing adverse effects in humans in a normal diet	21+	10
Documented as causing, or suspected of causing, adverse effects in humans from atypical use, abnormal diet, drug use, substance abuse, accident, or ignorance	93+	45
Documented as causing, or suspected of causing, adverse effects in domestic animals	84+	40
Documented as causing, or suspected of causing, adverse effects in experimental animals	161+	77

human food. However, their risks appear to be well known locally and some species and strains are more toxic than others. Native methods of processing exist to reduce the alkaloid content. It is not clear which varieties are part of a "normal" diet or how certainly they can be so classified.

The pattern of data in Table 14 is summarized in Table 10. These data lead to the following observations:

1. Even with our incomplete knowledge, 209 substances out of hundreds of thousands constitute less than one-tenth of 1% of the total number of constituents in food plants and microorganisms, and 21 constitute less than one one-hundredth of 1% of the total. Even if the number of known toxicants were to be several times higher, the conclusion is inescapable: the vast majority of food constituents—though not quite all—are safe under normal conditions of use and exposure.

2. Approximately 10% of the total number of toxicants, 21 of 209, have been shown to cause harm in humans when consumed in a normal diet. Since analytical chemistry moved from its "wet chemistry" to its "instrumental" age, there has been a steady and spectacular increase, already noted, in sensitivity and selectivity. Gas/liquid chromatographs, for example, are now approximately 100,000 times more sensitive than they were 30 years ago. The plant constituents now being isolated and identified typically are present at very low levels, near current limits of sensitivity. The bulk of those present at high concentration are already known.

Even at these low concentrations of current research interest, a few potent toxicants may be found to be threats to human health. No doubt many of these low-level constituents can be shown to cause adverse effects in conventional high-dose toxicity tests in experimental animals; however, virtually all will be detected in foods only at exceedingly low levels. Because of the great influence of dose on hazard, very few, and over time still fewer, of these low-level constituents can have any possible adverse implications for human health. Almost all simply will have no toxicological impact whatever. Thus, as our knowledge of all constituents grows, the proportion of human toxicants in the total will decline.

3. Nearly half have caused adverse effects in humans when ingested in circumstances other than a normal diet.

TABLE 11

SOLANINE CONTENT OF POTATO (mg/100 g)

Reference	Crop year(s)	Number of analyses	Average	Range	Range factor
1. Bomer and Mattis (1926)	1893–1922	79	8.1	1.7–19.7	12
2. Bomer and Mattis (1926)	1922	5	35.8	2.4–58.3	24
	1923	5	2.7	2.0–3.4	1.7
3. Wolf and Duggar (1946)	1938	32	5.9	1.8–13	7
4. Wolf and Duggar (1946)	—	10	12.0	3.7[a]–18.0[b]	5
Mean of means (weighted)				8.7	
Range of ranges				1.7–58.3	
Overall range factor				34	
Excluding the results from the unusually bad year, 1922 in Germany (No. 2 above):					
Mean of means (weighted)				7.6	
Range of ranges				1.7–19.7	
Overall range factor				12	

[a] 255 g average tuber weight.
[b] 31.3 g average tuber weight.

4. Observations of harm in domestic animals have been a useful means of intercepting and preventing possible human harm.

5. Some of the listed substances were tested in laboratory animals after having been suspected of causing toxicity in humans or domestic animals. Others were tested on the basis of expected structure/activity relationships. Conventional toxicological tests are designed to produce adverse effects at least at the highest dose given. Thus, the demonstration of adverse effects in experimental animals serves merely to confirm the general validity of the table.

Quantitative data on the amounts of toxicants in foods are far more sketchy than those available on nutrients. Those few that have been investigated present the same pattern of extensive variability as the nutrients. The toxic glycoalkaloids (GAs) found in potato provide an example. The principal GAs are α-chaconine and α-solanine, often referred to collectively as "solanine." Typical concentrations are summarized in Table 11.

One major source of variation in solanine content is genetic. Entry 3 in Table 11 supplies a partial indication of this. It reports average solanine contents of 32 different varieties grown in two Wisconsin locations in one year.

Tuber size and maturity have a major influence on solanine content. Solanine levels are highest in and near the skin of the potato and in the eyes and, therefore, are higher in small potatoes which have a higher surface to volume ratio. Entry 4 compares results of 10 different analyses of tubers ranging in average size from 31 to 270 g. Solanine concentrations consistently were inversely proportional to tuber size.

TABLE 12

IMPACT ON SAFETY OF THE VARIABILITY IN THE SOLANINE CONTENT OF WHITE POTATOES

Mean consumption, eaters only[a] (g person^{-1} day^{-1})	Solanine concentration from Table 11 (mg/100 g edible)	Amount of solanine consumed per day[b] (mg)	Percentage of LAEL (200 mg) in humans
74	Mean 7.6	5.6	3.
	Low 1.7	1.3	0.6
	High 19.7	14.6	7.3
	Abnormal High 58.3	43.1	22

[a] Data based on *Nationwide Food Consumption Survey among Individuals, 1977–78* (U.S. Department of Agriculture, 1979); personal communication with Arletta Beloian.

[b] Consumption × solanine concentration.

Growing conditions heavily influence GA content. Entry 2 in Table 11 compares results from five growing areas in Germany for 1922, a very poor growing year, with those for 1923, a very much better year. Tuber size was a factor, but sunshine and amount of rainfall apparently were major determinants of solanine content.

Finally, storage and handling—particularly, exposure to light, which causes "greening"—sharply affect solanine concentrations (Bomer and Mattis, 1926):

	Sample 1	Sample 2
After removal of skin and green portions	1.9 mg/100 g	7.9 mg/100 g
In skin and green portions	13.2 mg/100 g	15.0 mg/100 g

Several observations, (National Academy of Sciences, 1973; Liener, 1980) indicate that the lowest dose of solanine that produces adverse effects in humans—the lowest adverse effect level (LAEL)—is 200 mg (ca. 3 mg/kg). A recent review (Slanina, 1990) confirms the older data on concentrations of GAs presented in Table 11, but suggests, without specific documentation, that the LAEL may be as low as 100 mg per person. With the higher value, however, the extent of risk from solanine is summarized in Table 12.

For the average eater consuming 74 g/day potatoes with a solanine content at the mean (7.6 mg/100 g) the intake of solanine is 5.6 mg, which is 3% of the LAEL in humans. This provides a margin of safety of 33 (100% divided by 3%). At minimum solanine content the percentage of the LAEL is less than 1%. At high solanine content, the percentage of the LAEL is 7.3%, and the corresponding safety margin is 14. However, as the last entry in Table 12 illustrates, abnormally high, but actual, solanine concentrations, even for the average eater, increase the percentage of the LAEL to 22%, a safety margin of less than 5. For the heavy eater, with twice the potato consumption, the percentages of the LAEL would be doubled, and the corresponding safety margins halved. If the LAEL is in fact 100 mg, then the percentages in the last column would be doubled again, and the safety margins halved. It is clear why there continue to be occasional outbreaks of potato poisoning (Willimott, 1933; McMillan and Thompson, 1979).

During the 1970s the U.S. Department of Agriculture (USDA) developed a potato variety (Lenape) with unusually high solids content and, therefore, desirable process-

ing characteristics. It also derived late blight resistance from a wild ancestor, *Solanum demissum.* In the course of routine monitoring of incoming potatoes for glycoalkaloid solanine content, a food company found solanine levels several times normal in the Lenape variety. The company called the problem to the attention of the USDA and the Food and Drug Administration (FDA) and the variety was quickly withdrawn.

Because both acute and chronic cyanide toxicity are problems in areas where cassava is a major calorie source, cyanide content of cassava has been investigated extensively. Low-cyanogen varieties of cassava yield about 21–44 mg of hydrogen cyanide per kilogram of fresh root (Okeke and Oti, 1988), whereas varieties not selected for low cyanogen content may yield more than 20 times this amount of HCN (Montgomery, 1980). Thus, the range factor exceeds 20.

Those toxicants known to have caused harm in normal human diets deserve further comment. These substances are categorized by causative factor(s) in Table 13.

A striking aspect of this summary is that one-third of the 21+ toxicants capable of causing adverse effects in normal diets have been consumed as components of ordinary honey. There are at least two possible reasons for this at first surprising state of affairs. Quite possibly many people may be unprepared ever to be cautious about honey, because of the mythology that has always—even today—collected around it. Beyond that, we do not ordinarily feed honey to domestic or laboratory animals, and thus lack the warning these measures could have provided, and did provide for many other toxicants.

The honey toxicants, cicutoxin, and the coniines are examples of "pass-through" toxicants conveyed, respectively, by bees, milk cows, and "green" quail. These animals were relatively unaffected by toxicants that caused harm to humans who ate their food products.

As indicated earlier for both nutrients and toxicants, the importance of the plant source in the diet and the concentration of the constituent in the plant determine that constituent's impact on human health. There can be little question that because of these factors, the cyanogenic glycosides linamarin and lotaustralin and the neurotoxin solanine are responsible for far more instances of human illness and death than any other toxicants in Table 13 [see discussions in National Academy of Sciences, (1973) and Liener, (1980)].

The quinolizidine alkaloids in the lupines appear to protect the plant from fungal infection. Reduction of alkaloid levels has resulted in increased levels of mycotoxins (Cheeke, 1989). This is another example of the role of plant toxicants as pesticides and the need to weigh such trade-offs in evaluating human safety. It also illustrates the potential interplay among nutritional, microbiological, and toxicological attributes. Compositional change can alter significantly the nature and extent of the biological burden presented by a food.

Vicine and convicine are the proximate causes of favism, an acute hemolytic anemia, self-limiting in adults, but occasionally fatal in children. The underlying cause, however, is an inherited deficiency of glucose-6-phosphate dehydrogenase (G6PD), perhaps "the most common genetically determined enzymatic defect in human beings affecting . . . about 100 million people of all races" (Liener, 1980). While Tables 13 and 14 exclude substances hazardous only to those with uncommon inborn errors of metabolism, G6PD deficiency is hardly "uncommon."

The cucurbitacin in squash and cucumber can be dangerously high in some wild, not normally edible, varieties. On rare occasions, in producing seed for cultivated

TABLE 13

PLANT TOXICANTS DOCUMENTED AS CAUSING HARM IN NORMAL HUMAN DIETS

Reference No. in Table 14	Substance (category/name)	Plant source	Number of substances in category	Methods of risk reduction
	Honey toxicants		7	
23	Acetylandromedol	Rhododendron		
24	Andromedol	Andromeda		Monitoring, prohibition
25	Anhydroandromedol	Azalea family		of bee-keeping
26	Desacetylpieristoxin B			
96	Gelsamine	Yellow jasmine		
197	Tutin			
198	Hyenanchin	Tutu tree		
	Forage and meat/milk toxicants		4	
59	Cicutoxin	Water hemlock		Proper grazing and forage practices; avoidance
	Coniine			
	Methylconiine	Hemlock		
	Conhydrine			
	Toxicants from poor choice, handling, or processing of local diet		5+	
207	Hypoglycin A	Ackee fruit (immature)		Avoidance
71	Linamarin	Lima beans and		Selection and breeding,
72	Lotaustralin	cassava root		proper processing
131	β-*N*-Oxalylamino-L-alanine	Chick-pea		Reduced usage
183	(−)-Sparteine and related alkaloids	Lupine		Proper processing
	Plant genetic factors/poor handling		1	
188	Solanine	Potato		Selection and breeding, monitoring, proper handling
	Human genetic factors		2	
164	Vicine	Fava bean		Reduced usage
165	Convicine			
	Other		2	
67	Curcubitacin E	Squash, cucumber		Breeding Isolation
146	Nitrates	Spinach and other green, leafy vegetables		Proper fertilizing practices and handling; monitoring
Total			21+	

varieties, traces of pollen from a wild relative may contaminate the seed plot, causing the production of an unintentional "wide cross" that may carry the genes for high toxicant production. These are rare risks, but they do occur in conventional breeding programs.

Green leafy vegetables such as spinach, celery, and lettuce are highly useful foods, but contain, even under normal conditions, relatively high levels of nitrates. Intensive

fertilization with high-nitrate fertilizers can raise the nitrate content to hazardous levels. Nitrate is reduced to nitrite by enzymes in the leaf, by bacteria on the leaf surface, and by bacteria in the human alimentary tract. Nitrite can and has produced methemoglobinemia (National Academy of Sciences, 1973). Moreover, in the digestive tract, nitrite can react with free amines to form carcinogenic nitrosamines (Hotchkiss, 1989).

2.3.4. Managing the Risks of Natural Toxicants

The last column of Table 13 indicates the methods that have been employed to reduce to tolerable levels the risks of the toxicants listed there. These same methods have also been used to deal with many other toxicants, listed in Table 14, that have been known to affect domestic animals but not humans.

Chief among these risk management methods are plant breeding and selection, discussed at greater length in Chapter 3. The lupines, the chick-pea, and the fava bean offer the possibility of similar improvements in the future.

After genetic improvement, the broadest and most effective means of risk reduction are monitoring and proper postharvest practices. One cannot, of course, monitor for everything. Experience and judgment will be required to direct monitoring to maximize usefulness. Beyond these measures, a moderate, varied, and balanced diet keeps many other constituents such as caffeine, selenium, menthol, and glycyrrhizic acid at easily tolerable levels except for consumers with unusual sensitivities. Avoidance of faddism prevents toxicity from excessive vitamin intakes. Avoidance also deals best with the aethusin in fool's parsley, the coniines in "green" quail, and the djenkolic acid in djenkol beans. More extensive discussion of each of these substances can be found in the references listed for each in Table 14.

The overall conclusion from these 21+ known naturally occurring toxicants, out of hundreds of foods and hundreds of thousands of constituents consumed over many decades, is that our current protective measures have served us very well. Most of the harm from these known human toxicants occurs in circumstances in which those protective measures are not applied. This lends further support to the recommendation at the end of Chapter 1. Beyond that, however, naturally occurring toxicants are and will remain the primary safety concern accompanying products of any genetic modification, by traditional or newer means. To emphasize this point further, and to make easier the task of being systematically aware of the occurrence of natural toxicants, all of the toxicants listed in Table 14 are rearranged and listed by botanical family in Table 15. This reinforces the repeated injunction in Chapter 6 to consider "closely related species." Knowledge of these toxicants and their botanical origins is an essential tool for dealing effectively with them.

3. VARIABILITY FROM POSTHARVEST CHANGES: PROCESSING, HANDLING, AND STORAGE

The advantages derived from processing and preservation of foods are important and mostly obvious: lessened hazard from microbial pathogens, lessened spoilage, inactivation of heat-labile naturally occurring toxicants, year-round availability of foods, availability of foods in regions remote from areas of production, and increased

convenience. Unfortunately, disadvantages in terms of undesirable changes in nutritive value and sensory properties often accompany each of the methods used for the long-term preservation of foods.

Pertinent to this report are compositional changes that foods undergo during home or commercial processing, handling, and storage. These compositional changes can be substantial and, when added to the sizable variation in composition existing in plants at the time of harvest, the total range in composition for a given food type can be very large.

Thus, the consequences of genetic modification must be considered not only for the raw foodstuff, but in terms of the potential impact on processing characteristics and the food as consumed. Among the many potential compositional changes that could affect processing requirements, nutritional value, and safety are changes in pH and solids content. The examples that follow illustrate the size and nature of the compositional changes that can occur.

Commercial air drying of food can cause losses of vitamins C, A, and thiamine ranging from 5 to 70% depending on the food and conditions of drying (Muller and Tobin, 1980).

Commercial water blanching of eight common vegetables can result in losses of vitamin C ranging from 1 to 76% and losses of thiamine ranging from 1 to 80% depending on the product and the conditions (Fennema, 1988). Commercial canning of 11 different vegetables can result in the following losses of vitamins (vitamin, range): biotin, 0–78%; folacin, 35–84%; B_6, 0–91%; pantothenic acid, 30–80%; A, 0–84%; thiamine, 17–83%; riboflavin, 25–67%; niacin, 0–75%; ascorbic acid, 26–90% (Lund, 1988). Commercial sterilization of evaporated milk in cans results in about a 25% loss in lysine availability (Mottu and Mauron, 1967).

The foregoing data resulted from analyses of commercially processed foods. Few data have been gathered on home-processed foods. Furthermore, such home processing or preparation is often in addition to commercial processing. The relatively unsophisticated equipment available for home processing, the lack of process controls, and the very much higher incidence of foodborne illness from home-processed foods, compared with commercially processed foods, all suggest strongly that the range of variation in the nutrient content of home-processed food is likely to be even greater than in commercial packs.

Storage of fresh green beans, peas, and spinach for 48 hr at 20°C can cause losses of vitamin C ranging from 20 to 79% (Zacharias, 1962). In green beans and green peas stored for 12 months at −18°C, losses of nutrients can occur as follows (nutrient, range): thiamine, 0–32%; riboflavin, 0–8%; niacin, 0–8%; vitamin B_6, 0–21%; pantothenic acid, 30–50%; carotenes, 0–20% (Fennema, 1988). Losses of ascorbic acid in frozen raspberries, peaches, and strawberries can range from 10 to 40% after 12 months of storage at −18°C (Fennema, 1988). During storage of canned foods for 12 months at 25°C, losses of vitamins A, C, thiamine, and riboflavin will exceed 10% in green beans, green peas, sweet corn, peaches, and spinach (Kramer, 1974).

The content and the biological availability of the individual amino acids determine the nutritional value of proteins. Most animal proteins, except gelatin, are balanced for human nutritional needs. Plant proteins are typically low in one or more amino acids, usually methionine or lysine. Proteins from a single plant source therefore have less nutritional value than animal proteins. The measure of this value is the protein efficiency ratio (PER), which is the weight gain in rats divided by protein intake.

Casein, the high-quality protein of milk, normally has a value of 2.5. Lower PERs imply lower biological value.

The PER of cereal products can range from a low of 0.8 (corn, toasted at 150°C) to 1.8 (boiled wheat) depending on the product and the process (Morgan *et al.*, 1931).

Severe heat treatment, particularly under alkaline conditions, results in crosslinked amino acid residues, such as lysinoalanine. These reduce nutritional value. The lysinoalanine content of a wide range of foods can vary from 0 to 50,000 μg/g of protein depending on the food and the process (Sternberg *et al.*, 1975).

The proportion of aspartic acid existing as the nonessential D-enantiomers in untreated food proteins is about 2–3% and this value can increase to 9–17% in alkali processed products such as Coffee-Mate, Plus Meat, Fritos, and Breakfast Strips (Masters and Friedman, 1980).

On the positive side, the PER of soybean meal increases from 1.4 to 2.4 when it is heated for 30 min in steam at 100°C (Rackis, 1974). There are numerous other instances where antinutritive substances and, of course, pathogenic microoganisms in foods are greatly reduced in concentration or eliminated by moderate heat treatments (National Academy of Sciences, 1973). Processing has been one of the principal means of reducing or eliminating risks from natural toxicants (see Table 13 and Managing the Risks of Natural Toxicants in Section 2.3).

These examples clearly show that substantial changes in the concentration of important constituents of food can occur during home or commercial processing, handling, and storage.

4. SUMMARY

Most traditional foods are highly complex mixtures that vary widely in composition as a result of genetic and environmental factors, postharvest handling, and normal processing and preparation. Knowledge of this composition and its variability is very unevenly distributed among the various classes of food constituents. Yet knowledge of all this, where appropriate, to a considerable level of detail, is necessary for assessing the importance of individual constituents and the significance of any changes in them resulting from genetic modification, cultural practices, or processing and handling procedures.

Useful microorganisms have long played an important role in the production of traditional foods. Their contribution continues to expand in scope and quality. Many other microorganisms, usually from the environment of the food source, are incidental but harmless food contaminants, without either known value or health risk. Still others are toxigenic or pathogenic bacteria and fungi and are such major threats to human health that they constitute the largest of the hazards in the food supply. These hazardous microorganisms and the poisonous mushrooms (higher fungi) are listed in Table 16. If useful genetic elements are to be sought from these harmful organisms, great care will be necessary in ensuring the safety of the resulting expression products.

The inherent constituents of higher plants include most of the essential and useful nutrients. More than 99% of the other hundreds of thousands of inherent constituents present neither health benefits nor any practical risks whatever. Yet, a very large proportion of all plants, including almost all of those used as human food, contain at least traces of naturally occurring toxic constituents, and knowledge of these continues to

grow. Those that involve irreversible adverse effects, or that we consume with narrow margins of safety, are listed in Table 14. These natural toxicants will appropriately be the primary focus of concern in evaluating the safety of foods produced by genetic modification. This concern should extend to the toxicants found in normally nonedible portions of food plants and to closely related nonfood species. To assist in that process, the natural toxicants in Table 14 have been rearranged by botanical family in Table 15. Although we have already seen, and continue to seek, reduction of the risks from these natural toxicants, they form the only available and practical benchmarks of acceptable toxicological safety for inherent constituents in our food supply.

The health impact of variations in concentration of both nutrients and toxicants depends in each case on the importance in the diet of each food source, and the range of concentration of each constituent of interest in that food source. These data are essential in evaluating safety and nutritional value.

Postharvest handling and processing, including home preparation, add to the variation in the levels of nutrients and toxicants.

IFBC recommends that all of these factors that determine the normal range of variation in the composition of foods must be taken into account in evaluating the direct and indirect impact of genetic changes on the safety and nutritional value of food.

5. APPENDIXES

Appendix A. Toxicants Occurring Naturally in Foods from Plants and Microorganisms Used in Food Production

Thousands of papers and several excellent books have appeared dealing with toxicants occurring naturally in plants, animals, and microorganisms. There is little consistency in the use of the term *toxicant* in these publications, and many of the substances discussed in them are not toxicants but structurally, chemically, or biologically related substances.

Table 14 was compiled largely from three sources (National Academy of Sciences, 1973; Liener, 1980; Cheeke, 1989) with some additions of material from other sources where noted. *It is intended to be comprehensive and representative, but it is by no means complete.* Potential new additions appear in the literature every year.

Because this report deals with genetic modification of plants and microrganisms used in food production, Table 14 is limited to toxicants from those sources. It attempts to sift out of an enormous mass of literature, data particularly relevant to genetic modification of plants and microbes used for human food.

Inclusions:

1. Substances documented as toxic when eaten
2. Toxicants from plants that are at least occasional sources of human food, and from closely related plants (Also included, to the extent known, are toxicants from the normally inedible portions of food plants.)
3. Toxicants from plants that are used only for animal food and forage, but only if the toxicants are, or might reasonably be expected to be, passed through and occur in the animal products that are used as human food (e.g., toxins in honey and in milk)

TABLE 14

NATURAL TOXICANTS IDENTIFIED IN FOOD PLANTS AND MICROORGANISMS

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
1	Aethusin		L458	Fool's parsley (Umbelliferae)			X						
	Aliphatic Nitro Compounds		C II 143-160	Found in 296 species & varieties of Astragalus (Leguminosae)				Death in cattle and sheep	Less toxic in rats which do not hydrolyze the glucosides.		neurotoxin		
2	3-nitro-1-propanic acid (NPA)												
3	3-nitro-1-propanol (NPOH)												
	Amino Acids (Normal)	Protein	N132							(Ratio:) Adv. effect level Nutr. reqmt.(rat)			
4	*Methionine			—		--			1.5% [a]	3			—
5	*Tryptophan			|		--			2%	16			|
6	Histidine			|		--			2%	8			|
7	Tyrosine			|		--			3%		X		|
8	Cystine			|		--			3%				|
9	*Phenylalanine			|		--			4%	4			|
10	*Leucine			|		--			2.5%	4			Dose
11	*Isoleucine			Many		--			5%	10			Dependent
12	*Valine			Edible		--			5%	10			Non-
13	*Lysine			Plant		--			5%	6			Specific
14	*Threonine			Seeds		--			5%	10			Adverse
15	Serine			|		-			4%				Effects
16	Arginine			|		--			4%	20			|
17	Glycine			|		--			4-5%				|
18	Aspartic acid			|		--			5%				|
19	Proline			|		--			5%				|
20	Alanine			|		--			5%				|
21	Glutamic acid			—		--			7%				—

22	L-alpha-amino-beta-methylaminopropionic acid	N 165	Cycad nut		X over-reliance when food short (war-time)	X		"Alzheimer-like CNS syndrome	
	Andromeda toxicants	N 495	Rhodendron, Azalea, Andromeda, Kalmia (spp)	X			X		neurotoxin
23	Acetylandromedol		100						
24	Andromedol		2						
25	Anhydroandromedol		3						
26	Desacetylpieristoxin B								
	Antifertility Agents Contraceptive & Interceptive:	C IV 53-81		Prevent Conception		X			
27	Coronaridine		Apocynaceae						
28	Lithospermic Acid		Boraginaceae						
29	Rottlerin		Euphorbiaceae						
30	Xylohydroquinone		Leguminosea (garden pea)						
	Abortifacients & Uterine Stimulants:		225 species have been shown to have uterine stimulating effects		cause abortion when consumed as teas	X			
31	Berberine		Menispermaceae						
	(Cytisine, see # 177)		Compositae						
	(Serotonin, see #154)		Bromeliaceae						
32	Hordinine		Graminae						
	(D-Lupinene, see #174)		Leguminoceae						
33	Nicotine		Lycopodiaceae						

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
34	Palmatine			Berberidaceae									
35	Plumbagin			Plumbaginaceae									
36	Protopine			Papaveraceae									
37	Reserpine			Apocynaceae									
	(Sparteine, see #181)			Leguminosae									
38	Stachydrine			Labiatae									
39	Tricosanthin			Cucurbitaceae									
	(Tyramine, see # 150)			Cruciferae									
40	Vasicine			Acanthaceae									
	Antispermatogenic, Spermicidal Semen Coagulating												
41	Aristolic acid (Gossypol, (see # 124)			Aristolochiaceae					Gonadal pathology in rabbits				
	Estrogenic					Prevent Ovulation	Prevent Ovulation						
42	Coumestrol			Leguminoseae									
	Cytotoxic					Sterility	Sterility						
43	Demicolcine			Liliaceae									
	(Podophyllotoxin, see # 47)			Berberidaceae									
	(Vascine, see # 40)			Acanthaceae									
44	Vinblastin			Apocynaceae									

	Anthroquinones		N314									
45	Desaspidin			Male fern		X		200-600 LD_{50}				Anthelminthic Carthartic
46	Filicin			Male fern		X		90 sub-chron.				Carthartic
47	Podophyllotoxin			Mayapple		X		90 LD_{50}				Carthartic
48	Arsenic	Crustacea 42-174 Oysters 3-10 Sea fish 2-6 Drinking water up to 1.4	N 47	Plants >1		X (only from water high in As)		200 sub chr.	X			growth depr. goitre deaf-mutism
49	beta-Asarone		N 453	Calamus (sweet flag) ~10-180				500-10000 chronic		tumors		growth depr. incr. mortality
	Atractylosides		N 291 C II 253-263	Thistle (Actratylis gummifera)		X Oxidative phosphorylation inhibitor	X Deaths	X Hypoglycemia Deaths				
50	Atractyloside (AT)			Coffee								
51	Carboxy-AT (CAT)											
52	Benzo(a) pyrene		N 534	algae				X		carcinoma		
53	Caffeine		N 179	coffee 600-900 in beverage cola ~142	tea 400-600 in beverage	X		X	X			caffeinism-sleeplessness
54	Canavanine		N 169	Canavalia ensiformis Dioclea		X		200 ?				
55	Capsaicin		N 452	Red peppers (Capsicum annuum)								irritant, vessicant

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Invers. Toxicity	Unusual Unexpected Toxicity	Other effects
	Cardiac Glycosides		C II 61-96 R	Family Apocynaceae			Death (Wood used as skewers) Poisoning from undercooked meat of animals dying from CG poisoning	Death	X	X	X	X	
56	Narin			Nerium					100 LD_{50}				neurotoxin
57	Carotatoxin		L 458	Carrots (umbelliferae)									
58	Chlorogenic acid	Coffee	N 317 L 454	Sunflower seed ~1%					growth depression at 3%				protease lipase & amylase inhibitor
59	Cicutoxin		N 179	Water hemlock (Cowbane) (snakeroot)		X* *19th century only		X					CNS stimulant convulsant
60	Coniine & related alkaloids:-	Quail	N 464	Hemlock (conium maculatum)		(Hemlock mistaken for Anise seeds, Parsely)	X Fatal dose ~100 mg.						CNS
61	Methyl Coniine												
62	Conhydrine												
63	Copper	Oysters, Crustacea, Organ Meats 200-400	N 50	Leafy plants 10-15						X			
64	Coumarin		N 453	Cassie Lavender Lovage Woodruff					X 2000 & 5000 ppm (diet) 25, 50, 100 mg/kg		X carcinoma ?		liver damage growth depr.
	Coumestans												
65	Coumestrol		N 552	Soybeans					X				estrogen
66	4-O-methylcoumestrol		N 551	Soybeans					X				estrogen
67	Curcurbitacin E(and other related compounds)		K	Squash		Purgative (high toxin strains)							

	Cyanogenic Glycosides	N 299										
68	Acacipetalin		South African acacias				death					
69	Amygdalin		Almond Apricot	Cherry other rosaceae		X	death					
70	Dhurrin		Sorghums Bamboo			X		X				
71	Linamarin		Lima bean &		X (High cyanide varieties or poorly proc.)					X	tropical ataxic neuropathy	
72	Lotaustralin		Cassava									
73	Prunasin		Cherry laurel			death						
74	Sambunigrin		elderberry Acacia sp.				death					
75	Triglochinin		Arrow grass									
76	Vicianin		Common vetch									
77	Cycasin (methylazoxymethanol beta-glucoside)	N 531 L 350	Cycad nuts up to 4%		—				carcinoma		carcinoma	jaundice hemorrhage part. paralysis
	Cyclopropene fatty acids	N 196	Cottonseed 0.1 to 0.5% of salad oil	Kapok					growth suppression, death			
78	Malvalic acid											
79	Sterculic acid											
	Cysteine sulfoxides	N 223										
80	(+)-S-allyl-L-cysteine sulfoxide (alliin)		garlic						depressed iodine uptake (goitrogens)			
81	(+)-S methyl-L-cysteine sulfoxide		onion	turnip & cruciferae								
82	(+)-S propyl-L-cysteine sulfoxide		onion									
83	(+)-S trans-1-propenyl-L-cysteine sulfoxide		onion									

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects +Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
84	L-alpha, gamma-Diaminobutyric acid		N 155	Everlasting pea	Leguminosae Cruciferae				Neurotoxin				
85	Dihydrokawain		N 467	Kava-kava (Piper methysticum)			soporific, tranquilizer 500 mg/day		soporific 50-100				
86	Dihydromethysticin												
87	L-3, 4-Dihydroxyphenylalanine (L-DOPA)		N 163	Broad bean (Vicia faba)			Hemolysis (see pyrimidine glycosides & favism)						
88	Dioscorine		N 180	yam (Dioscorea hyspida)	other yam				65-LD_{50}				CNS depressant
89	Djenkolic acid		N 466 L 438	Djenkol beans 1-2% "black" var. 3-4%			X kidney failure, urinary blockage						
90	Erucic acid		N 189	Mustard seed oil 20-40% Rapeseed oil 20-50%					myocarditis 15% of diet				
91	Ethanol (Ethyl alcohol)		N 179				CNS depressant						
92	Ethyl Acrylate		A	Pineapple	Raspberry				forestomach tumors in rats		X		
93	Fagopyrin		N 564	Buckwheat				photosensitizer	photosensitizer (rat)				
94	Photofagopyrin												
95	Fluorine		N 72	Tea 100			Drinking water: <1 no effect 2-6 tooth mottling >10 skeletal & muscular fluorosis		X	~10			
96	Gelsamine	Honey	N 501	Yellow jasmine									neurotoxin

	Glucosinolates				
97	Allyl (Sinigrin)		Cannot yet be excluded as contributing to endemic goiter in certain parts of the world	Growth depression in swine, cattle, and poultry	Death in rats
98	Benzyl (Glucotropaeolin)				
99	3-Butenyl (Gluconapin)	2,3,12,19[b] (0-69,000)			
100	(R)-2-Hydroxy-3-butenyl (Progoitrin)				
101	(S)-2-Hydroxy-3-butenyl (epi-Progoitrin)				
102	(R)-2-Hydroxy-4-pentenyl				
103	p-Hydroxybenzyl (Sinalbin)				
104	4-Hydroxybenzyl	9 (22,-52,000)			
105	2-Hydroxybutyl-3-enyl	3,4,5,18 (0-28,000)			
106	4-Hydroxy-3-indolylmethyl	16 (1,200-1,900)			
107	2-Hydroxypentyl-4-enyl	15,18			
108	3-Indolylmethyl (Glucobrassican)	2,3,4,5,16,19 (0-990)			
109	1-Methoxy-3-indolylmethyl	4,5 (70-570)			
110	4-methoxy-3-indolymethyl	3 (97-547)			
111	5-Methylsulfinylbutyl	3,4 (9-390)			

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
112	4-Methylsulfinylbutyl (Glucoraphannon)			19 (178-2,230)									
113	3-Methylsulfinylpropyl (Glucoibenn)			2,3,4,5 (1-1,290)									
114	5-Methylsufinylpental			3,5 (0-257)									
115	5-Methylsulfinylpentyl (Glucoalysin)												
116	4-Methylthio-3-butenyl												
117	4-Methylthiobutyl			5,18 (9-380)									
118	5-Methylthiopentyl			5,18 (4-330)									
119	3-Methylthiopropyl			4 (0-295)									
120	3-(N-Methoxyindolyl) methyl (Neoglucobrassican)			3,9,12,18 (0-2,700)									
121	4-Pentenyl (Glucobrassicanapin)			3,5,14,18 (5-7,200)									
122	2-Pherylethyl (Gluconasturlin)			2,3,18,19 (0-45,000)									
123	Prop-2-enyl												

124	Glycyrrhizic acid		N 452				excessive consumption ~100 g/d					adreno-mimetic (hypertension, heart enlarg., Na retention)
125	Gossypol		N 319	Cottonseed ~1%		-	Sterility in man @ 20mg/day for 2+ months	X	10-200 sub-chr. fatal in dogs 200 in pig min. effect in pigs X			multiple
	Hops Toxicants		N 550									Estrogen
126	Adlupulon	Beer		Hops								
127	Colupulon	Beer		Hops								
128	Lupulon	Beer		Hops								
129	Iodine		N 76	Seaweed	Vegetables ~0.3 Cereals ~0.1 Fruits ~0.04		X diets high in seaweed			X Because of intentional and incidental addition		
130	Iron		N 49		Dried legumes, cocoa, grains		X Hemochromatosis if poor nutrition +~200 mg Iron/d.					
	Lathyrogens		C III 169-201	50 Varieties of Lathyrogens								
131	beta-N-Oxalylamine-L-Alanine (BOAA)			Lathyrus Sativas (chick pea)		*Lathyrism* (spastic paralysis)		*Spastic* Paraparesis	*Spastic* Paraparesis			

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Invers. Toxicity	Unusual Unexpected Toxicity	Other effects
	Lectins		C III 29-71	Contained in 30% of Fresh/Proc. foods including Wheat, Ricin Seeds, Soyabean, Jack bean,Potato,Kid. bean			Growth Impairment	G.I. Pathology					
	(Heat sensitive Hemaglutenins)												
	Binding Types:												
132	Mannose/Glucose												
133	Acetyl/Glucosamine												
134	Acetylgalactosamine/Galact.												
135	Fucose												
136	D-Limonene		A	Orange	Allspice, apricot, bay, beans, blueberry, calamus, ginger, cocoa, coffee, tea, dill, tomato, gentian, grape, hops, corn, litchi, mango, papaya, passion fruit, black pepper, peppermint, saffron, tarragon, vanilla				kidney tumors in male rats				
137	Menthol		N 455	peppermint	cocoa, yarrow		heart fibrillation (excessive use)						
138	3-(4-Methylpentanoyl) furan		N 459	purple mint (*Perilla frutescens*)				Acute pulmonary emphysema	pulmonary lesions				
139	Mimosine		N 469	*Leucaena glauca* 1-4%			hair loss	X	alopecia, retarded growth, short life span rats @1%				

140	Myristicin	N 455	Nutmeg 300-600 Mace 300-600	black pepper, carrot, parsley, dill		vomiting, hallucinations, liver damage, death			
141	Nitrates	N 7		leafy vegetables	methemo-globinemia 2 mg/kg b.wt from high nitrate leafy vegetables or water	>8·15g fatal	X		
142	Nobiletin	N 323	tangerine mandarin orange						embryotoxin
143	Oxalate	N 394	Spinach 0.3-1.2% Rhubarb 0.2-1.3% Tea 0.3-2.0%	many vegetables	probably none	probably none	X		X (with vitamin D and calcium deficiency)
144	Phlorizin	N 324	Apple core and seeds 300-400			glucosuria 200-400 mg/kg b.wt (experiment)			Glucosuria 200-400 mg/kg b.wt
145	Phytates	N 359	Cereals nuts legumes	potato sweet potato artichoke blackberries strawberries figs		zinc deficiency [?] in diets low in Calcium, Vit. D, zinc			
	Phytoalexins [plant stress metabolites]	N 409 ApS							
146	Ipomeamarone		sweet potato (damaged)						liver toxicity
147	Ipomeamaranol								nephrotoxicity (?)
148	4-Ipomeanol						lung toxicity		lung toxicity

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Invers. Toxicity	Unusual Unexpected Toxicity	Other effects
149	8-Methoxypsoralen (xanthotoxin)			celery (damaged) or genetically enhanced			photosensitization (non-oral occupational exposure)						
150	Ptaquiloside		C II 239-251	Bracken fern Used as human food in Japan				Leukopenia Hemorrhage (Thiaminase induces thiamin def.)	Carcinogenic to rats				
	Pressor Amines		N 172										
151	Dopamine			Banana 8	Avocado 4-5								
152	Epinephrine												
153	Histamine	wine, beer											
154	Norepinephrine			Banana 2	Potato 0.1-0.2								
155	Octopamine			Citrus									
156	Serotonin (5-Hydroxytryptamine)			Plantain 45 Pineapple 20-35 Banana 28	Tomato 12 Red plum 10 Avocado 10		Heart lesions with high plantain/ serotonin diets			X			
157	Synephrine			Citrus									
158	Tryptamine			Blue plum 5 Tomato 4	Red, blue plum 2								

159	Tyramine	Cheese esp cheddar, Emmernthaler Camembert Boursault up to 2,170	Avocado 23 Orange 10 "Marmite" yeast & yeast ext. up to 2,250	Banana 7 Red Plum 6 Tomato 4			severe hypertension -Persons on antidepressant (tranylcypromine) therapy	
	Protease Inhibitors (PI)	C III 1-27	Protein Inhibitors are ubiquitous to animals and micro-organisms, probably serving as pest deterrents			Impaired Growth	Impaired Growth	Impaired Growth
	Types:							
160	Serine PI							
161	Sulphydryl PI							
162	Acid PI							
163	Metal PI							
	Pyrimidine Glycosides	C II 161-200			*Favism*:- Cataracts; Hemalytic crisis; Leukopenia; Death [causation in susceptible people appears to be related to G6PD deficiency and involves L-DOPA]			*Liver pathology* and Leukopenia

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Invevers. Toxicity	Unusual Unexpected Toxicity	Other effects
164	Vicine (Divicine)		C II 161-200	Faba Beans 7200			Death from faba beans	Liver pathology and leukopenia in rats	X	X	X		
165	Convicine (Isouramil)			Faba Beans 2,700			X	X	X	X	X		
	Pyrrolizidine alkaloids	Honey Milk Ackee Fruit	N 526 L 353 C I 1-86, R	Senecio Crotolaria Heliotropium Tarweed Borage Hound Tonque Echium Heliotrope Comfrey Dusty Miller Tangy Ragwort Common Groundsel Grain (Contam) 200 herbal teas			liver toxicity from "Bush teas" Venal occlusive disease Hepatic failure death	X liver & lung toxicity Death	X liver & lung toxicity Cirrhosis from milk with # 185 & 186 shown to be carcinogens		X Carcinogenicity		
166	Danaidal												
167	Danaidone												
168	Echinatine												
169	Heliotridine												
170	Indicine												
171	Monocrotaline												
172	Petasitinene												
173	Retroricine												
174	Seneciphyllin												
175	Senkirkine												

	Quinolizidine Alkaloids	C I 133, 168	Over 70 alkaloids in over 100 species in U.S. and Canada including: Fabaceae, *Chenopodiaceae* Berberidaceae Solanaceae		Malaise Nausea Ataxia Death	Death	Death	
	Bicyclic							
176	(-)-Lupinine							
	Tricyclic							
177	Albine							
178	Augustifoline							
179	Cytisine							
	Tetracyclic							
180	(-)-Anagyrine							
181	(+)-Lupanine							
182	(-)-Multiflorine							
183	(-)-Sparteine							
184	Rhodomyrtoxin	N 313	Australian finger cherry		Blindness		toxic (mice) 30	
185	Safrole	N 457 L 359	Sassafrass (root bark)	Star anise Mace Nutmeg Black pepper Anise			hepatic toxicant & carcinogen (rats)	carcinogen

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
186	Selenium		N 58	Wheat up to 1.3	other plants		dermatitis fatigue dizziness alopecia from home-grown, highly seleniferous food	X 10 ppm ("Blind staggers")	X anemia liver toxicity heart atrophy >5-10 ppm.			carcinogenicity (?)	
187	Sesamol		N 529	Sesame					increased benign lesions @ 1% (rats)				
188	Alpha-solanine Alpha-chaconine		N 289 L 447	potato 20-1000 ppm		G.I. disorders neurotoxin 2.8 mg/kg b.w.	G.I. disorders & neurotoxin in green potatoes & sprouts		590 LD_{50} in rats 1000 in mice-no effect	~8		anticholinesterase	
189	Steviol		N 474	Stevia rebaudiana								anti-androgen	
190	Tangeretin		N 323	Tangerine Mandarin Orange					embryotoxin				
	Tannins		N 327 L 453	Plants		none known: ADI = 560 mg. high intake, up to 1000 mg							
	Condensed tannins							X					
191	Sorghum tannin (Transkei)		N 329	Sorghum "Bird proof"			Esophageal cancer						
192	Condensed tannin			Sericea Lespideza cuneata					2% in rats 150 d., no effect	X			Protein binding
193	Condensed tannin			grapes				X	2% in rats 150 d., no effect				methyl donor depletion

	Hydrolyzable tannins								—	—
194	Tannic acid						LD_{50}>2,260			
195	Thujone, alpha, L, or (-)		N 458	Wormwood Oak moss Cedar leaf Sage		X	Convulsant CNS lesions			
196	iso-Thujone, beta, D, or (+)		N 458	Wormwood Oak moss tansy yarrow						
197	Tutin	Honey 10-67	N 499	Tutu tree	giddiness, delirium convulsions, coma, memory loss		LD_{50} rat~20 LD_{75} guinea pig 1.2			
198	Hyenanchin (alpha-hydroxytutin) (Mellitoxin)	Honey 10-67	N 499	Tutu tree	giddiness, delirium convulsions, coma, memory loss		LD_{50} rat~12; LD_{50} guinea pig 40-90			
199	Urethane (ethyl carbamate)	Fermented foods, bread, yoghurt; wine, beer usually <6.0 ppb.					carcinogen	X		teratogen

TABLE 14—*Continued*

Ref.	Name	Food if not plant source	Ref. Page	Primary plant source & conc (range) in ppm	Secondary source & conc (range) in ppm	Adv. effects -Humans -Norm. diet	Adv. effects -Humans -Atypical Use	Adv. effects domestic animals	Adv. effects laboratory animals	Narrower safety margin than normal	Irrevers. Toxicity	Unusual Unexpected Toxicity	Other effects
	Vitamins												
	Fat-Soluble Vitamins												
200	Vitamin A			yellow and dark green vegetables		None	multiple, serious effects, including liver damage, teratogenicity, diets high in polar bear or fish liver; excessive use of vitamin supplements. >1000 mcg. or 3000 I.U. per kg. b.wt./day	calf, pig similar to man	anorexia, weight loss, hemorrhage, teratogenicity	X ~50 adults- much less infants and children			
201	Vitamin E			wheat germ vegetable oils	all foods	none	variable and uncertain effects at high supplementation doses		non-specific degenerative changes in tissues				
202	Vitamin K			green leafy vegetables		none	vomiting, and more serious effects in infants K, only						
	Water Soluble Vitamins												
203	Vitamin C			citrus fruits, green vegetables	potato	none	oxalate kidney or bladder stones >5g./day increased requirement for Vit. C in newborns >400 mg/d		increased requirement for Vit. C in newborns, guinea pigs				

	Vitamin Inhibitors (Many antivitamins are known to exist and appear to be proteins, but have not yet been isolated or identified)	N 254						
204	Caffeic acid	N 260	Brackenfern		X			Anti-thiamine
205	Citral	N 442 H				X	X	Anti-Vitamin A
206	gamma-Glutamyl-1-amino-D-proline	N 265	Flaxseed		X	X		Anti-B_6
207	Hypoglycin A	N 472	Ackee	X				Anti-riboflavin
208	Niacytin	N 263	Wheat bran					Anti-niacin
209	Thiaminase	C III 73-91	Bracken Fern	Antinutritive until heat inactivated	Growth Impairment	Growth Impairment		

Key to References: AM – Ames, AS – ApSimon, C = Cheeke, H – Hall, K = Kirschman, L = Liener, N = National Academy of Sciences, R = Ridker.

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[a] mg/kg body wt unless otherwise stated.

[b] **1** = Broccoli, 2 = brussels sprouts, **3** = cabbage, **4** = cauliflower, **5** = charlock, **6** = crambe (Abyssinian), 7 = garden cress, **8** = horseradish, **9** = kale, **10** = kohlrabi, **11** = mustard (black/brown), **12** = mustard (yellow/white), **13** = radish, **14** = rape (Argentine), **15** = rapeseed (Ethopian), **16** = rutabaga, **17** = turnips, **18** = watercress.

4. Toxicants from microorganisms only if those microorganisms are used in the production of food

Exclusions:

1. Substances known to be toxic only by data from nonoral routes of exposure, for examples, injection, skin exposure, inhalation (This is not intended to minimize the importance of nonoral routes of exposure, but the principal focus of this report is food safety, rather than environmental or workplace safety.)
2. Toxicants from animals, except for inclusion 3
3. Contaminants, such as mycotoxins, that are not inherent constituents of food (see definition in Glossary) (These are therefore listed separately in Table 16; (also see discussion in Sections 2.2 and 2.3.2.)
4. Plants known to be toxic and that are eaten as food only by mistake or ignorance (e.g., poisonous mushrooms) or are consumed only for nonfood purposes (e.g., hallucinogens or other substances of abuse)
5. Normally nontoxic substances that are hazardous only to those with uncommon inborn errors of metabolism (e.g., phenylketonuria) or unusual sensitivities or intolerances (e.g., gluten intolerance)
6. Products of processing (e.g., lysinoalanine), as they are not really relevant to genetic modification, except insofar as genetic modification may lead to new products of processing
7. Suspected toxicants not yet well documented, isolated, or identified
8. Suspected toxicants for which there are no data based on ingestion

Appendix B. Natural Toxicants Identified in Food Plants and Microorganisms

The production of inherent constituents by a plant or microorganism reflects its evolutionary history. Large parts of that history are shared with related species and genera. A few inherent constituents appear to be unique to a particular species or genus. Far more are found, in varying quantity, in related species and genera. A large number, including D-limonene, coumarin, and some of the pressor amines, (see Table 15), are found, at least in traces, in many different families. One must thus look at least to the genus, and in many instances to the family, to know what natural toxicants one might reasonably expect to find. The arrangement by botanical family in Table 15 may both illustrate and ease that process.

Appendix C. Algal, Protozoal, Bacterial, and Fungal Toxicants and Toxins

Toxicants and toxins may inadvertently be introduced into food from several microbial sources: algae, protozoa, bacteria, and fungi. In addition, several genera of higher fungi, the mushrooms, produce toxicants that continue to cause many cases of human illness and death when, as frequently happens, a poisonous mushroom is mistaken for an edible one. This is a very active research field; Table 16 is representative, but necessarily incomplete.

TABLE 15

NATURAL TOXICANTS IDENTIFIED IN FOOD PLANTS AND MICROORGANISMS ARRANGED BY BOTANICAL FAMILY

Family	Ref. No. from Table 14	Name
Acanthaceae	40	Vasicine
Algae	52	Benzo[*a*]pyrene
Apocynaceae	27	Coronaridine
	37	Reserpine
	44	Vinblastin
	56	Nerin
Araceae	49	β-Asarone
	136	D-Limonene
Aristolochiaceae	41	Aristolic acid
Berberidaceae	34	Palmatine
	47	Podophyllotoxin
	176	(−)-Lupinine
	177	Albine
	178	Augustifoline
	179	Cytisine
	180	(−)-Anagyrine
	181	(+)-Lupanine
	182	(−)-Multiflorine
	183	(−)-Sparteine
Boraginaceae	28	Lithospermic acid
	166	Danaidal
	167	Danaidone
	168	Echinatine
	169	Heliotridine
	170	Indicine
	171	Monocrotaline
	172	Petasitinene
	173	Retrorsine (Senecionine)
	174	Senecciphyllin
	175	Senkirkine
Bombacaceae	79	Sterculic acid
Bromeliaceae	92	Ethyl acrylate
	156	Serotonin (5-hydroxytryptamine)
Cannabaceae	126	Adlupulon
	127	Colupulon
	128	Lupulon
Cereals	129	Iodine
Cereals and nuts	145	Phytates
Chenopodiaceae	143	Oxalate
	176	Lupinine
	177	Albine
	178	Augustifoline
	179	Cytisine
	180	(−)-Anagyrine
	181	(+)-Lupanine
	182	(−)-Multiflorine
	183	(−)-Sparteine
Compositae	50 and 51	Atractylosides
	58	Chlorogenic acid
	136	D-Limonene
	145	Phytates
	166	Danaidal
	167	Danaidone
Compositae (*cont.*)	168	Echinatine
	169	Heliotrine
	170	Indicine
	171	Monocrotaline
	172	Petasitinene
	173	Retrorsine (senecionine)
	174	Seneciphyllin
	175	Senkirkine
	179	Cytisine
	189	Steviol
	195	Thujone—α, L, or (−)
	196	Isothujone—β, D, or (+)
Convolvulaceae		Phytoalexins (plant stress metabolites)
	145	Phytates
	146	Ipomeamarone
	147	Ipomeamaranol
	148	4-Ipomeanol
Coriariaceae	197	Tutin
	198	Hyenanchin (α-hydroxytutin) (mellitoxin)
Cruciferae	80–83	Cysteine sulfoxides
	84	L-α,γ-diamino-butyric acid
	90	Erucic acid
	97–123	Glucosinolates
	159	Tyramine
Cucurbitaceae	39	Tricosanthin
	67	Cucurbitacin E (and related compounds)
Cycadaceae	22	L-α-Amino-β-methyl-aminopropionic acid
	77	Cyasin (methylazoxymethanol β-glucoside)
Dioscoreaceae	88	Dioscorine
Ericaceae	23	Acetylandromedol
	24	Andromedol
	25	Anhydroandromedol
	26	Desacetylpieristoxin B
	136	D-Limonene
Euphorbiaceae	29	Rottlerin
	72	Lotaustralin
Fresh/processed foods		Lectins (heat-sensitive hemagglutinins)
		Binding types
	132	Mannose/glucose
	133	AcetylGlucosamine
	134	Acetylgalactos-amine/galactos-amine
	135	Fucose

TABLE 15—*Continued*

Family	Ref. No. from Table 14	Name
Fruits	129	Iodine
Gramineae	32	Hordinine
	70	Dhurrin
	130	Iron
	136	D-Limonene
	191	Sorghum tannin (Transkei)
	205	Citral
	208	Niacytin
Juncaginaceae	75	Triglochinin
Labiateae	38	Stachydrine
	136	D-Limonene
Lamiaceae	64	Coumarin
	137	Menthol
	138	3-(4-Methyl-pentanoyl) furan
	195	Thujone—α, L, or (−)
Lauraceae	136	D-Limonene
	151	Dopamine (3-hydroxytyramine)
	152	Epinephrine
	159	Tyramine
	185	Safrole
Leguminoseae (Fabaceae)	2	3-Nitro-1-propionic acid (NPA)
	3	3-Nitro-1-propanol (NPOH)
	30	Xylohydroquinone
	42	Coumestrol
	54	Canavanine
	64	Coumarin
	65	Coumestrol
	66	4-*O*-methyl-coumestrol
	68	Acacipetalin
	71	Linamarin
	74	Sambunigrin
	84	L-α,γ-Diamino-butyric acid
	87	L-3,4-Dihydroxy-phenylalanine (L-DOPA)
	89	Djenkolic acid
	124	Glycyrrhizic acid
	130	Iron
	131	β-*N*-Oxalylamine-L-alanine (BOAA)
	136	D-Limonene
	139	Mimosine
	145	Phytates
	164	Vicine (divicine)
	165	Convicine (isouramil)
	166	Danaidal
	167	Danaidone
	168	Echinatine
	169	Heliotrine
Leguminoseae (Fabaceae) (*cont.*)	170	Indicine
	171	Monocrotaline
	172	Petasitinene
	173	Retrorsine (senecionine)
	174	Seneciphyllin
	175	Senkirkine
	176	(−)-Lupinine
	177	Albine
	178	Augustifoline
	179	Cytisine
	180	(−)-Anagyrine
	181	(+)-Lupanine
	183	(−)-Sparteine
	191–193	Condensed tannin
Liliaceae	43	Demecolcine
	80	(+)-*S*-Allyl-L-cysteine sulfoxide (alliin)
	81	(+)-*S*-Methyl-L-cysteine sulfoxide
	82	(+)-*S*-propyl-L-cysteine sulfoxide
	83	(+)-*S*-*trans*-1-propenyl-L-cysteine sulfoxide
Linaceae	206	γ-Glutamyl-1-amino-D-proline
Loganiaceae	96	Gelsamine
Lycopodiaceae	33	Nicotine
Malvaceae	78	Malvalic acid
	125	Gossypol
Menispermaceae	31	Berberine
Moraceae	136	D-Limonene
	145	Phytates
Musaceae	151	Dopamine (3-hydroxytyramine)
	152	Epinephrine (5-hydroxytryptamine)
	154	Norepinephrine
Myristicaceae	136	D-Limomene
	140	Myristicin
	185	Safrole
Myrtaceae	136	D-Limonene
	184	Rhodomyrtoxin
Oleaceae	136	D-Limonene
	158	Tryptamine
Papaveraceae	36	Protopine
Pedaliaceae	187	Sesamol
Pinaceae	195	Thujone-α, L, or (−)
Piperaceae	85	Dihydrokawain
	86	Dihydromethysticin
	136	D-Limonene
	185	Safrole
Plantaginaceae	156	Serotonin (5-hydroxy-tryptamine)

TABLE 15—*Continued*

Family	Ref. No. from Table 14	Name	Family	Ref. No. from Table 14	Name
Plants	48	Arsenic	Sapindaceae	174	Seneciphyllin
	160–163	Protease inhibitors	(*cont.*)	175	Senkirkine
		(PIs)		207	Hypoglycin A
	186	Selenium	Seaweed	129	Iodine
Plants, leafy	63	Copper	Solanaceae	55	Capsaicin
	141	Nitrates		136	D-Limonene
Plants and seeds	4–21	Amino acids		145	Phytates
Plumbaginaceae	35	Plumbagin		154	Norepinephrine
Polygonaceae	93	Fagopyrin		158	Tryptamine
	94	Photofagopyrin		176	(−)-Lupinine
	143	Oxalate		177	Albine
Polypodiaceae	45	Desaspidin		178	Augustifoline
	46	Filicin		179	Cytisine
	150	Ptaquiloside		180	(−)-Anagyrine
	204	Caffeic acid		181	(+)-Lupanine
	209	Thiaminase		183	(−)-Sparteine
Rosaceae	69	Amygdalin		188	Solanine
	73	Prunasin	Sterculiaceae	53	Caffeine
	92	Ethyl acrylate		130	Iron
	136	D-Limonene		136	D-Limonene
	144	Phlorizin	Theaceae	53	Caffeine
	145	Phytates		95	Fluorine
Rubiaceae	50	Atractyloside (AT)		136	D-Limonene
	51	Carboxy-AT (CAT)		143	Oxalate
	53	Caffeine	Umbelliferae	1	Aethusin
	64	Coumarin		57	Carotatoxin
	136	D-Limonene		59	Cicutoxin
Rutaceae	136	D-Limonene		60	Coniine
	142	Nobiletin		61	Methylconiine
	155	Octopamine		62	Conhydrine
	157	Synephrine		64	Coumarin
	159	Tyramine		136	D-Limonene
	190	Tangeretin		149	8-Methoxypsoralen
	203	Vitamin C			(xanthotoxin)
Sapindaceae	136	D-Limonene		185	Safrole
	166	Danaidal	Useaceae	185	Thujone—α, L, or (−)
	167	Danaidone		186	Isothujone—β, D, or (+)
	168	Echinatine			
	169	Heliotrine	Vegetables	129	Iodine
	170	Indicine	Vitaceae	191–193	Condensed tannin
	171	Monocrotaline		136	D-Limonene
	172	Petasitinene	Wine, beer	153	Histamine
	173	Retrorsine	Yeast	159	Tyramine
		(senecionine)	Zingiberaceae	136	D-Limonene

Algae and Diatoms

Certain toxic dinoflagellates (protozoa) and toxic diatoms (algae) sometimes undergo explosive growth or "bloom" in ocean waters, causing the so-called "red tides." These organisms produce a variety of potent toxicants (listed in Table 16). The organisms are at the bottom of the food chain, and are consumed in large quantities by

TABLE 16

ALGAL, PROTOZOAL, BACTERIAL, AND FUNGAL TOXINS

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
Algae and Diatoms						
Dinoflagellates, e.g. Gonyaulax catenella and G. tamarensis	saxitoxin and derivatives	Paralytic shellfish poisoning (PSP) parasthesias, breathing and other muscular difficulties, paralysis, possible respiratory failure.	Clams and other bivalves	Finfish, dolphins, humans		6,7,11,13
Dinophysis, Porocentrum	okadaic acid, dinophysis toxins, pectenotoxins, yessotoxin	Diarrheic shellfish poisoning (DSP) abdominal cramps, nausea, diarrhea	toxin-bearing marine finfish, e.g. snapper, grouper, mackerel	humans		6
Tycodiscus brevis	brevetoxins	Neurotoxic shellfish poisoning (NSP) parasthesias of lips, tongue, throat, muscular pain, dizziness, reversal of hot & cold sensations, vomiting, diarrhea.	shellfish	humans		6,7
Nitishia pungens	domoic acid	Amnesic shellfish poisoning (ASP) Abdominal pain, vomiting, diarrhea, confusion, memory loss, convulsions, coma	mussels	humans		6
Bacteria						
Clostridium botulinum	partially heat-stable toxins, A, B, E, F, (C,E)	nausea, vomiting, weakness, double vision, vertigo, death	many foods especially home processed, if inadequately processed and handled.	humans (A, B, E, F), domestic & wild animals (D, E)		1,9

Staphylococcus aureus	enterotoxin	nausea, vomiting, abdominal cramps, prostration	many foods, including meat and meat products, dairy products, cream filled pastries if contaminated or mishandled	humans	1930	1,9
Lower Fungi (genera)						
Absidia	?	estrogenic abortive		laying chickens		10
Alternaria	altemariol	teratogenic	dry beans, soy beans, corn, sorghum, barley			2, 3, 10
	altemariol-monomethylether	teratogenic				
	tennazonic acid	convulsant, emetic, hemorrhagic				2,3,8,10,11,12
Aspergillus	(* see also Penicillium)					
	*aflatoxins B_1, B_2, G_1, G_2	liver damage, cancer	grains, peanuts, many foods	many domestic, wild & experimental animals, humans(?)	1959	10
	aflatrem	tremors				3,10
	aflavinine	tremors				10
	anthraquinones	hemorrhage	seeds, flour	cattle, poultry, rabbits		10
	ascladiol	ulceration				10
	asperthecin					10

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
	asp-hemolysin	hemorrhage, hemolysis, skin irritation				3
	austamide					10
	austdiol					10
	austocystins					10
	avenaciolide					10
	aversin	liver damage				3,10
	candidulin					10
	*chrysophanol	mutagen				3
	*citrinin	kidney damage	dry beans, soybeans, grains			10
	*cyclopiazonic acid	kidney & liver damage, neurotoxicity				3,10
	*cytochalasins B D E	cytotoxic, acute tox., teratogenic acute tox., hemorrhage	peanuts peanuts peanuts			3,10
	(see also Chaetomium) 5,6-dimethoxy-sterigmatocystin	mutagenic				3
	*emodin	g.i. effects, mutagen				3
	flavipin					10
	fumagillin					10,12
	*fumitremorgins A,B,C	tremors				3,10
	*gliotoxin	kidney damage				3,10
	helvolic acid					10

*kojic acid	convulsant	home stored foods		9,10
malformin C	acute tox.			10
maltoryzine	neurotoxicity		cattle	10
5-methoxy-sterigmatocystin	mutagenic			3
naptho-gamma-pyrones				3
nidulin				10
nidulotoxin				10
*beta-Nitropropionic acid	liver damage			3
nomidulin				10
*ochraloxins	liver damage	grains, peanuts,many foods	chickens, lambs, heifers, pigs	7,9,10,13
oryzacidin				10,12
[oxalic acid]				3,10
paspalinine	tremors			3
*[patulin]	neurotoxicity		cattle	10
secalonic acid D	kidney, liver, lung damage, hemorrhage			3
sterigmatocystin	kidney, liver damage	grains, peanuts, many foods		2,9,10,13
terreic acid	liver damage			3
territrems A,B	convulsions, tremors			3
*terrein				10
TR-1 toxin	tremors			3
TR-2 toxin	tremors			3,10

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
	tryptoquivaline & derivatives	tremors				3,10
	tryptoquivalone & derivatives	tremors				3,10
	*verruculogen	tremors				10
	*viomellein	kidney & liver damage				3
	viriditoxin					10
	xanthoascin	heart & liver damage				3
	*xanthocillin X, Y_1, Y_2	acute tox., liver damage				3,10
	*xanthomegnin	kidney & liver damage				3
Byssochlamys	byssochlamic acid [patulin] (see Aspergillus)	hemorrhage	fruit juices			10,12
Cephalosporium	cephalosporin P_1					10
	crotocin (see Tricothecium)					15
	ophiobolins A,B,C,D	nutrient intake effects				3
Cercospora	C.beticola toxin(s)	hemolysis				3

Chaetomium	chaetochromin				14
	chaetoglobosins				14
	chaetocin				10
	chetomin	acute tox.,g.i. effects, hemorrhage			3 10 14
	cochliodinol				14
	chrysophanol	mutagenic			3
	cytochalasin B	cytoxic	peanuts		2,3
	D	acute tox., teratogenic	peanuts		2,3
	E	acute tox., hemorrhage	peanuts		2,3
	isocochliodinol				14
	mollicelins C,E,G,H	mutagenic			3,14
	neocochliodinol				14
	oosporein				10
	sterigmatocystin & related compounds (see Aspergillus)				14
Cladosporium	epicladosporic acid				10,12
	fagicladosporic acid				10
Claviceps	ergot alkaloids	vasoconstriction, hallucinations, gangrene of the extremities	rye	humans, domestic animals	11,12
	paspalinine				
Cochliobolus	ophiobolins A,B,C,D	effects on nutrient intake			3
Curvularia	cytochalasins B,D,E (see Chaetomium)				3
Cylindrocarpon	roridin H				15
Dendrodochium	dendrodochin				10
Diplodea	D. maydis toxins	neurotoxins			3
	diplodiol	anorexia, lethargy			3
	D. zeae toxins	neurotoxins			10
Dreschlera	chrysophanol (see Aspergillus)				3
	ophiobolins (see Cochliobolus)				3
Engleromyces	cytochalacins B,D,E (see Aspergillus)				3

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
Epicoccum	flavipin					10
Fusarium	tricothecenes -		peanuts, moldy			15
	3-acetyldeoxynivalenol	acute tox., g.i.effects, hemorrhage	supermarket foods			3,15
	15-acetoxy-T-2-tetraol	skin irritation				3
	citreoviridin	neurotoxin	cereals, esp. oats			10
	deoxynivalenol (vomitoxin)	acute tox., g.i.effects, hemorrhage				3,15
	diacetoxyscirpenol	acute tox.,leuckopenia, anemia	seeds	pigs, horses, sheep		3,10,15
	4,15-diacetylnivalenol	skin irritation				
	3alpha,4beta-dihydroxy-15-acetoxy-8alpha-(3-hydroxy-3-methyl-butyryloxy)-12,13-epoxytricothec-9-ene	skin irritation				3 3
	fusarenone					
	fusarenon-X	acute tox., carcinogenic, emetic leucopenia, mutagenic, teratogenic, skin irritation				
	fusariogenin	hemorrhagic	overwintered grains esp.millet	humans (Ukraine, Siberia)		10,13
	HT-2 toxin	skin irritation				3,15
	3'-hydroxy T-2 triol					15
	3'-hydroxy HT-2 toxin					15
	monoacetylneosolaniol	skin irritation				3
	neosolanio	blood cell count altered				3,15
	nivalenol	mutagenic, skin irritation				3,15
	T-2 toxin	acute tox., carcinogenic, teralogenic, g.i. effects, hemorrhage, liver damage, leucopenia, neurotoxic				3,13,15
	3alpha,4beta,15-trihydroxy-8alpha-(3-hydroxy-3-methylbutyryloxy)-12,13 epoxytricothec-9-ene	skin irritation				3
	moniliformin	muscular weakness, lung disease				3
	sporafusarenes A,B	kidney and liver damage				3
	zearalenone		stored corn	pigs, poultry		10,13

Giberella	zearalenone		stored corn	pigs, poultry	10
Gliocladium	gliotoxin	kidney damage			10
	viridin				10
Gloeotinia					10
Hemispora					10
Helminthosporium	cytochalacins B,D,E (see Aspergillus)				3
	ophiobolins A,B,C,D (see Cochliobolus)				3
Hormiscium	cytochalacins B,D,E (see Aspergillus)				3
Metarrhizium	cytochalacins B,D,E (see Aspergillus)				3
Micronectriella	T-2 toxin (see Fusarium)				
Mucor	?	estrogenic abortive		laying chickens	10
Myrothecium	*tricothecenes*				15
	muconomycin				10
	satratoxins F,G,H	skin irritation			3
	roridin A,D,E				15
	verrucarin A,B	anemia, leucopenia, skin irritiation			3,10,15
	verrucarol				10
Neurospora					10
Nigrosabalum	cytochalacin B,D,E (see Aspergillus)				3
Oospora	oosporein		peanuts		10
Paecilomyces					10

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
Penicillium	(*=see also Aspergillus)					
	*aflatoxins B_1, B_2, G_1, G_2	liver damage, carcinogenic	peanuts, corn & many foods	many domestic & wild animals, man(?)		2
	brevianamide					4
	carolic acid		cereals			10
	chaetogobosin C					4
	*chrysophanol	mutagenic				3
	citreoviridin	tremors				3,4,9,10
	citrinin	kidney damage	peanuts,corn;grains	pigs		9,10,12
	citromycelin					
	costaclavin					10
	cyclochlorotin					4,10
	*cyclopiazionic acid	acute toxicity, kidney & liver damage, neurotoxic				3,4,10,12
	*cytochalacins B	cytotoxic				3
	D	acute toxicity, teratogenic				3
	E	acute toxicity, hemorrhage				3
	decumbin	g.i. effects				3,10
	emodic acid					10
	*emodin	g.i. effects, mutagenic				10
	erythroskyrin					4
	frequentic acid					10
	*lumitremorgin A,B	tremors				4,10
	glaucanic acid	hemorrhage	cereal products	chickens		10
	glauconic acid	hemorrhage				10
	*gliotoxin					10
	griseofulvin		dry beans, soybeans, grains			2,4
						3
	hadacidin	teratogenic				10
	helenin					
	islandicin	mutagenic				10,12
	islanditoxin					3,4
	janthitrems A, B, C	tremors				4
	*kojic acid					4,10,12
	luteoskyrin					4,10,12
	mycophenolic acid					10
	*beta-nitropropionic acid	liver damage				10
	notatin					10
	ochratoxins	liver damage	grains, peanuts and many foods			2,4,8,10

palitantin				10
[patulin]				9,10,12
paxilline	tremors			3,10
penicillic acid	cardiotoxin	dry beans & soy beans, moldy meals,cereals		2,4,10
phoenicin				10
P.islandicum Sopp toxins	carcinogenic			3
P.lanosum toxins	g.i.effects, kidney damage			3
P.paraherquei toxins	tremors			3
penitrems A, B, C, E	tremors			3,10
PR toxin	acute tox., kidney & liver damage			3,4,12
puberulic acid				10
roquefortine C	convulsions			3,4,12
rubratoxin A				4,9,10,12
B	acute toxicity, liver damage, hemorrhage, teratogenic	seeds	pigs, horses, geese	3,4,12
rugulosin				4,10
rugulovasin A, B				4
secalonic acid				3,4,10
simatoxin	acute toxicity, liver damage			3
skyrin				10
spinulosin				4,10
*terrein		cereals		
terrestric acid				4,10
*verruculogen	tremors	cereals		4,10
viridicatic acid				10
viridicatin				10
viridicatum toxin	cardiotoxic, kidney & liver damage	cereals		3,4
*viomellein	kidney & liver damage			3,4
xanthocillin X, Y, Y_2,	acute toxicity, liver damage			3,10
xanthomegnin	kidney & liver damage		cattle sheep	3,4
?	neurotoxin			10
Periconia	?		cattle	10
Piptocephalis				10

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects	Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
Pithomyces	sporidesmins A,B,C,D,E,F,G,H,I	kidney & liver damage, photosensitization		sheep		3,10
Phoma	decumbin (see Penicillium)					3
	cytochalicin B, D, E (see Penicillium)					3
	P.sorghina toxins	hemolysis				3
Phomopsis	cytochalicin C, D, E (see Penicillium)					3
	P.leptostromiformis toxins	cardiotoxic, liver damage				
Rhizoctonia	stalramine	parasympathomimetic				10
Rhizopus	Rhizopustoxins	kidney & liver damage				
Rosellinia	cytochalasin B, D, E (see Penicillium)					3
Sclerotium						10
Scopulariopsis	Scopulariopsis toxins	hemorrhage				3
Sporidesmium (see Pithomyces)						10
Stachybotrys	satratoxins F, G, H (see Myrothecium)					3,12,15
	Verrucarin A (see Myrothecium)					
	stachybotryotoxin	hemorrhage		horses		10
Stemphilium	stemphone					10
Thamnidium						10
Trichoderma	tricothecenes (?)	g.i.effects				10

Tricothecium	tricothecenes		
	crotocin		15
	T-2 toxin (see Fusarium)	acute tox., carcinogenic, teratogenic, liver damage, hemorrhage, neurotoxic, g.i. effects, leucopenia	3
	tricothecin		10
	tricothecolene		10
Tricophyton	viomellein (see *Penicillium*)		3
	xanthomegnin (see Penicillium)		
Verticillium	oosporein (see Oospora)		10
Verticimonosporium	satratoxins F, G, H (see Myrothecium)		3
	verrucarin A (see Myrothecium)		3
	vertisporin		15
Wallemia			10
Zygosporium	cytochalasins B, D, E (see Penicillium)		3

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects					Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
Higher Fungi										
Genus	Toxicant	D	G	N	P[a]	Adverse Effects				References
Agaricus, e.g. arvensis	?		X			nausea, vomiting, abdominal cramps, diahrrea				1,13
Amanita, including biosporigera, brunnescens, phalloides, tenuifolia, verna, virosa	amatoxins, alpha, beta, gamma phallotoxins				X	6-48 hr. latent period, abdominal pain, vomiting diarrhea, thirst, anurea, followed by apparent recovery followed by weakness, pain, liver, kidney & muscle damage, coma, death (30-+50%)				

		D	G	N	P		
Amanita, including muscaria, pantherina	ibotenic acid muscimol			X		1-2 hr latent period, drowsiness, dizziness, hyperactivity, excitability, confusion, delerium, and in children, convulsions, coma	1,7,13
Boletus, e.g. piperatus	?		X			(see Agaricus)	
Chlorophyllum e.g. molybdites	?		X			(see Agaricus)	
Clytocybe, including dealbata	muscarine			X		15-30 min. latent period, para-sympathetic effects, sweating, salivation, lachrymation, followed by abdominal pain, diarrhea, blurred vision, labored breathing	1, 7,11

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects					Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
		D	G	N	P					
Conocybe, including cyanopus	psilocin,psilocybin			X		onset rapid, hallucinations, inappropriate behavior similar to alcohol intoxication, in small children, fever, convulsions, death				1,7,11
Copelandia	psilocin,psilocybin			X		(see Conocybe)				
Coprinus atramentarius and other species	coprine		X			no effects unless alcohol is ingested within 48 hrs; produces disulfiram-like reactions including headache, flushing, parathesias, nausea, vomiting, tachycardia				7,13

		D	G	N	P		
Cortinarius, including orellanus	orellanine				X	latent period 3-14 days; intense thirst, excessive urination, nausea, headache, muscular pain, chills, spasms, loss of concoiusness, kidney failure, death (15%)	1,
Entoloma, e.g., lividum	?		X			(see Agaricus)	1,
Gallerina, including autumnalis, marginata, venenata	anatoxins, alpha, beta, gamma, phallotoxins				X	(see Amanita)	7,11,13
Gyromitra, including esculenta, gigas	gyromitrin				X	6-10 hr. latent period, abdominal fullness, severe headache, vomiting diarrhea liver damage death (2-4%)	1,7,13

TABLE 16—*Continued*

Causative Organism	Toxicant or Toxin	Disease or Adverse Pharmacological Effects				Food or Feed Affected	Species Affected	First Recognized as Food-Borne Human Pathogen	References
		D	G	N	P				
Inocybe, including geophylla	muscarine			X	(see Clitocybe)				1
Oomphalotus, e.g. illudens	?		X		(see Agaricus)				
Panaeolus spp.	psilocin, psilocybin			X	(see Conocybe)				1,7
Paxillus, e.g. involutus	?		X		(see Agaricus)				1
Pluteus spp.	psilocin, psilocybin			X	(see Conocybe)				
Psilocybe spp.	psilocin, psilocybin			X	(see Conocybe)				1,7,11
Russula, e.g., emetica	?		X		(see Agaricus)				1
Tricholoma, e.g., pardinum	?		X		(see Agaricus)				1
Verpa, e.g., bohemica	?		X		(see Agaricus)				

Note: See following page for references and footnotes for this table.

shellfish, such as oysters and mussels, and by finfish. The bivalves are relatively tolerant of the toxicants, but animals further up the food chain, such as fish, dolphins, and humans, are not. Fish kills and human shellfish poisonings are the result. These toxicants can be avoided only by closing shellfish beds during periods of "bloom."

Bacteria

Diseases from bacteria are the largest of the foodborne hazards. A few, such as botulism and salmonellosis, have been recognized for many years. Others are of far more recent knowledge.

Botulism and staphylococcal food poisoning clearly are caused by preformed toxins (see Table 16) that are produced when the responsible organisms grow in food before it is consumed. There are seven serologically distinct types of botulinum toxin designated by the letters A through G. Likewise, *Staphylococcus aureus* produces five serotypes of enterotoxin designated by letters A through E. Humans are exquisitely

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[a] Type of poisoning: D = disulfiram-like, G = GI irritant, N = neurological, P = protoplasmic (life threatening).

sensitive by the oral route to both of these preformed toxins. This is why we must be diligent in maintaining a food supply free of these organisms and toxins.

The other bacteria that cause foodborne disease obviously produce toxins, but it is not clear that the bacteria produce them in food nor is it known whether humans are susceptible to those toxins by the oral route. *Bacillus cereus* and *Clostridium perfringens* are believed to release their toxins when large numbers of cells are swallowed and undergo sporulation in the intestinal tract.

Pathogenic species and serotypes of *Vibrio, Escherichia coli, Shigella, Salmonella, Yersinia, Campylobacter,* and *Listeria* are believed to cause their typical symptoms only after they invade the body tissues and establish an infection. Presumably, *Aeromonas hydrophila, Plesiomonas shigelloides,* and other less established pathogens act in much the same way.

Lower Fungi

The class Ascomycetes contains most of the common molds and other fungi that grow on sources of organic matter, living or dead. Ergot, a fungus that attacks rye, has been recognized as a hazard for many years. The aflatoxins, discovered in 1959, were the first of the many mycotoxins now known to be produced by the filamentous fungi. Table 16 of the table lists many of the fungal sources and their reported toxic metabolites. Those underscored in the second column are among the best known, most widely distributed, or most significant sources of risk. The field is growing so rapidly that only a few, such as those underscored, have been studied in detail, and many, including those in parentheses, probably do not meet the fairly narrow definition of "natural toxicant" employed with respect to organisms used for human food. As the tabulation shows, however, many others have already been established as a source of harm to wild and domestic animals and humans.

Higher Fungi

The mushrooms, both edible and poisonous, belong to the class Basidiomycetes. Those listed in Table 16 are among the better known of the toxic genera.

The toxicants in mushrooms fall into four broad classes denoted in the table (pages S70–S74) by the following letters:

D—Disulfiram-like toxicants interfere with the metabolism of alcohol in a manner similar to disulfiram (Antabuse). Species that contain them are generally nontoxic unless alcohol is consumed within 72 hr of eating the mushroom. They are seldom life threatening.

G—Gastrointestinal irritants produce nausea, vomiting, cramps and diarrhea shortly after eating. They are seldom life threatening, but debilitated, very young, or very old patients may need supportive therapy.

N—Neurotoxins produce several characteristic sets of signs and symptoms. The distinguishing aspect is italicized below. The seriousness and extent of the other symptoms depend on the dose:

- *Prompt and profuse sweating, salivation, lacrimation,* abdominal pain, nausea, vomiting, diarrhea

• *Drowsiness, dizziness,* sleep, followed by hyperactivity, excitability, illusions, delirium

• *Psychotropic effects similar to those of alcohol intoxication* and rarely, except in children, fever, convulsions, and coma

P—Protoplasmic poisons are of several types. They have long latent periods, cause generalized destruction of cells and, in the doses normally encountered, frequently cause organ failure (typically liver and kidney) and death.

Because edible and poisonous species are so easily confused, avoidance is the only sensible course for those who are not truly expert.

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Chapter 3: Methods of Genetic Modification and Their Use

1. INTRODUCTION

The form and composition of each plant, animal, and microorganism are the result of the interaction between its innate genetic constitution, or genotype, and the environment. The product, or phenotype, is what we see, feel, taste, and analyze. The edible portions of crop plants and livestock and of food microorganisms are very often changed after harvesting by cooking, mixing with other products, and processing. In this chapter we discuss the nature of the genotype, factors that influence natural or evolutionary change, methods of traditional and nontraditional genetic modification, and the role that nontraditional genetic modification is expected to play in the agricultural and food industries.

1.1. The Basis of Genetic Variability

The chemical composition of plant parts consumed as food—proteins, carbohydrates, fats and oils, and fiber—is determined genetically. Some proteins serve a structural role; others are enzymes that catalyze chemical reactions in the cell. These enzymes govern the synthesis of the other constituent parts of the plant. The coded instructions for making enzymes and structural proteins reside in the DNA (deoxyribonucleic acid) found primarily in the nucleus of the cells.

1.1.1. Structure of DNA

DNA is a very long, thin molecule with a backbone composed of alternating sugar groups and phosphate groups. Attached to each sugar is one of four nucleotide bases; adenine, guanine, thymine, and cytosine. These are commonly represented by the letters A, G, T, and C. The order of these bases provides the genetic information, with each series of three encoding an amino acid.

DNA molecules consist of two strands that spiral around each other to form a double helix. Each nucleotide position in one strand is matched in the other strand by a complementary nucleotide. A is always paired with T, and C is always paired with G. Prior to cell division, DNA molecules replicate by separation of the strands, each single strand then serving as a template for formation of a complementary strand to yield a new double-stranded molecule. Because of the exact nucleotide pairing, the two double-stranded molecules that result from replication are identical. At cell division each daughter cell receives one of them. Their identity ensures that all the

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cells of the organism carry the same genetic information, apart from changes resulting from mutation.

1.1.2. DNA, RNA, Protein and Biosynthetic Pathways

The many enzymes responsible for directing each step in a biosynthetic pathway in a cell are each governed by a gene, a unique sequence of DNA. Each gene consists of from several hundred to a thousand or more nucleotide bases. When a gene is active, or switched on, the DNA codons of one of the two strands are first transcribed into RNA (ribonucleic acid). RNA differs from DNA in having a different sugar (ribose instead of deoxyribose), and the base thymine is replaced by uracil. The RNA transcript consists of a single strand and is often short-lived in the cell. Because it carries a message represented by the codon sequence in the original DNA, it is called messenger RNA (mRNA). The mRNA moves from the nucleus to small particles in the cytoplasm called ribosomes. One end of the mRNA becomes attached to a ribosome which then begins to move along it. As it does so, individual amino acids are selected and assembled into a chain reflecting the codon sequence in the DNA. The translation of the nucleic acid sequence into an amino acid sequence requires the participation of other forms of RNA called transfer RNAs (tRNAs). These are small RNAs that become attached to specific amino acids. As the ribosome translates each codon, it picks out from the pool of 20 different amino acids complexed to their tRNAs the one it requires. Each amino acid is linked to the previous one, forming a chain that grows in length.

Protein molecules are large and complex. They are usually made up of 20 different amino acids and become folded into three-dimensional structures. This complexity of structure means that individual proteins can have very different and highly specific functions as enzymes. An additional level of complexity results because the average cell has thousands of biosynthetic pathways whose coordinated expression is vital for efficient cell growth and function. The mechanism of regulation depends on regions that are present in most genes which control their function in relation to the changing environment within the cell.

Proteins that are not enzymes are part of plant cell structures such as the nuclei and organelles such as mitochondria and chloroplasts. Others, the storage proteins, are deposited in seeds, often with starch or fat, as reserves that are mobilized to support germination and seedling development.

Through their control of biosynthetic pathways, genes and the enzymes they code for determine the nature of each biochemical pathway and its product. Although these products contribute to the desirable phenotype, occasionally they are poisonous or harmful. Humans, in developing crops, have avoided such poisonous plants or plant products.

1.1.3. Central Importance of Accuracy

DNA is remarkably stable in large part because of the existence of repair mechanisms that correct errors resulting from deletion or mismatching of paired bases. Occasionally, however, one or more bases may change, causing mutations in the coded information. Several different agents, called mutagens, are known to increase the

frequency of mutation in nature and in the laboratory. They include ionizing radiation (X rays, radon gas, and exposure to radioactive isotopes), ultraviolet light, and certain chemicals. Mutations also arise rather frequently when plants are cultured on artificial media as undifferentiated cells. The mutants that arise from cell culture of somatic tissue are referred to as somaclonal variants.

A mutation results when a codon sequence is modified, either spontaneously or by a mutagen, and is not correctly repaired by the cell. From the food processing standpoint, some mutations are beneficial in that protein modification may result in useful changes to the end product. Such changes in protein structure are the basis of "protein engineering" as discussed later in this chapter. The movement of transposable elements may also cause mutation by the disruption of the sequence where the transposon becomes located. A mutated gene sequence may convey its altered phenotype by inhibiting transcription or translation or by producing a gene product that is nonfunctional. In diploid cells the deficiency caused by a mutation may often be covered by the unmutated sequence in the homologous chromosome. In this case, the defect will show only if the normal gene is absent, which is the case when two copies of the mutant are present, one on each homolog. Some mutants are dominant and are expressed even in the presence of an unmutated homolog. If the function lost by mutation is vital, the cell will die. If it regulates cell growth and division, the cell may begin dividing out of control and form a tumor. If the change in coding sequence is minor, substituting one amino acid for another at a point in the protein structure that is not crucial for function, the mutant may be indistinguishable from the normal or wild-type form or only slightly impaired, for example, forming an enzyme usually with less than normal activity.

1.2. Genetic Variability in Nature

Differences among individuals within a group may be observed at the level of DNA and protein but are commonly seen as variations in phenotype involving color, size, and shape of tissues and organs. Plant populations may show differences in a wide range of traits including plant height, size of seeds and fruits, floral and leaf attributes, environmental adaptability, insect and disease resistance, and variation in protein composition.

1.2.1. Examples of Variation

Rick *et al.* (1977) evaluated naturally occurring populations of *Lycopersicon pimpinellifolium*, the "currant tomato," for morphological features and protein composition. *L. pimpinellifolium* is found in the dry areas of coastal Ecuador and Peru. Forty-three populations representing its range of distribution were chosen for evaluation. The survey revealed not only morphological differences but variation among 11 enzyme proteins. The variant proteins are called "isozymes" and are detected by their different mobilities when separated by electrophoresis. Isozymes are naturally occurring forms of enzyme proteins, and for the most part have no effect on the phenotype or adaptability of plant populations. The genetic makeup of the currant tomato populations differed from one end of the geographic range to the other, with the central

area of the distribution much more variable than either the northern or southern regions.

1.2.2. Influence of Environment on Variation

The form, appearance, and chemical composition of any organism are products of the interaction of its genetic makeup with the environment. Genetic variability in a population allows it to respond to changes in the environment. If appropriate variants that are better adapted to the changed conditions are not present, and the shifts in environment are major, the population will not survive.

Extremes of temperature and humidity, such as frost and drought, can stunt growth and kill crop plants. Excessively saline or toxic soils may have similar effects. Other environmental factors are more subtle. Day length, for example, may control flowering in crops such as wheat and corn. Even crops like tomato which are not sensitive to day length become yellow and chlorotic if grown in continuous light without dark periods. For the crops of any region, these factors are well understood by farmers in their attempts to grow marketable produce. They use cultivars that are locally adapted and that afford some resistance to unpredictable climatic extremes.

Little is known of the precise effects of environmental variation on the expression of individual genes in food crops. Work with experimental systems, often microorganisms, has shown that some genes have functions that are temperature sensitive and that are not expressed either above or below certain critical temperatures. Current research is also exploring the mechanisms whereby plants vary their development in response to light intensity, wavelength, and day length. Environmental factors have a major impact on foods by controlling yield and food quality.

1.3. Factors That Influence Evolution

Five processes are responsible for evolutionary change (Stebbins, 1988): mutation, genetic recombination, selection, genetic drift, and reproductive isolation.

1.3.1. Mutation

Heritable changes in genetic material are by definition mutations. Mutations include chromosomal abnormalities such as translocations and changes in number; more subtle changes such as nucleotide substitutions, deletions, or duplications; and movement of DNA from one location to another (see Section 2.1.7). Mutations provide a genetic basis for variation and evolutionary change. In the short term, populations remain remarkably stable in spite of mutations. This is because unrepaired, heritable mutations occur infrequently and because many mutants are lethal or are of no immediate benefit to the organism. Thus, many of the genes arising from mutations do not contribute to evolutionary change.

1.3.2. Recombination

Paralleling the reproduction of animals, each plant starts life as a fertilized egg, receiving one set of chromosomes from the paternal parent through the pollen and

one set from the maternal parent through the egg. In a given species, geneticists name the chromosomes by size: chromosome one is the largest and so on. The plant has two copies of chromosome 1, one from the maternal parent and one from the paternal parent. Each chromosome contains a double helix of DNA. Before each cell divides, a process termed *mitosis,* each chromosome duplicates itself: the two strands of the helix separate, and each strand serves as the template for construction of an exact replica of its partner strand. As a consequence of this process, the cell contains twice the usual number of chromosomes. During mitosis, the chromosomes are precisely partitioned into each daughter cell so that each contains exactly the same set of chromosomes as the starting cell. The nearly error-free processes of DNA replication and chromosome partitioning ensure that each cell in the plant has the same set of chromosomes.

During the formation of sperm and egg in the flowers of the plant, a special type of chromosomal division termed *meiosis* occurs that produces cells with half of the usual number of chromosomes, that is, just one copy of chromosome 1, one of chromosome 2, and so on. This is important, because the union of sperm and egg will restore the normal chromosome number. The chromosome is the physical unit of inheritance: all of the genes on a particular chromosome are transmitted together to daughter cells. During production of the sex cells, recombination of genetic information occurs on the chromosomes. To do this, the maternal and paternal copies of chromosome 1, for example, pair and exchange material to create new combinations of genes. As a consequence of this genetic recombination, each chromosome in a particular sperm or egg can transmit some genes from the maternal and some from the paternal parent. Furthermore, because there are thousands of genes on each chromosome, the exchanges involve different groups of genes in each cell; as a consequence, each sperm and egg contains a unique combination of traits. This process of recombination of parental genomes is the major source of variation in higher plants.

1.3.3. Selection

Darwin, in his book *The Origin of Species* (1872), described the process of selection as follows:

> Variations, however slight and from whatever cause proceeding, if they be in any degree profitable to the individuals of a species . . . will tend to the preservation of such individuals, and will generally be inherited by the offspring. The offspring, also, will thus have a better chance of surviving. . . . I have called this principle, by which each slight variation, if useful, is preserved, by the term Natural Selection.

1.3.4. Genetic Drift and Reproductive Isolation

Random genetic drift refers to chance, nondirectional fluctuations in the frequencies of different forms of genes, or alleles, in a population. Genetic drift results because real populations are limited in size and gene frequencies may change due to random chance. Reproductive isolation may result from biotic factors, for example, seed dispersal by other organisms, or abiotic forces, such as variation in soil fertility. Genetic drift and reproductive isolation decrease genetic variation.

IFBC recognizes that variation in wild and domesticated plants is normal and results from environmental and genetic influences. Selective forces, either natural or by humans, result in shifts in the genetic compositions of populations.

2. TRADITIONAL GENETIC MODIFICATION

2.1. Traditional Methods of Introducing Variability

2.1.1. Hybridization

Hybridization is the most widely used method of introducing variability into crop plants. In many ways plant breeding and hybridization are synonymous. Hybridization is the process whereby crosses are made between different cultivars or species to give unusual or improved types. The use of interspecific crosses, is less typical and is discussed below. In a standard breeding program, the plant breeder begins by selecting parents that as closely as possible show genetic variability only for the characters of interest. Usually only two parents are selected, but in some cases three or more parents are used. The idea behind the use of the more complex mating schemes is to increase the number of possible alleles in the population to maximize the opportunities for effective cultivar development. This is particularly helpful in cases where many genes are responsible for a particular trait. Once the hybrid populations are formed, the breeder will choose from one of a number of possible breeding schemes to develop a new and improved cultivar. Further improvement or genetic advance is dependent not on new genes, but on new combinations of genes based on the breeder's starting materials. The recombining of the parental gene pools was discussed earlier and was referred to as recombination.

2.1.2. Mutagenesis

Following early pioneering studies on the deliberate induction of mutations, there was much excitement among plant breeders who saw mutation induction as a great opportunity to increase the variability of their stocks. Much activity in this area followed but eventually it diminished because induced mutations, like spontaneous mutations, were almost always deleterious. Further, most of the variation that breeders were interested in already existed in modern stocks or wild species. Also, the mutations that occurred were random in their effect, and thus it was nearly impossible to target any particular trait. Mutation breeding, as a result, has been largely ignored as a crop improvement tool. Most breeders are too busy capitalizing on existing variability to be bothered with the long and difficult process of mutation breeding. Some successful examples of mutation breeding include cultivars of wheat, barley, peas, soybean, tomato, cotton, and rice. Traits improved by mutation breeding include yield, lodging, disease resistance, and adaptability. Many geneticists have concluded that spontaneous and induced mutations are not significantly different from one another (Stubbe, 1967). For example, the entire spectrum of genetic variability observed in barley as the result of spontaneous mutation and recombination from traditional plant breeding has been recreated using induced mutation techniques. In addition,

identical phenotypes from both spontaneous and induced mutations have been observed in other crops such as corn and tomato.

2.1.3. Wide Hybridization

Genetic variability may be increased by making crosses with different species or genera. This procedure is frequently referred to as wide hybridization. As mentioned earlier, crop improvement usually involves the hybridization of select modern cultivars and subsequent selection of individuals that contain desirable attributes from both parents. The choice of parents is critical in that each parent should contain a minimum number of undesirable characteristics. In wide crosses, the number of undesirable characteristics is very large.

Where the trait of interest cannot be found in modern germplasm the breeder is forced to look at more exotic sources. The first preference is to use germplasm of the same species, perhaps primitive land races or old cultivars. If the desired variation is not found the search is extended to closely related species and, as a last resort, depending on the crop, species within a closely related genus. The tomato demonstrates the importance of wide hybridization in crop improvement. Genes for resistance to at least 30 diseases have been identified in wild tomato species (Rick *et al.*, 1987) and 16 have been used in commercial cultivars (see Table 17).

The ability to intercross species is sometimes limited by genetic differences between the species; this is referred to as sexual compatibility. The wider the cross the more difficult hybridization is to achieve. Some *in vitro* procedures, including embryo culture and protoplast fusion, have made it possible to hybridize sexually incompatible species. Nontraditional genetic modification, including recombinant DNA techniques, hold much promise for effecting gene transfer among species that cannot otherwise be hybridized.

2.1.4. Novel Variation

Wide hybridization can sometimes result in novel or unexpected phenotypes. High levels of β-carotene (provitamin A) resulted when a green fruited wild species, *L. hirsutum*, was crossed with a standard red fruited tomato cultivar. Although the resultant orange fruited hybrid had enhanced nutritional value it was not produced commercially because of its unacceptable color.

2.1.5. Problems Associated with Interspecific Crosses

Even though exotic germplasm seems an obvious way of increasing variation, it is used only as a last resort. Exotic germplasm introduces such problems as hybrid inviability and sterility. Wild parents also carry much unwanted genetic information. Eliminating undesirable traits while retaining the desired features is a major portion of the breeding effort. The task of selecting desired recombinant individuals, carrying only the desired characteristic, can last for decades. The use of linked markers, such as isozymes and restriction fragment length polymorphisms (RFLPs), should make the transfer of useful traits from wide hybrids easier.

TABLE 17

RESISTANCE IN WILD SPECIES OF *Lycopersicon* AND *Solanum* OF SOME ECONOMICALLY IMPORTANT DISEASES OF TOMATO

Disease	Responsible organism	Source of resistance
Bacteria		
Bacterial canker[a]	*Clavibacter michiganese*	*L. hirsutum, peruvianum, pimpinellifolium*
Bacterial speck[a]	*Pseudomonas tomato*	*L. pimpinellifolium*
Bacterial spot	*Xanthomonas vesicatoria*	*L. esculentum* var. *cerasiforme*
Bacterial wilt[a]	*Pseudomonas solanacearum*	*L. pimpinellifolium*
Fungi		
Collar rot	*Alternaria solani*	*L. hirsutum, peruvianum, pimpinellifolium*
Leaf mold[a]	*Cladosporium fulvum*	*L. esculentum* var. *cerasiforme*
Fruit anthracnose[a]	*Colletotrichium coccodes*	*L. esculentum* var. *cerasiforme*
Target leaf spot	*Corynespora cassiicola*	*L. pimpinellifolium*
Didymella canker	*Didymella lycopersici*	*L. hirsutum*
Fusarium wilt[a]	*Fusarium oxysporum f. sp. lycopersici*	*L. pimpinellifolium*
Phoma blight	*Phoma andina*	*L. hirsutum*
Late blight[a]	*Phytophthora infestans*	*L. pimpinellifolium*
Phytophthora fruit rot	*Phytophthora parasitica*	*L. pimpinellifolium*
Phytophthora root rot	*Phytophthora parasitica*	*L. esculentum* var. *cerasiforme*
Corky root[a]	*Pyrenochaeta lycopersici*	*L. peruvianum*
Septoria leaf spot[a]	*Septoria lycopersici*	*L. esculentum* var. *cerasiforme, hirsutum, pimpinellifolium*
Gray leaf[a]	*Stemphylium*	*L. pimpinellifolium*
Verticillium wilt[a]	*Verticillium albo-atrum*	*L. esculentum* var. *cerasiforme*
Dahlia wilt	*Verticillium dahliae*	*L. peruvianum*
Nematodes		
Potato cyst	*Globodera pallida*	*L. hirsutum*
Sugarbeet	*Heterodera schactii*	*L. pimpinellifolium*
Root-knot[a]	*Meloidogyne incognita*	*L. peruvianum*
Viruses		
Spotted wilt[a]	TSWV	*L. pimpinellifolium*
Tobacco mosaic[a]	TMV	*L. peruvianum*
Tomato yellow leaf curl	TYLCV	*L. cheesmanii*
		L. hirsutum, peruvianum, pimpinellifolium
Cucumber mosaic	CMV	*L. peruvianum, S. lycopersicoides*
Curly top[a]	CTV	*L. peruvianum*
Potato Y[a]	PYV	*L. esculentum* var. *cerasiforme*

Source. Rick *et al.* (1987). Reprinted with permission from Acta Horticulturae.

[a] Resistance has been incorporated into cultivars.

2.1.6. Changes in Chromosome Number or Structure

Variability in wild and domesticated populations can also be increased by changes in chromosome number or structure. Chromosome number is a useful character in

plant taxonomy. It is usually constant for each species but may vary among species in the same genus. Cultivated strawberry has 56 chromosomes, but some related wild species have 14, and others 28. The basic number of chromosomes in the genus *Fragaria*, to which strawberry belongs, is 7. Cultivated strawberry has 8 sets and is therefore octoploid. Polyploids are sometimes more vigorous and higher yielding and may have greater environmental stability than diploids.

In nature, polyploids may arise by chromosome doubling of the parents followed by hybridization, by hybridization followed by somatic doubling, or through the union of unreduced gametes. The second method is commonly used by breeders to produce polyploids.

Genetic variation can also arise by the addition or subtraction of portions of the genome or by structural changes within or between chromosomes such as deletions, translocations, and inversions. These types of chromosomal abnormalities are exploited in wheat breeding and allow the substitution of parts of chromosomes from related wild species.

2.1.7. Transposable Elements

During the years 1942–1956 Barbara McClintock described the behavior of some unstable mutants of corn that exhibited unusual colors and color patterns. She proposed that these traits resulted from the presence of mobile genetic elements—pieces of DNA that move on the corn chromosomes. Movement to a new position was revealed by a change in the expression of another gene occupying the site. If the mobile element moved away, expression of the resident gene was restored. This work, for which McClintock received the Nobel Prize in 1983 (Anonymous, 1983), was remarkable in that it depended on rigorous careful observation and experimentation and preceded the major discoveries in molecular biology which were to describe it at the DNA level.

The importance of McClintock's findings was realized when similar behavior was observed in bacteria and the mechanisms of gene movement were elucidated. The mobile elements, called transposons, are activated by a gene coding for an enzyme, a transposase, which cleaves them from the DNA. They move preferentially into nearby sites, apparently chosen at random, on the same chromosome and produce an effect similar to a mutation. When a transposon moves it leaves behind a short sequence of bases forming a characteristic footprint. Transposons are relatively short pieces of DNA, from several hundred to more than ten thousand nucleotide pairs in length. Transposons responsible for genetic variation have been studied in microorganisms, as well as corn and snapdragon (*Antirrhinum*), and have been tentatively identified in several other plant and animal genera. The extent to which transposons are responsible for variation in other crop plants is not known. Transposons provide a useful tool for isolating genes by the methods of molecular biology and have been introduced from corn into other plants such as tobacco and tomato by methods discussed later (see Section 3.2).

Some issues raised by the application of recombinant DNA technology, which we discuss later, are also encountered in traditional genetic modification. For example, despite the evidence for transposon activity in several crop plants there have been no safety issues related to transposon movement.

IFBC recognizes that traditional methods of introducing genetic variability, although successful in the past, are limited by crossing barriers, inability to induce directed genetic changes by mutagenesis, and inefficient selection procedures.

2.2. Plant Breeding

Two key factors are required for useful genetic modification. The first is the availability of sources of variation. The second is ability to select individuals that contain the desired genetic change. Selection is frequently the most difficult aspect of crop improvement, due to the masking effect of the environment on genetic composition.

Differences in color, shape, texture, and the presence or absence of certain characters are qualitative and are generally easy to classify. Variability that grades gradually from one extreme to the other is quantitative and cannot usually be assigned to discrete classes. Many characters of economic importance such as yield, period of development, height, and vigor are quantitative in nature. Quantitative characters are often very sensitive to the environment. For quantitative traits the task of selection is proportionately more difficult.

2.2.1. Crop Stability

When farmers sow seeds they expect to harvest a crop that can be marketed. Barring environmental factors, they take for granted that a crop cultivar grown before will perform more or less the same way each time it is planted. The consumers assume that the food they buy will taste like other samples they have always eaten. The characteristics of the harvested product depend on the genetic makeup of the plant. For example, wheat with good bread-making quality has grains with a protein content of 12–14% and the component proteins that contribute the correct viscoelastic properties to bread doughs. The grain must have very little α-amylase, an enzyme that promotes stickiness in the dough. These and other properties are dependent on the interaction between genetic makeup, or genotype, and the environment in which the plant is grown.

The modern-day farmer's yearly crop expectations depend on the efforts of plant breeders, on agronomic improvements such as optimal planting densities, herbicides, pesticides, growth regulators, and fertilizers, and, of course, on the environment. The farmer and plant breeder strive for crop stability. Their desire is to produce, year after year, a product that performs at an acceptable level despite variations in environment.

2.2.2. Principles of Crop Improvement

Plant and animal breeding is a form of evolution depending in large part on the same rules that regulate the evolution of natural species but with one important difference: natural selection has been replaced largely by human selection. Modern plant breeders work with the end products of a long period of natural selection. Humans have accelerated and changed this process and in numerous instances acted contrary to natural selection by preserving mutants that would not survive without

human intervention. Natural selection and artificial selection have provided the modern plant breeder with a liberal heritage of plant material.

The three major objectives of modern plant breeding programs are to increase yield, improve quality, and reduce production costs. The most important element of the latter goal is to breed for resistance to pests and diseases.

2.2.3. Pest and Disease Resistance

Food crops are subject to major losses, in the field and after harvest, caused by pests and diseases. To control loss, farmers commonly apply pesticides, use resistant cultivars, follow cultivation practices designed to limit pest and disease development, or combine all three approaches by practicing integrated pest management. Pesticides can be very effective in reducing crop damage and spoilage. However, breeders have emphasized development of cultivars with inherited disease resistance which provide alternatives that are cheaper than pesticides and have less environmental impact. Resistant cultivars also reduce pesticide usage and the potential risk of their residues in food, even though the risks to human health from these residues are much less than those from microbial contamination and natural toxicants.

Fortunately, each crop is susceptible to only a narrow spectrum of fungi, bacteria, and viruses. For example, wheat and maize are not susceptible to the same pathogens, and of the diseases that affect cereals virtually none affect horticultural crops. Although plant breeders have been developing disease-resistant cultivars for many years, there is very limited understanding of the molecular basis for most disease resistance.

In spite of the lack of knowledge of the biochemical and physiological basis of resistance to insect pests and diseases, plant breeders have successfully transferred disease resistance from the wild species by developing direct screening methods. Examples of crop resistance to insects include apples resistant to the woolly aphid (*Eriosoma lanigerum*), wheat resistant to Hessian fly (*Mayetiola destructor*), and grape vines resistant to phylloxera (*Phylloxera vitifoliae*).

It is difficult to develop insect resistant cultivars. For example, a tomato wild relative, *L. hirsutum*, contains a natural insecticide, tridecanone, produced by the hairs on the leaves of this species. *L. hirsutum* contains about 72 times as much tridecanone as do susceptible commercial cultivars of tomato, and is resistant to larvae of the Colorado potato beetle and the tomato hornworm which are killed by the compound. Tridecanone also has an adverse effect on tomato fruitworm larvae, but the fruitworm will acclimate to the compound. It has not yet proved possible to introduce the high levels of this compound into the cultivated tomato by breeding.

If it were possible to introduce high levels of tridecanone into tomatoes (by either traditional or rDNA methods), there could be a legitimate concern about possible toxic effects, even though the compound produced is manifestly "natural." We have a long history of imparting (and taking away) natural disease and pest resistance in crops through breeding. In these cases, the molecular basis of the resistance trait is usually unknown, but the approach has been used successfully for many years. A rDNA approach requires much fuller knowledge of the molecular basis of the resistance trait. From a regulatory point of view, it would appear that such an rDNA approach would be more predictable than the traditional approach.

2.2.4. Breeding for Quality or Composition

Quality is governed by composition but is often complex in its inheritance, and screening can be difficult. The best tasting, most nutritious cultivar is unlikely to succeed unless it has yield, disease resistance, and the other characteristics essential to growers, processors, and shippers. However, progress in breeding winter wheat in the United Kingdom illustrates what can be done in improving both quality and yield. Better baking quality resulted in part from selection for grains with hard endosperm coupled with adequate protein content. But stringent selection for particular glutenin and gliadin protein subunits present in the endosperm was critical. The structure and composition of these protein molecules determine the viscoelastic properties of bread doughs. Choosing the best and most effective ones from among the many available is most important. As a result of breeding better varieties the percentage of home grown wheat in British loaves rose from 30 in 1970 to more than 80 in 1984 (Day *et al.*, 1985).

Plant foods, especially vegetables and fruits, are usually harvested when the edible portion is undergoing a rapid change in composition. Stage of maturity is not easily categorized and thus sampling becomes a major source of error. It is difficult to determine whether sampling differences are due to the environment, stage of maturity, or genetic variation. Therefore, even when constituents can be easily measured, the data obtained may not represent genetic potential.

The problem can be illustrated by considering vitamin C, or ascorbic acid, in tomatoes. Malewski and Markakis (1971) found that ascorbic acid concentration decreased slightly during the second week after pollen was shed, increased rapidly until just before full red color development, and markedly decreased during senescence. The rate of loss of ascorbic acid after ripeness is determined by genotype and environmental effects. Also, seasonal and weekly variations accompany environmental effects (Shivrina, 1937). Light intensity is an important factor in the ascorbic acid fluctuations associated with season and location since ascorbic acid concentration is correlated positively with the intensity of the light reaching the fruits. For instance, fruits on plants with dense foliar coverage, such as fruit collected from unsupported vines, are usually lower in ascorbic acid than those on plants with sparse foliar coverage. Also, greenhouse-grown tomatoes are lower in ascorbic acid than are field grown tomatoes.

2.2.5. GRAS (Generally Recognized as Safe) and Plant Breeders

In the early 1970s, FDA considered the possibility that changes in food composition that might result from traditional genetic modification of crop plants could affect the GRAS status of the resulting foods.

The FDA cited six incidents which raised questions of safety as the possible reasons for including new cultivars under GRAS regulations (Spiher, 1974): (1) a 60% increase in solanine content of potatoes grown from seed tubers treated with 1000 rads of gamma radiation to break dormancy; (2) the development of a high-solids potato cultivar with high solanine content; (3) the hypothesis that potatoes resistant to late blight develop additional chemicals that are teratogenic; (4) the production of the toxic chemical ipomeamarone by sweet potatoes under certain environmental condi-

tions; (5) the development of cultivars of food plants resistant to insect attack; and (6) unexpected changes in plant composition due to other varietal changes (the example given was reduced vitamin C in tomatoes due to mechanical harvesting).

FDA indicated that an increase in toxicants of 10% or more compared with the parent containing the least toxicant or a decrease in a principal nutrient of 20% or more will require that appropriate analytical data be supplied to the FDA in a GRAS affirmation petition.

Despite the concern of plant breeders over regulation by the FDA, the vast majority of new plant varieties have not been formally reviewed under GRAS regulations or required premarket approval from the FDA.

2.2.6. Disease and Insect Resistance—Toxins

There has been concern that new plant cultivars with improved disease or insect resistance may owe their resistance to the presence of compounds that are toxic not only to plant pathogens or insects but also to humans. There are a few instances where research data support this supposition; although problems are possible, the weight of historical evidence would suggest they are manageable. Glycoalkaloids, for example, may be a factor contributing temporary resistance of young potato and tomato leaves to the disease early blight incited by *Alternaria solani*.

Tomatoes were slow to gain acceptance as a food largely because of superstitions that the fruits were poisonous. Their membership in the poisonous nightshade family created great reluctance to eat the fruits. In some areas, these superstitions persisted into the 20th century. The main steroidal glycoalkaloid in tomatoes is α-tomatine. There is large variation in α-tomatine content among the tomato species. The content of this glycoalkaloid is greatest in the young fruit and declines as fruits mature. At the beginning of color development, α-tomatine apparently is not present in fruits of any cultivar or wild species.

Other alkaloids present in the leaves and stems of plants protect against insects. Leptines, demissine, and, to some extent, solanine, reduce feeding on potatoes by larvae of the Colorado potato beetle. α-Tomatine is toxic to several pathogenic microorganisms and insect pests of tomato (Juvik *et al.*, 1982).

In celery several psoralens, a class of compounds called furanocoumarins, were implicated in cases of photodermatitis among grocery store personnel working with produce. Investigation showed that the problem was caused by a celery variety, not named in the report, which was grown on certain farms (Seligman *et al.*, 1987). This variety had concentrations of psoralen some 10 times greater than varieties not causing the problem. The skin of workers who trimmed or weighed these materials was repeatedly exposed to sap containing the compound. Subsequent exposure to ultraviolet light resulted in severe dermatitis. Psoralens have been shown to play a role in pest resistance in plants (R. C. Beier, in press).

2.2.7. Other Toxins

As previously described (Chapter 2, Section 2.3.3) scientists became highly sensitized to the importance of screening breeding lines for naturally occurring toxicants after the potato variety Lenape was discovered to have a higher-than-normal tuber

glycoalkaloid content and was removed from commercial trade. As a result of the Lenape episode research on glycoalkaloid in potato tubers was expanded and problems associated with them have been largely overcome by breeding.

Trypsin inhibitors and ipomeamarone are both found in sweet potatoes. However, neither component is a problem to humans when sweet potatoes are properly prepared. Boiling for 15 min destroys trypsin inhibitor activity. Peeling and trimming blemished areas, as well as cooking, reduce ipomeamarone to insignificant levels (Reitz and Caldwell, 1974).

Plants containing cyanide-yielding glycosides are not uncommon, with the following types being consumed regularly by humans: cassava, sweet potato, yam, maize, millet, bamboo, sugarcane, peas, beans (especially lima or butter bean), kernel of almond, lemon, lime, apple, pear, cherry, apricot, prune, and plum (Montgomery, 1980). Cyanogenic glycosides (CGs) yield hydrogen cyanide (HCN) on treatment with acid or suitable enzymes, and these enzymes are endogenous to many of the plant types just mentioned. In intact, undamaged plant tissue the HCN-generating enzymes are inactive (compartmentalized) and generation of HCN does not occur. Bruising, slicing, or macerating the raw tissue releases the enzymes and they will then act on the CGs causing release of HCN in amounts that can have debilitating or lethal effects on humans (Conn, 1973). The seriousness of this problem is demonstrated by the fact that the cyanogen (cyanide-yielding) content of lima beans imported into the United States is monitored and controlled (Montgomery, 1980).

The risk of poisoning can be reduced by breeding cultivars that are low in CGs (Conn, 1981). Cassava has received particular attention because of its great importance as a food crop. Low-cyanogen cultivars of cassava yield about 21–44 mg HCN/kg of fresh root (Okeke and Oti, 1988), whereas cultivars not selected for a low cyanogen content may yield more than 20 times this amount of HCN (Montgomery, 1980).

2.2.8. Nutrients

Breeders have devoted little attention to improving the nutritional value of plants, spending far more time on yield, appearance, and pest and disease resistance. For example, despite the great genetic potential for increasing the concentrations of vitamins A and C in fruits and vegetables, the limited efforts to breed and commercialize cultivars with high nutritional value have, in general, not been successful. Only a few cultivars bred for high vitamin content have been released and listed in seed catalogs since 1940; however, almost none of these gained widespread use. The major exceptions are carrot and sweet potato cultivars with higher carotene (provitamin A) levels, and these have probably been widely accepted because of visual appeal rather than nutritional value. There is no evidence indicating that a lack of attention to nutrient level by plant breeders has had an adverse effect on the nutritional value of newer cultivars.

Carrots are the most important plant source of provitamin A (β-carotene) in the United States, providing about 14% of the total vitamin A intake (Senti and Rizek, 1975). Typical U.S. carrot cultivars contain 60 to 150 ppm total carotene. Selection has been successful in increasing the carotene content of one population (β_3) to 270 ppm and another (HCM) to an average of 475 ppm; some roots contain more than 700 ppm carotene. Both of these high carotene populations combine germplasm from the United States with oriental germplasm (Simon, 1988).

Tomatoes are an important dietary source of provitamin A and vitamin C because they are consumed in large quantities. With respect to provitamin A, a great difference exists in the β-carotene content of cultivated and wild tomato species. Differences in β-carotene content of more than 100 fold were found in progeny from crosses between the cultivated tomato and the high β-carotene wild species *L. hirsutum*. From a cross between a tomato cultivar and *L. hirsutum*, cultivars have been developed that are 10-fold higher in β-carotene than current cultivars.

Tomato cultivars with twice the normal vitamin C level have been developed, but none of these has achieved commercial importance. In spite of considerable effort to develop cultivars with higher vitamin C levels, few have been released. There have been repeated charges that newly released cultivars have lower vitamin C levels than traditional cultivars. A careful comparison of vitamin C levels of cultivars released over a long period shows that vitamin C content of tomato cultivars has steadily increased. Cultivars released in 1972 averaged 25% more vitamin C than those released in 1952 (Matthews *et al.*, 1973).

IFBC recognizes that although the primary objective of plant breeders has been yield and pest resistance, plant breeders through selection of breeding materials, roguing of test plots, and monitoring of the ultimate commercial product have very effectively conserved nutritional quality and safety.

2.2.9. Breeding Methods

Most breeders are extremely conservative in their choice of parental plant material. If this material is not well adapted and close to the desired endpoint, the breeder will have to spend much additional time and effort to improve the crop. For this reason most breeders select modern cultivars, produced by themselves or their competitors, intercross them, and select segregants that are an improvement from the original parents. Only when the needed variation is missing from adapted cultivars do plant breeders turn to older or primitive cultivars and wild relatives.

Effective breeding programs depend very heavily on methods that allow few people to handle large amounts of material and information rapidly and accurately. Most breeders therefore use specially designed equipment for planting, cultivation, recording data in the field, harvesting, and testing large numbers of product samples in the laboratory. As a consequence breeders work closely with agricultural engineers, computer programmers, biochemists, and industry personnel to develop the most efficient systems that are possible. In the end the program that can screen the most material most effectively is successful.

2.3. Limitations of Plant Breeding

There are four major limitations to plant breeding: (1) genetic variability, (2) ability to select desirable types, (3) generation time, and (4) tight linkage with undesirable characters. The new technologies (Section 3.2) involving recombinant DNA have the potential to help the breeder improve crops in each of these areas.

2.3.1. Genetic Variability

The use of exotic germplasm, though valuable, can be difficult and time consuming. The exotic gene pool is also limited in that only relatively closely related species can be utilized. It is not possible to cross tomato and eggplant, for example, even though they are in the same family. By the use of recombinant DNA methods, however, it is now possible to move corn genes into tomato or bacterial genes into crop plants. Thus, crossing barriers no longer limit the exchange of genetic information and recombinant DNA methods have the potential to expand greatly the breeder's germplasm pool. The limitation now becomes the identification of desirable genes.

2.3.2. Selection of Desirable Types

Selection has been one of the biggest problems for breeders, particularly for environmentally influenced quantitative traits. Methods of identifying individuals that contain the desired gene or genes based on detection of molecular markers may well improve the selection process.

2.3.3. Generation Time

Most of the annual crops such as corn, wheat, rice, and tomatoes can be put through a breeding cycle two or three times a year. Perennial crops, such as coffee, dates, and citrus, require many years before a single cycle of selection can be made. Even the incorporation of a single trait can take the lifetimes of several plant breeders. The ability to modify such crops by the use of recombinant DNA methods has great potential for shortening the time it takes to develop new cultivars.

2.3.4. Linkage

Backcrossing is an integral part of most plant breeding programs. It is used widely to incorporate desirable monogenic traits into elite germplasm. A problem frequently arises in backcross breeding because it is difficult to eliminate undesirable traits that are closely associated or linked to the trait of interest. Linkage is usually more of a problem as the gene donor source diverges from the recipient or recurrent parent. Recombinant DNA (rDNA) methods are more precise than traditional methods in that the transferred DNA sequences are well defined.

IFBC recognizes that recombinant DNA methods offer unique opportunities for crop plant improvement. These include the incorporation of novel traits from diverse organisms and improvements in the efficiency and precision of crop improvement.

3. NONTRADITIONAL GENETIC MODIFICATION

3.1. Tissue Culture Methods

Biotechnology does not displace conventional plant breeding, but simply allows the process to proceed at a more rapid pace. The two phases of plant breeding are

creation of genetic variability and selection for improved gene combinations. From these gene combinations are selected breeding lines for the development of new varieties. Varietal traits have a genetic basis, inherited from one generation to another through seed, and perform uniformly under defined growth conditions.

During recent years, plant breeding procedures have been further refined with improved selection techniques and statistical analysis. Moreover, determined efforts to preserve natural genetic variability represented in thousands of plant seeds have led to the establishment of germplasm centers for certain crops. The well-orchestrated use of available germplasm with the new tools of tissue culture, somaclonal variation and gametoclonal variation, somatic cell hybridization, cellular selection procedures, and recombinant DNA will provide expanded opportunities for the rapid production of new breeding lines and hence new varieties.

3.1.1. Clonal Propagation

Clonal propagation allows the large-scale reproduction of "carbon copies" of superior genetic varieties. A wide variety of plant species can be clonally propagated from leaf, stem, or root tissue. Examples of tissue culture-propagated crops include strawberry, asparagus, and oil palm (Morris, 1983). Current research focuses on mechanized industrial-scale clonal propagation using new technology. The technical steps involved in clonal propagation are illustrated in Fig. 1. Current and future applications for clonal propagation are (1) propagation of special parent plants, such as male steriles, for use in hybrid breeding programs; (2) mass propagation of hybrid plants for crops whose hybrid seed is difficult or expensive to produce; (3) more rapid development of improved perennial crops with long generation times, such as fruit, forest, and coffee trees; and (4) production of disease-free planting stock.

3.1.2. Somaclonal and Gametoclonal Variation

In contrast to clonal propagation, which faithfully produces genetic carbon copies, regeneration of plants from callus, leaf tissue explants, or plant protoplasts (wall-less cells) by means of tissue culture can result in the recovery of somaclonal variants (Evans and Sharp, 1983). Somaclonal variants have been recovered in tomato (Evans and Sharp, 1983), potato (Shepard, 1982), and sugarcane (Larkin and Scowcroft, 1981). In these crops, somaclonal variants have been produced and selected for new breeding lines with new agronomic and processing benefits. Among the variants of tomato observed were those with changes in fruit color, plant architecture, and harvesting characteristics. The steps involved in somaclonal and gametoclonal variation are illustrated in Fig. 2.

The genetic variability recovered in plants regenerated from tissue culture probably reflects both preexisting cellular genetic differences and tissue culture-induced variability. For example, geranium plants obtained from *in vitro* root and petiole cuttings and plants regenerated from callus were quite variable relative to parent plants in plant and organ size, leaf and flower morphology, essential oil constituents, fasciation, pubescence, and anthocyanin pigmentation (Skirvin and Janick, 1976). Long-

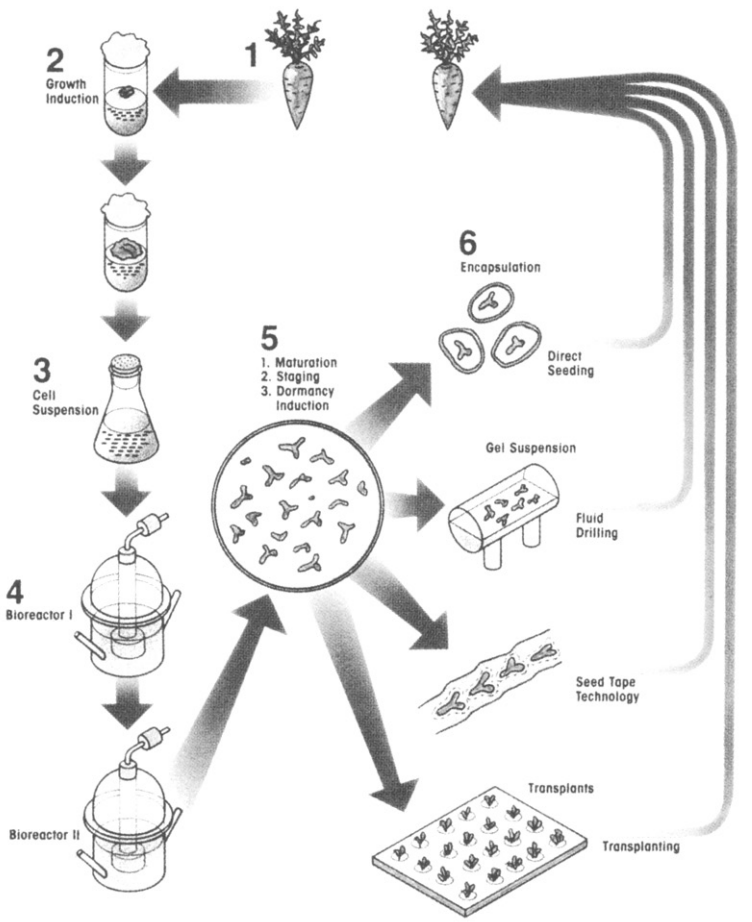

FIG. 1. Steps in clonal propagation. (1) A unique genetic variant or hybrid plant is selected. (2) Cell cultures are established. (3) Cells may be transferred to liquid medium during first scaleup to increase the number of regenerated plants. (4) Cells can then be transferred to bioreactors for further scaleup. (5) Embryos produced in bioreactors or cell suspension cultures can be staged for reliable production of plants. At this point, it is necessary to develop methods that permit induction of dormancy if artificial seed delivery systems are to be used. (6) Young plants are removed from tissue culture and transferred to the greenhouse, nursery, or field. This step is delicate and may require up to 1 month even for annual crops. Experimentation is proceeding to develop efficient delivery systems; these include encapsulation, use of gel suspensions, and use of seed tapes. *Source:* Sharp *et al.* (1984). Reprinted with permission from *Food Technol.*, 1984, 38(2), 112–119. Copyright by Institute of Food Technologists.

term cell cultures often contain tissue culture-induced variability in chromosome number that results in commercially useless variants of sexually propagated species but that may be useful in asexually propagated crops such as sugarcane and potato.

Some sugarcane clones with altered chromosome number were found to have useful disease resistance (Heinz *et al.*, 1977). Other types of variation may be due to stable, single gene changes, such as those characterized in tomato by Evans and Sharp (1983), or to chromosomal rearrangements, such as that observed in newly disease resistant potato clones isolated by Shepard (1982).

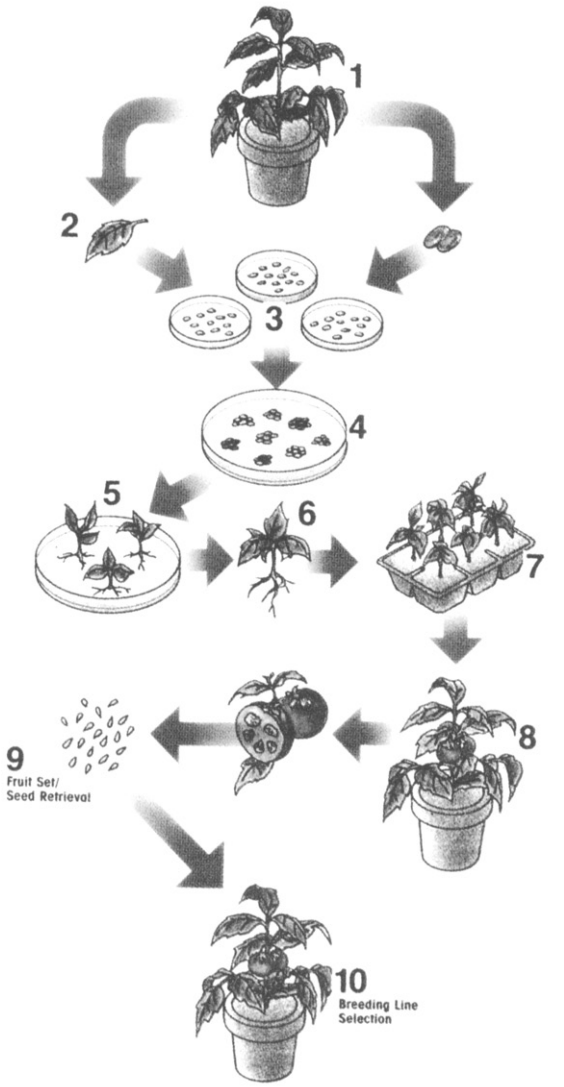

FIG. 2. Steps in somaclonal and gametoclonal variation. (1) Suitable donor plant material is selected, i.e., leaf, stem, or other somatic tissue for somaclonal variation or anthers for gametoclonal variation. (2) Tissue (explants) of the plant capable of plant regeneration and suitable for recovery of genetic variants is removed from the donor plant. This explant is disinfected prior to introduction into tissue culture. (3) The explant is placed onto a culture medium specifically prepared both for the induction of variation and for cell growth. (4) The tissue grows to form an unorganized cellular mass (callus). (5) The callus is, in some cases, transferred to a second culture medium to permit shoot regeneration. Regenerated shoots are then transferred to a culture medium to induce root formation. (6) Young plants are removed from tissue culture and acclimated to greenhouse conditions. (7) Young regenerated plants are transferred to the greenhouse and transplanted to larger pots or vessels as necessary. (8) Regenerated plants are raised to maturity in the greenhouse or field. (9) Fruit is collected from the regenerated plants, and seed is retrieved from this fruit to permit evaluation of the progeny of the regenerated plants. (10) New variants are identified in field, and the seed collected from the regenerated plants is subjected to evaluation. This ten-step procedure can be completed in less than a year for annual crops, such as tomatoes and tobacco. (*Note:* The chromosome number of plants derived from anthers must be doubled to obtain seed.) Reprinted with permission from *Food Technol.*, 1984. **38**(2), 112–119. Copyright by Institute of Food Technologists.

3.1.3. Protoplast Fusion Technology

Protoplast fusion, the fusion of wall-less cells, permits the development of unique hybrid plants impossible to achieve via conventional sexual hybridization (Evans, 1983a). Such new hybrids used in a breeding program may permit development of new plant varieties that are otherwise not possible. Interspecies somatic hybrid plants have been produced in the following genera: *Datura, Daucus, Nicotiana, Petunia, Brassica,* and *Solanum.* These hybrids represent new combinations of genetic material (Evans, 1983b). The steps involved in protoplast fusion are illustrated in Fig. 3.

Several intergeneric hybrid plants have been recovered: *Solanum* + *Lycopersicon* (Melchers *et al.*, 1978), *Atropa* + *Datura* (Krumbiegel and Scheider, 1979), *Daucus* + *Aegopodium* (Dudits *et al.*, 1980), *Arabidopsis* + *Brassica* (Gleba and Hoffman, 1980), and *Nicotiana* + *Atropa* (Gleba *et al.*, 1982). While most of these are sterile and morphologically abnormal, stable plants can be recovered in which a small amount of genetic information has been transferred from one species into a cultivated crop (Dudits *et al.*, 1980).

The primary limitation in using somatic hybridization products for crop improvement is certainly the inability to regenerate plants from protoplasts. Numerous hybrids can be proposed to complement cereal and legume breeding programs but very little success has been reported in plant regeneration from these important crops. The limitation of plant regeneration from protoplasts thus precludes short-term application of protoplast fusion to cereals and legumes.

3.1.4. Development of Proprietary Plant Varieties

Hybrid seed production and molecular fingerprinting are two means of protecting new plant breeding lines. Commercial F_1 hybrids are automatically protected, as growers that save seed of the F_1 hybrid no longer have the uniform hybrid characteristics in their seed; F_2 seed segregates and produces nonuniform plantings containing many undesirable plants. Hence, when growers use hybrid seed, they must return each year to purchase new hybrid seed.

Molecular fingerprinting by means of isozymes or RFLPs yields a banding pattern that uniquely reflects the breeding line, allowing accurate identification of varieties protected by the Plant Variety Protection Act or by Plant Utility Patents.

3.2. Recombinant DNA Methods

The most widely known method of nontraditional genetic modification of microbes and plants is commonly referred to as genetic engineering or recombinant DNA technology.

Recombinant DNA methods of introducing additional genetic variability from diverse organisms offer unique opportunities for crop plant improvement (Gasser and Fraley, 1989; Goodman *et al.*, 1987). Recombinant DNA technology is a collection of methods used for the *in vitro* separation, isolation, and remodeling of DNA, and consequently the information that it contains, followed by its introduction into cells. The first of the genetic engineering procedures was described in the early 1970s and these methods are continuously being improved and extended to permit further un-

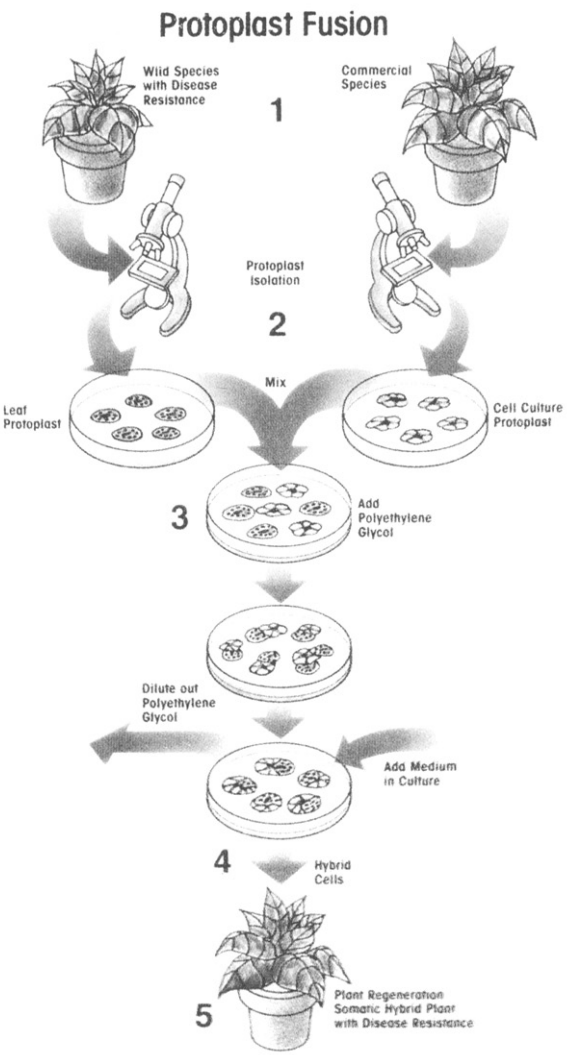

FIG. 3. Steps in protoplast fusion: (1) Plant species or varieties to be combined are identified. This selection is based on attempts to recover hybrids not possible to produce using conventional sexual hybridization. (2) Protoplasts are isolated from plant cells of each of the two parents. To optimize the release of protoplasts, it may be necessary to environmentally or chemically pretreat the plant or to use cells from plans at certain stages of development. Alternatively, liquid cell cultures can be established from one or both of the parents and used as donor material for protoplast fusion experiments. This is important for visual identification of hybrids. (3) Protoplasts of the two parents are mixed and fused using a multistep chemical treatment, including treatment with polyethylene glycol (PEG). (4) Following chemical treatment, fused protoplasts must be distinguished from unfused parent protoplasts. This selection of cell hybrids can be accomplished by using visual, physiological, or genetic markers. (5) Cell hybrids are grown in culture medium appropriate for regeneration of new hybrid plants. Reprinted with permission from *Food Technol.*, 1984, **38**(2), 112–119. Copyright by Institute of Food Technologists.

derstanding and control of the genetic information present in all living things. Although a detailed explanation of all of the techniques of rDNA technology is beyond the scope of this description, a description of the basic principles and the results of

their application is essential to identifying potential issues related to the safety of food ingredients and foods produced using these methods. For a more detailed introductory discussion of the basics of rDNA methods see Drlica (1985), Watson *et al.* (1983), or Wu *et al.* (1989).

3.2.1. Cloning and Transferring a Gene

Most of the steps involved in the rDNA process are carried out in a common laboratory strain of the bacterium *Escherichia coli.* The initial steps are the same whether the final product of the rDNA process is an enzyme purified from a genetically modified bacterium, an improved strain of yeast with a new ability to ferment an additional sugar, or a genetically altered crop plant that is resistant to a virus disease. There are four steps:

1. Cloning (isolating) a DNA segment containing the gene of interest by joining it to a vector DNA[1] (Fig. 4)
2. Trimming the cloned DNA segment to its smallest usable length that contains sufficient information for production of the expression product
3. Editing the genetic information by exchanging control regions, such as "start" and "stop" signals, to create chimeric genes (see Section 3.2.4) as required
4. Moving the vector into a suitable host organism

For genetic modification of microbes, the vector is now ready to be introduced into the host microbe, usually a bacterium, where the vector will continue to reproduce itself along with the cell and carry out the instructions of the cloned gene.

For genetic modification of plants, one vector system uses a second bacterium as a host, *Agrobacterium tumefaciens,* which can transfer DNA and genes into a plant cell. When the *Agrobacterium* containing the vector DNA is then mixed with plant cells or tissues from plants or seedlings, the cloned genes are transferred into the plant cells where they become part of the plant's genetic material. The bacteria are then removed and genetically modified plant cells carrying the added cloned genes are selected.

Until recently, modified plants could be produced only from those species susceptible to *Agrobacterium* or those that readily regenerated from protoplasts following free DNA delivery treatments (electroporation, calcium phosphate, microinjection). This has limited the recipient plant species to the plants such as those listed here.

tomato	alfalfa	white clover	sugarbeet
potato	peas	soybean	pear
celery	lettuce	cotton	cucumber
tobacco	sunflower	cabbage	asparagus
carrot	rape	rice	apple
walnut	bean	broccoli	eggplant

The techniques for creating modified plants are rapidly evolving. A procedure developed by a researcher at Cornell University and the Geneva, New York, Experi-

[1] Vector DNA—usually a plasmid or circle of DNA able to copy and reproduce itself and the inserted DNA in *E. coli.* A vector often contains DNAs that enable it to reproduce itself in a second host.

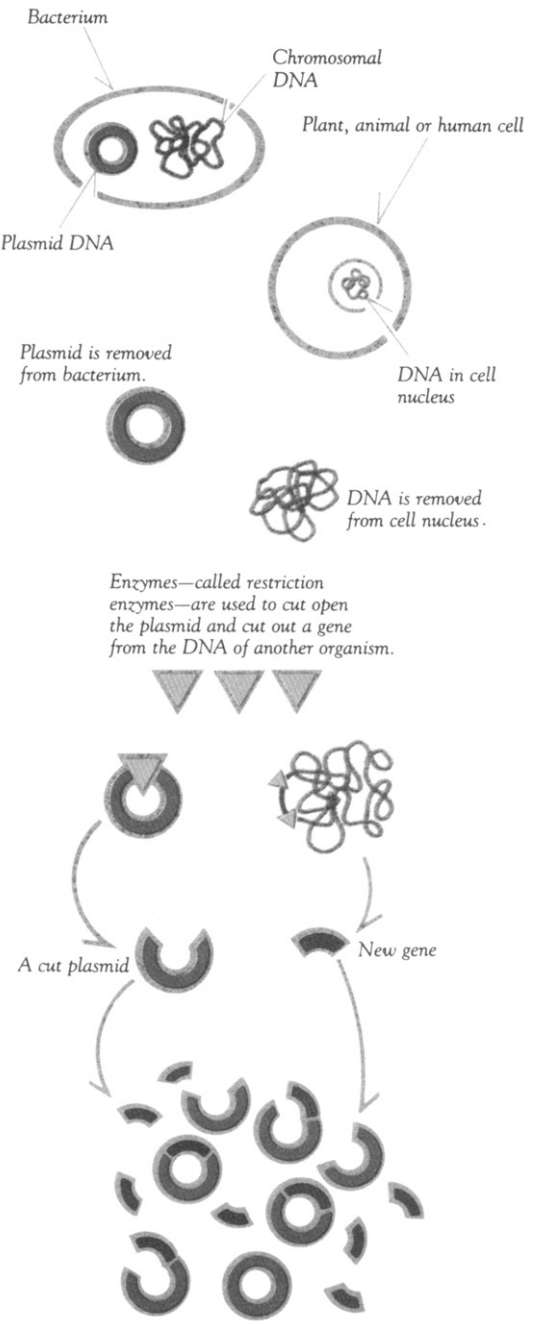

FIG. 4. Cloning DNA in *Escherichia coli*. Reprinted with permission from Monsanto Company.

mental Station, uses a cartridge to shoot tiny particles of tungsten coated with DNA into intact plant cells in meristems, and can lead to modified offspring. This ballistic method obviates the need for sophisticated tissue culture techniques and may permit the introduction of traits into species that have previously been recalcitrant to genetic engineering. Within the next few years, it should be possible to produce modified plants in all species including the grasses which constitute the major grain crops of the world. (As we go to press, corn has been transformed and regenerated.)

3.2.2. Selecting Cells That Contain the Cloned Genes

Since not every cell exposed to the vector will receive and incorporate the cloned gene, and since one cannot determine by their appearance which cells have the cloned gene, the vectors for microbial and plant genetic modification contain selectable marker genes. Selectable marker genes provide a growth advantage to genetically modified bacterial or plant cells under specially chosen laboratory conditions. The marker is essential to identify and/or select the cells containing the introduced genetic material against a background of hundreds of thousands of nonmodified cells. In microbes, these markers permit continuous maintenance of stable lines during growth and production. In plant genetic modification, the kanamycin resistance trait is the tool most commonly used to identify the cells with the added genes. However, other marker systems, including some not involving antibiotic resistance, are under development.

3.2.3. Placement of the Introduced Genetic Material in the Modified Cell

Either the introduced genetic material and vector may be present outside the main body of the cell's DNA and able to reproduce separately as for the plasmid in *E. coli*, or the DNAs may be physically joined to the cell's DNA. The location of the DNA is determined by a number of factors including the source of the DNAs, the host, and the type of vector or DNA transfer method. In a microbial host, three distinct locations are possible. If the gene of interest came from the same or a very closely related microbe, it can combine with the same gene in the microorganism's DNA and replace the resident gene (homologous recombination). Or it can remain in the plasmid vector and reproduce to a high copy number to increase the number of genes and expression product in the microbe. Finally, the gene and vector DNAs could combine at random locations in the host microorganism's or plant's DNA. In plants modified by currently available rDNA methods, the site of insertion is random. In microbes, the frequency at which either type of insertion occurs will depend on the particular microbial host, and the particular type can be readily identified. In either the replacement or random insertion product, the DNA arrangement is stable. The inserted DNA cannot move to other locations.

3.2.4. Native versus Chimeric Genes

A plant receiving DNA from another plant usually has no difficulty in understanding the DNA message because the control signals of the gene are "readable" by plants

in general. If, however, a bacterium receives DNA from a plant, the bacterium cannot understand and use the instructions in the gene unless the signals at the beginning of the gene are first changed by the addition or substitution of bacterial control signals that tell the cell it is a bacterial gene. When a gene contains modified or substituted control signals joined to portions of the native genetic information, the gene is referred to as "chimeric" and its information has been interpreted for the new host.

Increasingly complex genetic modifications using genetic material from different hosts require increasingly complete levels of knowledge concerning the structure of the DNA introduced (the most detailed and complete being the nucleotide sequence). It is possible to add additional copies of a plant's own gene to increase the amount of expression product or to transfer a gene from one plant to another, for example, from potato to tomato. In both these examples, the native plant gene could be directly transferred with no further editing and remodeling.

As a further example, we could transfer the DNA and gene that encode a sugar-degrading enzyme from one related *Bacillus* bacterium species to another by selecting for a *Bacillus* with the new enzyme activity. The information in the DNA and in the gene from one will be understood in the other without further remodeling or editing of the instructions for making the enzyme because these bacteria are closely related. In contrast, the DNA signals that tell a tomato plant to make an RNA and enzymes (proteins) are not the same as those from a *Bacillus* bacterium. To make a *Bacillus* protein in a tomato plant, the DNA with the coding sequence information must be joined to DNA from another source with information that signals the tomato that this is a tomato gene. This chimeric or interpreted gene contains signals isolated and recombined from different sources. In many applications, the genes for additional traits were actually obtained from bacterium or other nonplant sources or were modified to change the RNA or protein product of the gene. This required the construction of chimeric genes designed to express in plants. In most cases, researchers have used the cauliflower mosaic virus (CaMV) 35S promoter to produce an RNA in plants. The promoter fragment is only 325 nucleotides in length and encodes neither a protein nor a determinant of the cauliflower mosaic virus disease. The CaMV promoter is active at all times in nearly all cells of the modified plants and is most active in the cells of vascular and epidermal tissues. Additional useful promoters that are active in particular tissues at certain times are being isolated in anticipation of providing increased control over expression of the introduced gene.

Next the coding sequence DNA is joined to the promoter. The chimeric gene is completed by the addition of polyadenylation signals obtained from several different plant genes. These signals do not encode proteins and contribute to the stability of the RNA made from the chimeric gene.

3.2.5. Characterization of Cloned and Inserted Genetic Material: Standard Genetic Practice

When using rDNA processes, the scientist characterizes the genetic construct in several ways prior to introducing it into and producing the final host plant or microbe. The following is routinely known about the genetic material:

- The physical and functional limits of the coding region, and its size and structure
- The physical extent of the signal DNA regions

- Functional properties of signals such as promoters where the sequence, relative strength, and start of transcription are known from published literature or direct determinations

After the genetic material is introduced and an individual genetically modified plant or microbe has been selected, the following additional information may be obtained:

- Quantitative data on the levels and consistency of the expression products from the introduced gene
- Copy number of the introduced gene and vector sequences

In addition to this basic information concerning the genetic structure of the genetically modified plant or microbe, its phenotypic properties are compared against criteria determined appropriate for similar varieties or strains produced by traditional genetic modification techniques. Taken together this information comprises a set of procedures or standard genetic practice that should be followed to provide the core information about an rDNA modified organism.

3.2.6. Issues Raised by the Application of rDNA Technology

3.2.6.1. Antibiotic resistance as a selectable marker. As discussed above a selectable marker is necessary to identify or select the cells that receive cloned DNAs; however, the use of these agents and markers has raised questions concerning human health and environmental safety. We now discuss these issues using kanamycin resistance as an example.

1. Will the use of a kanamycin resistance marker increase the use of antibiotics on the farm? No. Kanamycin is used only in media in the laboratory and kanamycin is not used in the open environment.
2. Can the plant gene for kanamycin resistance be transferred to bacteria in the environment? Transfer of genes from plants to bacteria has never been documented. The kanamycin resistance gene is permanently incorporated into the plant DNA and would not be transferred to bacteria by any known biological mechanism. The process that transferred and inserted the added DNA into the plant DNA requires approximately a half dozen *Agrobacterium* proteins made by bacterial genes in the *Agrobacterium* cell. These *Agrobacterium* genes are not transferred into the plant cell, and if they, were they would not be recognized as genes by the plant cell and would not produce proteins. Thus, the rDNA-modified plant does not contain genetic information required for transfer of the marker gene to bacteria.

Even if transfer from plant to bacteria were to occur in the field at a frequency of 1 in 1,000,000 (which is about the frequency in bacteria of spontaneous mutation to kanamycin resistance), the increase in number of bacteria in the soil that are kanamycin resistant would be insignificant. Samplings of soil bacteria have shown that one in 100,000 are already resistant to kanamycin: this is ten times more than we estimate would become newly resistant if transfer occurred at all.

It is very unlikely that bacteria could acquire DNA from the environment as plant material decays. Even if this DNA were incorporated into the bacterial cell it would not be recognized as bacterial DNA unless the cell first replaced the plant promoter

sequences with bacterial promoter sequences. This physical method of DNA uptake followed by genetic modification within the bacterial cell is not considered to be significant relative to other mechanisms by which bacteria become resistant to kanamycin in the environment. Thus, it is extremely unlikely that such bacteria would become resistant to kanamycin by acquiring DNA from the environment. In any case, unless there was strong selection pressure for kanamycin resistance they would have little or no environmental significance.

3. Are the gene products of antibiotic resistance genes safe to consume? As for any gene product, this will depend on the characteristics of the gene product itself and the level of dietary exposure to it. The decision tree in Chapter 6 should be interpreted to include in the term *introduced genetic material* any selectable marker. Thus, these materials would be subject to the same type and extent of safety evaluation as any other expression product from introduced DNA, as indicated in the decision tree.

Although this discussion has focused on kanamycin resistance, it should be noted that all of the other selectable markers and nonselectable marker genes for plants, such as β-glucuronidase, are subject to direct selection only in the laboratory, are permanently inserted in the plant DNA, and are chimeric plant genes similar to the kanamycin marker.

3.2.6.2. Potential secondary effects of the DNA insertion process. The random process by which DNAs become inserted into the host's genetic material has raised questions concerning the potential of this process to activate or inactivate genes of the host leading to changes in host expression products. These possible secondary or unintended effects of the genetic modification methods are discussed more fully here. Methods for dealing with their potential consequences in the absence of use experience constitute a significant portion of the chapters on safety assessment (Chapters 4–6).

The introduction of DNAs into plants using nontraditional genetic modification techniques results in the insertion of DNAs at one or more random locations within the nuclear DNA of the plant cell. At the time of this writing, March 1990, neither multiple copy vectors nor gene replacement by homologous recombination (gene targeting) have been accomplished in nontraditionally modified plants. The insertion of DNAs at random locations occurs following all methods of DNA introduction whether by *Agrobacterium* or by a physical means (DNA coated particle bombardment, microinjection, or DNA uptake following electrical permeabilization).

The possible consequences of random insertion events are (1) "position effect" control of the level of expression of the introduced DNAs; (2) no significant effect on the host phenotype; and (3) alteration of expression of a native gene, either inactivation or activation.

"Position effect" refers to a documented phenomenon that the level of expression of an introduced gene may vary with insertion site. The factors contributing to it are not understood at this time; however, with regard to assessment of food safety, the level of gene expression is stable and is inherited by offspring of the modified plants in a consistent manner.

There may be no significant effect on the plant phenotype after gene insertion. The vast majority of the plant genome is either nonsense or redundant DNA. Insertion into nonsense areas would be expected to have no effect.

The random insertion events could, as described above, activate or inactivate genes of the host at a certain estimated frequency. The total number of insertion events of all kinds can be estimated from the copy number of inserts as described earlier for standard genetic practice.

The situation with transposons described earlier (Section 2.1.7.) is analogous to that described for DNA insertion during nontraditional genetic modification. This, with other evidence, suggests that there is only a remote chance, if any, for adverse changes due to the random insertion of DNA. This low probability, coupled with prudent safety evaluation of engineered foods, further reduces the potential for adverse health effects associated with random insertion.

In traditional crop improvement it is not unusual to find genes, especially those from diverse genetic sources, that have unusual or pleiotropic effects. This means that aside from the expected effect of the gene, there are other effects that appear to be unrelated. An example is the *Mi* gene for resistance to root knot nematode in tomato. Associated with this trait is soft-fruitedness, an undesirable attribute for processing tomatoes. Despite these effects the benefits of *Mi* are significant and cultivars with *Mi* are used in nematode infested areas. Another example of pleiotropy is *hp*, a mutant that has fruit with high lycopene content. Despite its attractiveness, however, this gene is not used because it extends maturity and decreases plant vigor. Lastly, the otherwise desirable high-color tomato mutant og^c, or *crimson*, is not widely used by the industry because it is associated with low provitamin A levels.

Genetic linkage also produces secondary effects. When useful traits are introduced into modern cultivars from distantly related or primitive materials, large extraneous linked segments of the chromosome may be brought along with the desired gene. In general, the more backcrosses that are made away from the donor parent the smaller the linked segment becomes. Nevertheless, undesirable traits are frequently associated with the linked DNA. Even after 11 backcross generations (excessive backcrossing by most standards) approximately half the length of the chromosome (ca. 50 map units) was still shown to be associated with the selected disease resistance trait in a recent study using RFLPs in tomato (Young *et al.*, 1988). In contrast, if rDNA techniques were to be used, the resulting introduced segment would be much smaller than 1 map unit.

These examples demonstrate that some concerns over nontraditional breeding using rDNA technology are already addressed with traditionally modified crops. The traditional system of cultivar development and evaluation adequately guards against potential hazards associated with gene insertion, pleiotropy, and linkage effects. Food plants developed using rDNA methods will go through similar field and laboratory testing procedures.

IFBC recognizes that apart from any potential health effects from the expression product(s) of introduced DNA, there is no new human health risk associated with the random insertion of DNA into a plant or microbial genome using rDNA techniques since both crop plants and food microorganisms may contain active transposons or undergo chromosomal recombination.

Gene inactivation (by insertion into the coding region of a gene) is approximately ten times more likely than gene activation (by insertion into the control region of gene) based on the relative sizes of control and coding regions alone. Further, insertion in either orientation could inactivate a gene, while a proper orientation of inserted sequences is required for gene activation. The activation of most of the genes

in the plant is inconsequential either because they are already activated or the product of the gene does nothing to affect composition of the food. In any case any risk posed by activation is addressed by the decision trees.

After unusual individuals are screened out on the basis of phenotype and the remaining tranformants are incorporated into a breeding program, the chances of producing a cultivar with an activated or inactivated gene are further reduced. In addition, according to the decision tree in Chapter 6, any new cultivar would undergo a safety evaluation and be screened for levels of important inherent constituents, further reducing the potential for a cultivar with deleterious secondary effects reaching the market place.

3.2.6.3. Genetic change resulting from the use of tissue culture. The location of the DNA is not the only source of secondary or unintended effects in nontraditionally genetically modified plants. All these plants are produced by some steps involving organ, tissue, or cell culture. As described earlier in this chapter in Section 3.1 on protoplasts and tissue culture, these procedures have the potential to introduce genetic change at a low frequency that depends on plant species and the variety being cultured, the culture conditions, and time in culture. Since these conditions, time of culture and response of the tissues, are dependent on the plant variety, we cannot, at this time, standardize conditions to eliminate this potential source of variability. However, the relevant question is not what that estimated frequency might be but rather whether the food produced is safe for consumption. The analysis for potential relevant changes due to the gene insertion suggested in Chapter 6 will also identify changes as a consequence of tissue culture, if any.

The rDNA technologies discussed above are tools for the editing of genetic instructions to make interpreted, chimeric genes that direct cells to produce expression products often not previously found in that cell. Random, nondirected genetic change could lead to similar changes but only over the protracted evolutionary time scale. To accomplish this editing in the laboratory, the scientist must know the exact limits of the coding region and the endpoints of the signal DNAs. These are most accurately determined from the nucleotide sequences of these DNAs. This detailed knowledge of the genetic information introduced by rDNA technologies provides and permits a level of comfort not previously possible. It would be contradictory if we were to accept readily foods produced by the traditional methods of genetic modification (that entail thousands of recombination events and where much less detailed information is available) but be hesitant to accept the products of a much more precisely controlled process that does not have a long history of human use.

Most of the concerns raised about nontraditional genetic modification relate to unintended effects of the gene insertion or production of the expression product, which may be the protein itself or, if an enzyme, the products of the reaction the enzyme catalyzes. We do know more about the genes than has traditionally been known, but even with the nontraditional methods we do not know every detail. In the absence of experience of use we must provide a detailed description of the process and consequences of the process for the initial products. Case-by-case evaluation must be applied in a logical manner and the questions to be addressed should not build on one another inappropriately without underlying scientific justification. As more knowledge of the nontraditional methods accumulates, some of these questions will become irrelevant while others must continue to be considered. Each new gene expression product in the food supply will require examination of basic questions of

health and safety. As an example, the detailed examination of the DNA introduction process should decrease as we have gained more experience with these processes. This would be similar to the way that foods produced through traditional methods such as plant breeding are treated.

IFBC recommends that academic, government, and industrial scientists working in areas of nontraditional genetic modification be encouraged to publish their results in refereed journals to facilitate the exchange of information concerning the safety of foods derived from these processes.

The IFBC proposes in Chapter 6 that recognition of the accumulated knowledge be formalized by a listing of approved and acceptable sources or elements of genetic material. This listing will encompass vectors, gene signals used in expression systems, and marker genes and their expression products as data accumulate on their scientifically based acceptance and/or history of safe use. IFBC has already placed on this list the DNA sequences that do not produce proteins (these include noncoding sequences) since they produce no expression product in the food ingredient or food that contains them.

3.3. Protein Engineering

Protein engineering allows one to change specific regions of a single protein by a process termed site-directed mutagenesis. In this procedure the exact DNA sequence of the gene of interest is determined, a target region of the DNA is selected for mutagenesis, and specific changes are introduced into the DNA sequence. One method for introducing these alterations in the DNA sequence uses a DNA replication primer synthesized to include the nucleotide changes. The primer is annealed to a single-stranded DNA template containing the native gene. *In vitro* DNA replication from the primer results in a newly synthesized DNA strand which carries the altered region. The mutant and parental strands segregate *in vivo* following transformation of the vector into a host cell and subsequent replication of the vector. Other approaches rely on methods which enhance misincorporation of nucleotides during the synthesis of DNA or on replacement of a target region with a synthetic double-stranded DNA with the appropriate alteration. Generally, only one (or a few) amino acid change will result from the new DNA sequence. The resulting protein "variant" will differ from the native protein at only the selected regions.

The variants are structurally quite similar to the native proteins. Functional activity of the protein will depend on where in the protein the amino acid changes were introduced. Rationale for site-directed mutagenesis is generally based on improving some aspect of the protein by specifically altering certain amino acid residues involved in functionality.

In the case of enzymes, for example, single amino acid alterations have been shown to affect specific activity, thermal stability, substrate specificity, and a number of kinetic properties. However, since these are single amino acid changes, it is quite likely that these variants would arise naturally, and if one had the appropriate screening techniques (and an infinite number of samples) naturally occurring organisms expressing these traits could be found. In this sense, the site-directed mutagenesis process differs from the traditional natural isolate screening and mutagenesis/selection programs, which have been employed for years, only in the ability to preselect the variant of interest.

4. THE APPLICATION OF GENETIC MODIFICATION IN THE AGRICULTURE AND FOOD INDUSTRY

Food and agricultural technology is now being greatly enhanced by the advent of rDNA technology and other procedures for genetic modification. These will bring a new urgency and focus to the chemical and biological characterization of not only new, but traditional foods as well.

Two important features of new methods of genetic modification are (1) the ability to shorten and compress the time required for developing new varieties of food sources and (2) the broadening of genetic sources for generating new food products by introducing genes from unrelated species.

Extended premarket opportunities to detect any possible adverse characteristics of new varieties will remain an important part of their development. Capturing the benefits of the new techniques, while still providing adequate assurance of safety, will require development and application of effective scientific assessments of nutrition, safety, and wholesomeness of the new products.

Traditional genetic modification has played a central role in providing the great variety and abundance of wholesome foods available today. The nontraditional methods of genetic modification will be used to improve crops and to advance food processing providing ultimate benefits to the consumer in more nutritious, lower-cost foods.

The first of the food processing aids and food ingredients derived from genetically modified microbes will replace classically derived (selection and mutation) strains of microorganisms. These microorganisms are currently used to produce enzyme preparations employed to manufacture high-fructose corn syrups, cheese, fruit juices, wine, beer, bread and other products.

One of the first applications of genetically modified microorganisms has been to increase the gene copy number, and hence the yield, of enzymes identical to those produced by classically derived strains. Other genetically modified organisms have been developed that express enzymes with improved properties such as amylases with improved pH, thermal stability, or other desirable properties. Genetically modified organisms are also under development and regulatory review as more economical sources of calf chymosin, a milk coagulant, used for cheese production.

In the future it is anticipated that enzyme manufacturers will focus on a small number of host microbes for production of enzymes. This will greatly simplify safety and regulatory concerns because it will be possible to characterize completely the genome of these organisms. Thus, for instance, when enzymes with desirable properties are discovered in relatively obscure organisms, it should be possible to isolate the coding gene and transfer it to a production organism which has a history of safe use.

The first crop plants from nontraditional genetic modification will carry new traits or properties that decrease the input that the grower must make to achieve the same level of productivity. The seeds of these crops will carry genes that allow plants to make, on their own, proteins that control certain insect pests, enabling growers to decrease their use of chemical insecticides. Genes for resistance will come from two general sources: soilborne bacteria such as *Bacillus thuringiensis* (Bt protein) and insect-resistant plants, such as legumes that make insect-active protease inhibitors. Such genes could be designed so the resistance proteins will be expressed only in those portions of the crop that the insect eats.

This research could produce corn resistant to such insect pests as the corn borer and corn root worm. Field tests have already shown transgenic tomato plants that make the Bt protein control tomato pinworm (*Keiferia ycopersicella*), a significant pest of tomatoes in Mexico, a major tomato-producing area. Under current agricultural practice, as many as 12 insecticide applications are made during a single growing season to try to control pinworm. Effective control of pinworm by Bt protein-producing transgenic plants would benefit growers, because the decrease in pesticide applications and decrease in crop loss due to insect damage will result in cost savings; processors, because there will be fewer pesticide and insect fragments in processed tomatoes; and consumers, because their potential exposure to chemical insecticide residues will be reduced.

One company is developing cucumber plants resistant to virus attack; the plants make a virus protein, the coat protein, that interferes with virus infection. Virus infection is a particularly serious problem in Mexico where cucumbers for fresh-packed pickling are grown. The important viruses are zucchini yellow mosaic, watermelon virus 1, and watermelon virus 2, and cucumber mosaic virus. There are benefits to growers, processors, and consumers:

- Virus resistance would result in consistent yield and fruit quality. Virus infestation can quickly devastate a field.
- Virus resistance would result in a steady supply of consistent fresh picked cucumbers from Mexico to U.S. processing plants.
- There would be a decreased need for insecticides to control the insect vectors that spread the virus. The final product should cost less because the supply of cucumber would be more dependable.

Several companies are developing crop plants tolerant to nonselective herbicides that normally kill the crop along with the weeds. Tolerance is being developed through the introduction of genes for a target protein with reduced sensitivity to herbicide action or for a protein that inactivates the herbicide. Herbicide-tolerant crops increase the options for selecting environmentally benign herbicides that are more rapidly degraded and provide greater flexibility in designing weed control programs for both major and minor acreage crops. They also provide the grower with benefits of improved safety for the crop, broader annual and perennial weed control, increased yield potential, and reduced weed control expenses.

Several groups are examining the possibility of regulating the level of polygalacturonase (PG) in ripening tomato fruit. This protein is thought to promote fruit softening, and decreasing PG may extend the shelf life of the tomato. To reduce the level of this enzyme, several laboratories have developed transformed tomato plants that contain the PG gene in an antisense orientation driven by the cauliflower mosaic virus 35S promoter. The antisense RNA binds to the PG RNA and prevents synthesis of PG protein. Transformed plants show a 10-fold reduction in the level of PG protein compared with normal tomatoes.

If these transformed plants result in tomato fruits that are more stable, with an extended shelf life, the following benefits could be provided to growers, processors and consumers:

1. Processing tomato growers currently harvest their tomato crop at around 95% ripe. If the PG antisense tomatoes demonstrate a significant extension of firmness/shelf life on the vine, it should be possible to harvest when the field is 100% ripe. The

increased percentage of ripe fruit should translate into increased profit to the growers due to the decreased percentage of unusable green tomatoes.

2. Fresh market tomato growers currently harvest their crop at the mature green stage of fruit development. PG antisense tomatoes should make it possible to harvest the fruit at a later stage of development when they have a much superior flavor. Fresh market PG antisense tomatoes should capture a greater portion of the market share due to their increased color, flavor, and shelf life and reward the grower with the higher price that these premium quality tomatoes would command.

3. Food processors of PG antisense processing tomatoes can anticipate benefits due to an increased percentage of ripe tomatoes, therefore, increased red color (lycopene) content of the processed tomatoes, making a better appearing product. Also, because the pectin component of the fruit cell wall may not be degraded, it is possible that the processed product will have increased consistency. Currently, the tomatoes are heat treated to inactivate PG before crushing. An additional benefit may result from energy savings during processing of tomatoes with reduced polygalacturonase (cold break versus hot break processing).

4. The processor of PG antisense tomatoes will benefit from decreased manufacturing costs. The consumer of PG antisense fresh market tomatoes will benefit primarily from the increased flavor and the increased shelf life of the vine-ripened product.

The examples just listed result from nontraditional methods and include cases where genes for traits from bacteria or sexually incompatible plants have been introduced into crops. These methods will ultimately lead to foods with improved nutrition, taste, and cooking properties as the limits of the techniques are extended and identification of genes for these attributes are identified. Some of the first steps are being made.

Improved quality fruits and vegetables will be developed using genetic modification techniques to control developmental regulation and expression of plant genes involved in carbohydrate and hormone biosynthesis. The development and commercialization of fruits and vegetables with improved flavor, texture, and postharvest shipping qualities could result in the following benefits to growers, processors, and consumers: expansion of existing markets and development of new business opportunities for growing food crops instead of commodity grains, increased freshness and prolonged shelf life, reducing spoilage losses in the distribution and processing systems; improved nutritional composition, and reliable supplies of consistent high quality products.

Research to produce corn with higher nutritional quality proteins for animal feed and use in human food products is also being done. Elevation of specific amino acids, such as lysine and tryptophan, will enhance the nutritional value of corn-based food products. The genes for more nutritional corn protein will most likely originate from microorganisms already used in food and from various edible plants.

The traditional methods of plant breeding have led to the development and commercialization of oil seed rape (canola) with modified oil composition during the 1980s resulting in the following benefits to processors and consumers: increased levels of monounsaturated fatty acids, decreased levels of saturated fatty acids, improved shelf life and flavor, reduced costs for refining and hydrogenation, increased flexibility for end product uses, and improved nutritional quality for animal feed energy

sources. Canola with further improvements in oil composition is being developed using nontraditional genetic modification techniques to control developmental regulation and expression of plant genes involved with fatty acid biosynthesis. The ultimate oils produced will be the result of both traditional and nontraditional genetic modification. The combined use of older and new methods will lead to many of the foods produced by genetic modification in the future.

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Chapter 4: Safety Evaluation of Foods and Food Ingredients Derived from Microorganisms

1. INTRODUCTION

Microbes have been an important part of food preparation for millennia. They are consumed directly, and are in fact essential, in familiar foods such as cheese, bread, and yogurt as well as in a variety of Oriental foods such as natto and tempeh. Products of microbial fermentation have a long history of safe use in beer, wine, soy sauce, and vinegar preparation. Desirable microorganisms are also used simply as tools to produce food ingredients. Among these are alcohol, food acids, proteins, enzymes, fat, vitamins, and flavors. In most of these cases, the microorganisms and their products are not present in sufficient quantity to make a substantial contribution to the product's overall nutrient composition, however, consideration has been and still is being given to producing microorganisms for use in food and feed as sources of protein, fat, and vitamins. This application is largely dependent on economics, that is, the cost of the substrate on which the organism is grown. Much effort in recent years has gone into developing ways to produce microorganisms using various widely available materials as the substrates. Not surprisingly, enzymes produced by microorganisms have been used successfully for decades in food and food preparation.

2. NATURALLY OCCURRING MICROORGANISMS USED TO PRODUCE FOOD OR FOOD INGREDIENTS

One must assume that microorganisms grew in the foods of early humans and produced undesirable changes, which we now regard as spoilage. Some time later in the course of history, humans learned to use microorganisms deliberately to produce desirable changes in food.

No doubt our ancestors recognized that cooked meat spoiled less readily than raw meat. By adding salt to shredded cabbage they were able to produce sauerkraut. Adding salt to chopped meat produced a zesty tangy sausage, not a stinking slimy mess. By holding cucumbers in salt brine they obtained firm and tasty pickles. The same was true for green olives. Milk became sour and separated into whey and curd, the forerunner of cheese. Grape juice underwent spontaneous alcoholic fermentation, and if the product were held long enough it changed to vinegar. All of this was known long before we had heard about microbes. Humans simply learned by intuition and accident how to select for growth of certain types of microorganisms and produce desirable changes while inhibiting growth of unwanted types.

Pasteur's disproof of the theory of abiogenesis and his unequivocal demonstration of microorganisms as a leading cause of disease and the primary agent of decomposi-

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TABLE 18

TRADITIONAL AMERICAN FERMENTED FOODS AND THE ORGANISMS USED IN THEIR PRODUCTION IN THE UNITED STATES BEFORE 1958

Food	Microorganisms	See note
Bread	*Saccharomyces cerevisiae*	4
Sourdough bread	*S. cerevisiae* plus various lactic acid-forming bacteria	4
Beer and ale	*S. cerevisiae* or *Saccharomyces carlsbergensis*	5
Wine	*S. cerevisiae* var. *ellipsoideus*	5
Vinegar	*S. cerevisiae* var. *ellipsoideus* plus various acetic acid-forming species of *Acetobacter* or *Bacterium*	5
Soy sauce	*Aspergillus oryzae* plus various salt-tolerant yeasts and lactic acid bacteria	1, 5
Sauerkraut; pickles and green olives	*Leuconostoc mesenteroides, Lactobacillus brevis,* and *Lactobacillus plantarum*	4
Fermented sausage	Various lactobacilli; *Pediococcus cerevisiae*	2, 4
Cultured buttermilk; butter	*Streptococcus cremoris* or *Streptococcus lactis* and *Leuconostoc dextranicum* or *L. citrovorum*	3, 4
Yogurt	*Streptococcus thermophilus* and *Lactobacillus bulgaricus*	3, 4
Bulgarian buttermilk	*L. bulgaricus*	3, 4
Acidophilus milk	*Lactobacillus acidophilus*	3, 4
Cheeses		
Cottage, Cream, Neufchâtel	*S. cremoris* or *S. lactis* and *L. dextranicum* or *L. citrovorum*	3, 4
Cheddar, Edam, Gouda	*S. cremoris* or *S. lactis*	3, 4
Swiss	*S. thermophilus, L. bulgaricus, L. lactis,* or *L. helveticus;* and *Propionibacterium shermanii*	3, 4
Blue, Roquefort, Stilton	*S. lactis* or *S. cremoris* and *Penicillium roqueforti*	3, 4
Brick, Limburger	*S. lactis* or *S. thermophilus, Mycoderma, Geotrichum* spp., and *Bacterium linens*	3, 4
Camembert	*S. lactis* or *S. cremoris, Mycoderma, Geotrichum* spp., and *Penicillium camemberti*	3, 4

Notes

1. One large producer of fermented soy sauce has identified the organisms used as *Aspergillus oryzae* or *Aspergillus sojae; Pediococcus halophilus; Saccharomyces rouxii;* and *Candida (Torulopsis) versatilis* and *Candida etchellsii* (Sugiyama, 1984).
2. Various lactic acid bacteria are now available commercially for this purpose.
3. Now called *Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis,* and *Lactobacillus delbrueckii* subsp. *bulgaricus.*
4. The microorganisms become an integral part of the food.
5. The microorganisms grow and produce their typical changes but are removed in whole or in part by centrifugation, filtration, or washing before the food is consumed. Thus, in usual circumstances, only their soluble products are consumed with the food.

tion of organic material led eventually to extensive studies of the organisms responsible for food fermentations. This made it possible to isolate and identify the desirable microbes and to add them deliberately as starter cultures. Using known organisms greatly decreases the likelihood of aberrant fermentations and ensures better quality products.

Table 18 lists many of our traditional fermented foods and the organisms used in their production (Foster *et al.,* 1957; Frazier, 1958). The long history of use of these

organisms and the widespread consumption of these foods and beverages testify to their safety. These organisms meet the criterion of "common use in foods in the United States before 1958." They may therefore reasonably be "generally recognized as safe" (GRAS).

Table 19 lists foods, food ingredients and enzymes that were produced industrially by microorganisms in the United States before 1958 (with the exceptions in notes 2 and 3).

Fermented foods have been produced in Oriental countries for centuries. Table 20 gives a partial list of the better known products. Some of these (e.g., Shoyu) have become important articles of commerce in Europe and North America.

3. MICROORGANISMS ASSOCIATED WITH FOOD

The common occurrence of harmless microorganisms in food is discussed at some length in Chapter 2. The fact that a specific microorganism is recognized in the published scientific literature as a harmless common contaminant in foods is relevant to establishment of its safety for use as a source of food ingredients. For instance, in the preamble to a GRAS affirmation regulation (Food and Drug Administration, 1983) the GRAS status of an enzyme product of *Bacillus licheniformis* was partially based on published information establishing that *B. licheniformis* is widely recognized as a harmless contaminant found in many foods.

4. MUTAGENESIS AND SELECTION OF MICROORGANISMS USED TO PRODUCE FOOD AND FOOD INGREDIENTS

Mutagenesis and selection techniques were first widely used in the 1940s with strains of *Penicillium* for the improvement of antibiotic production (Jacobson, 1981; Elander, 1982). In the intervening years remarkable improvements have been achieved using this technique in numerous other microorganisms of industrial importance including those used in the production of food ingredients such as citric acid, tryptophan, lysine, glutamic acid (Jacobson, 1981), and enzymes (Aunstrup *et al.*, 1979).

Mutations occur spontaneously in microbial populations; however, the observed frequency of a particular spontaneous mutation is usually lower than 10^{-5}. One would therefore have to examine as many as 100,000 colonies to observe a single mutation. Where a new phenotype can be selected for (such as growth on starch for an amylase positive mutant) even very infrequent spontaneous mutations can be detected easily. Frequently, however, it is not possible to select for a particular phenotype, and cells must be screened using various screening assays. These screening assays are often linked to computer analysis and automated methodology to screen large populations.

The proportion of mutants in a bacterial population can be increased by using mutagens—physical (e.g., ultraviolet irradiation), chemical (e.g., hydroxylamine, nitrosoguanidine), or biological (e.g., phage MU-1) agents. Some induce primarily base substitutions, others are efficient deletion mutagens, whereas still others can cause frameshifts (Jacobson, 1981).

The dose of the mutagen can alter the degree of mutation (Elander and Chang, 1979). Heavy doses can produce major changes in the morphology or biochemistry

TABLE 19

EXAMPLES OF FOODS, FOOD INGREDIENTS, AND ENZYMES PRODUCED INDUSTRIALLY BY MICROORGANISMS PRIOR TO 1958

Product	Microorganisms	See note
Microorganisms themselves	*Saccharomyces cerevisiae*	1
	Saccharomyces carlsbergensis	1
	Geotrichum candidum	2
	Cryptococcus (Torulopsis) utilis	1
	Candida arborea	1
	Torula pulcherrima	1
Fats	*Torulopsis pulcherrima*	3
	Geotrichum candidum	3
	Endomyces vernalis	3
Vitamins	*S. carlsbergensis*	5
	Aspergillus fisheri	5
	Clostridium acetobutylicum	4
	Eremothecium ashbyii	4
	Ashbya gossypii	4
	Streptomyces spp.	4
Dextran	*Leuconostoc mesenteroides*	
Lactic acid	*Lactobacillus delbrueckii*	
	Lactobacillus bulgaricus	
	Lactobacilus plantarum	
	Bacillus coagulans	
Citric acid	*Aspergillus niger*	
Enzymes		
Amylases	*Aspergillus oryzae*	
	Rhizopus delemar	
	Mucor rouxii	
	Bacillus subtilis	
Invertase	*S. cerevisiae*	
Pectinases	*Aspergillus* spp.	
	Penicillium spp.	
Proteases	*Aspergillus oryzae*	
	Bacillus subtilis	
Glucose oxidase	*Aspergillus* niger	

Notes

1. Yeasts are often consumed as sources of protein or vitamins. They may be obtained as by-products of the brewing industry or they may be produced directly for food use when inexpensive sources of fermentable carbohydrate are available.
2. The mold *Geotrichum candidum* has been used in some countries as a source of protein and vitamins during wartime.
3. These organisms were used in Germany and Sweden as sources of fat during World Wars I and II.
4. Used primarily for the vitamins of the B complex.
5. Used for fat-soluble vitamins.

of the organism. Small doses can result in subtle changes in the phenotype of an organism. Sequential mutagenesis with small doses of mutagens has been used successfully in yield improvement programs (Elander and Chang, 1979).

Mutagenesis and selection constitute a random process and do not necessarily require an extensive knowledge of the genetics of the microorganism to be successful.

TABLE 20

SOME ORIENTAL FOODS PRODUCED BY MICROBIAL ACTION

Nature of food product	Microorganisms	Substrate	
Tempeh	*Rhizopus* sp.	Soybeans	Solid
Sufu	*Actinomucor elegans, Mucor* sp.	Soybeans	Solid
Ragi	*Mucor* sp., *Rhizopus* sp., yeast	Rice	Solid
Tea fungus	*Acetobacter* sp., two yeasts	Tea extract and sucrose	Liquid
Miso	*Aspergillus oryzae, Saccharomyces rouxii*	Rice and other cereals	Paste
Shoyu	*Aspergillus oryzae*, Lactobacilli. *Hansenula* sp., *Saccaromyces* sp.	Soybeans and wheat	Liquid
Ang-kak (red rice)	*Monascus purpurea*	Rice	Solid
Natto	*Bacillus subtilis*	Soybeans	Solid
Nata	*Acetobacter* sp.	Fruit juices	Gel

Source. Adapted from Hesseltine (1965).

These have been used extensively to optimize strain properties such as development of a constitutive mutant that does not require an expensive or undesirable inducer and elimination of objectionable by-products such as antibiotics or undesirable enzymatic side activities (Aunstrup *et al.*, 1979).

There is little doubt that genetic modification of producer strains by mutagenesis coupled with rational selection procedures has been the most important single factor contributing to the success of the fermentation industry in producing food ingredients, pharmaceuticals, industrial enzymes, and other chemicals. In the future it is anticipated that the ability to move well-defined genes from a large number of donor microorganisms into a relatively small number of genetically well-studied host organisms will lead to a better understanding of the complex cellular regulatory control that has been modified to yield higher production in improved mutants (Elander, 1982). This will lead to an increasingly rapid development of the use of microorganisms to produce useful products, including food products.

5. EVALUATION OF FOOD INGREDIENTS DERIVED FROM GENETICALLY MODIFIED MICROORGANISMS

Recently, the advent of biotechnology has given us the ability to use microbes and enzymes in new and better ways. For example, cheesemaking has traditionally relied on the enzyme rennin, prepared from calf stomach. Biotechnology has enabled the efficient preparation of this same enzyme from microbes engineered with the rennin-encoding gene.

According to a National Academy of Sciences (1987) report there is no evidence of a unique hazard from the transfer of genes between organisms. Nonetheless food and food ingredient manufacturers and suppliers, and the federal agencies responsible for food safety regulation, are committed to ensuring the public that the products

FIG. 5. Decision tree for evaluating relative safety of food ingredients derived from genetically modified microorganisms.

	If *Yes* Proceed to	If *No*
1. Does the microbe end up in food?	2	4
2. Is the organism free of transferable antibiotic resistance genes? (see Appendix A)	4	3
3. Does the resistance gene code for resistance to a substance used in control of disease agents in human or veterinary medicine?	Table 21, part D	4
4. Are the vectors characterized and free of attributes that would render them unsafe for constructing microorganisms to be used to produce food-grade products? (see Appendix B)	5	Table 21, part D
5. Does the DNA insert code for a substance safe for use in food? (see Appendix C)	6	Table 21, part D
6. Is the microbe free of DNA from an intermediate host which could code for a toxic product? (see Appendix D)	Table 21, part A	Table 21, part D

of biotechnology are safe for consumption. The decision tree developed in this document is modeled after an earlier one developed by Pariza and Foster (1983). It has been widely accepted by the scientific community for determining safety assessment criteria for microbial enzyme preparations used in food. The Pariza and Foster approach has been extended in this section to cover food ingredient products obtained from genetically modified microorganisms.

6. DECISION TREE FOR EVALUATING RELATIVE SAFETY OF FOOD INGREDIENTS DERIVED FROM GENETICALLY MODIFIED MICROORGANISMS

The focus of the decision tree is on the safety of the organism and the products it produces. It is assumed that if the organism is nontoxigenic and nonpathogenic, then foods or food ingredients produced from the organism under current Good Manufacturing Practices will be safe to consume. Whole foods produced from microorganisms can best be evaluated by using the decision tree in Chapter 6.

As currently developed, the decision tree (Fig. 5) extends the Pariza and Foster approach (Table 21) to genetically modified organisms and represents a conservative guide to safety evaluation. No organism or product can be accepted without testing for toxin production, and in most cases this will involve animal studies. It is expected that the proposed scheme will evolve as the safety data base on new organisms from biotechnology expands.

A number of microorganisms such as some species of *Bacillus, Saccharomyces, Lactobacillus* and *Aspergillus* have a documented history of safe use in food. Thus, we regard the transfer of a gene from a nonpathogenic, nontoxigenic source to a similarly safe host, especially one that is already part of the food chain, as a safe

TABLE 21

GUIDELINES FOR DETERMINING THE SAFETY OF FOOD INGREDIENTS DERIVED FROM MICROORGANISMS[a]

A. Decision tree	If *yes* Proceed to	If *no*
1. Is the test material free of antibiotics?[b]	A.2	D
2. a. For bacteria and yeast:		
i. Is the test material free of toxins[c] known to be produced by other strains of the same species?	A.3	D
ii. If there are no known toxins[c,d] produced by other strains of the same species, is the no-observable-effect level (NOEL) in a single oral challenge sufficiently high to ensure safety[e–g]	B	D
b. For molds, is the test material free of detectable levels of aflatoxin B_1, ochratoxin A, sterigmatocystin, T-2 toxin, zearalenone, and any other toxins known to be produced by strains of the same species?[h]	C	D
3. Is the NOEL in short-term feeding studies sufficiently high to ensure safety?[e–g]	ACCEPT	D

B. Special considerations for certain yeasts and bacteria:

1. If the source culture is a well-known, widely distributed, nonpathogenic yeast, e.g., certain species of the genus *Saccharomyces*, or if it belongs to a bacterial species that is well characterized, commonly present in foods, has a history of safe use in food ingredient manufacture, and has never been implicated in foodborne disease, e.g., *Bacillus coagulans, Bacillus licheniformis, Micrococcus lysodeikticus,* and *Bacillus subtilis* (Buchanan and Gibbons, 1974), the test material can be *ACCEPTED* at this point.
2. Test material from other bacteria and yeasts must be considered under part A.3.

C. Special considerations for certain molds:

1. If the source culture is well characterized, commonly present in food, has a history of safe use in food ingredient manufacture, and has never been implicated in foodborne intoxication or disease, e.g., *Aspergillus oryzae, Aspergillus niger,* and *Rhizopus oryzae* (Beckhorn *et al.* 1965; Fennel, 1976; Moskowitz and Cayle, 1974; Riemann and Bryan, 1979; Rogers, 1977; Roland, 1981; Scott, 1980; Stoloff *et al.*, 1977), the test material can be *ACCEPTED* at this point.
2. Test material from all other species of molds must be considered under part A.3.

D. Disposition of materials that fail any decision tree requirements: A negative answer to question 1, 2, or 3 signifies the presence of an undesirable substance and the material is not acceptable for use in food. If the undesirable substance can be removed, the purified material must be passed through the system again, beginning at the point of the original negative answer.

Source. This table is essentially reproduced from Pariza and Foster (1983). See original source for further discussions and rationale.

[a] These guidelines are intended for crude culture extracts, for whole cultures, and for concentrated enzyme or other microbially derived fractions which, when diluted, become preparations suitable for marketing.

[b] As determined by (Anonymous, 1981) or comparable methods.

[c] For the purposes of these guidelines, the term *toxin* refers to a substance which is regarded by experts as a cause of food poisoning, intoxication, or illness when ingested. Examples are staphylococcal enterotoxins, botulinal neurotoxins, and mycotoxins.

[d] Certain cultures in this category are acceptable on the basis of single acute oral toxicity test, as explained in part B.1. Cultures that fall under part B.2 can go directly to part A.3 without an acute oral toxicity test. This is permissible because the subchronic feeding specified in part A.3 is more rigorous and more meaningful than the acute oral toxicity test embodied in part A.2.a.ii.

[e] Expressed as mg/kg body wt and determined using appropriate animal species.

[f] Estimated mean consumption level is calculated from the sum of the intakes for each food category in which the material is expected to be used. An example of such determination is (USDA mean portion size) × (Market Research Corporation of American eating frequency for the entire population) × (the usual level of use expressed as total organic solids (TOS) for microbial preparation in question) (Anonymous,

system either for enzyme or ingredient production or for direct use in a food product. In some cases the vector used has also been determined to be safe on the basis of full sequencing and characterization. In these cases, the exact structure of the new genetic construct is known and should be considered safe. pBR322 and pUB110 are examples of such vectors (see Appendixes A and B).

In cases where an entire gene is deleted from a microbe in current use, usually additional safety testing may not be necessary. For instance, deletion of a sporulation gene from a *Bacillus* strain used for α-amylase production should not raise any safety issues about the α-amylase itself.

Mutations important in industrial yield improvement programs (Elander and Chang, 1979; Elander, 1982) are usually the result of the alteration of a regulatory gene for production of a given product or cellular function. It is not possible to convert an organism into a toxin producer by mutagenesis if it lacks the gene(s) for synthesizing the toxin in question. It is important to keep in mind, however, that under certain growth conditions, toxigenic strains may not express the toxin. Organisms that have a history of use in food processing are preferred. New microbial isolates should be evaluated under a variety of growth conditions for the ability to produce toxins elaborated by other strains in the same species. It is not possible to establish absolutely that a strain is nontoxigenic solely from data on toxin expression. Therefore, in cases where a new, less familiar host, vector, or gene is used we propose that the material be tested as suggested by Pariza and Foster (1983).

To date the Food and Drug Administration has accepted for filing six GRAS petitions (CPC International, Ltd., 1986; Enzyme Bio-Systems, Ltd., 1988; Pfizer, Inc., 1988a; Gist-Brocades, Inc., 1989; Genencor, Inc., 1989; Novo Laboratories, Inc., 1990) and one food additive petition (Pfizer, Inc., 1988b) concerning food ingredients derived from rDNA-modified microorganisms. In response to the Pfizer petitions (1988a, b), the regulations were recently amended (Food and Drug Administration, 1990) to affirm that the use of a chymosin preparation derived by fermentation from *E. coli* K-12 is generally recognized as safe (GRAS). The rest of the above petitions are currently under review by the Agency. In addition, a number of other GRAS petitions for products from genetically modified microorganisms have been submitted and are currently under prefiling review by the agency.

According to a paper prepared for the 18th session of the Codex Alimentarius Commission (Berkowitz and Maryanski, 1989), there is no evidence of unique hazards associated with rDNA technology and that potential risks which may occur are the same kind as those associated with conventional methods. Safety evaluation should be based on accumulated experience and scientific knowledge of the characteristics of the finished food substance.

1972, 1982). TOS is defined as the sum of the organic compounds, excluding diluents, contained in the final microbial preparation (Pariza and Foster, 1983).

[g] The term *sufficiently high* refers to appropriate multiples of the estimated mean human consumption level. Where the product is an incidental additive or processing aid (e.g., an enzyme) the NOEL should be at least 100 times the estimated mean human consumption level. Where the product is itself a food (e.g., yogurt) or a major food component (e.g., mycoprotein) it may not be possible to test at this high a level. In these cases, safety may be established by feeding the highest level compatible with the maintenance of adequate nutritional requirements and consideration of the questions outlined in the decision tree for whole foods and complex mixtures (Fig. 7).

[h] As determined by Patterson and Roberts (1979) or comparable methods.

With regard to the safety evaluation of improved production microorganisms to produce substances that are already marketed, Berkowitz and Maryanski stated that the safety evaluation should focus on the following factors:

(i) the identity of the host organism;
(ii) any evidence of pathogenicity or toxin production;
(iii) the function of the inserted gene(s);
(iv) the identity of organisms that contribute genetic material to the final construct;
(v) characterization of the inserted genetic material to ensure the absence of sequences that may encode harmful substances;
(vi) insertional and genomic stability;
(vii) chemical specifications;
(viii) dietary use and exposure and other relevant information.

The IFBC agrees that these criteria are relevant to the safety evaluation of such microorganisms.

7. APPENDIXES

Appendix A. Antibiotic Resistance Genes

Is the organism free of transferable antibiotic resistance genes?

Antibiotic resistance genes, often originally from transposons, are integral parts of most common vectors. These marker genes allow cells transformed with the vector to be distinguished from nontransformed cells. Many of these resistance genes, especially those of therapeutic importance, were originally isolated from plasmids.

The use of antibiotic resistance genes as selectable markers in microorganisms has been questioned since antibiotic resistance is common in bacteria that cause disease in humans and animals and is usually determined by plasmids (Saunders, 1984). The prevalence of such plasmids and the range of drugs to which they confer resistance have increased greatly in the past 30 years (Hughes and Datta, 1983). The mechanisms (conjugation, transformation, and transduction) by which bacteria exchange genes have been reviewed (Saunders, 1984). The human bacterial flora had the potential to transfer genes long before resistance became a problem (Hughes and Datta, 1983; Saunders, 1984). The reported incidence of bacteria that harbor plasmids conferring resistance is normally higher in countries where the use of antibiotics is not controlled, and in hospitals as compared to the community at large (Falkow, 1975; Saunders, 1984). The proportion of strains resistant to specific drugs can also be related to changes in antibiotic policy within hospitals (Buckwold and Ronald, 1979; Saunders, 1984). These findings strongly suggest that there is a causal relationship between antibiotic use (and overuse) and the evolution of a resistant bacterial flora (Saunders, 1984). The preceding strongly indicates that the development of antibiotic resistance among bacterial populations is not due to the availability of plasmids, but rather is the genetic consequence of imposing selective pressure on these populations by the introduction of therapeutic antibiotics into clinical use.

Cloning vectors containing resistance genes as selectable markers are usually constructed such that the resistance genes are no longer transposable. The resistance genes on such vectors can be considered to be stably associated with the vector.

If the rDNA organism does not enter the food product or if the organism is not deliberately released to the environment, then the presence of antibiotic resistance

genes should also not be a concern. This is because the expression products of such genes do not add toxic components to the food supply and, more importantly, the genes themselves will not be transferred to other organisms. In many cases the recombinant microorganism is used in a contained fermentation facility to produce an enzyme or other food ingredient. The recombinant microorganism is then removed from the commercial product. The residual microbial biomass is treated so as to inactivate the production microorganism before it is disposed of by spreading on agricultural land, in sanitary landfills, or other appropriate means. The small numbers of recombinant microorganisms that may enter the environment under these conditions should be of no consequence (National Academy of Sciences, 1987).

In cases where the microorganism does enter the food product or will be released directly to the environment, then the presence of antibiotic resistance genes may be a concern. In such cases the extent to which the presence of the genes will compromise the use of antibiotics to control disease agents in human or veterinary medicine must be evaluated. This is considered further in Appendix B.

Appendix B. Characterization of Vectors

Are the vectors characterized and determined to be safe for genetically modifying microorganisms to be used to produce food-grade products?

The key issue is the gene product itself and its safety in food applications. The vector will have no negative safety impact on the final product unless (1) it produces toxic substances that are seen in the final product; (2) it affects the production of toxic substances by the host production strain that are seen in the final product; or (3) it contains a mobile antibiotic resistance gene that could ultimately be transferred from the production strain to pathogens in the intestinal microflora. In cases where the production strain does not contact humans, animals, or other microorganisms, minimum safety concerns should exist with regard to the vector.

We would set a standard for a safe plasmid as one which after extensive use and testing in microbial systems is not known to generate any toxic material, or one for which there is extensive evidence not to expect toxin to be generated. This would include, but not be limited to (1) plasmids with documented prior safe use in the preparation of a food product [thus far, this includes pBR322 and pUB110 used and evaluated in food enzyme production (Pfizer, Inc., 1988a; MacKenzie *et al.*, 1989a,b; Andersen *et al.*, 1987; Diderichsen and Christiansen, 1988; U.S. Food and Drug Administration, 1990)] and (2) plasmids whose complete DNA sequence is known and which have also been shown not to encode any protein toxin found in a species with which the plasmid is associated.

A well-characterized plasmid, one whose full DNA sequence is known and whose genes have been defined, should be the vector of choice. Currently, the best known plasmid is pBR322 which has been reviewed by Balbas *et al.* (1986). Plasmid pUB110 has also been characterized at this level (McKenzie *et al.*, 1986, 1987); several other yeast and *Aspergillus* plasmids have been characterized, but not as well as pBR322 and pUB110.

It should be possible either to use a plasmid derived from a nonpathogenic, nontoxigenic strain or to show that toxins produced by the strain from which the plasmid is obtained are not encoded by the plasmid. Hence, in the case of pUB110, obtained from *Staphylococcus aureus*, genes for several of the well known enterotoxins such

as A, B, and C have been cloned and sequenced, and it can be shown that pUB110 does not encode for any of these.

It should be noted, however, that knowledge of the DNA sequence of a plasmid cloning vector is not an assurance of safety. For example, the sequence of pBR322 has been corrected at least twice since its initial publication, and that of pUB110 at least once. The consequence of the corrections is that new potential reading frames to encode proteins are constantly being revised, and the assurances of today become tomorrow's questions. A second problem is that even given an apparently safe DNA sequence, a potential open reading frame may be difficult to correlate with a function. For example, authors still disagree over the nature of the actual product encoded by the pUB110 *alpha* gene as well as where the gene actually starts. However, when the protein sequence of a toxin or the DNA sequence of its gene is known, it can be stated with assurance that toxin production is not determined by a given plasmid (for example, there are no similarities between the sequence of pUB110 and the DNA sequence of the *Staphylococcus aureus* toxin B). While knowledge of the DNA sequence of a plasmid or construct represents a significant step in our understanding of its function, such information only increases the comfort level with which we can use the plasmid, and does not, by itself, provide absolute assurance of safety.

A partial list of plasmids certified for use in cloning experiments may be found in the NIH Guidelines (*Fed. Reg.* **51,** 16970–16971). The most complete list of available plasmids may be found in the series *Cloning Vectors* (Pouwels *et al.,* 1985 and supplements in 1986 and 1987); however, many more plasmids have become available since the 1987 list was assembled.

Other aspects related to the safety of a vector used in rDNA technology are (1) whether the strain carries genetically modified extrachromosomal DNA and (2) whether the gene of interest has been integrated into the chromosome.

I. In strains with extrachromosomal DNA one should consider two factors:
   A. The presence or absence of relevant human or animal antibiotic resistance marker genes. The concern is the possibility of compromising medical or veterinary antibiotic therapy if the antibiotic resistance gene is transferred to pathogenic intestinal microflora.
   B. The possibility that extrachromosomal DNA might increase the overall toxicity of the final product by the action of proteins produced from other coding regions.

To avoid these problems one has three options:

1. Take the extrachromosomal DNA from a microorganism that is known to be safe in food applications.
2. Use extrachromosomal DNA that is itself known to be safe (e.g., pUB110 or pBR322).
3. Use a vector that has been sufficiently characterized to determine the presence of other functional genes, if any, and the lack of toxicity of the gene's products (restriction analysis, Northern analysis, sequencing).

II. In strains with the gene of interest integrated into the chromosome one needs to consider three factors:
   A. Mobility of the insert within the chromosome and movement to extrachromosomal DNA with subsequent transfer to intestinal pathogens. This refers to the use of mobile transposons, which are short sections of double-stranded DNA that consist of more than 2000 base pairs. They are able to move within the genome, even between a chromosome and a plasmid transferring genes

relevant to the treatment of human or animal diseases. It is also possible, if a strain carries plasmids which have regions of homology with inserted DNA, that the gene could be transferred from the chromosome to a free plasmid by homologous recombination. The plasmid would need to be transferable and able to move by itself (self-mobilizable) for exchange to other organisms to be possible.

B. The nature of the genetic insert. This involves the presence of the gene of interest and any supporting DNA spacers, linkers, etc., and vector DNA.

C. The location of the insert, which may inactivate genes.

To resolve these issues one may do the following:

1. Inactivate the mobility of transposons, if used.
2. Eliminate the possibility that mobilizable plasmids are present which could "rescue" the inserted DNA from the chromosome.
3. Eliminate the possibility of transferring antibiotic resistance genes to the intestinal microflora.
4. Use homologous recombination for gene insertion.
5. Insert the gene of interest at the same site as the wild type or any other gene which in its absence does not affect the toxicity of the final product.

The Food and Drug Administration (1990) concluded that chymosin preparation from a recombinant strain of *E. coli* K-12 made in conformity with 21CFR § 184.1685 will not contain DNA encoding resistance to antibiotics at levels that would provide any safety concern. This conclusion was based on a gel electrophoresis/DNA hybridization experiment and a transformation assay submitted by Pfizer, Inc. (1988b) demonstrating that the enzyme preparation does not contain gene-size DNA fragments or transformable DNA. In the electrophoresis experiment, DNA fragments were sized on the basis of their differential rates of migration through the gel and quantitated on the basis of their level of hybridization with labeled complementary DNA. No DNA fragments large enough to contain an intact gene encoding antibiotic resistance were detected in the enzyme preparation.

In the transformation assay, bacterial cells were mixed with DNA under optimized conditions to see if they had picked up the antibiotic resistance encoded by the DNA. Cells mixed with the enzyme preparation did not become antibiotic resistant.

Appendix C. Safety of DNA Insert

Does the DNA insert code for a substance safe for use in food?

Safety evaluation should focus on the organism that embodies the final construct. The nature of the gene donor should not be of particular importance except as it may guide the assessment of safety of the final construct. For example, any toxic potential of the gene source organism should be addressed in the safety evaluation scheme.

Two considerations should guide safety evaluation of the DNA insert. First, it should be shown that the insert itself is safe; second, it should be shown that use of the insert does not produce a pleiotropic effect (secondary phenotypic alteration resulting from a single genetic change) (Tiedje *et al.*, 1989) that results in elaboration of a toxin.

The DNA insert is important in that it codes for a desirable product. Safety evaluation of the insert should focus on its expression product.

The possibility of a pleiotropic effect resulting in toxicity is greatly diminished by using a host organism that does not produce toxins. For prokaryotes a demonstration of nontoxicity is fairly easily accomplished because of the relative simplicity of the genome (Pariza and Foster, 1983). However, for eukaryotic microorganisms (especially molds) such a demonstration may be more difficult. There are many examples where potentially toxic products are elaborated by eukaryotes only under special conditions (Pariza and Foster, 1983). At other times, toxin is not produced. The products of the construct intended for use in food should therefore be tested for toxicity under the exact conditions that will be used for routine growth in the manufacturing plant. Toxicity should be evaluated using chemical tests for specific toxins as well as animal assays (decision tree, Fig. 5) (Pariza and Foster, 1983).

Appendix D. DNA from Intermediate Hosts

Is the microbe free of DNA from an intermediate host which could code for a toxic product?

Recombinant DNA procedures usually rely on an initial cloning of the gene of interest in what is termed an intermediate host. Due to extensive genetic knowledge and 40 years of laboratory experience with the organism, *Escherichia coli* is the most common (though certainly not the only possible) intermediate host. During construction of the recombinant vector, it is technically possible that small portions of the intermediate host DNA may be transferred along with the vector and the cloned gene. If the intermediate host is a nontoxigenic, nonpathogenic organism, it is not possible that these pieces (regardless of size) will render the production organism toxic. When the intermediate host is known to carry toxin genes, then it becomes imperative to show that any intermediate host DNA in the final construction does not code for a toxin. This proof could be based on an evaluation of the DNA sequence if the toxin has been cloned and its sequence is known. Alternatively, classical methods for showing lack of toxicity in the final product should be sufficient.

In cases where the intermediate DNA constitutes regulatory regions (i.e., promoters, terminators) which are themselves not expressed, no further testing would be necessary. Usually these regulatory regions are selected for use by design and have been completely sequenced, and it is clear that they do not code for proteins. If long regions which might potentially code for proteins are used, they could be confirmed to be nonfunctional by (1) lack of promoter regions upstream or (2) lack of mRNA complementary to the DNA.

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Chapter 5: Safety Evaluation of Single Chemical Entities and Simple Chemically Defined Mixtures

1. INTRODUCTION

The complexity of our food supply is such that simple generalizations are not valid, and valid generalizations are not simple. The separation we have made into "simple substances" and "complex mixtures" in Chapters 5 and 6 provides an example. IFBC believes it is a useful categorization because many complex mixtures are consumed in large volumes, such as foods themselves, or are major components of familiar foods, such as soluble fiber or a plant protein. Conversely, simple substances often are food additives, GRAS substances, or prior sanctioned substances that are used in foods at relatively low levels and low total consumption compared with major ingredients. Safety evaluation of whole foods and complex mixtures poses different problems and requires different handling compared with evaluation of substances that occur or are used at low levels in food. It is to deal with these latter materials that conventional toxicology and safety evaluation practices have been developed. IFBC recommends that the safety evaluation of single chemical entities and simple chemically defined mixtures continue to be based on these concepts.

Complexity arises because some simple substances, such as sucrose and high-fructose corn syrup, are used at high levels in food and therefore encounter many of the same safety evaluation problems as foods and complex major ingredients. Conversely, many complex mixtures, such as spices, essential oils, and papain, are used only at low levels. The safety evaluation of such food components becomes a blend of the problems and opportunities that accompany traditional natural foods and those that are associated with single ingredients used at low levels.

Processes involving genetically modified organisms may be used to produce a variety of discrete chemical substances or simple mixtures that may be used in food processing. These substances will usually be classified from a regulatory point of view as food additives or GRAS substances. They may range from highly purified single chemical entities (i.e., sweeteners) to simple chemically defined mixtures, for example, certain flavoring materials. Because the majority of these types of products derived via genetically modified organisms can be readily characterized analytically, their safety evaluation will tend to follow along traditional lines. The purpose of this chapter is to elaborate criteria that may be used to evaluate these products.

The characteristic feature of this evaluation is that it focuses on the product, with less emphasis on the process by which the product is derived. This is due to the fact that most of these substances can be purified to discrete, chemically identifiable ingredients which, for the most part, are unlikely to contain unsafe levels of undesirable

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components or impurities. This chapter contains a discussion of conditions under which products and processes would not be expected to present unresolved safety issues and would require no further review beyond that required for chemicals produced through traditional means.

In performing the safety evaluation, four principal parameters should be considered: (1) the method of production, (2) the product specifications, (3) the anticipated human exposure, and (4) the need for toxicological data on the product. It should be noted that specifications may serve two purposes. One is to control the presence of possible toxic impurities; the other is to ensure that the product is being produced under Good Manufacturing Practices.

The approach taken in this chapter to the evaluation of single chemicals and simple mixtures is to elaborate a procedure using these parameters that will permit categorization of products into two broad groups, those for which no safety concern would exist and those for which some degree of safety evaluation is warranted. If, during evaluation, a question regarding the safety of the product is raised pursuant to the application of any or all of these evaluation parameters, then a more detailed safety evaluation of the product would be required. Procedures for identifying those products that may present unresolved safety issues also are outlined in this chapter. In addition, the chapter contains a series of decision criteria and a decision tree (Fig. 6) that may be used in conducting a safety evaluation of new products.

2. PROCESSES AND PRODUCTS OF GENETIC MODIFICATION THAT WOULD NOT BE EXPECTED TO RAISE SAFETY ISSUES

The purpose of this section is to identify those products and processes of genetic modification that would not be expected to raise any significant safety concern or where no further reviews would be required beyond those required or practiced for food chemicals produced through traditional means.

It is recognized *a priori* that the criteria outlined below will apply principally to substances that are in current use (i.e., food additives, color additives, and GRAS substances) and that may be produced through a genetically modified system as an alternate method of manufacture. An existing product, newly produced through a genetically modified system, that passes all the conditions outlined below would be expected to meet or exceed any safety standard established for that product. It should be stressed that the newly produced product must satisfactorily meet *all four* of the criteria outlined below and failure to do so would require a further analysis of its acceptability in accordance with the conditions outlined later in Section 3. Products that would be categorized as acceptable would meet the following conditions:

1. The substance is a recognized food ingredient (e.g., food additive, GRAS substance, or prior sanctioned substance).

—and—

2. A review of the genetically modified production process and the starting materials provides a reasonable basis to presume the absence of new, unwanted constituents in the product. This review would comprise an evaluation of the genetic characteristics of the transformed production system, its genetic stability, process variation and control elements.

—and—

3. The final product meets existing specifications for identity and purity. Analytical fingerprint comparison of the product of the new manufacturing method with the traditional product demonstrates no new or unknown constituents that exist at a concentration that may pose a safety concern at anticipated exposure levels of the product.

—and—

4. The use pattern and exposure to the substance does not demonstrate that exposure levels would exceed the limits supported by the existing safety evaluation.

If, on the basis of this evaluation it is concluded that any or all of these conditions will not be met, the product will require further evaluation. The next section lists a series of conditions that may require products to undergo further evaluation.

3. IDENTIFICATION AND PRIORITIZATION OF PRODUCTS/ PROCESSES OF GENETIC MODIFICATION THAT PRESENT UNRESOLVED SAFETY ISSUES

In this section the general types of safety-related issues that may be encountered in the evaluation of products derived from genetically modified systems are discussed. It should be noted that if any or all of these conditions exist, then the product would require further safety evaluation:

1. The substance derived via a genetically modified system is not a recognized food ingredient but is a chemical entity not presently approved for use in food.

—or—

2. A review of the genetically modified process and starting materials leads to the conclusion that
   (i) data are not adequate to characterize the genetic material, its stability under usual process conditions, or to ensure consistency in the nature and amount of expression product(s);
   (ii) data are not adequate to ensure the safety or permit characterization of the starting material(s) and its potential to lead to the presence of unwanted impurities in the final product.

—or—

3. The product fails to meet existing specifications for identity and purity or none exist. Analytical characterization demonstrates the presence of new or increased levels of contaminants or by-products. These may be
   (i) known substances of no biological concern, but not included in the existing specification;
   (ii) unknown substances requiring further evaluation;
   (iii) known substances of possible safety concern.

—or—

4. An evaluation of the proposed use pattern and exposure levels to the substance demonstrates
   (i) the potential for a significant increase, compared with previous exposures, that exceeds the limits supported by the existing safety evaluation;

—or—

   (ii) data are not adequate to characterize the level of human exposure.

4. RESOLVING SAFETY ISSUES

As indicated earlier, a spectrum of possible unresolved issues may exist. The intent of this section is to provide practical guidance for dealing with each of these issues. It should be recognized that the resolution of scientific issues associated with new products cannot be divorced from their legal status. Requirements for safety evaluation also need to be considered in the light of existing regulations and guidelines, taking into consideration previous practices and precedents in product safety evaluation. The legal requirements for regulatory approval of new products are discussed in detail in Chapter 7.

4.1. Substances Not Previously Recognized for Use in Food

When genetically modified systems are used to produce substances that have not previously been recognized as food ingredients, the procedures for safety evaluation of these products will be similar to those required for the evaluation of products produced through conventional chemical techniques. An important step in the process of safety evaluation concerns the determination of the products' probable legal status as outlined in Chapter 7. Substances that are legally considered as food additives will require a food additive petition be prepared containing supporting documentation in accordance with the Food and Drug Administration (1982) or other safety evaluation guidelines (World Health Organization, 1987).

4.2. Specifications

If the product prepared by genetic modification techniques fails to meet the existing specifications for identity and purity for the traditional product, it might be considered unacceptable for use in food. This would require that action be taken to address this concern. It may be possible to further purify the end product through traditional chemical procedures to bring it into compliance with existing specifications and thus preclude the necessity of any further safety evaluation. Alternatively, consideration may be given to requesting a change in the specification to encompass the product produced by the genetically modified system. In establishing revised specifications for chemical products it must be recognized that various safety-related issues may have to be addressed. These relate to determining the safety of impurities or by-products that cannot be readily removed by good manufacturing practices. The presence of unavoidable impurities may present a range of problems:

- The chemical impurities in the product may be well known materials of no safety concern at anticipated exposure levels of the product.
- The chemical impurities may be known substances of possible safety concern requiring that a safety evaluation be conducted to ensure they pose no safety concern at anticipated exposure levels of the product.
- The chemical impurities may be unknown substances or substances for which only minimal safety data exist and which may require additional studies to ensure safety. Additional studies may not be required if the levels of the individual impurities

are below those that may pose a safety concern at anticipated exposure levels of the product.

4.3. Issues Related To The Genetically Modified Production System

In documenting the safety of any new or existing product produced through genetically modified systems there will be a requirement to adequately document the genetic origins and stability of the process. The genetic origins may be readily documented by providing data on the source of the biological material (e.g., cell or breeding line) or gene and the nature and extent of the genetic modifications that have been made to obtain the production line including a description of the regulatory and coding sequences as appropriate. The stability of the process is best handled by documenting the uniformity or range of variability in the final product rather than focusing on the theoretical or estimated stability of the methods used to produce them. It is also important to ensure the purity of starting materials used in the process and to determine the effect of process conditions on the purity of the desired expression product(s) and the nature and level of any chemical impurities and variations in these.

4.4. Exposure-Related Issues

For substances newly produced through genetically modified systems, there is a need to ensure that the proposed use and exposure are covered by the existing safety data, especially in instances where there is an anticipated increase in exposure. This will require documentation of the anticipated exposure in accordance with existing practices. If an analysis of anticipated exposure leads to the conclusion that the established safe intake may be exceeded, two courses of action may be considered:

- The level of use of the product may be limited to within the existing safety data base.
- The safety of the material may need to be reevaluated through appropriate techniques in order to achieve approval for an increase in the acceptable daily intake.

Procedures and practices for estimating the intake of food ingredients and for assessing changes in exposure are outlined in the Appendix to Chapter 6.

5. DECISION TREE FOR SINGLE CHEMICALS AND SIMPLE MIXTURES

The decision tree for single chemicals and simple mixtures is shown in Fig. 6. This decision tree utilizes the criteria and guidance developed earlier. The approach taken to the safety evaluation of new products is to determine first whether the product is currently approved for use in food (question 1). If the material is an already approved food ingredient, animal safety studies or other forms of safety evaluation would not normally be required, provided the product meets existing specifications for identity

and purity (question 2) and provided the existing specifications are adequate for the new process (question 3).

In addition, it will be necessary, as indicated in question 4, to assess the probable daily intake of the material to determine whether its proposed use level and consequent human exposure are supported by existing safety evaluations. If the anticipated or proposed use of the substance is such that it is not fully supported by the existing safety data, it may be necessary to limit the use to within those supported by existing data, or alternatively, to conduct further safety studies. Such studies would be aimed at developing documentation to support an increase in the acceptable daily intake (ADI). If the existing specifications are not adequate (question 3), it will be necessary to conduct a more detailed safety evaluation to ensure that the constituents pose no safety concern (question 5) at anticipated levels of exposure.

If the new product does not meet existing specifications and if the constituents are deemed to pose no safety concern, the specification is revised and attention is directed to question 4. If, on the other hand, the product is found to pose a safety concern, then the issue addressed in question 6 will require attention. It may be necessary to purify the product to remove offending substances or to reduce these substances to levels that would pose no safety concern.

FIG. 6. Decision tree for the safety evaluation of single chemicals and simple mixtures.

Describe the product and characterize it chemically, then proceed to answer the following series of questions:

	If: Yes — Go to	*If:* No — Go to
1. Is the product *currently approved* for use in foods?	2	*Develop specifications and safety evaluation* and go to 3 or reject
2. Does the product meet *existing specifications* for identity and purity?	3	5
3. Are the *existing specifications* adequate to ensure the absence and control of toxic constituents?	4	5
4. Do the intended or reasonably expected conditions of use of the product result in a pattern of intake that is supported by the safety data base?	Accept	Accept with use limitations or do *safety evaluation* (accept ADI, raise ADI, or reject)
5. Do the constituents pose *no safety concern?*	Revise specifications and go to 4	6
6. Can the undesired constituents be removed by processing?	Remove and go to 4	*Safety evaluation; revise specifications* and go to 4

Safety Evaluation means the entire process or the appropriate parts thereof, discussed in this chapter and in numerous publications including those elaborated by the Food and Drug Administration (1982) Cramer *et al.* (1978), Food Safety Council (1978), National Academy of Sciences (1969), and World Health Organization (1987). Safety evaluation may or may not require animal tests.

For newly proposed food ingredients (e.g., additives), it will be necessary, in all probability, to conduct detailed safety testing and evaluation to ensure that the product is safe for its intended use. Procedures for accomplishing this have been published (Food and Drug Administration, 1982; World Health Organization, 1987).

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Chapter 6: Safety Evaluation of Whole Foods and Other Complex Mixtures

1. INTRODUCTION

The process of safety evaluation of whole foods and other complex mixtures is considerably more complex than that of single chemical substances or simple mixtures. In the case of plant-derived foods and macroingredients produced through genetic modification, it will usually not be possible to develop a product specification in the sense that it can be derived for single chemical entities or simple mixtures. Here we are dealing with complex biological matrices with considerable natural variability as pointed out in Chapter 2. Unlike the case of single chemicals which can be purified, the process and source materials used in the production of genetically altered food sources are of more importance in the safety evaluation.

Many edible plants and macroingredients are either GRAS substances or accepted as common food, and the question to be addressed is whether compositional changes induced through genetic modification are sufficient to cause a change in regulatory status of the food which would require premarket approval. The critical feature of the safety evaluation of genetically modified foods is the need for documentation concerning the genetic change and the influence this has on the overall compositional characteristics of the food product.

Genetic modification of plants and the use of genetically altered organisms in the production of macroingredients requires a thoughtful analysis of appropriate procedures for safety evaluation, taking into consideration the regulatory classification of such products. *IFBC recommends that procedures for safety evaluation of such foods should be closely linked to existing agricultural and food processing practices as well as to the regulatory status of comparable traditional foods and ingredients.* Traditional foods as defined in Chapter 2 are plants, animals, and microorganisms and their products widely consumed as human food.

The extent to which safety evaluation of genetically modified foods is warranted will depend, to a significant degree, on the nature of compositional change of the product relative to its traditional counterpart. The extent and depth of analytical comparison must be guided by the fact that we have only limited knowledge of the total complement of inherent constituents that make up traditional foods. Unless there is some very good reason, based on safety or nutritional considerations, to go beyond our current knowledge of the principal inherent constituents of a food, the analytical comparison would normally be confined to an examination of the principal chemical characteristics, significant nutrient constituents, and nonnutrients such as endogenous plant and other toxins, typically associated with the food, its parents,

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or related species. In addition, identification of new intended constituents resulting from genetic manipulation will be required. A new constituent of food is any expression product present solely as a result of the introduction of new genetic material, but not any known or even unidentified constituent inherent to the food, its parents, or related species.

An important factor that provides guidance on the extent to which analytical examination might be required is the perceived safety concern. Some genetic changes might lead to extensive alterations in the plant genome; others are less likely to do so. Given the specificity of rDNA technology the latter case would apply in most genetic manipulations involving food plants. Thus, wholesale changes in constituent composition, outside the normal range, would not be expected to occur. This must be balanced, however, in the case of foods known to produce plant toxins, with a careful examination of the effect of genetic change on the extent of toxin production.

While the safety evaluation of newly produced foods cannot be based on analytical studies alone, it is not recommended that genetically modified foods be subjected to the extensive safety testing in animals akin to that required for direct food additives. It may be necessary in some circumstances to develop a safety profile, based on appropriate studies in animals, to ensure that the food possesses no unexpected toxicity. Studies in humans will be useful for organoleptic detection of possible changes in comparison with the traditional food product. Studies of these types may provide important leads in the analytical and safety evaluation of new foods. In addition, as is presently the practice with all new foods, the evaluation of the extent of anticipated human intake of the new product, in comparison with its traditional counterpart, will provide an important perspective on practical analytical detection limits for new constituents.

Evaluation of new products must embody the notion that analytical studies and biological evaluation proceed in a coordinated fashion, integrating the results of these various studies in a comprehensive and reasoned program of safety assessment. It is of paramount importance to recognize that neither analytical chemistry nor biological evaluation, by themselves, constitutes an adequate basis for product safety evaluation. Based on lessons from the past, the employment of only one avenue of evaluation is a recipe for endless pursuit of unobtainable objectives and may also miss real problems.

The approach taken in this chapter to the safety evaluation of whole foods and other complex mixtures is to outline a stepwise series of conditions and criteria that form the basis for product evaluation. These criteria should be applied in the light of past practices regarding the acceptability of traditional plant breeding. These are considered in the context of three principal evaluation elements. The first of these relates to documentation regarding the product lineage and the extent to which it is possible to assess the safety of the product on the basis of knowledge regarding the nature of genetic change. The second factor relates to an assessment of the degree of compositional change induced by genetic modification, in comparison with the traditional food, along with an assessment of the nature and amount of new constituents. In cases where compositional change is substantial, the product may no longer be considered acceptable in the regulatory context or a question may arise as to its continued regulatory status. The third factor relates to the degree of dietary exposure that might exist with a new product and whether it would be anticipated to change

due to increased consumer acceptance over the traditional product or as a result of new uses. The chapter concludes with a series of decision criteria and a decision tree (Fig. 7) for determining the safety of new food products.

2. EVALUATING PRODUCT SAFETY—GENERAL PRINCIPLES

The purpose of this section is to outline the conditions and criteria that constitute the critical decision elements in ensuring product safety.

2.1. Genetic Origins

IFBC recommends that the initial basis of the safety evaluation of a genetically modified food should begin with consideration of the lineage of all genetic materials present in the final food product. Any diverse organisms, including nonrelated or related but noncultivated relatives and particularly those without a history of safe dietary use that contributed genetic material to the final food product, should be fully described. Descriptions should include relevant donor taxonomic information, previous donor uses in or as food, and any nutritional or toxicological concerns associated with the donor. Genetically modified foods which contain only genetic material from sources already part of our present food supply and considered to have a history of safe use will require a lesser degree of evaluation than genetically modified foods whose parents have not been commonly consumed.

Although all plants contain substances which are deleterious if consumed at a sufficiently high dose, IFBC considers our food plants and selected wild relatives (those that have been used previously as sources of genetic variation in breeding programs) as safe sources of genetic materials for genetic modification because (1) there is a history of safe use of products containing genetic elements derived from these sources, (2) these sources have been sufficiently well characterized that we know what kinds of potential toxicants they contain and accordingly we know what to screen for, and (3) information is likely available on the toxicological properties of various substances contained therein.

While the foregoing statements would be difficult to document in detail, long experience provides adequate pragmatic justification for their validity. The consequences of not accepting these statements would be to deny the established safety record of experience of past plant breeding practices, as described in previous chapters, and to cast unfounded doubt on the safety and wholesomeness of the present food supply.

Novel genetic sources, although they undoubtedly contain many expression products which will be found safe to consume, have no such history of safe use and may be less well characterized.

Where sufficiently documented, any species that has been used previously as a source of genetic material for traditional breeding programs would not be considered by IFBC to be a novel source. Genetic material from any sources that are not novel would produce expression products that are probably already being consumed, and thus are not new constituents in the food supply. If an expression product from a novel source is identical to a substance that is already commonly consumed in food, that expression product would not be considered by IFBC to be a new constituent.

Fully characterized genetic material derived from nontoxigenic, nonpathogenic microorganisms that are not intentionally consumed as food but are commonly found in or on food as consumed would not be considered by IFBC as sources of new constituents (see Table 22). They, together with their expression products, would be considered to be acceptable constituents of food. The acceptability for food use of these and other genetic elements is discussed more fully in Table 22.

If a food is genetically modified, by whatever method, so that it produces a new constituent, that modification would trigger a more detailed safety evaluation. Quantification of the levels of new expression products (constituents) will aid in the dietary exposure assessment required by the decision tree for the safety evaluation of foods containing new constituents. Although the safety of any particular substance will not depend on its source *per se*, our lack of exposure experience with novel genetic sources and consequent possible lack of knowledge of potential constituents necessitate caution.

Traditional breeding programs continue to incorporate into crops useful genetic traits from nonfood sources. The historical record of safety supports the soundness of this approach even though it has not been feasible to identify either the exact genetic sequences introduced from the nonfood source or their expression products. However, an extra measure of safety can be included in any crop improvement program that uses nonfood sources of genetic material now that recombinant DNA methods are available. When recombinant DNA methods are properly used, the recombinant genetic material has been precisely identified, the amount of genetic material introduced is controlled, and the result can be fully characterized. Thus, if the level of knowledge concerning the genetic material permits, recombinant DNA should be the method of choice whenever any genetic resource that has not yet contributed to the food supply is used.

To aid in the safety evaluation of such a genetically modified food, the following types of data might be appropriate: each functional transcription unit (e.g., promoter, initiation sequence, structural gene, termination sequence) could be identified and characterized by its size, sequence, function, source, and location in the construct; any nonfunctional sequences (e.g., vector sequences, other spacers, or extraneous DNA) could be mapped, measured, and verified as nonfunctional; the number of copies of the introduced genetic construct could be carefully estimated; the new constituent could be quantified in the edible portion of the plant. Depending on the specific expression product(s) some of these data may not be relevant or necessary.

2.2. Product Composition

IFBC recommends that a food product be considered to present no safety concern if analytical studies indicate that the concentration of inherent constituents does not differ significantly from the concentration range typical of the traditional food, and any new constituent(s), if present, is already accepted for use in food under the anticipated conditions of use. The expression "no safety concern at anticipated exposure levels to the food product," as used here, is intended to mean the practical certainty that no harm will result under the conditions of exposure to the constituent or the whole

food. *On the other hand, IFBC recommends that further safety evaluation of a food product be required if (1) analytical studies demonstrate a significant change in the levels of inherent constituents of the food, or (2) the new constituent(s) is not an accepted food ingredient and its safety under conditions of use requires further evaluation.*

2.3. Exposure

IFBC recommends that a food product be considered to present no safety concern if use of the food would not be expected to alter significantly present intake of it or its constituents in comparison with the traditional product, and the proposed conditions of use of the new product would not reasonably be expected to lead to such an intake of the food that the total intake of any constituent would exceed the amount acceptable under the standard of safety appropriate for that constituent. Alternatively, if introduction of the new food product would be expected to lead to a significant change in use and/or exposure, this could raise nutritional/safety concerns. Where unusual exposure to the new constituent(s) may be expected to occur, further safety evaluation would be warranted.

3. RESOLVING SAFETY ISSUES

The purpose of this section is to provide some practical guidance on the resolution of the various safety issues implied by the conditions listed previously. Because of the well known difficulties associated with conducting and interpreting toxicity studies in which whole foods or macroingredients are fed to animals, the principal focus of this section will be on developing as complete as possible an understanding of the compositional changes induced through genetic modification as the primary basis for safety evaluation. This, coupled with a detailed evaluation of anticipated use pattern and exposure, provides a mechanism for both conducting a safety evaluation and identifying those products which will require some degree of safety testing. The safety evaluation of new genetically modified plant products, or macroingredients derived therefrom, has to be based on a comprehensive comparison with the traditional counterpart in regard to inherent and new constituents. This, coupled with documentation on the nature of the genetic change induced along with exposure assessment, provides the basis for a rigorous safety evaluation. Only in isolated circumstances would safety testing in animal studies be required since most safety questions can be answered on the basis of analytical studies on the product in question. However, as is presently the practice with traditionally bred cultivars, introducing new foods into the marketplace should continue to include preintroduction consumer evaluation. Informed consumer evaluation presents an effective means of detecting unexpected organoleptic qualities. In identifying practical means to deal with safety concerns raised by food products and macroingredients produced via genetically modified systems the following issues should be addressed.

3.1. Product Composition

IFBC recommends that the principal feature of the safety evaluation of genetically modified food products be a comparison of the composition of the new product with

that of its traditional counterpart in regard to the levels of inherent constituents. This does not mean that a detailed and exhaustive analytical comparison would be required in each case. Analytical methods that have adequate selectivity, sensitivity, and precision to ensure food safety and that can be performed at a reasonable cost are generally available. The reference point for analytical work on new foods is the traditional food. Thus, the analytical criteria for method acceptance would be based on the normal range of levels of inherent constituents and not on a method of maximum sensitivity. There are several classes of inherent constituents which would need evaluation in new genetically modified foods including nutrients, naturally occurring toxicants, and constituents that affect the processing of food.

3.1.1. Nutrients

The nutrient composition of commercial foods is known to vary considerably depending on environmental conditions, genetic factors, and production and processing practices (see Chapter 2). In fact, such variations may be considered to be normal fluctuations in composition which have existed for millennia. Nontraditional genetic modification techniques might be expected to contribute to this variation; however, the extent to which this will occur cannot be predicted in advance with certainty. As is the case with traditionally bred crops, cultivars using nontraditional genetic modification techniques should be evaluated individually to assess the possible impact of genetic changes on nutrient composition. The evaluation should focus on significant nutrients traditionally associated with the food in question and nutrients newly introduced through genetic modification techniques. Particular attention should be given to foods which contribute significantly to meeting dietary needs of the population and to those nutrients that are most likely to be underconsumed or present risks if overused. For example, significant reductions in the concentration of vitamin C in citrus fruits would be undesirable due to the important role fruits and fruit juices have traditionally played as dietary sources of this vitamin.

In evaluating a new genetically modified food, a comparison with its traditional counterpart will be necessary in order to determine whether the significant nutrients in the new food as consumed fall within the range typical of the product. If the new product is found to have essential nutrients in the same range as its traditional counterpart, no further nutritional evaluation of the product would be required. On the other hand, if there are substantial changes, particularly reductions in the concentration of significant nutrients such that they fall below the range typical of the food, further evaluation is warranted. This evaluation consists of assessing the contribution the affected food and nutrient makes to the dietary need. To accomplish this it will be necessary to obtain data on the anticipated intake of the new food. With these data in hand, it is possible to assess the contribution of the food/nutrient to the dietary need. Foods that contribute less than about 5% (see Chapter 2, Sections 2.1.2 and 2.1.3, for a perspective on these percentage figures) of the dietary need for a given nutrient may be considered as contributing only marginally to the dietary need. Above this level, and especially, for foods that contribute about 10% or more of the dietary need of a particular nutrient and may, therefore, be considered as significant sources of nutrients, a careful analysis of the impact of a reduced significant nutrient concentration in a new food would be required.

The great majority of genetic modifications will have narrowly specific objectives. For reasons of consumer recognition and acceptance it will usually be desirable to have as little change as possible in all other characteristics of a food beyond the aspect that is the focus of the modification. In most of these cases, analyses for significant nutrients, without nutritional feeding studies, will provide adequate assurance that nutritional quality has been maintained. This may not be the case, however, if the significant nutrients include some, such as iron or calcium, that vary a great deal in bioavailability. It will be necessary to consider the need for nutritional feeding studies when there are questions concerning the bioavailability of significant nutrients, multiple changes in composition, the reasonable possiblity of antinutrient constituents derived from one of the parental species, or the need to ensure the validity of later toxicological feeding studies.

3.1.2. Naturally Occurring Toxicants

As previously noted, natural toxicants are inevitably the primary concern of safety evaluation. This evaluation should focus on those toxicants that could reasonably be thought to be present because of their presence in any portion of the plants or microorganisms that were used as sources of genetic material. Chapter 2, its appendixes, and Tables 14–16 provide background and perspective for this examination.

The needed assurance of safety is a matter for thoughtful, perceptive, interdisciplinary consideration. It must provide the practical certainty that there will be no adverse effects (no safety concern) while avoiding an open-ended search for the unknown. This is not mere rhetoric; one cannot prove a negative.

There are several feasible, effective, and generally used measures that will provide fully adequate practical assurance for the absence of adverse effects.

1. It will be necessary to consider toxicants known to occur in other members of the same genus or family. There is no automatic checklist. Constituents such as D-limonene occur widely throughout the plant kingdom (see Chapter 2, Appendix A, and Table 14). But adequate toxicological advice will make clear that D-limonene, though necessarily included in Tables 14 and 15, poses no human risk and may well be an important anticarcinogen (Elegbede *et al.*, 1984). There is no sensible escape from such careful, specific guidance.
2. Human exposure has always played an essential role in the development of any new product or new food plant variety. Its primary purpose, heretofore, has been to measure organoleptic quality or aspects of functional value. But it has also served safety. Clearly, there is a cautiously enlarged role for gradually expanded, carefully monitored human exposure. The "sip and spit" test can be an invaluable detector and a guide to any further, more specific efforts at analysis or toxicological study and precedes further efforts at safety evaluation.
3. The combined professional judgments of the toxicologist, the analytical chemist, and the geneticist, among others, will suggest when some form of toxicological examination could be desirable beyond whatever data may already be available (see Section 5 of this chapter).

Beyond these steps, the same combined judgments will be needed to provide the direction and extent of any further analytical screening studies which, if done, must

both guide and be guided by these other considerations. Such screening studies must also have a clearly intended purpose. They must, moreover, have as a broad benchmark, the range of normal variation in closely comparable foods.

3.1.3. Constituents That Affect the Processing of Food

In most cases components of interest in food processing are macrocomponents such as total solids, acids, sugars, salts, and alcohols, and occasionally intrinsic features such as pH. The analytical technology is available to quantify the levels of these components and such assays should be done. The loss or change of levels of these compounds does not necessarily mean that the food is unsafe; however, the processor must be made aware of any such changes so that the processing of the new food can be done in a manner to ensure a safe product.

3.2. Exposure-Related Issues

Any safety evaluation of new genetically modified products will require careful documentation of the anticipated use pattern and exposure. A number of considerations must be taken into account in developing criteria for determining what constitutes a significant change in exposure to foods and their components:

1. The total amount of a particular food component (i.e., nutrient, toxicant) consumed in a fixed period
2. The pattern of use of the food within a fixed period
3. The biological (e.g., nutritional, physiological, toxicological) potency of the individual components
4. The biological availabilities of the components of interest in the particular food as consumed
5. The presence in the food itself of other components that modify the potency of compounds of biological value and interest
6. The above relationships between the components in a single food item and other foods in the total diet

A more detailed discussion of methodology relative to exposure analysis is presented in the Appendix to Chapter 6.

4. DECISION TREE FOR THE SAFETY EVALUATION OF WHOLE FOODS AND OTHER COMPLEX MIXTURES

IFBC recommends the decision tree as presented in Fig. 7 for assessing the safety of whole foods. In keeping with the general concepts for safety evaluation of whole foods as described elsewhere in this chapter, the three principal questions to be asked relate to the genetic origins of the new food, the effect of genetic modification on its

FIG. 7. Decision tree for the safety evaluation of whole foods and other complex mixtures.

Describe the product and characterize it in light of its genetic origins, then proceed to answer the following series of questions.[a] *Note:* Words in italic are defined.

Questions	*If:* *Yes/go to*	*If:* *No/go to*	*Comments*
1. Was the product developed only from genetic material derived from plants or microorganisms that are *traditional foods* or related nonfood species previously used as sources of genetic variation in developing and improving foods by traditional methods of genetic modification?	2	7[b]	For a fuller discussion of acceptable genetic elements see *Table 22. Traditional foods* are defined in the Glossary.
2. Are the constituents in the food product only *inherent constituents?*	3	4	*Inherent constituents* is defined in the Glossary.
3. Do these constituents (question 2) occur within the documented range for the parental traditional food?	5	5	Criteria for acceptable ranges of inherent constituents are presented in Chapter 6, Section 3.1.
4. Does the intake of *new constituent(s)* under intended or reasonably expected conditions of use present *no safety concerns?*	6	10	The terms *new constituent* and *no safety concern* are defined in the Glossary.
5. Can the intended or reasonably expected conditions of use result only in a pattern of intakes of individual inherent constituents that does *not alter significantly present intakes?*	6	Safety evaluation of constituents; go on to 6 or reject	The term *not alter significantly present intake* is defined in the Glossary. Safety evaluation refers to existing practices to ensure that a food product or constituent presents *no safety concern* (Food and Drug Administration, 1982; Food Safety Council, 1978; Cramer *et al.*, 1978; World Health Organization, 1987).

FIG. 7—*Continued*

Questions	*If: Yes/go to*	*If: No/go to*	*Comments*
6. Are the *significant nutrients* in the product within the expected range for the closely comparable *traditional foods* which the new food will replace?	Accept	Evaluate consequences and accept or reject	The term *significant nutrients* is defined in the Glossary.
7. Is available knowledge and documentation adequate to characterize the *introduced genetic material* in terms of its origin and expected expression products and to ensure its acceptability for use in food? (Table 22)	2 and 4	8	Introduced genetic material means any incorporated DNA. Documentation should be adequate to support its inclusion in Table 22.
8. Are the expression products of the *introduced genetic material inherent constituents of foods*?	9	Safety evaluation of new constituents; go to 4 or 10, or reject	*Foods* in this context means any food, not necessarily the traditional counterpart food.
9. Are the expression products of the *introduced genetic material* present at concentrations inherently found in foods?	2 and 4	Safety evaluation of new constituents; go to 4 or 10 or reject	*Foods* in this context means any food, not necessarily the traditional counterpart food.
10. Can the new constituents be removed, reduced to acceptable levels, or inactivated by processing?	2	Safety evaluation of new constituents and/or whole foods	Food processing may be used to reduce or remove undesirable constituents.

[a] Procedures for product characterization are discussed on pages S138–140. In essence this consists of a description of the genetic origins of the food and an analytical profile of the food in comparison with its traditional counterpart.

[b] If the material is a new macroingredient such as single-cell protein, safety evaluation would be required along with the development of process and product specifications.

composition in relation to its traditional counterpart, and the expected pattern of intake of (exposure to) the new food. The safety evaluation of any new food, derived via nontraditional genetic modification or produced through conventional plant breeding, must be linked to the safety of its counterpart traditional food.

TABLE 22

IFBC FINDINGS AND RECOMMENDATIONS FOR THE ACCEPTANCE FOR USE IN FOOD OF GENETIC ELEMENTS DERIVED FROM SOURCES THAT ARE NOT TRADITIONAL FOODS[a]

Findings

The following genetic elements, due to a history of safe use, are considered to be acceptable for use in food:

1. Uncharacterized genetic material presently consumed in food that was introduced from nonfood species used as sources of genetic variation in developing and improving foods using traditional methods of genetic modification and for which documentation of safe food product use is available
2. Fully characterized genetic material derived from nontoxigenic, nonpathogenic[b] microorganisms that are not intentionally consumed as food but are commonly found in or on food and that have an established and documented record of safe exposure and use

Recommendations

The following additional genetic elements are recommended to be acceptable for use in food due to the availability of widely accepted scientific rationale in support of such use:

1. Coding DNA from nonfood species that have already been used as sources of genetic variation in developing and improving foods using traditional methods of genetic modification and for which documentation of safe food product use is available
2. Fully characterized noncoding DNA from sources that are not traditional foods

Since noncoding DNA cannot produce any protein or other expression products, we need be concerned with only the intrinsic properties of such DNA's biochemistry and digestibility. IFBC is aware of no health risks, either functional or toxicological, from the ingestion of DNA based on its specific chemical characteristics. Only from the quantitative standpoint does the total intake of nucleic acids pose a potential health concern. This situation can arise with traditional foods, particularly those recognized for their high nucleic acid content including the glandular organ meats, liver, pancreas, kidneys, and embryos or germ of grains and legumes. If an individual consumes sufficient quantities of nucleic acids so that the total dietary intake exceeds the individual's capacity to eliminate uric acid, the metabolite of nucleic acids, then the disease gout results. The use of noncoding DNA in genetic modification programs would not significantly increase the amount of DNA in the food and, thus, would not increase the incidence of gout. There is no a priori reason why noncoding DNA from a nonfood source should be less safe to use in a food plant than noncoding DNA from a food source.

[a] Food products containing such genetic elements are not considered safe a priori but should be evaluated using the decision tree in Chapter 6 (Fig. 7).

[b] Microorganisms that are nontoxigenic and nonpathogenic to humans and animals and, therefore, considered to be safe for use in food.

The phrase *traditional food* is intended to include those microorganisms consumed as food, such as *Lactococcus bulgaricus* and *Streptococcus thermophilus* in yogurt, but it excludes organisms or products consumed only coincidentally or as carryover from processing. Additionally, if documentation is sufficient to support it, other nontoxigenic, nonpathogenic microorganisms that have a history of unintentional, yet safe, consumption as food may be considered to be acceptable sources of material for genetic modification. Table 22 and Chapters 2 and 4 contain a fuller discussion of traditional foods and accepted food-related microorganisms.

Inherent constituents as defined in this chapter must be readily identifiable by generally available and widely accepted instrumental analysis. They need not be individually identified nor is there a requirement to identify all constituents. They must be typical of the parents and of closely related species. The amount of any constituent may vary greatly and a specific constituent may occasionally be undetectable.

Question 1 of the decision tree relates to the determination of the genetic origins for the new genetically modified food. If the food was developed *only* from plants or microorganisms that are traditional foods or related nonfood species traditionally used as breeding material sources, its safety evaluation is less complex than if it was derived in part from nontraditional food species. Related species of organisms not themselves used as food have long played a prominent role in the development of useful new varieties of food crops and are known to present no safety concerns although monitoring would be appropriate. A discussion of and recommendations regarding other acceptable genetic elements are presented in Table 22. An important facet of the safety evaluation, discussed in questions 2 and 3, consists of determining whether the food consists only of inherent constituents and whether these occur within the documented range for the traditional food. Analytical studies on the new food (discussed in Section 3.1) will be required to make this determination. The analytical examination should include a determination of the levels of significant nutrients and known naturally occurring toxic factors. Beyond this, constituents that affect processing of the food might be examined. Provided the inherent constituents (including significant nutrients, question 6) are found to occur at levels typical of the traditional food and at levels not associated with adverse effects and the intake (exposure) as discussed in question 5 is not altered significantly in relation to the traditional food, further safety evaluation of these inherent constituents would not be required. If a new constituent, as discussed in question 4, is present in the food whether by design or unexpectedly detected in the analytical screening, a determination will have to be made regarding its safety. If the material is a recognized and accepted food ingredient, it would be expected to present less of a safety concern than if it is a substance previously unknown to occur in the edible portion of the plant. Question 4 addresses this issue and asks the question whether the intake of the new constituent, under intended conditions of use of the food, would be expected to raise a safety concern. In conducting a safety evaluation of new constituents, the reader is referred to several general references listed in the decision tree and elsewhere in the chapter regarding the safety assessment of food constituents.

Question 6 is intended to ensure that all nutrients of which the raw product is a significant source are examined and that there has been no major change that would raise questions of nutritional inadequacy or, much more rarely, of toxicity. The importance of a particular food as a source of a specific nutrient will depend both on the concentration of the nutrient in the food and on the amount of the food that is consumed (Chapter 2, section 2.1.3).

There is a very large variation in the nutrient content of the individual foods depending on ripeness and many other factors. In commercial processing and distribution, however, many foods are extensively "pooled", i.e., lots of the same food from areas, varieties, and seasons are blended prior to retail sale.

Where foods, for example, orange juice, are extensively pooled, *IFBC recommends that the standard for a significant nutrient (one that food supplies, in the average diet, 10% or more of the dietary need) be the mean value reported in the literature plus or minus 20%*. References such as *USDA Handbook No. 8* (1976–1984) or Souci *et al.*, (1981) may be used to obtain mean values.

If the food is not extensively pooled, for example, fresh potatoes, *IFBC recommends that the standard should be the mean reported in the literature plus or minus*

two standard deviations or 75% of the reported range, where a standard deviation is not available.

If a nutrient in a food supplies less than 5% of the average dietary need, the nutrient may be considered nonsignificant for the purpose of this evaluation.

The range from nonsignificant (less than 5%) to significant (more than 10%) is a judgmental area. If the nutrient is not consumed in adequate amounts by some segments of the population, reductions may be of concern and the 5% level should be observed. If the nutrient is in ample supply the 10% level may be more appropriate. Special food consumption patterns may need to be taken into account.

The term *reject*, in the decision tree (question 6) need not necessarily mean total inability to use. It may, depending on applicable regulations, result in use but only with fortification or other appropriate public health measures.

If the intent of the genetic modification is to increase the level of a particular nutrient in a food, this requires no specific evaluation unless the normal range (usual mean ± 2 SD) is exceeded; however, certain trace elements, for example, selenium, fluorine, sodium, and manganese, and even certain fat-soluble vitamins cannot be increased substantially without raising health concerns. Amino acid ratios may require attention. The multiple interrelationships of many nutrients suggest that a major increase in any essential nutrient, though quite probably beneficial, requires detailed expert evaluation.

If the answer to question 1 indicates that some of the genetic material came from nonfood sources, question 7 then addresses the level of knowledge and confidence in the inserted genetic material. If there is sufficient documentation, that is, published information, to establish that a specific source or category of genetic material, over a very broad range of applications, performs only the intended function(s), carries no known signficant risk of undesired secondary effects, and poses no problems touching on safety or nutritional adequacy, then it should be proposed for addition to the list of acceptable genetic elements in Table 22 by a note to a journal of suitably wide circulation. In that case the question should be answered "yes."

A negative answer to question 1, earlier, establishes that the new genetic material was not from a traditional food and a negative answer to question 7 establishes that it was not yet well enough known to merit inclusion in Table 22.

The purpose of question 8 is to acertain whether or not there is routine significant exposure to the expression products of the introduced genetic material from *other* food sources. If the answer is "yes," question 9 will address concentration (i.e., exposure). If the answer is "no," then the next step is an appropriate more detailed safety evaluation.

If, as indicated in question 9, the expression products of the introduced genetic material are present at concentrations generally found in foods, the product may be acceptable. As indicated in questions 2, 3, and 4, it should be noted that inherent constituents in a new food may occur outside the documented range and still be acceptable as long as the pattern of exposure indicates that present levels of intake from all sources would not be altered (question 5).

If a new constituent presents a safety concern or is not found in the present food supply (question 8), or is outside the concentration inherent in the food supply (question 9), question 10 provides an alternative to detailed safety evaluation. The problem may be averted by removal, reduction, or deactivation. If this is not possible, a more detailed safety evaluation may be required.

In evaluating genetically modified products via the decision tree it will be noted that their safety evaluation is geared principally to an evaluation of their inherent constituents as a means of ensuring the safety of the whole food as consumed. Accordingly, the decision tree does not include a formal requirement for safety/biological testing of the final product. Nevertheless, a prudent manufacturer, who has the ultimate responsibility for product safety may, depending on the particular product being dealt with, undertake some degree of testing of the final product in animals and/or humans prior to placing the product on the market. Whenever such testing is considered, the specific approach, type, and methods of testing must be very carefully customized to the particular product keeping in mind the rationale of this overall document. The safety/nutritional testing of whole foods and other complex mixtures is discussed in Section 5.

5. ISSUES RELATED TO THE SAFETY TESTING OF WHOLE FOODS AND COMPLEX MIXTURES SUCH AS MACROINGREDIENTS

5.1. Background Comments

The safety testing of substances to be consumed in relatively large quantities differs from the evaluation of low-level-use substances such as flavoring agents, colors, and most other food additives (World Health Organization, 1987). For example,

1. The maximum concentration which can be fed to animals may closely approximate the intended level of human use.
2. Some substances with nutritional significance may replace traditional foods with a potential for nutritional imbalances.
3. Processing impurities and minor constituents assume greater significance because of relatively higher intake.
4. Many are complex mixtures, as most foods are.
5. Some are metabolized into normal body constituents.

Past experience has demonstrated that toxicity testing of whole foods and macroingredients in animal studies may present a number of problems not encountered in traditional toxicity studies with food additives. When large amounts of dietary components, including both nutritive and nonnutritive substances, are incorporated into the diet of animals at levels of several percent, it is common to find spurious responses in animal feeding trials. These responses may at first glance be considered of toxicological significance but on further inspection are usually the result of dietary nutrient imbalance or physiological perturbation induced by the test material when fed at excessive exposure levels. An example of this phenomenon is the induction of enlarged colon in animals fed high levels of osmotically active substances such as xylitol, sorbitol, polydextrose, and certain modified starches (Roe, 1989). It is important to separate these physiological responses and their toxicologic sequelae from genuine toxicological effects which may result from contaminants such as heavy metals and adventitious toxic factors that may occur naturally in certain foodstuffs and complex mixtures. While posing no threat to health at usual human exposure levels, exagger-

ated exposure in animals may reveal the expected adverse effects from these contaminants. As a result of these problems, it will usually not be possible to use studies in animals to establish an acceptable daily intake (ADI) for whole foods and macroingredients in the traditional sense employed for xenobiotics. This is because it will usually not be possible to include sufficient test material in the diet of animals to achieve the usual 100-fold safety factor/ADI approach used by regulatory agencies for food additives and similar materials. This is particularly true for food materials that may be used at several percent in human diets.

To an extent these problems limit the usefulness of traditional animal studies in assessing the safety of food materials. On the other hand, animal studies may serve a valuable purpose as screening tests to ensure the food material contains no unexpected acute toxicity at usual exposure levels and as an evaluation of the nutritional adequacy of the product. It must be recognized that if animal studies are employed in the safety evaluation of whole foods and macroingredients, the traditional 100-fold safety factor approach to establishing acceptable human exposures will have limited validity. Often a safety factor of only 2- to 10-fold may exist between the feeding levels in animals (the no-observed adverse effect level) and the anticipated human exposure level. Perhaps the concept that should be used in extrapolating the results of such studies to humans is to recognize that, excluding adventitious contaminants, foods and macroingredients are *per se* nontoxic and that large safety factors are not necessary. The support for this concept comes from the recognition that any common foodstuff when fed at 10 to 100 times the usual exposure level might be expected to induce adverse physiological and possibly toxic effects (Hall, 1977). This matter has been discussed by the World Health Organization (1987) with the resulting statement:

> When establishing an ADI, the traditional concept of a 100-fold safety factor cannot operate when the human consumption level is high and feeding studies do not produce adverse effects (except for effects arising from the physical properties of the additive, such as its bulk and hydrophilicity), even when the substance is added to the diet in the maximum possible proportion, consistent with reasonable nutrition. In such cases, new approaches are indicated, including setting the ADI on the basis of a smaller safety factor, which may be permissible when factors such as similarity to traditional foods, metabolism into normal body constituents, lack of overt toxicity, etc., are considered.

Because of the practical limitations of animal studies, many authors (MAFF, 1984) have suggested that increased use be made of studies in humans as a means of assessing the acceptability of new foods and ingredients. This concept has merit provided the limitations surrounding the design and interpretation of human trials are recognized by the regulatory agencies and taken into consideration in regulatory decisions.

As pointed out previously, the safety evaluation of whole foods derived through genetic modification techniques must begin with the development of documentation concerning the manufacturing process, including the genetic origins of organisms used in production. If the foodstuff is intended to replace a traditional food component, as might be expected with many food products of genetic modification, it is important to assess the potential nutritional implications of this substitution. The nutritional effects of new products must take into consideration the contribution the product itself makes to nutritional status as well as any micronutrients (i.e., vitamins

and minerals) it contains. In addition, the impact that the foodstuff has on nutrient utilization of other dietary components requires careful analysis. Because of the well known role of antinutritional factors in certain foods and food ingredients (Walker and Quatrucci, 1988; Scheuplein, 1990), this area of concern deserves to be evaluated prior to the conduct of any toxicological investigations with the foodstuff itself. Such evaluations should take into consideration the proposed use pattern and anticipated human exposure to the test substance, so as to obtain an accurate measure of any potential adverse nutritional consequences under conditions comparable to actual use conditions.

5.2. Nutritional and Safety Testing of New Foods

When testing high- consumption ingredients, palatability must be determined to arrange for consumption at the highest levels possible, consistent with nutritional status considerations. If a palatability problem is encountered, it may be necessary to increase gradually the amount of the test substance to the required level; this is usually advisable in any case. There are practical limits to the amounts of certain foods that can be added to animal diets without adversely affecting the animal's nutrition and health.

To ensure that the nutritional status of the test animal is not distorted or compromised, the test and control diets should have the same nutritive value in terms of both macronutrients (e.g., protein, fat, carbohydrate, and total calories) and micronutrients (e.g., vitamins and minerals). When feeding substances at high levels, it is essential to formulate diets from individual ingredients rather than adding the test material to a standard laboratory diet. This will ensure that the same nutrient levels are in both control and test diets. Comprehensive nutrient analyses of the test and control diets must be performed to ensure that they are comparable nutritionally. Nutritional studies may be advisable before toxicological studies are planned to ensure that test diets are correctly balanced. Without nutritional balance, excessive exposure may result in imbalances and adverse sequelae, without reflecting the true effects of levels more likely under conditions of use. Secondary toxic effects are not uncommon under these conditions.

It is particularly important that the variables for assessing the safety of the substance, such as body weight, food and water consumption, hematological parameters, ophthalmology, blood chemistry, urine analysis, fecal analysis, and mineral and vitamin excretion levels, are chosen carefully to include monitoring of all possible effects which may accompany high levels of consumption.

While metabolic studies are useful in assessing the safety of high-consumption additives, with complex mixtures such as food, determination of the metabolic fate of every constituent could not be a practical reality. If, however, contaminants or minor components are suspected or documented as the cause of toxicity, their metabolism should be investigated. Consideration also should be given to the secondary effects of new constituents (many have interactions with other agents). For example, nutrients and nonnutrients can have profound effects on the metabolism of xenobiotics and on dietary contaminants.

When biochemical and metabolic studies show that the test food is completely broken down in the gastrointestinal tract to substances that are common dietary or

body constituents, then further toxicity studies may not be necessary. This is particularly the case if the breakdown into these common constituents occurs under the conditions of normal consumption of the material, if the material contributes only a small proportion of these common constituents in the daily diet, and if side reactions giving rise to toxic products do not occur.

Urine and fecal analyses often provide important information relating to changes in normal excretory functions caused by the test substance. The gut flora for example can be markedly altered with potential loss of minerals or vitamins which, in turn, can have a detrimental effect on the health of the animal used in the study. If the substance is not degraded or is only partially degraded by the digestive enzymes of the stomach or the small intestine, appreciable concentrations may appear in the distal gut compartments and change the absorption of other dietary constituents. This may also result in changes in the composition and metabolic activity of the intestinal flora. Because of species-dependent anatomical differences in the digestive tract and because of considerable differences in the composition of the basal diet, such effects may occur only in humans but not in rodents, or vice versa. Short-term biochemical studies should therefore be performed in animals and in humans in which variables likely to be affected by the test substance are examined in detail. It is especially important to determine if eventual effects are progressive or transient, and whether they occur in subjects exposed to daily intake of the substance. A thorough knowledge of the nutritional and biochemical literature can serve as a guideline.

5.3. Special Issues Related to Macroingredients and Fermentation Products

Complex mixtures such as single-cell protein and major food ingredients derived through fermentation technology present unique challenges for safety evaluation. The principal difference between these products and genetically altered whole foods is that they do not have a traditional counterpart which can be used for comparative purposes in the safety evaluation. Thus the criteria outlined above that are used for evaluation of whole foods are not wholly applicable. Nonetheless, the general principles apply, since the source materials, method of manufacture, composition, and exposure still constitute the principal evaluation criteria but the evaluation must include other factors as well. On the basis of well-studied examples from the past, it is now clear that the evaluation of these products requires that careful attention be given to ensuring the purity of starting materials and that the production process follows appropriate good manufacturing practice. The organism used to produce the product must be well characterized in terms of genetic stability. Care also must be taken in the conditions of growth to control and, if possible, prevent/eliminate the production of undesirable expression products such as toxins or antimetabolites. A specification on the product should be drafted. Careful attention to these details will greatly assist in ensuring uniformity of composition of the product. Due to the fact that these products do not have traditional counterparts their safety evaluation will often incorporate the need for toxicity testing. Past experience has indicated that the testing of such products in classical animal tests presents numerous pitfalls that warrant close scrutiny in the design and interpretation of these tests. The problems encountered in the evaluation of these products, which have been the subject of several reviews and

guidelines respecting their safety evaluation, have been recently published (MAFF, 1984; World Health Organization, 1987). In addition, a consideration of factors concerning the safety and nutritional evaluation of these products is presented later.

Potentially hazardous contaminants, such as mycotoxins and heavy metals, and other substances of concern must be kept to a minimum with toxicological evaluations closely related to well-defined materials. Products from different processing methods must be considered separately. The introduction of a new substance and its effect on the nutrient composition of the diet as a whole should be identified, particularly with respect to such groups as children, the elderly, and "captive populations," e.g., hospital patients and schoolchildren. To prevent adverse affects on the nutritional quality of the diet, fortification with vitamins, minerals, or other nutrients may be necessary.

The nutritional value of a macroingredient should be assessed initially from its chemical composition of both macronutrients and micronutrients. The possible influence of other components in the macroingredients, such as antinutritional factors (e.g., inhibitors of enzyme activity or mineral metabolism) on the keeping quality and nutritional value of the remainder of the diet must also be established.

Depending on the nature and intended uses of the macroingredients, studies in animals may be needed to supplement the chemical studies. If the macroingredient is intended to be an alternative to a significant portion of dietary protein, tests on quality of the protein will be necessary. *In vivo* studies will also be needed when it is appropriate to determine (1) the availability of vitamins and minerals in the macroingredient in comparison with the food it would replace; and (2) any interaction the macroingredient might have with other items of the diet that would reduce the diet's overall nutritional value. If the macroingredient is expected to play an important role in the diet, it may be necessary to verify that the results of animal studies can be extrapolated to human beings by measuring the availability of nutrients to human subjects. In the case of proteins, assurance must be provided that allergenicity will not be a significant problem.

After the appropriate animal tests have been done and a tentative acceptable exposure level set, human volunteer studies to test for human tolerance should be designed. Following simple organoleptic evaluation, the first human study should involve the feeding of a single meal containing the macroingredient at a known dose level to one volunteer at a time. If no harmful effects are observed with several volunteers, studies involving the feeding of the novel food for a short period (initially about 4 weeks with follow-up studies of longer duration) should be performed.

Different diets incorporating different levels of the macroingredient should be related to the anticipated levels of human exposure. The closest attention should be paid to matching groups with respect to age, height, weight, sex, alcohol intake, and smoking habits. In addition to having normal control groups, it may be useful to organize studies in which the test groups are fed diets incorporating and not incorporating the macroingredient in sequential periods, so that each volunteer acts as her or his own control; blind crossover trials are the most satisfactory.

Once it has been determined that the macroingredient is tolerated well by volunteers at fixed dietary levels, it may be useful to feed it *ad libitum*, for a short period, to assess its acceptability. As noted above, allergenicity studies on the macroingredient may be considered because of its composition (e.g., if it is highly protein-

aceous) or because the results of animal or human feeding studies suggest that the food might produce hypersensitivity in some people.

Important information regarding allergenicity can be gained by monitoring the health of production workers coming into contact with the macroingredients as well as laboratory staff, research personnel, and other employees in the manufacturing plant. To detect possible allergenicity of the macroingredients in the general population, it will generally be essential to monitor a large number of people using traditional immunological methods, such as the human repeat insult patch test (HRIPT) and other accepted techniques.

Where they are required or deemed necessary, large-scale acceptability and marketing trials should be undertaken only after the macroingredient's safety has been demonstrated by the studies indicated above. It may be most useful to restrict the trial to a defined geographical area. The local medical services responsible for the area in which the substance is tested should be alerted so that they may take it into account when evaluating any unusual disease patterns that may appear during or after the test period. Because large numbers of people will be involved in the trials, it may be possible to obtain information about rare food intolerance (e.g., allergic reactions) that may not have been observed in earlier human studies. The extent to which health monitoring should be performed will depend on the nature of the substance and the results of previous toxicological investigations.

6. APPENDIX: CRITERIA TO DETERMINE WHAT CONSTITUTES A SIGNIFICANT INCREASE IN EXPOSURE

6.1. Introduction

The major data bases and accepted procedures available for use today in the United States for estimating food consumption are discussed here. While the system has worked reasonably well for food additives and GRAS materials, additional factors need to be considered in the evaluation of the replacement of one whole food, a complex mixture, with another.

Presently, our foods are considered to be acceptably safe and wholesome despite the fact that most every one of them contains components known to be toxic to humans or animals in certain circumstances. However, only when a food itself has been found to be unsafe, has the attention of regulatory agencies such as FDA focused on the problem and dealt with it as an adulterated and unsafe food product.

For the numerous reasons discussed further in this chapter, chemically complex new food products cannot be tested and evaluated for wholesomeness in the same way as an individual nutrient or potential toxicant. Throughout the exercise of chemically and biologically evaluating complex foods, the key component is comparison with traditional counterpart products. Knowledge of the use and exposure patterns of specific traditional counterpart food types is therefore extremely important. Knowledge about patterns of use of traditional foods (e.g., crop types, varieties, effects of growing locations and conditions) much more specific than has been needed before or is available now may well be required.

Detailed knowledge of compositional variability across the traditional counterpart crop types, under various growing conditions, will play a critical role in determining whether or not the new product presents any safety or nutritional concern. Such compositional knowledge within food types and across various foods must be the basis for making judgments as to the significance of potential exposures from new products.

6.2. Factors to Consider in Assessing Exposure

A number of factors must be taken into account in developing criteria for determining what constitutes a significant change in exposure to foods and their components:

- The total amount of a particular food component (i.e., nutrient, toxicant) consumed in a fixed period (Note! The 90th percentile consumer generally uses about twice the amount of the mean consumption.)
- The pattern of use of the food within a fixed period
- The biological (e.g., nutritional, physiological, toxicological) potency of the individual components
- The biological availabilities of the components of interest in the particular food as consumed
- The presence in the food itself of other components which modify the potency of compounds of biological value and interest (e.g., antioxidants, anticarcinogens, antivitamins, antimutagens, goiterogens, chelating agents)
- The above relationships between the components in a single food item and other foods in the total diet

Decision making as to the wholesomeness of a particular food, in light of these many variables, is further complicated by numerous difficulties in availability, types, and use of the necessary data bases:

- No preferred data base or estimation approach is universally valid for all situations.
- The biological testing (i.e., nutritional, physiological, toxicological) data bases are basically different from the human dietary food intake data bases (e.g., continuous versus short-term exposures, fixed dietary composition versus free choice variability of diet).
- Intake estimate data bases (e.g., designed to evaluate nutritional, commodity use or marketing trends) do not explicitly correlate with specific use patterns of the food.
- All available assumptions and approaches must be evaluated. Where feasible, checks of validity external to this estimation process should be undertaken so that the degrees of overestimation and underestimation can be estimated and understood.

For each food group containing a component of interest, a food intake value is multiplied by the component concentration value to obtain the intake for that food group and then the amounts from each of the food groups are summed over all food groups to obtain a total dietary additive intake.

6.3. Available Data Bases

A primary impetus for collection of food consumption data in the United States originated in 1958 with the enactment of the Food Additives Amendment to the Food, Drug, and Cosmetic Act. Under this amendment, FDA was required to consider "probable" consumption of a food additive.

The legal requirement for FDA to handle food intake data bases under due process has resulted in legal challenges and formal adjudications. Accordingly, FDA has developed a set of principles for estimating food additive intakes (Modderman, 1986).

In response to a presidential consumer message in 1969 for reevaluation of the safety of substances generally recognized as safe (GRAS), the FDA requested the National Academy of Sciences to develop and test a format and survey procedure that could be used to elicit information from industry on the extent of consumer exposure to the GRAS substances. The National Academy of Sciences (1972) report describes in detail the various data bases, methods of analysis, and strengths and weaknesses of various assumptions, in addition to presenting volumes of data resulting from the survey. Knowledge of the approaches used in this GRAS survey (National Academy of Sciences, 1976) should be extremely helpful to anyone contemplating an evaluation of food or food component consumption patterns—a necessary exercise for introduction of any novel food.

The food consumption data used in the GRAS survey included Market Research Corporation of America Third National Household Menu Census, conducted in 1967–1968, available commercially, which determined the eating habits of 4000 families (12,857 individuals) with each family participating for 14 days; and the *Nationwide Food Consumption Survey, 1977–78* (U.S. Department of Agriculture, 1984), which determined, by the recall method, the daily food intake of a representative sample of 14,500 men, women, and children in the United States. The U.S. Department of Agriculture's most recent survey data were collected in 1985.

FDA sponsored a Federation of American Societies for Experimental Biology (FASEB) expert panel to evaluate the issues and approaches involved in estimating human exposure (FASEB, 1988). The report of this panel provides an excellent update on available data sources. FDA now also maintains an ongoing market basket survey termed the "Total Diet Study" (Pennington and Gunderson, 1987) which measures 11 nutrients, pesticide residues, and industrial and environmental contaminants.

6.4. Assessing Significant Changes in Exposure

These principles for guiding FDA's premarket safety evaluation of chemicals intentionally added to food (e.g., a new biotechnologically introduced food component) tailor the intake estimates to toxicological concerns. These principles are listed here:

1. There is no preferred data base or estimation approach that is universally valid for all food additive use situations.
2. For specific application in food additive safety assessment, the type of estimate of food additive intake must correlate with the toxicological assessment.
3. Estimation of additive intakes are derived from food intake data bases that do not explicitly correlate with the specific food uses of the additive.

4. Estimates of food additive intake are made by all available approaches using different data bases.

The most broadly accepted convention for expressing the amount of a food chemical component (e.g., food additive) that can safely be consumed by humans has been established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA) as the acceptable daily intake (ADI) expressed in mg/kg/day (World Health Organization, 1987). This acceptable intake can, of course, be reached by many combinations of concentrations of use, varieties of foods, and consumption patterns of each.

The initial determination as to significance of a change in exposure to a food component should be an estimate of the proportion of the ADI expected to be utilized by the total uses following introduction of the new product.

If the total use after introduction of the new product is not expected to exceed the ADI, the change in use will not be significant and can be allowed.

On the other hand, should the ADI appear to be exceeded by introduction of the new product, caution would be needed in proceeding. Then, either more definitive titration of dosages in new studies on the specific toxicity test on which the ADI has been set, or a closer approximation of consumer patterns of the new and existing products may yield an opportunity for revision of the ADI and/or actual consumption estimates followed by introduction of the new product.

The other major benchmark to which a new food product or ingredient should be related is the consumption of the item of interest as part of the traditional counterpart food and foods in general. Unless a substantive safety issue has been established for the compound itself and its presence in the food supply, warranting its reduction or no increase, then an increase in appearance of a new component over that already existing in the traditional counterpart food (e.g., 50–200%) or in the diet in general (e.g., 15–20%) should be considered nominal and of no concern.

Essentially, therefore, the use level determinations for single ingredients and simple known mixtures would be handled in the same way food additives and GRAS substances have been handled.

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Chapter 7: General Legal and Regulatory Issues

1. INTRODUCTION

This report addresses the safety evaluation of foods and food ingredients produced through genetic modification and other techniques of biotechnology. Regardless of legal requirements, it will be standard practice for the developers of biotechnology-derived foods to carefully evaluate and document their safety, on a case-by-case basis, to ensure public safety and market acceptance. It also will be necessary, however, for the food products of biotechnology to satisfy all applicable legal and regulatory requirements. This chapter addresses how compliance with U.S. food safety laws can be achieved.

In the United States, the safety of most foods and food ingredients is regulated by the Food and Drug Administration (FDA) under various provisions of the Federal Food, Drug, and Cosmetic Act (FDC Act).[1] The existing food safety laws provide FDA with a comprehensive, flexible set of tools for regulating the safety of every component of the food supply. Current law has worked well over the years to ensure the safety of the North American food supply, and it is the policy of the U.S. government to use existing laws to regulate the food products of biotechnology.[2]

The overriding objective of current food safety laws is, of course, to ensure that consumers are not harmed by the foods they eat. To achieve this objective, the law provides an array of safety standards and enforcement tools FDA can use to act against foods that are potentially harmful to health. In some cases, FDA has authority to review the safety of a food substance prior to marketing. A key premise of the law, however, is that safety standards and regulatory procedures should be tailored to the nature of the food substance in question and the potential safety questions it may pose. This reflects the policy judgment of the U.S. Congress that foods should be regulated as thoroughly as necessary to ensure safety but not in a manner that unnecessarily interferes with production of an abundant, wholesome, and economical food supply.

This congressional policy judgment is reflected in the structure of the law itself and in FDA's implementation of the law over the years. For example, the law recognizes that the natural food supply contains many substances that, when isolated and consumed in large amounts, are toxic, but that are not harmful when consumed as inherent constituents of food. FDA is empowered to act against such substances, but only if it finds that they render the food "ordinarily injurious" to health.[3]

The law provides that substances added to food through human intervention receive a greater degree of scrutiny, but it is in this area that the law gives FDA substantial discretion in choosing the safety standard and regulatory procedure to apply.

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For example, substances that are "added" to food not for the intentional purpose of accomplishing a function in the food, but as the unintended or unavoidable consequence of some human activity, are regulated under a provision that permits FDA to act if it finds that the "added" substance "may render [the food] injurious to health."[4] FDA has interpreted the scope of this provision expansively over the years to achieve the goal of ensuring safety without imposing requirements more stringent than necessary for that purpose.[5]

Substances added intentionally to accomplish a function in the food are subject to yet another safety standard and may be required to undergo premarket review and approval by FDA. Even here, however, Congress stated its intent to foster innovation in food technology, as well as ensure safety.[6] It sought to accomplish both goals by adopting a protective but realistic safety standard and by not requiring premarket approval when it is not required to ensure safety, e.g., when the food substance is "generally recognized as safe" (GRAS).[7] As FDA has interpreted and applied the law over the years, formal premarket approval has generally been reserved for new chemicals and new uses of chemicals that are not GRAS. FDA has also developed special procedures and practices for the regulation of GRAS substances. All of this will be discussed more fully later in this chapter.

The food products of biotechnology, including products of nontraditional genetic modification, should fit in well with the existing pattern of food safety regulation. If a food substance derived without genetic modification would require premarket approval by FDA, a genetically modified version would also. If a nongenetically modified food or food ingredient would not require formal FDA approval, its genetically modified counterpart probably would not.

Inevitably, important new technologies may pose new regulatory challenges. Biotechnology is no different. It may be necessary to devise new procedures to handle new situations. This chapter suggests one such procedure for genetically modified food plants that have been the subject of nontraditional genetic modification. In every case, however, regulation of the food products of biotechnology can be grounded soundly in existing law and practice. The next section of this chapter describes in more detail how current law operates with respect to the major categories of food substances. The concluding section explains how current practices would carry over to products of biotechnology.

This will not be a legal treatise on food safety law and will not address every detailed aspect of how current law would operate in the biotechnology area. Its purpose is instead to convey the basic concepts and procedures FDA applies—and should continue to apply—to ensure the safety of food. More detailed discussion of some legal and regulatory issues, as well as a brief discussion of the law in other countries, is provided in the Appendix.

2. FDA'S CURRENT APPROACH TO IMPLEMENTING THE FOOD SAFETY LAWS

The food supply can be divided roughly into four major categories of food substances: (1) agricultural commodities or "whole foods"; (2) processed derivatives of agricultural commodities, most of which are "simple chemically defined mixtures,"

as that term is used in previous chapters; (3) "biological" ingredients and processing aids; and (4) chemical additives, most of which are "single chemical entities." Biotechnology has the potential to produce products in each of these categories, making it appropriate to organize our description of current practices in food safety regulation around them.

2.1. Agricultural Commodities

This category consists of edible products from plants (i.e., such "whole foods" as fruits, vegetables, grains, and other produce). Whole foods are subject to regulation by FDA under section 402(a)(1) of the FDC Act.[8] Section 402(a)(1) establishes two different safety standards—one for substances that are inherent natural constituents of the food and one for substances that are "added." Naturally occurring constituents violate section 402(a)(1) and render the food legally "adulterated" only if they make the food "ordinarily injurious to health." "Added" substances are subject to a more rigorous safety standard. They render the food legally adulterated if they "may render it injurious to health." This safety standard for added substances is violated if there is a "reasonable possibility" that any consumer will be injured by consuming the food.[9]

Whole foods are not required to undergo any premarket review or approval by FDA. Under the law, however, any person who introduces food into commerce is responsible for ensuring that it complies with all requirements of the FDC Act, including the requirement that it meet the applicable safety standards. FDA has enforcement powers under the statute that permit it to seize adulterated food, seek a court order preventing its further distribution, and criminally prosecute firms and individuals responsible for its distribution.[10]

Section 402(a)(1) is used most frequently by FDA to remove from commerce food that is unintentionally contaminated by manmade chemicals, such as polychlorobiphenyls (PCBs), mercury, and lead, or by natural contaminants, such as aflatoxin. FDA has only rarely needed to use this section against inherent natural constituents, but it is available in the event a naturally poisonous food, such as a poisonous mushroom, is encountered in the marketplace. Section 402(a)(1) is also available to regulate the safety of new strains and varieties of plants used or to produce whole foods, such as fruits and vegetables. As explained in Chapter 3, thousands of new strains and varieties have been developed by plant breeders and others who have found ways to transfer the useful properties of one plant to another by conventional plant breeding techniques. If such plant breeding were to introduce to the edible portion of the plant a new toxic substance or elevate to harmful levels an existing natural toxin, FDA could act to prevent sale of the food by showing a "reasonable possibility" that the food would be harmful. There is no record of FDA ever having had to use this authority in such a situation. In one instance described in Chapters 2 and 3, involving elevated levels of solanine in a new potato variety, the mere existence of the authority backed up FDA's suggestion to the U.S. Department of Agriculture that the new variety not be commercialized.

2.2. Processed Derivatives of Agricultural Commodities

For centuries, agricultural commodities have been processed to produce such staple food materials as vegetable oils, sugars, starches, milled grains, protein sources

(e.g., whey, casein, and soy), and natural spices and flavors. These are not typically "whole foods" capable of being consumed alone. They are more commonly chemical mixtures and are used very broadly as ingredients of food.

If a particular lot of one of these materials were found to contain an unintentional contaminant that posed a safety concern, FDA would ordinarily use section 402(a)(1) to remove that lot from commerce. However, the material itself, used intentionally as a component of food, is regulated by FDA under an entirely different section of the statute.

In 1958, Congress enacted the Food Additives Amendment to the FDC Act in response to the increasing use of chemical additives in food and the widely recognized need to ensure adequate premarket safety testing of new food ingredients.[11] This law adopted a new safety standard for "food additives" and required that food additives be proven safe to FDA's satisfaction prior to marketing. This meant proving to a "reasonable certainty" that "no harm" would result under the additive's intended conditions of use.[12]

In addition to ensuring the safety of food additives, the new law also sought to foster progress in food technology and to avoid testing and premarket review by FDA when it was not necessary to ensure safety.[13] Congress recognized that the safety of many food ingredients, including both chemical additives and processed derivatives of traditional whole foods, had already been established based on their long history of safe use in food. Congress concluded that it would be wasteful and disruptive to force these ingredients through a program of testing and FDA approval and thus excluded from the definition of "food additive" (and from the requirement of premarket approval) substances that are "generally recognized as safe" (GRAS).[14]

Congress provided that GRAS status could be achieved in two different ways. Both require general recognition among qualified experts that the substance is "safe," i.e., that there is a reasonable certainty no harm will result under intended conditions of use. For substances introduced after enactment of the Food Additives Amendment, the general recognition of safety must be based on "scientific procedures," which means reliance on the same quantity and quality of scientific evidence that would be required to prove safety if the material were being evaluated by FDA as a "food additive."[15]

For substances used prior to 1958, however, general recognition of safety can be based on scientific procedures or "experience based on common use in food."[16] Most processed derivatives of agricultural commodities are GRAS on this latter basis. They had been used in food for many years prior to 1958 without adverse effects. They, thus, were exempt from the premarket approval requirements of the new law.

As a legal matter, the GRAS exemption is self-executing: food manufacturers considering the use of an ingredient are legally free to make their own determination that the substance is GRAS and, on that basis, use it without seeking FDA approval. They run the risk, however, that FDA will reach a different conclusion and challenge use of the substance on the ground that it is an unapproved food additive.[17] Foods containing unapproved food additives are deemed "adulterated" and thus unlawful.[18]

FDA has made substantial efforts to define the universe of GRAS substances. FDA recognizes that it would be difficult, if not impossible, to identify every GRAS substance,[19] but in the period immediately following enactment of the Food Additives Amendment FDA published several lists of substances it considered GRAS. Most

of these remain codified as GRAS substances in FDA's regulations.[20] FDA has also published criteria for GRAS status and procedures for filing petitions to obtain FDA affirmation of GRAS status.[21] FDA has affirmed the GRAS status of many materials on its own initiative, including many of the staple ingredients that fall in the category of processed derivatives of agricultural commodities.[22] Processed derivatives of agricultural commodities developed after 1958 have typically been regulated as GRAS substances. These include materials, such as canola oil and high-fructose corn syrup, that had no significant food use prior to 1958, as well as more traditional materials, such as whey, for which new forms and processing methods have been developed since 1958. Many but not all of these materials have been the subject of GRAS affirmation petitions and have had their GRAS status affirmed by FDA.[23]

2.3. Biological Ingredients and Processing Aids

Enzymes, yeast, and other microorganisms have a long history of use in food production. Traditionally, they have been used in the production of such foods as cheese, bread, beer, and yogurt and in numerous other fermentation processes. They also play an important role in many of the newer food production processes. Enzymes, for example, play a critical role in the production of high-fructose corn syrup and various hydrolyzed or predigested protein products (such as those used in certain infant formulas).

Most materials in this category are used only in the production process and leave little if any residue in the food. This is the case with most of the enzyme systems used to hydrolyze proteins and to produce such ingredients as high-fructose corn syrup. Other materials are added directly to the food and remain as a component of the finished product. Microbial cultures used in yogurt production and certain yeast products fall into this category.

Based on their long history of safe use, many biological ingredients and processing aids have achieved GRAS status. Some were included on FDA's original GRAS lists, but others were not. For example, many of the most commonly used food production enzymes, such as trypsin and β-amylase, were not listed, but FDA has long acquiesced in their use and is moving toward formal affirmation of their GRAS status.

A number of microorganisms and enzyme systems, and new uses of old ones, have come into food use since 1958. In many cases, these have entered the market as GRAS substances and have been the subject of GRAS affirmation petitions, which have resulted in the issuance by FDA of GRAS affirmation regulations.[24] In other cases, the sponsor has chosen to file a food additive petition, and FDA has approved their use in food additive regulations.[25] In either case, the same quantity and quality of scientific evidence are required to obtain FDA approval.

2.4. Chemical Additives

Hundreds of chemicals are used in food to accomplish many purposes. These include antioxidants and other preservatives, emulsifiers, thickening agents, flavors, artificial sweeteners, and essential nutrients. These are the components of the food supply Congress intended to regulate under the Food Additives Amendment of 1958.

Many of these substances had been used in food for years and were ones that Congress recognized should avoid additional testing and FDA review. A good many were included on FDA's original GRAS lists and have been regulated as GRAS substances ever since.[26] This includes many natural and synthetic flavor substances, essential nutrients (from ascorbic acid to zinc sulfate), and other additives such as phosphoric acid, glyceryl monostearate, and the antioxidants BHA and BHT. In 1969, FDA embarked on an extensive review of the safety of many of the ingredients on these lists and found that it was able to affirm the GRAS status of virtually all of them based on contemporary safety standards and information.[27]

For many substances on the market in 1958, FDA was not able to make the GRAS finding. These were thus classified as "food additives" and were subject to the safety testing and FDA approval requirement of the new law. Some were subsequently approved by FDA; others were removed from the market.

Chemical additives in this category—mostly single chemical entities—that have entered the market after 1958 without any prior use in food have typically been regulated as food additives and have been approved by FDA prior to use. FDA approval requires the filing of a food additive petition containing extensive information on the physical and chemical properties of the additive, its intended use, and its safety. If, on evaluation, FDA agrees that the proposed use is safe, the agency will issue a food additive regulation describing the additive and the conditions under which it may be used.[28]

The majority of new chemicals approved through this process have been indirect food additives: substances that do not perform a function in the finished food but rather enter food incidentally by virtue of their use in contact with food, typically in food packaging.[29] Some important direct additives have also passed through the process, however, including various preservatives, alternative protein sources, and the sweetener aspartame.[30]

Under the Food Additives Amendment, FDA regulates the additive, not the process by which it is produced. Information about the manufacturing process may be relevant to evaluating the safety of the additive, and FDA requires submission of such information. On occasion, FDA will describe one or more features of the manufacturing process in the regulation approving the additive, if this is necessary to ensure safety. If the manufacturing process is not prescribed, however, manufacturers are free to use any process so long as the resulting additive meets all of the identity and purity requirements of the applicable regulation and does not introduce new substances that themselves would require food additive approval.

Pesticide residues in food are an important category of chemical additives. They are regulated not by FDA but by the Environmental Protection Agency (EPA). Only certain pesticides in processed foods are regulated as "food additives," but virtually all pesticide residues, including those present in or on raw agricultural commodities, must be the subject of an EPA-promulgated tolerance or be exempted from the requirement of a tolerance.

3. PROPOSAL FOR FOOD PRODUCTS OF BIOTECHNOLOGY

U.S. food law is complicated because the food supply is complicated. Congress and FDA have devised a system of regulation that tailors legal and regulatory require-

ments to the nature of the particular food substance and the safety questions it poses. This system has successfully accommodated tremendous progress in food technology while maintaining and enforcing high standards of safety. It can do the same for the food products of nontraditional genetic modification. The following discussion is not intended to answer every question about how such products should be regulated, but it is intended to show that the existing system can work to ensure the safe and lawful marketing of these products.

As stated at the outset, a careful safety review by the manufacturer is assumed for all products to protect public health and ensure market acceptance. This would have to be done without regard to legal requirements. As has been the case with nongenetically modified food substances, however, this safety review will also play a critical role in satisfying legal requirements, including determining which legal/regulatory category appropriately applies.

3.1. Agricultural Commodities

Nontraditional genetic modification will be doing for agricultural commodities what cross-breeding and other traditional techniques have done for centuries. It will modify the genetic composition of food plants to change or enhance one or more agronomic, processing, nutritional, or other plant characteristic. It is theoretically possible to modify a plant so that it will produce a chemical that itself has a discrete function in the finished food. This could include substances and functions that have traditionally been regulated as food additives or GRAS substances (e.g., sweeteners or preservatives) or chemicals that perform pesticide functions and have been regulated as pesticides by EPA (e.g., *Bacillus thuringiensis* toxin).

The more typical application of nontraditional genetic modification to agricultural commodities, however, will not be to add some discrete chemical that remains functional in the finished consumer product. It will instead be for purposes identical to conventional plant breeding and strain development: to alter or enhance some agronomic or processing function. Examples include enhancing disease and drought resistance, increasing solids content, and improving transportability. The genetically controlled expression products that produce these characteristics are typically proteins that may be present in the food as consumed, but they do not function in the finished food in the way food additives and GRAS substances typically do.

The IFBC proposes that the regulation of genetically modified food plants derived by nontraditional genetic modification be patterned directly on existing law and practice. If the purpose of the modification is to introduce as an expression product of the transferred gene a functional chemical entity that, if introduced exogenously, would be regulated as a food additive or GRAS substance, it would be regulated in the genetically modified plant as a food additive or GRAS substance.[31]

If the purpose of the genetic modification is not to introduce a functional chemical entity but to affect some agronomic or processing function, the plant would be regulated in the first instance under section 402(a)(1) of the FDC Act, just as whole foods are regulated today. To ensure compliance with the law, the edible portion of the plant would have been evaluated by its developer to determine whether the genetic modification had introduced substances that might render the food injurious within

the meaning of section 402(a)(1). If such substances were found, the modified plant would be legally adulterated and could not be sold.

It is also possible that the genetic modification could have an effect on the composition of the plant's edible portion great enough to warrant regulation beyond section 402(a)(1). A careful compositional comparison of the edible portion of the modified plant and its traditional counterpart will have been undertaken routinely as part of the scientific safety evaluation of the modified plant. If this comparison reveals the presence in the modified plant of significant levels of substances not previously found in any food or if the modified plant has a nutritional profile or balance of macronutrients (e.g., fiber and amino acids) that is significantly outside the range typical of other related or generally similar and commonly encountered food plants, and these changes raise a question of safety or nutritional adequacy, FDA might choose to regulate the edible portion of such a plant as a food additive or GRAS substance. In this case, FDA's data requirements and safety evaluation would appropriately be tailored to the safety or nutrition question that made such regulation necessary.

New strains and cultivars of agricultural commodities do not typically require premarket approval by FDA. Such commodities derived through nontraditional genetic modification would also not require premarket approval, except as just described. Moreover, there is currently no procedure for systematically making FDA aware of new strains and varieties of food plants. It may be desirable, however, to have such a procedure for plants that have been the subject of nontraditional genetic modification as a means of keeping FDA informed and fostering acceptance of new technology in the marketplace. *The IFBC recommends that FDA consider establishing a voluntary premarket notification system for genetically modified food plants.*

Under such a system, a manufacturer would have the option of submitting to FDA at some specified time prior to marketing, and in accordance with FDA guidelines, a package of information documenting its safety evaluation of the modified plant and why no food additive or GRAS regulation is required. This information would be available to the public in accordance with established FDA policies and procedures under the Freedom of Information Act which protect essential trade secrets but provide for disclosure of most safety information. The submission would not trigger or require formal FDA review and approval of the modified plant, but it would ensure that the agency was informed about the product and provide an opportunity for FDA to express any objection or concerns it might have in advance of marketing. FDA would also have the option of issuing a letter stating that it is aware of the product and, on the basis of information submitted, does not object to its marketing.

3.2. Processed Derivatives of Agricultural Commodities

Products in this category might be affected by nontraditional genetic modification in two ways. First, they might be derived from genetically modified plants, such as a vegetable oil derived from a grain crop genetically modified to be drought resistant. Second, they might be derived by processes involving genetically modified microorganisms, such as enzymatic hydrolysis using an enzyme from a genetically modified microbe. Like their traditionally derived counterparts, these products would be regulated as food additives or GRAS substances.

In the first case, if the composition of the oil or other edible product were the same as that from the traditionally derived plant, the product would have the same regulatory status as its traditional counterpart. This simply reflects the fact that they would be, in fact, the same thing. Thus, if the traditional product were GRAS or an approved food additive, so too would be the new product. *If the product derived by nontraditional genetic modification were compositionally different from its traditionally derived counterpart, it would have to be evaluated to see whether it nevertheless still fell within an existing FDA food additive regulation or GRAS affirmation. If it did not, it would require its own food additive petition or GRAS determination.*

In cases involving new processes that rely on genetically modified microbes, the safety of the processed derivative of an agricultural commodity would be ensured through regulation of the microbe or the enzyme system derived therefrom, as discussed below.

3.3. Biological Ingredients and Processing Aids

The impact of nontraditional genetic modification has already been seen in this product category. Genetically modified microbes have been developed that more efficiently and reliably produce enzymes used in food processing. The potential also exists for using genetically modified microbes to produce edible protein and other food ingredients or to perform various food processing functions. *Again, the IFBC proposes that these products be regulated on the same basis as their conventional counterparts: as food additives or GRAS substances.*

In many cases, microbial systems that have been the subject of nontraditional genetic modification will be used simply as a new process to produce an ingredient already approved by FDA. As with the use of any new process, the decision whether to require a petition and the choice between a food additive and GRAS petition would depend on the facts.

If the *traditionally* produced ingredient were already the subject of a food additive or GRAS regulation and it could be determined that the ingredient produced by a nontraditionally modified microbe complied fully with all identity and purity requirements of that regulation and introduced no new substances not covered by the regulation, the manufacturer would have the option of marketing the microbially produced ingredient under that regulation without the need for a petition. This is consistent with the rules currently governing the introduction of new conventional processes for manufacturing established ingredients. If the microbially produced ingredient were compositionally different from the approved ingredient or otherwise did not comply with the existing regulation, a food additive or GRAS petition would likely be required,[32] with the choice depending on whether the traditionally produced ingredient were subject to a food additive or GRAS regulation.

3.4. Chemical Additives

Most products of the nontraditional genetic modification in this category will likely be produced using genetically modified microbial production systems. As discussed earlier, these would be regulated by FDA as food additives or GRAS substances and

might or might not require a petition, depending on the circumstances. Pesticidal substances produced by a genetically modified plant would be regulated by EPA and be subject to the requirement for a tolerance or a tolerance exemption.

3.5. Independent GRAS Determinations and Flexible FDA Procedures

Food products of biotechnology other than whole foods will typically be regulated as food additives or GRAS substances. The law and current FDA practice recognize that producers of food ingredients have the option of making independent determinations that an ingredient is GRAS and marketing it on that basis without premarket review by FDA.[33] This approach to market entry is rarely followed in the case of new chemical entities that lack any history of food use and are intended for direct addition to food. It is not unusual, however, for companies to make independent GRAS determinations with respect to new or expanded uses of existing ingredients or for ingredients produced by a new process.

Many of the innovations made possible by biotechnology will involve processes that have little or no impact on the composition or safety of the ingredient. Many of these will be appropriate candidates for GRAS status, but it would be an enormous drain on FDA resources if all of these were the subject of GRAS affirmation petitions.

The IFBC suggests that FDA affirm the practice of making independent GRAS determinations with respect to specified types of biotechnology-derived food products and that it also establish an informal procedure for informing FDA of these determinations as discussed in the Appendix, Section 5.4. This could be similar to the voluntary premarket notification procedure IFBC proposes for whole foods. It would provide FDA with information on the many process changes about which it might otherwise remain unaware, and it would provide the agency an opportunity to raise any questions it might have or to request the filing of a formal petition.

Flexible procedures of this kind are desirable to facilitate regulatory scrutiny of biotechnology products without bogging the system down with formal petitions in cases where they are not necessary to ensure safety.

4. CONCLUSION

This chapter has described how regulation of the food products of biotechnology can be grounded in existing law and practice. This includes the responsibility U.S. food law places on those who make and sell food to ensure the safety of every product.

It also includes the tailoring of regulatory standards and procedures to the nature of the safety question posed by particular products and product categories. Thus, most food ingredients and processing aids produced through biotechnology would be regulated as "food additives" or GRAS substances, depending on the circumstances, and under the same standards and FDA review procedures as their traditionally derived counterparts.

Likewise, whole foods modified by nontraditional methods would typically be regulated under the same provision of law as new strains and varieties derived through traditional techniques. These would not require formal premarket review by FDA

unless they were so compositionally different from their traditional counterpart as to raise a safety or nutritional concern.

Finally, IFBC encourages FDA to consider some flexible, voluntary procedures for informing the agency about applications of biotechnology that might not require formal FDA review. This would help keep FDA informed about new technologies and products and contribute to public and market confidence in the food products of biotechnology.

5. APPENDIX

5.1. Introduction

This Appendix expands on the discussion in Chapter 7 by describing in greater detail the legal and regulatory issues governing food safety in the United States and other countries. At the outset, this Appendix cites the policy decision of the FDA to apply the same administrative review to products of biotechnology as is used for all other products. Then, key aspects of the laws and regulations in the United States are reviewed as they apply to food and food ingredients, including animal food. The basic requirements of the National Environmental Policy Act are discussed as they apply to the manufacture of food and food ingredients. The Appendix concludes with a brief review of the laws and regulations of other countries, to indicate the similarities of application of food safety requirements.

5.2. Regulating Products of Biotechnology

With the prospect of increasing numbers of products derived from the application of biotechnology, the regulatory agencies in the United States have considered the alternatives of continuing with the existing regulatory approach or developing a customized approach to regulating these products. The preferred regulatory approach, which has been adopted by the Office of Science and Technology Policy (OSTP) and the participating federal agencies, is the application, to the extent possible, of the existing laws and regulations to the products derived from biotechnology.[34] The FDA has embraced the OSTP Coordinated Framework for Regulation of Biotechnology and has stated that there is no need to amend the applicable laws and regulations to regulate the products within its jurisdiction, such as food and food products. FDA intends that its administrative review for products of biotechnology be the same process that is used for all other products, that is, one based on the intended use of each product on a case-by-case basis.

In addition to the FDC Act,[35] a number of other U.S. statutes apply to food-producing plants and animals which are genetically modified.[36] The U.S. Department of Agriculture (USDA) has provided a summary of its intended approach in carrying out its responsibilities under the various statutes.[37] In connection with the Coordinated Framework for Regulation of Biotechnology released by the OSTP, USDA published its final policy statement for regulation of biotechnology products. The Animal and Plant Health Inspection Service (APHIS) of the USDA has broad authority to coordinate the biotechnology regulatory activities for USDA as a whole.

The U.S. Environmental Protection Agency (EPA) has stated that microorganisms intended for use as pesticides are subject to FIFRA.[38] If a plant is genetically modified to contain a pesticide that clearly fits within the scope of FIFRA, EPA would also be expected to assert jurisdiction under both FIFRA and its responsibility to establish tolerance levels for pesticides or an exemption from a tolerance under sections 408 and 409 of the FDC Act.

5.3. General Principles Regarding Safety Assurance of Food in the United States

5.3.1. Review of U.S. Requirements (Food, Drug and Cosmetic Act)

The U.S. law governing food safety has gradually become more complex, to keep abreast of the developing science and technology. Several distinct categories of food and food ingredients have been established with appropriate methods of evaluation and control. Whole foods are placed into one category, although when a specific food is processed its category may change. Food ingredients fall into several categories depending on the history of use of the food ingredient, its functional use, and available information on safety.

The FDC Act defines the term *food* as articles used for "food or drink for man or other animals, chewing gum, and articles used for components of any other such article." (section 201(f)). The definition has been interpreted to include the requirement that a substance is not a food unless it is "consumed primarily for taste, aroma or nutritive value" to distinguish food from drugs and other products.[39] A substance may be considered as food, however, if it is generally recognized as food, regardless of the intended use of the substance.[40]

The first food law (Food and Drugs Act of 1906) stated that a food was adulterated if it contained "any added poisonous or other added deleterious ingredient which may render such article injurious to health." In 1938, when the law was substantially revised and became known as the Federal Food, Drug, and Cosmetic Act, the statute authorized the control of adulteration whether or not it resulted from added substances. Section 402(a)(1) of the 1938 FDC Act provides that a food is adulterated if it "bears or contains any poisonous or deleterious substance which may render it injurious to health." The section concludes with the statement: "[B]ut in case the substance is not an added substance such food shall not be considered adulterated under this clause if the quantity of such substance in such food does not ordinarily render it injurious to health." The standard of "may render it injurious to health" is considered a more stringent limitation than the standard of "ordinarily render it injurious to health." The law does not define the safety standards any further nor does it define the term *added,* but leaves those tasks for FDA and the courts.[41]

Other sections of the FDC Act contain related provisions. In section 406, tolerances may be established for "added" toxicants when their presence in food cannot be avoided or if their use is "necessary" to produce the food. Section 406 sets out the formal procedure for developing tolerances, with the provisions of section 402(a) applying when there is no tolerance level for a particular unavoidable, harmful, added substance, or when the established tolerance is exceeded.[42]

With respect to both sections 402 and 406, FDA has the burden of proving that a substance is "added" and that it causes the food to be "adulterated." As originally enacted, the FDC Act had no provision for preclearance of "added" ingredients. A food ingredient manufacturer or a food processor was free to market products without any advance testing. Only after a food product containing the food ingredient was on the market could FDA challenge the use of the ingredient. FDA was required to prove that the food was adulterated by reason of "any added poisonous or other added deleterious ingredient which may render such article injurious to health."

In 1958, Congress enacted the Food Additives Amendment to require that added ingredients be subject to an advance review by FDA and to shift the burden of proof on the safety of those ingredients onto the food industry. Section 402 was amended to provide that a food is adulterated "if it is, or it bears or contains, any food additive which is unsafe (i.e., has not had its safety demonstrated)."[43]

A "food additive" is defined as "any substance, the intended use of which results or may reasonably be expected to result, directly or indirectly, in its becoming a component or otherwise affecting the characteristics of any food" (section 201(s)). A food additive may not be used in connection with food unless there has first been published a regulation in conformance with section 409. Section 409 describes in detail the considerations which determine whether a food additive is suitable for regulation as an authorized ingredient for use in food. An exception to the definition of food additive was carved out for any substance which is

> generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures (or, in the case of a substance used in food prior to January 1, 1958, through either scientific procedures or experience based on common use in food) to be safe under the conditions of its intended use.[44]

Substances which fit within this definition are known as "generally recognized as safe" (GRAS) substances. By definition, GRAS substances do not require preclearance by FDA to be used as food ingredients.

A grandfather clause was included in the Food Additives Amendment that allows the continued use of a substance which has a "prior sanction," issued before January 1, 1958, by FDA or USDA stating that the substance is acceptable for food use.[45] The FDC Act also includes a definition of "color additive" and prohibits the use of a color additive unless there has first been published a regulation in conformance with Section 706.[46]

5.3.2. Review of Specific Application of U.S. Requirements

5.3.2.1. Determination of added substances. Whole foods are regulated on the basis of section 402 of the FDC Act, which defines the term *adulteration.* Of paramount importance here is whether the whole food contains any "added" substance, and if so, whether that added substance is poisonous or deleterious.

A substance is considered as added if it is present in food other than as an "inherent natural constituent" and is not intrinsically part of the food. A federal court has explained that substances present by reason of "acts of man" are added but those present by reason of "acts of nature" are not added.[47] The distinction is significant since added materials are held to a higher standard of safety. "[I]f a coffee processor subjects

coffee to a process in which the naturally occurring caffeine is removed and later replaced with an equal amount of identical caffeine, it seems clear that Congress would have the stricter health standard apply."[48]

FDA takes a broad view of what are considered added substances. Not only is a substance considered added if it is present as a consequence of contamination from a source which is manmade or which is otherwise caused by human conduct, but FDA also considers as added any substance which is not inherent in the food, whether or not there has been intervention by humans.[49] Courts have tended to take a slightly narrower viewpoint, but they still embrace within the scope of "added" indirect human-caused pollution. Consequently, pollutants from the air, pesticide residues, soil minerals, and minerals from fertilizers all fall within the scope of "added" substances.

If the substance is an "added" contaminant, FDA exercises its prosecutorial discretion to recommend court proceedings under section 402(a)(1), guided by informal action levels. FDA may also establish tolerances by formal rulemaking under section 406, which provides that "Any poisonous or deleterious substance added to any food, except where such substance is required in the production thereof or cannot be avoided by good manufacturing practice shall be deemed to be unsafe" unless a tolerance has been set.[50] Contaminants are not food additives because they perform no functional purpose in food; FDA has concluded that Congress could not have meant to bring within the category of food additive any substance which could not possibly meet the standard of approval for a food additive.[51]

5.3.2.2. Definition of "safe" for intentionally added ingredients. The evaluation of the safety of intentionally added components has gone through a process of development, beginning with the 1906 Act. The authoritative interpretation of the "may render injurious" standard in section 402(a)(1) is the Supreme Court's opinion interpreting that provision of the 1906 Act in *United States v. Lexington Mill and Elevator Co.*, 232 U.S. 399 (1914). The Supreme Court rejected the argument by industry that food could be condemned only if it was shown by the government actually to injure health, and the Court rejected the argument by the government that food must be condemned if it contained even a harmless amount of a substance that would be poisonous or deleterious at a higher level:

> It is not required that the article of food containing added poisonous or other deleterious ingredients must affect the public health, and it is not incumbent upon the Government in order to make out a case to establish that fact. The act has placed upon the Government the burden of establishing, in order to secure a verdict of condemnation under this statute, that the added poisonous or deleterious substances must be such as may render such article injurious to health. The word "may" is here used in its ordinary and usual signification, there being nothing to show the intention of Congress to affix to it any other meaning . . . In thus describing the offense Congress doubtless took into consideration that flour may be used in many ways, in bread, cake, gravy, broth, etc. It may be consumed, when prepared as a food, by the strong and the weak, the old and the young, the well and the sick; and it is intended that if any flour, because of any added poisonous or other deleterious ingredient, may possibly injure the health of any of these, it shall come within the ban of the statute. If it cannot by any possibility, when the facts are reasonably considered, injure the health of any consumer, such flour, though having a small addition of poisonous or deleterious ingredients, may not be condemned under the act.[52]

When Congress subsequently enacted Section 409 as a component of the Food Additives Amendment, the standard of safety for intentionally added ingredients no

longer was defined by section 402 but was brought under the purview of Section 409. As Congress explained:

> Safety requires a proof of reasonable certainty that no harm will result from the proposed use of an additive. It does not—and cannot—require proof beyond any possible doubt that no harm will result under any conceivable circumstance.[53]

FDA has proceeded to regulate food ingredients based on the general categories described in the FDC Act: food additives, GRAS substances, and color additives. In this connection, the FDA has defined "safe" as follows:

> [T]here is a reasonable certainty in the minds of competent scientists that the substance is not harmful under the intended conditions of use. It is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of the use of any substance. Safety may be determined by scientific procedures or by general recognition of safety. In determining safety, the following factors shall be considered:
>
> (1) The probable consumption of the substance and of any substance formed in or on food because of its use.
>
> (2) The cumulative effect of the substance in the diet, taking into account any chemically or pharmacologically related substance or substances in such diet.
>
> (3) Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.[54]

5.3.2.3. GRAS ingredients. The regulation describing eligibility for GRAS status details FDA's requirements, whether based on experience derived from common use in food prior to January 1, 1958, or based on scientific information.[55] While the determination of GRAS status may be made by the manufacturer or user of the substance (based on the general recognition of safety of the substance) without consulting FDA, FDA will evaluate the GRAS status of a substance based on a petition filed in conformance with 21 C.F.R. § 170.35. Pertinent aspects of a petition for affirmation of GRAS status are chemical definition of the substance, evidence of the historic human consumption of the substance in food, levels of consumption, intended use, and data relating to and attesting to the safety of the substance. If, after review of the petition, FDA concludes that the data support GRAS status of the substance, FDA will publish a regulation in the *Federal Register* affirming the GRAS status.

FDA has provided regulations which give general guidance regarding the criteria for determining whether a substance is GRAS. General recognition of safety through experience based on common use in food prior to January 1, 1958, "may be determined without the quantity or quality of scientific procedures required for approval of a food additive regulation. . . . [I]t shall ordinarily be based upon generally available data and information."[56] FDA has confirmed that for a substance to be affirmed as GRAS on the basis of a history of common use in food "there must be consensus among the community of qualified experts that the use of the substance is safe. For such a consensus to be possible, information about the use of the substance must be generally available. General availability is the result of documentation of the information, usually by publication."[57] In addition to being generally available, "information on the history of use of a substance must be verifiable. . . . [A]n independent source that confirms the history of the use of the ingredient must be available."[58] A general agreement on the interpretation of the evidence can "occur only when similarly qualified experts share an understanding of the concept of safety."[59] In lieu of surveying the scientific community and examining their views as to whether a substance is safe,

FDA considers itself qualified to perform the task. "The experts at FDA are selected from the community of experts who are qualified to evaluate the safety of food ingredients, and, therefore, the opinions of FDA are representative of those held by the larger community."[60]

FDA carefully circumscribes the group of food ingredients that it considers as GRAS by reason of common use in food. Over the years, FDA has defined the category as open only to those substances that were in fact in common use prior to 1958, that have been in continued use virtually unchanged, that have been subject only to conventional processing as practiced prior to 1958, and only for those food uses and functional uses which were in common use prior to 1958.[61] In one case, a court interpreted the requirement by stating that common use in food was not satisfied by use in only one manufacturer's food prior to 1958.[62] The affirmed level of use is commonly limited by FDA to the historic level of consumption that is demonstrated by information which is published or otherwise readily available.[63] A significant increase in use of a particular food ingredient, a change in composition of the food ingredient, or a change in manufacturing method could trigger a loss of the GRAS status by reason of common use in food.[64]

If a GRAS ingredient is manufactured by a new process, the regulatory question is whether this causes the GRAS status to be changed. FDA has noted that for an otherwise GRAS substance "A change in manufacturing process may or may not require a food additive regulation, depending on the information available about it. In any event, consideration must be given to the new process, to determine whether additional specifications or limitations are required to assure that the new version of the ingredient is not different from the version that has been determined to be GRAS."[65]

For several years following the enactment of the Food Additives Amendment, FDA provided only little guidance regarding the standards for determining whether a particular food ingredient was GRAS by reason of scientific procedures. Finally, in 1976, FDA published explanatory regulations, which stated that:

> General recognition of safety based upon scientific procedures shall require the same quantity and quality of scientific evidence as is required to obtain approval of a food additive regulation for the ingredient. General recognition of safety through scientific procedures shall ordinarily be based upon published studies which may be corroborated by unpublished studies and other data and information.[66]

In this connection, FDA has suggested that "there will be at least some gap between the gathering of the scientific knowledge necessary to provide the toxicological underpinning for general recognition of safety and the dissemination to and assimilation by the scientific community of this material that is necessary for general recognition of safety to exist."[67]

Whether a substance is affirmed by the FDA as GRAS by reason of common use in food or by reason of scientific procedures, the category of use is commonly defined in the regulation, thereby limiting the affirmed GRAS status to the regulated uses.[68] FDA often considers processing information significant with regard to a GRAS substance as it may serve to identify the substance and provide information on its safety. The burden is on the manufacturer to demonstrate that the ingredient being manufactured is GRAS and to determine whether a change in manufacturing method has changed that GRAS status.[69]

FDA has also stated that "credible proof of some harm will undercut efforts to prove no harm, even if there is not enough proof to make out a certain case of harm."[70] FDA has stated that the demonstration of a genuine dispute among experts will refute a general finding of safety.

> General recognition of safety requires not only the general availability of appropriate evidence on the substance but also general agreement on the interpretation of the evidence. FDA believes that this general agreement can occur only when similarly qualified experts share an understanding of the concept of safety.[71]

By the terms of the FDC Act, the determination of GRAS status, whether GRAS by common use in food or by scientific procedures, is not solely a prerogative of FDA. GRAS status is achieved by virtue of the history of use of the substance prior to 1958 or by virtue of the scientific data regarding the substance. A determination of the GRAS status of a substance may be made independently by the manufacturer, by a scientific consultant to the manufacturer, by a specially convened group of scientific experts, or by a more formalized evaluation procedure, such as the Expert Panel of the Flavor and Extract Manufacturers' Association.

A private determination should be undertaken with full knowledge of the criteria used by FDA when it affirms the GRAS status of a food ingredient. A failure to require conformance with the standards of FDA places the private determination at risk, not only from the viewpoint of FDA but also from the viewpoint of the public health.

5.3.2.4. Food and color additive petitions. The requirements for a food additive petition are set forth in 21 C.F.R. § 171.1 and the requirements for a color additive petition are set forth in 21 C.F.R. § 71.1. In both cases, the petition should contain information on the chemical identity of the substance, anticipated level of consumption, demonstration of product functionality, analytical methods used to determine specifications, safety tests, and tolerances. Whether evaluating a petition for affirmation of GRAS status based on scientific procedures, a food additive petition, or a color additive petition, FDA relies on the same guidelines to establish the appropriate level of safety testing.[72]

A food additive which is not currently the subject of a food additive regulation and which is not exempt under section 201(s) of the FDC Act should not be marketed until a food additive petition is filed for the use of the food additive and a regulation published. Otherwise, the use of the food additive in food will cause the food to be adulterated within the meaning of section 402(a)(2)(C). In evaluating food additive petitions, FDA considers the method of manufacture of the additive and the analytical controls to ensure that it is a reproducible composition.[73] For food additives, however, the manufacturing process is not generally specified in the regulation because under section 301(j) of the FDC Act confidential production information may not be disclosed. Consequently, the burden is on the manufacturer to prove that the manufacturing method used is consistent with Good Manufacturing Practices, that the product meets the applicable specifications, and that there are no impurities or contaminants in the product which cause it to be unsafe.[74]

5.4. Proposal for Procedural Evaluation Options

Currently, the role of FDA in the evaluation of the safety of a particular food or food ingredient is dependent to a great extent on the potential regulatory status of

that item. In the case of food generally, FDA does not have a premarket review but rather maintains an oversight over all food. Should a producer of a food product consider FDA review desirable, FDA is available to assist in the evaluation. For example, in the case of foods derived from biotechnology, FDA has expressed interest in and willingness to review and evaluate the safety of such products. Whether to contact FDA for a review and comments is a question for careful consideration, since marketing of an adulterated product is a violation of the FDC Act.

With respect to food ingredients, FDA plays a more active role, due to the statutory requirements. When an ingredient is prior sanctioned or GRAS, a private determination may be made, without the requirement of an FDA review, but FDA has historically been willing to consult informally with manufacturers concerning these determinations. To achieve formal FDA affirmation of GRAS status, food additive, or color additive, however, a formal petition containing all relevant data on the ingredient must be filed. FDA undertakes a detailed review of the petition, which by necessity involves several different scientific disciplines. The review of a petition is generally a prolonged process, requiring an average of several years until a regulation is issued.

Because of the potential spectrum of products which may result from the use of biotechnology, consideration should be given to adoption of a flexible regulatory review process, one which would provide a range of evaluation procedures differing in degree of formality and extensiveness of review. The nature of the review process appropriate for a particular product might reflect such considerations as the identity of the host organism, any evidence of pathogenicity or toxin production, the function of the inserted genes, the identity of the organisms that contribute genetic material to the final construct, characterization of the inserted genetic material to ensure the absence of sequences that may encode harmful substances, insertional and genomic stability, chemical specifications, and dietary use and exposure.

The level of review applicable to a specific product would be dependent on criteria designed to ensure that safety is adequately evaluated and documented without imposing review and petition processes that are unnecessary to ensure safety. The criteria for selecting one level of review over another would develop as time progresses. Conceivably the criteria would change as the food industry, the public, and the FDA accumulate information and experience as more products are developed through biotechnology and undergo regulatory review.

Implementation of an effective and flexible range of review processes should be considered a desirable goal, to be achieved in time as experience permits. Manufacturers of food ingredients, who must satisfy the food additive or GRAS requirements of the law, would benefit greatly from an informal review process that would, in appropriate cases, help ratify judgments that an ingredient is GRAS and does not require a formal food additive or GRAS petition. As mentioned in section 3.1, an informal, flexible process should also assist developers of whole food products, who may desire some indication or assurance of a product's acceptance by FDA. The range of possible review processes might contain the following:

1. *Private determination:* With respect to foods, GRAS substances, and regulated additives which are being manufactured by a different process, a private determina-

tion would be appropriate. The determination may include consultation with scientific experts or be accomplished by a group of experts convened for that purpose.

2. *Product Introduction Letter (no FDA response requested):* With respect to foods, GRAS substances, and additives which are being manufactured by a different process, a Product Introduction Letter (PIL) would be a means to advise FDA of the food or food ingredient which has been developed using genetic modification. The PIL would contain information adequate to advise FDA of the nature of the product and the modifications which were employed. The PIL could include the results of an evaluation by scientific experts. FDA would not be expected to reply to the PIL, although were FDA to have a concern, it would be appropriate for FDA to so advise the submitter of the PIL.

3. *Product Introduction Letter (FDA response requested):* With respect to foods, GRAS substances, and additives which are being manufactured by a different process, a Product Introduction Letter requesting an FDA response would be a means to advise FDA of the nature of the product and the genetic modifications which were employed, as well as to obtain FDA concurrence that no other action would be necessary. If FDA had no concerns with the information provided in the PIL, FDA would respond with a No Objection Letter, a letter with comments, or a request that the submitter of the PIL follow some other regulatory procedure.

4. *Notice of Safety Determination:* Under this option, the submitter would prepare and submit to FDA a Notice of Safety Determination, which would contain a data package sufficient in detail to establish the basis for a determination that the product, if a food, was not adulterated or, if a food ingredient, was GRAS. On receipt of the Notice, FDA would publish the Notice in the *Federal Register* with opportunity for public comment (30 days). The information contained in the Notice would be available to the public for review. Within 60 days of publication of the Notice, FDA would indicate its view regarding the information contained in the Notice by making no response, providing a No Objection Letter, or requesting that the submitter of the Notice follow some other regulatory procedure.

5. *Petition for affirmation of GRAS status:* When appropriate, a petition for affirmation of GRAS status could be prepared pursuant to the requirements set forth in 21 C.F.R. § 170.35.

6. *Food additive petition:* When appropriate, a food additive petition could be prepared pursuant to the requirements set forth in 21 C.F.R. § 171.1.

7. *Color additive petition:* When appropriate, a color additive petition could be prepared pursuant to the requirements set forth in 21 C.F.R. § 71.1.

An important point to be reemphasized is that the foregoing list is simply a broad outline which presents the general characteristics or main features of a review process. While the implementation of an effective and flexible range of review is a desirable goal, only through experience as products from biotechnology are developed will the process be refined.

5.5. Animal Feed for Animals Consumed as Food

The terms of the Food, Drug, and Cosmetic Act authorize regulation of animal feed and pet food under the same statutory provisions that apply to human food.

FDA has promulgated regulations regarding animal feed and pet food that parallel the regulations for human food, with such differences as would be appropriate to distinguish between the requirements for humans and animals. "Animal feed" is for food-producing animals or animals consumed as food, and "pet food" is for the animals not consumed as food but owned as pets. Commencing with Part 570 of Title 21 in the Code of Federal Regulations, FDA describes the criteria for "generally recognized as safe" ingredients, food additives, and prior sanction ingredients for use as animal feed and pet food. For example, "common use in food" for GRAS status for animal feed or pet food "means a substantial history of consumption of a substance by a significant number of animals in the United States."[75].

The provisions of Section 402 regarding the adulteration of food apply also to animal feed. Likewise, the provisions of section 406 may be employed to establish tolerances.

The regulations provide a format for a food additive petition (21 C.F.R. § 571.1) and for a petition for affirmation of GRAS status (21 C.F.R. § 570.35). In both cases, while the format is the same as that for a petition with respect to a food ingredient for human food, the data to be supplied in the petition must necessarily focus on the specific animal species. As in the case with ingredients for human food, a private determination may be made for an ingredient to be used in animal food. A formalized procedure has been developed by the American Association of Feed Control Officials. This organization publishes lists of ingredients for use in animal feed, in effect a GRAS list for animal feed.

Accordingly, for most purposes, the regulatory interpretations of the FDC Act as applied to food for human use would apply to food for animal use. A significant consideration in the evaluation of the safety of animal feed is whether any adverse effects arise from consumption of that animal as human food.

5.6. U.S. Environmental Issues Relating to Food Products Prepared by Genetic Modification

5.6.1. Review of FDA Requirements

Products derived from genetic modification can be perceived to create unique impacts on the environment. Within the United States, a complex regulatory procedure already exists; it is comprehensive enough to embrace any and all concerns relating to products derived from biotechnology and adequately implemented to control the impact on the environment. Under the National Environmental Policy Act (NEPA) of 1969, FDA and USDA are required to prepare a "detailed statement by the responsible official" on the environmental impact of every major federal action significantly affecting the quality of the human environment. Both organizations have identified those circumstances which are considered as major federal actions. In addition, these agencies have standard procedures to guide the presentation of information from industry and other sources to assist them in making evaluations on environmental impact. Typically, an "environmental assessment" (EA) is conducted first to determine whether a proposed action will have sufficient impact on the environment to justify development of a full "environmental impact statement" (EIS).

The format for an EIS is contained in the regulations of the Council of Environmental Quality (CEQ). The CEQ has the responsibility for overseeing the implementation of NEPA by the federal agencies. In Part 25 of Title 21 of the Code of Federal Regulations, FDA outlines the procedures that it follows to comply with NEPA. The environmental review normally commences after an industry-initiated submission of an application or petition for approval of a product, although FDA has the responsibility to evaluate any action within its jurisdiction which may significantly affect the quality of the human environment. The sponsor must include with the application or petition either a claim for one of the FDA promulgated categorical exclusions from the requirement of an EA or an EA prepared in a standard or an approved abbreviated format. In addition to the information contained in the regulations in Part 25, FDA has a supplementary document describing the environmental review process and the data gathering process.[76] To date, FDA has received several EAs in connection with petitions that have been filed for approval of enzyme preparations derived from genetically modified microorganisms.

By their very nature, activities which do not call for any action by FDA are categorically excluded from the need for an EA. For example, FDA has categorically excluded from an EA the

> affirmation of a food substance as generally recognized as safe (GRAS) for humans or animals on FDA's initiative or in response to a petition, under Part 182, 184, 185, or 582, if the substance is already marketed for the use for which affirmation is sought and data available to the agency do not establish that, at the expected levels of exposure, the substance may be toxic to organisms in the environment.[77]

Information from FDA indicates that there are specific points that should be set forth in an EA for a food or food ingredient derived from genetically modified sources.[78] The EA should describe the genetic constructions used to make the organism. The physical containment procedures should be described, along with a reference to compliance with any state and local requirements. This information would include statements on whether waste streams are treated to inactivate the organisms and whether any special precautions are taken to minimize releases as a result of nonroutine or accidental situations. Information should be provided regarding any traits that would limit the survival, growth, or activity of the organism if it were released into the environment. The EA should also indicate any characteristics of the modified organism that could result in adverse environmental effects.

On reviewing the EA, FDA would decide whether the data indicates a significant impact on the environment. If the action will not significantly affect the quality of the human environment, a finding of no significant impact (FONSI) will be prepared by FDA, and the preparation of an EIS is not required. If warranted because of the anticipated environmental impact, steps for preparation of an EIS will be undertaken by FDA, pursuant to the regulations in Part 25.

5.6.2. Review of USDA Requirements

Under the authority of the 1957 Federal Plant Pest Act and the 1912 Plant Quarantine Act, APHIS, an agency of USDA, reviews and regulates the importation, interstate movement, and release into the environment of genetically modified plants and

microorganisms if the donor organism, recipient organism, vector, or vector agent used in engineering the organism belongs to one of the taxa listed in the regulations[79] and is also a plant pest. Such genetically engineered organisms are called "regulated articles." Because the requirements of NEPA also apply to authorizations by APHIS to permit the release of regulated articles, APHIS carries out an environmental assessment prior to issuing such a permit, based on information the applicant is required to submit as well as supplemental information available in the literature. Applicants are required to submit the necessary environmental data in their application from which APHIS prepares the environmental assessment. The information which would be appropriate for consideration by APHIS in evaluating the environmental impact is similar to that which is specified by FDA for environmental assessments.

In preparing their environmental assessment, APHIS analyzes the impact of a release on the physical environment, human health risks, and impact on wildlife, endangered and threatened species and other nontarget flora and fauna.

On review of the information, APHIS announces the results of its evaluation. This could include a finding of no significant impact on the environment, if appropriate.[80]

5.7. Common or Usual Names of Genetically Modified Products

Food and food ingredients must be described by their common or usual name. In the case of food ingredients prepared using genetic modification, they should be identified by the rules applicable to ingredients manufactured by any other process. FDA has a historic approach to the process of naming products which would continue to be applicable to genetically modified food and food ingredients.

In the case of a food, the name

> shall accurately identify or describe, in as simple and direct terms as possible, the basic nature of the food or its characterizing properties or ingredients. The name shall be uniform among all identical or similar products and may not be confusingly similar to the name of any other food that is not reasonably encompassed within the same name. Each class or subclass of food shall be given its own common or usual name that states, in clear terms, what it is in a way that distinguishes it from different foods. (21 C.F.R. § 102.5(a)).

The guiding rule in determining the name of a product is that it should not be misleading to the consumer. The name should reflect the functional effect relevant to the product and provide such information necessary as not to mislead the consumer. For example, a food or food ingredient which has been genetically modified to incorporate or enhance some functional attributes may have the same name as products which are not so modified, but the modification may be appropriately addressed on the label, as is the case with other products, i.e., a high-vitamin C vegetable. A food which has been genetically modified to incorporate a food additive not normally contained in that food may have a common or usual name which refers to that ingredient. Likewise, genetically modified food which may raise special concerns for consumers with health problems should also be correctly identified; i.e., a food product genetically engineered to include aspartame would be labeled with the warning required by the FDA for those who are phenylketonurics.

The current regulatory considerations for selecting a name of a food and food ingredient have adequately served the needs of consumers throughout the years of develop-

ment of more technically sophisticated food products. The names which have been selected have adequately and effectively described the food products. There is no apparent need for any other approach in the development of common or usual names for products from biotechnology.

5.8. General Principles Regarding Safety Assurance of Food in Other Jurisdictions

5.8.1. Canada

In Canada, the Food and Drugs Act defines "food" to "include any article manufactured, sold or represented for use as food or drink for man, chewing gum, and any ingredient that may be mixed with food for any purpose whatsoever."

In terms of basic legislative prohibitions, the Food and Drugs Act prohibits the sale of food that

> (a) has in or upon it any poisonous or harmful substance;
> (b) is unfit for human consumption;
> (c) consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance;
> (d) is adulterated; or
> (e) was manufactured, prepared, preserved, packaged or stored under unsanitary conditions.

In common with statutes developed in the British legal tradition, the Food and Drugs Act permits the promulgation of regulations for carrying the purpose and provisions of the Act into effect. Under these regulation-making powers, a separate division (Division 16) of the Regulations has been established to deal with premarket clearance of food additives. In regulatory terms, a "food additive" is defined as follows:

> any substance, the use of which results, or may reasonably be expected to result in it or its by-products becoming a part of or affecting the characteristics of a food, but does not include:
>
> a. any nutritive material that is used, recognized or commonly sold as an article or ingredient of food;
> b. vitamins, mineral nutrients and amino acids other than those listed in Division 16;
> c. spices, seasonings, flavoring preparations, essential oils, oleoresins and natural extractives;
> d. agricultural chemical, other than those listed in the tables to Division 16;
> e. food packaging material and components thereof; and
> f. drugs recommended for administration to animals that may be consumed as food.

Division 16 of the Food and Drug Regulations contains tables of positively listed food additives which are organized in terms of functionality (i.e., food colors, pH-adjusting agents, and preservatives). The regulations provide for amendment to the tables through the use of formal submission or petition to the Health Protection Branch of the Department of National Health and Welfare. Such submissions are required to contain the following information:

1. A description of the food additive, including its chemical name and the name under which it is proposed to be sold, its method of manufacture, its chemical and physical properties, its composition and its specifications and, where that information is not available, a detailed explanation

2. A statement of the amount of the food additive proposed for use and the purpose for which it is proposed, together with all directions, recommendations, and suggestions for use
3. Where necessary, in the opinion of the Director, an acceptable method of analysis suitable for regulatory purposes that will determine the amount of food additive and of any substance resulting from the use of the food additive in the finished food
4. Data establishing that the food additive will have the intended physical or other technical effect
5. Detailed reports of tests made to establish the safety of the food additive under the conditions of use recommended
6. Data to indicate the residues that may remain in or on the finished food when the food additive is used in accordance with Good Manufacturing Practices
7. A proposed maximum limit for residues of the food additive in or on the finished food
8. Specimens of the labeling proposed for the food additive
9. A sample of the food additive in the form in which it is proposed to be used in foods, a sample of the active ingredient, and, on request, a sample of food containing the food additive

If the petition is approved via the normal regulatory process, the substance is then listed in the appropriate table together with a statement of the foods in which it may be used and the maximum authorized level of use. The specifications for the purity of the food additive may be set forth in the regulations, but if not, then the specifications for that substance found in the *Food Chemicals Codex*, Third Edition (National Academy Press, 1981), are applicable.

5.8.1.1. Comparison between Canadian and American legislative and regulatory provisions. In the United States, the terms *food additive* and *color additive* have been given statutory definitions under the Federal Food, Drug, and Cosmetic Act. This is not the case in Canada where the definition of a "food additive" falls within the basic definition of "food." In effect, this means that in Canada a food additive is a special class of ingredient that requires premarket clearance before it can be used in or on a food product.

The fact that Canada does not have a GRAS list is an important distinction between the Canadian and American regulatory structures. This means that the tables found in Division 16 of the Food and Drug Regulations contain many substances that have GRAS status under American law. This also means that in Canada a regulatory amendment must be made to change the provisions for using a substance that may have GRAS status in the United States.

It should be noted also that the exclusions from the food additive definition noted above mean that food ingredients, vitamins and minerals, spices, seasonings, flavorings, preparations, food packaging materials, agricultural chemicals, and residues of veterinary drugs are regulated separately, outside of the premarket clearance structure used for food additives.

Until March 23, 1989, food irradiation was treated as a food additive and was, in fact, specifically included within the definition of "food additive." However, the regulatory amendments of March 23 created a separate division in the Regulations devoted specifically to the control of food treated with ionizing radiation. These new

regulations, which still require premarket clearance of food subject to irradiation, treat irradiation as a process rather than a food additive.

5.8.1.2. Products of Biotechnology. Products of biotechnology will, in the main, be regulated on a case-by-case basis within the existing framework described above. However, this would not preclude the development of specific criteria or approaches for areas such as the safety assessment or characterization of such entities.

5.8.2. European Community

The Council of the European Communities (EC) has published a Directive which is intended to provide for the development of a single authorization of food additives throughout the EC.[81]

The Directive applies to 24 specified categories of food additives. For purposes of the Directive, the term *food additive* means

> any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food results, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods.

The Directive states that it does not apply to processing aids, substances used in the protection of plants and plant products in conformity with EC rules relating to plant health, flavorings for use in foodstuffs, and substances added to foodstuffs as nutrients (for example, minerals, trace elements, and vitamins). The Directive defines "processing aid" as

> any substance not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or their ingredients, to fulfill a certain technological purpose during treatment or processing and which may result in the unintentional but technically unavoidable presence of residues of the substance or its derivatives in the final product, provided that these residues do not present any health risk and do not have any technological effect on the finished product.

Based on this Directive, EC will adopt a positive list of authorized additives. This list will include a statement of the foods in which each additive may be used and the conditions under which each may be added, with appropriate specifications.

At the time of this writing, the EC was considering the adoption of a Council Regulation on novel food ingredients and novel food processes. The regulation would encompass "food ingredients or foods which have been produced by a novel process (and) contain genetically modified organisms," "food ingredients manufactured by cell tissue culture," foods or ingredients "containing chemical compounds which are new to the food supply," foods with significantly altered nutritional value or metabolic behavior, and ingredients whose new or expanded uses are likely to result in a significantly increased dietary exposure. The proposal outlines the types of products that would require premarket notification to the EC, and indicates the types of information to be submitted, including a description of the product and the manufacturing process, and the results of the safety evaluation. If the novel food ingredients or foods contain genetically modified organisms, then the notification would also need to include an environmental risk assessment.

5.8.3. Japan

In Japan, food and food ingredients are regulated by the Ministry of Health and Welfare pursuant to the Food Sanitation Law, first enacted in 1947.[82] The Ministry implements the Law pursuant to a Cabinet Order and regulations which have been promulgated by the Ministry.[83] The Law draws a line between food and food ingredients in Article 2, with "food" being defined as "articles used as food or drink for human beings" and "additives" being defined as "articles used in or on foods in the process of manufacturing foods, or for the purpose of processing or preserving foods by means of adding, mixing, infiltrating or by other means." A special category of additives, called "chemical synthetics," are defined as "substances obtained through chemical means by causing chemical reactions other than decomposition to elements or compounds." Chemical synthetics are not permitted for use in food unless they are included on the positive list promulgated by the Ministry.[84]

The Law in Article 4 prohibits the sale of foods or additives "which contain or are contaminated with toxic or harmful substances or are suspected to contain or are suspected to be contaminated with toxic or harmful substances, except in the case that the Minister of Health and Welfare designates them as not harmful to human health." The Minister has in the implementing regulations provided that certain toxic or harmful substances are to be considered as not harmful to health and therefore permitted in food: (1) those substances naturally contained or attached in or on the food or additive and present in a very small quantity or treated by some measures; and (2) substances used in processing food or additives which are unavoidable. Also, the Minister of Health and Welfare is authorized to prohibit the sale of an "article which has never generally been eaten or drunk by human beings, and there is no evidence that the said article is harmless for human consumption and the said article is going to be sold in the market" when the Minister considers the action "necessary to prevent the occurrence of human hazard on food sanitation grounds." The Minister in Article 7 is authorized to establish standards for methods of manufacturing and processing, and to establish specifications of components of foods or additives for sale. These specifications are published in the Official Formulary of Food Additives.

5.8.4. Codex Alimentarius Commission

The Codex Alimentarius Commission was formed in 1962 as a voluntary association of nations under the auspices of the United Nations. Funding for the activities of the Commission is provided jointly by the Food and Agriculture Organization and the World Health Organization. The main activity of the Commission is to develop internationally acceptable food standards: this is accomplished by commodity committees. Those countries which are members of the Commission generally follow the standards as they are adopted. When a country adopts a standard, the standard is applied to domestic commerce as well as to international trade. The standards incorporate elements such as limits on levels of pesticide residues, food additives, and contaminants.

The Commission adopted definitions to guide the activities of the Codex Committee on Food Additives and Contaminants (CCFAC):

Processing aid means a substance or material not including apparatus or utensils and not consumed as a food ingredient by itself, intentionally used in the processing of raw materials, foods or other ingredients to fulfill a certain technological purpose during treatment or processing and which may result in the non-intentional but unavoidable presence of residues or derivatives in the final product.

Food additive means any substance not normally consumed as a food in its own right and not normally used as a typical ingredient of food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport, or holding of such food results, or may be reasonably expected to result (directly or indirectly) in it or its by-products becoming a component of or otherwise affecting the characteristics of such food. The term does not include contaminants or substances added to food for maintaining or improving nutritional qualities.[85]

Each food standard contains a list of authorized food additives, which are drawn from information developed by the CCFAC. The CCFAC was charged by the Commission with the responsibility for establishing tolerances for individual food additives in specific food items and the preparation of lists of food additives. In the course of determining whether a given food additive may be used in a commodity, three fundamental criteria are taken into account: (1) need and technological function, (2) safety of the food additive, (3) consumer protection (other than safety).[86]

To provide assistance to the CCFAC in evaluating the safety of food additives, a Joint FAO/WHO Expert Committee on Food Additives (JECFA) was created to formulate general principles governing the use of food additives and consideration of suitable uniform methods for evaluating the safety of food additives. Based on a priority listing prepared by the CCFAC, JECFA reviews food additives based on toxicological data and also from the viewpoint of specifications. JECFA establishes a temporary or full ADI (acceptable daily intake) or an ADI (not limited) for such food additives which, when used in food within limits specified, do not pose any health hazards. An ADI is defined as the acceptable daily intake for humans taken daily in the diet over a lifetime without appreciable risk to the health of the consumer. JECFA also declares some food additives as not suitable for use in food for toxicological reasons. JECFA has developed a comprehensive approach in this evaluation process.[87] To make its evaluations, JECFA takes into consideration such information as (1) method of manufacture, (2) functional use, (3) impurities, (4) estimates of daily intake, (5) reactions and fate in food, and (6) toxicological data.

One of the problems facing the CCFAC and JECFA in applying the definition of "food additive" is the phrase "not normally consumed as food." JECFA has concluded that there is no simple guideline distinguishing foods from food additives. Each substance must therefore be considered separately. The distinction is significant, because whenever a substance is considered to be a food additive, toxicological evaluation is required to ensure safety and to establish an ADI. While new definitions which would allow a clear distinction between food additives and processing aids are under discussion, there is yet no consensus for changing the original definition which reads:

Food additive means any substance not normally consumed as food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food results or may be reasonably expected to result (directly or indirectly) in it or its byproducts becoming a component of or otherwise affecting the characteristics of such foods. The term does not include "contaminants" or substances added to foods for maintaining or improving nutritional qualities.[88]

At the 21st session of the CCFAC (held March 1989), the Committee discussed the development of foods and food ingredients from biotechnology and agreed to seek the advice of the Codex Alimentarius Commission as to how best to proceed in this area. In this discussion, a paper prepared by Dr. J. Maryanski (FDA) and Dr. D. Berkowitz (USDA) was reviewed, with numerous delegates suggesting that a novel food should be examined to determine whether or not it should be considered as a food ingredient or additive. This issue remains under discussion by the Codex Alimentarius Commission.

5.9. Conclusion

This Appendix reviews the laws and regulations governing food and food ingredients in the United States and in less detail those of select other countries and international organizations. While some basic similarities exist, the differences between the various countries limit the harmonization of the regulation of biotechnology throughout the world. It is hoped that this discussion will promote a better understanding of the various requirements and encourage the development of a common approach.

ENDNOTES

1. 21 U.S.C. § 321 *et seq.* As discussed later, the Enviromental Protection Agency (EPA) plays an important role in regulating pesticide residues in food. The U.S. Department of Agriculture regulates meat and poultry products, which are beyond the scope of this report.

2. Office of Science and Technology Policy (OSTP), Executive Office of the President, "Coordinated Framework for Regulation of Biotechnology." 51 *Fed. Regist.* 23,303 (June 26, 1986).

3. 21 U.S.C. § 342(a)(1).

4. Ibid.

5. By interpreting the term *added* broadly, FDA has minimized the number of food substances subject to the more lenient "ordinarily injurious" safety standard. *See* 21 C.F.R. § 109.3(c) and (d); 42 *Fed. Regist.* 52,814 (Sept. 30, 1977). On the other hand, by regulating under section 402(a)(1) substances that are foreseeably present in food as a result of human conduct but lack a function in the food itself, FDA has avoided subjecting such substances to a "food additive" regulatory standard they could not meet. *See* 39 *Fed. Regist.* 42,743 (Dec. 6, 1974).

6. Food Additives Amendment of 1958, codified at 21 U.S.C. §§ 321(s) and 348. In enacting the Food Additives Amendment, Congress declared that its purpose was twofold:

> (1) to protect the health of consumers by requiring manufacturers of food additives and food processors to pretest any potentially unsafe substances which are to be added to food; and
> (2) to advance food technology by permitting the use of additives at safe levels.

H. Rep. No. 2284, 85th Cong. 2d Sess. at 4–5 (1958)).

7. Substances that are added intentionally to food to perform a function in the food are excluded from the statutory definition of "food additive" and thus from the requirement of premarket approval if they are "generally recognized . . . to be safe." 21 U.S.C. § 321(s).

8. 21 U.S.C. § 342(a)(1).

9. *United States v. Lexington Mill & Elevator Co.*, 232 U.S. 399 (1914).

10. 21 U.S.C. §§ 332, 333, and 334.

11. *See* Chemicals in Food Products: Hearings Pursuant to H. Res. 323 before the House Select Comm. to Investigate the Use of Chemicals in Food Products, 81st Cong., 2d Sess. (1951); Chemicals in Foods and Cosmetics: Hearings Pursuant to H. Res. 74 and H. Res. 447 before the House Select Comm. to Investigate the Use of Chemicals in Foods and Cosmetics, 82d Cong., 1st Sess. (1952).

12. The House committee primarily responsible for developing the Food Additives Amendment stated in its report on the bill:

> safety requires proof of a reasonable certainty that no harm will result from the proposed use of an additive. It does not—and cannot—require proof beyond any possible doubt that no harm will result under any conceivable circumstance.
>
> This was emphasized particularly by the scientific panel which testified before the Subcommittee. The scientists pointed out that it is impossible in the present state of scientific knowledge to establish with complete certainty the absolute harmlessness of any chemical substance. . . . Reasonable certainty determined in this fashion that an additive will be safe, will protect the public health from harm and will permit sound progress in food technology.

H. Rep. No. 2284 at 4–5 (1958). FDA has codified the "reasonable certainty of no harm" safety standard in its regulations. 21 C.F.R. § 170.3(i).

13. *See* note 6.

14. *See* 21 U.S.C. § 321(s).

15. 21 U.S.C. § 321(s); 21 C.F.R. § 170.30(a)–(c).

16. *Ibid.*

17. This risk can be minimized or avoided by making the independent GRAS determination on a sound scientific basis and in a manner consistent with FDA's standards. One example of how the GRAS concept can operate is the systematic evaluation of the potential GRAS status of flavors conducted under the auspices of the Flavor and Extract Manufacturers Association (FEMA). FEMA convenes an expert panel of toxicologists, pharmacologists, and others with expertise relevant to food safety, and this panel determines whether particular flavor substances, both natural and manmade, are GRAS for their intended use. FEMA then publishes its conclusions and the basis for them, providing FDA a preprint of each of its publications. FDA is not bound by FEMA's determinations, but it has generally acquiesced in this procedure for nearly 30 years because it adequately ensures the safety of flavors and avoids the unnecessary burden of FDA reviewing hundreds of GRAS and food additive petitions.

18. 21 U.S.C. §§ 342(a)(2)(C) and 348.

19. 21 C.F.R. § 182.1(a).

20. 21 C.F.R. Part 182.

21. 21 C.F.R. § 170.30 and 170.35.

22. *See* e.g., 21 C.F.R. §§ 184.1277 (dextrin) 184.1321 (corn gluten), 184.1322 (wheat gluten), and 184.1339 (guar gum).

23. *See*, e.g., 21 C.F.R. §§ 184.1555(c) (canola oil) and 184.1979 (whey).

24. *See*, e.g., 21 C.F.R. §§ 184.1027 (mixed carbohydrase and protease enzyme product) and 184.1372 (insoluble glucose isomerase enzyme preparations).

25. *See* 21 C.F.R. Part 173, Subpart B (Enzyme Preparations and Microorganisms).

26. The original FDA GRAS list was adopted shortly after enactment of the Food Additives Amendment. *See* 23 *Fed. Regist.* 9511 (1958) and 24 *Fed. Regist.* 9368 (1959). Much of the original list remains codified in 21 C.F.R. Part 182.

27. Federation of American Societies for Experimental Biology, Final Report, FDA 223-75-2004, "Evaluation of GRAS Monographs (Scientific Literature Reviews)," April 30, 1980.

28. 21 C.F.R. Parts 172–179. FDA defines "safe" to mean a "reasonable certainty" that a substance will not be harmful under its intended conditions of use. 21 C.F.R. § 170.3(i).

29. 21 C.F.R. Parts 174–178.

30. *See*, e.g., 21 C.F.R. Part 172 Subpart B (Food Preservatives), 21 C.F.R. § 172.340 (fish protein isolate) and 172.804 (aspartame).

31. This would not require that the whole plant be considered as a food additive or GRAS substance but only the functional chemical entity (e.g., the sweetener or preservative) that is produced in the plant as a result of the genetic alteration.

32. As a legal matter, the option also exists to make an independent GRAS determination and market on that basis without petitioning FDA. See discussion later in text.

33. *See*, e.g., 21 C.F.R. § 184.1(b)(1).

34. The Office of Science and Technology Policy published the proposed Coordinated Framework for Regulation of Biotechnology on December 31, 1984 (49 *Fed. Regist.* 50856) and a final Framework was published June 26, 1986 (51 *Fed. Regist.* 23303).

35. 21 U.S.C. § 301–392.

36. The U.S. Department of Agriculture has responsibilities under the Federal Meat Inspection Act (FMIA)(21 U.S.C. §§ 601–695), Poultry Products Inspection Act (PPIA) (21 U.S.C. §§ 451–470), Egg Products Inspection Act (EPIA) (21 U.S.C. §§ 1031–1056), Virus–Serum–Toxin Act (VSTA) (21 U.S.C. §§ 151–158), Federal Plant Pest Act (FPPA) (7 U.S.C. §§ 150aa–150jj), Plant Quarantine Act (PQA) (7 U.S.C. §§ 151–164, 166, 167), Animal Quarantine Act (21 U.S.C. § 111 *et seq.*), Organic Act (OA)(7 U.S.C. § 147a), Federal Noxious Weed Act (FNWA) (7 U.S.C. § 2801 *et seq.*), Federal Seed Act (7 U.S.C. § 551 *et seq.*), Plant Variety Protection Act (PVPA) (7 U.S.C. § 2321 *et seq.*). The Environmental Protection Agency has responsibility for implementing the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) (7 U.S.C.§§ 136–136d, 136p, 136s, 136w 136y) and the Toxic Substances Control Act (TSCA) (15 U.S.C. § 2601 *et seq.*). FDA and USDA have responsibilities under the National Environmental Policy Act (NEPA) (42 U.S.C. §§ 4321, 4331–4335, 4341–4347). These statutes all need to be evaluated in connection with any specific intended product to ensure that the requirements are satisfied.

37. 51 *Fed. Regist.* 23336 *et seq.*

38. 51 *Fed. Regist.* 23315.

39. *Nutrilab v. Schweiker*, 713 F.2d 335 (7th Cir. 1983).

40. *U.S. v. Technical Egg Products, Inc.*, 171 F.Supp. 326 (N.D. Ga 1959).

41. Economic considerations may also result in the determination that a food is "adulterated." Section 402(b) states that a food shall be deemed to be adulterated "(1) if any valuable constituent has been in whole or in part omitted or abstracted therefrom; or (2) if any substance has been substituted wholly or in part therefor; or (3) if damage or inferiority has been concealed in any manner; or (4) if any substance has been added thereto or mixed or packed therewith so as to increase its bulk or weight, or reduce its quality or strength, or make it appear better or of greater value than it is."

42. *Young v. Community Nutrition Institute*, 475 U.S. 1123 (1986).

43. Section 402(a)(2)(C).

44. Section 201(s).

45. Section 201(s)(4).

46. Section 706 of the FDC Act describes in detail the considerations which determine whether a color additive is suitable for regulation as an authorized ingredient. The section also sets forth the procedure to be followed by the FDA in making that determination.

47. *U.S. v. Anderson Seafoods, Inc.* 447F. Supp. 1151 N.D. Fla. 1978. "[T]he legislative history makes clear that the terms seek . . . to establish a standard based upon the necessary and inherent normal condition of the food."

48. *U.S. v. Anderson Seafoods, Inc.*

49. 21 C.F.R. 109.3(c), and preamble 42 *Fed. Regist.* 52815 (Sept. 6, 1977).

50. An intentionally added non-GRAS substance is exempt from section 402(a)(1) only if a regulation has been promulgated under the authority of section 406, 408(a), 409(a), or 512(k). 39 *Fed. Regist.* 42747.

51. 39 *Fed. Regist.* 42747–42748.

52. 232 U.S. at 411.

53. H.R. Rep. No. 2284, 85th Cong. 2d Sess. 4–5 (1958); S. Rep. No. 2422, 85th Cong. 2d Sess. 6 (1958).

54. 21 C.F.R. § 170.3(i).

55. 21 C.F.R. § 170.30.

56. 21 C.F.R. § 170.30.

57. 50 *Fed. Regist.* 27294–27295.

58. Ibid.

59. Ibid.

60. Ibid.

61. 21 C.F.R. § 170.30(d).

62. *U.S. v. Article of Food . . . Coco Rico*, 752 F.2d 11 (1st Cir. 1985).

63. In certain circumstances, a food commonly used in a foreign country prior to 1958 may be considered as GRAS within the meaning of section 201(s). 50 *Fed. Regist.* 27295.

64. Such changes could also affect the GRAS status of a post-1958 substance whose safety had been established on the basis of "scientific procedures."

65. See 39 *Fed. Regist.* 34194–34195.

66. 21 C.F.R. § 170.3.

67. 39 *Fed. Regist.* 34194.

68. *See generally* 39 *Fed. Regist.* 34194–34195.

69. 41 *Fed. Regist.* 53600–53601.
70. 45 *Fed. Regist.* 61474.
71. 50 *Fed. Regist.* 27295.
72. "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food." Docket No. 80N0446.
73. 21 C.F.R. § 171.1(a).
74. *See* 41 *Fed. Regist.* 53600.
75. 21 C.F.R. § 570.3(f).
76. *Environmental Assessment Technical Handbook,* National Technical Information Service (No. PB87–175345/AS).
77. 21 C.F.R. § 25.24(b)(7).
78. Buzz L. Hoffmann, Presentation at Third International ABC Biotechnology Meeting, March 30, 1989.
79. 51 *Fed. Regist.* 23336 *et seq.*, which was followed by a final rule "Introduction of Organisms and Products Altered or Produced through Genetic Engineering Which Are Plant Pests or Which There Is Reason to Believe Are Plant Pests," 52 *Fed. Regist.* 22892, codified at 7 C.F.R. Part 340.
80. Environmental Assessment and Finding of No Significant Impact with respect to a controlled field test of genetically engineered tobacco plants, issued November 16, 1987 with respect to permit 87-229-01 and issued November 27, 1989, with respect to permit 87-229-02. For plant pests, USDA published proposed rules in 51 *Fed. Regist.* 23352–23366 which became final rules at 7 C.F.R. Part 340, 52 *Fed. Regist.* 22892.
81. Council Directive of 21 December 1988 (89/107/EECD). Official Journal of the European Communities, No. L 40/27 (February 11, 1989).
82. Food Sanitation Law, as of 12th Revision, 1972 (Law No. 233, December 24, 1947).
83. The Cabinet Order for the Enforcement of Food Sanitation Law, As of 18th Revision, 1987 (Cabinet Order No. 229, August 31, 1953); The Enforcement Regulation of Food Sanitation law, As of 81st Revision, 1987 (Ministerial Ordinance No. 23, July 13, 1948). For purposes of this discussion, reference is made to a translation by Y. Furusawa, published by Japan Food Hygiene Association.
84. See Table 2 to The Enforcement Regulation of Food Sanitation Law.
85. *Codex Alimentarius,* Vol. XIV, 1st ed. (1983), Joint FAO–WHO Food Standards Programme.
86. Codex Committee on Food Additives, XIV Session, CX/FA 80/16 (1980).
87. Principles for the Safety Assessment of Food Additives and Contaminants in Food, Environmental Health Criteria 70, World Health Organization (1987).
88. Codex Alimentarius Commission, *Procedural Manual,* 7th ed., p. 31.

REGULATORY TOXICOLOGY AND PHARMACOLOGY 12, S190–S196 (1990)

Glossary[a]

Abiogenesis Theory claiming that living organisms could be derived from nonliving substances (spontaneous generation). This theory was disproved by Pasteur.

Activation Significant increase in gene expression, or the initiation of expression of a gene that formerly was not expressed.

Aflatoxin Acutely toxic and carcinogenic metabolites of some strains of *Aspergillus flavus* and *A. parasiticus*.

Aneuploid Containing more or less than the normal diploid number of chromosomes.

Antibiotic Substance derived from a fungus or bacterium that inhibits the growth of other microorganisms. Many antibiotics are used as drugs in treating disease.

Antisense RNA RNA that is transcribed from the noncoding, or antisense, strand of DNA for a given gene and is therefore complementary in nucleotide sequence to the normally produced messenger RNA (mRNA). Antisense RNA forms a duplex with mRNA by the pairing of complementary nucleotide bases, and this inhibits translation of the mRNA into protein.

Backcrossing Technique in plant breeding for recovering the characteristics of one parent (the recurrent parent) while intentionally losing the vast majority of characteristics from another parent (the donor) that was a source of a particular trait. After the initial cross and selection of individuals displaying the trait of interest from the donor, all further generations are crossed only to the recurrent parent until an acceptable facsimile of the recurrent parent is obtained that still retains the trait of interest from the donor.

Bacterium Single-celled, prokaryotic organism that reproduces by binary fission (splitting into two equal cells.)

Biotechnology Use of biological processes to produce products.

Callus Unorganized mass of plant cells growing in tissue culture.

Carcinogenic Causing cancer.

CFR Code of Federal Regulations (United States).

Chimeric gene Gene containing modified or substituted control signals joined to portions of the native genetic information. (see Chapter 3).

Chromosome Linear body in the cell nucleus that is composed of DNA (containing genes) plus surrounding proteins. In a chromosome, the DNA is present as two complementary strands whose bases pair in the center: guanine pairs with cytosine, and adenine pairs with thymine.

Clone Group of genes, cells or organisms derived from one common ancestor. Members of the clone are genetically identical to each other and to the parent.

Cloning See *gene cloning*.

Codex Alimentarius Commission Organization under WHO and FAO joint sponsorship which convenes governments to elaborate standards for foods and food ingredients used in international trade.

Coding sequence In a gene, the sequence of nucleotide bases that determines an amino acid sequence for a protein or a nucleotide sequence for an RNA molecule.

[a] The following reference was consulted during preparation of the Glossary: Industrial Biotechnology Association (1988). *Biotechnology at Work: Glossary of Terms*. IBA, Washington, DC.

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Codon Sequence of three nucleotide bases in DNA or RNA that specifies an amino acid or represents a signal to start or stop a function.

Constitutive mutant Organism mutated in such a way that it is continually synthesizing a specific protein.

Contaminant Noninherent material intruding into the product from an outside source.

Conventional toxicological tests Methods devised for use in experimentally characterizing the qualitative and quantitative toxic potential of single chemicals (e.g., pharmaceuticals, food additives, pesticides, and environmental contaminants) as outlined in various references cited in Chapter 6 (i.e., Food and Drug Administration, 1982; Food Safety Council, 1980; National Academy of Sciences, 1969).

Crude culture extract Fermentation product that is less refined than a commercial enzyme preparation and often used for toxicological studies.

Cultivar Form of a cultivated crop plant with distinct features introduced by breeding or other genetic modification.

Currently approved substances In the United States, prior sanction substances, GRAS substances, and food or color additives; in other countries, substances in accepted use under the laws of those countries.

Cyanogenic glycoside Naturally occurring toxicant in lima bean and cassava (see Chapter 2).

Cytotoxic Property of inhibiting or preventing the functioning of cells, or causing their destruction.

Deoxyribonucleic acid (DNA) Complex biochemical substance of which genes are made and which carries hereditary information in most living systems. DNA is composed of alternating phosphate groups and deoxyribose with one of four attached nucleotide bases: adenine, thymine, cytosine and guanine. The sequence of bases in the DNA determines what expression product, if any, will be derived from the DNA. DNA that does not determine the sequence of any expression product is noncoding DNA.

Diploid Containing two sets of chromosomes. The sets are maternal and paternal in origin. Each chromosome is matched with a homolog derived from the other parent.

DNA See *deoxyribonucleic acid.*

DNA insert Piece of foreign DNA that is introduced into a vector molecule using recombinant DNA techniques.

Elite germplasm Plant materials, often proprietary, used by a breeder to develop cultivars or hybrids.

Ergot Fungal disease of rye and other cereals resulting in the production of toxic alkaloids.

Estimated mean human consumption level Estimation of the intake of a particular food component based on portion size, eating frequency, and use level.

Eukaryote Organism composed of one or more cells with nuclei bounded by a membrane.

Existing specifications Specifications that define food grade. This includes those provisions necessary for use in safety evaluation. See also *specification.*

Expression product Specific RNA, protein or polypeptide coded for by the DNA sequence in a gene (primary expression product). If the protein is an enzyme, a biochemical reaction product (e.g., a sugar, fatty acid, or vitamin) resulting from the enzymatic activity is also considered to be an expression product of the DNA (secondary expression product).

Extrachromosomal DNA Self-replicative genetic element separate from the chromosome(s) of a cell, e.g., a plasmid.

Favism Disease induced in some individuals by eating *Vicia faba* beans or inhaling pollen. Susceptible persons lack sufficient quantities of the glucose-6-phosphate dehydrogenase enzyme.

Fermentation Process of growing microorganisms for the production of chemicals, pharmaceutical compounds or biomass. Large tanks, called fermenters, contain the microorganisms and the nutrients they require for growth.

Fetotoxic Causing damage to the unborn young.

Fingerprinting Technique used to uniquely characterize individuals or foods based on their partial protein, DNA, or chemical composition. This technique has applications for plant variety identification, compositional comparison of two genetic lines, and evolutionary studies.

Food Defined in the U.S. Food, Drug and Cosmetic Act as articles used for "food or drink for man or animals, chewing gum, and articles used for components of any other such article." In a broader context, food should be considered as anything sold or consumed as such. Under U.S. law any person who introduces food into commerce is responsible for ensuring that it complies with all applicable safety standards.

Food additive (1) In general, any minor ingredient added to food to achieve a specific (technical) effect. (2) Under U. S. food law, a term of art that excludes from the preceding definition many intentionally added food substances such as color additives and additives that are "generally recognized as safe."

Foodborne disease Any illness caused by food consumption.

Food poisoning Illness resulting from consumption of food that contains toxic chemicals which are usually, but not necessarily, toxic by-products of certain microorganisms.

FR *Federal Register* (United States).

Frameshift Mutation resulting when the genetic code is read beginning at the second or third base of a codon.

Gametoclonal variation Phenotypic expression of genetic changes resulting from clonal propagation of gametophytic tissue, as with anther culture.

Gene Smallest portion of a DNA molecule that contains sufficient heritable information to direct the production of a protein or a molecule of transfer or ribosomal RNA, or to perform a regulatory function.

Gene cloning Isolating a DNA segment by cutting it out of the parent chromosome and joining it to a vector DNA *in vitro*.

Genetic construct Gene sequence of a genetic element formed using recombinant DNA techniques.

Genetic drift Random changes in a population composition caused by suboptimal population sizes.

Genetic engineering Directed modification of the genome to produce desired changes in the characteristics of an organism.

Genetic modification Addition, deletion, substitution, rearrangement, or recombination of heritable genetic material. Processes for achieving genetic modification include plant and animal breeding, cell and tissue culture, cell and protoplast fusion, mutagenesis, and recombinant DNA with transformation.

Genome Total hereditary material (DNA) of a cell.

Genotype Genetic complement of an organism.

Glycoalkaloid Naturally occurring toxicant found in the potato family (Solanaceae).

GMP See *Good Manufacturing Practices*.

Good Manufacturing Practices (GMP) Those means of ensuring that products are made and handled in a sanitary manner; in a way designed to preclude the formation of undesirable by-products, as well as contamination, deterioration, mixup, and mislabeling, and in a way that avoids the introduction of unusual or unexpected impurities.

GRAS Generally recognized as safe (refer to Chapter 7).

Hallucinogenic Causing hallucinations.

Haploid Containing half the number of chromosomes typical of somatic (vegetative) cells, as a result of meiosis. Pollen and egg cells are haploid.

Hemagglutinin Substance that causes aggregation of red blood cells.

Homologous recombination Process of DNA exchange in which introduced DNA is substituted for native DNA containing identical or very similar (homologous) nucleotide base sequences at the edges of the exchanged regions. Homologous recombination can occur in a cell or *in vitro*.

Homolog (homologous chromosome) Set of chromosomes that are similar in their length and linear order of genes.

Hybrid Offspring of two genetically distinct parents.

Inactivation Significant decrease in gene expression.

Inducer External substance that enhances the expression of a gene.

Inherent constituent In a food, any component naturally and endogenously present in an organism used for food including the normally edible as well as inedible portions. See also *significant constituent*.

Intermediate host Microorganism that is used as a host during construction of a DNA insert but is not the final host for the insert.

Intoxication Illness resulting from consumption of food containing toxic products of microbial action.

Introduced genetic material Any DNA incorporated into a parental cell through a process of genetic modification.

In vitro Outside of the living body, for example, in a test tube or in laboratory tissue culture.

Marker gene Gene with a detectable or selectable phenotype that is engineered into a vector to allow detection of neighboring sequences (a gene or genes of interest) in a new genetic element.

Meiosis Cellular process that results in the number of chromosomes in gamete-producing cells being reduced to one-half their original number.

Messenger RNA Form of ribonucleic acid (RNA), transcribed from DNA, that carries instructions to a ribosome for the synthesis of a particular protein.

Microbial biomass Cell material of microorganisms.

Mitosis Partitioning of identical sets of chromosomes into two daughter cells during cell division. Each resulting cell is diploid, as was the parent cell.

Mold Filamentous fungus.

Monogenic Of, relating to, or controlled by a single gene.

mRNA See *messenger RNA*.

Mutagen Agent (e.g., ultraviolet radiation, X rays, certain chemicals) that increases the frequency or extent of mutation.

Mutagenesis Process that results in the modification of a DNA sequence (refer to Chapters 3 and 4).

Mutation Change in the DNA sequence caused by deletion, addition or alteration of bases.

Mycotoxin Toxic substance produced by fungi.

Native gene Gene that occurs naturally in a specific organism.

Natural toxicant Any substance that occurs in food as a consequence of biosynthesis in the organism or absorption by the organism from its natural occurrence in the environment, provided that the toxic effects that the substance causes in humans, domestic animals, or experimental animals either are irreversible, e.g. carcinogenicity, teratogenicity, certain neurotoxicities *or* occur with narrow margins of safety, i.e., at low multiples (approximately 25 or less) of ordinary exposures.

Neurotoxin Poison that acts on the nervous system.

New constituent Any expression product present solely as a result of the introduction of new genetic material but not any known or even unidentified constituent inherent to the food, its parents, or related species.

NOEL See *no-observable-effect level*.

Noncoding DNA DNA sequences that cannot produce an expression product.

No-observable-effect level In an animal toxicity study, the highest dose level at which no significant toxicological effects are observed.

Normal diet Foods that are customary, accepted, and familiar to the locality and culture, not including those items consumed only during unusual deprivation or those that are of primarily ceremonial or religious significance.

Northern analysis Nucleic acid hybridization method used to identify specific RNA sequences with a DNA probe. The RNA is isolated from cells and cut using enzymes, and the resulting RNA pieces are separated by gel electrophoresis and transferred to a membrane filter. When the DNA probe is added to the filter, the probe binds to any RNA with base sequences complementary to those of the probe.

No safety concern (lack of adverse impact) Appropriate standard of safety applicable to the food product, i.e., the constituents do not ordinarily render the food product injurious to health and there is a reasonable certainty that no harm will result from ingestion of the food or food constituent under the proposed or intended conditions of use of the product.

Not alter significantly present intake Proposed conditions of use of the new product would not reasonably be expected to lead to such an intake of the food that the total intake of any constituent would exceed the amount acceptable under the standard of safety.

Nucleotide sequence Order of the bases (adenine, thymine, guanine, cytosine) in a strand of DNA. This term also refers to RNA, where the base uracil is present rather than thymine. See also *coding sequence.*

Ochratoxin Toxic product of the fungus *Aspergillus ochraceous* and several other species of *Aspergillus* and *Penicillium* which has been reported to cause serious damage to the kidneys of animals that consume feed on which these molds have grown.

Organoleptic evaluation Description and measurement, using a panel of human subjects, of the nature and intensity of the appearance, taste, odor, flavor and other characteristics of a food as perceived under conditions of intended use.

Pass-through toxicants Toxicants that are found in the diet of wild or domestic animals and that persist in the flesh, milk, or other product of the animal used for human food.

Pathogen Any virus or microorganism that causes disease.

Pathogenic Capable of producing disease.

Phenotype Observable characteristics, resulting from an interaction between an organism's genetic makeup and the environment.

Phytoalexins Subset of the substances that are produced by plants in response to stress or infection by a pathogen and that may contribute to disease resistance.

Plasmid Small, circular piece of extrachromosomal DNA that carries certain genes and is capable of replicating independently in a bacterial cell. Plasmids are normally not essential for growth but can be stably inherited.

Pleiotropic effect Production of several unrelated changes in the characteristics of a cell or organism by a single genetic change.

Point mutation Change in a single nucleotide base of the DNA sequence.

Polyploid Having more than two homologous sets of chromosomes.

Position effect Phenomenon in which the level of expression of an introduced gene may vary with the site of insertion in the chromosome.

Primer Short piece of DNA that promotes DNA synthesis by providing a site for the action of the enzyme, DNA polymerase, to add nucleotides to one end of the primer. The added nucleotides complement the native nucleotide sequence to which the primer is attached.

Prokaryote Cellular organism whose DNA is not enclosed by a nuclear membrane (bacteria, blue-green algae).

Promoter DNA sequence that is located in front of a gene and controls gene expression. The promoter is the binding site of RNA polymerase on the DNA molecule and serves as the starting point of the synthesis of messenger RNA.

Protease Enzyme that breaks down protein.

Protoplast Plant cell whose wall has been removed by enzymatic or mechanical means.

rDNA See *recombinant DNA technology.*

Recombinant DNA technology Processes of cutting and recombining DNA molecules to remove segments from or otherwise modify an organism's genetic material, or to combine segments of DNA from different types of organisms.

Recombinant microorganism Microorganism containing DNA from two or more sources.

Recombination Breakage and reunion of DNA that result in new combinations of genes in offspring.

Rennin Milk-curdling enzyme used in commercial cheesemaking. Also referred to as chymosin or rennet.

Reproductive isolation Process that restricts genetic exchange and thus furthers evolutionary divergence between populations in the same habitat.

Restriction analysis Use of endonuclease enzymes to cut DNA at specific sites and thereby aid in the determination of the base sequence of the DNA.

Restriction fragment length polymorphism (RFLP) Observable difference between individuals in the size of enzymatically produced DNA fragments. Such analysis is useful in the fingerprinting or characterization of genetically distinct individuals.

RFLP See *restriction fragment length polymorphism.*

Ribonucleic acid (RNA) Nucleic acid composed of alternating phosphate groups and ribose with one of four attached nucleotide bases: adenine, guanine, cytosine, and uracil.

Ribosome Cytoplasmic particle composed of RNA and protein that is part of the protein synthesizing machinery of the cell.

Risk Probability of adverse effects, their nature, and their severity over a range of exposures.

Risk/benefit Decision-assisting approach that attempts to identify, estimate, and weigh all the risks and benefits associated with a particular action and to determine whether, overall, the benefit would be worth the associated risk.

RNA See *ribonucleic acid.*

Rogning Elimination of undesirable, individual plants.

Safety Reasonable certainty that no harm will result under expected conditions of use.

Safety evaluation Process by which knowledge of a material's intrinsic toxicity, occurrence, pattern, and level of exposure generally, as well as its concentration in the product of interest and its intended use level, is reviewed to determine the conditions under which the material can safely be used. (refer to Chapter 6, Sections 4 and 5).

Selectable marker Gene whose expression product allows its host cell to grow preferentially in a defined laboratory culture medium, or a gene that ensures survival of a cell or organism exposed to an otherwise lethal environment. See also *marker gene.*

Sequencing Determining the order of nucleotides in a DNA or RNA molecule or that of amino acids in a polypeptide chain.

Short-term feeding study Feeding study lasting less than 90 days.

Significant constituents Essential nutrients and nonnutrient components such as naturally occurring toxic factors typically associated with the food, its parents, or related species.

Significant nutrients In the context of this report, essential nutrients found in a food recognized as a source that contributes about 10% or more of the recommended daily allowance (RDA).

Significant risk Deemed to be posed by a food material for which the margin of safety between the toxic dose and typical exposure levels is a multiple of approximately 30 or less.

Site-directed mutagenesis Modification of a DNA sequence at a location that is precisely controlled (See Chapter 3).

Somaclonal variation Phenotypic expression of genetic changes observable after growth of plant tissue in cell or tissue culture.

Somatic Relating to the vegetative characteristics of the mature organism, as distinct from gametic or gametophytic.

Specification Recognized standard of identity, performance, and quality which foods, food ingredients, and adjuncts used in food processing must meet to be acceptable for their intended uses and applications. It is not sufficient, however, for an end product merely to meet the specifications. Food-grade materials must also be produced under *Good Manufacturing Practices (GMP).*

Staphylococcal enterotoxin Heat-resistant toxic protein produced by certain strains of *Staphylococcus aureus* when they grow in food. The toxin causes violent vomiting and diarrhea when consumed by humans.

Sterigmatocystin Carcinogenic mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi.

Structure/activity relationship Consistent and, therefore, within limits, predictable association between the chemical structure of a substance and its pharmacological or toxicological effect on living organisms.

Sub-chronic feeding test Ninety-day toxicology study in an appropriate animal species.

T-2 toxin One of many trichothecene mycotoxins produced principally by species of *Fusarium* fungi.

Teratogenic Producing malformations in the unborn young.

Terminator sequence DNA sequence that signals the end of a gene and thus the synthesis of messenger RNA.

Tissue culture *In vitro* growth of plant cells in a sterile nutrient medium.

Toxic Capable of chemical disruption of the normal biological processes of living organisms.

Toxicant Substance that has been shown to present some significant degree of possible risk when consumed in sufficient quantity by humans or animals.

Toxicity testing Use of experimental procedures to determine the levels at which exposure to a material leads to adverse effects in test subjects, the characterization of such induced effects, and the elucidation of mechanisms of action by which the effects were induced.

Toxin (1) Toxic peptide or protein capable of eliciting antibody production, and produced by a microorganism, plant, or higher animal, also (2) Synonymous with toxicant.

Traditional foods Plants, animals and microorganisms and their products that are widely consumed as human food by at least certain cultures or population groups.

Transformant Cell or individual organism whose genetic makeup has been altered by the introduction of foreign (nonnative) DNA.

Transformation Process whereby a cell permanently incorporates foreign DNA into its genome.

Translocation Change in chromosome structure resulting from the rearrangement of chromosome segments.

Transposon Short, mobile piece of DNA that can insert itself into different sites in the chromosome. Transposon insertion can cause a mutation which may or may not be observable.

Vector In the context of this report, DNA used to introduce other DNAs, especially genes of interest, into food plants or microorganisms. A vector is usually a small, circular piece of DNA that is able to incorporate and reproduce cloned genetic material and be transmitted to another cell.

Wild-type Organism isolated from nature.

Xenobiotic Substance not found in nature. The word implies that the chemical or physical structure of the substance strongly suggests that it is unlikely ever to be found in nature.