Chapter 11

Scientific Issues in Food Safety

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In an ideal world, the food products we eat would contain no hazardous components and would be completely safe. We do not live in an ideal world. It is impossible to eliminate all potential food hazards, but food risks can be minimized by controlling microbial hazards, toxic constituents, and the nutritional value of foods. Scientists generally agree that when it comes to food safety, the number one concern is the problem of microbial contamination, followed closely by the effects of nutritional imbalance. The risks posed by toxic constituents such as pesticide residues, environmental contaminants, natural toxins, and chemical food additives, viewed as most important by the public, are generally considered by scientists to present lower risks (19, 28, 37).

It is impossible to precisely measure the number of foodborne illnesses that occur as a result of microbial contamination each year in the United States. Hundreds of thousands of cases are documented, including thousands of deaths. Due to underreporting, these documented cases represent only a fraction of the number of actual cases that occur. The Centers for Disease Control and the Food and Drug Administration (FDA) estimate that up to 33 million cases of foodborne illnesses occur annually, and some studies have placed the estimate as high as81 million (27, 55). This staggering number costs the U.S. economy billions of dollars in lost productivity each year. Some of the major genera of bacteria responsible for foodborne illnesses include *Salmonella, Shigella, Campylobacter, Listeria, Escherichia, Bacillus, Staphylococcus. Vibrio,* and *Clostridium* (botulism) (4).

The nutrient composition of foods affects their safety both directly and indirectly. Diets high in saturated fat, cholesterol, salt, sugar, and calories may be associated with an increased risk of cardiovascular disease and cancer. Failure to eat an appropriate diet indirectly may affect health by diminishing the body's capacity to prevent certain diseases such as cancer (17, 33). Additionally, the fad diets followed by many Americans can be dangerous.

Toxic constituents are either inherent to the food (produced naturally by plants and animals, particularly marine animals), result from microbial infections, or result from human activities such as environmental pollution or chemicals used in the production and processing of whole foods and food products. Public attention focuses primarily on toxins arising from human activities, and it is not surprising that the public views these constituents as posing the most severe risks. However, scientists generally feel that the levels of these constituents present in foods are generally low enough that the risks posed by them are less than those posed by microbial contamination, nutritional imbalances, and natural toxicants (19, 28, 37).

In part, this situation results from the extensive regulation of toxic constituents. Indeed, the food safety laws place heavy emphasis on the premarket approval of food additives and pesticide use. These laws also seek to minimize microbial contamination via extensive inspection of food establishments and sampling and laboratory analysis of foods for microbes. However, there are numerous ways in which a food may become contaminated, and it is an ongoing battle to try to minimize these occurrences.

The development of new technologies used to produce food products has raised new public concerns about food safety. This chapter will present some of the scientific issues pertinent to those concerns. The chapter will begin with a discussion of how conventional food products are assessed for safety. A discussion of issues raised by new food products produced with biotechnology will follow. The chapter will close with a discussion of the applicability of traditional safety assessment procedures to these new products.

FDA ASSESSMENT OF FOOD AND FEED ADDITIVES AND ANIMAL DRUGS

As discussed in chapter 10, FDA does not perform premarket evaluations on whole foods, only on food, feed, and color additives and new animal drugs. The FDA has the responsibility of assessing the safety of substances added to food and livestock feed and of drugs administered to animals used for human food. The FDA assesses the safety of food additives for human consumption and for quality control. Feed additives are evaluated for safety to the animal. Residue levels of feed additives or metabolizes related to the additive in edible animal products must be determined and assessed for their safety to humans. Animal drugs are treated in a similar manner to feed additives—they must be safe and effective for the animal, and any residues left in edible animal products must be safe for human consumption.

For products that might have an environmental impact (animal drugs in particular), an environmental impact assessment is also needed.

The basis for a safety assessment of additives and drug residues for humans relies on determining the toxicity of the additive or drug and the likely levels of human exposure to the substance. Human safety assessments require attention to the levels of toxic substances present. In 1564, the physician Paracelsus stated "Everything is poison. There is nothing without poison. Only the dose makes a thing not a poison. This concept of dosage still underlies toxicity assessments today.

Ingestion of excessive quantities of any substance, even one necessary for survival, can lead to death. Vitamin A is a necessary nutrient in small quantities, but is highly toxic in large quantities (24). Sometimes the acceptable consumption range is narrow as is the case with vitamin A. Therefore the dose is a fundamental determinant of toxic potential. The dose that a human is likely to consume will depend on the toxicity of the compound for the individual consuming the food, the level of the compound in food, and the levels of intake of the food. Exposure levels will vary by individual and by cultural, economic, and geographic factors. People have the ability to detoxify and/or excrete a large variety of potentially toxic compounds (10, 58, 59). However, in the elderly, children, and infirm those abilities may be compromised, raising their susceptibility to toxins in foods.

Firms seeking the approval of a food additive must submit a petition that contains information about the chemical identity of the substance, the anticipated level of consumption of the additive, and documentation of the efficacy of the additive for its intended use. Firms must also provide analytical methods to detect the additive and any related metabolizes that might result from use of the additive in food. Firms must also submit toxicity testing data.

Toxicological testing is conducted to ensure that the product is safe for its intended use and is required not only for the substance itself, but for any other substance that may form in and on food as a result of the use of the additive. Metabolic and pharmacokinetic studies are required to assess the fate of the test substance in the body. These studies help to identify metabolizes that might pose toxic risks.

Photo credit: U.S. Department of Agriculture, Agricultural Research Service

Microbiologist checks growth medium **for visual evidence of harmful foodborne bacteria.**

The extent of testing required depends on the chemical structure of the ingredient, and on its intended level of use and consequent human exposure. Compounds whose chemical structures are such that they are unlikely to pose toxicological risks and those with low human exposure potential require only limited toxicological testing.

Full toxicological testing is required for high-use substances, especially when the chemical structure is judged not to lend itself to rapid and complete metabolism to innocuous end products. Full toxicological testing of food ingredients includes acute, subchronic, and long-term (including carcinogenicity) testing; impacts on reproduction; teratogenicity (ability to cause birth defects) testing; and genotoxicity (ability to mutate genetic material) testing. These tests are performed in multiple species.

Toxicology testing of additives is conducted by administering large doses of the test substance to an animal. The amount administered to animals is in increments so

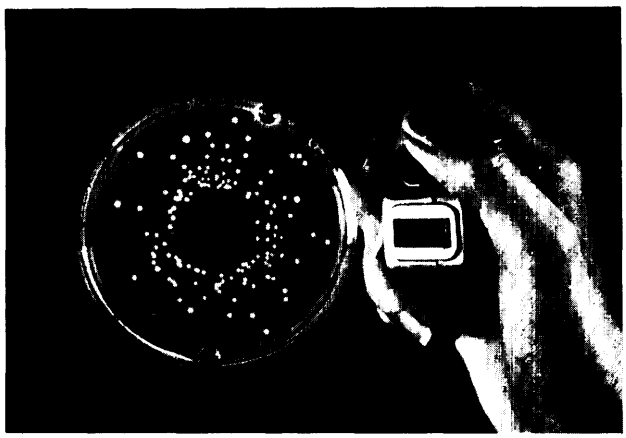


Photo credit: U.S. Department of Agriculture, Agricultural Research Service

Fluorescent light illuminating colonies of bacteria in growth medium aids researchers in counting organisms present in studies that help ensure food safety.

that it can be determined what maximum daily dose can be administered without producing evidence of toxicity (i.e., the no observed effect level or NOEL). To be acceptable for inclusion in the diet of humans, food additives must have a margin of at least 100-fold between the highest estimated human exposure and the NOEL (20, 30).

If the product is intended for use in a food-producing animal, it must also be tested for human safety in a manner similar to food additives. The manufacturer must develop analytical methods to detect and measure feed additives, drug residues, and other potential metabolizes related to the additive or drug, in edible animal products. Residue levels are usually determined for muscle, liver, kidney, and fat, and where applicable, in skin, milk, and eggs (yolk and egg white). The length of time required for residues to be eliminated from animal products must also be determined (54).

The extent of testing required is tied to the degree of concern (anticipated hazard). This provides a flexible and scientifically valid procedure for assessing the safety of food ingredients, and allows for the safety evaluation of a wide range of food additives. It can be used to evaluate chemically synthesized or microbially derived additives, and drug residues resulting from medicated feeds or direct application to livestock. However, this method is not appropriate to evaluate the safety of whole foods, because toxicity is determined by feeding test animals large quantities of the ingredient. It is not possible to feed large quantities of whole foods needed to induce toxicity without so radically changing the metabolism of the test animal as to invalidate the results of the test. This constraint has not been a problem in the past, primarily because whole foods have traditionally been viewed as Generally Recognized as Safe (GRAS), and have not undergone formal toxicity testing. This situation may change with the development of new biotechnology products (20).

Quality Control

The FDA requires manufacturers to submit an extensive dossier of information pertaining to the method of manufacture of food or feed additives and of animal drugs. Detailed studies of the chemistry and purity of substances under the proposed conditions of manufacture, and information pertaining to their intended use is required. Petitions must include a description of the methods, facilities, and controls used to manufacture, process, and package the new product in sufficient detail to demonstrate that the methods will preserve the identity, strength, quality, and purity of the ingredient. Methods used in the synthesis, extraction, isolation, or purification must be described. Analytical procedures must be available that are capable of determining the active components with reasonable accuracy and of assuring the identity of such components, These procedures must have adequate sensitivity to determine the amount of the new ingredient in the final product.

Animal Safety Assessment

If the new product is a veterinary drug or feed additive used for livestock, the safety and efficacy of the product for the animal must be documented. Evidence must be provided that the drug or additive performs as claimed under the conditions of use specified in the petition. Animal drugs and feed additives must be tested for toxicity in all species of animals for which they will be used. Similar to food additives, the level of toxicity testing depends on the perceived risk of the substance. Drugs and additives may require acute, subacute, and chronic toxicity testing. Drug side effects must be evaluated. Reproductive effects may also be examined.

Environmental Assessment

The National Environmental Policy Act (NEPA) of 1969 requires Federal agencies to prepare a statement of the environmental impact of every major Federal action that significantly affects the quality of the human environment. Typically, the environmental review begins when industry submits a food additive petition, although FDA has the responsibility to evaluate any action within its jurisdiction that may significantly affect the environment.

Firms must either file for categorical exclusion from the requirements or submit an environmental assessment (EA).

Categorical exclusions include any actions under FDA authority that do not result in the production, distribution, or introduction of substances into the environment. Such actions might include inspection requests, changing labels, etc. Additionally, some additive and drug petitions seeking GRAS affirmation may also be excluded from the EA requirement. Examples would include products already marketed for the use for which the affirmation is sought and which are not toxic to organisms in the environment at expected levels of exposure (21 CFR 25.24(b)(7)).

Environmental assessments include, for example, data concerning the identification of the substances, physical containment procedures, waste stream treatment procedures, fate of the substance in the environment, and any special precautions taken to minimize release as a result of nonroutine or accidental situations. Information on traits that would limit survival, growth, or activity of organisms if released into the environment should be included. Verification of compliance with State and local requirements is needed (21 CFR 25.3 la). If the EA indicates that there might be adverse environmental impacts, then a full environmental impact statement may be required.

EPA ASSESSMENT OF RESIDUE TOLERANCES

The Environmental Protection Agency (EPA) has responsibility for determining the safety of pesticide residues in or on food for humans, or feed for domestic animals that are used for human food. Before a pesticide can be registered for use on a food or feed crop, either a tolerance or an exemption from the requirement of a tolerance must be established. A tolerance is the maximum level of pesticide residues that can be present in or on raw agricultural commodities, food, or feed transported in interstate commerce. Tolerances or exemptions from the requirement of a tolerance, must be established for each active and inert ingredient contained in the pesticide and for each raw commodity, processed commodity, and livestock species that might contain residues of the pesticide.

In a manner similar to FDA risk assessments of food additives, the EPA conducts a risk assessment to establish, or exempt from the requirement, a pesticide residue tolerance in food and feeds. This assessment includes identifying the existence and type of hazards that may be caused by pesticides; evaluating the relationship between the amount of the pesticide administered and the incidence of any adverse effects; and determining probable human exposure to the pesticide (53).

For pesticides used on raw agricultural commodities, [1] EPA tries to determine whether or not the pesticide can be used in such a manner that it is reasonably certain that no injuries will result in humans even after a lifetime of exposure. The risk assessment is based on the toxicology and residue data submitted by the petitioner.

Several kinds of data must be included when a petition is submitted for the establishment of a tolerance or exemption from a tolerance (21 U.S. C. 346a (d)). Required data include:

1. the name, chemical identity, and composition of the pesticide chemical;
2. the amount, frequency, and time of application of the pesticide chemical;
3. full reports of investigations made with respect to the safety of the pesticide chemical;
4. the results of tests on the amount of residue remaining, including a description of the analytical methods used;
5. practicable methods for removing residue in excess of any proposed tolerance;
6. proposed tolerances for the pesticide chemical if tolerances are proposed, and
7. reasonable grounds in support of the petition.

Petitioners also may be required to submit an analytical grade standard sample of the pesticide so that the adequacy of the residue detection method can be evaluated.

Residue chemistry data are designed to provide the information necessary to determine the site, nature, and magnitude of residues in or on food or feed. The purpose of the data is to identify what chemical residues are present and in what quantities. These data, along with information on use patterns of the pesticides, are used to determine dietary exposure levels. Information required includes qualitative data on the metabolism and degradation of the pesticide, quantitative data on the

[1] Raw agricultural commodities are considered to be fresh fruits, vegetables, grains, nuts, eggs, raw milk, and meats as opposed to foods that have been processed, fabricated, or manufactured by cooking, freezing, dehydrating. or milling among other processes (40 CFR180.1(e)).

magnitude of the residue in plant or animal tissues, and analytical methods to detect residues.

Residues present when a crop is harvested may not be identical to the applied pesticide. Environmental and host plant factors can degrade or metabolize an applied pesticide to form a variety of metabolizes. Plant metabolism data is collected to identify any types of pesticide residues that actually remain in agricultural crops as a result of these transformations. Field trials are conducted to determine the magnitude of the identified residues under conditions that simulate the way the pesticide will be used commercially. These data provide information about the kinds of residues likely to be present in raw agricultural commodities, as well as the amount of residues expected after pesticides are used in an approved manner.

Pesticide residues, degradation products, and metabolites all are tested for toxicity. Acute toxicity testing is required of all residues and provides information on the health hazards likely to arise from a single exposure to any toxic components associated with the pesticide. Changes in behavior, body weight, clinical symptoms, mortality, and tissue pathology among other symptoms are noted. Additional subchronic and chronic toxicity testing, oncogenicity testing, teratogenicity testing, neurotoxicity testing, and reproductive and fertility testing may be required depending on the pattern of *use* for the pesticide, its physical or chemical properties, the expected exposure of nontarget organisms, and the results of the acute toxicity testing.

As with FDA testing of pesticides, EPA toxicity testing involves feeding test animals large quantities of the pesticide to determine the dosage level at which the pesticide shows no observable or measurable effects in treated animals when compared to control animals (the no observed effect level, NOEL). Because of uncertainty in extrapolating data from test animals to humans, the NOEL is divided by a safety factor to determine the maximum levels considered safe for human consumption. The safety factor may vary depending on the type of data submitted and the chemical evaluated, with a factor of 100 the minimum generally used.

The EPA calculates a total amount of residues that a person can be exposed to in the daily diet. Based on residue data obtained from field testing, a petitioner may propose a safe tolerance level for humans. This proposed tolerance is multiplied by the number of commodities treated with the pesticide and the average consumption of the commodity by the general public. Similar exposure levels also may be calculated for specific groups that may be particularly sensitive to a pesticide, such as pregnant women and infants.

The EPA then compares the maximum level of residues considered safe with the total theoretical exposure level. If the maximum level considered safe is greater than the total theoretical exposure level, then usually the proposed tolerance level is established as the tolerance level of the pesticide in raw agricultural commodities. If, however, the maximum level considered safe is less than the total theoretical exposure level, EPA may reject the proposed tolerance level, or request further review.

With raw agricultural commodities, it maybe possible to establish a tolerance for pesticides that are carcinogenic. Additional risk assessments to determine the additional cancer risk will be conducted. Usually, if the additional cancer risk is less than 1 in a million, the proposed tolerance for the pesticide will be accepted.

Livestock feeding studies are required whenever residues result in or on crops used as feed items. Animal metabolic studies are conducted to determine the types and levels of residues present in edible animal tissues, such as meat, poultry, milk, or eggs.

Processing studies are required to determine whether residues in raw agricultural commodities can concentrate or degrade when those commodities are processed. If residues do not concentrate on processing, the tolerance established for the raw commodity applies to all processed food or feed derived from the commodity. If, however, residues concentrate on processing, a pesticide tolerance level must be established for the processed product (51).

Exemptions from the requirement of a tolerance can be granted if it appears that no hazard to public health will result from residues of a pesticide. Data that may be required to support an exemption include residue chemistry, product chemistry, and toxicology data, including subchronic toxicity, teratology, and mutagenicity studies.

Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA regulates microbial pesticides used in food and feed crops as well as chemical pesticides. To register a microbial pesticide, toxicity testing is required. Such testing might include acute oral, pulmonary, dermal, and intravenous administration. Additional subchronic and chronic toxicity testing may be required, as well as oncogenicity, mutagenicity, teratogenicity, and pathogenicity studies. The EPA has established protocols for such testing (57). Generally, however,

microbial pesticides have been exempted from the requirement of a tolerance.

POTENTIAL FOOD SAFETY CONSIDERATIONS INVOLVING BIOTECHNOLOGY-DERIVED FOODS

Currently, there is no evidence that whole foods, food or feed additives, animal drugs, or pesticides produced with biotechnology methods create greater food safety risks than these same products produced with traditional methods. However, biotechnology results in a new class of products, with which we have little experience. This lack of experience, combined with the novelty of the types of genes that can potentially be transferred, has raised concerns about the safety of such products.

Speculation about the potential food risks associated with biotechnology products has focused on the same general areas of concern as apply to traditional food products—namely, microbial contamination, nutritional imbalances, and presence of toxic constituents. The major new concern is whether or not the new technologies increase the potential for microbial contamination, whether or not they could lead to nutritional imbalances, and whether or not they might add new toxins or increase the levels of existing naturally occurring or synthetic toxins in food.

Potential To Affect Microbial Contamination of Foods

Several factors play a role in the growth of microbial organisms in food. Factors such as pH, type and concentration of acid, water activity, concentration of sodium chloride and other electrolytes, availability of nutrients and growth factors, and the levels of microbial growth inhibitors all function to inhibit or enhance the potential for microbial contamination and growth. Any change in the composition of a food that affects one or more of these factors will influence the chances of that food causing illness (37).

Products produced using biotechnology could potentially alter some of these factors in ways that could increase the potential for microbial contamination. For example, the development of low-acid fruits and vegetables might increase the possibility of botulism. Most tomatoes have a pH of 4.5 or lower, but some low-acid varieties are pH 5 or greater. When canned or processed, such low-acid foods are more likely to support the growth and toxin production of *Clostridium botulinum* than are high-acid varieties.

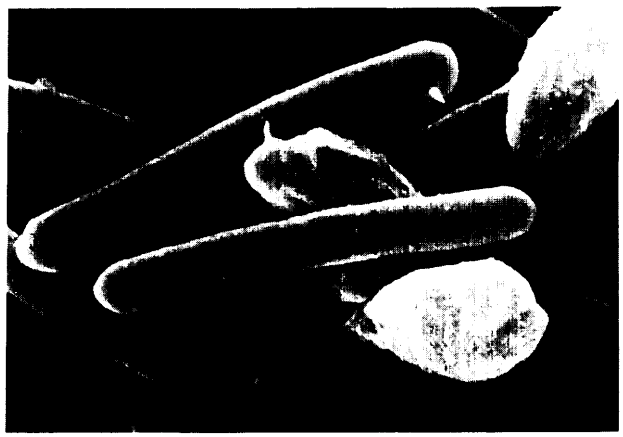


Photo credit: U.S. Department of Agriculture, Agricultural Research Service

Clostridium botulinum is a toxin-producing food spoilage organism and dangerous human pathogen. The sac-like dormant spores can survive conditions that are lethal for the rod-shaped bacterial cells. Magnification is about 8,500 times.

Removal of substances that act as microbial growth inhibitors may also increase the potential for contamination. For example, there is some evidence that caffeine in coffee beans may suppress aflatoxin production (3 1). Suppression of caffeine production in coffee beans could increase the potential for contamination. It may also be possible to introduce nutrients into foods previously lacking in sustenance. If the introduced nutrient is a required growth factor for a particular microbe, its introduction might enhance the potential for infection by the microbe.

Changes in factors that affect microbial growth might also be in the direction that inhibits the growth of pathogens. For example, delayed softening of tomatoes may decrease the potential for mold and bacterial growth. Nor is the potential for such events to occur limited to foods produced with biotechnology. Low-acid tomatoes produced with traditional methods are currently being marketed. But new technologies do warrant an awareness of the potential to enhance microbial contamination.

Potential To Affect Nutritional Content of Foods

Whole foods are compositionally highly complex. They contain carbohydrates (e. g., starches, sugars, gums, cellulose, etc.), fats, proteins, minerals, vitamins, enzymes, genetic materials (e.g., DNA, RNA), waxes, plant pig-

ments, essential (volatile) oils (e.g., peppermint and citrus oils), alkaloids, and many other compounds. The levels and types of these constituents present vary significantly between species due to different genetic composition, but even within the same species, or even variety, the levels can vary substantially as result of different environmental factors. Different soil types, sunlight, rainfall, temperature, and agricultural practices such as irrigation, planting date, maturity at harvest, and storage conditions substantially can alter the level of food constituents present (20).

Information is available concerning the normal levels of major nutrients in several food products. In general, levels of nutrients vary by two- to three-fold in foods, although higher levels of variation are seen. For example, the level of beta-carotene (a precursor of Vitamin A) in carrots ranges from O to 370mg per 100 grams of tissue (42). In general, such nutrient variation does not pose severe problems for humans, because humans eat a wide range of food products, even in situations where there is heavy dependence on one source of food for most of the calories in the diet. Thus, diet quality is measured by the sum total of everything eaten, and is not generally based on a single crop or product. Decreases in the nutritional value of one crop may not be significant unless that one product is the major or only source of that nutrient for the population. Likewise, increases in a nutrient compound may not be significant unless the population eats large quantities of the food containing the compound and/or the compound has a narrow range of toxicity acceptability (35).

Because of the diversity of food products available in the United States, alternative sources of major nutrients are available. However, some foods do constitute the major source of particular nutrients. For example, Americans rely on milk and milk products as the major food source of calcium, on oranges and orange juice to provide most of the vitamin C consumed, and on carrots to provide the precursors of vitamin A. Furthermore, some nutrients (e. g., vitamins and minerals) can be toxic in high levels, and a significant increase could potentially pose some risks. Thus, an evaluation of the levels of nutrients in individual food types and the amount of the different food types eaten will determine if there is likely to be a nutritional impact.

Nutritional impact depends not only on the amount of the nutrient present in the food, but also on how much of the food is consumed by an individual, and the individuals physiological state. If technological changes significantly alter the types or amounts of foods eaten, nutritional risks could result from this changed behavior. For example, technological changes that alter the growing season or geographical region where foods are grown could alter the seasonal or quantitative availability of some foods such that consumers may eat more or less of that particular food. Technology could change the quality of the food (e. g., decreased fat in meats, altered taste of the food) such that consumption patterns would change. Uncertainty about technologies used to produce some foods may cause some consumers to avoid that food. For example, surveys have indicated that if bovine somatotropin (bST) is used to produce milk, some consumers will decrease their consumption of milk and milk products, potentially with a consequent reduction in calcium consumption. Thus, use of technologies that result in significant changes in consumption patterns may also create nutritional risks.

Whereas some biotechnology research is conducted for the purpose of altering the nutritional composition of foods (i.e., efforts to decrease the fat content in meat products and to increase lysine in corn), potential may also exist to inadvertently alter a critical nutrient biosynthetic pathway. If an important nutrient like vitamin C was inadvertently reduced in oranges, this potentially could have significant nutritional implications. Some nutrients are needed in small quantities but are toxic in large quantities. Increases in the levels of these nutrients could be significant from a food safety standpoint. Nutrient levels in normal foods differ significantly. Additionally, changes in acidity or solidity, for example, may alter the ability of food constituents to be utilized as nutrients. Similar to the development of new varieties using traditional breeding practices, which can also alter the level of important nutrients, careful attention should be paid to whether there are significant changes in the level of important nutrients between a biotechnology-derived food and its traditional counterpart, or whether the change is within the normal range of nutrient variation for foods.

Potential To Affect Toxic Constituents of Foods

In addition to the potential impacts of biotechnology on microbial contamination and nutrient composition of foods, there is concern that new biotechnology-derived food products may have new and/or increased levels of toxic compounds. Postulated mechanisms for this increased toxicity include:

1. the transferred gene(s) code for toxic compounds,
2. the transferred gene stimulates the production of secondary compound(s) that are toxic,
3. the marker genes used for identification of transformed cells code for toxic compounds,
4. the production of naturally occurring toxins increases unexpectedly as a result of the undirected insertion of the transferred gene into the host genome (the so-called pleiotropic effects), or
5. plants unexpectedly accumulate environmental toxins (e.g., heavy metals) in edible tissues (likely only to be a problem for plants developed to grow in contaminated soils) (16, 20, 30, 44).

When transferring genes into host organisms, a genetic construct consisting of the gene to be transferred, marker sequences to identify those organisms that have been genetically transformed, and regulatory elements such as promotors and enhancers that control the operation of the transformed gene are all inserted into the new organism. Additionally, in many transformations, vectors are used to insert the gene construct, and it is possible that some of the vector DNA could also be inserted into the host organism. Some groups have expressed concerns that the expression products coded for by these genes may be toxic.

Primary and Secondary Gene Products

When a gene is transferred into a host organism it produces a gene product (protein). This gene product may be the final active product, or it may act as an enzyme or hormone that mediates the production of other compounds. Gene products may therefore have direct, primary effects and indirect, secondary, or compensatory effects. Most proteins are generally nontoxic, and no known proteins exhibit mutagenesis or carcinogenesis. A few highly specialized proteins (i.e., cytotoxins, enterotoxins, and neurotoxins) are acutely toxic, but these proteins are generally well characterized as to their source and mode of action. Proteins, unlike some chemicals, are constantly being degraded in an organism and do not accumulate in tissues (21, 22).

Most proteins are readily degraded during digestion but a few do not. These exceptions are characterized by fairly well-understood chemical interactions that stabilize parts of the protein molecules, thereby enabling them to survive digestion partially intact. Most of these protein fragments are excreted in the feces, but some enter the blood stream where they may elicit immunological reactions. Allergic reactions have been documented to protein products found in many common foods including nuts, peanuts, chocolate, barley, rice, wheat, citrus, melons, bananas, tomatoes, spinach, corn, potatoes, and soybeans. In some cases, the immunological agent is known to be a protein, while in other cases a glycoprotein (a compound containing both a carbohydrate and a protein) is involved. With glycoproteins, it is not known whether it is the protein portion or the carbohydrate portion that is causing the immune response. Both components could be affected by genetic modification (21, 22).

In cases where a gene product mediates another chemical reaction, indirect effects from gene transfers may occur. For example, the primary gene product might be an enzyme that catalyzes production of another product. Alternatively, the gene product might be a protein hormone that itself produces biological effects as well as stimulates production of other compounds that have biological functions. As an example, somatotropins (growth hormones) are protein hormones that elicit several physiological responses in the body, such as enhancing protein accretion in immature animals. Somatotropins also stimulate the production of other compounds such as insulin-like growth factors (IGFs), which also elicit physiological responses. Thus, in this example, the primary gene product would be somatotropin and the secondary gene product would be IGF.

Unlike the primary gene expression product which is a protein, secondary products do not necessarily have to be proteins. Enzymes can catalyze the production of other proteins or the production of other classes of chemicals such as carbohydrates, fats, etc. Protein hormones may also stimulate the production and release of steroidal hormones. Concern has been expressed that these nonprotein secondary metabolizes may be chemically more stable, may accumulate in body tissues (most notably fat) and may be easier to absorb through the digestive system than protein products. Thus, indirect effects of gene transfer may be an important food safety consideration if the organism compensates for, or responds to, the primary gene product by producing increased levels of a compound that displays oral toxicity (22). Food safety evaluations must include an assessment of both primary and secondary products.

Marker Genes

Various marker genes, including antibiotic resistance genes, are used in biotechnology as a means of distinguishing between cells that have been genetically transformed and those that have not—only the transformed cells will contain the resistance gene. Many first-generation transgenic plants use as a marker the gene for

neomycin phosphotransferase, an enzyme that converts the antibiotic kanamycin to an inactive form. Some groups have expressed concern over the inclusion of such marker genes in genetic constructs. Possession of a particular antibiotic resistance gene allows an organism to grow in the presence of normally toxic levels of the particular antibiotic the enzyme deactivates. Thus, the organism is resistant to that antibiotic.

The primary issues raised with respect to the use of antibiotic resistance genes as markers are not those of toxicity per se. Rather, the issues focus on whether or not potential exists for the transfer of antibiotic resistance from food products to intestinal bacteria during digestion (thus creating a strain of intestinal bacteria resistant to kanamycin) and whether the presence of these genes will interfere with therapeutic antibiotic administration.

Evidence to date does not suggest that naturally occurring antibiotic resistant organisms transfer resistance in the intestinal environment. Usually DNA rapidly degrades in the presence of acids and enzymes contained in the gastro-intestinal tract, significantly decreasing the likelihood of an intact gene being transferred. Additionally, the promoter sequences used with the kanamycin resistant gene are plant promotors rather than bacterial promotors; plant promotors do not function in bacteria so there is some question as to whether the gene would be active even if transferred. Additionally, kanamycin resistance is relatively common among soil microorganisms, and thus it is likely that humans are already consuming the gene. The likelihood of the transfer of kanamycin resistance seems remote.

A more pertinent concern with respect to the use of antibiotic resistance markers is the possibility that they could interfere with therapeutic antibiotic administration if the food containing the resistance enzyme is ingested with antibiotic administration. This may not be significant for kanamycin, but the issue must be addressed. The FDA has been petitioned for an advisory opinion concerning the use of the kanamycin resistance marker in transgenic plants. To date, FDA has not issued that opinion.

In addition to the kanamycin resistant marker, other marker genes can and are being used. Such genes include those that code for herbicide tolerance and the lacZY color marker. Concerns have been expressed that these markers might also create novel gene products or associated secondary metabolizes. Once again, the expression products and the genes coding for these markers are well characterized and have been studied for many years, so it seems unlikely that they would cause safety problems.

In general, the marker genes currently used code for gene products that are well characterized and have been part of the food system for many years. Use of such markers significantly reduces the potential for food safety problems. Additionally, new research showing that these genes can be successfully removed from transgenic crops may eliminate many of the concerns associated with the use of marker genes.

Vector Material

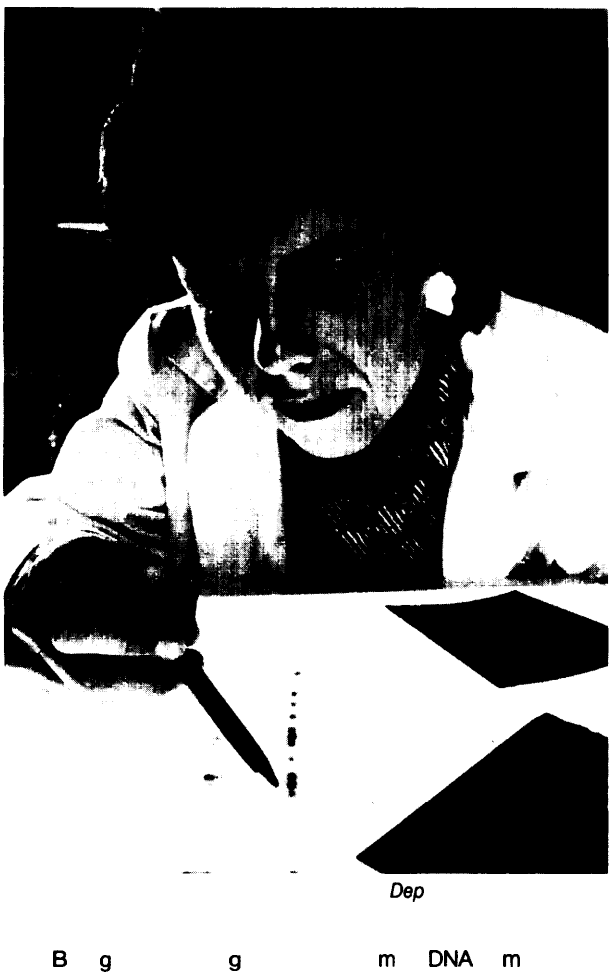
Viral or bacterial plasmid vectors are sometimes used to transfer genes from one organism to another. Some groups have expressed concern that these vector sequences may code for toxic substances. The likelihood of this occurring is significantly decreased by using vectors derived from microbes that do not produce toxic substances or that are not closely related to microbes that produce toxic substances. Additionally, use of plasmids from bacteria that have a long history of use in food products decreases the likelihood that the vector used will code for toxic substances. It is a commonly accepted practice to use vectors with these characteristics in genetic engineering (e. g., the Ti plasmid of Agrobacterium tumefaciens). Thus, the probability of vector toxin production is low.

Unexpected Pleiotropic Effects

A primary concern raised with respect to biotechnology products is the potential for pleiotropic effects. Pleiotropic effects are secondary changes in metabolism (i.e., phenotypic alterations) that result from a single genetic change (50). Genetic material contained in cells is composed of sequences of DNA that code for gene product (the coding regions), sequences of DNA involved in controlling gene expression (regulatory sequences), and sequences of DNA for which there is no readily apparent function (the noncoding regions). The majority of the plant genome consists of this noncoding DNA.

As discussed in chapter 2, when genes are introduced into a plant, there is little control over where the gene is inserted. The new gene can be inserted into the coding regions of a host gene, into the noncoding regions of a host gene, or within regulatory regions of a host gene. This undirected insertion of the gene raises the possibility that

- the site of insertion will affect the level of expression of the introduced gene itself,
- the site of the insertion is such that host organism genes will be activated or inactivated, or



. the site of insertion will be such that there will be no inadvertent effects on the host organism (20).

Gene expression levels (i.e., the amount of gene product actually produced) vary depending on a number of factors, including the number of copies of the gene incorporated into the host and the position of the gene within the host genome. The mechanism by which insertion site affects expression levels is not fully understood; however, the insertion site and expression levels are passed on to the offspring in a consistent manner (20, 41). Expression levels of the gene may be too high or too low or absent altogether. Depending on the nature of the gene, overexpression may be detrimental to the host organism itself, or pose food safety risks for human consumption. Underexpression, and particularly no expression, may pose no technical safety issues, but may still raise concerns among a public uncertain about the process of biotechnology itself. This situation has arisen with transgenic animals in which the gene was incorporated into the host genome, but not expressed. Some consumer groups have opposed the slaughter of these experimental animals. The Food Safety and Inspection Service (FSIS) is in the process of formulating guidelines for the slaughter of these types of animals.

Insertion of the foreign gene into noncoding regions of the host DNA may cause no disruption of any of the host genes. These regions do not code for gene expression products, so disruption of these sequences is unlikely to result in the activation or inactivation of host genes. The more likely consequence would be production of the expression product of the inserted gene itself, rather than any unexpected pleiotropic effects (20, 21).

The third, and likely most significant possible pleiotropic effect of gene transfer, involves the activation or inactivation of *host* organism genes as a result of undirected insertion of the foreign gene. The foreign gene may insert into the coding sequences of a host organism gene or into the regulatory sequences of a host organism gene.

Insertion of foreign genetic material into a regulatory sequence of the host organism could destroy the ability of the regulatory sequence to control the expression of host organism gene(s). The foreign gene construct that is inserted into the regulatory sequence of the host gene, moreover, contains a regulatory sequence itself, which could affect the expression of the host gene(s). The foreign regulatory sequence may be activated under different circumstances than the host organism's regulatory sequence, thus altering the expression of the host organism genes in terms of amount, timing, and/or tissue location. The significance of this occurrence may, in part, be influenced by whether or not the promotor sequence used is inducible (controlled by specific stimuli) or constitutive (turned on all the time) (20, 21, 56).

If the foreign gene is inserted into the coding region of a host gene, then the most likely outcome would be the inactivation of the host gene. This is because the foreign gene must be inserted in the proper place and in the proper direction (i. e., the sense direction) for activation to occur. Insertion into improper sites (even if in the proper direction) or in the backwards direction (i.e., the antisense direction) will cause the gene to be deactivated. The probability that the foreign gene will be inserted in the improper position or wrong direction is higher than the probability that the gene will be inserted into the proper position and direction (20).

Host gene inactivation could present food safety risks if, for example, it led to decreased levels of nutritional

components. Inactivation of enzymes or hormones that play key roles in biosynthetic pathways could lead to the use of alternate pathways and the potential buildup of some secondary metabolizes.

If the insertion of foreign genetic material is such that host organism genes are activated (i.e., the insertion is in the regulatory rather than coding region), several possible outcomes could result, some of which could pose food safety risks. Activation of host genes could result in increased levels of naturally occurring toxins or the appearance of those toxins in plant tissues where they do not normally appear. For example, a toxin normally produced only in the leaves of a plant now may be produced in the seeds as well. Other possibilities include the increased uptake and concentration of environmental contaminants by the organism (6, 11, 14, 23, 30, 32, 36). It is more probable that foreign gene insertion will lead to host gene deactivation rather than activation, although the probability of host gene activation is not zero.

Ten times more DNA is contained in the coding regions of genes than in the regulatory regions. Therefore, if gene insertion is truly random, gene inactivation by insertion into a coding region of a gene is about 10 times more likely to occur than gene activation by insertion into a regulatory region of a gene. However, it is possible that gene insertion may occur preferentially in selected areas of the genome (e. g., in active genes) rather than in a completely random fashion. If this is the case, host gene activation might occur with a higher probability.

The potential activation of genes that code for natural toxins in the organism is of particular concern. Plants are known to contain hundreds of toxic compounds, and it is likely that they contain many more that have not been identified. For example, roasted coffee is known to contain at least 826 volatile compounds that could potentially have toxic effects (1). At least 148 naturally occurring food compounds have been demonstrated to have acutely toxic effects in experimental animals, livestock, or humans when consumed.

In humans, most of the toxic effects of food have occurred as a result of abnormal diets or substance abuse, but at least 14 food compounds can be acutely toxic under certain circumstances even when consumed in quantities within the range of normal dietary intake (table 1 l-l). For example, the solanine content of white table potatoes normally ranges from 2 to 20 mg/10Og of tissue, but abnormal weather conditions can raise the concentration. Just 100 mg of solanine is enough to evoke death in some individuals. Low cyanogen varieties of cassava, if improperly prepared, are capable of yielding 20 to 40 mg of hydrogen cyanide per kilogram of cassava. However, some varieties of cassava can yield 20 times that much hydrogen cyanide, which is enough to be deadly. Increased levels of these known naturally occurring toxins as a result of biotechnology would certainly present food safety risks (20, 23, 26, 30, 32, 60).

Table n-l-Naturally Occurring Toxins in Foods That Have Been Documented To Have Acutely Toxic Effects on Humans Consuming Normal Diets

Toxic compound	Food source
Acetylandromedol	Honey
Andromedol	Honey
Anhydroandromedol	Honey
Desacetylpieristoxin	Honey
Gelsamine	Honey
Tutin	Honey
Hyenanchin	Honey
Cicutoxin	Milk (19th century America from water hemlock)
Hypoglycin A	Ackee fruit
Linamarin	Lima beans and Cassava
Lotaustralin	Lima beans and Cassava
Solanine	Potatoes
Curcurbitacin E	Squash, Cucumber
Nitrates	Spinach, and other green leafy vegetables

SOURCE: International Food Biotechnology Council, 1990.

Sufficient knowledge has accumulated regarding naturally occurring acutely toxic plant toxins to provide assurance that the food supply contains either safe levels of these toxins or can be processed in a way that minimizes or eliminates their acute health effects. However, much less is known regarding the role in chronic disease of naturally occurring plant toxins currently consumed (30). A large number of naturally occurring compounds frequently found in foods appear to be mutagens and possible carcinogens. For example, of the 826 volatile compounds contained in coffee, only 21 have been tested for chronic effects, and 16 of them were found to be carcinogenic in rodents (1, 2, 3). Potentially, the number of such substances could reach into the thousands, we know neither their identities, their normal concentrations, nor their long-term impacts on human health (1, 2, 3, 20).

Compared to plants, substantial literature on microorganisms and their toxins relative to foodborne illness exists. Considerable information concerning the genetic and/or environmental determinants of microbial toxin expression is also available. This information could be used to structure strategies for determining the safety of microbially derived food products (7, 20, 30, 38). The microbial toxins of primary concern are those that are

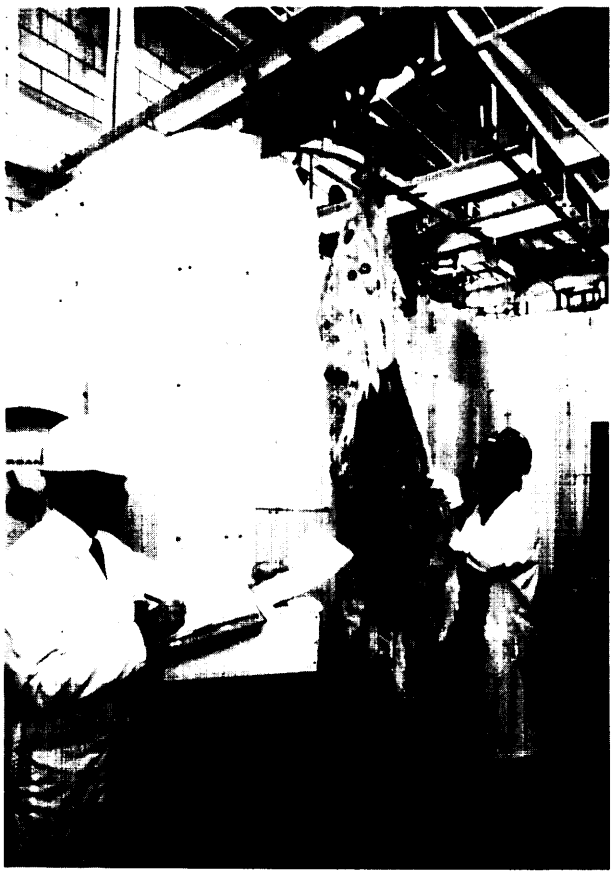


Photo credit: U.S. Department of Agriculture, Agricultural Research Service

Microbiologist obtains samples for microbial analysis from carcass, Production of toxins by animals consumed for food purposes is rare.

active orally and are known to be produced by organisms related to those used in food processing.

The production of toxins by animals consumed for food purposes is rare and is generally limited to a few marine species such as the puffer-fish (6, 23, 32). Severe insertional effects are likely to be uncommon because such impacts would probably lead to the death of the embryo. Insertional effects could potentially affect the manner in which an environmental toxin is detoxified, or could increase the accumulation of contaminants such as heavy metals, pesticides, or orally active compounds in edible tissues. A significant change in the accumulation of such compounds is likely to be detrimental to the animal itself; thus, the health of the animal serves as a preliminary screen for toxic effects (5).

Plietotropic effects might also result from using tissue-culture techniques to regenerate genetically transformed cells. When the genetic material is transferred into a cell, the cell must first be regenerated into a whole plant using tissue-culture techniques before additional breeding can occur. Plants regenerated from cell tissue culture have sometimes shown striking differences among themselves and from the parent cells from which they were regenerated. The process of separating mature plants cells and regenerating those cells into whole plants releases a pool of genetic diversity inherent in the plant. This process is referred to as somaclonal variation, and it is being used in traditional breeding programs to identify new traits that might be of agricultural interest. Because transgenic cells are regenerated into whole plants, it is possible that some unexpected gene products might be expressed in the mature plant. Frequently, the gene expression of these somaclonal variants is not stable, and they are not inherited by subsequent generations, but this does not hold in all cases (34).

For whole foods that rely almost exclusively on the use of tissue culturing in the breeding program, these effects might be significant. For many transgenic plants (i.e., grain crops), however, they may not be. Transgenic crops will not immediately go from the laboratory to the dinner table. In some cases, backcrossing with traditionally bred lines may be needed. Even if the gene is transferred to a well-adapted parent line, the stability of the gene and the agronomic performance will need to be determined. Thus, if a key enzyme is deactivated, an essential pathway disrupted, or detrimental somaclonal variants occur, it is likely that the crop will not perform well in field trials and will be screened out and never commercialized. Likewise, as a result of the Lenape incident, screening has improved for compounds known to be acutely toxic to humans if consumed in high enough quantities. While these methods do not guarantee that all unexpected and undesirable effects will be detected prior to commercial release, it is likely that many of the more significant ones will be.

Unexpected results can and do happen frequently as a result of traditional breeding. This situation is not unique to biotechnology. Indeed, many of the issues raised today concerning biotechnology are the same concerns raised in the 1970s with respect to the development of new crop varieties by traditional breeding and the use of chemicals and irradiation to mutate microorganisms. The majority of these unexpected effects that occur have not been demonstrated to cause severe food safety risks, although on rare occasions there are exceptions. Unexpectedly high levels of toxic compounds have occurred as a result of traditional breeding. The classic example is the development of a new potato variety (Lenape) in the 1970s.

This new variety had better processing characteristics and enhanced disease resistance over traditional varieties. It also had significantly elevated levels of solanine, which were fortunately discovered before any illnesses resulted (20, 30, 61). However, this example involves a crop that contains a known, acutely toxic compound. Most crops do not have such compounds. Therefore, even if unexpected effects do occur as a result of biotechnology, they may not present significant food safety risks just as they do not when they occur during traditional breeding. Clearly, particular attention should be paid to those crops known to produce highly toxic compounds.

APPLICABILITY OF CURRENT SAFETY ASSESSMENT METHODOLOGIES TO THE PRODUCTS OF BIOTECHNOLOGY

The FDA does not routinely review for the safety or toxicity of food, only for food additives. Food additives generally are synthetically produced in batch quantities and added to foods. Because of the way these compounds are produced, a safety assessment approach has been established that administers large quantities of the additive to animals to determine at what level any toxic effects may occur. Concentrations of additives must be well below the level at which any toxic effects may have occurred. This type of approach will be difficult to apply to genetically modified whole foods.

Whole foods are complex mixtures of chemicals, not single chemicals. It is not possible to feed whole foods in quantities sufficient for toxicity assays without simultaneously producing gross disturbances in the nutrient balance and physiology of the test animal, which invalidates the results of the test. Experiments involving whole foods fed at the levels approximating the intended use for humans lack the sensitivity to detect anything but the most potent toxins. Thus, conventional procedures of toxicological investigation lack the sensitivity necessary to ensure the safety of genetically modified whole foods under chronic use conditions. It is for these reasons that the safety evaluation of genetically modified whole foods requires that innovative new approaches be developed.

Many first-generation transgenic crops involve the transfer of a single gene, often derived from a different species than the host (transformed) plant. The foreign gene may or may not significantly alter inherent biosynthetic pathways in the transformed plant. In the future, however, genetically engineered crops will likely be more sophisticated. Multiple genes will be transferred. Host plant biosynthetic pathways may be significantly altered such that the levels of several naturally occurring compounds in the transformed plant will be altered.

Recently, for example, it was announced that the first protein plant hormone has been identified (13). This hormone mediates several metabolic reactions within the plant. Research is being conducted to identify additional plant protein hormones and to possibly clone and transfer the genes that code for these hormones. Thus, future transgenic plants may display significant compositional differences from those available today. Current safety assessment methods that rely on testing individual components will be increasingly inadequate as a method to assess the safety of these more complex genetically engineered plants.

Finally, as discussed above, the potential food safety risks that may result from the use of biotechnology in food production fit two general categories—those that can be anticipated based on the structure and known metabolic activity of the gene product, and unexpected results, such as the enhanced production of naturally occurring toxic substances, that might result from the undirected insertion of the gene.

For all these reasons, a new approach to safety evaluations is needed in the era of biotechnology. The new approach has two key elements:

1. knowledge of the genetic modification practices used and the inferences this has for product safety, and
2. compositional studies designed to evaluate whether changes in composition of food products might lead to safety concerns under the intended conditions of use (30).

Understanding the genetic modification practices used and the inferences this has for product safety provides information concerning the types and nature of gene products likely to be present. Compositional studies yield information on any unexpected effects that may occur as a result of the genetic modification. Since the effects are unexpected, one does not know what kinds of gene products to evaluate for toxicity. The way to obtain this information is to compare the transgenic organism to its conventional counterpart and note any significant changes in the amounts of common constituents associated with the foodstuff and for identifying any new constituents that may have been introduced by the genetic modification process (30). Knowing what these changes are

provides a basis on which to conduct a safety evaluation of the new food product.

Knowledge of the Genetic Modification and Inferences

An analysis of the safety of the gene products will require understanding the type and nature of products expressed, any toxic effects of these products, and the levels at which they occur in the food. These are the same issues that must be addressed when evaluating the safety of conventional food additives. As with the safety assessment of traditional food additives, the assessment of biotechnology-derived foods must begin with the identification of the types and nature of gene products present. Such information can be obtained by evaluating the genetic construct itself and understanding the metabolism of the product of the inserted gene.

Evaluating the genetic construct itself includes analyzing both the product of the newly transferred gene and the products of any other genetic material transferred with the desired gene (e. g., marker sequences, regulatory sequences, vector sequences). The information needed to evaluate the genetic construct includes:

- the physical size, structure, and functional limits of the coding region;
- the physical extent and functional properties of the regulatory DNA regions (e. g., where the regulatory sequence occurs relative to the coding sequence, the relative strength of the regulatory sequence;
- the starting signal for transcription of the gene); and
- the structure and function of the marker sequences (20).

This information is needed whether the host organism is a microorganism, a plant, or an animal. In some cases, this information is already available in the public literature, but if it is not, it usually becomes available as a result of the genetic engineering process itself, or it can be obtained relatively easily with genetic engineering techniques.

An understanding of how the inserted gene functions in the plant is also needed. An ideal situation is to have stable and predictable gene expression. Information useful in determining gene expression in the plant includes an estimate of the number of gene copies inserted, whether they are inserted into the chromosomes or other organelles that contain genetic material (i.e., the mitochondria or chloroplasts), and whether gene expression is inducible or constitutive (turned on all the time). Tissue location (plant part) and concentration of gene expression products during the plant's life cycle should be determined. And any evidence of the gene moving to other locations within the genetic material should be evaluated (56).

The mode of action of the gene product also should be assessed. In general, with food additives and pesticides, these compounds and any degradation products are traced in the plant by radioactively labeling the compounds. Such an approach may not be adequate to identify metabolic products inherently produced in a plant as a result of genetic engineering. New analytical methods and greater understanding of basic plant metabolism will be needed to identify endogenous plant metabolizes that result from genetic engineering.

Once the gene products have been characterized, their potential to produce toxic effects must be addressed. The material used for the toxicity testing should represent as closely as possible the expression product as it actually occurs in the plant. It is preferable to develop methods that could assess the toxicity of the whole food, i.e., the form in which it is eaten. However, such methodology is not currently available. An alternative approach is to isolate and purify the gene product from the plant in sufficient quantities to conduct traditional toxicity testing (i.e., administering large doses of the substance to a test animal). Isolating sufficient quantities of primary and secondary gene products from whole foods, however, may be difficult in some cases. The gene product must be extracted from the food. In some cases, methodology for such extraction may not be available, and new analytical techniques will need to be developed.

If the gene product does not undergo significant post-translational modifications in the plant, an alternative approach to obtaining sufficient quantities of the gene product for toxicity testing might be to produce and purify the product from a microbial system. Even if post-translation modifications occur, knowledge of the sequence of the gene allows for the use of computer algorithms to identify other proteins with related sequences, taking into account any post-translational processing that might occur to alter the protein. Once the protein family has been identified, it may be possible to establish a history of safe consumption of closely related proteins in other foods. This does not guarantee the safety of any specific protein, but each new case does not have to be treated as being entirely novel; the relationship of a protein to other proteins with a similar function provides additional information that can sharpen the focus of the safety evaluation (5).

Box II-A—FDA Safety Review of a Food Enzyme Derived From a Genetically Modified Bacterium

Rennet, an enzyme preparation isolated from the forestomach of calves is used to clot milk in the cheesemaking process. The principal enzyme contained in rennet is chymosin. The FDA affirmed rennet as GRAS for use in food in 1983. However, this source of the chymosin enzyme is expensive for food processors, and an appropriate substitute would be beneficial to the industry.

In February 1988, Pfizer Central Research (Pfizer Co.) petitioned FDA to affirm as GRAS, a chymosin preparation obtained from genetically inserting a chymosin gene into a bacterium. This genetically modified bacterium was then used to bacterially ferment large quantities of chymosin, which could be used in place of rennet. Chymosin **was the** first biotechnology-derived food additive reviewed by FDA.

During the review, FDA viewed the chymosisn preparation as a product consisting of an active enzyme plus any impurities that may have been introduced during fermentation and processing. The FDA was interested in determining whether the cloned chymosin gene yielded a protein enzyme of the same structure and function as is contained in rennet.

The cloned gene was sequenced and other analytical tests performed to establish the chemical identity of the resulting enzyme. This cloned chymosin enzyme was tested to determine if it had the same functional activity as chymosin derived from rennet; its ability to clot milk was tested under various conditions of temperature, salt concentration, and PH. This information was used to determine that the cloned chymosin enzyme was indistinguishable from that contained in rennet. The safety of the chymosin enzyme preparation was also tested by feeding large quantities of the preparation to laboratory animals. No adverse effects were detected.

The FDA also examined the safety of the bacterium into which the chymosin gene was inserted. The bacterial strain used has been used widely as a laboratory organism for at least 30 years without any reported incidents of illness. The strain does not colonize the gut of man or animals, even when present in high concentrations; does not produce toxins; and lacks the characteristics necessary for pathogenicity. Additionally, the process used to purify the enzyme destroys the bacteria and removes most of the microbial material from the final product. Because the bacterial strain used contained an antibiotic resistance gene as a marker, FDA also sought to ensure that this gene was destroyed during purification and that there was no possibility of the gene being transferred to bacteria contained in the human gut.

Chemicals used in the purification process were also **evaluated** to determine if they presented safety concerns. Compounds used in processing were those already approved as food additives or were GRAS. The resulting chymosin preparation was considerably purer than the rennet preparation currently in use.

After review of test data and published literature pertinent to the use of chymosin in food, FDA affirmed the GRAS status of biotechnology-derived chymosin in March 1990.

SOURCES: Federal Register, vol. 55, No. 57, Mar. 23, 1990, pp. 10932-10936. Eric L. Flamm, "How FDA Approved Chymosin: A Case History," Bio/Technology, vol. 9, April 1991, pp. 349-351.

For microorganisms used as a source of simple chemical additives, the safety assessment includes identifying: the host organism, any evidence of pathogenicity or toxin production, the function of the inserted gene, and the identity of any organisms that contributed genetic material to the final construct. In addition, characterization of the inserted genetic material is needed to ensure the absence of sequences that may encode harmful substances. Insertional and genomic stability, chemical specifications, dietary use and exposure, and other relevant information must also be evaluated. Safety evaluation of the insert itself focuses on its expression product. In addition, the fermentation process is evaluated for variation and control elements. The purity and identity of the final product should be maintained throughout the production process. This approach was taken with the FDA review of chymosin, the first chemical additive produced by genetically modified bacteria to be approved (box 1 l-A).

In keeping with the approach that chemicals with the highest potential risks must undergo the most extensive toxicity testing, the use of genetic elements that have a safe history of use in food could require a less rigorous evaluation than is necessary if genetic elements foreign to the food supply are used. "Safe" genetic elements

might consist of genetic material from nonpathogenic, nontoxigenic microorganisms that are commonly associated with or found in foods; and genetic elements, characterized or uncharacterized, used as source material for the genetic modification of food species via conventional breeding procedures (20).

Assessment of Potential Unexpected Effects

While some of the traditional safety assessment practices may be used to identify the toxicity of primary and secondary gene products, the evaluation of the potential impacts of gene insertion effects will require a different approach. The major difficulty encountered is documenting the effects of undirected insertion since one does not know what compounds could be produced or what expression levels could be enhanced.

The way to determine whether unexpected expression products or nutritional deficiencies have in fact occurred, is to compare the compositional changes of a genetically modified organism with that of a traditional organism, or a selected reference organism. Bacteria commonly used in food production are generally well characterized, and the possibility of production of toxic compounds is very low if the host bacterium does not normally produce toxins. Demonstration of unexpected results in more complex organisms, such as plants, will be complicated by the large size of the genome and the fact that toxic products may only be produced under special conditions (38).

To compare transgenic plants to traditional or reference plants requires knowing the normal range of the latter's nutritional components, and identifying any naturally occurring toxic compounds that have significantly increased levels in genetically modified plants. The inadequacy of the information concerning whole food composition of traditional foods limits the ability to make such comparisons at the present time (30).

While knowledge concerning the normal range of toxic compounds in raw foods is limited, even less is known about the normal range of such toxins in processed foods. While food processing and cooking often lowers the levels of toxic factors, sometimes this processing and cooking has the opposite effect. For example, high temperature that kills organisms also can thermally transform normal components of foods, such as proteins, carbohydrates, and lipids into toxic materials (45, 46, 47). Thus, pyridines, which are mutagenic compounds, can be formed by cooking meats. Acid and alkali treatment and fermentation processes also can result in toxic compound production (23). Data collected on toxin levels usually consists of determining whether or not particular regulatory limits have been reached. This type of data is not the type needed to predict levels of toxins that may occur during processing. Additionally, the methods used are generally not sensitive enough to detect and quantify extremely low levels of toxicants in foods (30).

In addition to the issues of toxicity and nutritional deficiencies, genetic modification has raised the issue of allergenicity of the gene product. The possibility exists to alter the structure of endogenous proteins or introduce new proteins into foods (16). One approach to determining allergenicity is to allow limited distribution and carefully monitor for allergic response (29). Other possibilities might be to use double antibody screening procedures, in which food materials (or extracts) are used as antigens to which human blood plasma (containing antibodies) is added. Complexes formed by the interaction of antibodies and antigens are detected using a second antibody labeled with fluorescent materials to bind to the initial antigen-antibody complex. This method can be used as a general means of detecting potential allergenic effects of food products (12). This approach is most useful for proteins to which sensitive individuals have already been exposed; it is not particularly useful for new proteins.

Similar to the FDA, the EPA may face analytical difficulties in their attempts to develop tolerances for pesticidal products created using the new tools of biotechnology. Historically, EPA has worked with chemical rather than biological substances. Biological pesticides have heretofore been restricted to microbial pesticides, not whole plants. Whole plants are considerably more complex than microbial pesticides, which in turn are much more complex than chemical pesticides. Identifying, isolating, and assessing the toxicity of endogenously produced pesticides creates new analytical challenges. Identifying the appropriate test material for toxicology testing, and synthesizing radioactively labeled materials to conduct metabolism studies will require the development of new methodologies.

EPA guidelines for establishing a tolerance level for transgenic plant pesticides have not yet been developed. Determining the type, nature, and level of residues in whole plants, and then testing those residues for toxicity will create analytical challenges. EPA has indicated that its assessment will focus on the pesticide product and its active ingredient, although at present, it has not clarified whether that means that EPA will regulate the gene itself, the gene product, or both. EPA also has not yet clarified

whether regulations will be applied to the seed or the whole plant.

EPA has suggested that for the purposes of product assessment, pesticidal products produced in transgenic plants might be divided into two categories—proteinaceous products and nonproteinaceous products. EPA expects that the information and data needed to assess the safety of proteinaceous products will, in general, be less than that required for nonproteinaceous products, because proteins are susceptible to acid and enzymatic digestion (56).

An example of the types of problems that may be encountered with whole plants genetically engineered to contain pesticidal compounds is illustrated by some of the technical difficulties encountered with the registration of plant extracts as pesticides. Plant extracts contain many chemical compounds, several of which maybe pesticidal. Additionally, the quantities and types of these compounds can vary substantially depending on soil type, temperature, rainfall, etc. To register plant extracts, EPA requires composition and product chemistry data for all chemical compounds in the extract. Toxicology tests representing the entire range of possible compositions must be conducted and tolerances may need to be established for all compounds (9). Needless to say, it can be time consuming, expensive, and difficult to register plant extracts as pesticides.

It is reasonable to expect that in the future, fundamental biosynthetic pathways in plants will be altered such that several potential pesticidal compounds may be present, a situation that may be analogous to plant extracts. Because EPA has not clarified its policy with respect to these types of products, it is speculative how EPA will address such products. However, if EPA does treat these biotechnology products similarly to plant extracts, this may create significant obstacles to the development of many of these types of biotechnology products.

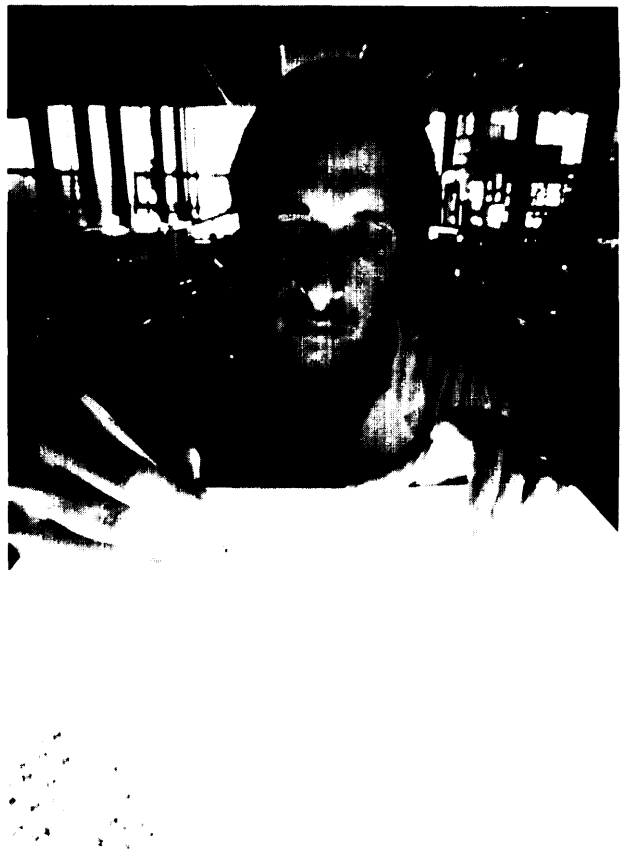


Photo credit: U.S. Department of Agriculture, Agricultural Research Service

Chemist evaluates a screening assay for residues. New analytical methodology will need to be developed for biotechnology-derived foods.

RESEARCH NEEDS

New analytical methodology must be developed to measure the normal range of toxic and nutritional components in foods needed for comparison with biotechnology-derived foods. Whole food composition analysis is a complex task due to large numbers of potentially toxic materials that may be present in raw foods and the constantly changing nature of the processed food market. Monitoring levels of key toxic components will require a large number of assays for many different compounds, sometimes at quite low levels. Many traditional analytical methods, such as titrations and calorimetry, can be used to assay classes of compounds, such as reducing sugars or proteins, but by themselves these methods cannot be used to quantify individual members of those classes in mixtures of compounds. Food safety assays for determining individual compounds in complex mixtures are needed (30).

The analytical process starts with the preparation of the food sample followed by extraction by chemical class. Most modern analytical separation and detection techniques require clean samples free of interfering compounds. Most food samples are mixtures of multiphase materials with extremely complex chemical compositions, and the quantitative extraction of a given chemical class can be quite difficult. The development of adequate plant extraction techniques has lagged behind the other analytical techniques of food analysis. Those wishing to

use modem analytical separation and detection tools often find that the companion sample extraction techniques are inadequate, untested, or nonexistent. For example, the present methods of determining amino acid composition of foods with high sugar and starch contents is unsatisfactory-sugar and starches cause extensive losses of the amino acids in the sample preparation step (hydrolysis). The lack of proper extraction techniques is frequently the primary bottle neck to obtaining good data on the levels of the components in foods and feeds. In most cases the compounds of interest must be separated from other similar components in foods and feeds before they can be quantified. Once the extraction and separation of chemical classes has been accomplished, techniques and instrumentation for the analytical separation and detection of individual compounds are available (30).

New assay procedures must be validated before they can be widely used for food safety analysis. Validation has been defined as the process of determining the suitability of methodology for providing useful analytical data (48). Validation generally consists of 1) estimating acceptable performance parameters in a laboratory, 2) demonstrating successful performance in limited interlaboratory studies, and 3) demonstrating successful performance in collaborative studies. Performance parameters assessed include accuracy (how well the methodology measures true values), reproducibility, specificity, sensitivity (lowest levels detected), and scope (number of analytes to which the procedure can be applied (8).

New analytical methodology is needed not only to determine the initial safety of food products, but to conduct follow-up regulatory compliance and monitoring. For example, with pesticides, the toxicity of the pesticide initially must be determined. Once a pesticide is approved, methods are needed to verify that it does not exceed tolerance levels in marketed food products. Conditions under which new products are developed differ significantly from the routine conditions that exist in the day-to-day and year-to-year production and processing of foods. Genetic drift of new genetically modified species; changes in cultivation conditions or in processing conditions; and transportation or storage conditions might alter levels of toxic materials. Routine quality assurance measures should be developed. Often quick, inexpensive, and reliable analytical techniques are not available for widescale sample testing (30).

There is often a significant delay in the development of new analytical methods and their general use in food safety regulation. Nonselective, insensitive, and time-consuming assays for which validation protocols are inadequate or unavailable may be the only assays available for some compounds. New assay procedures are being developed, but they must be validated before they can be widely used for food safety regulation. Additionally, new developments in automated chemical analysis can help reduce the time and expense of manual assays. These new methods have not been rapidly adopted for food analysis, however (18, 25, 30, 43, 49, 52, 53).

Quality control and regulatory compliance personnel may work under less-than-ideal conditions, have less formal analytical training, and use less sophisticated instrumentation than food scientists working in research. The assays developed need to be rugged (i.e., require minimal training and skill on the part of the analyst and give good results even when there are small deviations from the assay protocols), completed quickly at low unit cost, and provide the necessary accuracy. Assays need to generate low levels of false positives (i.e., doesn't identify a compound as being present when it is not) and yet not have high levels of false negatives (i.e., doesn't miss compounds that are present). Assays must be accepted by the professional analytical community, regulatory community, and legal community. Formal validation usually will be required (30). In addition, compliance monitoring of genetically modified organisms may require the development of statistical sampling methods that differ for those used for pesticides and other chemical additives.

Compliance assays are particularly pertinent in that there exist no analytical techniques capable of identifying whether a food **or** feed crop has been genetically modified. Nor is it clear that any such methodology can be developed on a generic level. Development of assays for selected genetic alterations may be possible (i.e., if a given genomic sequence is known to always be present or absent in a given species, then its loss or appearance would be reasonable evidence that genetic modification had occurred). Probe technologies do exist to determine the existence or absence of specific DNA or RNA sequences and proteins, but the types of DNA sequences that conceivably could be engineered into plants is potentially great, and this procedure may not be very efficient (30).

The absence of a means of identifying if a food or feed crop has been genetically modified is made more significant by the fact that the United States imports large quantities of food and feeds yearly. The United States is by no means the only country capable of genetically modifying food crops. If the United States enacts standards that are more strict than other countries, then the general population may not feel that the assurances of

other countries is sufficient proof that the crops they are exporting are not genetically engineered. A verification methodology may be needed.

The lack of analytical systems for quality control and regulatory compliance assays of genetically modified foods and the lack of sufficient numbers of adequately trained analysts could pose major problems in the assessment of the safety of genetically modified foods. Furthermore, the training of food analysts lags far behind that of other fields (30).

Although this chapter has focused on potential food safety risks that might arise from using biotechnology to produce food products, it should be pointed out that biotechnology itself can be used to develop analytical methodologies that might improve the safety of foods. Biotechnology products can be used to monitor plant and animal products for food safety. Nucleic acid probes and monoclinal antibodies can be used to analyze raw materials, ingredients, and finished products for pathogenic organisms, bacterial or fungal toxins, chemical contaminants (i.e., pesticides, heavy metals), and biological contaminants (i. e., hormones, enzymes). Detection kits to monitor several pesticide and antibiotics, and some microorganisms such as *Salmonella,* are commercially available. Additionally, animal cell cultures may partially replace whole animal systems to test for acute toxicity. Biosensors may be used to monitor food processing, packaging, transportation, and storage (15, 39).

New analytical methodologies still are needed to assay the safety of genetically engineered foods. Such methodologies also could be used for other food-related issues, such as current attempts to analyze the anticancer properties of certain food ingredients that occur in foods such as garlic, broccoli, etc. (i.e., the designer foods project currently in progress) (40). Much research is needed to develop new methodologies. Primary attention should be given to:

- The development of acceptable alternatives to animal feeding tests for safety assessment. Because of the inability to feed high levels of whole foods to animals to determine toxicity, in vitro tests and chemical/biochemical assays need to be developed.
- The development of rapid, accurate methods for assaying food components of particular interest.
- The development of comprehensive food composition databases. It will not be possible to determine acceptable limits of variation in composition of new foods without knowing what kind of variation now exists in traditional foods.
- A greater understanding of basic molecular biology of plant development. This information will be helpful in designing genetic strategies to improve composition or food characteristics. Greater knowledge of the organization of plant genomes would be helpful in assessing the positional effects if any, Improved methods of toxicological assessment of proteins and/or whole foods would constitute an important advance in the safety review of foods.
- The development of fixed algorithms for computations and report generation to reduce the human error in food safety assessments and research.

Research must be conducted in many areas to develop the analytical methodology needed to assess the safety of food products produced with biotechnology. The regulatory agencies responsible for assuring safety must set priorities for their own in-house research programs. Additionally, it would be useful for these agencies to work with the major research funding agencies (i.e., NIH, NSF, and USDA) to support the research and training of food analysts needed to assess the safety of biotechnology food products.

SUMMARY

The key scientific issues raised by the genetic modification of foods are with respect to the activity of the inserted gene and the site of insertion of the gene into the host genetic material. Assessment of the activity of the inserted gene includes assessing the safety of the gene product itself and any secondary products whose production might be stimulated by the presence of the gene product (e. g., if the gene product is an enzyme or hormone that mediates the production of other compounds).

The gene product itself is a protein. Proteins are generally nontoxic, readily degradable in the host organism, and easily digestible by humans. The major concern with respect to the gene product itself may be that it results in increased allergenic responses rather than toxic effects. Secondary products stimulated by the presence of the gene product, however, may not be proteins. An increased understanding of plant physiology, the physiological impacts of the inserted gene, and the possible development of new analytical techniques is needed to identify any secondary compounds produced so they can be assessed for safety.

Other genetic material inserted into a host organism in addition to the selected gene might include vector

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material and marker genes used to identify those cells that incorporate the selected gene. The current use of vectors and marker genes that are well characterized, nontoxic, and already widely present in the food system is not expected to result in significant food safety problems when used to genetically modify foods.

At present, researchers cannot completely control the location where a selected gene inserts into the host's genetic material. There is a possibility that the insertion site of the selected gene will be such that it activates or deactivates host organism genes (possibly resulting in pleiotropic effects). Some host organisms used as food (e.g., most food crops, some microorganisms, and some marine animals) naturally produce compounds that could potentially display toxic effects in humans if consumed in sufficient quantity. If the insertion site of the selected gene in the host organism is such that it increases the production of these potentially toxic compounds, food safety issues could arise.

Because these insertional effects would not be predicted based on the knowledge of the physiological activity of the selected gene, one approach to detecting whether or not any of these effects have in fact occurred would be to compare the composition of the genetically modified organism with its traditional counterpart. However, our understanding and knowledge of the identity and normal levels of toxic compounds in the foods we currently eat and extraction methodologies are insufficient to perform an extensive comparison. Comparison of the levels of major nutrients and some widely known acutely toxic compounds between biotechnology-derived foods and their traditional counterparts could probably be made. But plant compounds have never been identified nor evaluated to determine if they cause long-term toxic effects in humans.

A question that must be decided is whether or not those comparisons that cannot currently be made are significant from a food safety standpoint. The development of new crop varieties using traditional breeding and cell culture techniques can also result in similar pleiotropic effects. To date, no evidence exists that the development of new crop varieties has significantly decreased the safety of the food supply. It may also be the case that new food products produced with biotechnology will present no food safety risks greater than those already generated by the foods we eat every day. It will be the task of the agencies responsible for food safety to identify those biotechnology-derived food products that may present increased food risks.

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