PN-ACS-257

Ministry of Agriculture & Land Reclamation
US Agency For Intl. Development
Agriculture Policy Reform Program
Reform Design and Implementation

وزارة الزراعة واستصلاح الأراضي
الوكالة الأمريكية للتنمية الدولية
مشروع إصلاح السياسات الزراعية
وحدة تصميم وتنفيذ السياسات

Ministry of Agriculture and Land Reclamation

AGRICULTURE POLICY REFORM PROGRAM

Reform Design and Implementation Unit (RDI)

USAID CONTRACT NO. 263-C-00-97-00005-00

Report No. 134

Regulating the Commercialization of Transgenic Plants for Food and Feed in Egypt: Food Safety Assessment

RDI REPORTS

APRP

Reform Design and Implementation Unit

Development Alternatives Inc. Group: Office for Studies & Finance, National Consulting Firm Development Associates, Cargill Technical Services, The Services Group, Training Resources Group, Purdue Universities, University of Maryland

PN-ACG-257

Report No. 134

Regulating the Commercialization of Transgenic Plants for Food and Feed in Egypt: Food Safety Assessment

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May 2001

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Abbreviations Used in This Report

AGERI	Egyptian Agricultural Genetic Engineering Research Institute
ANZFA	Australia New Zealand Food Authority
CFIA	Canadian Food Inspection Agency
CLFF	Egyptian Central Laboratory for Food and Feed
EOS	Egyptian Office of Standards
FAO	Food and Agriculture Organization of the United Nations
FDA	United States Food and Drug Administration
LRAPHMF	Egyptian Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods
MALR	Egyptian Ministry of Food, Agriculture, and Land Reclamation
MOH	Egyptian Ministry of Health
NBC	Egyptian National Biosafety Committee
OECD	Office of Economic Cooperation and Development
SCFS	Egyptian Supreme Committee for Food Safety
VRC	Variety Registration Committee
WHO	World Health Organization

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Executive Summary

Scientists in both the public and private sector in Egypt are currently using the techniques of genetic engineering to develop new, improved varieties of crops for eventual use by Egyptian farmers. Up until now, this work has been restricted to research, but soon some new varieties will become ready for commercial release. Such release will need to be regulated to ensure safety.

Egypt's National Biosafety Committee (NBC) uses the Biosafety Regulations and Guidelines to guide its work in regulating the use of genetically engineered plants in Egypt. These Guidelines focus mainly on controlled testing of genetically engineered plants proposed for planting in Egypt. The Guidelines, however, are underdeveloped when it comes to specifying requirements for approving the release of such plants for commercial purposes. This report proposes a draft amendment to the Guidelines (Annex I) to remedy this deficiency, with particular emphasis on the necessity of (a) food and feed safety assessments, and (b) environmental safety assessments, as conditions for approval.

This report proposes detailed guidelines for the food and feed safety assessment of food derived from genetically engineered plants (Annex II). These guidelines describe the data and test results to be supplied by the applicant to the NBC, including (1) characteristics of the genetically engineered plant and the effects of the transformation on known toxicants, anti-nutrients, or nutrients, (2) the sources of new genetic materials and their characterization in the genetically engineered plant, and (3) safety assessment of the gene products in the genetically engineered plant.

Similar guidelines will need to be developed for the assessment of environmental safety.

Egyptian laboratories have the capacity to conduct most of this food safety testing, although in some cases additional equipment may be necessary.

It is up to the NBC to consider these proposed amendments to the Biosafety Regulations and Guidelines, adapt them to Egyptian needs, and arrange for their official approval. Once the guidelines are finalized and adopted, the NBC will be able to assess the food and environmental safety of new genetically engineered plants proposed for commercial release in Egypt and when appropriate, recommend their registration to the Variety Registration Committee.

It is important to note that the work of the NBC focuses on evaluating genetically engineered plants for the purpose of planting in Egypt. It does not focus on evaluating the safety of imported genetically engineered commodities, such as maize, that are directly consumed by people or livestock. The import of these commodities is governed by a separate decree (No. 242/1997) that prohibits the import of foods derived from genetically engineered plants, unless their safety is

confirmed. Exactly how this safety is to be confirmed is presently under-defined in Egypt. In practice, the current policy, based on a recommendation of the Supreme Committee for Food Safety, allows food commodities from genetically engineered plants to be imported only if the importer provides certification of safety from a foreign governmental agency and documentation of its commercial status in the country of origin. It is not clear, however, if this policy is fully implemented, given difficulties in identifying which shipments include genetically modified commodities.

1. Introduction

Genetically engineered plants have become an important source of raw material for food produced in a number of countries throughout the world. Global acreage of genetically engineered crops totaled 44 million hectares in 2000, primarily in the United States, Argentina, Canada, and China (James, 2000). This technology holds much promise as one of the tools for improving crops. Egypt, having long recognized the potential benefits of this technology, has been conducting genetic engineering research for crop improvement in its Agricultural Genetic Engineering Research Institute (AGERI), an institute of the Agricultural Research Center within the Ministry of Agriculture and Land Reclamation (MALR). After several years of research, some of AGERI's projects are on the verge of commercialization.

In order to move to the commercialization phase of this technology in Egypt, appropriate policies, regulations and guidelines must be established. This report examines the background for these policies, regulations, and guidelines, specifically with respect to the evaluation of food safety. It also proposes regulations and guidelines for food safety assessment, discusses the capacity of particular laboratories to conduct the necessary experiments in connection with these assessments, and makes recommendations for upgrading the capacity of these laboratories to enable them to conduct the required testing. Finally, this report also examines the issue of labelling of foods derived from genetically engineered plants, and provides questions to be considered when formulating and implementing labelling regulations.

2. Current Food Safety Policies, Procedures and Practices Related to the Introduction of New Foods and Feeds in Egypt, and the Regulation of Genetically Enhanced Food and Feed Products

Food safety regulation in Egypt is based upon two primary laws: Law No. 10/1966, Concerning Food Control, and Law No. 48/1941 as amended by Law No. 281/1984, Prohibition Against Adulteration and Deception of Foods. In practice, these laws make food safety the responsibility of various ministries, but with the Ministry of Industry's Egyptian Office of Standards (and specifically the Egyptian Codex Committee within this office) as the focal point. Assurance of safety of foods and feeds in Egypt primarily hinges on enforcing adherence to a set of standards codified in either the Egyptian Product Standards, International Organization for Standardization (ISO) Standards, European Standards (EN), British Standards (BS), German Standards (DIN), French Standards (NF), American Standards (ANS), Japanese Standards (JIS), or Codex Standards. Domestically, food safety is assured by certifying production facilities for the safe production of foods—which means eliminating known hazards (e.g., microbial, pesticide, and heavy metal contamination)—coupled with sampling products for testing by central laboratories in the Ministries of Health (MOH) and Agriculture and Land Reclamation (MALR). Imported products are subject to the same scrutiny as domestic products, with the obvious exception of facility inspections.

Food safety policies and regulations in Egypt are geared toward assuring both safety and quality by eliminating contaminants and nonconforming products. Efforts to reduce contaminants focus on testing for levels of known toxins such as pesticides, heavy metals, aflatoxins, and microbial contamination. Efforts to reduce

nonconforming products aim at increasing the consistency of quality by paying strict attention to production and expiration dates, as well as confirming that adherence to product and quality standards set by the Egyptian Office of Standards or the other standard-setting bodies mentioned above are met.

Quality and safety assurance is viewed as the government's responsibility. The government, through its central laboratories that test for safety and quality, supervises compliance with food safety and quality standards. The Ministry of Health is responsible for assuring food safety through its Food Control Department. Inspectors from this agency inspect food processing plants and sample products, which are sent to the Central Laboratory for Food within MOH. This laboratory analyzes the samples for compliance with standards set by the EOS.

Quality control of all feedstuffs, both domestic and imported, is the responsibility of MALR's Central Laboratory for Food and Feed (CLFF). This includes commodities that may be used for food as well as feed (e.g., corn and soybeans). Also within MALR, the Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods (LRAPHMF) is responsible for monitoring foods for pesticide residues. Imports as well as exports are sampled on a daily basis, and the Ministry of Agriculture takes remedial action whenever pesticide residue amounts above threshhold levels are detected.

The mandate of the LRAPHMF presents an interesting situation on testing commodities for pesticides. This laboratory tests for the presence of a number of conventional pesticides, heavy metals, and microbes. However, this laboratory does not routinely test for the presence of biopesticide residues. Whether or not such testing will be done is a matter that should be clarified, since it will have an impact on organically grown and genetically modified crops developed in Egypt, as well as commodities and seeds imported into the country. Plants engineered to be resistant to a pest might be viewed as producing a pesticidal compound, as defined by the Manual for Agricultural Pesticides Registration, Handling, and Inspection in Egypt. According to MALR's Ministerial Decree No. 663/1998:

> Pesticide means <u>any substance or mixture of substances intended for preventing, destroying or controlling any pest</u>, including vectors of human animal disease, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or which may be administered to animals for the control of insects, arachnids or other pests in, or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit, and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. [Emphasis added]

Moreover, biopesticides are viewed under the same registration umbrella as chemical pesticides, even though they clearly should not be viewed in the same way as chemical pesticides with demonstrated toxicity. Thus, plants producing a pesticidal compound *could be* interpreted as falling under the monitoring mandate of LRAPMHF.

In conflict with MALR's Ministerial Decree No. 663/1998, Ministerial Decree No. 1648/1998 (MALR) makes commercial approval of genetically engineered plants (presumably including plants expressing pesticidal traits) a matter for the Variety Registration Committee. However, it is unclear whether such genetically engineered plants remain free from pesticide oversight after they are commercialized and the products from such plants enter the food stream. This important issue must be resolved, since not only will it apply to domestically produced genetically engineered plants expressing pesticidal traits, but also to crops—particularly corn—currently being imported from countries (notably the U.S. and Argentina) that grow genetically engineered crops that express pesticidal traits. There is scientific support for the position that these plants should not be dealt with as a pesticide matter. However, this position needs to be officially affirmed.

Because the focus of food safety assessments is on contaminants, there is no system in place to assess the safety of food *per se*. Thus, no regulations are currently in place to assess the inherent safety of any food or feed, whether traditional or truly novel. For example, a novel food product (from an Egyptian point of view) is currently made from the noxious weed, *Pueraria montana*, commonly known as kudzu (http://www.geocities.com/mferg265/Kudzu_Jelly.html). If an importer were to market in Egypt a jelly made from this plant, no prior testing for endogenous toxins, anti-nutrients, carcinogens, or allergens would be required. Only those known toxins that would appear in the food as a result of contamination or incorporation of a prohibited food additive would be detected. Of the certificates required for all imported food products, only two would apply: a Health Certificate and a Free Sale Certificate (FAS/USDA Attache Report #EG0023). Neither of these certificates provides an assurance of safety, because they would not be based on any studies that assess the inherent safety of this novel food. It is of some assurance to know that this novel food is consumed in the country of origin, with no reports of adverse effects. However, even this assurance would be little comfort to some who would argue that safety information and experience obtained with one population is not predictive of safety for Egypt.[1]

Due to the lack of requirements for pre-market safety assessment of any novel foods, no assessment of the safety of foods derived from genetically engineered plants is currently being conducted, although many Egyptian scientists recognize the need to conduct them. The ultimate goal of these scientists is to establish the safety of these foods through independent safety assessments conducted in Egypt, thus allowing their importation into the country. At present, these types of food are officially prohibited from entry into Egypt as a consequence of Ministerial Decree No. 242/1997 (MOH), which is paraphrased in Madkour, et al. (2000):

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[1] The lack of pre-market testing requirements for novel foods is not unique to Egypt. In fact, the United States, the country of origin in this example, does not require pre-market safety testing for this food either. However, the FDA would consider producers of this novel food to be subject to the same obligations to conduct safety assessments as producers of food derived from genetically engineered plants (FDA, 1992). However, in contrast to a non-genetically engineered novel food, the FDA will be requiring mandatory consultations for producers of foods derived from genetically engineered crops (FDA, 2001).

> This decree prohibits importing any foodstuff produced through genetically modified (GM) organisms, unless its safety is confirmed. The act also necessitates that a certificate should accompany any imported seeds from the country of origin, confirming that these seeds were not produced from GM plants. GM plants or seeds can be imported if previously approved for use in the country of origin, and having been deemed safe.

Therefore, Decree No. 242/1997 currently prohibits the importation of foods derived from any genetically engineered plants, since their safety has not been officially confirmed by any Egyptian Authority. In practice, however, the current policy, based on a recommendation of the Supreme Committee for Food Safety (SCFS), allows food from genetically engineered plants to be imported, as long as the importer provides certification of safety from a foreign governmental agency, and documentation of its commercial status in the country of origin. It is not clear whether even this policy is fully implemented.

If extensive safety testing is planned to arrive at the safety confirmation required by Decree No. 242/1997, the scope of this testing must be defined more precisely than it is at present. At one extreme, the safety of all foods (i.e., all processed foods derived from transgenic plants) could be tested. At the other extreme, the safety testing would be conducted on the original raw material (i.e., transgenic events). If the intent is the first extreme, then such testing would be formidable. It is doubtful whether any country in the world is equipped for such a level of effort. If the intent is the latter extreme, then the undertaking would still be daunting. There already exist a large number of transgenic lines approved for commercialization in countries (principally the U.S.) from which Egypt imports significant quantities of commodities. For example, Egypt imported 3.6 million tons of corn in 1998/99 and 4.4 million tons in 1999/2000 from the United States (Dr. Akila Hamza, personal communication; FAS/USDA Attache Report No. EG1006). The U.S. does not require segregation of genetically engineered products, and therefore, the safety testing of at least thirteen different corn products would be required (http://www.vm.cfsan.fda.gov/~lrd/biocon.html). Argentina is also a source of corn (http://www.netlink.de/gen/Zeitung/970920.htm), some of which would also be genetically engineered. Egypt also imports soybeans from the U.S. and Argentina (http://www.netlink.de/gen/Zeitung/970920.htm). Both countries grow genetically engineered varieties of this crop on an extensive scale, and therefore, soybean meal, soybean oil, and other soy-derived products coming from these countries most likely have been produced from sources containing genetically engineered beans. These transgenic lines have been commercialized since 1995. Finally, wheat is a significant import commodity for Egypt. Egypt is the second largest wheat buyer in the world, importing 4 million tons annually (http://metimes.com/2K/issue2000-15/bus/wheat_czars_dispute.htm) for a total value of approximately L.E. 2.17 billion (US$ 564 million). While no genetically engineered wheat is presently on the market, such wheat lines will be commercialized in 2001-2002 (Monsanto Annual Report, 2000). Because of the large number of existing and soon-to-be marketed commodities derived from genetically engineered plants in countries that are important sources of these commodities, a significant effort on the part of major laboratories in Egypt would be required to meet the goal of independently assessing the food safety of these commodities. This goal could very easily command the resources of entire laboratories for a number of years.

The sole mechanism currently in place for assessing the food safety of genetically engineered plants is to delegate the responsibility for this task to the National Biosafety Committee (NBC) and the Supreme Committee for Food Safety (SCFS) as a part of variety registration procedures outlined by Decree No. 1648/1998 (MALR). This system covers approvals for seed destined for planting only, and not seeds imported as commodity grain. Decree No. 1648/1998 apparently "confirms the authority and responsibility of CASC for releasing genetically modified as well as conventional seeds" (Madkour et al., 2000). The NBC has the authority to review data from food and feed safety studies provided by an applicant. After review and a favorable finding, the NBC forwards its approval to the Variety Registration Committee (VRC) after which the transgenic variety enters the process review for seed registration. As part of this seed registration process, Decree No. 702/1999 (MALR) also requires the DNA fingerprinting of all new crop varieties in order to confirm identity, and to provide a reference. The DNA fingerprinting is to be conducted by a certified laboratory to be identified by the VRC Secretariat.

Therefore, while a procedure for commercializing the seed of genetically engineered plants by both domestic and foreign entities has been established, no policies or regulations are currently in place that govern the procedures for safety testing of genetically engineered food and feed. The existing procedure for commercialization of seeds for planting is inadequate, since specific requirements and guidelines governing the assessment of safety have not been formulated.

Furthermore, while it is not a stated objective of this study, it should be pointed out that policies and regulations regarding the demonstration of environmental safety are also not in place. The protocol for the commercial registration of transgenic plants, provided in Annex 4 of Madkour et al. (2000), states that "...[t]he applicant should submit all relevant studies carried out to ensure [the] biosafety status of this new variety, including environmental biosafety, food safety, ensuring absence of any risk hazard on human, animal, plant, environment [emphasis added]." Policies and guidelines for establishing enviromental safety are required as well.

3. Regulations for Commercializing Genetically Engineered Plant Varieties: Assessing Safety for Food and Feed

As discussed above, Egypt's regulation of genetically engineered plant varieties that will eventually be made into food and feed is primarily the responsibility of the National Biosafety Committee, established under Ministerial Decree No. 85/1995 (MALR). The NBC oversees adherence to the Biosafety Regulations and Guidelines adopted via Ministerial Decree No. 136/1995 (MALR), which cover greenhouse and contained field releases of genetically engineered plants. The mandate of the NBC is described in the Biosafety Regulations and Guidelines as follows:

> The purpose of the national committee is to establish policies and procedures to govern the use of mode[rn] biotechnology in the country. This includes publishing the National Biosafety Committee guidelines (NBC Guidelines) to be followed at the national level. The committee would also provide technical advice to the regulatory authorities and the institutions responsible for the development of biotechnology in the country (MALR, 1994, p. 12).

Therefore, the NBC plays a central role in providing technical advice and formulating guidelines—including guidelines for commercialization (MALR, 1994, p.13)—to be followed by regulatory authorities, but does not appear to have regulatory authority itself. As previously noted, regulatory authority for commercialization of transgenic plants has been given to the VRC under Ministerial Decree No. 1648/1998 (MALR) as described above.

A more detailed description of the process for commercialization is provided by Madkour et al. (2000) based on the protocol outlined in the decree (Annex IV):

> For varieties produced within Egypt, the process is as follows:
>
> 1. The applicant completes a permit application form providing details of the genetic material introduced, the process used for inserting it, and other relevant information. The applicant also provides data from food and feed safety studies and evidence supporting a determination of low or negligible environmental risk. Where applicable, the applicant provides documents indicating approval of similar GMO's for release in their country of origin.
> 2. The application form is submitted to the NBC, which after examination and approval forwards it to the Variety Registration Committee for their preliminary approval to proceed with standard field trials conducted at several locations. The VRC assigns a team of qualified inspectors drawn from relevant ARC units and/or private certified laboratories to supervise cultivation, ensure adherence to any biosafety requirements, confirm the new phenotype, and evaluate agronomic performance.
> 3. The NBC has the right to confirm the nature of the genetic modification by taking samples from the field for molecular analysis.
> 4. After successful completion of the field trials and submission of a report to the NBC, the NBC authorizes the applicant to submit an application to the Variety Registration Committee for final approval to commercially release the new variety. Pending this, three years or seasons of agronomic performance trials are conducted under the supervision of the VRC.
>
> The process for securing commercial release approval for crops genetically engineered outside of Egypt has an added step. The applicant must first obtain a permit for importing the initial seed material from the Supreme Committee for Food Safety, MOH. The permit is then presented to the NBC and the Variety Registration Committee, after which the seed is imported into the country. From this point forward, the remaining steps in the approval process are exactly the same as for GMO's developed within Egypt.

While Ministerial Decree No. 1648/1998 (MALR) refers to processes for commercialization, these processes should be clarified and further refined to provide an easily discernible process for the commercial release of seed for transgenic plants. For example, the process as described above appears to require the submission of food and feed safety studies prior to conducting experimental scale field trials in Egypt. This requirement appears to be applicable in practice only to the situation in which transgenic plant material approved for commercialization (or close to commercialization) in another country is being considered for testing and eventual commercial release in Egypt as well. Only in this situation would there have been the type of food and feed safety assessment required in step 1 of the above process. Certainly, for several transgenic crops that have been developed and field tested in Egypt, no food and feed safety testing has been conducted. Further, there are no

specific guidelines concerning the types of data that should be required to allow the NBC to assess the food safety of the transgenic plant under consideration. Finally, the decision-making process within which the applications are considered is not clearly defined. These deficiencies have been previously pointed out by Madkour et al., (2000).

In addition to these specific deficiencies, an overarching philosophy for regulation must be established. While this area is beyond the scope of the present report, the importance of such a philosophy cannot be underestimated. Without such a philosophy guiding the actions of the NBC and other regulatory bodies, coordinating regulations that govern genetically engineered organisms will be a difficult task to accomplish.

4. A Proposed Regulation

In response to the need for regulations covering the commercialization of genetically engineered varieties, and for specific guidelines to assess the food safety of these varieties, we have developed a draft amendment to the existing Biosafety Regulations and Guidelines. This draft is presented for consideration in Annex I of this report. Guidelines for implementing food and feed safety assessments mandated in the regulation are provided in Annex II. We have also provided an illustration of the application of these guidelines to two genetically engineered insect resistant potato lines produced by AGERI and Michigan State University in Annex III. Finally, examples of assessment procedures carried out by various countries and by international organizations are provided in Annex V.

The proposed guidelines are necessarily flexible to accommodate the wide range of traits that could be presented to the NBC for consideration. The NBC should have the authority to determine which tests should be necessary to demonstrate food safety. Thus, the potato example provided in Annex III should not be viewed as the benchmark process for all safety testing of food derived from transgenic plants. Not all such food needs to be tested by feeding studies. For example, some transgenic plants have been produced in other countries that express the coat protein of viruses that normally infect these plants (see Tricoli et al., 1995). As a consequence of the expression of these coat proteins, the plants are resistant to viruses. As part of the food safety assessment of these plants, no toxicology or allergenicity tests were conducted. Instead, data were gathered from produce in markets to demonstrate the presence of these viral proteins in the food supply in much higher concentrations than the amounts found in transgenic plants (Quemada and Tricoli, 1994). Since these proteins were shown to be a normal part of the food supply, no toxicology or allergenicity tests were required.

The safety analysis guidelines presented in this report were designed to be consistent with internationally accepted guidelines and principles. Examples of these principles are contained in such documents as the proposed Codex Alimentarus guidelines (Codex Ad Hoc Intergovernmental Task Force on Foods Derived From Biotechnology, 2000), reports on consultations conducted by the WHO and FAO (FA0, 1996, 2001; WHO, 2000), the Organization for Economic Cooperation and Development (OECD, 2000), and the FDA. These documents agree on the following points, among others, regarding food safety assessment:

1. The process of genetic engineering itself is not inherently more risky than the process of conventional breeding. Therefore, the evaluation of safety should focus on the product or final trait and the unintended (both predicted and unpredicted) effects resulting from the transformation. This principle is echoed in the existing Egyptian Biosafety Regulations and Guidelines.
2. Traditional toxicological methods and risk assessment approaches used for drugs, food additives, or pesticides are difficult and have questionable application to whole food testing, thus necessitating an approach that relies on establishing substantial equivalence between the genetically engineered plant and/or food from which it is derived and an appropriate comparator (ideally an isogenic, non-genetically engineered version of the same plant).
3. Establishing substantial equivalence between a genetically engineered plant and the appropriate non-genetically engineered plant as a comparator is the most widely accepted and most practical approach to assessing safety. A finding of substantial equivalence provides a valid basis upon which to conclude that the genetically engineered plant is as safe as the non-genetically engineered plant to which it has been compared. It is implicitly recognized in this analysis that a finding of complete safety is not an endpoint that can be scientifically reached for any food, whether genetically engineered or traditionally derived.
4. Beyond establishing substantial equivalence, a safety evaluation should focus on those aspects of the equivalence analysis where substantial equivalence has not been demonstrated.

It is noted that a proper safety assessment must employ all sources of available information; relevant information from other scientific work cannot be ignored. Therefore, proper safety evaluations cannot be based on testing alone. As with all science, relevant information obtained by other means are part of the entire base of data on which a safety assessment is made.

5. Existing Facilities and Human Resource Capacities for Evaluating Food Safety in Egypt

To generate the data to satisfy the information requirements mentioned in the guidelines, significant laboratory resources must be employed. Fortunately, Egypt has laboratories that have most of the capacity to conduct the type of testing or data gathering proposed. Consequently, the Egyptian system of risk assessment should not necessarily be viewed as a model for developing nations, since Egypt appears to have facilities that are not common to most developing countries. Because of its scientific capability, Egypt is in a position to establish itself as a resource to provide services and information to benefit other countries in the region.

The four laboratories visited while preparing this report illustrate the baseline capacity of Egyptian laboratories. Along with a description of these laboratories, the following discussion includes recommendations for their role in a system of food safety assessment and monitoring.

A. Laboratories

1. Laboratory of Dr. Nabih Ibrahim, Food Technology Research Institute, Ministry of Agriculture and Land Reclamation

This laboratory conducts research into various aspects of food and food processing to enhance the quality and uses of different foods. The foods studied include bread, fish, meat, plant oils, horticultural products, and milk. Dr. Ibrahim's laboratory is used as a reference laboratory to settle disputes between the government and importers regarding analysis of imported foods. They can conduct *in vivo* and *in vitro* work to assess nutritional aspects and functional attributes of foods, but they do not presently conduct toxicology studies. Specifically, they can test for—among other things—vitamin and mineral content, protein profile, oil profiles, and crude fiber. The laboratory presently has no capability to conduct nucleic acid analyses.

A student in Dr. Ibrahim's laboratory is planning to conduct tests in Germany to assess some aspects of food safety of the insect resistant potato developed by AGERI. These tests will be conducted to fulfill requirements for a doctoral thesis. The work will be divided into two parts. First, a study will be conducted to determine current use of genetically modified crops for food in Egypt. Second, two *in vitro* methods of safety evaluation will be applied to the insect resistant potato, with the intention of replacing or reducing animal studies. The first study is an immunological assay to check for allergenic potential. The assay will employ rat basophil leukemia (RBL) cells. IgE production will be induced by feeding or injecting the extracts of the potato or the novel protein itself into a live rat. If IgE production is successfully induced, serum will be collected from the rat and applied to the RBL cell culture along with the novel protein. The response in terms of mediator release will be followed colorimetrically. A second option being considered is the use of chip technology to check for so-called unintended effects. A pattern of transcribed mRNA from genetically engineered potatoes will be compared with the non-genetically engineered parent variety to determine whether any differences are detected. These new approaches, while interesting and of potential utility in assessing safety, have not yet been validated, and their value in assessing safety has yet to be demonstrated.

Dr. Ibrahim is familiar with the overall process for assessing food safety as generally practiced worldwide and which are included in draft guidelines being considered by the Codex Alimentarus (Codex Ad Hoc Intergovernmental Task Force on Foods Derived From Biotechnology, 2000). He is very interested in the issue of food safety testing, including food derived from transgenic plants. He is planning to dedicate a new floor in a building now under construction to food safety testing. He is concerned about demonstrating food safety as a means of allowing the importation of commodities containing transgenic plants or products derived from them.

2. Laboratory of Dr. Akila Saleh Hamza, Central Laboratory for Food and Feed

The laboratory is responsible for quality control of all feedstuffs (i.e., corn, soybean, meat, and bone meal) either imported or produced locally in Egypt. All primary staff members are trained abroad and are accredited by international accreditation agencies. Operations are financed through fees charged to the companies whose products are tested. Consequently, there are sufficient funds to purchase up-to-date equipment and to maintain it. Testing capabilities include testing for minerals (trace elements and heavy metals), vitamins, protein quantity and quality, mycotoxins and food nutritional value. Dr. Hamza has a laboratory capable of analyzing human blood chemistry. The lab could also conduct toxicology studies and analyze the digestibility of foods both *in vivo* and *in vitro*. Current nucleic acid testing capacity consists of one PCR machine, with a second machine being purchased. Dr. Hamza also is very interested in food safety testing, with the same goal as Dr. Ibrahim—the safety testing of food derived from transgenic plants in order to enable importation. Dr. Hamza believes it is necessary for an Egyptian agency to conduct its own tests, since data from other non-governmental sources outside of Egypt have proven unreliable in the past (i.e., foods from non-genetically engineered sources).

3. Laboratory of Dr. Yousef El-Dawoudy, Office of Plant Quarantine

This laboratory is dedicated to the testing of potatoes for the presence of brown rot. Therefore, the techniques in this lab serve that goal only. The lab employs immunofluorescent methods (manual reading of slides), and is beginning to develop PCR methods for detecting the brown rot pathogen.

Because the analysis of brown rot is the focus in this laboratory, and because of the importance of this analysis for Egyptian exports, it would not be advisable to divert the focus from its important mission. However, the other three laboratories have the mandate and capability to fulfill various aspects of a regulatory framework for dealing with foods derived from genetically engineered plants.

4. Laboratory of Dr. Salwa Dogheim, Laboratory of Residue Analysis of Pesticides and Heavy Metals in Foods

Dr. Dogheim's laboratory is given the task of surveying local markets, imported goods, and goods for export, for the presence of pesticides, aflatoxins, heavy metals, and contaminating microbes. Testing is done for the government (which provides approximately 60% of funding) as well as for private company clients. The laboratory has accreditation from European and U.S. accreditation bodies. While the laboratory's focus is on pesticides, Dr. Dogheim recognizes the potential need for acquiring technology and training to analyze food samples for the presence of nucleic acid sequences or proteins indicating that the food was derived from genetically engineered plants. The current operating budget for the lab is approximately L.E. 6 million for a staff of 16 analysts and approximately 120 support personnel.

B. Recommendations

The laboratories of Drs. Ibrahim and Hamza seem best positioned to conduct the work connected with safety testing of transgenic plants. Because of the wider range of testing already being conducted in Dr. Hamza's laboratory (including the

capacity to conduct toxicology tests) it appears logical for her laboratory to take primary responsibility for conducting any safety assessments required by the NBC. Dr. Ibrahim's laboratory can play a supporting but important role. It should not be forgotten that consideration of methods for safety testing of genetically engineered plants has progressed further in Dr. Ibrahim's laboratory, and expertise gained by his graduate student in Germany will be a valuable asset to his laboratory, and to the overall ability to assess the food safety of genetically engineered plants in Egypt.

It is also noted that there is at least one laboratory under the umbrella of MOH that was not visited and which could be recruited to assist in executing the safety studies required by the NBC. The role of MOH laboratories should not be ignored, particularly when studies involving medical procedures, such as some allergenicity tests, would be required. It is conceivable that these laboratories already have the expertise and equipment necessary for carrying out such tests, but their involvement in matters of medical and health concerns could also lend a more acceptable stamp of validity to conclusions from their test results.

Despite the broad capabilities existing in both Dr. Ibrahim's and Dr. Hamza's laboratories, a significant investment is needed to render one or both laboratories capable of obtaining all of the information that might be required to satisfy NBC requirements. Specifically, facilities, equipment, and expertise to conduct the full range of molecular biology work are lacking in both laboratories. Therefore, at least one fully equipped molecular biology laboratory should be established, with the capability (equipment and personnel) to conduct standard molecular biology procedures such as Southern, northern, and western blots; sequencing; molecular cloning; and various other techniques for manipulating nucleic acids, determining DNA structure, and measuring gene expression. Most if not all of this capability can be found at AGERI, but that expertise is devoted to the continuing development of technology. Assigning AGERI laboratories to conduct safety testing would not only be viewed as a conflict of interest but would also divert the laboratories from their primary mission. A replication of the analytical capabilities (and not the genetic engineering capability) found at AGERI would be desirable.

The present policies of the European Union (EU) are of extreme importance to Egypt, since this region is a major market for agricultural goods. As Egypt's market in the EU expands, and the use of genetic engineering to produce new plant varieties in Egypt begins and develops, compliance with EU regulations covering foods derived from genetically engineered varieties will be an increasingly important trade issue. Particularly important will be EU labelling requirements for foods containing or derived from genetically engineered plants. The ability to test for the presence of genetically engineered components will be critical to any system of compliance with labelling requirements. Furthermore, it is apparent that labelling requirements are being seriously considered domestically as well. The ability to test for the presence of genetically engineered components will be critical for enforcement of any Egyptian labelling regulations. Thus, for external as well as internal reasons, a laboratory that can function to certify the presence or absence of genetically engineered material in foods will be an important part of the food regulatory system. The laboratory of Dr. Salwa Dogheim appears to be ideally suited for such a task, both in terms of physical facilities and mandate.

The accuracy of results necessary for certification of genetically engineered components depends upon facilities that are able to keep contamination from other confounding sources to an absolute minimum. The way Dr. Dogheim's laboratory is constructed, and the standard operating procedures that are instituted in it best meet this goal. Furthermore, the mandate of Dr. Dogheim's laboratory is one of monitoring. The monitoring of genetically engineered content is consistent with this mandate. If Egyptian regulatory policy becomes clearer with respect to biopesticides such as *Bacillus thuringiensis*, Dr. Dogheim's laboratory might also be required to monitor the presence of these types of pesticides (even though the safety of these types of pesticides would not necessarily be a concern).

The extent of facilities for accomplishing the monitoring task will depend upon policy decisions regarding labelling (see below). One alternative would be to assay for the presence of introduced DNA, despite the fact that the presence of the DNA itself provides little indication from a safety point of view. If safety is a primary issue, then it would be more appropriate to assay the level of gene products, since it is the product of a gene and not the gene itself that would be cause for any potential safety concern. Most likely, it would be necessary to establish the capacity to conduct a combination of assays for assessing not only the presence of a gene but also the level of gene expression. Thus, Dr. Dogheim's laboratory would have to be provided with the equipment and personnel to conduct standard molecular biology analysis, a capability that does not presently exist in the laboratory. To be consistent with EU labelling requirements, the ability to conduct PCR assays, preferably real-time PCR, would be desirable. In order to maximize accuracy, a newly built separate section of Dr. Dogheim's laboratory would be required, with rooms to enable the separation of sample preparation from sample detection and analysis, and preferably a dedicated area for cleaning and maintaining non-disposable supplies and equipment. Training in laboratories certified to conduct assays for genetically engineered content would be necessary.

The assays conducted in Dr. Dogheim's laboratory would presumably be focused on food products. If similar testing would be required for feed products, then it is likely that another laboratory would be required to conduct those assays. From the standpoint of laboratory mandate, such a function would appear to fall within the purview of Dr. Hamza's laboratory. Dr. Hamza's laboratory would therefore need to be equipped as described for Dr. Dogheim. Thus, in addition to work related to safety assessments, monitoring might also become a function of this laboratory. Consequently, it would be important to rationalize the activities and responsibilities shared between Dr. Hamza's and Dr. Ibrahim's laboratories.

6. Food Labeling Laws and Regulations in Egypt and Their Application to Genetically Enhanced Food and Feed

Present food labelling requirements are not very extensive and are geared primarily to provide the consumer with information regarding product identity and quality. The following information is required on food labels of imported (and presumably also domestic) food products (FAS/USDA Attache Report No. EG0023):

1. Name and address of manufacturer,
2. Brand or trademark, if appropriate,

3. Country of origin,
4. Type of product and grade,
5. Name and address of importer,
6. Production and expiration dates,
7. Product use instructions (optional),
8. Product ingredients,
9. Storage instructions or temperature,
10. Net weight,
11. Gross weight and total number of packages per case or carton, and
12. Percentage of any preservatives.

The labeling of final products made from ingredients derived through genetic engineering is now being contemplated, but the details of regulations have not yet been determined. The consensus among the scientists and government officials interviewed for this report was that labeling for GMO content is an inevitable requirement, primarily because the EU—Egypt's most important export market—requires it. While the government appears to believe that labeling of the final product can be implemented easily, industry would not find it trivial to comply. The necessary system of traceability (i.e., a means of determining the source of transgenic material), which is a necessary component of a reliable and enforceable labeling regulation, would require operating systems that apparently do not yet exist in Egypt. For this report, it was not possible to estimate the cost of compliance, since the possible regulatory requirements are not yet sufficiently defined to arrive at the level of understanding of the burden imposed necessary to calculate that cost.

Identifying the goals of a labelling requirement is important. Clearly, one goal is compliance with EU regulations; the economic incentive behind this goal is significant and compelling. However, this reason need not drive labelling requirements for domestically consumed goods. Another goal would be to inform the public. But what purpose would this information serve? Is it simply to provide information that the public wants, or is it to warn them about a hazard to which they might be exposed? Is a label truly the best way to inform the public about the genetically modified content of their food? How would the information be conveyed for foods that are unpackaged (e.g., food in restaurants)? Will it be necessary to label foods derived from genetically engineered plants even though the introduced DNA or resulting protein is no longer detected in the final product? Will the labelling of foods containing genetically engineered components convey a connotation of hazard (or possibly of product superiority) that is unsupported by scientific evidence? Should the motivation for labelling be purely science based? These are questions that ought to be considered during the formulation of labelling requirements, at least for domestic purposes, so that labelling is based on reasoning that makes sense for Egypt rather than on a reaction to the decisions of other countries.

Identifying labelling goals also has a bearing on the scientific methods used to support those goals. If the goal is simply to inform the public of the content of the food they are consuming—regardless of safety concerns—then a method for detecting foreign DNA (expressed or not) might be appropriate. If the goal is to alert the public to a specific new ingredient, then an assay for the protein encoded by the gene or the final end product of a pathway affected by the encoded protein (e.g., a new secondary compound, carbohydrate, or oil) might be more suitable. If a labelling requirement is

imposed, monitoring for genetically engineered material can be conducted as part of the product testing procedures carried out by the government particularly within the system of monitoring for pesticides and heavy metals (Dr. Salwa Dogheim's laboratory) and/or quality control of food and feeds (Dr. Akila Saleh Hamza's laboratory).

7. Conclusion

Because of Egypt's recognition of the value of genetic engineering as a tool to improve crops, this country finds itself in the position of being a regional leader in implementing this technology. The next steps in applying this technology are critical to its successful development in Egypt; it is therefore important that these steps are taken carefully and executed correctly. Promulgation by the NBC of regulations and guidelines that provide scientifically sound assessments of food safety is one of the steps along this path.

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Annex I

Proposed Section III of Biosafety Regulations and Guidelines: Procedure for Commercial Approval of Genetically Modified Plants in Egypt

The applicant is required to make available to the National Biosafety Committee the information and data described below. The NBC will review and assess this information to inform its decision on the application.

1. Prior to submission of an application for registration of a genetically engineered variety to the Variety Registration Committee, the applicant must

   a. Consult with the National Biosafety Committee regarding the food and environmental safety assessment that must be conducted. The National Biosafety Committee will make recommendations with the advice and consent of the Supreme Committee for Food Safety. For foreign applicants, the NBC can decide whether to accept results of tests accepted and approved by regulatory agencies of other countries.
   b. Arrange for the testing and collection of data required by the National Biosafety Committee.
   c. Obtain the approval of the National Biosafety Committee, which will be forwarded to the Variety Registration Committee.

2. The information submitted for assessment of food safety should be consistent with the guidelines set forth in Appendix VIII.

3. The information submitted for assessment of environmental safety shall be consistent with the guidelines set forth in Appendix IX.

4. The National Biosafety Committee and the Supreme Committee on Food Safety will review the information submitted, and decide

   a. To approve the application, or
   b. Require further tests and consultations where additional requested data are reviewed. Additional tests may be requested especially when the results of the first assessment reveal a safety concern that needs to be resolved.

5. Further consultations shall take place until one of the following endpoints are reached:

   a. The National Biosafety Committee approves the application, with concurrence by the Supreme Committee for Food Safety;
   b. The National Biosafety Committee rejects the application because the plant is judged to be unsafe;
   c. The National Biosafety Committee and/or the applicant conclude that remaining safety issues cannot be resolved scientifically; or
   d. The applicant withdraws the application.

6. The applicant may resume the application for approval, at which time the process for approval begins at the point at which it was terminated.

7. Upon approval, the National Biosafety Committee forwards its decision and recommendation in accordance with MOA Decree No. 1648/1998.

8. The National Biosafety Committee may call on experts in relevant scientific fields to assist in the review of the data presented, or to serve in a general advisory capacity during the assessment of specific applications.

9. The decision of the National Biosafety Committee stating the basis for their decision shall be published in printed form, and made accessible via the internet or other publicly available media that the Minister of Agriculture deems appropriate.

10. After approval, the National Biosafety Committee will forward a copy of the applicant's information package to laboratories that require it to develop the necessary testing to detect transgenic material if mandated by any future labelling or quality standards regulations.

Annex II

Proposed Appendix VII to the Biosafety Regulations and Guidelines: Guidelines for Food and Feed Safety Assessment of Foods Derived from Genetically Engineered Plants

These guidelines follow the generally accepted principle, expressed by various regulatory agencies and international bodies, such as the Codex Alimentarus Commission, which states that

> the safety of foods derived from new plant varieties, including recombinant DNA plants, is assessed relative to a similar product having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a particular food, the intention is to identify new or altered hazards relative to a conventional counterpart. (Codex Ad Hoc Intergovernmental Task Force on Foods Derived From Biotechnology, 2000)

This approach employs the concept of "substantial equivalence" as the starting point for safety assessment. It assumes that a genetically engineered plant used as food is not inherently riskier than any other novel plant used as food, and that this approach can (and should) be followed for all novel foods. It also recognizes that demonstration of complete safety is not possible for any food, whether genetically engineered or not. A safety assessment should judge whether a new food (genetically engineered or not) is substantially equivalent to a comparable food presently being consumed, and is therefore at least as safe as the food to which it is being compared. The areas where substantial equivalence does not exist are then the foci of further safety assessment.

Further, these proposed guidelines assume that the safety of foods derived from a genetically engineered plant should focus on the plant itself. Since a genetically engineered plant can be the raw material for a variety of different foods prepared in a variety of different ways, analysis of each end product would be an impossible task. However, during the safety assessment of foods derived from a genetically engineered plant, the ways in which the plant will be prepared for food must be kept in mind to identify the areas necessary for a proper safety assessment.

These proposed guidelines are compatible with internationally accepted data requirements expressed by various regulatory agencies and international bodies, summarized and exemplified by the following list published by the Codex Alimentarus Commission (Codex Ad Hoc Intergovernmental Task Force on Foods Derived From Biotechnology, 2000):

A. Description of the new variety;
B. Description of the host plant and its use as food;
C. Description of the donor organism(s);
D. Description of the genetic modification(s);
E. Characterization of the genetic modification(s);
F. Safety assessment:
   a. introduced substances (non-nucleic acid substances);

b. compositional analyses of key components;
c. metabolic evaluation;
d. food processing; and
e. nutritional modification.

G. Other considerations.

The WHO (2000), ANZFA (1998), FAO (1996), Health Canada (1994) and the FDA (1992) are among other agencies that recognize these general data requirements as sufficient for a food safety assessment.

Finally, the guidelines should not be viewed as rigid requirements. The NBC may decide to waive requirements, or to impose additional requirements, if scientific evidence exists to support the decision.

The following guidelines for data requirements and safety assessment are therefore proposed:

I. Characteristics of the Genetically Engineered Plant and the Effects of the Transformation on Known Toxicants, Anti-nutrients, or Nutrients

1. A comprehensive description of the genetically engineered plant, including the following information:

   A. Taxonomy, such as species and variety name, of the non-genetically engineered plant. The designation of the genetically engineered line or lines should also be provided.
   B. Other plant species or varieties that have contributed to the genetically engineered plant's genetic background. For example, if the original plant genotype (before transformation) was the result of traditional hybridization of two compatible species, this information should be provided. Information in this category would typically be that which a breeder would know.
   C. Relevant information on the genetically engineered plant's background genotype and phenotype, including any known toxicity or allergenicity. Such information should be provided not only for the genetically engineered plant, but also for the related species and for plants that have made or may make a significant contribution to the genetic background of the genetically engineered plant. For example, in the case where a breeding line known to produce a high level of alkaloids was part of the pedigree of the line that was transformed, this information should be provided. As another example, in the case of genetically engineered peanuts, information regarding allergies normally caused by non-genetically engineered peanuts should be discussed.
   D. History of safe use for consumption as food. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (e.g., which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, and what important macro- or micro-nutrients it contributes to the diet).

2. A safety assessment carried out by the NBC based on this information might proceed by a series of questions and answers, as follows:
   A. Does the non-genetically engineered species or plant variety have a history of safe use? **If yes, go to B. If no, go to C.**
   B. Does the information on the non-genetically engineered species or related species indicate the need for analytical or toxicological tests of the genetically engineered plant? **If yes, go to C.** (The tests would be determined by the NBC with input from the Supreme Committee for Food Safety, and would focus on the levels of the toxicants or anti-nutrients that are known to exist in the non-genetically engineered plant species or variety.) **If no, go to D.**
   C. Do test results provide evidence that toxicant levels in the genetically engineered plant do not present a safety concern? **If yes, then go to D. If no, then reject the application.**
   D. Is the concentration and bio-availability of important nutrients in the genetically engineered plant within the range normally seen in the non-genetically engineered species or variety? **If yes, then conclude no unintended and unpredicted effects on known toxicants, anti-nutrients, or nutrients. If no, then consult with the NBC for any additional testing necessary to satisfy safety concerns.**

II. Sources of New Genetic Materials and Their Characterization in the Genetically Engineered Plant

1. Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:
   A. Its scientific name, taxonomic classification, and usual or common name.
   B. Information on pathogenicity or other potential toxic concerns, particularly the relationship to known pathogens or known producers of toxins, allergens or anti-nutrients within the same family.
   C. Information on the past and present uses, if any, in the food supply and exposure route(s) other than intended food uses (e.g., possible presence as contaminants). The information should include typical methods of processing and the impact of this processing on reducing or enhancing effects from potentially harmful constituents.
2. Information on all genetic material potentially delivered to the genetically engineered plant:
   A. The specific method used for transformation.
   B. The vector DNA, including the source (e.g., plant, microbial, viral, synthetic), identity and expected function in the plant.
   C. Intermediate host organisms including the organisms (e.g., bacteria) used to produce or process DNA for transformation of the host organism.
   D. The DNA to be introduced (other than the vector), including:
      i. the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA;
      ii. the size and identity;
      iii. the location and orientation of the sequence in the final vector/construct;
      iv. the function.
3. Molecular and biochemical characterization of the DNA actually inserted into the plant genome, including:
   A. The number of insertion sites;

B. The organization of the inserted genetic material at each insertion site, including sequence data of the inserted material and of the surrounding region if deemed necessary. Information should be presented regarding whether the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration;
C. Information on all known open reading frames, including those that could result in fusion proteins;
D. Any new substances in the genetically engineered plant as a consequence of the introduced DNA, including:
   i. The gene product (e.g., a protein or an untranslated RNA)
   ii. The gene product's function and any resulting new substances expected from that function;
   iii. The phenotypic description of the new trait(s);
   iv. The level and site of expression in the plant of the introduced gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions. This information should indicate if the newly introduced trait(s) are expressed as expected, in the appropriate tissues, in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;
   v. If the function of the introduced sequence(s)/gene(s) is to regulate the expression of a specific endogenous mRNA or protein, the level and expression pattern of this alteration relative to the original;
   vi. Data to demonstrate whether modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;
   vii. Data to demonstrate that all introduced traits are stably expressed and inherited.
   viii. If evidence exists suggesting that a gene in the host plant has been affected by the insertion event, that evidence should be provided. If any new proteins are produced as a result, those new proteins should be characterized.

In addition to this information, which provides the technical background to understand the nature of the genetic modification, the following specific safety data should be provided:

III. Safety Assessment of Gene Products in the Genetically Engineered Plant

1. Information relating to the safety of the new gene products:
   A. The concentration of the substance in the parts of the genetically engineered plant used as food. This information will be already determined in II.D.iv. above.
   B. In the case of proteins, information on amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g., protease inhibitors or lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems.
   C. Information to demonstrate that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to the

genetically engineered plants, if those plants do not normally express those compounds.

D. Toxicology studies if needed. Conventional toxicology studies would not be required in all cases. For example, they would not be necessary when the substance or a closely related substance that has been consumed safely in food made from the non-genetically engineered plant is found in similar amounts in the genetically engineered plant. A specific example of such as case would be the engineering of plants to express viral coat proteins. Such proteins are known to be a normal part of the human diet, occurring at much higher concentrations in the normal human food supply. Appropriate oral toxicity studies may be carried out in cases where the protein has biological activity in humans or animals, but is not similar to proteins that have been safely consumed in food, and has not previously been consumed safely in food. Careful consideration should be paid to the design of such studies, since toxicology studies designed to assess the safety of specific compounds such as food additives or pesticides might not be appropriate for whole foods.

E. Information relating to the allergenicity of the new products (particularly proteins) produced in the genetically engineered plant. Guidance on the process for assessing the allergenicity of the new product has been provided by the FAO and WHO (FAO, 2001; WHO, 2000), in the form of a decision tree.

2. Information relating to the nutritional value of the genetically engineered plant relative to the original, non-genetically engineered plant. An analysis should be done of the nutrients which are typically expected to be found in the plant when used as food. Guidance for the types of nutrients to measure in various foods can be found in reference texts, as well as in specifications that have either been or can be set by the Egyptian Office of Standards.

3. Any other information that might indicate a scientific reason for a safety concern.

As these requirements illustrate, a proper safety assessment need not rely solely upon the results of testing. Information from existing scientific literature, databases, and other relevant and reliable sources of scientific information cannot be ignored and should be used to assess the safety of a genetically engineered plant. The existing body of information could be judged sufficient to conclude that specific toxicology or allergenicity tests would not be required.

4. A series of questions and answers that could be used by the NBC to assess the safety of the donor(s) follows:

A. Is food from the donor(s) commonly allergenic? **If yes, go to B. If no, go to C.**

B. Can it be demonstrated that the allergenic determinant has not been transferred to the genetically engineered plant? **If yes, go to C. If no, go to D.**

C. Do characteristics of the donor species, related species, or progenitor lines indicate a need for analytical or toxicological tests? **If yes, go to E. If no, conclude that there is no concern for toxicity.**

D. Require allergenicity tests, using protocols such as the FAO/WHO decision tree and other subsequent refinements as guidance. **If tests show no**

evidence of allergenicity, proceed with toxicology considerations beginning at C. If allergenicity tests show the potential for allergenicity, then reject the application or decide to approve under special restrictions such as labelling, and proceed with toxicology assessments at C.

E. Do test results provide evidence that toxicant levels in the genetically engineered plant do not present a safety concern? **If yes, conclude that there is no concern for toxicity. If no, then reject application.**

5. The following is a series of questions and answers that could be used by the NBC to assess the safety of new proteins produced in the genetically engineered plant:
   A. Will the new protein be present in food made from the genetically engineered plant? **If yes, go to B. If no, conclude that there are no safety concerns.**
   B. Is the new protein derived from a food source, or substantially similar to an edible protein? **If yes, go to C. If no, go to D.**
   C. Is food from the donor commonly allergenic? **If yes, go to E. If no, go to F.**
   D. Does the biological function of the introduced protein raise any safety concern, or is the introduced protein reported to be toxic? **If yes, reject application or consider restricted approval for specific purposes. If no, go to H.**
   E. Can it be demonstrated that the allergenic determinant has not been transferred to the genetically engineered plant? **If yes, go to F. If no, go to I.**
   F. Is the introduced protein reported to be toxic? **If yes, reject application, or consider restricted approval for specific purposes. If no, go to G.**
   G. Will the intake of the donor protein in the new variety be generally comparable to the intake of the same or similar protein in donor or other food? **If yes, go to H. If no, go to D.**
   H. Is the introduced protein likely to be a major constituent in the human or animal diet? **If yes, consider the potential impact on nutrition, and approve application if nutritional impact is neutral or positive. If negative, consider reasons for or against approval before final decision. If no, conclude that there are no safety concerns.**
   I. Conduct allergenicity tests as required by the NBC, using the FAO/WHO decision tree and other subsequent refinements as guidance. **If tests show no evidence of allergenicity, proceed with toxicology considerations beginning at F. If allergenicity tests show potential for allergenicity, then reject the application or decide to approve under special restrictions such as labelling, and proceed with toxicology assessments at F.**

6. The following is a series of questions and answers that could be used by the NBC to assess the safety of new or modified fats or oils produced in the genetically engineered plant:
   A. Has there been an intentional alteration in the identity, structure, or composition of fats or oils in the genetically engineered plant? **If yes, go to B. If no, conclude that there are no safety concerns.**
   B. Have intentional alterations been in a fat or oil that will be a major constituent in the human or animal diet? **If yes, consider the potential impact on nutrition, and go to C if nutritional impact is neutral or positive. If negative, reject or decide to proceed to C. If no, go to C.**

C. Are any unusual or toxic fatty acids produced in the new variety? **If yes, reject application, or consider restricted approval for specific purposes. If no, conclude that there are no safety concerns.**

7. The following is a series of questions and answers that could be used by the NBC to assess the safety of new or modified carbohydrates produced in the genetically engineered plant:
   A. Has there been an intentional alteration in the structure, composition, or level of carbohydrates in the genetically engineered plant? **If yes, go to B. If no, conclude that there are no safety concerns.**
   B. Have any structural features or functional groups been introduced into the carbohydrate that do not normally occur in food carbohydrates? **If yes, reject application, or consider restricted approval for specific purposes and proceed to C. If no, go to C.**
   C. Have there been any alterations that could affect digestibility or nutritional qualities in a carbohydrate that is likely to be a major constituent of the human or animal diet? **If yes, reject application, or consider restricted approval for specific purposes. If no, conclude that there are no safety concerns.**

Annex III

Application of Food Safety Guidelines to the Food and Feed Safety Assessment of Potato Variety "Spunta," Genetically Engineered to be Resistant to Potato Tuber Moth

Background

AGERI has cooperated with researchers at Michigan State University in developing a potato that has been genetically engineered to resist attack by the potato tuber moth, *Phthorimaea operculella* Zeller, the primary insect pest of cultivated potato in tropical and subtropical regions. A codon-modified *Bacillus thuringiensis cry-5* gene, encoding a delta-endotoxin protein was introduced into the variety "Spunta" via *Agrobacterium tumefaciens*-mediated transformation. In addition to the *cry5* gene, the potato also expresses the marker genes, *npt2*, encoding the enzyme neomycin phosphotransferase from *E. coli* as a selectable marker.

To illustrate how the guidelines proposed in Annex I would be applied, the following discussion describes the type of information that could be used to comply with the guidelines and arrive at a satisfactory safety assessment of the *cry5* potato.

I. Characteristics of the Genetically Engineered Plant and the Effects of the Transformation on Known Toxicants, Anti-nutrients, or Nutrients

1. A comprehensive description of the genetically engineered plant, including the following information:

   A. Taxonomy, such as species and variety name, of the non-genetically engineered plant. The designation of the genetically engineered line or lines should also be provided.

   The genetically engineered plant is a potato, *Solanum tuberosum* var. Spunta. Transgenic lines are designated Spunta-G2 and Spunta-G3.

   B. Other plant species or varieties that have contributed to the genetically engineered plant's genetic background. For example, if the original plant genotype (before transformation) was the result of traditional hybridization of two compatible species, this information should be provided. Information in this category would typically be that which a breeder would know.

   The pedigree of Spunta would be described in this section.

   C. Relevant information on the genetically engineered plant's background genotype and phenotype, including any known toxicity or allergenicity. Such information should be provided not only for the genetically engineered plant, but also for the related species and for plants that have made or may make a significant contribution to the genetic background of the genetically engineered plant.

Phenotypic characteristics that were reported for registration of this variety with the Variety Registration Committee would be relevant information to be included here. If Spunta is a variety known for a particularly important trait, that trait information would be provided.

D. History of safe use for consumption as food. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (e.g., which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, and what important macro- or micro-nutrients it contributes to the diet).

A discussion of the history of *Solanum tuberosum* as a plant used for food for thousands of years would be provided here. The history of use of potato as a food in Egypt would also be included, with information regarding the approximate date of introduction into the country as a novel food, and any history of known toxicity and/or allergic reactions to the food in Egypt. For example, since potatoes contain steroidal glycoalkaloids that have been responsible for human poisoning (Zitmak, 1977), this fact should be mentioned here, along with any specific documentation of human poisonings in Egypt. Typical methods of preparation and processing for use in Egypt would be provided as well. If potatoes are a significant crop used for feed in Egypt, that fact should also be noted. If there are any national standards for identity and quality of potato, such as nutrient values set by the Egyptian Office of Standards, they would be indicated in this section.

2. A safety assessment of Spunta G2 and Spunta G3 carried out by the NBC based on this information might proceed by a series of questions and answers, as follows:

A. Does *Solanum tuberosum* var. Spunta have a history of safe use? **If yes, go to B.**

B. Does the information on *Solanum tuberosum* or related species indicate the need for analytical or toxicological tests of the genetically genetically engineered plant? **If yes, go to C.**

Because of the known occurrence of steroidal glycoalkaloids in potato, analytical tests to measure the level of this toxicant in Spunta G2 and Spunta G3 would be warranted.

C. Do test results provide evidence that toxicant levels in the genetically engineered plant do not present a safety concern? **If yes, then go to D. If no, then reject the application.**

The level of glycoalkaloids in Spunta G2 and Spunta G3 should be compared with non-genetically engineered Spunta. If standard protocols have been established for determining glycoalkaloid content during testing for seed

variety registration, those protocols should be followed. If this is the case, the possibility of involving the Variety Registration Committee should be considered to avoid duplication of work during the registration process. If no standard tests have been established, a statistically sound replicated series of trials should be established to determine the range of glycoalkaloid levels in the transgenic and non-transgenic Spunta. If levels of either Spunta G2, Spunta G3, or Spunta exceed maximum acceptable glycoalkaloid levels (e.g., see Sinden, 1991), the varieties should not be marketed. Such measurements will allow for safety assessment of non-genetically engineered Spunta itself if this parameter has not been measured before.

D. Is the concentration and bio-availability of important nutrients in the genetically engineered plant within the range normally seen in the non-genetically engineered species or variety? **If yes, then conclude no unintended and unpredicted effects on known toxicants, anti-nutrients, or nutrients. If no, then consult with the NBC for any additional tests to be performed to satisfy safety concerns.**

If analysis of important nutrients that have been established for variety registration purposes, or by the Egyptian Office of Standards are necessary, these nutrients should be measured, using any protocols established by the Variety Registration Committee. If no standard tests have been established, a statistically sound replicated series of trials should be established to determine the range of nutrient levels in the transgenic and non-transgenic Spunta. In the absence of specifications for nutrient levels, then the nutrients and other components suggested by Love (2000) could be measured: total solids and sugars (important traits), protein and vitamin C (desirable).

II. Sources of New Genetic Materials and Their Characterization in the Genetically Engineered Plant

1. Information on the donor organism(s) and, when appropriate, on other members closely related to the donor organism(s), including:

   A. Its scientific name, taxonomic classification, and usual or common name.

   The *Bacillus thuringiensis* (donor of the cry5 gene) should be described, including the strain. The other donors that should be described are *Escherichia coli*, the contributor of the *npt2* gene; Cauliflower Mosaic Virus, the contributor of the promoter and terminator sequences for the *cry5* gene; and *Agrobacterium tumefaciens*, the contributor of the promoter and terminator for the *npt2* gene, the T-DNA and possibly other backbone sequences.

   B. Information on pathogenicity or other potential toxic concerns, particularly the relationship to known pathogens or known producers of toxins, allergens or anti-nutrients within the same family.

   If any of the donors mentioned above are known to be pathogenic or to produce toxins, that information should be reported here.

C. Information on past and present uses, if any, in the food supply and exposure route(s) other than intended food uses (e.g., possible presence as contaminants). The information should include typical methods of processing and the impact of this processing on reducing or enhancing the effects from potentially harmful constituents.

Information on the use of any of the donors mentioned above in the food supply. If the bacteria are known to be present to a certain degree in food, that information should be supplied. Information on the use of *Bacillus thuringiensis* as a pesticide and its presence as a residue on food, if known, should also be reported. The literature on the food and environmental safety of *Bacillus thuringiensis* (e.g., McClintock et al., 1995; WHO, 1999) should be provided here.

2. Information on all genetic material potentially delivered to the genetically engineered plant:

A. The specific method used for transformation.

Transformation was mediated by *Agrobacterium tumefacens*. Since this method is standard, published procedures could be referenced here, with any additional information provided if there was any variation that might pose a safety concern.

B. The vector DNA, including the source (e.g., plant, microbial, viral, or synthetic), identity and expected function in the plant.

A detailed map of the vector plasmid would be provided. In the case of Spunta G2 and Spunta G3, the map of pSPUD5 would be given along with an indication of the origin of the various elements in the map.

C. Intermediate host organisms including the organisms (e.g., bacteria) used to produce or process DNA for transformation of the host organism.

Since engineering of pSPUD5 was probably conducted in *E. coli*, the *E. coli* host strains should be reported.

D. The DNA to be introduced (other than the vector), including:
   i. the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA;
   ii. the size and identity;
   iii. the location and orientation of the sequence in the final vector/construct;
   iv. the function.

This information would be included in the detailed map provided in II.2.B. above. If the actual DNA to be inserted differs from the entire vector, the intended fragment would be indicated.

3. Molecular and biochemical characterization of the DNA actually inserted into the plant genome, including:
   A. The number of insertion sites;
   B. The organisation of the inserted genetic material at each insertion site, including sequence data of the inserted material and of the surrounding region if deemed necessary. Information should be presented regarding whether the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration.

      Information for A and B would be provided by a thorough Southern blot analysis of Spunta G2 and Spunta G3. The National Biosafety Committee would decide whether sequencing of the inserts would be necessary to resolve any specific safety concerns.

   C. Information on all known open reading frames, including those that could result in fusion proteins.

      Southern analysis, and certainly a complete sequencing of all inserts, would reveal whether events occurring during transformation resulted in the production of new open reading frames, as well as the preservation of the structure of intended open reading frames. A description of these reading frames should be provided.

   D. Any new substances in the genetically engineered plant as a consequence of the introduced DNA, including:
      i. The gene product (e.g., a protein or an untranslated RNA);
      ii. The gene product's function and any resulting new substances expected from that function;
      iii. The phenotypic description of the new trait(s);
      iv. The level and site of expression in the plant of the introduced gene product(s), and the its metabolite levels in the plant, particularly in the edible portions. This information should indicate if the newly introduced trait(s) are expressed as expected, in the appropriate tissues, and in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;
      v. If the function of the introduced sequence(s)/gene(s) is to regulate the expression of a specific endogenous mRNA or protein, provide the level and expression pattern of this alteration relative to the original;
      vi. Data to demonstrate whether modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;
      vii. Data to demonstrate that all introduced traits are stably expressed and inherited;
      viii. If evidence exists to suggest that a gene in the host plant has been affected by the insertion event, that evidence should be provided. If any new proteins are produced as a result, those new proteins should be characterized.

Expression data that are obtained during the normal course of the characterization of Spunta G2 and Spunta G3 would be reported here. Since the promoters used to drive the expression of the Bt gene and *npt2* are constitutive, expression is expected in most tissues. However, expression levels might vary between tissue types as well as at different stages of the plant's life cycle. Any observations of altered phenotypes (other than the intended alteration), or any available mapping data that indicates the insertion into another gene, should be taken into consideration. In addition to the regulatory function of the promoters and terminators already described, and the function of the *cry5* gene to express the delta-endotoxin which confers resistance to potato tuber moth, the function of the *npt2* gene to express the neomycin phosphotransferase which confers resistance to the selective agent, kanamycin, would also be described. If sequences outside the T-DNA are incorporated, information on any expression of genes or regulatory sequences and their known function (or lack of function) in plants would be needed.

While, strictly speaking, the expression of the *npt2* gene is not involved in producing the intended trait, the npt2 protein must be considered in this document, since this case might provide the first opportunity for the NBC to review data concerning its safety. Therefore, the level of the npt2 protein in various tissues, and particularly in tubers, should be reported. If there are any other functional genes revealed by the molecular characterization of Spunta-G2 or Spunta-G3, the level of expression of those genes should be reported as well.

III. Safety Assessment of Gene Products in the Genetically Engineered Plant

1. Information relating to the safety of the new gene products:

   A. The concentration of the substance in the parts of the genetically engineered plant used as food. This information will be already determined in II.D.iv. above.

   B. In the case of proteins, information on amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g., protease inhibitors, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems.

   A computer search of protein sequence databases such as PIR, SwissProt, and TrEMBL would be conducted to determine sequence similarity to proteins that might pose safety concerns. A search strategy similar to that proposed by the FAO for sequence similarity searching for allergens (FAO, 2001) could be adapted.

   In vitro studies to determine the stability of the cry5 protein to heat/processing, or to degradation by low pH and digestive enzymes would also be useful in determining whether a safety concern exists. Proteins that are labile under these conditions would be regarded as posing no safety concern.

C. Information to demonstrate that gene codings for known toxins or anti-nutrients present in the donor organisms are not transferred to the genetically engineered plants, if those plants do not normally express those compounds.

If any of the donor organisms used to donate genetic material to Spunta G2 or Spunta G3 are known to produce toxins, then the genes encoding those toxins should be identified and information should be provided to the NBC to show that those genes were not transferred.

D. Toxicology studies, if needed.

At least three options could be followed at this point. First, one could rely on the existing data, based on extensive literature concerning the safety of microbial Bt preparations, to support the argument that the cry5 protein in the transgenic Spunta potato could be deemed safe. Examples of these data include reviews of the literature by the World Health Organization (WHO, 1999), McClintock, et al. (1995), and Betz et al. (2000). These reviews of the literature refer to numerous studies supporting the lack of toxicity of Bt proteins. This information, in conjunction with the data obtained to answer III.B. above, could be judged sufficient to establish the safety of the cry5 protein. Despite the scientifically sound nature of this option, it might not be a politically viable choice, since this potato will most likely be the first genetically engineered crop to be commercialized in Egypt.

Consequently, a second option could be considered: measuring the level of cry5 protein in various forms in which the potatoes might be consumed. For example, it is most likely that the potatoes will be consumed as a cooked food, either prepared in the home or as a processed food. Determination of identifiable cry5 protein in representative foods (e.g., boiled or fried, as in a potato chip) would determine whether there is a need to conduct further safety assessment. If no cry5 protein could be measured, then the need for further testing would be eliminated.

Finally, a third option would be conducting actual feeding studies involving the cry5 protein. This option would appear to be necessary only if significant amounts of the cry5 protein are found in cooked potato preparations, or if significant consumption of raw potato in Egypt's human or animal diet is evident. Feeding studies could be accomplished in various ways: First, feeding studies could be done with whole potatoes. Consideration should be given here to the form in which the whole potatoes should be tested. While raw potatoes might provide the greatest amount of cry5 protein, it might not be the relevant form for assessing safety (see the third option above). Further, the use of raw potatoes as a food source in feeding studies presents methodological problems that might invalidate the study. For example, when used in rat feeding studies, the relatively low amount of protein in potatoes could itself be the source of pathological effects such as reduction of growth rate, development, hepatic metabolism, and immune function (LeMoullac et al., 1992; Konno et al., 1993). Alternatively, whole protein from potatoes could be extracted from transgenic and nontransgenic lines, and incorporated into a standard laboratory feed. This approach might be difficult to

accomplish given the amounts of protein—particularly cry5 protein—that would be needed for statistically valid tests. In this case, care would have to be taken to characterize the protein well. The co-purification of other compounds must be avoided. A third approach would be to express the protein in bacteria in order to isolate sufficient amounts to incorporate in feeding studies. If this approach is taken, experiments must be conducted to demonstrate the equivalence of the bacterial and plant-produced proteins. Other guidelines and considerations for the design of feeding studies are given by the WHO (WHO, 2000).

The safety of the npt2 protein should also be considered. Ample data and direct assessments, including feeding studies, exist that can be relied upon to support the safety of the npt2 protein (WHO, 1993; FDA, 1994). Therefore, since the appropriate determinations have already been made with this protein, it would not be necessary to repeat the safety testing of this protein in Egypt.

E. Information relating to the allergenicity of the new products (particularly proteins) produced in the genetically engineered plant. Guidance on the process for assessing the allergenicity of the new product has been provided by the FAO and WHO (FAO, 2001; WHO, 2000), in the form of a decision tree.

The FAO/WHO decision tree could be used as guidance to determine if allergenicity testing is appropriate, and if so, what types of testing would be sufficient to establish safety.

2. Information relating to the nutritional value of the genetically engineered plant relative to the original, non-genetically engineered plant. An analysis should be done of the nutrients that are typically expected to be found in the plant when used as food. Guidance for the types of nutrients to measure in various foods can be found in reference texts, as well as in specifications that either have been or could be set by the Egyptian Office of Standards.

   The data relevant to this question are discussed in section I.2.D. above.

3. Any other information that might indicate a scientific reason for a safety concern.

   If there is specific information unique to Spunta G2 or Spunta G3 that might raise a safety concern, that information should be provided to the NBC.

4. A safety assessment of the Bt protein in Spunta G2 and Spunta G3 might be carried out by the NBC based on this information and through a series of questions and answers as follows (the same procedure could be applied to the npt2 protein):

   A. Is food from the donor(s) commonly allergenic? **No, neither *Bacillus thuringiensis, Agrobacterium tumefaciens, Escherichia coli,* nor Cauliflower Mosaic Virus are made into food, and therefore are not commonly recognized to be allergenic. Go to C.**

While the donors, particularly the bacteria, are not commonly known to be allergenic—no normal population of individuals are known to be sensitized (FAO, 2001)—and therefore present a reduced concern for allergenicity, additional information compiled to satisfy III.B. above could be employed in the FAO/WHO decision tree. The source of the genes, as mentioned above, is not recognized to be allergenic, and therefore, a sequence similarity search according to specific criteria (e.g., those proposed by the FAO) (FAO, 2001) would be recommended.

Assuming no sequence similarity to known allergens is found, then tests to assess stability to digestion and/or processing could be conducted. Tests for stability to digestion could be done by assessing the timecourse of degradation of the cry5 protein in simulated gastric fluid (see FAO, 2001 for additional guidance). A comparison of this timecourse with the known timecourse of degradation of Bt proteins already approved for food and feed consumption in other countries will allow the NBC to judge whether a concern exists. Tests for stability to processing could be done by assessing whether the cry5 protein remains intact after boiling and/or frying, or after any other common method of preparing potatoes in Egypt.

Assuming that the Bt protein is found to be unstable to digestion and/or to processing, then a conclusion could be reached that there is no evidence of allergenicity. However, other scenarios are possible. For example, if sequence similarity is found to a known allergenic protein, then serum screening, skin prick tests, or in a few cases *in vivo* human testing (e.g., double-blind, placebo-controlled food challenges) might be warranted. The latter extreme, as with any experimentation involving human testing, would have to pass scrutiny by an institutional review committee to assess the necessity and ethics of such testing.

B. Can it be demonstrated that the allergenic determinant has not been transferred to the genetically engineered plant? **If yes, go to C. If no, go to D.**

C. Do characteristics of the donor species, related species, or progenitor lines indicate a need for analytical or toxicological tests? **No. While pathogenic strains of *E. coli* are known, the donor strains are non-pathogenic, and the safety of the *npt2* gene derived from *E. coli* has been established (WHO, 1993; FDA, 1994). The other donors are not known to be toxic. Therefore, the NBC could conclude that analytical or toxicological tests are not warranted.**

D. Require allergenicity tests, using protocols such as the FAO/WHO decision tree and other subsequent refinements as guidance. **If tests show no evidence of allergenicity, proceed with toxicology considerations beginning at C. If allergenicity tests show potential for allergenicity, then reject the application or decide to approve under special restrictions such as labelling, and proceed with toxicology assessments at C.**

E. Do test results provide evidence that toxicant levels in the genetically engineered plant do not present a safety concern? **If yes, conclude that there is no concern for toxicity. If no, then reject application.**

5. Additional questions and answers that could be used by the NBC to further assess the safety of the Bt protein (as well as the npt2 protein) produced in the genetically engineered plant:

A. Will the new protein be present in food made from the genetically engineered plant? **If yes, go to B. If no, conclude that there are no safety concerns.**

The information obtained to assess the stability of the Bt protein in processed foods would be applied to answer this question, as well as information on any use of potato as a raw food.

B. Is the new protein derived from a food source, or substantially similar to an edible protein? **No, *Bacillus thuringiensis* is not commonly a food source. However, it could be argued that the cry5 protein is substantially similar to an edible protein, since it has sequence similarity to other cry proteins commonly in the food supply in certain countries. Despite this argument, one could choose to go to D.**

C. Is food from the donor commonly allergenic? **If yes, go to E. If no, go to F.**

D. Does the biological function of the introduced protein raise any safety concern, or is the introduced protein reported to be toxic? **No, in order to exhibit its toxic properties, the Bt protein functions specifically in alkaline environments and binds to specific receptors in insect gut epithelia. Neither of these conditions is met in mammals, and therefore the biological activity of these toxins in humans poses no safety concern. Go to H.**

E. Can it be demonstrated that the allergenic determinant has not been transferred to the genetically engineered plant? **If yes, go to F. If no, go to I.**

F. Is the introduced protein reported to be toxic? **If yes, reject application, or consider restricted approval for specific purposes. If no, go to G.**

G. Will the intake of the donor protein in the new variety be generally comparable to the intake of the same or similar protein in donor or other food? **If yes, go to H. If no, go to D.**

H. Is the introduced protein likely to be a major constituent in the human or animal diet? **No, the level of expression of the *cry5* gene is not enough to cause the cry5 protein to be a major constituent of the human or animal diet. Conclude that there are no safety concerns.**

I. Conduct allergenicity tests as required by the NBC, using the FAO/WHO decision tree and other subsequent refinements as guidance. **If tests show no evidence of allergenicity, proceed with toxicology considerations**

beginning at F. If tests show the potential for allergenicity, then reject the application or decide to approve under special restrictions such as labelling, and proceed with toxicology assessments at F.

Annex IV

Procedure for Commercial Registration of Genetically Engineered Plant Varieties as Outlined in MALR Ministerial Decree No. 1648/1998*

This protocol provides framework for the steps and procedures that must be observed by private or public companies and institutions concerned with the production of the genetically modified plant varieties, whether those companies are Egyptian or foreign. The protocol outlines steps required before permitting the handling of those varieties on a commercial scale.

Step 1.

The company or the cooperating organization willing to commercialize a genetically modified plant variety must apply to the Secretariat of the National Biosafety Committee for a permit application, a special form for handling genetically modified plant material. The address of the NBC Secretariat follows:

Agricultural Genetic Engineering Research Institute
Agricultural Research Center
9, Gamaat El –Qahera St., Giza 12619

Step 2.

The applicant must fill in the form to provide necessary information on the genetically engineered plant material which in this case are the plant variety, the genetic modification technique used, and other related data as itemized in the permit application. The applicant should submit all the pertinent studies that indicate the bio-safety level of the GM variety: environmental safety and food safety (with an assertion that no risk of any kind is evident on humans, animals, plants and all the other components of the environment). The applicant shall also submit a document that confirms the use of the GM plant variety in the country of origin.

Step 3.

The NBC Secretariat shall submit the completed form at the first consecutive meeting of the Committee for consideration and a decision on whether or not to approve the handling of the variety applied for and the level of its handling (i.e., open field testing, limited field testing or testing inside greenhouses).

Step 4.

If and when the NBC approves the handling of the GM plant variety and specifies the level of that handling, the following procedures shall be applied:

***(Unofficial translation with minor modifications for ease of reading in English)**

1. If the GM plant variety has been produced in Egypt, the applicant will be allowed to initiate testing only at the set level of handling. The NBC may inspect the testing sites by itself or by whomever it designates for this task (e.g., AGERI/ARC). Inspection shall be undertaken at any time NBC deems appropriate to ascertain conformity with the prerequisite technical standards. The committee has the right to draw samples from the genetic material for molecular analysis to confirm the nature of genes introduced and the degree of gene expression in the GM plant variety.

2. If the GM plant variety has been produced outside Egypt and the original producer thereof (or his representative in Egypt) is willing to initiate testing within the approved level of handling, the applicant must obtain a permit for importation of a limited quantity of the planting material (normally seeds) as a preliminary step for testing (i.e., field experimentation) within the approved level of handling.

   The following procedures are to be observed:

   1. The foreign company, or its Egyptian agent, shall apply to the "Supreme Committee on Food Safety," a standing committee within MOH, for an import permit, having authenticated its application by the NBC. The application may take the form of a Material Transfer Agreement (MTA) or any other form, provided that transparency and clarity are fulfilled.

   2. If the import permit has been issued by the Supreme Committee for Food Safety and the foreign company (or its agent in Egypt) has initiated the testing operation at the set level of handling, the NBC shall, either by itself or by whomever it officially designates (e.g., AGERI/ARC), inspect the testing/ experimentation sites at anytime it deems appropriate to ensure compliance with the needed technical standards. The Committee shall have the right to draw samples from the genetic materials for analysis at the molecular level in order to elicit the nature of genes introduced into (the genetic structure of) the plant variety and to detect the degree of gene expression in that variety.

Step 5:

Having completed field testing (i.e., open or limited) of the GM plant variety in Egypt, and having confirmed that the variety meets all biosafety and environmental considerations, the applicant willing to commercialize the GM plant variety can file an application for registration of the GM plant variety with the Secretariat of the VRC (Variety Registration Committee) of MALR. Special forms are accessible from the Secretariat's headquarters at the following address:

The Central Administrations for Seed Certification
Agricultural Research Center 9, Cairo University St.
Giza, 12619

Annex V

Food Safety Assessment Procedures of WHO, CFIA, and FDA

codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

JOINT OFFICE: Viale delle Terme di Caracalla 00100 ROME Tel: +39(06)5701 Telex: 625825-625853 FAO I Email: Codex@fao.org Facsimile: +39(06)5705.4593

Agenda Item 5

CX/FBT 01/5
December 2000

JOINT FAO/WHO FOOD STANDARD PROGRAMME

CODEX AD HOC INTERGOVERNMENTAL TASK FORCE ON FOODS DERIVED FROM BIOTECHNOLOGY

Second Session

Chiba, Japan, 25-29 March 2001

CONSIDERATION OF PROPOSED DRAFT GUIDELINE FOR THE CONDUCT OF SAFETY ASSESSMENT OF FOODS DERIVED FROM PLANTS OBTAINED THROUGH MODERN BIOTECHNOLOGY *AT STEP 4*

BACKGROUND

1. The Codex *Ad Hoc* Intergovernmental Task Force on Foods Derived from Biotechnology (CTFBT) held its First Session in Chiba from 14-17 March 2000 and agreed to establish an *Ad Hoc* Working Group to develop specific guidance on the risk assessment of foods derived from biotechnology (ALINORM 01/34, para.35).

2. In June 2000, the 47th Session of the Executive Committee approved, at Step 1, the development of the text mentioned above, its precise title being still to be determined (ALINORM 01/3, para.43 and Appendix III).

REPORT OF THE *AD HOC* WORKING GROUP

3. The *Ad Hoc* Working Group met twice in Tokyo, Japan, from 5-7 July and from 30 October to 1 November 2000. The invitation was sent to all participating Members and Observers of the First Session of CTFBT as well as other Members and international organizations that indicated their interest.

FIRST MEETING OF THE AD HOC WORKING GROUP, TOKYO, 5-7 JULY 2000

4. Delegates from 21 Members and 16 observers attended the First Meeting of the Working Group. The Working Group reviewed a preliminary text of the Proposed Draft Guideline for the Conduct of Safety Assessment of Foods Derived from Recombinant-DNA Plants, while, at the same time,

considering the Proposed Draft General Principles for the Risk Analysis of Foods Derived from Modern Biotechnology[1].

5. The Working Group had an in-depth discussion on the proposed draft Guideline (risk assessment document), and there was a general consensus that priority should be given to the development of guidance for the risk assessment of genetically modified plants, rather than other categories of foods, given that plant products were already being placed on the market and there was urgency in this area. The Working Group took note of the title of the document referring to "safety assessment", instead of "risk assessment", aimed at highlighting the distinction between the conventional risk assessment dealing with discrete chemicals and the assessment of whole foods including foods derived from biotechnology.

6. The Working Group agreed that the proposed draft Guideline would need to be redrafted by a smaller drafting group, taking full account of the recommendations of the Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology held in Geneva from 29 May to 2 June 2000[2]. Australia, Canada, Japan, United Kingdom and the United States volunteered to participate in the redrafting of the proposed draft Guideline.

7. The group of volunteer countries had a three-day meeting in early September in Tokyo and drafted a revised version of the proposed draft Guideline. The revised proposed draft Guideline was placed on the Codex website to invite comments from all interested Members and Observers.

SECOND MEETING OF THE *AD HOC* WORKING GROUP, TOKYO, 30 OCTOBER – 1 NOVEMBER 2000

8. The Second Meeting of the *Ad Hoc* Working Group was held with the participation of delegates from 16 Members and 13 observers to review the proposed draft documents for the second time.

9. With regard to the proposed draft Guideline (risk assessment/ safety assessment document), the *Ad Hoc* Working Group noted that the proposed draft Guideline, as redrafted by the drafting group, drew most of its texts from the Report of the Joint FAO/WHO Expert Consultation on Biotechnology held in Geneva, Switzerland from 29 May to 2 June 2000, and therefore reflected scientific advice provided by the joint FAO/WHO expert consultation, including the answers to the five specific questions previously put forward by the First Session of CTFBT (ALINORM 01/34, paras.37, 38, Appendix III).

10. The *Ad Hoc* Working Group was informed that FAO and WHO were planning to convene, in January 2001, a second Expert Consultation on Foods Derived from Biotechnology, focusing on the issue of allergenicity. Even so, the *Ad Hoc* Working Group decided to continue the discussion and development of the proposed draft Guideline, including the section on the assessment of allergenicity, with the understanding that subsequent amendments could, if required, be done by the Second Session of CTFBT, in order to accommodate the outcome of the second FAO/WHO expert consultation.

11. Several amendments were made to the proposed draft Guideline to ensure the consistency with the proposed draft Principles document and to clearly depict the concept of safety assessment. The title of the document was amended to bring it in line with the work in progress within the Codex Committee on Food Labelling, although it was agreed that the scope of the proposed draft

[1] For the latter Principles, please refer to CX/FBT 01/4.

[2] The report is available as "Safety Aspects of Genetically Modified Foods of Plant Origin. Report of the Joint FAO/WHO Expert Consultation on Biotechnology (WHO/SDE/PHE/FOS/00.6)" at http://www.who.int/fsf/GMfood/ FAO-WHO_Consultation_report_2000.pdf. and http://www.fao.org/WAICENT/FAOINFO/ECONOMIC/ESN/gm/biotec-e.htm

Guideline, at least for the time being, should be limited to recombinant-DNA plants. The proposed draft Guideline as amended is attached as Annex to the present document.

REQUEST FOR COMMENTS

12. The Proposed Draft Guideline for the Conduct of Safety Assessment of Food Derived from Recombinant-DNA Plants is attached as Annex for comments at Step 3. The comments submitted will be considered by the Task Force at its Second Session when discussing the attached Proposed Draft Guideline at Step 4.

Annex

PROPOSED DRAFT GUIDELINE FOR THE CONDUCT OF SAFETY ASSESSMENT OF FOODS DERIVED FROM PLANTS OBTAINED THROUGH MODERN BIOTECHNOLOGY

(At Step 3 of the Elaboration Procedure)

SECTION 1 - SCOPE

1. This Guideline supports the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology and addresses safety and nutritional aspects of foods derived from plants that have a history of safe use as sources of food and that have been modified to exhibit new traits.

2. The Codex principles of risk analysis, particularly those for risk assessment, are primarily intended to apply to discrete chemical entities such as food additives and pesticide residues or a specific chemical or microbial contaminant; they are not intended to apply to whole foods as such. Indeed, few foods have been assessed scientifically in a manner that would fully characterise all risks associated with the food. Further, many foods contain substances that would likely be found harmful if subjected to conventional approaches to safety testing. Thus, a more focused approach is required where the safety of a whole food is being considered.

3. This approach is based on the principle that the safety of foods derived from new plant varieties, including recombinant DNA plants, is assessed relative to a similar product having a history of safe use, taking into account both intended and unintended effects. Rather than trying to identify every hazard associated with a particular food, the intention is to identify new or altered hazards relative to a conventional counterpart. This process is commonly referred to as a "safety assessment".

4. Safety assessment falls within the risk assessment framework as discussed in Section 3 of the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology. If a new or altered hazard, nutritional or other safety concern is identified by the safety assessment, the risk associated with it would first be assessed to determine its relevance to human health. Following the safety assessment or further risk assessment, the food would be subjected to risk management considerations in accordance with the Principles for the Risk Analysis of Foods Derived from Modern Biotechnology before it is considered for commercial distribution.

5. The Guideline describes the recommended approach to making safety assessments of foods derived from recombinant DNA plants where a conventional counterpart exists, and identifies the data and information that are generally applicable to making such assessments. While this Guideline is designed for foods derived from recombinant DNA plants, the approach described could, in general, be applied to foods derived from plants that have been altered by other techniques.

SECTION 2 - DEFINITIONS

6. The definitions below apply to this Guideline.

- "Recombinant DNA Plant" - means a plant in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles.

-["Conventional Counterpart" - means a related plant variety for which there is experience of establishing safety based on common use as food.]

SECTION 3 - INTRODUCTION TO SAFETY ASSESSMENT

7. Traditionally, new varieties of food plants have not been systematically subjected to extensive chemical, toxicological, or nutritional evaluation prior to marketing, with the exception of foods for specific groups, such as infants, where the food may constitute a substantial portion of the diet. Thus, new varieties of corn, soya, potatoes and other common food plants are evaluated by breeders for agronomic and phenotypic characteristics, but generally, foods derived from such new plant varieties are not subjected to the rigorous and extensive food safety testing procedures, including studies in animals, that are typical of chemicals such as food additives or pesticide residues that may be present in food.

8. Animal studies are a major element in the risk assessment of many compounds such as pesticides, pharmaceuticals, industrial chemicals and food additives. In most cases, however, the substance to be tested is well characterised, of known purity, of no particular nutritional value, and, human exposure to it is generally low. It is therefore relatively straightforward to feed such compounds to animals at a range of doses some several orders of magnitude greater than the expected human exposure levels, in order to identify any potential adverse health effects of importance to humans. In this way, it is possible, in most cases, to determine levels of exposure at which adverse effects are not observed and to set safe upper limits by the application of appropriate safety factors.

9. Animal studies cannot readily be applied to testing the risks associated with whole foods, which are complex mixtures of compounds characterised by wide variation in composition and nutritional value. Due to their bulk and effect on satiety, they can usually only be fed to animals at low multiples of the amounts that might be present in the human diet. In addition, a key factor to consider in conducting animal studies on foods is the nutritional value and balance of the diets used, in order to avoid the induction of adverse effects which are not related directly to the material itself. Detecting any potential adverse effects and relating these conclusively to an individual characteristic of the food can therefore be extremely difficult. Another consideration in deciding the need for animal studies is whether it is appropriate to subject experimental animals to such a study if it is unlikely to give rise to meaningful information.

10. Due to the difficulties of applying traditional toxicological testing and risk assessment procedures to whole foods, a more focused approach is required for the safety assessment of foods derived from food plants, including recombinant DNA plants. This has been addressed by the development of a

multidisciplinary approach for assessing safety which takes into account both intended and unintended changes that may occur in the plant or in the foods derived from it, using the concept of *substantial equivalence.*

11. The concept of substantial equivalence[1] is a key step in the safety assessment process. However, it is not a safety assessment in itself; rather it represents the starting point which is used to structure the safety assessment of a new food relative to its conventional counterpart. This concept is used to identify similarities and differences between the new food and its conventional counterpart. It aids in the identification of potential safety and nutritional issues and is considered the most appropriate strategy to date for safety assessment of foods derived from recombinant DNA plants. The safety assessment carried out in this way does not imply absolute safety of the new product; rather, it focuses on assessing the safety of any identified differences so that the safety of the new product can be considered relative to its comparator.

UNINTENDED EFFECTS

12. In achieving the objective of conferring a specific target trait (intended effect) to a plant by the insertion of defined DNA sequences, additional traits could, in some cases, be acquired or existing traits could be lost or modified (unintended effects). The potential occurrence of unintended effects is not restricted to the use of *in vitro* nucleic acid techniques. Rather, it is an inherent and general phenomenon that can also occur in conventional breeding. Consequently, unintended effects in recombinant DNA plants may arise through the insertion of DNA sequences and/or they may arise through subsequent conventional breeding of the recombinant DNA plant. Unintended effects may be deleterious, beneficial, or even neutral with respect to the health of the plant or the safety of foods derived from the plant. Nevertheless, careful consideration should be given to reducing the possibility that a recombinant DNA plant has an adverse effect on human health.

13. Unintended effects may result from the random insertion of DNA sequences into the plant genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites. For example, the expression of enzymes at high levels may give rise to secondary biochemical effects or altered metabolic flux.

14. Unintended effects due to genetic modification may be subdivided into two groups: those that are "predictable" and those that are "unexpected". Many unintended effects are largely predictable based on knowledge of the inserted trait and its metabolic connections or of the site of insertion. Due to the expanding information on plant genome and the increased specificity in terms of genetic materials introduced through recombinant DNA techniques compared with other forms of plant breeding, it may become easier to predict unintended effects of a particular modification. Molecular biological and biochemical techniques can also be used to analyse potential changes at the level of gene transcription and message translation that could lead to unintended effects.

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[1] The concept of *substantial equivalence* has been elaborated in several international fora, such as the joint FAO /WHO expert consultations (2000 and 1996) and OECD (1993). Related references include: WHO (2000): Safety aspects of genetically modified foods of plant origin, Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology; FAO (1996): Biotechnology and food safety, Report of a Joint FAO/WHO Consultation. FAO Food Nutrition Paper 61; and OECD (1993): Safety evaluation of foods derived by modern biotechnology, Concepts and principles.

15. The safety assessment of foods derived from recombinant DNA plants involves methods to detect such unintended effects and procedures to evaluate their biological relevance and potential impact on food safety. A variety of data and information are necessary to assess unintended effects because no individual test can detect all possible unintended effects or identify, with certainty, those relevant to health. These data and information, when considered in total, provide assurance that the food is unlikely to have an adverse effect on human health. The assessment for unintended effects takes into account the agronomic/phenotypic characteristics of the plant that are typically observed by breeders in selecting new varieties for commercialization. These observations by breeders provide a first screen for plants that exhibit unintended traits. New varieties that pass this screen are subjected to safety assessment taking into account several factors; these may include, but are not limited to:

A) molecular characterization, including stability of the introduced DNA;

B) chemical analyses of key nutrients, anti-nutrients, toxicants, vitamins, minerals, and other compounds that are typical of the plant or food;

C) alterations of metabolites; and

D) any effects due to food processing.

FRAMEWORK OF SAFETY ASSESSMENT

16. The safety assessment of a food derived from a recombinant DNA plant follows a stepwise process of addressing relevant factors that include:

A) Description of the new variety;

B) Description of the host plant and its use as food;

C) Description of the donor organism(s);

D) Description of the genetic modification(s);

E) Characterization of the genetic modification(s);

F) Safety assessment:

a) introduced substances (non-nucleic acid substances);

b) compositional analyses of key components;

c) metabolic evaluation;

d) food processing;

e) nutritional modification; and

G) Other considerations.

17. In certain cases, the characteristics of the product may necessitate development of additional data and information to address issues that are unique to the product under review.

18. Experiments intended to develop data for safety assessments should be designed and conducted in accordance with sound scientific concepts and principles as well as Good Laboratory Practice. Primary data should be made available to regulatory authorities at request. Data should be obtained using validated methods and analysed using appropriate statistical techniques. The sensitivity of all analytical methods should be documented.

19. The goal of each safety assessment is to provide assurance that the food does not cause harm when prepared, used and/or eaten according to its intended use. The expected endpoint of such an assessment will be a conclusion regarding whether or not the new food is as safe and nutritious as the conventional counterpart against which it has been compared and for which there exists a history of safe use. In essence, therefore, the outcome of the safety assessment process is to define the product under consideration in such a way as to enable risk managers to make informed and proportionate decisions.

SECTION 4 - GENERAL CONSIDERATIONS

DESCRIPTION OF THE NEW VARIETY

20. A description of the new plant variety being presented for safety assessment should be provided. This description should identify the crop, the transformation event(s) to be reviewed and the type and purpose of the modification. This description should be sufficient to aid in understanding the nature of the food being submitted for safety assessment.

DESCRIPTION OF THE HOST PLANT AND ITS USE AS FOOD

21. A comprehensive description of the host plant should be provided. The necessary data and information should include, but need not be restricted to:

A) taxonomic information, such as species and variety name of the host plant;

B) a record of other plant species that have contributed to the host plant's genetic background;

C) relevant information on the host plant's genotype and phenotype, including any known toxicity or allergenicity; and

D) history of safe use for consumption as food.

22. Relevant phenotypic information should be provided not only for the host plant, but also for related species and for plants that have made or may make a significant contribution to the genetic background of the host plant.

23. The history of use may include information on how the plant is typically cultivated, transported and stored, whether special processing is required to make the plant safe to eat, and the plant's normal role in the diet (e.g. which part of the plant is used as a food source, whether its consumption is important in particular subgroups of the population, what important macro- or micro-nutrients it contributes to the diet).

DESCRIPTION OF THE DONOR ORGANISM(S)

24. Information should be provided on the donor organism(s) and, when appropriate, on other members of the corresponding genus. It is particularly important to determine if the donor organism(s) or other members of the family naturally exhibit characteristics of pathogenicity or toxin production, or have other traits that affect human health (e.g. presence of antinutrients). The description of the donor organism(s) should include:

A) its usual or common name;

B) scientific name;

C) taxonomic classification;

D) information about the natural history;

E) information on pathogenicity or other potential toxic concerns, particularly the relationship to known pathogens or known producers of toxins, allergens or anti-nutrients within the same family; and

F) information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).

DESCRIPTION OF THE GENETIC MODIFICATION(S)

25. Sufficient information should be provided on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide the necessary information for the analysis of the data supporting the characterization of the DNA inserted in the plant.

26. The description of the transformation process should include:

A) information on the specific method used for the transformation (e.g. *Agrobacterium*-mediated transformation);

B) information, if applicable, on the DNA used to modify the plant (e.g. helper plasmids), including the source (e.g. plant, microbial, viral , synthetic), identity and expected function in the plant; and

C) intermediate host organisms including the organisms (e.g. bacteria) used to produce or process DNA for transformation of the host organism;

27. Information should be provided on the DNA to be introduced, including:

A) the characterization of all the genetic components including marker genes, regulatory and other elements affecting the function of the DNA;

B) the size and identity;

C) the location and orientation of the sequence in the final vector/construct; and

D) the function.

CHARACTERIZATION OF THE GENETIC MODIFICATION(S)

28. In order to provide clear understanding of the impact on the composition and safety of foods derived from recombinant DNA plants, a comprehensive molecular and biochemical characterization of the genetic modification should be carried out.

29. Information should be provided on the DNA inserted into the plant genome; this should include:

A) the characterization and description of the inserted genetic materials;

B) the number of insertion sites;

C) the organisation of the inserted genetic material at each insertion site, including sequence data of the inserted material and, where appropriate, of surrounding region; and

D) identification of any open reading frames including those that could result in fusion proteins.

30. Information should be provided on any introduced substances in the recombinant DNA plant; this should include:

A) the gene product (e.g. a protein or an untranslated RNA);

B) the gene product's function;

C) the phenotypic description of the new trait(s);

D) the level and site of expression in the plant of the introduced gene product(s), and the levels of its metabolites in the plant, particularly in the edible portions; and

E) the amount of the target gene product(s) if the function of the introduced sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.

31. In addition, information should be provided:

A) to demonstrate whether the arrangement of the genetic material used for transformation has been conserved or whether significant rearrangements have occurred upon integration;

B) to demonstrate whether deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function;

C) to demonstrate that the intended effect of the modification has been achieved and that all introduced traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance. It may be necessary to examine the inheritance of the DNA insert itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly;

D) to demonstrate that the newly introduced trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene;

E) to indicate whether there is any evidence to suggest that a gene in the host plant has been affected by the insertion event; and

F) to confirm the identity and expression pattern of any new fusion proteins.

SAFETY ASSESSMENT OF INTRODUCED SUBSTANCES (NON-NUCLEIC ACID SUBSTANCES)

Assessment of possible toxicity

32. *In vitro* nucleic acid techniques enable the introduction of DNA which can result in the synthesis of new substances in plants. These can be conventional components of plant foods such as proteins, fats, carbohydrates, vitamins which are novel in context of that recombinant DNA plant. Conventional toxicology studies are not considered necessary where the substance or a closely related substance has been consumed safely in food, taking into account its exposure, for the reasons described in Section 3.

33. In other cases, the use of conventional toxicology studies on the new substance will be necessary. This may require the isolation of the new substance from the recombinant DNA plant, or the synthesis or production of the substance from an alternative source, in which case the material should be shown to be structurally, functionally and biochemically equivalent to that produced in the recombinant DNA plant.

34. The safety assessment of the introduced substance should identify the concentration of the substance in the edible parts of the recombinant DNA plant, including, as appropriate, variations and mean values. Current dietary exposure and possible effects on population sub-groups should also be considered. In the case of proteins, the assessment of potential toxicity should focus on amino acid sequence similarity between the protein and known protein toxins and anti-nutrients (e.g. protease inhibitors, lectins) as well as stability to heat or processing and to degradation in appropriate representative gastric and intestinal model systems. Appropriate oral toxicity studies may be carried out in cases where the protein is present in the food, is not similar to proteins that have been safely consumed in food, and has not previously been consumed safely in food.

35. The introduced trait should be shown to be unrelated to any characteristics of donor organisms that could be harmful to human health. Information should be provided to ensure that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to recombinant DNA plants that do not normally express those toxic or anti-nutritious characteristics. This assurance is particularly important in cases where a recombinant DNA plant is processed differently from a donor plant, since traditional processing techniques associated with the donor organisms may deactivate anti-nutrients or toxicants.

36. Additional *in vivo* or *in vitro* studies may be needed on a case-by-case basis to assess the toxicity of introduced substances. The types of studies depend on the original source of the introduced substances and their function. Such studies may include assays of metabolism, toxicokinetics, chronic toxicity/carcinogenicity, impact on reproductive function, and teratogenicity.

37. The safety assessment should take into account the potential accumulation of any substances, toxic metabolites, contaminants, or pest control agents on plants that might result from genetic modification.

Assessment of possible allergenicity (proteins)[2]

38. When the protein(s) resulting from the inserted gene is present in the food, it should be assessed for potential allergenicity in all cases. The following decision tree strategy can be applied in this assessment (see the attached Chart).

39. When the transferred gene is obtained from a source with a known history of allergenicity, the assessment should focus initially upon the immunochemical reactivity of the introduced protein with IgE from the serum of individuals with known allergies to the source of the transferred genetic material. In cases where no evidence of immunochemical reactivity is obtained, skin prick tests with extracts containing the introduced protein and double-blind placebo-controlled food challenges (DBPCFC) with the new food should be conducted, if appropriate, on individuals with known allergies to the source of the transferred genetic material, in order to provide confirmation that the introduced protein is not allergenic. This series of tests provides adequate evidence regarding the allergenicity (or lack thereof) of introduced proteins expressed by genes obtained from known allergenic sources.

40. When the transferred gene is obtained from a source with no known history of allergenicity, the decision-tree approach relies upon various criteria used in combination, since no single criterion is sufficiently predictive. The current criteria include the amino acid sequence similarity of the introduced

[2] This part will be revised, as necessary, in light of the 2nd Joint FAO/WHO Consultation on Foods Derived from Biotechnology, Allergenicity of genetically modified foods, 22-25 January, 2001.

protein to known allergens, the immunochemical reactivity of the introduced protein with IgE from serum of appropriate, allergic individuals when amino acid sequence similarities are found, and the stability of the introduced protein to degradation in appropriate representative gastric and intestinal model systems.

41. The incorporation of two additional criteria to the decision-tree approach might be useful when the source of the genetic material is not known to be allergenic.

A) the level of the protein in food; and

B) the functional properties of the protein (e.g. storage protein)

42. These criteria taken together offer reasonable evidence as to whether or not the protein is allergenic, is cross-reactive with known allergens, and has a potential to be a food allergen.

43. The introduced proteins in foods derived from recombinant DNA plants should be evaluated for any possible role in the elicitation of gluten-sensitive enteropathy, if the introduced genetic material is obtained from wheat, rye, barley, oats, or related cereal grains.

44. The transfer of genes from commonly allergenic foods and from foods known to elicit gluten-sensitive enteropathy in sensitive individuals should be discouraged unless it is documented that the transferred gene does not code for an allergen or for a protein involved in gluten-sensitive enteropathy.

COMPOSITIONAL ANALYSES OF KEY COMPONENTS[3]

45. Analyses of concentrations of key components[4] of the recombinant DNA plant and, especially those typical of the food, should be compared with an equivalent analysis of a conventional counterpart grown and harvested under the same conditions. In some cases, a further comparison with the recombinant DNA plant grown under its expected agronomic conditions may need to be considered (e.g. application of an herbicide). The statistical significance of any observed differences should be assessed in the context of the range of natural variations for that parameter to determine its biological significance. The comparator(s) used in this assessment should ideally be the near isogenic parental line. In practice, this may not be feasible at all times, in which case a line as close as possible should be chosen. The purpose of this comparison, in conjunction with an exposure assessment as necessary, is to establish that substances that are nutritionally important or that can affect the safety of the food have not been altered in a manner that would have an adverse impact on human health.

46. The location of trial sites should be representative of the range of environmental conditions under which the plant varieties would be expected to be grown. The number of trial sites should be sufficient to allow accurate assessment of compositional characteristics over this range. Similarly, trials should be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature. To minimise environmental effects, and to reduce any effect from naturally

[3] See for example OECD Consensus Documents on Canola and Soybean for a discussion of key components specific to these crops.

[4] Key nutrients or key anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates as nutrients or enzyme inhibitors as anti-nutrients) or minor compounds (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in the plant, such as those compounds whose toxic potency and level may be significant to health (e.g. solanine in potatoes if the level is increased, selenium in wheat) and allergens.

occurring genotypic variation within a crop variety, each trial site should be replicated. An adequate number of plants should be sampled and the methods of analysis should be sufficiently sensitive and specific to detect variations in key components.

METABOLIC EVALUATION

47. Some recombinant DNA plants may have been modified in a manner that could result in new or altered levels of various metabolites in the food. Consideration should be given to the potential for the accumulation of metabolites in the food that would adversely affect human health. Safety assessment of such plants requires investigation of residue and metabolite levels in the food and assessment of any alterations in nutrient profile. Where altered residue or metabolite levels are identified in foods, consideration should be given to the potential impacts on human health using conventional procedures for establishing the safety of such metabolites (e.g. procedures for assessing the human safety of chemicals in foods).

FOOD PROCESSING

48. The potential effects of food processing, including home preparation, on foods derived from recombinant DNA plants should also be considered. For example, alterations could occur in the heat stability of an endogenous toxicant or the bioavailability of an important nutrient after processing. Information should therefore be provided describing the processing conditions used in the production of a food ingredient from the plant. For example, in the case of vegetable oil, information should be provided on the extraction process and any subsequent refining steps.

NUTRITIONAL MODIFICATION

49. The assessment of possible compositional changes to key nutrients, which should be conducted for all recombinant DNA plants, has already been addressed under 'Compositional analyses of key components'. However, foods derived from recombinant DNA plants that have undergone modification to intentionally alter nutritional quality or functionality should be subjected to additional nutritional assessment to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

50. Information about the known patterns of use and consumption of a food, and its derivatives should be used to estimate the likely intake of the food derived from the recombinant DNA plant. The expected intake of the food should be used to assess the nutritional implications of the altered nutrient profile both at customary and maximal levels of consumption. Basing the estimate on the highest likely consumption provides assurance that the potential for any undesirable nutritional effects will be detected. Attention should be paid to the particular physiological characteristics and metabolic requirements of specific population groups such as infants, children, pregnant and lactating women, the elderly and those with chronic diseases. Based on the analysis of nutritional impacts and the dietary needs of specific population subgroups, additional nutritional assessments may be necessary. It is also important to ascertain to what extent the modified nutrient is bioavailable and remains stable with time, processing and storage.

51. The use of plant breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile in two ways. The intended modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have

the same effect. Although the recombinant DNA plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile should be determined.

52. When the modification results in a food product with a composition that is significantly different from its conventional counterpart, it may be appropriate to use alternative conventional foods (i.e. foods whose nutritional composition is closer to that of the food derived from recombinant DNA plant) as appropriate comparators to assess the nutritional impact of the food.

53. Because of geographical and cultural variation in food consumption patterns, nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural population than in others. Some food plants serve as the major source of a particular nutrient in some populations. The nutrient and the populations affected should be identified.

54. Some foods may require additional testing. For example, animal feeding studies may be warranted for foods derived from recombinant DNA plants if changes in the bioavailability of nutrients are expected or if the composition is not comparable to conventional foods. Also, foods designed for health benefits may require specific nutritional, toxicological or other appropriate studies. If the characterization of the food indicates that the available data are insufficient for a thorough safety assessment, animal studies could be requested on the whole foods if properly designed.

SECTION 5 – OTHER CONSIDERATIONS

USE OF ANTIBIOTIC RESISTANCE MARKER GENES

55. Alternative transformation technologies that do not result in antibiotic resistance marker genes in foods are encouraged in the future development of recombinant DNA plants, where such technologies are available and demonstrated to be safe.

56. Gene transfer from plants and their food products to gut microorganisms or human cells is considered a rare possibility because of the many complex and unlikely events that would need to occur consecutively. Nevertheless, the possibility of such events cannot be completely discounted[5].

57. In assessing safety of foods containing antibiotic resistance marker genes, the following factors should be considered:

A) the clinical and veterinary use and importance of the antibiotic in question;

(Certain antibiotics are the only drug available to treat some clinical conditions (e.g. vancomycin for use in treating certain staphylococcal infections). Marker genes encoding resistance to such antibiotics should not be used in recombinant DNA plants.)

B) whether the presence in food of the enzyme or protein encoded by the antibiotic resistance marker gene would compromise the therapeutic efficacy of the orally administered antibiotic; and

[5] In cases where there are high levels of naturally occurring bacteria which are resistant to the antibiotic, the likelihood of such bacteria transferring this resistance to other bacteria will be orders of magnitude higher than the likelihood of transfer between ingested foods and bacteria.

(This assessment should provide an estimate of the amount of orally ingested antibiotic that could be degraded by the presence of the enzyme in food, taking into account factors such as dosage of the antibiotic, amount of enzyme likely to remain in food following exposure to digestive conditions, including neutral or alkaline stomach conditions and the need for enzyme cofactors (e.g. ATP) for enzymatic activity and estimated concentration of such factors in food.)

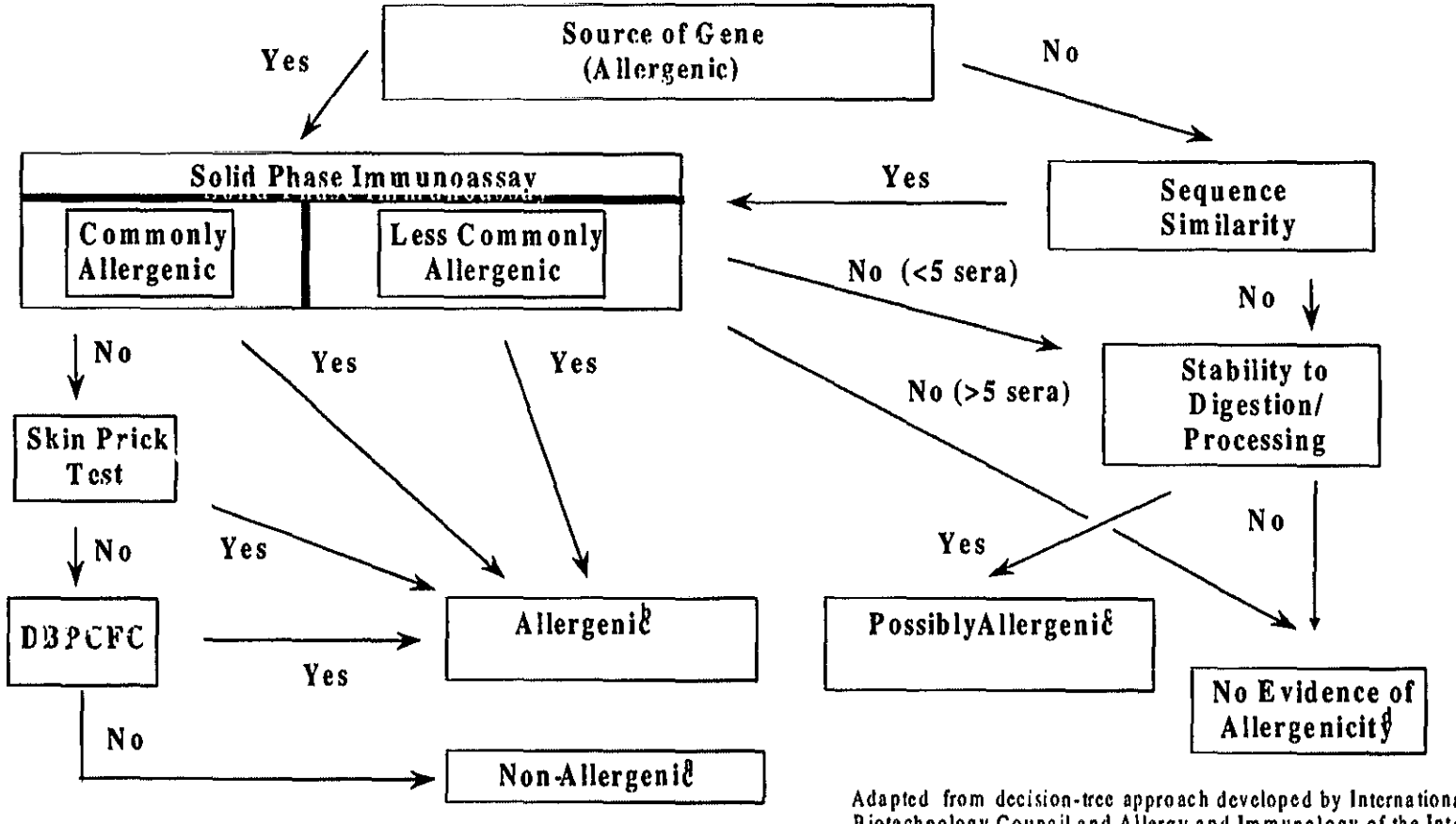
C) safety of the gene product, as would be the case for any other introduced gene product.

58. If evaluation of the data and information suggests that the presence of the antibiotic resistance marker gene or gene product presents risks to human health, the marker gene or gene product should not be present in the food. In general, antibiotic resistance genes used in food production that encode resistance to clinically important antibiotics should not be present in widely disseminated foods.

REVIEW OF SAFETY ASSESSMENTS

59. The goal of the safety assessment is a conclusion as to whether the new food is as safe as and no less nutritious than the conventional counterpart against which it was compared. Nevertheless, the safety assessment should be reviewed in the light of new scientific information that calls into question the conclusions of the original safety assessment.

Assessment of the Allergenic Potential of Foods Derived From Modified Plants



Adapted from decision-tree approach developed by International Food Biotechnology Council and Allergy and Immunology of the International Life Sciences Institute (Metcalfe *et al*, 1996).

Footnotes to the Chart

a. The combination of tests involving allergic human subjects or serum from such subjects would provide a high level of confidence that no major allergens were transferred. The only remaining uncertainty would be the likelihood of a minor allergen affecting a small percentage of the population allergenic to the source material.

b. Any positive results obtained in tests involving allergenic human subjects or serum from such subjects would provide a high level of confidence that the introduced protein was a potential allergen. Foods containing such introduced proteins would need to be labelled to protect allergic consumers.

c. An introduced protein either with no sequence similarity to known allergens or derived from a less commonly allergenic source with no evidence of binding to IgE from the serum of a few allergic individuals (<5), but that is stable to digestion and processing should be considered a possible allergen. Further evaluation would be necessary to address this uncertainty. The nature of the tests would be determined on a case-by-case basis.

d. An introduced protein with no sequence similarity to known allergens and that was not stable to digestion and processing would have no evidence of allergenicity. Similarly, an introduced protein expressed by a gene obtained from a less commonly allergenic source and demonstrated to have no binding with IgE from the serum of a small number of allergic individuals (>5 but <14) provides no evidence of allergenicity. Stability testing may be included in these cases. However, the level of confidence based on only two decision criteria is modest. Other criteria might also be considered such as the level of expression of the novel protein.

GUIDELINES FOR THE SAFETY ASSESSMENT OF NOVEL FOODS

VOLUME I

Preamble and Guidance Scheme for Notification

Food Directorate
Health Protection Branch
Health Canada

September 1994

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1. INTRODUCTION

Developments in food science and biotechnology are resulting in the introduction into the Canadian marketplace of novel foods and foods developed using novel processes. While such developments may add to the economic well-being of Canadians and provide a greater choice to the consumer, the safety of some of these foods has yet to be established. Concern has been expressed particularly with respect to the possible introduction of harmful substances into the food supply, including: the introduction of new toxicants; increased levels of existing toxicants; and, the reduction of nutritional value.

Existing regulations under the *Food and Drugs Act* use premarket notification and assessment to address food safety issues in several areas. These areas include the safety assessment of food additives; the establishment of maximum residue limits (MRL) for pesticides; the safety assessment of foods treated with ionizing radiation; and, the notification process for infant formulae. Such activities will not be affected by the approaches expressed in this document. However, premarket notification requirements do not presently exist for many novel foods, including the products of biotechnology.

Not all "new" food products will require notification or assessment. However, certain novel foods that have not been previously available in the Canadian marketplace, or foods produced by novel processes may require notification prior to sale. The Branch has proposed new regulations intended to ensure that these novel products receive oversight. A notification does not mean that a safety assessment of a novel food by the Branch will be required in all cases. However, information demonstrating the safety of the product may be requested. In support of these proposed Novel Food Regulations, guidelines have been developed which identify the safety assessment criteria for genetically modified microorganisms and genetically modified plants (*Guidelines for the Safety Assessment of Novel Foods, Volume II: Genetically Modified Microorganisms and Plants*).

2. PURPOSE

This preamble will provide assistance to producers and processors respecting pre-market notification in regard to novel products and products from novel processes.

3. RELEVANT PRODUCTS AND PROCESSES

Annex I of this guideline contains a number of definitions that are relevant to the concept of novel foods. The proposed definition of novel food is presented there, **but may be subject to revision as development of the regulation continues**. However, the current proposal requires further clarification to ensure that only those products for which notification is required receive the necessary evaluation, without requiring notification for all new products.

Novel foods may include:

- products and processes that have previously not been used before as food or to process food in Canada[1]

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[1] Newness of the product in the Canadian marketplace is of importance. However, use of the novel product in a jurisdiction with a similar food safety system would become an important consideration with respect to the evaluation of the product or process.

- food containing microorganisms that have not previously been used as food or to process food,

- foods that result from genetic modification and exhibit new or modified characteristics that have previously not been identified in those foods, or that result from production by organisms exhibiting such new or modified characteristics, or

- food that is modified from the traditional product or is produced by a process that has been modified from the traditional process.

These principles are further clarified in a series of charts that appear in Annex II. These charts are presented for guidance only and should not be considered a rigid checklist. The questions that are embodied in these charts lead to endpoints that in some cases require notification to the Food Directorate. The information requirements for a notification include:

- the name under which the novel food will be sold,

- the name and address of the principle place of business of the manufacturer and the importer if applicable,

- a statement of the nature of the novel food, its process of manufacture, its intended uses and history of consumption if used as food in another country.

- the name and nature of the novel food process used to produce a food that would not in or of itself be considered a novel food,

- as applicable, information about the possible displacement of existing foods and the nutritional impact thereof,

- the written text of all labels to be used in connection with the novel food, and

- the name and title of the person who signed the notification and date of signature.

In addition, information demonstrating the safety of such products as food may be requested by the Director.

Additional guidance was viewed as necessary for the safety assessment of certain novel foods. As a first step, Volume II of this guideline has been prepared to provide guidance for the safety assessment of genetically modified plants and microorganisms. Other guidelines may be developed as necessary to address similar issues.

The Guidelines are flexible due to the broad range of products being developed. A determination of the need for notification and the safety assessment of novel products will be conducted on a case-by-case basis, and will be based on the comparison of the novel substance to an analogous traditional food, where such exists. Notification may not be required if the modification to the product or process is not significant, or if a high degree of similarity to a traditional product exists. This concept of **substantial equivalence** is similar to the policy developed by the Organization for Economic Cooperation and Development (OECD).[2] Furthermore, not all

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[2] OECD, 1993. Safety Evaluation of Foods Derived By Modern Biotechnology. Concepts and Principles. Organization For Economic Cooperation and Development. Paris. 79 pages.

information requirements outlined in the Guidelines may be appropriate for all products. Therefore, developers are encouraged to consult the Food Directorate in the early stages of product development in order to reach agreement on whether notification is required, and what information is appropriate to the evaluation of the safety of the particular product.

ANNEX I

DEFINITIONS

Biotechnology

is the application of science and engineering in the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms. (*Canadian Environmental Protection Act*)

Food

includes any article manufactured, sold or represented for use as food or drink for man, chewing gum, and any ingredient that may be mixed with food for any purpose whatever. (*Section 2, Food and Drugs Act*)

Ingredient

is an individual unit of food that is combined as an individual unit of food with one or more individual units of food to form an integral unit of food that is sold as a prepackaged product. (*Section B.01.001, Food and Drug Regulations*)

Food Additive

is any substance the use of which results, or may be reasonably expected to result in it or its by-product becoming a part or affecting the characteristics of a food, but does not include

a) any nutritive material that is used, recognized or commonly sold as an article or ingredient of food,
b) vitamins, mineral nutrients and amino acids other than those listed in the tables to Division 16,
c) spices, seasonings, flavouring preparations, essential oils, oleoresins and natural extractives,
d) agricultural chemicals, other than those listed in the tables to Division 16,
e) food packaging materials and components thereof, and
f) drugs recommended for administration to animals that may be consumed as food.
(*Section B.01.001, Food and Drug Regulations*)

Genetic Modification

is any change to the heritable traits of an organism achieved by intentional manipulation. This includes, but is not limited to: recombinant nucleic acid techniques, somaclonal variation, electroporation, artificially induced mutagenesis, and the like.

Genetically Modified Organism

an organism which is constructed or intentionally changed, in its genetic make-up.

Recombinant Nucleic Acid Technology

is the precise transfer of spliced genes between different organisms of the same or different species. This can include the transfer of synthetic genes.

Substantial Equivalence

as described in *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles* (OECD, 1993) substantial equivalence embodies the idea that existing organisms used as food or as a source of food can be used as the basis of comparison when assessing the safety of the human consumption of a food or food component that has been modified or is new.

If one considers a modified traditional food about which there is extensive knowledge on the range of possible toxicants, critical nutrients or other relevant characteristics, the new product can be compared with the old in simple ways. These ways can include, *inter alia*, appropriate traditionally performed analytical measurements or crop-specific markers, for comparative purposes. The situation becomes more complex as the origins/composition/exposure experience decreases, or if the new products lack similarity to old established products or, in fact, have no conventional counterpart. (*Organization for Economic Cooperation and Development)*

Safety Assessment

refers to the concepts described in the document *Risk Management in the Health Protection Branch* (Health Canada, 1990) and encompasses hazard identification, risk estimation, and risk evaluation and management.

Novel Food

is a food that has not previously been used as food, results from a process that has not previously been used for food in Canada, or has been used as food, but has been modified such that:

(a) the food results from genetic manipulation and exhibits one or more characteristics that were not previously identified in that food, or the food results from production by a genetically manipulated organism exhibiting such new characteristics,

(b) the food contains microorganisms that have not previously been used as a food or to process food, or

(c) the food is modified from the traditional product or is produced by a process that has been modified from the traditional process.

Organism

any unicellular or multicellular biological entity capable of reproduction or replication and viruses.

Microorganism

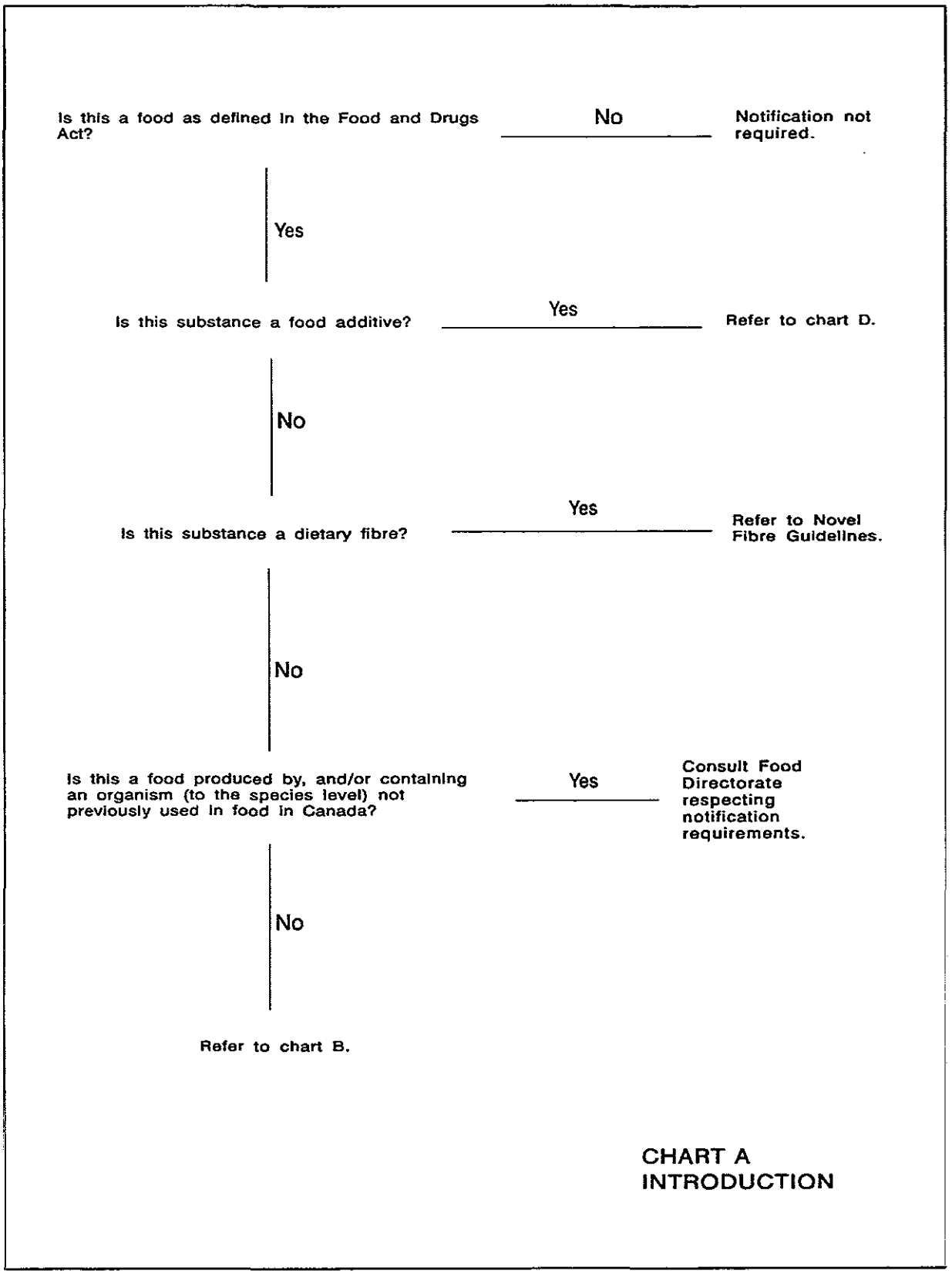
is any bacteria, mycoplasma, chlamydia, rickettsia, protozoa, fungi, algae, viruses, parts of these microorganisms and any combination thereof. (*Canadian Environmental Protection Act*)

ANNEX II

GUIDANCE SCHEME

FOR NOTIFICATION OF NOVEL FOOD PRODUCTS

65


Is this a food as defined in the Food and Drugs Act?
No
Notification not required.
Yes
Is this substance a food additive?
Yes
Refer to chart D.
No
Is this substance a dietary fibre?
Yes
Refer to Novel Fibre Guidelines.
No
Is this a food produced by, and/or containing an organism (to the species level) not previously used in food in Canada?
Yes
Consult Food Directorate respecting notification requirements.
No
Refer to chart B.
CHART A
INTRODUCTION

Has genetic modification been used in the development of the food or its source?

No → Refer to chart C.

Yes

Has a new characteristic been introduced or has the phenotype of the organism or the product composition been substantially altered?

Yes → Consult Part 2 (microorganisms) or Part 3 (plants) of Volume II of the Guidelines respecting notification requirements.

No

Notification not required.

CHART B
BIOTECHNOLOGY
PRODUCTS

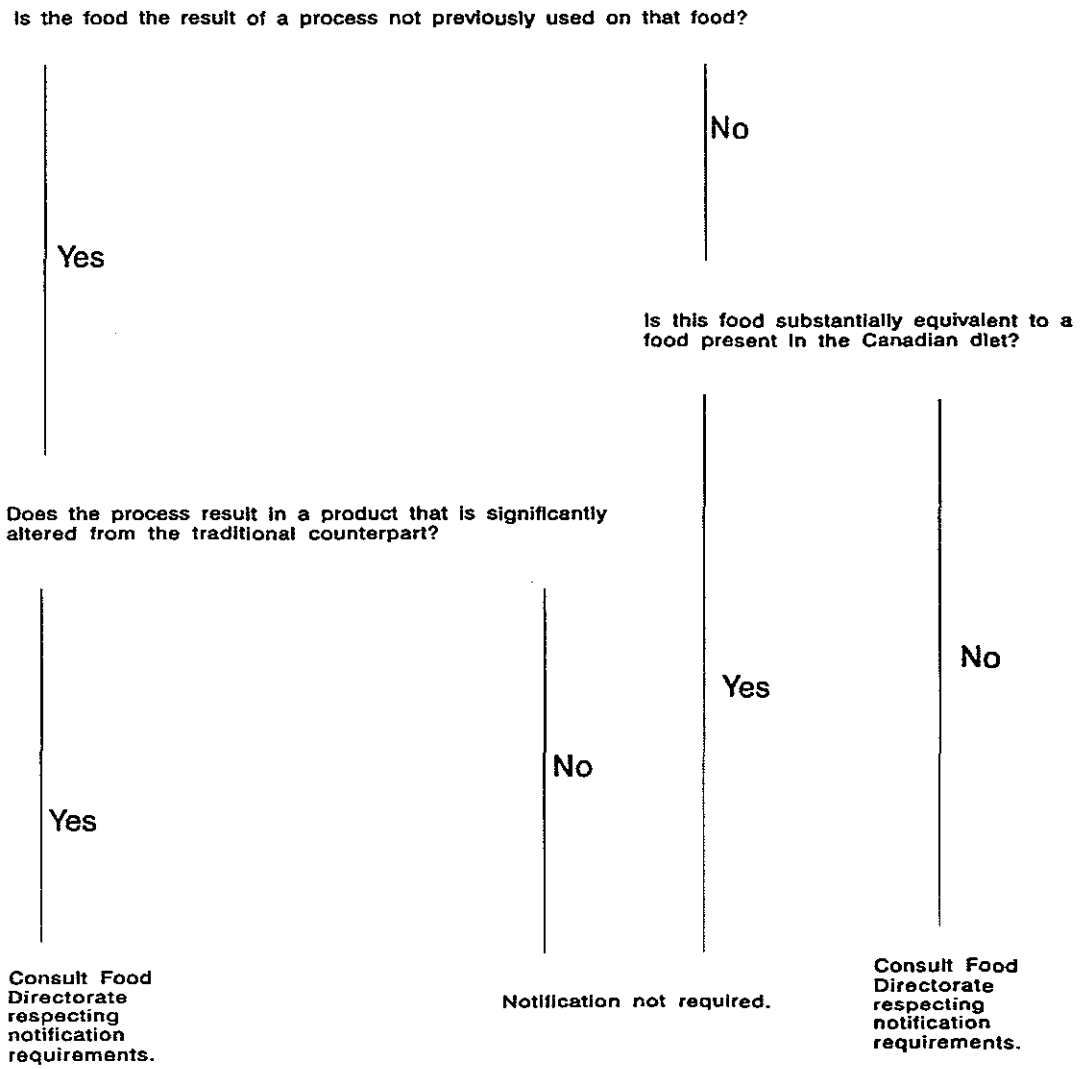


CHART C
FOOD PROCESSES

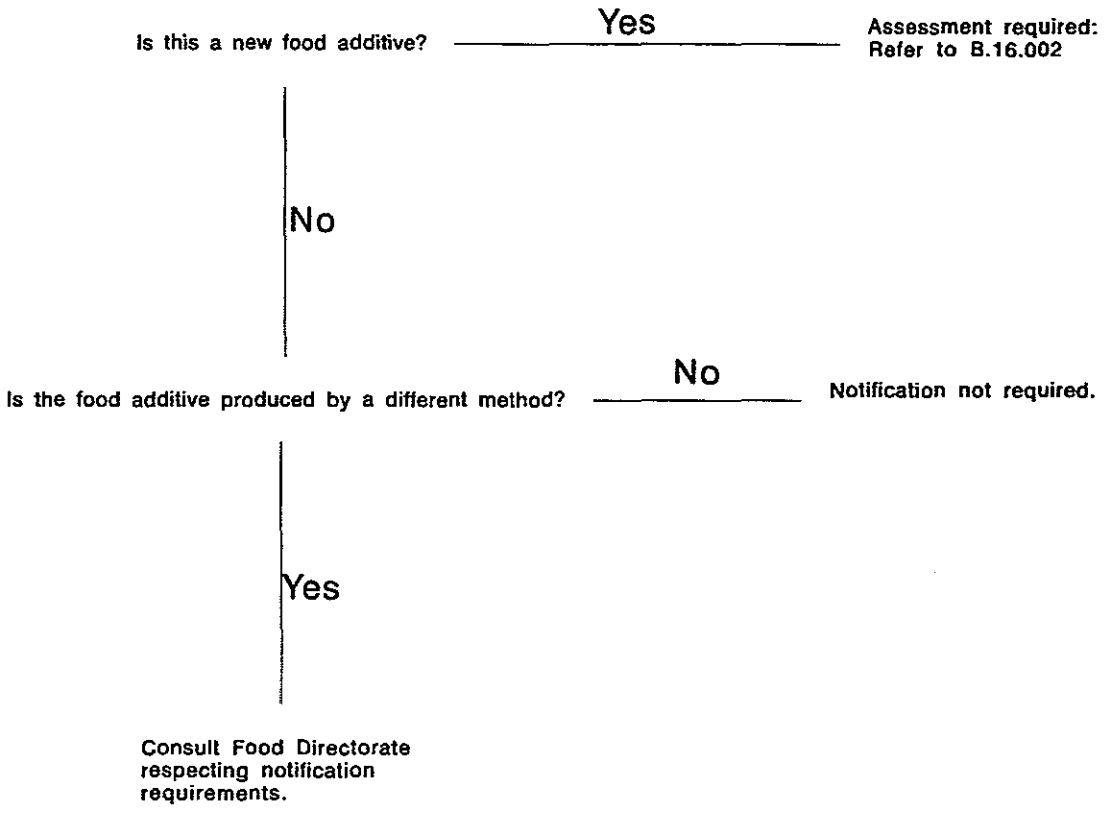


CHART D
FOOD ADDITIVES

GUIDELINES FOR THE SAFETY ASSESSMENT OF NOVEL FOODS

VOLUME II

Genetically Modified Microorganisms and Plants

Food Directorate
Health Protection Branch
Health Canada

September 1994

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1. INTRODUCTION

Novel whole foods and food constituents result increasingly from changes to the genetic make-up of microorganisms, plants, and animals which are modified to improve the agronomic, production, processing or nutritional characteristics. In many instances, these modification processes represent faster, more efficient mechanisms for achieving changes than traditional breeding. It is generally agreed that the application of genetic modification does not inherently increase or decrease the risk associated with an organism. However, the wide variety of modifications possible through genetic manipulation, and the potential for the introduction of toxic compounds, unexpected secondary effects, and changes in nutritional and toxicological characteristics may give rise to safety concerns. In the context of the proposed novel food regulations, it is considered important that an appropriate mechanism be developed for the safety assessment of foods derived through the application of genetic modification technology.

In keeping with generally accepted approaches, the emphasis of the safety assessment will be on the product and not on the process used to develop it. However, to ensure that appropriate concerns are addressed, a clear understanding of the methods used to develop the product is necessary. The fewer uncertainties regarding the nature of a novel product or its method of manufacture that remain, the more likely that nutritional and toxicological concerns will be easily addressed.

In the case of food constituents consisting of single chemical products or well-defined mixtures, procedures for safety assessment are well developed and internationally accepted. Novel food additives and conventional additives produced by genetically modified organisms will be required to meet the existing data requirements outlined in section B.16.002 of the *Canadian Food and Drug Regulations*. Specifications for identity and purity, developed for products from traditional sources, may not be entirely adequate to ensure the safety of products derived from genetically modified organisms. Therefore, additional specifications/parameters may need to be developed.

The safety assessment of whole foods derived from genetically modified microorganisms, plants and animals is more complex than evaluation of single chemical food constituents or defined chemical mixtures. In assessing the safety of whole foods, knowledge of the previous use as a food, the level of complexity of the whole food, and the breadth of the modification will be determining factors in establishing information requirements for the evaluation. Where appropriate, the basis for these safety assessments will be comparison of the molecular, compositional, toxicological and nutritional data for the modified organism to those of its traditional counterpart. In cases where the genetic modification is well defined, with specific effects, the safety assessment may be limited to information provided on the development and production of the modified organism and a comparison of the composition of the modified product to the unmodified product. For poorly characterized changes, or cases in which a genetically modified organism is determined to be significantly different from its traditional counterpart, a more comprehensive review may be required for the novel product. This review may include a toxicological and nutritional assessment of the product, including a combination of *in-vitro* and *in-vivo* tests applied on a product-specific basis. Where there are potential concerns related to the allergenicity of the novel food product, the Food Directorate should be consulted to determine the approach to be taken in order to mitigate any concerns.

In all cases, the degree of exposure to the modified organism or its metabolic products will be an important factor in determining the extent of the data required for a meaningful safety assessment.

A guiding principle in the safety assessment will be comparison of molecular, compositional and nutritional data for the modified organism to those of its traditional counterpart, where such exists. It is expected that once substantial equivalence to an existing food product can be established, no additional safety testing would be required. Where similarity or degree of equivalence cannot be be established, a more extensive safety assessment may be necessary. It is recognized that availability of compositional data for traditional foods is often limited and may be unavailable for new products. Thus, there is a need to develop international databases on the composition of traditional food stuffs to serve as a basis for comparison.

Initial assessments will necessarily be on a case-by-case basis. It may be possible, once sufficient experience is gained, to define more explicit criteria that may preclude the need for the detailed evaluation of specific products.

Developers are encouraged to consult with the Food Directorate at the earliest possible date as new products or modified existing products are developed in order that potential concerns might be addressed.

1.1 OBJECTIVES

These Guidelines outline the information to be considered in assessing the safety of novel whole foods and food constituents developed through the use of genetically modified organisms. They are intended to provide a basis for dialogue between petitioners and the Health Protection Branch. It was not intended to explicitly define in this document all of the data that might be required in the course of a safety assessment.

1.2 NOTIFICATION

Notifications for novel foods derived from or including genetically modified microorganisms or plants should be directed to:

Office of Food Biotechnology
Food Directorate
Health Protection Branch
Health Canada
4th Floor West
Sir Frederick Banting Research Centre
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

2. GENETICALLY MODIFIED MICROORGANISMS AND THEIR PRODUCTS

2.0.0 FOREWORD

Microorganisms have been an important component of food for millennia. They may be consumed as inocula in fermented milk, meat or vegetable products or their metabolites may be used in food and in food processing. More recently, microorganisms have also been consumed directly as food in the form of single cell protein.

It is recommended that the following information be included for assessing the acceptability of genetically modified microorganisms and their products that are intended for use in or as a food.

It is important to note that not all information requirements outlined below may be appropriate to all cases. Applicants are encouraged to consult the Food Directorate early in product development in order to reach agreement on what information is appropriate to the evaluation of the safety of the product.

2.1.0 DEVELOPMENT AND PRODUCTION OF THE MODIFIED ORGANISM

Sufficient data should be submitted to characterize the modified microorganism and permit comparison with its conventional or unmodified counterpart. Most of the questions regarding characterization of the modified organism can be answered by data that may have been generated in the developmental stage.

The genetically modified microorganisms referred to here are those developed by recombinant nucleic acid technology and other methods of DNA introduction, such as protoplast fusion in eukaryotic cells, ballistic microinjection, and electroporation. Microorganisms developed by deletion, rearrangement or suppression of native DNA should also be considered. In addition, those microorganisms that have undergone genetic modification by intentionally induced mutagenesis (i.e. through the application of techniques such as chemical treatment and ultra-violet irradiation), resulting in alteration of the phenotype or composition, may also be included. However, the degree of similarity to existing products should be taken into account in this determination.

The data to be submitted are to include, but not necessarily be limited to, those outlined here. Of special concern may be modified microorganisms where a parent or vector originates from a species known to produce toxic compounds. Wherever possible, transformation markers which generate safety concerns should not be present in the final food product. The acceptability of such markers however, will be evaluated on a case-by-case basis.

2.1.1 Host, Donor and Intermediate Host Orgamisms

Detailed information on the natural history of both donor and host organism should be considered. Such information should include, but not be limited to: known toxin production, relationship to toxin producers in the same genus, pathogenicity, previous food and/or medicinal use.

a) Identification

- taxonomic designation of the microorganism to the species level and where applicable, to include subspecies and strains, accompanied by technical data substantiatiating this designation.
- other names (synonyms, common usage, strain numbers, culture collection accession number) associated with the microorganism
- origin (environmental/clinical/food isolate, culture collection) of the microorganism
- strain development and enhancement history of the microorganism.

b) pathogenicity of genus and species

c) evidence pertaining to the potential for production of any toxic compounds

d) history of extended safe use, particularly in foods, of the subject microorganism and closely related strains

2.1.2 Introduced or Modified DNA

a) function of the introduced or modified DNA

b) location and extent of any deletion

c) location and orientation of any rearrangements

d) for all introduced DNA, evidence for:

- source and description of all introduced DNA
- sequence of introduced DNA, or restriction map where relevant
- characterization of the vector, where one is used
- lack of sequences known to code for toxic compounds
- limitation of insert to sequences required for intended function
- limitation of the effect of the introduced DNA to that intended
- absence or inactivation of potentially harmful markers
- absence of unnecessary intermediate host DNA

e) for all modifications not involving the introduction of foreign DNA

- description of the modification
- evidence that the modification is limited to that required for the intended functions
- identification of the genes affected by the modification, where appropriate

2.1.2.1 Regulation of Expression

A description of how the inserted gene(s) are regulated in the modified host is required (indicate if the gene is inducible or constitutive, and detail the mechanism of regulation). If inducible, information should be provided on:

a) the nature or mechanism of the induction e.g. chemically, developmentally

b) constancy of regulation and expression

Where native DNA is modified, without the introduction of foreign DNA, or where the expression of native gene(s) is (are) modified, regulation of target gene(s) should be considered as above.

2.1.3 The Modified Host

a) detailed description of the method of construction (introduced DNA) or other manipulation to achieve the genetic modification

b) purpose i.e. target function

c) metabolic profile (phenotypic comparison with parent organism)

d) taxonomic designation

e) biological activity, growth, physiological characteristics

f) potential pathogenicity

g) potential for production of toxic compounds

h) description of how the microorganism strain is being preserved and maintained.

i) documentation for:

- consideration of the potential for secondary effects of the modification on biochemistry, physiology and secondary metabolism e.g. no activation of cryptic (dormant) genes
- stability of the genetic construct under typical process conditions, including data to document the uniformity or range of product variability
- mobilisability of the introduced/modified DNA e.g. frequency with which the inserted/introduced DNA can be transferred from the original recipient

2.1.3.1 Expressed Material/Effect

Newly expressed material, either introduced or modified native material, should be characterised.

Where the result of the modification is the production of a novel protein, this material should be characterised as to identity, functionality and, where appropriate, similarity to products from traditional sources.

The net effect would, in some cases, not be the production of novel proteinaceous material, but would affect regulation, transcription or translation of native gene products. Examples of these effects include production of antisense mRNA or blocking of the production of regulatory enzymes. In these cases, the sensitivity and specificity of the desired action should be established. Altered regulation and expression of non-target genes in the host should be investigated in assessing the safety and nutritional acceptability of food produced from the modified organism.

2.1.3.2 Metabolism

Where genetic modifications alter the expression of traditional constituents or metabolites of the microorganism, information about the possible secondary effects on related pathways should be provided.

2.1.4 Methodology

Much of the data to be generated on modified microorganisms relates to the expression of inserted or natural genes. This information should be generated using the most appropriate current techniques such as nucleic acid hybridization, Restriction Fragment Length Polymorphism (RFLP) analysis, sequence analysis, monoclonal antibody typing and specific chemical analyses. The use of molecular biological techniques is recommended for determining several parameters including: gene expression kinetics and level of expression, inserted or blocked genes, specificity of expression, and fidelity of transcription and translation of gene products. Alternative methods may be appropriate as new technology is developed.

2.2.0 PRODUCT INFORMATION

2.2.1 Microorganisms Used in or as Food

For genetically modified microorganisms proposed for use in or as food, the following information would be necessary in addition to that outlined in Sections 2.1.0-2.1.3:

- description of the product, and detailed information on its proposed use including, where appropriate, process flow diagrams, standard operating procedures and quality control/quality assurance programmes that ensure production in accordance with good manufacturing practices.

- the growth characteristics and metabolic profile should be determined in the food in which the organism is to be used. Detailed technical data should be provided on composition, based on the analysis of typical production material. These data should document the variability in composition of the product to be offered for sale and upon which the safety assessment is based. Novel constituents, other than the product of the intentional modification, will require characterization.

- analytical investigation should include an examination of the principal chemical characteristics, significant nutrient constituents and non-nutrients such as endogenous toxins typically associated with the organisms in question or related organisms.

2.2.2 Microbial Products Used In Food

This section deals specifically with the products of genetically-modified organisms that are used in food.

Data requirements have been established for the evaluation of food additives, including enzymes, and have traditionally formed the basis for the assessment of other food constituents such as flavours. A comparable data base will be needed, where appropriate, to assess the safety of food constituents produced by genetically-modified organisms.

Additional data may also be requested, depending on the nature of the genetic modification, history of the organisms involved, degree of chemical characterization and anticipated level of exposure. These additional data will be determined on a case-by-case basis.

2.2.2.1 Products Identical To Permitted Food Additives

Products represented as identical to permitted food additives must be accompanied by adequate data to demonstrate that there is no significant change in composition of the product, when compared to that from a presently-accepted source. The technical specifications and supporting database should include detailed data on the identity and composition of the product when it is made in accordance with the established process. These data should document the uniformity or range of variability in composition of the final product and detail the analytical methods and sampling procedures used in their development.

If the composition of the proposed additive is judged not to be identical to that of a permitted food additive, then additional safety data may be required on a case-by-case basis. The required data will be a function of the potential dietary exposure, and the nature and degree of difference of the additive with respect to that obtained from an accepted source.

2.2.2.2 Products Which Represent New Food Additives

In addition to information outlined in this document, the submission must meet the preclearance requirements of section B.16.002 of the *Canadian Food and Drug Regulations*. The submission must include the following information:

- description, chemical name, trade name, method of manufacture and specifications/composition
- purpose, area of use and proposed level of use
- analytical method to determine the additive in food
- efficacy data justifying functionality and level of use
- safety data (includes toxicology data and intake estimates)
- residue data in cases where the additive is removed, destroyed or reactive

2.2.2.3 Microbial Products Produced *In-situ*

The assessment of microbial products, such as food additives produced *in-situ*, will require consideration of the data outlined in Sections 2.2.1 and 2.2.2. Where appropriate, the purified product will be subject to the assessment criteria in place for those products produced by traditional processes. Data documenting any other changes in cellular constituents or by-products that may be imparted to the food by the modified organism would also be required. The specific data to be required may be product-dependent.

2.3.0 DIETARY EXPOSURE

Estimates of dietary exposure to modified microorganisms and microbial products, used in or as food, may play a key role in determining the extent of the required toxicological and nutritional data. An organism or its metabolites, that are removed from the final food product or are only a minor constituent, may be of less concern than for significant components of a food. Complete details should be supplied on the levels of the modified organism and/or its products in the finished food. This information may be considered in developing an estimate of overall dietary exposure, in combination with the anticipated use pattern and the dietary intake of the food in question by the average consumer and population subgroups. If these data indicate that there is significant exposure to the food or food constituent from a genetically modified source, or a change in use and/or exposure for a related traditional component, this would be considered in the safety assessment. In the case of substances covered by existing safety data (e.g. permitted food additives) an estimate of anticipated increases in exposure would be considered as one factor in determining the adequacy of the existing safety assessment.

2.4.0 NUTRITIONAL DATA

The introduction of a significant dietary item may require an assessment of the nutritional consequences and implications for the population as a whole and/or specific subgroups (e.g. children) who may consume extreme amounts. The evaluation is needed in order to ensure that the nutritional status of consumers is not unduly jeopardized by:

- substitution of dietary components of known nutrient value (on which nutrition and dietary recommendations are based), with less nutritious varieties

- distortion of nutrient intakes as a result of unusual levels of particular nutrients or presence of anti-nutrients that could affect the nutritional value of the remainder of the diet.

It is expected that the development of food products or products containing novel food constituents from genetically modified sources would, where appropriate, include the generation of nutrient data that would be of value in assessing nutritional impact. Such information should include but not be limited to the following;

2.4.1 Nutrient Composition

a) proximate composition e.g. ash, moisture content, crude protein, crude fat, crude carbohydrate

b) content of true protein, non-protein nitrogenous material (e.g. nucleic acids and aminoglycosides), amino acid profile, unusual amino acids should be determined if their presence is suspected (e.g. d-amino acids from bacterial proteins)

c) quantitative and qualitative composition of total lipids, i.e. saponifiable and non-saponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids

d) composition of the carbohydrate fraction e.g. sugars, chitin, tannins, non-starch polysaccharides and lignins

e) qualitative and quantitative composition of vitamins, i.e. complete vitamin analysis

f) presence of naturally occurring or adventitious anti-nutritional factors e.g. phytates, trypsin inhibitors, etc.

g) storage stability with regard to nutrient degradation

The nutritional value may be assessed initially from the nutrient composition data. Unusual or unanticipated components should be subjected to further analysis.

2.4.2 Nutrient bioavailability

Many of the nutritional concerns may be amenable to resolution on the basis of chemical analysis of the product and comparison with the commodity to be replaced. "Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate.

In situations where the food from a genetically modified source may be a major component of the Canadian diet, and therefore a supplier of important dietary nutrients, animal studies may be needed in assessing nutritional adequacy.

2.5.0 TOXICOLOGY DATA

If concerns remain after assessment under the preceding sections, toxicity studies would be required as necessary, on the whole food, food constituent or specific component in question. These studies would most likely be necessary when there is appreciable estimated dietary exposure to new or altered components. In view of the diversity of products derived from modified microorganisms that may be used in or as food, it is not possible to precisely define the type or degree of toxicity tests that would be required in all instances. Toxicity testing requirements may be based, in part, on the assessment of the data submitted under sections 2.1 to 2.4.

2.5.1 Laboratory Animal Studies

Laboratory animal studies may be designed to address both nutritional and toxicological concerns. The length and types of these studies would be determined based upon the information available for the product.

Food constituents produced by modified microorganisms and proposed for use in food may be evaluated on the basis of toxicological data presently considered for similar products from traditional sources. Traditional approaches to toxicological studies are generally applicable to the assessment of individual compounds or simple mixtures and are directed at supporting the establishment of an acceptable daily intake (ADI) for the compound(s) under investigation. The studies are designed to assess the test material's potential to elicit short-term, chronic, carcinogenic, genotoxic, reproductive and teratogenic adverse effects. Data from pharmacokinetic studies (absorption, distribution, metabolism and excretion) should be considered when designing the various toxicity studies. Internationally accepted protocols are available for these studies, for example the protocols recommended by the OECD.

The application of standard laboratory animal testing protocols to thf toxicological evaluation of whole foods or major food constituents is problematic. For example, the incorporation of an appropriate amount, from a traditional safety testing standpoint, of a whole food into a laboratory animal's diet cannot normally be accomplished without encountering nutritional and/or

palatability problems. Modifications to the standard approach in order to address these issues may need to be considered.

2.5.2 Allergenicity Considerations

The potential for allergenic response would be considered on the basis of the history of the host and donor organisms and the modification undertaken. Where the potential for allergenicity exists, the petitioner should consult Food Directorate.

3. GENETICALLY MODIFIED PLANTS AND THEIR PRODUCTS

3.0.0 FOREWORD

Plants may be consumed as food or used to produce materials which are used in food or food processing. The variety of ways by which plants can be modified, and the degree of modification that can be produced, preclude standardization of the means to assess safety. The methods and extent of genetic modification, in part, determine both the type and quantity of information required to make an assessment.

The point in the development of the new variety at which data are generated is central to the assessment of safety. It is expected that for many "novel plants," the final product will be the result of repeated backcrosses between the initially-modified plant and the host variety. Some data generated in the initial stages would be accepted for an assessment of the final product. This would specifically relate to information on the method of modification, the stability of the transformed plant and molecular biology. The detailed data on the chemical and toxicological characterization should be generated with genetically stable, converted lines which are representative of the final food product.

It is important to note that not all information requirements outlined below may be appropriate to all cases. Applicants are encouraged to consult the Food Directorate early in product development in order to reach agreement on what information is appropriate to the evaluation of the safety of the product.

The following information is recommended for assessing the acceptability of genetically modified plants and their products intended for use in or as a food. Once a genetically modified plant is determined to be acceptable, further variety development using traditional breeding techniques would not result in varieties requiring notification.

3.1.0 DEVELOPMENT AND PRODUCTION OF THE MODIFIED PLANT

Sufficient data should be submitted to characterize the modified plant and permit comparison with the conventional or unmodified counterpart. Most of the questions regarding characterization of the modified plant can be addressed by data that may have been generated in the developmental stage. The presence and level of toxic compounds from novel plants developed from parents or vectors known to express these substances are of special concern. Wherever possible, transformation markers which generate safety concerns should not be present in the final food product. If selectable markers are present in the final food, they will be evaluated for safety.

3.1.1 Host and Donor Organisms

Detailed information on the natural history of both donor and host organisms should be considered. Specific information could include, but not be limited to, known toxin production, relationship to toxin producers of the same genus, previous food and/or medicinal use.

3.1.2 Modification Process

Sufficient information on the process used to effect the genetic modification should be provided to enable an assessment of both safety and potential secondary effects. Detailed information should be provided on source, purity and stability of all inserted material.

The modification process may include, but not be limited to, the use of recombinant nucleic acid procedures, noninsertional plasmid borne genes, viral vectors or other single or multiple vector systems, and minichromosomes. Novel plants that are developed using physical or chemical mutagenesis, somaclonal variation, embryo rescue, protoplast fusion or other methods producing wide genetic crosses, may also be included. However, the degree of similarity to existing products should be taken into account in this determination.

Information on all elements of the transformation/modification system should be provided, including identification of all known regulatory elements and coding sequences. Vector construct and method of modification/transformation should also be provided. The transformation/modification system should be mapped to a degree consistent with available technology, preferably to the level of base sequence.

The source of all elements in the construct and all available information on food uses for those elements should be provided. The potential for transformation/modification of exposed organisms should be assessed.

Where the transformation involves large pieces of genetic information (e.g. chromosome exchange, genome mixing), or changes not amenable to molecular analysis (e.g. chemical or radiation mutagenesis), the absence of detailed molecular information may necessitate more complete chemical and toxicological characterization of the product. The required information may depend on the existing information on the host, its history of food use, production of toxic compounds, etc..

3.1.2.1 Stage, Temporal and Site-Specific Expression

A description of whether the inserted gene(s) are inducible or constitutive should be provided. For inducible gene(s), the inducing agent should be identified. Where there is an intention for restriction of expression of the inserted gene(s), detailed information on expression may be necessary. The mechanism whereby expression is restricted should be detailed, along with information assuring stability of the restriction of expression.

3.1.3 The Modified Host

The modified plant should be assessed with respect to growth and genetic stability. The potential for secondary effects on biochemistry, physiology and secondary metabolism of the host plant species should be determined. Where secondary effects are identified, these should be characterized.

Where pesticidal properties, increased tolerance to environmental stresses, herbicides or plant pathogens has been transferred, as much information as possible should be provided concerning the "mechanism of action" and the consequences on the composition of the final plant e.g. accumulation of natural toxins, pesticide residues etc.

3.1.3.1 Expressed Material/Effect

Newly expressed material, either introduced or modified native material should be characterised.

Where the result of the modification is the production of novel proteinaceous material, this material should be characterised to identity, functionality and where appropriate similarity to products from traditional sources.

The expression product may alternatively not be novel proteinaceous material but might affect the regulation (transcription or translation) of native gene products. Examples of this include production of antisense mRNA or blocking the production of regulatory enzymes. In such instances, the sensitivity and specificity of the desired action should be established. Altered regulation or expression of non-target genes in the host should be investigated in assessing the safety and nutritional acceptability of food produced from the modified plant.

3.1.3.2 Metabolism

Where genetic modifications alter the expression of a traditional plant constituent, sufficient information on the anabolic or catabolic pathways should be provided to enable an assessment of possible secondary effects on related pathways and metabolite production.

3.1.4 Methodology

Much of the information to be generated on modified plants relates to the expression of inserted or natural genes. It is expected that this information may be generated using current techniques such as nucleic acid hybridization, monoclonal antibody typing and specific chemical analyses. The use of molecular biological techniques is recommended for determining several parameters including gene number, location and orientation, expression kinetics and level of expression of inserted or blocked genes, tissue or temporal specificity of expression, fidelity of transcription and translation of gene products. Alternative methods may be appropriate as new technology is developed.

The characteristics of the modified plant should be compared to those of the unmodified host, taking into account known ranges for those characteristics in that crop variety. Specific experiments should incorporate the unmodified crop for comparison purposes.

3.2.0 PRODUCT INFORMATION

A review of the literature for all of the information relevant to a safety assessment of the host plant and related varieties used in the development of the modified plant should be provided. This should include a critical assessment of the ability to produce potentially toxic compounds, available toxicology data, history of safe use of the host plant and related varieties used in the development of the modified plant.

Information for plants modified to introduce, for example: pesticidal properties, resistance to plant pathogens, and tolerance to pest control agents (such as herbicides) and environmental stresses (such as cold, drought, and contaminants) should be accompanied (as appropriate) by data concerning the accumulation, metabolism and fate of plant pathogen metabolites, potentially toxic contaminants and pest control agents permitted for use on the crop plant. An understanding of the mechanism by which the modified phenotype

operates may determine the need for additional data. If the detailed mechanism is not known, it is expected that a more extensive investigation of potential residue levels and metabolism in the modified plant may be necessary.

If novel constituents other than those resulting from the intentional modification of genetic material are identified, further studies would be required to characterize the product.

3.2.1 Plants Used As Food

The following information, in addition to that outlined in Sections 3.1.0-3.1.3, would be necessary to conduct a safety assessment of plants presently used as food and those proposed for use as food:

- a description of the plant material, detailed information on its proposed use, including details on processing and quality control/-quality assurance programs, as appropriate.

- information comparing the composition of the novel food or food constituent to that of the unmodified host, and, if necessary, other varieties of the host type based on analysis of representative samples e.g. from representative growing areas over more than one growing season. These data should demonstrate the uniformity or variability of the composition of the final product and include the analysis/characterization of the gene products (e.g. in the case of proteins, any post translational modifications are of interest).

The analytical comparison may include an examination of the principal chemical characteristics, significant nutrient constituents and non-nutrients. Such non-nutrients include: endogenous plant and other natural toxicants typically associated with the food, its parents or related species.

Further information for analytical characterisation of food crops modified to be resistant to plant pathogens or tolerant to pest control agents (e.g. herbicides) or environmental stresses (e.g. cold, drought, salinity and contaminants) may necessarily be determined on a case-by-case basis.

3.2.2 Plant Products Used In Food

This section deals specifically with the products of genetically-modified plants that are used in food.

Data requirements have been established for the evaluation of food additives, including enzymes, and have traditionally formed the basis for the assessment of other food constituents such as flavours. Comparable information may be needed, where appropriate, in assessing the safety of food constituents from genetically modified plants and plant materials.

Data over and above that required for the evaluation of a food additive, from traditional sources, may be requested depending on the nature of the genetic modification, history of the plants involved, the degree of chemical characterization and anticipated level of exposure. The additional data required will be determined on a case-by-case basis.

3.2.2.1 Products Identical To Permitted Food Additives

Products represented as identical to permitted food additives must be accompanied by adequate data to demonstrate that there is no significant change in composition of the product, when compared to that from a presently-accepted

source. The technical specifications and supporting database should include detailed data on the identity and composition of the product based on the analysis of typical production material produced in accordance with the established process. These data should document the uniformity or variability in composition of the final product and detail the analytical methods and sampling procedures used in their development.

If the composition of the proposed additive is judged not to be identical to that of a permitted food additive then additional safety data will be required, on a case-by-case basis. The required data will be a function of the potential dietary exposure, and nature and degree of difference with respect to the additive from an accepted source.

3.2.2.2 Products Which Represent Novel Food Additives

In addition to information on the genetic modification of the plant, the submission must meet the data requirements of section B.16.002 of the *Canadian Food and Drug Regulations*. The submission must include the following information;

- description, chemical name, trade name, method of manufacture and specifications/composition
- purpose, area of use and proposed level of use
- analytical method to determine the additive in food
- efficacy data justifying functionality and level of use
- safety data (includes toxicology data and intake estimates)
- residue data in cases where the additive is removed, destroyed or reactive

3.3.0 DIETARY EXPOSURE

Estimates of dietary exposure to the modified plant materials may play a key role in determining the extent of the toxicological and nutritional data required for a safety assessment. Plant materials or associated metabolites that are removed from the final food product may be of less concern than those representing significant components of a food. Complete details should be supplied of the amounts of the plant material and/or its products, in the finished food. This information will be considered, in combination with the anticipated use pattern and the dietary intake of the food in question by the average consumer as well as population subgroups, in developing an estimate of overall dietary exposure. If these data suggest that there will be significant exposure to the food from a genetically modified source or change in use and/or exposure for a related traditional food product this would be considered in the safety assessment. In the case of substances covered by existing safety data (e.g. permitted food additives or agricultural chemicals) documentation of anticipated increases in exposure would be considered as one factor in determining the adequacy of the existing safety assessment.

3.4.0 NUTRITIONAL DATA

The introduction of novel or nontraditional plants into the Canadian food supply requires an assessment of the nutritional consequences and implications for the population as a whole and/or specific subgroups (e.g. children) who may consume extreme amounts. The evaluation is needed in order to ensure that the nutritional status of consumers is not unduly jeopardized by:

- substitution of dietary components of known nutritive value (on which nutrition and dietary recommendations are based), with less nutritious varieties

- distortion of nutrient intakes as a result of unusual levels of particular nutrients or the presence of anti-nutrients that could affect the nutritional value of the remainder of the diet.

It is expected that the development of genetically-modified food products or products with constituents from genetically-modified sources would include, where appropriate, the generation of nutrient data that would be of value in assessing nutritional impact. Nutrient information on the genetically modified plant should focus on the dietary importance of food from that plant and may include:

3.4.1 Nutrient Composition

a) proximate composition e.g. ash, moisture content, crude protein, crude fat, crude carbohydrate

b) content of true protein, non-protein nitrogenous material (e.g. nucleic acids and aminoglycosides), amino acid profile - unusual amino acids should be determined if their presence is suspected (e.g. d-amino acids from bacterial proteins)

c) quantitative and qualitative composition of total lipids, i.e. saponifiable and non-saponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids

d) composition of the carbohydrate fraction e.g. sugars, chitin, tannins, non-starch polysaccharides and lignins

e) qualitative and quantitative composition of vitamins, i.e. complete vitamin analysis

f) presence of naturally occurring or adventitious antinutritional factors e.g. phytates, trypsin inhibitors etc.

g) storage stability with regard to nutrient degradation

The nutritional value may be assessed initially from the nutrient composition data. Unusual or unanticipated components should be subjected to further analysis.

3.4.2 Nutrient Bioavailability

Many of these concerns are amenable to resolution on the basis of chemical analysis of the product and comparison with the commodity to be replaced. Fingerprinting of the product by such techniques as HPLC, GC-MS, and conventional analytical methods is recommended.

Where the food from a genetically modified source is a source of important dietary nutrients, animal studies may be needed as evidence of nutritional adequacy.

3.5.0 TOXICOLOGY DATA

If concerns remain after assessment under the preceding sections toxicity studies would be required as necessary, on the whole food, food constituent or specific component in question. These studies would most likely be necessary when there is appreciable estimated dietary exposure to new or altered components. In view of the diversity of products derived from modified plants that will be used in or as food, it is not possible to precisely define the type or degree of toxicity tests that would be required in all instances. Toxicity testing requirements will be based, in part, on the assessment of the data submitted under sections 3.1 to 3.4.

3.5.1 Laboratory Animal Studies

Laboratory animal studies may be designed to address both nutritional and toxicological concerns. The length of these studies would be determined based upon the information available for the product.

Food constituents produced by modified plants and proposed for use in food will be evaluated on the basis of toxicological data presently considered for similar products from traditional sources. Traditional approaches to toxicological studies are generally applicable to the assessment of individual compounds or simple mixtures and are directed at supporting the establishment of an acceptable daily intake (ADI) for the compound(s) under investigation. The studies are designed to assess the test material's potential to elicit short-term, chronic, carcinogenic, genotoxic, reproductive and teratogenic adverse effects. Data from pharmacokinetic studies (absorption, distribution, metabolism and excretion) should be considered when designing the various toxicity studies. Internationally accepted protocols are available for these studies, for example those developed by the OECD.

The application of standard laboratory animal testing protocols to the toxicological evaluation of whole foods or major food constituents is problematic. For example, the incorporation of an appropriate amount, from a traditional safety testing standpoint, of a whole food into a laboratory animal's diet cannot normally be accomplished without encountering nutritional and/or palatability problems. Modifications to the standard approach in order to address these issues may need to be considered.

3.5.2 Allergenicity Considerations

The potential for allergenic response would be considered on the basis of the history of the host and donor organisms and the modification undertaken. Where the potential for allergenicity exists, the petitioner should consult Food Directorate.

federal register

Friday
May 29, 1992

Part IX

Department of Health and Human Services

Food and Drug Administration

Statement of Policy: Foods Derived From New Plant Varieties; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. 92N-0139]

Statement of Policy: Foods Derived From New Plant Varieties

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is issuing a policy statement on foods derived from new plant varieties, including plants developed by recombinant deoxyribonucleic acid (DNA) techniques. This policy statement is a clarification of FDA's interpretation of the Federal Food, Drug, and Cosmetic Act (the act), with respect to new technologies to produce foods, and reflects FDA's current judgment based on new plant varieties now under development in agricultural research. This action is being taken to ensure that relevant scientific, safety, and regulatory issues are resolved prior to the introduction of such products into the marketplace.

DATES: Written comments by August 27, 1992.

ADDRESSES: Submit written comments to the Dockets Management Branch (HFA-305), Food and Drug Administration, rm. 1-23, 12420 Parklawn Dr., Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Regarding Human Food Issues: James H. Maryanski, Center for Food Safety and Applied Nutrition (HFF-300), Food and Drug Administration, 200 C St. SW., Washington, DC 20204, 202-485-3617. Regarding Animal Feed Issues: William D. Price, Center for Veterinary Medicine (HFV-221), Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301-295-8724.

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I. Background and Overview of Policy

New methods of genetically modifying plants are being used to develop new varieties that will be sources of foods. These methods, including recombinant DNA techniques and cell fusion techniques, enable developers to make genetic modifications in plants, including some modifications that would not be possible with traditional plant breeding methods. This policy discusses the safety and regulatory status of foods derived from new plant varieties, including plants developed by the newer methods of genetic modification.

FDA has received numerous inquiries from industry, government agencies, academia, and the public requesting clarification of the regulatory status of foods, such as fruits, vegetables, grains and their byproducts, derived from new plant varieties developed using recombinant DNA techniques. The questions that FDA has received center on issues such as whether the agency will conduct premarket review of these new foods, whether such foods introduced into interstate commerce would be challenged by FDA on legal grounds, which new plant varieties might come under the jurisdiction of FDA, what scientific information may be necessary to satisfy FDA that such foods are safe and comply with the law, whether petitions would be required by the agency, and whether special labeling would be required.

Representatives of the food biotechnology industry have expressed to FDA the need for strong but appropriate oversight by Federal agencies to ensure public confidence in foods produced by the new techniques. FDA has received several specific comments and suggestions from the industry and from the public concerning Federal oversight of foods developed through new methods of genetically modifying plants (Refs. 1 through 4). The agency has considered these and other documents, including scientific research papers, in developing this notice, and is setting forth this policy statement to clarify its interpretation of the act with respect to human foods and animal feeds [1] derived from new plant varieties, [2] including but not limited to plants developed by new methods of genetic modification.[3]

Under this policy, foods, such as fruits, vegetables, grains, and their byproducts, derived from plant varieties developed by the new methods of genetic modification are regulated within the existing framework of the act, FDA's implementing regulations, and current practice, utilizing an approach identical in principle to that applied to foods developed by traditional plant breeding. The regulatory status of a food, irrespective of the method by which it is developed, is dependent upon objective characteristics of the food and the intended use of the food (or its components). The method by which food is produced or developed may in some cases help to understand the safety or nutritional characteristics of the finished food. However, the key factors in reviewing safety concerns should be the characteristics of the food product.

[1] "Food" means (1) Articles used for food or drink for man or other animals, (2) chewing gum, and (3) articles used for components of any such article (section 201(f) of the act (21 U.S.C. 321(f))). "Food" includes human food, substances migrating to food from food-contact articles, pet food, and animal feed (21 CFR 170.3(m)). "Animal feed" means "an article which is intended for use for food for animals or other than man and which is intended for use as a substantial source of nutrients in the diet of the animal, and is not limited to a mixture intended to be the sole ration of the animal" (section 201(x) of the act (21 U.S.C. 321(x))).

[2] "Variety" is used here as a general term to describe subgroups (whether varieties or cultivars) of plants within a species developed for desirable traits.

[3] "Genetic modification" means the alteration of the genotype of a plant using any technique, new or traditional. "Modification" is used in a broad context to mean the alteration in the composition of food that results from adding, deleting, or changing hereditary traits, irrespective of the method. Modifications may be minor, such as a single mutation that affects one gene, or major alterations of genetic material that affect many genes. Most, if not all, cultivated food crops have been genetically modified.

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rather than the fact that the new methods are used.

The safety of a food is regulated primarily under FDA's postmarket authority of section 402(a)(1) of the act (21 U.S.C. 342(a)(1)). Unintended occurrences of unsafe levels of toxicants in food are regulated under this section. Substances that are expected to become components of food as result of genetic modification of a plant and whose composition is such or has been altered such that the substance is not generally recognized as safe (GRAS) or otherwise exempt are subject to regulation as "food additives" under section 409 of the act (21 U.S.C. 348). Under the act, substances that are food additives may be used in food only in accordance with an authorizing regulation.

In most cases, the substances expected to become components of food as a result of genetic modification of a plant will be the same as or substantially similar to substances commonly found in food, such as proteins, fats and oils, and carbohydrates. As discussed in more detail in section V.C., FDA has determined that such substances should be subject to regulation under section 409 of the act in those cases when the objective characteristics of the substance raise questions of safety sufficient to warrant formal premarket review and approval by FDA. The objective characteristics that will trigger regulation of substances as food additives are described in the guidance section of this notice (section VII.).

The guidance section also describes scientific considerations that are important in evaluating the safety and nutritional value of foods for consumption by humans or animals, regardless of whether the food is regulated under section 402(a)(1) or section 409 of the act. The guidance section outlines a "decision tree" approach to safety assessment of foods derived from new plant varieties that FDA believes is compatible with current practice among scientists knowledgeable in this area. The guidance section also identifies certain scientific questions that may raise sufficient safety concern to warrant consultation with FDA.

Finally, this notice addresses FDA's responsibility under the National Environmental Policy Act (NEPA) and the food labeling provisions of the act as such provisions affect labeling of foods derived from new plant varieties.

This policy statement reflects FDA's current judgment based on the new plant varieties now under development in agricultural research. FDA invites comments on this document. Because scientific developments in this field are occurring rapidly, FDA will refine its policy, if circumstances warrant, in a future Federal Register notice. Additionally, FDA plans to announce in a future Federal Register notice a workshop to discuss specific scientific issues. FDA invites comment on topics that might be addressed at such a workshop.

II. Responsibility for Food Safety

FDA is the primary Federal agency responsible for ensuring the safety of commerical food and food additives, except meat and poultry products. FDA works closely on food safety matters with the U.S. Department of Agriculture (USDA), which regulates meat and poultry products, and with the U.S. Environmental Protection Agency (EPA), which regulates pesticides and sets tolerances for pesticide residues in food. FDA's authority is under the act, the Public Health Service Act, and FDA's implementing regulations codified in title 21 of the CFR. The act gives FDA broad authority to initiate legal action against a food that is adulterated or misbranded within the meaning of the act.

Producers of new foods have an obligation under the act to ensure that the foods they offer consumers are safe and in compliance with applicable legal requirements. Because in some cases the regulatory jurisdiction of a new food product including those produced using innovative methods may not be clear, producers can informally consult with FDA prior to marketing new foods to ensure that the safety and regulatory status of a new food is properly resolved.

Elsewhere in this issue of the Federal Register, FDA announces the filing of the first request by a producer for consultation with FDA concerning a new plant variety developed by recombinant DNA techniques. The request submitted by Calgene, Inc., (Calgene) concerns the FLAVR SAVR™ tomato, a new variety claimed to exhibit improved fruit ripening and other properties. Because Calgene made this request prior to the finalization of this policy statement, FDA advised the firm to submit the information about the tomato initially as a request for advisory opinion under § 10.85 (21 CFR 10.85) to permit the agency to consider the status of the new variety, and to utilize an evaluation process that is open to public comment and permits the agency to make its decision known to the public. Future requests for FDA consultation should be made consistent with the principles outlined in this notice. Thus, FDA does not anticipate that future requests of this nature will be filed under § 10.85

III. Scope of This Document

This notice discusses scientific and regulatory considerations for foods derived from new plant varieties. This notice does not address foods and food ingredients regulated by FDA that have been derived from algae, microorganisms, and other nonplant organisms, including: (1) Foods produced by fermentation, where microorganisms are essential components of the food (e.g., yogurt and single cell protein); (2) food ingredients produced by fermentation, such as many enzymes, flavors, amino acids, sweeteners, thickeners, antioxidants, preservatives, colors, and other substances; (3) substances produced by new plant varieties whose purpose is to color food, and (4) foods derived from animals that are subject to FDA's authority, including seafood. FDA is considering whether to address these issues in future Federal Register notices.

Finally, the principles discussed in this notice do not apply to "new drugs" as defined by section 201 (p) of the act (21 U.S.C. 321(p)), "new animal drugs" as defined by section 201(w) of the act (21 U.S.C. 321(w)), or to "pesticide chemicals" as defined by section 201(q) of the act. As discussed in section IX., EPA is responsible for pesticide chemicals, including those produced in plants as a result to genetic modification.

IV. Scientific Issues Relevant to Public Health

Plant breeding is the science of combining desirable genetic traits into a variety that can be used in agriculture. The desired traits can be broadly divided into two classes: Those that affect agronomic characteristics of the plant, and those that affect quality characteristics of the food. Agronomic characteristics include those affecting yield; resistance to diseases, insects, and herbicides; and ability to thrive under various adverse environmental conditions. Quality characteristics include those affecting processing, preservation, nutrition, and flavor.

The genetic modification techniques used to develop new plant varieties constitute a continuum. Traditional breeding typically consists of hybridization between varieties of the same species and screening for progeny with desired characteristics. Such hybridizations only can introduce traits found in close relatives. Breeders have developed or adopted a number of techniques to expand the range of

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genetic variation available to them. These techniques introduce variation either by using mutagenesis to alter the genome or by introducing or modifying DNA segments, including DNA segments derived from other organisms.

Mutagenic techniques include both random mutagenesis, resulting from treatment with chemical and physical mutagens, and somaclonal variation, whereby, with the use of tissue culture techniques, plants are regenerated from callus or leaf tissue explants. The regenerated plants often have properties not found in the progenitor plant, reflecting both preexisting cellular genetic differences and tissue-culture induced mutations. The mutations range from single gene changes to chromosomal rearrangements. Mutagenesis techniques are limited, however, by their inability to target a desired trait. Somaclonal variants also frequently are unstable or infertile.

Techniques for gene transfer between plants that belong to different species or genera fall under the general heading of "wide crosses." These "crosses" have been accomplished using hybridization, and protoplast fusion. Traditional wide crosses involve hybridization between closely related species or genera, frequently requiring the use of special techniques such as embryo rescue and chromosome doubling to overcome physical or genetic barriers to the production of fertile progeny. They permit the transfer of genetic traits that are not present in close relatives of the modern plant varieties but are found in more distant wild relatives. Traits that confer resistance to a number of diseases have been introduced this way.

All of the techniques described above require extensive back crossing with the parent line [4] to eliminate mutations unlinked to that responsible for the desired phenotype and undesirable traits in extraneous genetic material introduced along with that encoding the desired trait.

Recombinant DNA techniques involve the isolation and subsequent introduction of discrete DNA segments containing the gene(s) of interest into recipient (host) plants. The DNA segments can come from any organism (microbial, animal, or plant). In theory, essentially any trait whose gene has been identified can be introduced into virtually any plant, and can be introduced without extraneous unwanted genetic material. Since these techniques are more precise, they increase the potential for safe, better-characterized, and more predictable foods.

DNA segments introduced using the new techniques insert semi-randomly into the chromosome, frequently in tandem multiple copies, and sometimes in more than one site on the chromosome. Both the number of copies of the gene and its location in the chromosome can affect its level of expression, as well as the expression of other genes in the plant. To ensure homozygosity and to enhance the stability of the line and the ability to cross the trait into other lines, the breeder will often perform a limited number of back crosses to ensure that the plant line has the new trait inserted in only one location in the chromosome.

Additionally, as with other breeding techniques, the phenotypic effects of a new trait may not always be completely predictable in the new genetic background of the host. Therefore, it is common practice for breeders using recombinant DNA techniques to cross the new trait into a number of hosts to find the best genetic background for expression of the new trait. Currently, for most crops only a few lines or varieties of any species are amendable to the use of recombinant DNA techniques. Once the desired trait is introduced into a line amenable to the technique, it must then be crossed by traditional means to other desired lines or varieties.

Regardless of the particular combination of techniques used, the development of a new plant variety typically will require many site-years (number of sites x number of years of plant testing) of performance trials before introduction into agricultural practice. These range from as few as 10 to 20 site-years for some plants to 75 to 100 site-years for others (some 5 to 10 years). The time of evaluation and the size and number of sites will vary as necessary to confirm performance; to reveal vulnerabilities to pests, diseases, or other production hazards; to evaluate stability of the phenotype; to evaluate characteristics of the food; to evaluate environmental effects; and to produce the required amount of seed before the new plant variety can be grown commercially by farmers. In the course of this intensive assessment, individual plants exhibiting undesirable traits are eliminated.

Recombinant DNA techniques are used to achieve the same types of goals as traditional techniques: The development of new plant varieties with enhanced agronomic and quality characteristics. Currently, over 30 different agricultural crops developed using recombinant DNA techniques are in field trials. Food crops have been developed using these techniques to exhibit improved resistance to pests and disease and to chemical herbicides. For example, a plant's ability to resist insect infestation reportedly has been improved by transferring bacterial genetic material that encodes proteins toxic to certain insects (e.g., *Bacillus thuringiensis delta* endotoxin). Other plants have been given viral coat-protein genes that confer cross-protection to viral pathogens.

Other new plant varieties have been developed that exhibit traits for improved food processing, improved nutritional content, or enhanced protection against adverse weather conditions. For example, genetic modifications of plant enzymes involved in fruit ripening may yield tomatoes with improved ripening characteristics, texture, and flavor. Scientists have used recombinant DNA techniques to transfer genetic material for the production of seed storage protein conferring improvements in nutritional balance of important amino acids in the new plant varieties. Scientists have also identified genes in certain fish that encode proteins that conferee increased resistance to cold. Copies of those genes have been introduced into agricultural crops with the goal of producing new plant varieties that show improved tolerance to cold weather conditions.

These examples illustrate only a few of the many improved agronomic and food processing traits currently being introduced into plants using recombinant DNA techniques. Any genetic modification technique has the potential to alter the composition of food in a manner relevant to food safety, although, based on experience, the likelihood of a safety hazard is typically very low. The following paragraphs describe some potential changes in composition that may require evaluation to assure food safety.

A. Unexpected Effects

Virtually all breeding techniques have potential to create unexpected (including pleiotropic [5] effects. For example, mutations unrelated to the desired modification may be induced; undesirable traits may be introduced along with the desired traits; newly introduced DNA may physically insert into a transcriptionally active site on the chromosome, and may thereby inactivate a host gene or alter control of

[4] A line is a group of individuals from a common ancestry. It is a more narrowly defined group than a variety. (Breeding Field Crops, J.M. Poehlman, Van Nostrand Reinhold, New York, 1987.

[5] Pleiotropic effects refer to multiple effects resulting from a single genetic change.

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its expression; the introduced gene product or a metabolic product affected by the genetic change may interact with other cellular products to produce a deleterious effect. Plant breeders using well established practices have successfully identified and eliminated plants that exhibit unexpected, adverse traits prior to commercial use.

B. Known Toxicants

Plants are known to produce naturally a number of toxicants and antinutritional factors, such as protease inhibitors, hemolytic agents, and neurotoxins, which often serve the plant as natural defense compounds against pests or pathogens. For example, most cereals contain protease inhibitors, which can diminish the nutritive value of proteins. Many legumes contain relatively high levels of lectins and cyanogenic glycosides. Lectins, if not destroyed by cooking or removed by soaking, can cause severe nausea, vomiting, and diarrhea. Cyanogenic glycosides can be hydrolyzed by specific enzymes in the plant to release cyanide if food from the plant is improperly prepared. The levels of cyanogenic glycosides in cassava and some legumes can lead to death or chronic neurological disease if these foods are eaten uncooked. Cruciferae contain glucosinolates which may impair thyroid function. Squash and cucumber contain cucurbiticin, an acute toxicant. Chickpeas contain lathyrogens, which are neurotoxins.

Many of these toxicants are present in today's foods at levels that do not cause acuate toxicity. Others, such as in cassava and some legumes, are high enough to cause severe illness or death if the foods are not properly prepared. FDA seek to assure that new plant varieties do not have significantly higher levels of toxicants than present in other edible varieties of the same species.

Plants, like other organisms, have metabolic pathways that no longer function due to mutations that occurred during evolution. Products or intermediates of some such pathways may include toxicants. In rare cases, such silent pathways may be activated by mutations, chromosomal rearrangements, or new regulatory regions introduced during breeding, and toxicants hitherto not associated with a plant species may thereby be produced. Similarly, toxicants ordinarily produced at low levels in a plant may be produced at high levels in a new variety as a result of such occurrences. The likelihood of activation of quiescent pathways or increased expression from active pathways is considered extremely low in food plants with a long history of use that have never exhibited production of unknown or unexpected toxins, since the genetic changes that can lead to such events occur during growth and are induced with traditional breeding manipulations. In the few cases where toxicants have been raised to unsafe levels in a commercial plant variety, the toxicants were known to occur in significant levels in one of the parent species. Except in rare cases, plant breeders using well established practices have successfully identified and eliminated plants that express unacceptably high levels of toxicants prior to commercial use.

C. Nutrients

Another unintended consequence of genetic modification of the plant may be a significant alteration in levels of important nutrients. In addition, changes in bioavailability of a nutrient due to changes in form of the nutrient or the presence of increased levels of other constituents that affect absorption or metabolism of nutrients must be considered for potential nutritional impact.

D. New Substances

Because plant breeders using the new techniques are able to introduce essentially any trait or substance whose molecular genetic identity is known into virtually any plant, it is possible to introduce a protein that differs significantly in structure or function, or to modify a carbohydrate, fat or oil, such that it differs significantly in composition from such substances currently found in food.

E. Allergenicity

All food allergens are proteins. However, only a small fraction of the thousands of proteins in the diet have been found to be food allergens. FDA's principal concern regarding allergencity is that proteins transferred from one food source to another, as is possible with recombinant DNA and protoplast fusion techniques, might confer on food from the host plant the allergenic properties of food from the donor plant. Thus, for example, the introduction of a gene that encodes a peanut allergen into corn might make that variety of corn newly allergenic to people ordinarily allergic to peanuts.

Examples of foods that commonly cause an allergenic response are milk, eggs, fish, crustacea, mollusca, tree nuts, wheat, and legumes (particularly peanuts and soybeans). The sensitive population is ordinarily able to identify and avoid the offending food. However, if the allergen were moved into a variety of a plant species that never before produced that allergen, the susceptible population would not know to avoid food from that variety.

In some foods that commonly cause an allergic response, the particular protein(s) responsible for allergenicity is known, and therefore the producer may know whether the transferred protein is the allergen. However, in other cases, the protein responsible for a food's allergenicity is not known, and FDA considers it prudent practice for the producer initially to assume that the transferred protein is the allergen. Appropriate in vitro or in vivo allergenicity testing may reveal whether food from the new variety elicits an allergenic response in the potentially sensitive population (i.e., people sensitive to the food in which the protein is ordinarily found). Producers of such foods should discuss allergenicity testing protocol requirements with the agency. Labeling of foods newly containing a known or suspect allergen may be needed to inform consumers of such potential.

A separate issue is whether any new protein in food has the potential to be allergenic to a segment of the population. At this time, FDA is unaware of any practical method of predict or assess the potential for new proteins in food to induce allergenicity and requests comments on this issue.

F. Antibiotic Resistance Selectable Markers

In gene transfer experiments, only a small percentage of the recipient plant cells will actually take up the introduced genes, and many desirable traits (i.e., those that specify the intended technical effect) are not easy to detect before the plant has fully developed. Scientists, therefore, enhance their ability to isolate plant cells that have taken up and stably incorporated the desired genes by physically linking the desired gene to a selectable marker gene, such as a gene that specifies the production of a substance that inactivates antibiotics.

The kanamycin resistance gene is one of the most widely used selectable marker genes. The kanamycin resistance gene specifies the information for the production of the enzyme, aminoglycoside 3'-phosphotransferase II. The common name for this enzyme is kanamycin (or neomycin) phosphotransferase II. The kanamycin phosphotransferase II enzyme modifies aminoglycoside antibiotics, including kanamycin, neomycin, and geneticin (G418), chemically inactivating the antibiotic and rendering the cells that produce the kanamycin resistance gene product refractory or resistant to the

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antibiotic. Plant cells that have received and stably express the kanamycin resistance gene survive and replicate on laboratory media in the presence of the antibiotic, kanamycin. Plant cells that did not take up and express the introduced kanamycin resistance gene will be killed by the antibiotic. By linking the selectable marker gene to another gene that specifies a desired trait, scientists can identify and select plants that have taken up and express the desired genes.

The kanamycin resistance gene has been used as a selectable marker in more than 30 crops to develop varieties that exhibit improved nutritional and processing properties, resistance to pests and diseases, tolerance to chemical herbicides, and other agronomic properties. Once the desired plant variety has been selected, the kanamycin resistance gene serves no further useful purpose, although it continues to produce the kanamycin phosphotransferase II enzyme in the plant tissues. Thus, while the kanamycin resistance gene is a research tool that is important for developing new plant varieties through the current recombinant DNA techniques of gene transfer, both the kanamycin resistance gene and its product, the kanamycin phosphotransferase II enzyme protein, are expected to be present in foods derived from such plants, unless removed through recently developed techniques (Ref. 5).

Selectable marker genes that produce enzymes that inactivate clinically useful antibiotics theoretically may reduce the therapeutic efficacy of the antibiotic when taken orally if the enzyme in the food inactives the antibiotic. FDA believes that it will be important to evaluate such concerns with respect to commercial use of antibiotic resistance marker genes in food, especially those that will be widely used. FDA is now evaluating this and other issues with respect to the use of the kanamycin resistance marker in food. (See 56 FR 20004, May 1, 1991.)

C. Plants Developed to Make Specialty Nonfood Substances

New genetic modification techniques may develop plants that produce nonfood chemicals, such as polymers and pharmaceuticals. In many cases, the plant will not subsequently be used for food. In such cases, the developer must ensure that food-use varieties of the crop do not cross with or become mixed with the nonfood-use varieties. This is not a new issue for breeders and growers. For example, some varieties of rapeseed oil are grown for industrial oil use, and have high levels of toxicants, such as erucic acid and glucosinylates, while other varieties are grown for food use and have low levels of these substances. Similarly, potatoes grown for industrial uses can have higher levels of solanine than those grown for retail food use. The producer of the oil or potato must ensure that the edible plant variety is not adulterated within the meaning of the act. Developers of crops designed to produce specialty nonfood substances have a comparable obligation.

If plants (or materials derived from plants) used to make nonfood chemicals are also intended to be used for food, producers should consult with FDA to determine whether the nonfood chemical would be a food additive requiring an authorizing regulation prior to marketing for food use.

H. Issues Specific to Animal Feeds

Unlike a food in the human diet, an animal feed derived from a single plant may constitute a significant portion of the animal diet. For instance, 50 to 75 percent of the diet of most domestic animals consists of field corn. Therefore, a change in nutrient or toxicant composition that is considered insignificant for human consumption may be a very significant change in the animal diet.

Further, animals consume plants, plant parts, and plant byproducts that are not consumed by humans. For example, animals consume whole cottonseed meal, whereas humans consume only cotton seed oil. Gossypol, a plant toxicant, is concentrated in the cotton seed meal during the production of cotton seed oil. Because plant byproducts represent an important feed source for animals, it is important to determine if significant concentrations of toxicants or other harmful plant constituents are present in new plant varieties.

Nutrient composition and availability of nutrients in feed are important safety considerations for animal health. For example, if a genetic modification in soybeans caused an increase in phytin content, the soybean feed may need to be supplemented with phosphorous to avoid problems of animal health.

V. Regulatory Status of Foods Derived From New Plant Varieties

A. The Statutory Framework for New Foods and Food Ingredients

The United States today has a food supply that is as safe as any in the world. Most foods derived from plants predate the establishment of national food laws, and the safety of these foods has been accepted based on extensive use and experience over many years (or even centuries). Foods derived from new plant varieties are not routinely subjected to scientific tests for safety, although there are exceptions. For example, potatoes are generally tested for the glycoalkaloid, solanine. The established practices that plant breeders employ in selecting and developing new varieties of plants, such as chemical analyses, taste testing, and visual analyses, rely primarily on observations of quality, wholesomeness, and agronomic characteristics. Historically, these practices have proven to be reliable for ensuring food safety. The knowledge from this past experience coupled with safe practices in plant breeding has contributed to continuous improvements in the quality, variety, nutritional value, and safety of foods derived from plants modified by a range of traditional and increasingly sophisticated techniques (Ref. 1 at xvi). Based on this record of safe development of new varieties of plants, FDA has not found it necessary to conduct, prior to marketing, routine safety reviews of whole foods derived from plants.

Nevertheless, FDA has ample authority under the act's food safety provisions to regulate and ensure the safety of foods derived from new plant varieties, including plants developed by new techniques. This includes authority to require, where necessary, a premarket safety review by FDA prior to marketing of the food. Under section 402(a)(1) of the act, a food is deemed adulterated and thus unlawful if it bears or contains an added poisonous or deleterious substance that may render the food injurious to health or a naturally occurring substance that is ordinarily injurious. Section 402(a)(1) of the act imposes a legal duty on those who introduce food into the market place, including food derived from new crop varieties, to ensure that the food satisfies the applicable safety standard. Foods that are adulterated under section 402(a)(1) of the act are subject to the full range of enforcement measures under the act, including seizure, injunction, and criminal prosecution of those who fail to meet their statutory duty.

FDA has relied almost exclusively on section 402(a)(1) of the act to ensure the safety of whole foods. Toxins that occur naturally in food and that render the food ordinarily injurious to health (such as poisons in certain mushrooms), and thus adulterated, rarely required FDA regulatory action because such cases are typically well known and carefully avoided by food producers.

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FDA regards any substance that is not an inherent constituent of food or whose level in food has been increased by human intervention to be "added" within the meaning of section 402(a)(1) of the act. See *United States* v. *Anderson Seafoods, Inc.*, 622 F. 2d 157 (5th Cir. 1980). Added substances are subject to the more stringent "may render [the food] injurious" safety standard. Under this standard, the food is adulterated if, by virtue of the presence of the added substance, there is a "reasonable possibility" that consumption of the food will be injurious to health. *United States* v. *Lexington Mill & Elevator Co.*, 232 U.S. 399 (1914). The "may render injurious" standard would apply to a naturally occurring toxin in food if the level of the toxin in a new plant variety were increased through traditional plant breeding or some other human intervention. Section 402(a)(1) of the act would have been the legal basis under which FDA could have blocked marketing in the 1970's of a new variety of potato that had been found during its development to contain elevated and potentially harmful levels of solanine as a result of a cross with an inedible wild potato.

Section 402(a)(1) of the act is most frequently used by FDA to regulate the presence in food of unavoidable environmental contaminants such as lead, mercury, dioxin, and aflatoxin. FDA regulary establishes action levels and takes enforcement action to prevent the sale of foods that contain unacceptable levels of such unintended and undesired contaminants.

Section 402(a)(1) of the act was signed into law in 1938 and has its origins in a similar provision in the Federal Food and Drugs Act of 1906. Until 1958, this authority was the principal tool relied upon by FDA to regulate the safety of food and food ingredients. In 1958, in response to public concern about the increased use of chemicals in foods and food processing and with the support of the food industry, Congress enacted the Food Additives Amendment (the amendment) to the act. Among other provisions, the amendment established a premarket approval requirement for "food additives." The basic thrust of the amendment was to require that, before a new chemical additive (such as a preservative, antioxidant, emulsifier, or artificial flavor) could be used in food processing, its producer must demonstrate the safety of the additive to FDA. Congress recognized under this new scheme that the safety of an additive could not be established with absolute certainty or under all conditions of use. Congress thus provided for a science-based safety standard that requires producers of food additives to demonstrate to a reasonable certainty that no harm will result from the intended use of the additive. See 21 CFR 170.3(i). If FDA finds an additive to be safe, based ordinarily on data submitted by the producer to the agency in a food additive petition, the agency promulgates a regulation specifying the conditions under which the additive may be safely used. Food additives that are not the subject of such a regulation are deemed unsafe as a matter of law, and the foods containing them are adulterated under section 402(a)(2)(C) of the act (21 U.S.C. 342(a)(2)(C)) and are thus unlawful.

In enacting the amendment, Congress recognized that many substances intentionally added to food do not require a formal premarket review by FDA to assure their safety, either because their safety had been established by a long history of use in food or because the nature of the substance and the information generally available to scientists about the substance are such that the substance simply does not raise a safety concern worthy of premarket review by FDA. Congress thus adopted a two-step definition of "food additive." The first step broadly includes any substance the intended use of which results in its becoming a component of food. The second step, however, excludes from the definition of food additive substances that are GRAS. It is on the basis of the GRAS exception of the "food additive" definition that many ingredients derived from natural sources (such as salt, pepper, vinegar, vegetable oil, and thousands of spices and natural flavors), as well as a host of chemical additives (including some sweeteners, preservatives, and artificial flavors), are able to be lawfully marketed today without having been formally reviewed by FDA and without being the subject of a food additive regulation. The judgment of Congress was that subjecting every intentional additive to FDA premarket review was not necessary to protect public health and would impose an insurmountable burden on FDA and the food industry.

Congress' approach to defining food additives means, however, that companies developing new ingredients, new versions of established ingredients, or new processes for producing a food or food ingredient must make a judgment about whether the resulting food substance is a food additive requiring premarket approval by FDA. In many cases, the answer is obvious, such as when the ingredient is a man made chemical having no widely recognized history of safe use in food. Such an ingredient must be approved prior to its use by the issuance of a food additive regulation, based on information submitted to FDA in a food additive petition.

In other cases, the answer is less obvious, such as when an established ingredient derived from nature is modified in some minor way or produced by a new process. In such cases, the manufacturer must determine whether the resulting ingredient still falls within the scope of any existing food additive regulation applicable to the original ingredient or whether the ingredient is exempt from regulation as a food additive because it is GRAS. The GRAS status of some substances is recognized in FDA's regulations (21 CFR parts 182, 184, 186, 582, and 584), but FDA has not attempted to include all GRAS substances in its regulations.

FDA has traditionally encouraged producers of new food ingredients to consult with FDA when there is a question about an ingredient's regulatory status, and firms routinely do so, even though such consultation is not legally required. If the producer begins to market the ingredient based on the producer's independent determination that the substance is GRAS and FDA subsequently concludes the substance is not GRAS, the agency can and will take enforcement action to stop distribution of the ingredient and foods containing it on the ground that such foods are or contain an unlawful food additive.

FDA considers the existing statutory authority under sections 402(a)(1) and 409 of the act, and the practical regulatory regime that flows from it, to be fully adequate to ensure the safety of new food ingredients and foods derived from new varieties of plants, regardless of the process by which such foods and ingredients are produced. The existing tools provide this assurance because they impose a clear legal duty on producers to assure the safety of foods they offer to consumers; this legal duty is backed up by strong enforcement powers; and FDA has authority to require premarket review and approval in cases where such review is required to protect public health.

In the Federal Register of June 26, 1986 (51 FR 23302) (the June 1986 notice), FDA, in conjunction with the Office of Science and Technology Policy in the Executive Office of the President, described FDA's current food safety authorities and stated the agency's intention to regulate foods produced by

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new methods, such as recombinant DNA techniques, within the existing statutory and regulatory framework. This notice reaffirms that intention. The following paragraphs explain briefly how the current framework will apply specifically to foods derived from new plant varieties, including plants developed by recombinant DNA techniques.

B. The Application of Section 402(a)(1) of the Act

Section 402(a)(1) of the act will continue to be FDA's primary legal tool for regulating the safety of whole foods, including foods derived from plants genetically modified by the new techniques. Section 402(a)(1) of the act will be applied to any substance that occurs unexpectedly in the food at a level that may be injurious to health. This includes a naturally occurring toxicant whose level is unintentionally increased by the genetic modification, as well as an unexpected toxicant that first appears in the food as a result of pleiotropic effects. Such substances are regarded by FDA as added substances whose presence adulterates the food if present at a level that "may render" the food injurious to health.

It is the responsibility of the producer of a new food to evaluate the safety of the food and assure that the safety requirement of section 402(a)(1) of the act is met. In section VII., FDA provides guidance to the industry regarding prudent, scientific approaches to evaluating the safety of foods derived from new plant varieties, including the safety of the added substances that are subject to section 402(a)(1) of the act. FDA encourages informal consultation between producers and FDA scientists to ensure that safety concerns are resolved. However, producers remain legally responsible for satisfying section 402(a)(1) of the act, and they will continue to be held accountable by FDA through application of the agency's enforcement powers.

C. The Application of Section 409 of the Act

When Congress enacted the amendment in 1958, it did not explicitly address the possible application of the food additive approval process to foods derived from new plant varieties. As previously discussed, such foods have historically been regulated successfully under section 402(a)(1) of the act. The new methods of genetic modification have focused attention, however, on the possibility that intended changes in the composition of food resulting from genetic modification might be of a nature sufficient as a legal and public health matter to trigger regulation of a component of the food under section 409 of the act.

As discussed above, the food additive definition broadly encompasses any substance that has an intended use in food, unless the substance is GRAS. It was on this basis that the June 1988 notice indicated that, in some cases, whole foods derived from new plant varieties, including plants developed by new genetic modification techniques, might fall within the scope of FDA's food additive authority. Indeed, FDA's regulations have long recognized that it might be appropriate in some circumstances to review the GRAS (and implicitly food additive) status of foods or substances of natural biological origin that have a history of safe use but which subsequently have had "significant alteration by breeding and selection." (See 21 CFR 170.30(f).) As already discussed, however, FDA has rarely had occasion to review the GRAS status of foods derived from new plant varieties because these foods have been widely recognized and accepted as safe.

FDA has reviewed its position on the applicability of the food additive definition and section 409 of the act to foods derived from new plant varieties in light of the intended changes in the composition of foods that might result from the newer techniques of genetic modification. The statutory definition of "food additive" makes clear that it is the intended or expected introduction of a substance into food that makes the substance potentially subject to food additive regulation. Thus, in the case of foods derived from new plant varieties, it is the transferred genetic material and the intended expression product or products that could be subject to food additive regulation, if such material or expression products are not GRAS.

In regulating foods and their byproducts derived from new plant varieties, FDA intends to use its food additive authority to the extent necessary to protect public health. Specifically, consistent with the statutory definition of "food additive" and the overall design of FDA's current food safety regulatory program, FDA will use section 409 of the act to require food additive petitions in cases where safety questions exist sufficient to warrant formal premarket review by FDA to ensure public health protection.

With respect to transferred genetic material (nucleic acids), generally FDA does not anticipate that transferred genetic material would itself be subject to food additive regulation. Nucleic acids are present in the cells of every living organism, including every plant and animal used for food by humans or animals, and do not raise a safety concern as a component of food. In regulatory terms, such material is presumed to be GRAS. Although the guidance provided in section VII. calls for a good understanding of the identity of the genetic material being transferred through genetic modification techniques, FDA does not expect that there will be any serious question about the GRAS status of transferred genetic material.

FDA expects that the intended expression product or products present in foods derived from new plant varieties will typically be proteins or substances produced by the action of protein enzymes, such as carbohydrates, and fats and oils. When the substance present in the food is one that is already present at generally comparable or greater levels in currently consumed foods, there is unlikely to be a safety question sufficient to call into question the presumed GRAS status of such naturally occurring substances and thus warrant formal premarket review and approval by FDA. Likewise, minor variations in molecular structure that do not affect safety would not ordinarily affect the GRAS status of the substances and, thus, would not ordinarily require regulation of the substance as a food additive.

It is possible, however, that the intended expression product in a food could be a protein, carbohydrate, fat or oil, or other substance that differs significantly in structure, function, or composition from substances found currently in food. Such substances may not be GRAS and may require regulation as a food additive. For example, if a food derived from a new plant variety contains a novel protein sweetener as a result of the genetic modification of the plant, that sweetener would likely require submission of a food additive petition and approval by FDA prior to marketing. FDA invites comments on substances, in addition to proteins, carbohydrates, and fats and oils, that in the future may be introduced into foods by genetic modification.

Section VII. of this notice provides guidance to producers of new foods for conducting safety evaluations. This guidance is intended to assist producers in evaluating the safety of the food that they market, regardless of whether the food requires premarket approval by FDA. This guidance also includes criteria and analytical steps that producers can follow in determining whether their product is a candidate for food additive regulation and whether consultation with FDA should be pursued to determine the regulatory

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status of the product. Ultimately, it is the food producer who is responsible for assuring safety.

FDA has long regarded it to be a prudent practice for producers of foods using new technologies to work cooperatively with the agency to ensure that the new products are safe and comply with applicable legal requirements. It has been the general practice of the food industry to seek informal consultation and cooperation, and this practice should continue with respect to foods produced using the newer techniques of genetic modification.

VI. Labeling

FDA has received several inquiries concerning labeling requirements for foods derived from new plant varieties developed by recombinant DNA techniques. Section 403(i) of the act (21 U.S.C. 343(i)) requires that a producer of a food product describe the product by its common or usual name or in the absence thereof, an appropriately descriptive term (21 U.S.C. part 101.3) and reveal all facts that are material in light of representations made or suggested by labeling or with respect to consequences which may result from use (21 U.S.C. 343(a); 21 U.S.C. 321(n)). Thus, consumers must be informed, by appropriate labeling, if a food derived from a new plant variety differs from its traditional counterpart such that the common or usual name no longer applies to the new food, or if a safety or usage issue exists to which consumers must be alerted.

For example, if a tomato has had a peanut protein introduced into it and there is insufficient information to demonstrate that the introduced protein could not cause an allergic reaction in a susceptible population, a label declaration would be required to alert consumers who are allergic to peanuts so they could avoid that tomato, even if its basic taste and texture remained unchanged. Such information would be a material fact whose omission may make the label of the tomato misleading under section 403(a) of the act (21 U.S.C. 343(a)).

FDA has also been asked whether foods developed using techniques such as recombinant DNA techniques would be required to bear special labeling to reveal that fact to consumers. To date, FDA has not considered the methods used in the development of a new plant variety (such as hybridization, chemical or radiation-induced mutagenesis, protoplast fusion, embryo rescue, somaclonal variation, or any other method) to be material information within the meaning of section 201(n) of the act (21 U.S.C. 321(n)). As discussed above, FDA believes that the new techniques are extensions at the molecular level of traditional methods and will be used to achieve the same goals as pursued with traditional plant breeding. The agency is not aware of any information showing that foods derived by these new methods differ from other foods in any meaningful or uniform way, or that, as a class, foods developed by the new techniques present any different or greater safety concern than foods developed by traditional plant breeding. For this reason, the agency does not believe that the method of development of a new plant variety (including the use of new techniques including recombinant DNA techniques) is normally material information within the meaning of 21 U.S.C. 321(n) and would not usually be required to be disclosed in labeling for the food.

The guidance section (section VII.) of this notice discusses certain circumstances where questions may arise about the proper labeling of foods derived from new plant varieties. FDA requests comments on the labeling of foods derived from new plant varieties, including plants developed with recombinant DNA techniques.

VII. Guidance to Industry for Foods Derived From New Plant Varieties

A. Introduction

This guidance section describes many of the scientific considerations for evaluating the safety and nutritional aspects of food from new plant varieties derived by traditional methods (such as hybridization or mutagenesis), tissue culture methods (such as somaclonal variation and protoplast fusion), and recombinant DNA methods. Although some of the safety considerations are specific to individual technologies, many safety considerations are similar regardless of the technology used. This guidance section does not attempt to delineate acceptable practices for each specific technology. FDA expects plant breeders to adhere to currently accepted scientific standards of practice within each technology. This guidance section is based on existing practices followed by the traditional plant breeders to assess the safety and nutritional value of new plant varieties and is not intended to alter these long-established practices, or to create new regulatory obligations for them.

This guidance section describes food safety and nutritional concerns, rather than performance characteristics for which the new plant varieties may have been developed. However, this guidance section cannot identify all safety and nutritional questions that could arise in a given situation and, while comprehensive, should not be viewed as exhaustive. In some cases, additional factors may need to be considered, while in other situations, some of the factors may not apply. Therefore, this guidance section also describes situations in which producers should consult with FDA on scientific issues, the design of appropriate test protocols, requirements for labeling, and whether a food additive petition may be required.

Genetic modifications of plants can have unintended or unexpected effects on the phenotype of the plant, such as poor growth or reduced tolerance to conditions of environmental stress, that are readily apparent and can be effectively managed by appropriate selection procedures. However, effects such as an alteration in the concentration of important nutrients, increases in the level of natural toxicants, or the transfer of allergens from one species to another may not be readily detected without specific test procedures. FDA believes that a scientific basis should exist to establish that new plant varieties do not exhibit unacceptable effects with respect to toxicants, nutritional value, or allergens. In cases where the host plant has little or no history of safe use, the assessment of new plant varieties should include evidence that unknown toxicants are not present in the new plant variety at levels that would be injurious to health.

In addition, by using recombinant DNA techniques, plant breeders are now capable theoretically of introducing essentially any trait (and thus substance) whose molecular genetic identity is known into virtually any plant due to the increased power and precision of recombinant DNA techniques. This guidance section, however, discusses only proteins, carbohydrates, and fats and oils, in the belief that these are the principal substances that are currently being intentionally modified or introduced into new plant varieties. Using the new techniques, it is possible to introduce a gene that encodes a protein that differs significantly in structure or function, or to modify a carbohydrate, or fat or oil, such that it differs significantly in composition from such substances currently found in food. FDA believes that plant breeders must carefully evaluate the potential for adverse effects that could result from the presence of these substances in new plant varieties.

Theoretically, genetic modifications have the potential to activate cryptic

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pathways synthesizing unknown or unexpected toxicants, or to increase expression from active pathways that ordinarily produce low or undetectable levels of toxicants. However, this potential has been effectively managed in the past by sound agricultural practices. The agency believes that the use of host plants with a history of safe use, coupled with a continuation of sound agricultural practice, will minimize the potential for adverse public health consequences that may arise from increased levels of unknown or unexpected toxicants.

This guidance section provides a basis for determining whether new plant varieties are as safe and nutritious as their parental varieties. The assessment scheme focuses on characteristics of the new plant variety, based on characteristics of the host and donor species, the nature of the genetic change, the identity and function of newly introduced substances, and unexpected or unintended effects that accompany the genetic change. The assessment focuses on the following considerations:

1. Toxicants known to be characteristic of the host and donor species;

2. The potential that food allergens will be transferred from one food source to another;

3. The concentration and bioavailability of important nutrients for which a food crop is ordinarily consumed;

4. The safety and nutritional value of newly introduced proteins; and

5. The identity, composition and nutritional value of modified carbohydrates, or fats and oils.

The scientific concepts described in this guidance section are consistent with the concepts of substantial equivalence of new foods discussed in a document under development by the Group of National Experts on Safety in Biotechnology of the Organization for Economic Cooperation and Development (OECD). This guidance section is also consistent with the principles for food safety assessment discussed in the Report of a Joint Food and Agriculture Organization/World Health Organization Consultation (Ref. 6).

B. Flow Charts

The flow charts presented in sections VII.D. through VII.F. (Figures 2 through 6) outline a series of questions related to the safety and nutritional value of foods derived from the new plant variety, and are intended to provide general guidance to breeders and developers. FDA intends that these flow charts be used in conjunction with other information and practices that breeders and developers rely on to develop new plant varieties. These reflect the current state of scientific information and are not intended as regulatory requirements. As new information is developed, FDA anticipates that the flow charts may require modification.

The summary flow chart (Figure 1) presented in this section is a synopsis of FDA's safety assessment process. It describes, in a general way, the assessment for unexpected or unintended effects that may arise as a result of the specific characteristics that are associated with the host plant and donor(s), as well as the assessment of the expected or intended effects. Because Figure 1 is a summary, it should not be relied upon for a safety assessment. The boxes labeled Figure 2, Figure 3, Figure 4, and Figures 5 and 6, respectively, refer to more specific flow charts that describe, in appropriate detail, the safety assessment from the perspective of the host, donor, and new substances that are introduced into the new plant variety.

Sections VII.D. through VII.F. address the scientific considerations pertaining to the host plant, donor(s), and new substances in more detail. Each section describes information that relates to the safety assessment, presents a flow chart that summarizes the safety assessment, discusses each of the questions in that flow chart, and describes the endpoints that are reached in that flow chart.

There are three endpoints in the flow charts in this notice: (1) No concerns, (2) new variety not acceptable, and (3) consult FDA. The notes to each individual flow chart discuss the interpretation of these endpoints in relation to that particular flow chart. In general, the interpretation of "no concerns" or "new variety not acceptable" is similar for each flow chart. The endpoint "consult FDA" means that producers may need to consult FDA on regulatory questions, such as whether a food additive petition or special labeling is needed, or on technical questions, such as appropriate testing protocols or specific scientific issues.

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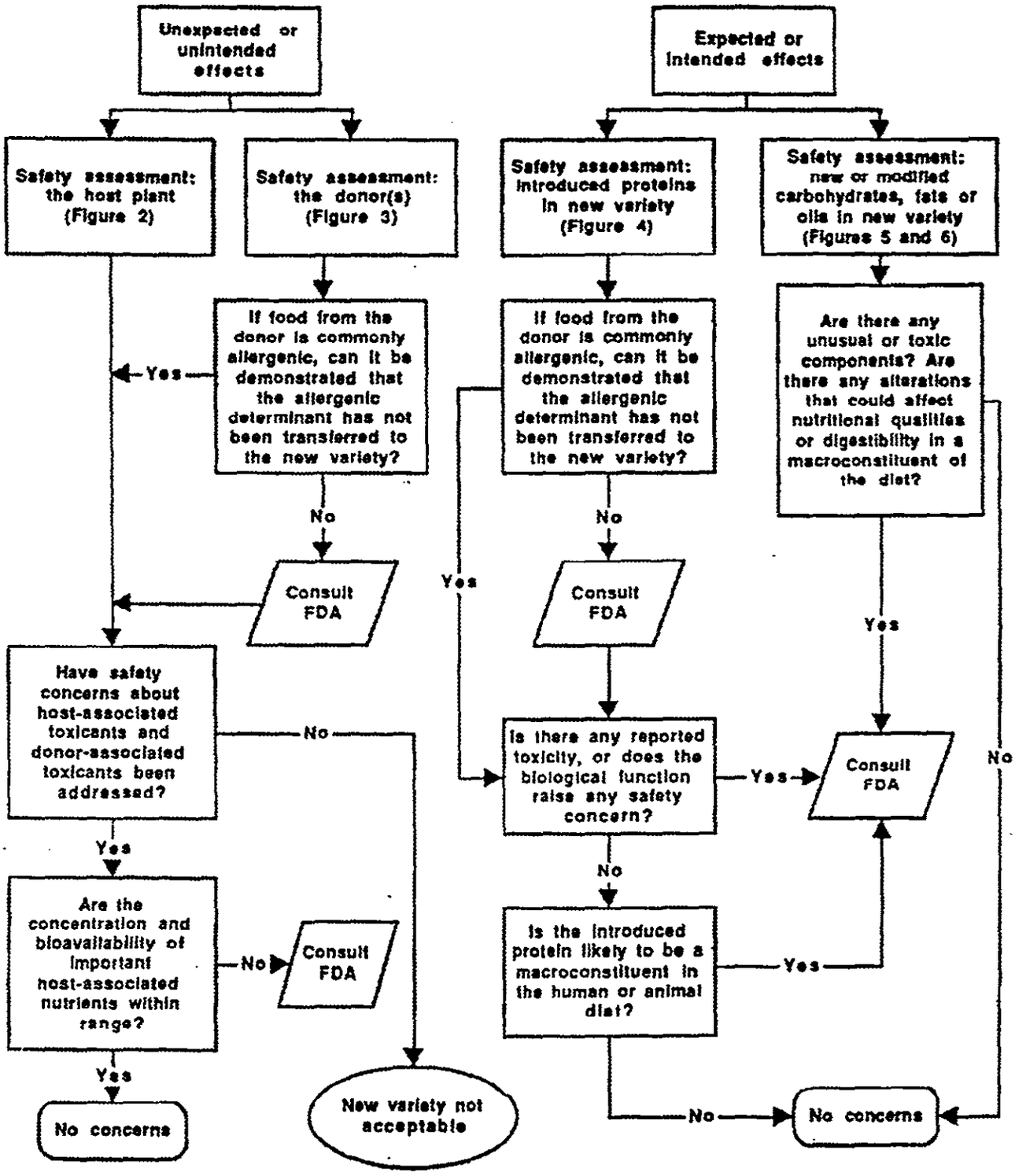

Figure 1. Safety Assessment of New Varieties: Summary

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C. Effects of Processing

Processing (e.g., cooking) may affect the safety of a substance. This is particularly important in the safety assessment of proteins transferred from one food source to another. For example, lectins, which are inactivated by cooking, would raise a safety concern if transferred from kidney beans, which are eaten cooked, to tomatoes, which may be eaten raw. The effects of any potential differences in food processing between the donor and the new plant variety should be carefully considered at each stage in the safety assessment.

D. The Host Plant

A premise basic to this guidance section is that a long history of safe use of the host species in food provides much information regarding the potential of new plant varieties to produce toxicants and antinutrients (substances that adversely affect the nutritional quality of food). In assessing the potential of the host plant to contribute unexpected harmful substances, producers should consider attributes of the host plant and its progenitors such as the following:

1. Taxonomy.
a. Variety name.
b. Known phenotypes and relevant genotypes.
2. Other species or varieties that have previously contributed genetic information to the host.
3. History of safe use.
a. Extent of previous experience.
b. The part of the plant used as food.
c. The presence and identity of potentially harmful constituents such as toxicants and antinutrients.
d. Typical methods of processing and the impact of this processing on the reduction or enhancement of effects from potentially harmful constituents.
4. The identity and level of nutrients for which the food is consumed

Figure 2

The numbers above each box in the flow chart refer to accompanying notes that immediately follow the flow chart.

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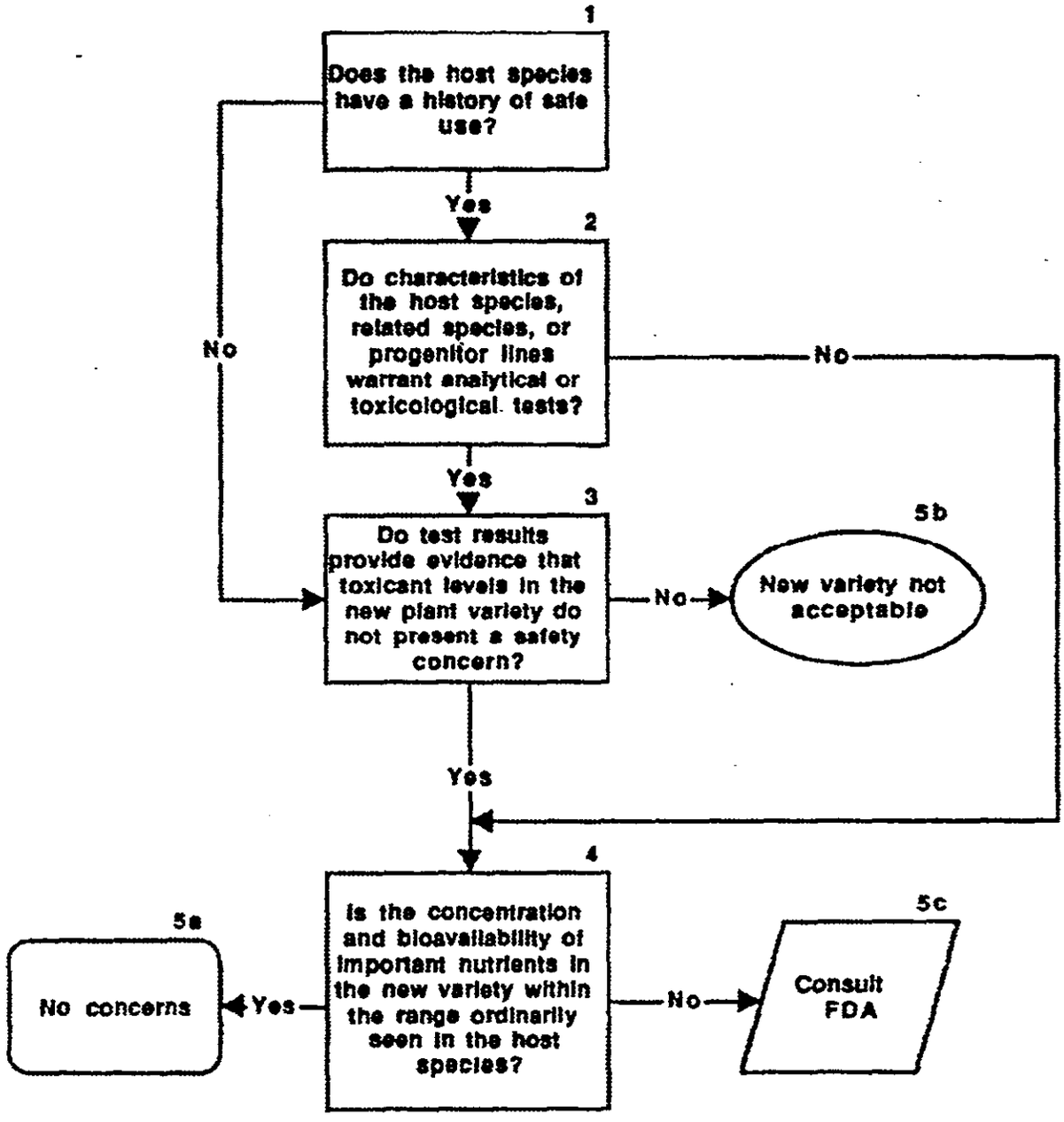

Figure 2. Safety Assessment of New Varieties: The Host Plant

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Notes to Figure 2

1—Does the host species have a history of safe use?

This guidance section is primarily designed for the development of new varieties of currently consumed food plants whose safety has been established by a history of use. If exotic species are used as hosts, testing may be needed to assure the safety and wholesomeness of the food.

2—Do characteristics of the host species, related species, or progenitor lines warrant analytical or toxicological tests?

It is not possible to establish a complete list of all toxicants that should be considered for each plant species. In general, the toxicants that are of highest concern in any particular species are those that have been documented to cause harm in normal or animal diets, or that have been found at unsafe levels in some lines or varieties of that species or related species.

In many cases, characteristic properties (such as a bitter taste associated with alkaloids) are known to accompany elevated levels of specific natural toxicants. If such characteristic provide an assurance that these toxicants have not been elevated to unsafe levels, analytical or toxicological tests may not be necessary.

3—Do test results provide evidence that toxicant levels in the new plant variety do not present a safety concern?

If a host plant or related species is known to contain toxicants whose presence must be assessed, analytical tests may be appropriate to establish that the toxicant levels are in a safe range. There is, however, a wide variation in the level of natural toxicants within and between varieties of a species, due to differences in genetic makeup and in environmental conditions during growth, harvest, and storage. Due to this natural variation, analytical tests, if necessary, should be performed using as a control the parental variety that has been grown, harvested, and stored under the same conditions as the new plant variety.

In some cases, analytical methods alone may not be available, practical, or sufficient for all toxicants whose levels are needed to be assessed. In such situations, comparative toxicological tests on the new and parental plant varieties may provide assurance that the new variety is safe. FDA encourages producers of new plant varieties to consult informally with the agency on testing protocols for whole foods when appropriate.

4—Is the concentration and bioavailability of important nutrients in the new variety within the range ordinarily seen in the host species?

If the native levels of important nutrients for which a food is widely consumed are not within the range ordinarily seen in the host species, appropriate labeling may be required. In addition, changes in bioavailability of a nutrient due to changes in form of the nutrient or the presence of increased levels of other constitutents that affect absorption or metabolism of nutrients must be considered for potential nutritional impact.

5—Endpoints in Figure 2.

5a—No concerns.

When this endpoint is reached, safety and nutritional concerns relative to the host plant will generally have been satisfied.

5b—New variety not acceptable.

This endpoint is reached when test results indicate that food derived from the new plant variety may be unsafe—e.g., if it contains unacceptable levels of toxicants.

5c—Consult FDA.

Producers should consult informally with FDA when the concentration or bioavailability of important nutrients is not within the range ordinarily seen in the host species. FDA will work with the producers on a case-by-case basis to address requirements such as labeling, or other issues relating to nutritional concerns.

E. The Donor(s)

In some cases, the donor will not have a history of safe use in food. For example, the donor may be a wild species that is related to the host plant, or may be a microorganism with no history of use in food. The potential of the donor(s) to contribute undesirable characteristics to the new plant variety should be assessed. In assessing the potential of the donor to contribute unexpected harmful substances, producers should consider attributes of the donor plant, or of fragments of genetic material from one or multiple donors, to the extent that such information is available (see Figure 3).

1. Donor Plants

Attributes of the donor plant and its progenitors, such as the following, should be considered:

1. Taxonomy.

a. Variety name.

b. Known phenotypes and relevant genotypes.

2. Other species or varieties that have previously contributed genetic information to the donor plant.

3. History of use (as applicable).

a. The part of the plant used as food.

b. The presence and identity of potentially harmful constituents such as toxicants, antinutrients, and allergens.

c. Typical methods of processing and the impact of this processing on the reduction or enhancement of effects from potentially harmful constituents.

2. Fragments of Donor Genetic Material

Attributes of each donor, and its progenitors when appropriate, such as the following, should be considered:

1. Taxonomy.

2. Other species or varieties that have previously contributed genetic information to the donor(s).

3. History of use (as applicable).

a. The part of the donor(s) used as food.

b. The presence and identity of potentially harmful constituents, such as toxicants, antinutrients, and allergens.

c. Typical methods of processing and the impact of this processing on the reduction or enhancement of effects from potentially harmful constituents.

d. The association of the transferred genetic material with harmful constituents.

4. Additional information consistent with currently accepted scientific practices, such as:

a. History and derivation of molecular constructs, such as passage through microbial hosts.

b. Known activities of any introduced regulatory sequences, such as environmental, developmental and tissue-specific effects on promoter activity.

c. The presence of extraneous open reading frames, and the potential for transcription and expression of these additional open reading frames.

Figure 3

The numbers above each box in the flow chart refer to accompanying notes that immediately follow the flow chart.

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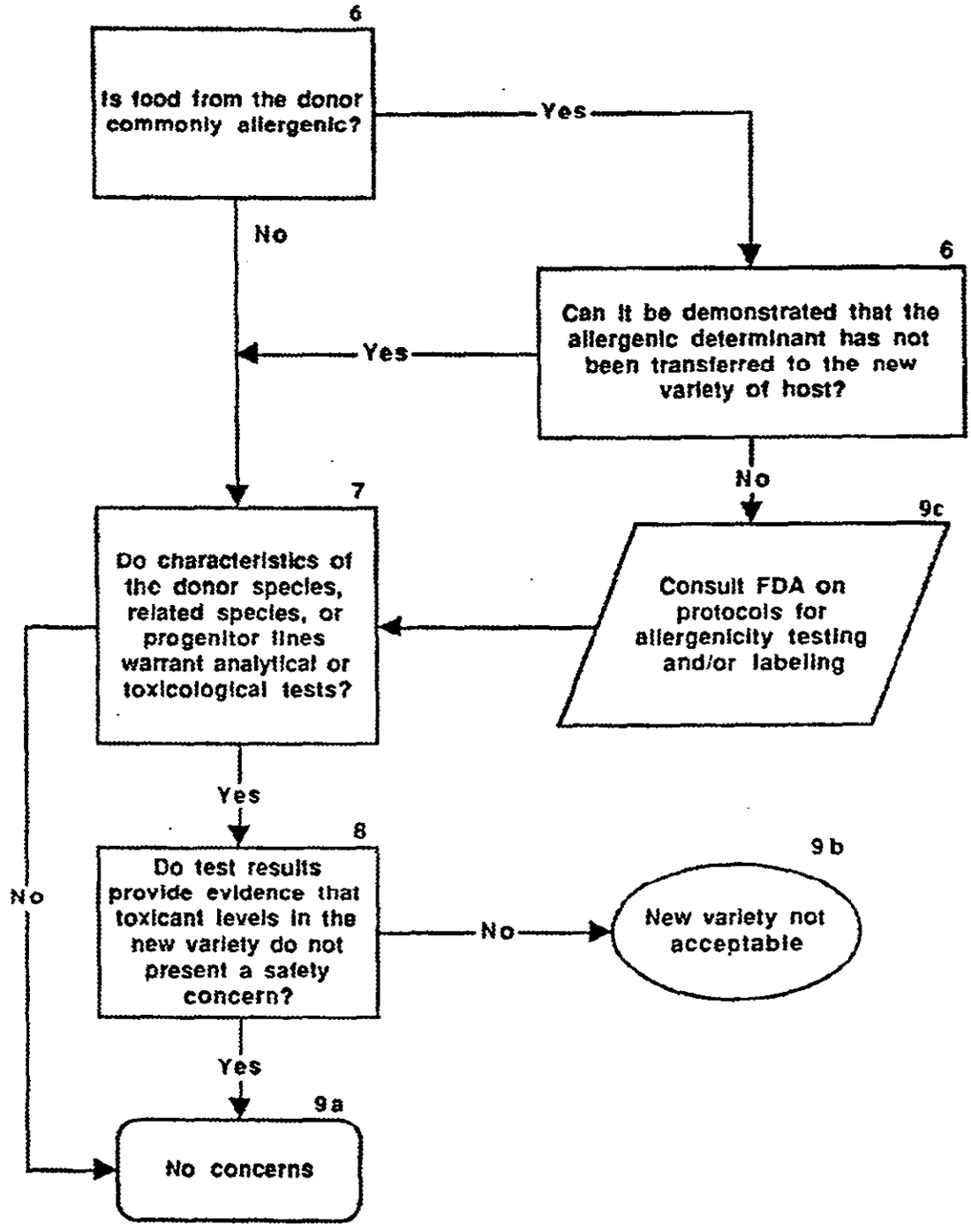

Figure 3. Safety Assessment of New Varieties: The Donor(s)

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Notes to Figure 3

6—Is food from the donor commonly allergenic? If yes, can it be demonstrated that the allergenic determinant has not been transferred to the new variety of host plant?

Some examples of foods that commonly cause an allergenic response are milk, eggs, fish, crustacea, molluscs, tree nuts, wheat, and legumes (particularly peanuts and soybeans). Allergens from these common sources may be knowingly or unknowingly transferred from a donor to a new variety of host plant. Knowledge of the identity of the allergenic determinant of the donor, coupled with appropriate knowledge of the genetic fragment that has been transferred from the donor to the new plant variety, may provide sufficient evidence that the allergenic determinant has not been transferred to the new variety of the host plant.

7—Do characteristics of the donor species, related species, or progenitor lines warrant analytical or toxicological tests?

It is possible that a toxicant present in the donor may be transferred to the host, e.g., during hybridization of a cultivated variety with a wild, poisonous relative. However, it is also possible to use a toxic donor safely. For example, a gene coding for an enzyme that is not toxic and does not yield toxic products may be isolated from pathogenic bacteria and safely transferred to a plant.

The potential that toxicants known to exist in the donor, related species, or progenitor lines will be present in the new plant variety should be addressed as described previously for the host plant (section VII.D.). Unless there is sufficient evidence that the toxicant has not been transferred to the new variety of host plant, such transfer should be assumed, and analytical and/or toxicological tests may be warranted.

8—Do test results provide evidence that toxicant levels in the new variety do not present a safety concern?

When the presence of donor-associated toxicants must be assessed, analytical or toxicological studies may provide assurance that the new variety is safe as described previously for the host species (section VII.D.). FDA encourages producers of new plant varieties to consult with the agency on testing protocols.

9—Endpoints in Figure 3.

9a—No concerns.

When this endpoint is reached, safety concerns relative to the donor will generally have been satisfied.

9b—New variety not acceptable.

This endpoint is reached when test results indicate that food derived from the new plant variety may be unsafe, e.g., if it contains unacceptable levels of toxicants.

9c—Consult FDA.

Appropriately designed tests may provide evidence that the suspected allergen in the donor was not transferred to the new plant variety, or is not allergenic in the new variety. Producers should consult informally with FDA on protocols that are designed to assess allergenicity. FDA will work with the producer on a case-by-case basis to address requirements such as labeling.

F. Substances Introduced Into the Host Plant From the Donor(s)

Safety assessment should address the specific risks associated with the new substances introduced from the donor(s) to a degree that is consistent with currently accepted scientific practices.

1. Proteins

Depending upon the circumstances, safety assessment of an introduced protein should be based on:

1. Presence and level in the food product.
2. Origin.
3. Known or suspected allergenicity.
4. Evidence of consumption in other foods at similar levels and under similar conditions of processing (e.g., eaten cooked or uncooked).
5. Effects of processing (e.g., cooking).
6. Biological function.
7. Known or potential toxicity.
8. Chemical differences and similarities to edible proteins.
9. The presence of host-specific posttranslational modifications.

Figure 4

The numbers above each box in the flow chart refer to accompanying notes that immediately follow the flow chart.

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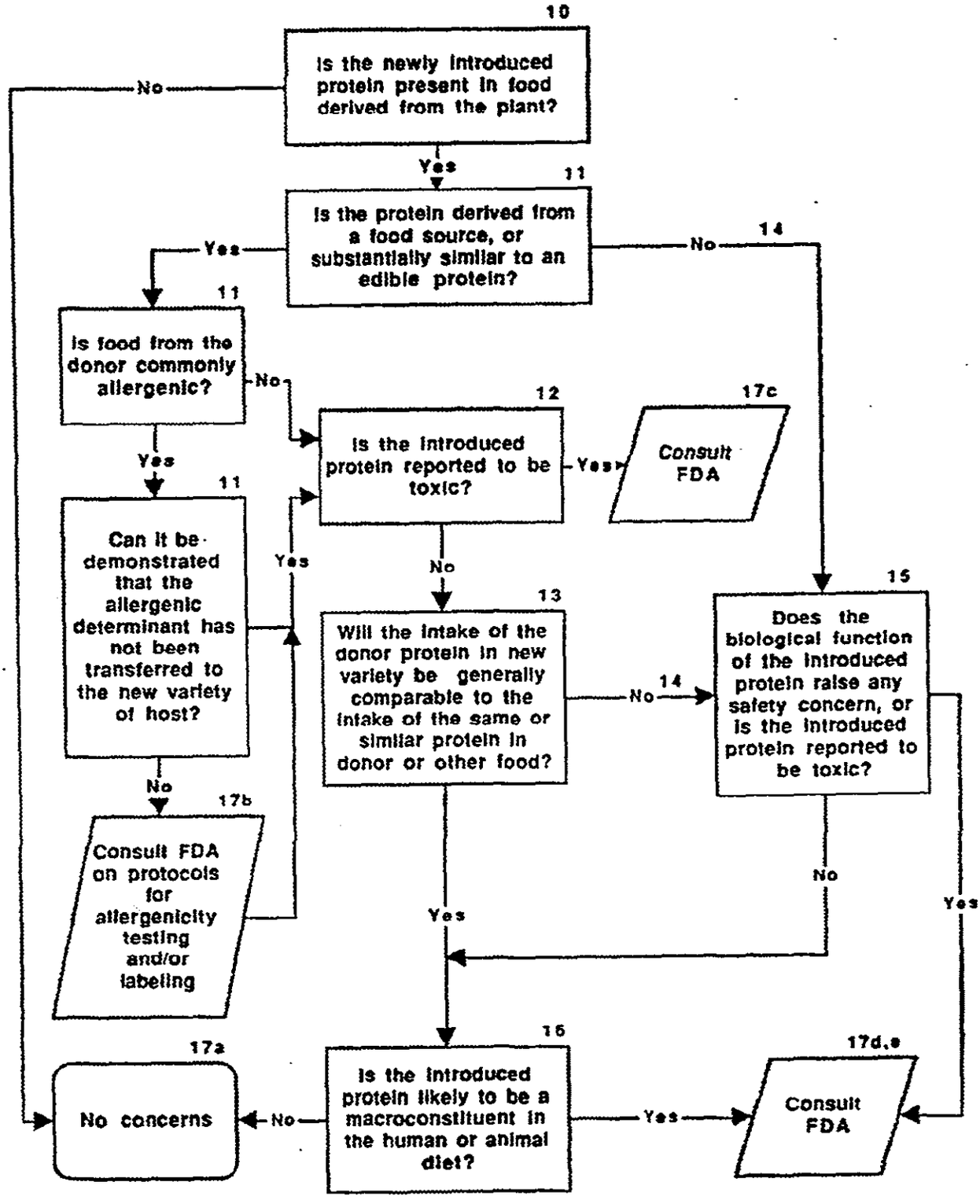

Figure 4. Safety Assessment of New Varieties: Proteins Introduced from Donor(s)

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Notes to Figure 4

10—Is the newly introduced protein present in food derived from the plant?

For example, an enzyme introduced to alter the fatty acid composition of an oil may be removed from the oil as a result of processing. Alternatively, an enzyme introduced to confer antibiotic resistance for use as a selectable marker may be present in food products.

11—If an introduced protein is derived from a food source, the question of allergenicity must be addressed in the same fashion as was discussed from the perspective of the donor as a whole.

12—Is the introduced protein that is derived from a food source, or is substantially similar to an edible protein, reported to be toxic?

For example, some lectins are toxic unless inactivated by cooking. If a protein whose safety is dependent on processing such as cooking has been transferred from a species that is commonly cooked before consumption to a species that may be eaten raw, safety questions may arise.

13—If the intake of an introduced protein that is derived from a food source, or that is substantially similar to an edible protein, is not generally comparable to the intake of the same or similar protein in the donor or other food, the biological function of the protein should be assessed.

14—The biological function of the introduced protein should be assessed if either of the following occur:

a. The introduced protein is not derived from a food source, or is not substantially similar to an edible protein; [6]

b. The intake of the introduced protein in the new variety is not comparable to the intake of the same or similar protein in the donor or other food.

15—Does the biological function of the introduced protein raise any safety concerns, or is the introduced protein reported to be toxic?

In general, proteins that function as enzymes do not raise concern [7] Exceptions include enzymes that produce substances that are not ordinarily digested and metabolized by vertebrates, or that produce toxic substances (e.g., the enzymes that convert cyanogenic glycosides to cyanide).

Other functions that could raise concern include any reported toxicity, such as known toxic activity toward vertebrates, known toxic activity toward nonvertebrates when the absence of toxic activity to vertebrates is not established, and unusual properties that indicate that the protein is significantly different from other proteins found in the diet. If the function of the protein is not known, see note 17d.

16—Is the introduced protein likely to be a macroconstituent in the human or animal diet?

From a nutritional standpoint, the amount and quality of total protein in the diet, rather than of any particular protein, is of greatest significance. However, while most individual proteins (e.g., enzymes) that might be introduced into food derived from plants will be present at relatively low concentrations, some proteins (e.g., seed storage proteins) [8] may become macroconstituents of the plant-derived food. Other proteins (e.g., enzymes used as selectable marker genes) may be introduced into many plants and therefore be consumed at a substantial level. Dietary exposure to such proteins should be considered.

17—Endpoints in Figure 4.

17a—No concerns.

When this endpoint is reached, safety concerns relative to intentionally introduced proteins will generally have been satisfied.

17b—Consult FDA: Allergens.

Producers should consult informally with FDA on protocols that are designed to assess allergenicity. FDA will work with the producer on a case-by-case basis to address requirements such as labeling.

17c—Consult FDA: Toxicity.

Producers should consult informally with FDA when a protein is reported to be toxic or when the safety of an introduced protein is dependent on processing such as cooking. FDA will determine on a case-by-case basis whether it will review the food additive status of these proteins, or whether the proteins are unacceptable in the new plant variety.

17d—Consult FDA: Function and toxicity.

Producers should consult informally with FDA on scientific issues and design of appropriate test protocols when the function of the protein raises concern or is not known, or the protein is reported to be toxic. FDA will determine on a case-by-case basis whether it will review the food additive status of these proteins.

17e—Consult FDA: Macroconstituents in the diet.

Producers should consult informally with FDA when a protein is expected to become a macroconstituent of the diet, whether as a result of its presence in high levels in one food or as a result of its use in many foods. FDA will determine on a case-by-case basis whether it will review the food additive status of these proteins.

2. Carbohydrates

Safety assessment of a new or modified carbohydrate should be based on the nature of the carbohydrate or modification.

Figure 5

The numbers above each box in the flow chart refer to accompanying notes that immediately follow the flow chart.

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[6] The issue of potential allergenicity of any new protein (as opposed to the allergenicity of a protein derived from a known source of allergens) is frequently raised. FDA recognizes that routine procedures for testing foods derived from new plant varieties for the presence of unknown allergens are not currently available. If the donor has no history of use in food, the issue of allergenicity cannot be addressed at this time. Comparison of gene sequences to data banks of known allergens may become increasingly useful as the information on such proteins expands. FDA invites comments on methods that may be available to address the issue of allergenicity of new proteins in foods.

[7] Pariza and Foster (Ref. 7) note that very few toxic agents have enzymatic properties. Exceptions include diphtheria toxin and certain enzymes in the venom of poisonous snakes.

[8] The nutritional content of seed storage proteins from some crops is particularly important in the case of animal feed, where one crop may furnish a substantial portion of the diet.

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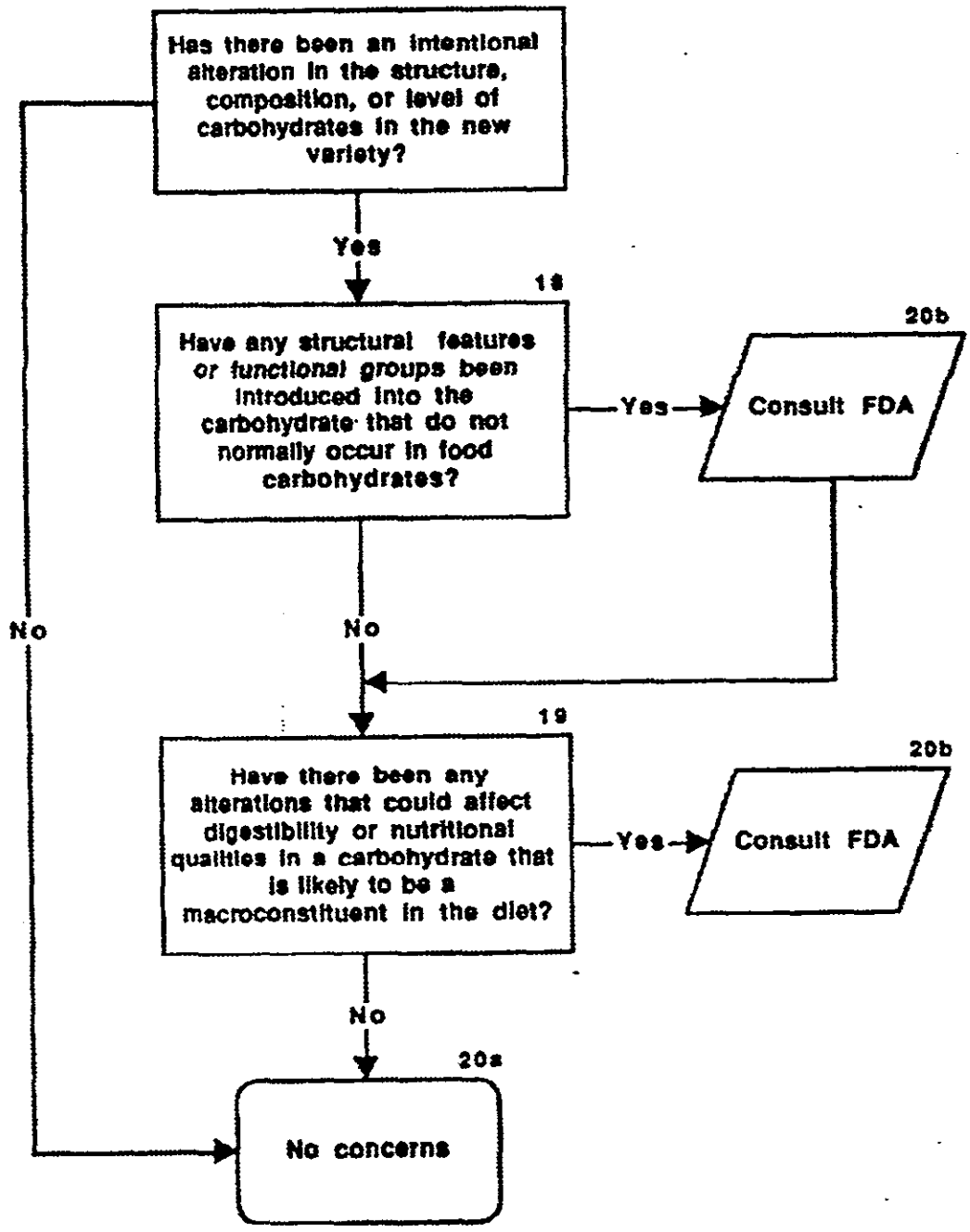

Figure 5. Safety Assessment of New Varieties: New or Modified Carbohydrates

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Notes to Figure 5

18—Have any structural features or functional groups been introduced into the carbohydrate that do not normally occur in food carbohydrates?

For example, developments that affect carbohydrates will frequently be modifications of food starches, presumably affecting the content of amylose and amylopectin, as well as the branching of amylopectin. Such modified starches are likely to be functionally and physiologically equivalent to starches commonly found in food and thus would not suggest any specific safety concerns. However, if functional groups or structural features that normally do not occur in food carbohydrates are introduced, such modifications should be evaluated with respect to any safety concerns that may arise.

19—Have there been any alterations that could affect digestibility or nutritional qualities in a carbohydrate that is likely to be a macroconstituent in the diet?

If a vegetable or a fruit is modified to produce high levels of an indigestible carbohydrate that normally occurs at very low levels, or to convert a normally digestible carbohydrate to an indigestible form, nutritional questions may arise.

20—Endpoints in Figure 5.

20a—No concerns.

When this endpoint is reached, safety and nutritional concerns relative to intentional modifications of food carbohydrates will generally have been satisfied.

20b—Consult FDA.

Producers may consult informally with FDA on scientific issues. FDA will determine on a case-by-case basis whether it will review the food additive status of these carbohydrates, and will work with the sponsor on a case-by-case basis to address requirements such as labeling.

3. Fats and Oils

Safety assessment of a new or modified fat or oil should be based on its composition and the presence of any unusual components at levels that would cause safety concern.

Figure 6

The numbers above each box in the flow chart refer to accompanying notes that immediately follow the flow chart.

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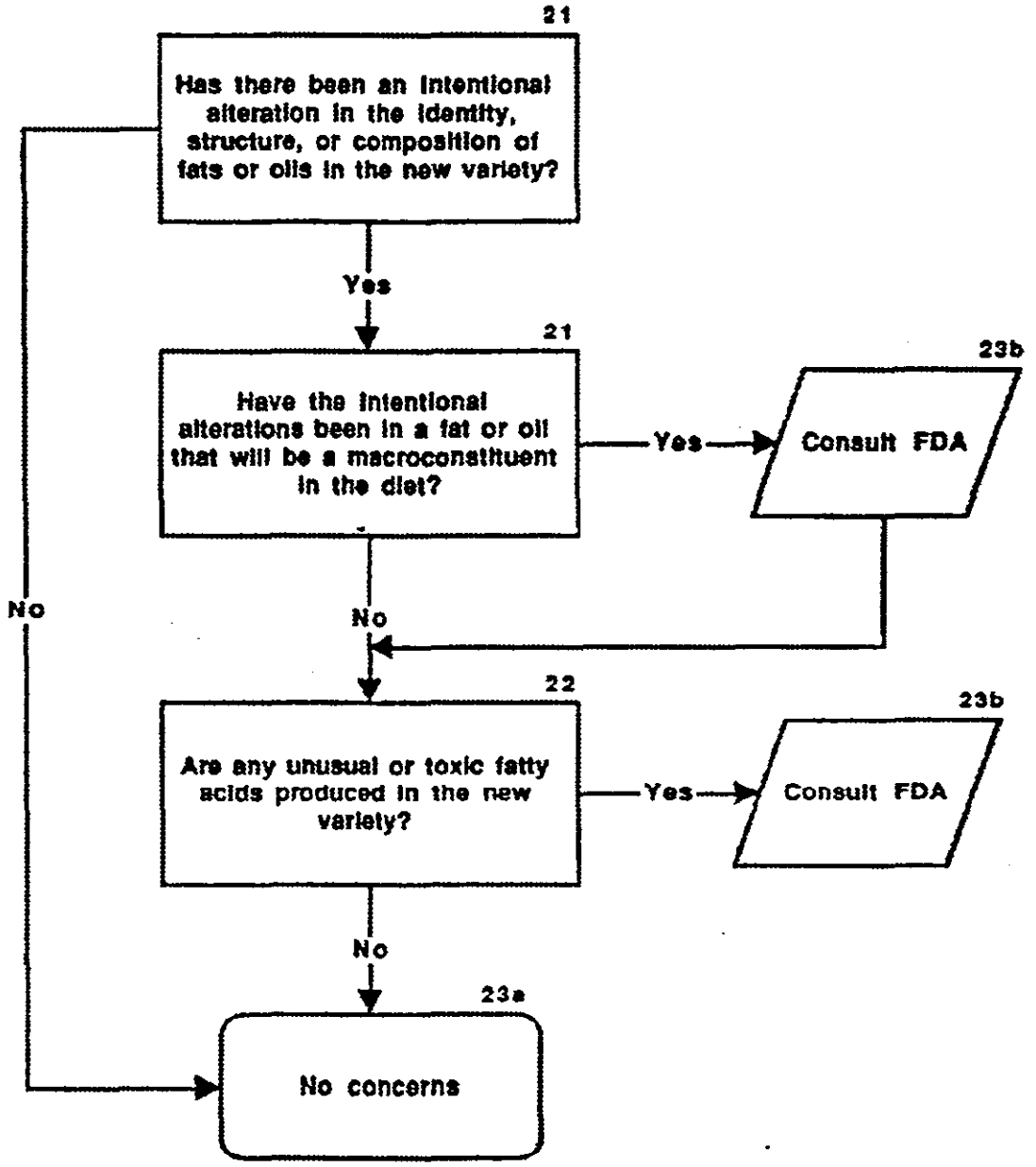

Figure 6. Safety Assessment of New Varieties: New or Modified Fats or Oils

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Notes to Figure 6

21—Has there been an intentional alteration in the identity, structure, or composition of fats or oils that are likely to be a macroconstituent in the diet?

Some alterations in the composition or structure of fats and oils, such as an alteration in the ratio of saturated to unsaturated fatty acids, may have significant nutritional consequences, or result in marked changes in digestibility. Other changes may produce a fat or oil that has been altered such that it is no longer representative of fats and oils from the host species.

22—Are any unusual or toxic fatty acids produced in the new variety?

For example, safety questions may arise as a result of the presence of fatty acids with chain length greater than C-22, fatty acids with cyclic substituents, fatty acids with functional groups not normally present in dietary fats and oils, and fatty acids of known toxicity (e.g., erucic acid).

23—Endpoints in Figure 6.

23a—No concerns.

When this endpoint is reached, safety and nutritional concerns relative to intentional modifications of fats and oils will generally have been satisfied.

23b—Consult FDA.

Producers may consult informally with FDA on scientific issues. FDA will determine on a case-by-case basis whether it will review the food additive status of these fats or oils, and will work with the sponsor on a case-by-case basis to address requirements such as labeling.

G. Toxicology

Feeding studies or other toxicological tests may be warranted when the characteristics of the plant or the nature of the modification raise safety concerns that cannot be resolved by analytical methods. FDA recognizes that feeding studies on whole foods have limited sensitivity because of the inability to administer exaggerated doses. Because of the difficulty of designing meaningful studies, FDA encourages companies to consult informally with the agency about test protocols.

H. Other Information

The information described below is not directly addressed in the flow charts but should be considered during the development of new plant varieties.

1. Nucleic Acids

Introduced nucleic acids, in and of themselves, do not raise safety concerns. Thus, for example, the introduction of a gene encoding an anti-sense ribonucleic acid (RNA) would not raise concerns about either the gene or the anti-sense RNA. Any safety considerations would focus on the intended effects of the anti-sense RNA. Hence, continuing the example, if the anti-sense RNA were used to suppress an enzyme, then just as for any other method intended to suppress an enzyme, such as deletion or nonsense mutations, the metabolic effects on the host plant of such enzyme suppression should be considered at the conceptual stage of development and monitored, when appropriate and feasible.

2. Metabolic Considerations

The effects of an intentional alteration of a biochemical pathway should be considered at the conceptual stage of development, and monitored when appropriate and feasible. For example, are there any toxic effects of a metabolic imbalance with respect to enzyme substrate depletion and product accumulation? Are any auxiliary pathways likely to be affected?

3. Stability

The genetic stability of the new plant variety and the inheritance of the introduced genetic material as a single Mendelian trait are important safety considerations. A safety assessment of food derived from early generations of the new variety may not be valid if the new genetic material is expressed at substantially different levels in subsequent generations. Factors that favor stability include a minimum number of copies of the introduced genetic material, and insertion at a single site.

I. Future Workshop on Scientific Issues

FDA recognizes the desirability of establishing consensus within the industry, the scientific community, and the public on the agency's scientific assessment approach to food safety presented in this guidance section. For this reason, FDA plans to announce, in a future **Federal Register** notice, a workshop to discuss specific scientific issues. The notice announcing the workshop will include a description of the scientific issues to be discussed. FDA invites comment on topics that might be addressed at such a workshop.

VIII. Environmental Consideration: Applicability of NEPA

NEPA requires FDA to consider in its decisionmaking the environmental impact of its major Federal actions that significantly affect the quality of the human environment. The promulgation of a food additive regulation is an agency action that ordinarily triggers the NEPA requirement for development of an environmental assessment (21 CFR 25.22(a)(10)) and, if the agency does not make a finding of no significant environmental impact, an environmental impact statement is prepared (21 CFR 25.21(b)).

The Council on Environmental Quality (CEQ) regulations (40 CFR 1500 through 1508) provide that in complying with NEPA, an agency should avoid unnecessary duplication and should tier its NEPA statements with those of other agencies to eliminate repetitive discussions of the same issues and to focus on the actual issues ripe for decision at each level of environmental review (40 CFR 1502.20 and 1508.28).

Other agencies, particularly USDA and EPA, may prepare NEPA and other environmental documentation before products are presented to FDA for a decision. FDA intends to rely on such documentation to the maximum extent possible.

Under regulations administered by the Animal and Plant Health Inspection Service (APHIS) in USDA (7 CFR part 340), the majority of plants developed by recombinant DNA techniques that are being commercially developed have been considered "regulated articles." The action that results in a permit for introduction of a regulated article into the environment is subject to NEPA review. At some stage of research and development of a regulated article, an interested party will request from APHIS a determination of the article's regulatory status. APHIS has informed FDA that when APHIS receives a petition or other request it intends to consult with other agencies. This should enable FDA to identify the type of data that would be useful if any subsequent environmental review is to be prepared for actions under FDA jurisdiction.

EPA has authority, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 *et seq.*), to regulate all pesticides, no matter how they are made or their mode of action. Under the act, EPA has authority to regulate pesticide residues in foods. Any relevant review that EPA conducts under FIFRA, the act, or any other of its statutes, involving an assessment of potential effects on human health and the environment will be available to FDA.

FDA intends to work closely with USDA and EPA to minimize duplication of environmental reviews. The agency will, to the extent possible, invoke the tiering provisions in the CEQ regulations and, in FDA's environmental assessments, rely on APHIS NEPA reviews and other such documents, as well as relevant environmental documents considered by EPA. Further,

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FDA will provide informal guidance on environmental issues to assist individuals who are preparing food additive petitions to meet FDA's requirements for environmental assessments.

FDA does not consider that the activities it may undertake with respect to foods from new plant varieties other than promulgation of food additive regulations, such as consultation with producers on safety issues and providing advice on the regulatory status of foods from new plant varieties, will constitute agency action under NEPA.

IX. Coordination With EPA: Pesticide Considerations

Questions have been raised concerning whether FDA or EPA would have jurisdiction when plants are modified to express pesticidal substances. FDA and EPA are agreed that substances that are pesticides as defined by FIFRA (7 U.S.C. section 136(u)), are subject to EPA's regulatory authority. The agencies also agree that FDA's authority under the act extends to any nonpesticide substance that may be introduced into a new plant variety and that is expected to become a component of food.

EPA and FDA are aware that there may be cases in which the jurisdictional responsibility for a substance is not clear. Because pesticides, as defined by FIFRA, are subject to EPA's jurisdiction, the agencies encourage producers who have such questions to contact EPA. FDA and EPA intend to consult closely on such jurisdictional questions, as well as on scientific matters where consultation will be helpful in resolving safety questions.

The agencies are also aware that, in some circumstances, evaluation of a particular substance introduced into a plant may require the expertise of both EPA and FDA. Both agencies agree that EPA will address under its regulatory jurisdiction the food safety issues associated with the pesticide, including marker genes used to confirm the presence of the pesticidal gene. Any food safety questions beyond those associated with the pesticide, such as those raised by unexpected or unintended compositional changes, are under FDA's jurisdiction and should be addressed under the policy set forth elsewhere in this notice.

Based upon the agencies' current knowledge, examples of substances that fall under FDA's authority include: (1) Substances intended to alter the nutritional composition of the food (e.g., amino acids or carbohydrates); (2) substances intended to enhance the plant's resistance to chemical herbicides (e.g., bromoxynil, glyphosate, and sulfonylurea); and (3) substances intended to alter the flavor or the texture of the food.

Similarly, based upon the agencies' current knowledge of new plant varieties being developed using the new technologies of gene transfer, EPA is in the process of evaluating how or if it will exert its oversight for the following examples subject to its jurisdiction under FIFRA and therefore not under FDA's jurisdiction: (1) Substances that are intended to kill insects (e.g., *Bacillus thuringiensis* delta-endotoxin);

(2) Substances intended to protect plants from viral, fungal, or bacterial infection (e.g., cecropin); and (3) substances that are plant regulators and thus "pesticides" under FIFRA.

X. Environmental Impact

The agency has determined under 21 CFR 25.24(a)(8) that this action is of a type that does not individually or cumulatively have a significant effect on the human environment. Therefore, neither an environmental assessment nor an environmental impact statement is required.

This action is intended to provide guidance to developers by describing the scientific considerations for the safe development of foods derived from new plant varieties.

XI. Comments

Interested persons may, on or before August 27, 1992, submit to the Dockets Management Branch (address above) written comments regarding this notice. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

XII. References

The following references have been placed on display in the Dockets Management Branch (address above) and may be seen by interested persons between 9 a.m. and 4 p.m., Monday through Friday.

1. Anonymous, "Biotechnologies and Food: Assuring the Safety of Foods Produced by Genetic Modification," International Food Biotechnology Council, Regulatory Toxicology and Pharmacology, Vol. 12, No. 3, Part 2 of 2 Parts, New York, December 1990.

2. Letter, Hopkins, D. D., R. J. Goldburg, and S. A. Hirsch to Dr. David Kessler, September 30, 1991, and enclosure, "A Mutable Feast: Assuring Food Safety in the Era of Genetic Engineering."

3. Letter, Richard D. Godown to James H. Maryanski, January 3, 1992; Letter, W. Douglas Crabb to Fred R. Shank, January 24, 1992.

4. Comments to Docket No. 90A-0418, Federal Register, May 1, 1991 (56 FR 20004).

5. Dale, E. C. and D. W. Ow, "Gene Transfer with Subsequent Removal of the Selection Gene from the Host Genome," Proceedings of the National Academy of Sciences USA, 88:10558–10562, 1991.

6. Anonymous, "Strategies for Assessing the Safety of Foods Produced by Biotechnology," World Health Organization, Geneva, 1991.

7. Pariza, M. W. and E. M. Foster, "Determining the Safety of Enzymes Used in Food Processing," Journal of Food Protection, 46:453–468, 1983.

Dated: April 2, 1992.

David A. Kessler,

Commissioner of Food and Drugs.

[FR Doc. 92-12860 Filed 5-28-92; 3:57 pm]

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