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]

[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

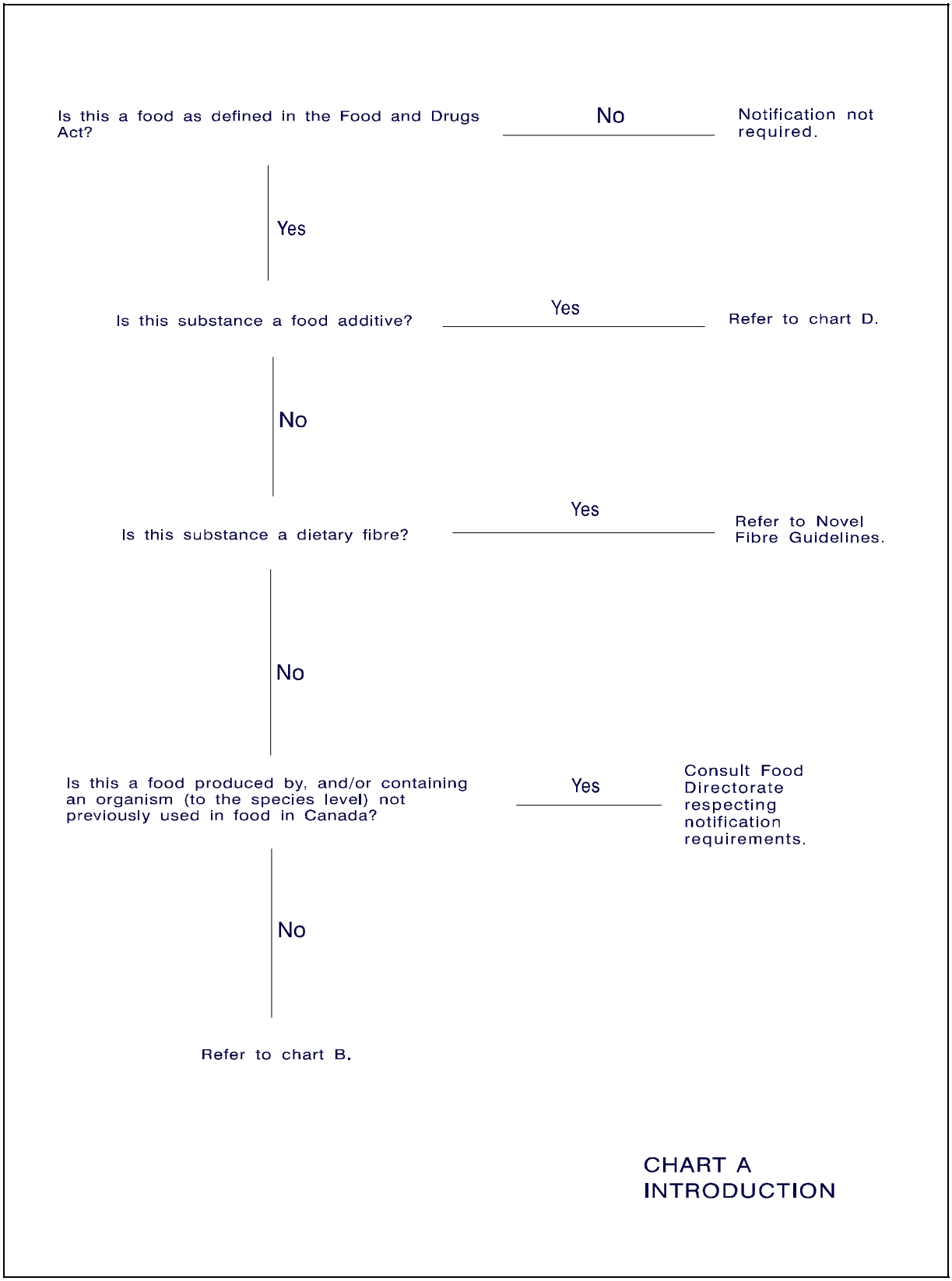

CHART A
INTRODUCTION

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

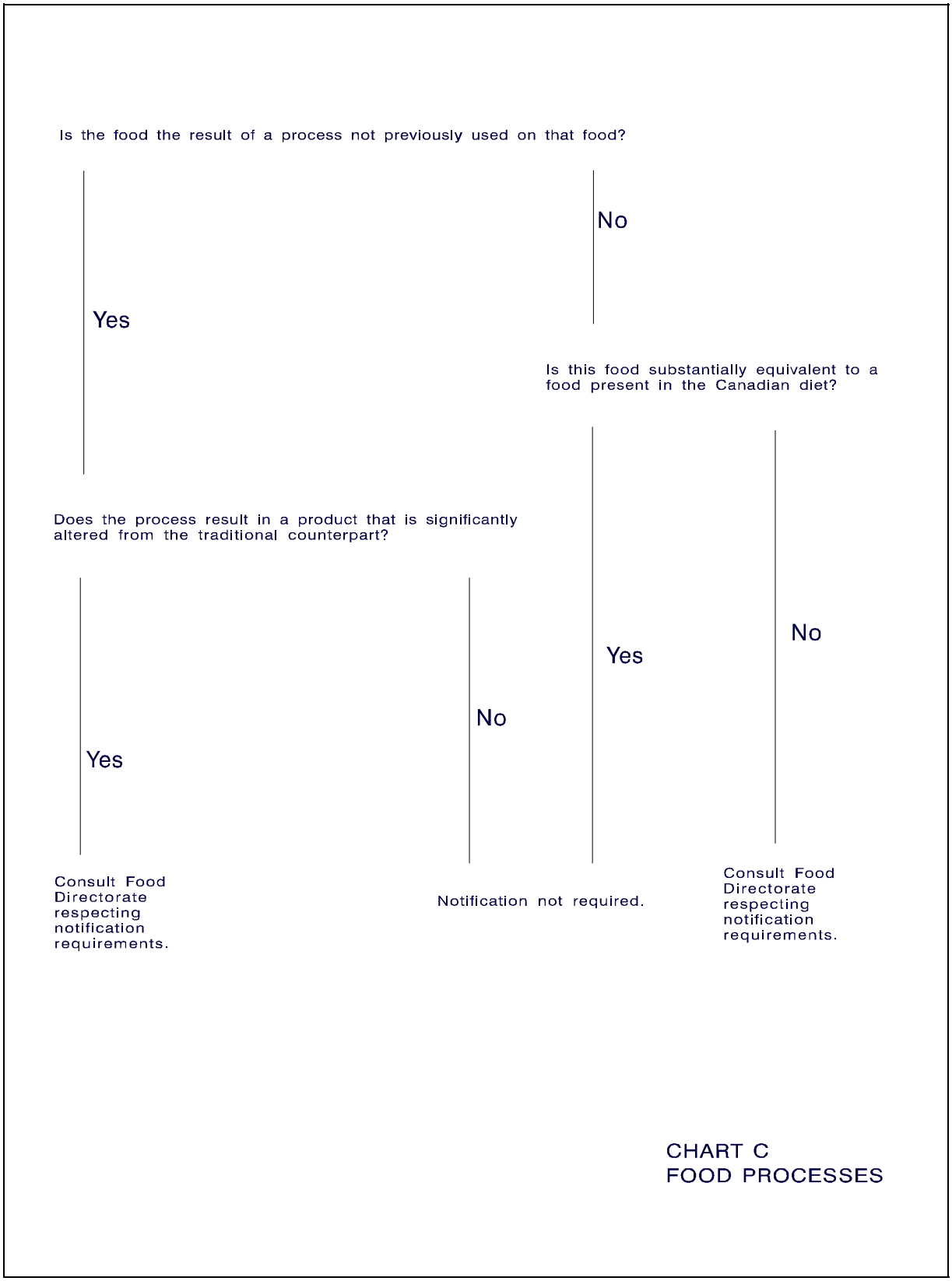

CHART C
FOOD PROCESSES

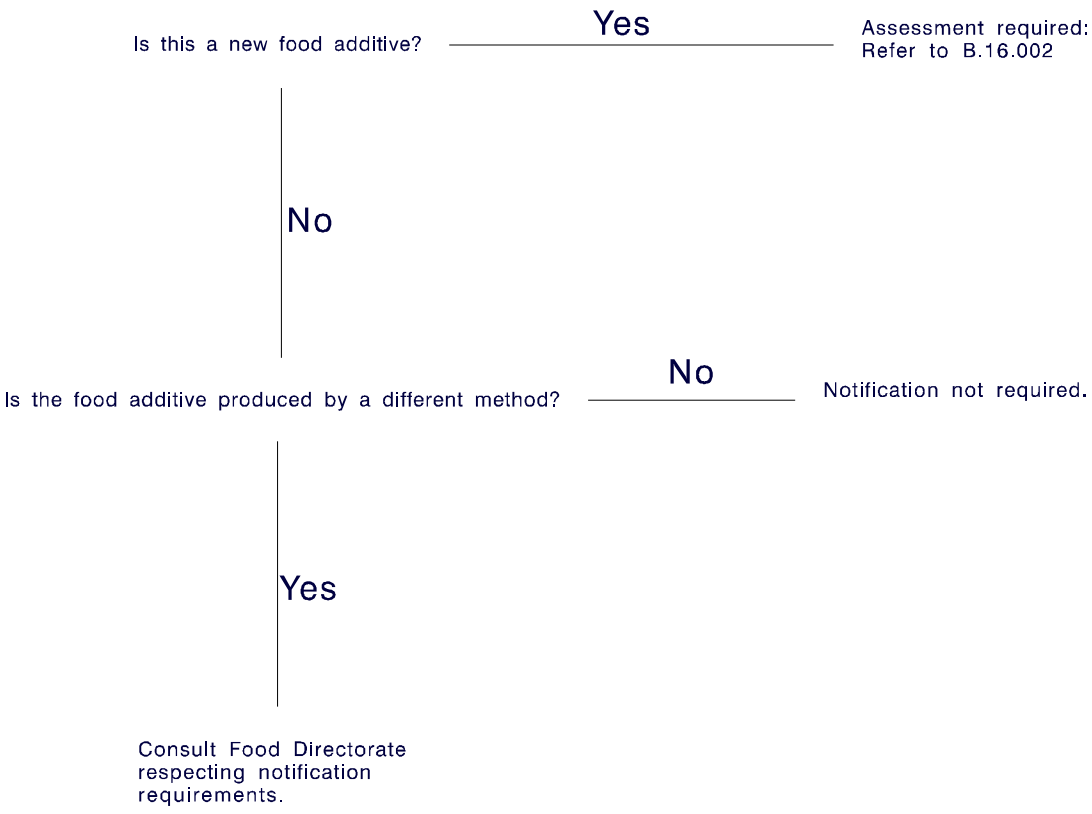

CHART D
FOOD ADDITIVES

GUIDELINES FOR THE SAFETY ASSESSMENT OF NOVEL FOODS

VOLUME II

Genetically Modified Microorganisms and Plants

Food Directorate
Health Protection Branch
Health Canada

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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.