Guidelines for the Safety Assessment of Novel Foods Derived from Plants and Microorganisms (DRAFT)

1. Introduction

1.1 Background

The globalization of the food supply, the demand for more food sources globally, and the rapid advances in food science and technology have resulted in the introduction of foods not previously available in the Canadian marketplace. Novel whole foods and food constituents may result from the importation of new products into Canada, the introduction of a new food source, the use of new processing techniques, and/or changes in the genetic make-up of the microorganisms, plants and animals from which foods are derived.

Advances in transportation technology and lower transportation costs have increased the variety of food and food products imported into Canada. Changing consumer food preferences driven by cultural and ethnic traditions as well as nutritional and health concerns, have also resulted in the diversification of our food supply. In addition, the increasing global population continues to drive the introduction of new food sources worldwide. Foods considered non-traditional in Canada may be widely consumed in other parts of the world. In some cases, adverse effects may be associated with their consumption or traditional methods may be needed to prepare the food prior to consumption. In these situations, consumers need to be informed of potential risks and appropriate preparation techniques. Foods derived from sources not previously used as human foods must be evaluated for safety as they may contain toxins, contaminants and/or anti-nutritional factors.

On a global level, new techniques for food preservation and processing continue to be developed to expand the shelf life of foods and food products, to reduce energy requirements for processing, and for many other purposes. As new processing techniques have the potential to alter the characteristics of a food, including nutritional and any toxic characteristics, human health impacts must be considered.

Genetic modifications to improve the agronomic, production, processing or nutritional characteristic of microorganisms, plants and animals may be achieved through traditional breeding techniques or modern gene technologies. The application of genetic modification through either traditional breeding or genetic engineering is not considered inherently to increase or decrease the risk associated with consuming the organism as a food. However, the wide variety of manipulations possible through genetic modification, and the potential for the introduction of toxic compounds, unexpected secondary effects and changes in the nutritional and toxic characteristics of the foodstuff may give rise to safety concerns.

Health Canada is responsible for establishing standards and policies governing the safety and nutritional quality of all food, including novel foods, sold in Canada. The mechanism by which Health Canada controls the sale of novel foods in Canada is a pre-market notification process specified under Division 28 of the *Food and Drug Regulations*.

The pre-market notification approach used for novel foods entails the submission of information to Health Canada regarding the product in question so that a determination can be made with respect to its acceptability as food prior to sale. Thus petitioners of novel foods must submit data of a sufficiently high calibre to meet the criteria specified by Health Canada.

The safety criteria for the assessment of novel foods outlined in the current document were derived from internationally established scientific principles developed through the Organization for Economic Cooperation and Development (OECD), Food and Agriculture Organisation (FAO), World Health Organisation (WHO) and the *Codex Alimentarius Commission*. These guidelines provide for the flexibility required to determine the need for notification and the safety assessment of the broad range of food products being developed. This flexibility is needed to allow novel foods and food products to be assessed on a case-by-case basis and to take into consideration future scientific advances.

1.2 Purpose of Guidelines

These guidelines define the criteria and basic information requirements that must be considered in assessing the safety of novel whole foods and food constituents. They are intended to provide a basis for dialogue between petitioners and the Health Products and Food Branch (HPFB). These guidelines are not intended to explicitly define all the data that might be required in the course of a safety assessment as further data requirements may be identified during the safety assessment process.

1.3 Scope

This document encompasses all novel whole foods, novel food products, and novel foods used as ingredients that are derived from **plant** and **microbial** sources. Safety assessment criteria for novel foods derived from **animals** are under development and will be available for external consultation in 2004.

Under Section **B.28.001** of the *Food and Drug Regulations*, a “novel food” is defined as follows:

"**novel food**" means

a) a substance, including a microorganism, that does not have a history of safe use as a food;

b) a food that has been manufactured, prepared, preserved or packaged by a process that

(i) has not been previously applied to that food, and

(ii) causes the food to undergo a major change; and

c) a food that is derived from a plant, animal or microorganism that has been genetically modified such that

(i) the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism,

(ii) the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism, or

(iii) one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism.

"genetically modify" means to change the heritable traits of a plant, animal or microorganism by means of intentional manipulation.

"major change" means, in respect of a food, a change in the food that, based on the manufacturer's experience or generally accepted nutritional or food science theory, places the modified food outside the accepted limits of natural variations for that food with regard to:

(a) the composition, structure or nutritional quality of the food or its generally recognized physiological effects;
(b) the manner in which the food is metabolized in the body; or
(c) the microbiological safety, the chemical safety or the safe use of the food.

2. Notification Procedure

2.1 Submission of a Novel Food Notification

Notifying Health Canada regarding the sale or advertisement for sale of a novel food may involve a one or two step process. In the first step, the manufacturer or importer of the novel food must notify the HPFB in writing of their intention to sell or advertise a novel food pursuant to section B.28.002 of the *Food and Drug Regulations*.

The notification package (4 copies) should provide the following as indicated in B.28.002 (2):

eferred to in paragraph (1)(a) shall be signed by the manufacturer or importer, or a person authorized to sign on behalf of the manufacturer or importer, and shall include the following information:

a) the common name under which the novel food will be sold;

b) the name and address of the principal place of business of the manufacturer and, if the address is outside Canada, the name and address of the principal place of business of the importer;

c) a description of the novel food, together with

i) information respecting its development,

ii) details of the method by which it is manufactured, prepared, preserved, packaged and stored,

iii) details of the major change, if any,

iv) information respecting its intended use and directions for its preparation,

v) information respecting its history of use as a food in a country other than Canada, if applicable, and

vi) information relied on to establish that the novel food is safe for consumption;

d) information respecting the estimated levels of consumption by consumers of the novel food;

e) the text of all labels to be used in connection with the novel food; and

f) the name and title of the person who signed the notification and the date of signing.

Upon receipt of the notification, a letter of acknowledgement in which the file number for the product is indicated, will be sent to the petitioner. This number, along with pertinent dates, should be used in all subsequent correspondence.

As stated in B.28.003, within 45 days of receiving this notification, HPFB will review the notification and provide in writing either no objection to the sale of the novel food for

consumption or a request that additional scientific data be submitted in order to assess the safety of the novel food.

If additional information is requested, the manufacturer or importer will be required to submit data for assessment. On the basis of the submitted safety data, HPFB will decide if the novel food is suitable for consumption. Manufacturers and importers of novel foods are at liberty to submit the scientific data necessary for the full safety assessment along with the basic information outlined in the first step of the notification.

It is important to note that, under B.28.002 and B.28.003, no person shall sell or advertise for sale a novel food unless the manufacturer or importer of the novel food has:

> (a) notified the Director (the Assistant Deputy Minister of Health Products and Food Branch) of their intention to sell or advertise for sale the novel food; and
>
> (b) received a letter indicating that the information submitted in support of the safety of the novel food for consumption is sufficient to permit the sale of the novel food in Canada (a letter of no objection).

2.2 Submission of a Safety Assessment Data Package

If the information provided in the notification for a novel food is not considered adequate to determine the novel food's safety, additional data supporting the safety of the food will be required**.** The type of information required to conduct the safety assessment of a novel food will depend on a number of factors such as the nature of the food, processing methods and the intended use. The approaches used to assess the safety of novel foods are outlined in these guidelines. However, the types of studies considered appropriate to demonstrate the safety of a novel food change with scientific knowledge and development. These guidelines are expected to be used in conjunction with information available in the scientific literature and from research and development conducted by the manufacturer.

Since novel foods represent a diverse range of products, not all data requirements outlined in this document will be appropriate for a specific submission. Petitioners should consider the novel characteristics of the product when addressing the criteria in these guidelines. Consultation with the Food Directorate in HPFB is encouraged during the development phase of a product to determine the data necessary to demonstrate the safety of the product. In addition, waiving of certain data requirements will be considered when accompanied by a sound scientific rationale.

The Regulations make it the responsibility of the manufacturer of a novel food to comply with requirements and to provide a full disclosure of the results of all studies undertaken and completed to support the safety of the novel food.

Within 90 days of receiving the safety assessment package, HPFB will review the data and provide in writing either a notice of no objection to the sale of the novel food for consumption or a request for additional scientific data to clarify outstanding issues.

2.3 When to apply

Written notification should be provided well in advance of the period when the manufacturer intends to market the product. Health Canada is obligated to respond regarding its acceptability for sale or whether further information is required for assessment within 45 days of receiving the notification.

2.4 Where to apply

Officially, manufacturers and importers are required to notify the Assistant Deputy Minister of the Health Products and Food Branch (HPFB). However, the Novel Foods Section has been established in the Food Directorate of HPFB to coordinate the safety evaluation of novel foods intended for human consumption in Canada. The notification and/or submission package should be addressed to:

Novel Foods Section
Food Directorate
Health Products and Food Branch
Health Canada
4th Floor West, Sir Frederick G. Banting Research Centre
Tunney's Pasture, Postal Locator 2204A1
Ottawa, Ontario. K1A 0L2

2.5 Standard Operating Procedure

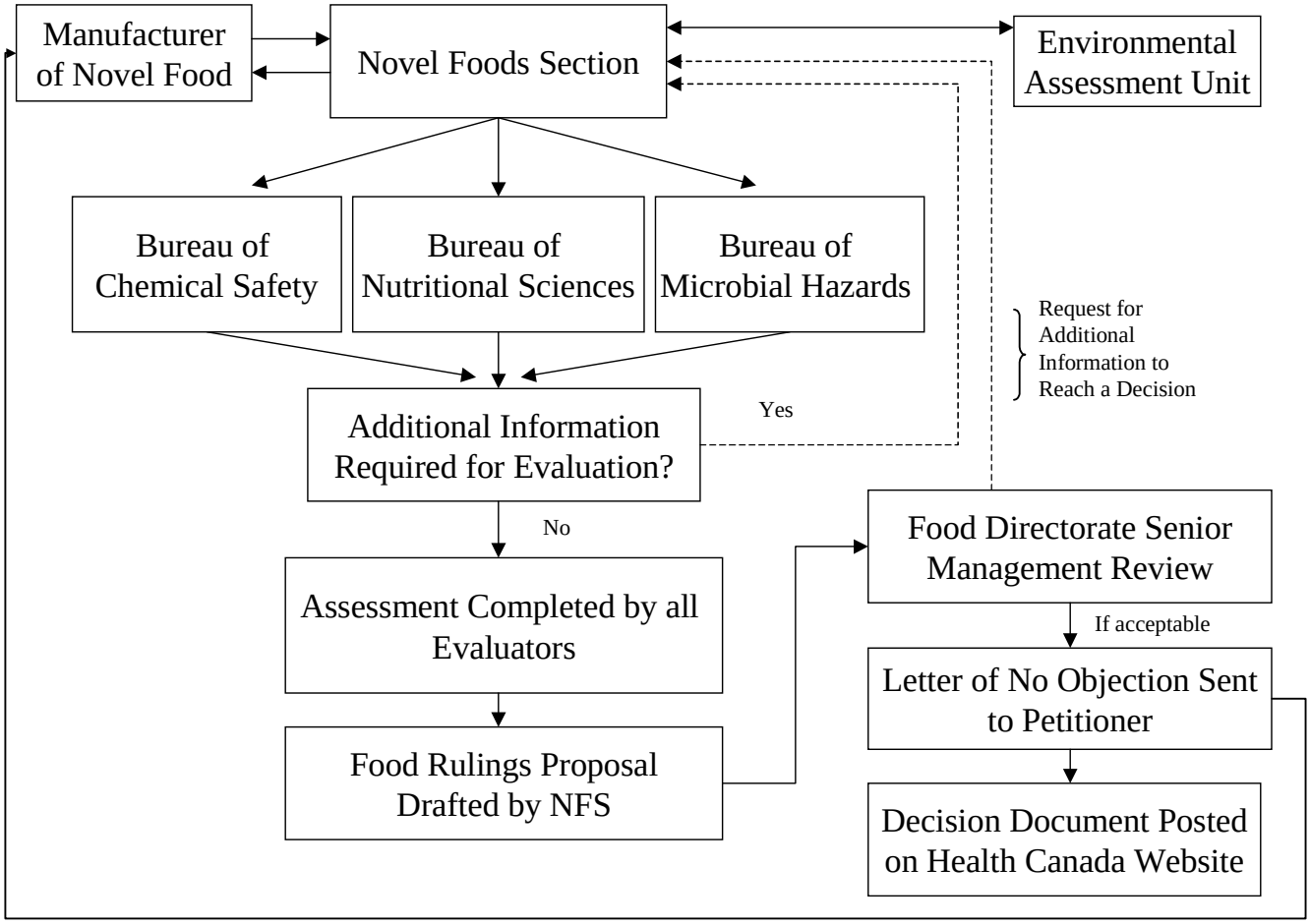
As the coordinating office, the Novel Foods Section (formerly Office of Food Biotechnology) is responsible for communicating with petitioners, receiving novel foods notifications and submission material and initiating the review process outlined in figure 1. The Novel Foods Section distributes the submission material to relevant Food Directorate bureaux, namely the Bureau of Chemical Safety, the Bureau of Nutritional Sciences, and the Bureau of Microbial Hazards for their respective reviews. In some cases, the Environmental Assessment Unit, Healthy Environments and Consumer Safety Branch will conduct environmental assessments of novel foods under proposed *Environmental Assessment Regulations* (EA Unit - see section 3.1). Evaluators have a period of 45 days to review a notification and 90 days to conduct a safety

assessment of a submission package as outlined in the regulation. All requests for additional information by evaluators are communicated through the Novel Foods Section which creates a single window approach to submission reviews. Any request for information resets the 90 day assessment time to allow for the review of the additional information once it is received from the petitioner. Submission of unsolicited additional data by a petitioner may also reset the 90 day review period.

At the completion of the safety assessment, if and only if all members of the evaluation team agree there are no health risks associated with the consumption of the novel food product in question, a proposal is drafted which contains a summary of the scientific reviews conducted by the relevant bureaux of the Food Directorate. This proposal is presented to the Food Rulings Committee consisting of Food Directorate senior management and representatives from other agencies or departments within the Canadian government. If found acceptable by the Committee, the petitioner is notified in writing by the Director General of the Food Directorate that, based on the evaluation of the submitted data, Health Canada has no objection to the sale of the novel food product as human food in Canada as specified in the letter.

Novel food decisions and summary documents are made available on the Health Canada website for all products for which Health Canada has issued a letter of no objection to the use as food in Canada (http://www.hc-sc.gc.ca/food-aliment).

Figure 1. Processing a novel food notification/submission in the Food Directorate.



3. Other Regulatory Considerations

3.1 Environmental Impact

Health Canada is in the process of developing Environmental Assessment Regulations for products regulated under the *Food and Drugs Act*, including novel foods. Until these new regulations are developed, information on the novel food's potential environmental and indirect human health impact will be required pursuant to the New Substances Notification Regulations under the *Canadian Environmental Protection Act* (CEPA).

Products that are regulated under other federal statutes listed in a CEPA schedule, such as the *Seeds Act* and the *Feeds Act* are exempted from regulation under CEPA. Therefore, if a novel food is derived from a plant for which an application has been submitted to the Canadian Food Inspection Agency (CFIA) for unconfined environmental release or for use as animal feed, this should be stated in the application to Health Canada.

A guidance document on current New Substances Notification Requirements for products regulated under the *Food and Drugs Act* is available on Health Canada's website at www.hc-sc.gc/ear-ree or upon request at 1-888-492-1104.

3.2 Plants with Novel Traits and Novel Feeds

The CFIA is responsible for the regulation of plants with novel traits to be cultivated in Canada. Under the *Seeds Act*, a new variety of a cultivated species that possesses a novel trait would be subject to Regulatory Directive Dir94-08 (*Assessment Criteria for Determinating Environmental Safety of Plants with Novel Traits*). More information on the regulations of plants with novel traits (PNT) is available through the CFIA's Plant Biosafety Office (phone number) or their website: (http://www.inspection.gc.ca/english/plaveg/pbo/pbobbve.shtml).

The Feed Section of the CFIA administers a national livestock feed program, under the authority of the Feeds Act and Regulations, to verify that livestock feeds, including novel feeds, manufactured or sold in Canada are safe, efficacious and labelled properly. Novel feeds consist of organisms or parts of products thereof that have not been evaluated and approved for use as livestock feed in Canada. Novel feeds may be from plant sources, including PNTs, that may be used as feed must be assessed by the Feed Section prior to their use as a livestock feed. More information on the regulation of novel feeds from plant sources is available at www.inspection.gc.ca/english/anima/feebet/bfeebete.shtml. Please refer to the *Guidelines for the Assessment of Novel Feeds: Plant Sources* for data requirements for a novel feed submission.

Livestock feed is an outlet for by-products and residual material of the food processing industry. By-products of foods derived from novel microorganisms must be assessed by the Feed Section prior to their incorporation into livestock feed. The draft *Guidelines for the Safety Assessment of Novel Feeds: Microbial Products* can be obtained by contacting the Feed Section of the CFIA.

3.3 Harmonization of Regulatory Approvals for Novel Foods and Novel Feeds derived from Plants with Novel Traits

Health Canada and the CFIA conduct interdepartmental consultations in order to coordinate the granting of their respective approvals to minimize the potential for unapproved food products to enter the Canadian marketplace. This approach will continue through a formalized process which will ensure the approvals of plants with novel traits are granted in a harmonized fashion.

Where products are intended for exclusive use as one of either food, feed or molecular farming (use of plants to produce industrial or therapeutic products), consultations among regulatory authorities will be required to assess any potential risks associated with release of the product in an unintended commodity stream. For these products, an identity preservation system or alternative will be essential to minimize the likelihood of such an event.

3.4 Post-Market Information

If the Department establishes that there is no objection to the sale of a novel food for human consumption, it will be permitted to enter the marketplace in the same manner as traditional food products and therefore subjected to the same post-market standards applicable to all foods in Canada. It remains the responsibility of a company to ensure that its products are in compliance with all applicable statutory and regulatory requirements.

At the current pace of technological advancement, it is expected that new information on previously approved products will be identified on occasion. Any post-market information obtained, which has potential health and safety implications, should be forwarded to Health Canada for consideration in order to ensure the continued safety and integrity of all novel foods available in the Canadian marketplace. The sale of a food that poses a hazard to the health of the consumer contravenes the provisions of the *Food and Drugs Act*.

Future novel food products may be composed of significantly different nutrient combinations or other novel food characteristics not previously encountered in the food supply. These foods may require post market monitoring to address potential long term health effects. In such cases, post market information may be a valid approach to include in the assessment of the overall safety of some products.

4. Information Requirements for Safety Assessment

The approach taken for the safety assessment of novel foods is based on the evaluation of these foods relative to conventional counterparts that have a history of safe use. This approach takes both intended and unintended effects into account. The intention is to identify new or altered hazards relative to the conventional counterpart. If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, it would be assessed to determine its relevance to human health. Following the safety assessment and, if necessary, further risk assessment, the food or component of food would be subjected to risk management considerations before it is considered for commercial distribution. Where no conventional counterpart exists for comparison, the safety of a novel food must be evaluated from data derived directly from historical experience or experimental studies with the food.

The safety assessment of novel foods follows a stepwise process of addressing relevant factors that include:

- History of use
- Dietary exposure
- Detail of novel process (if applicable)
- History of organism(s)
- Characterization of derived line/strain (if applicable)
- Genetic modification considerations (if applicable)
- Nutritional considerations
- Toxicology considerations
- Allergenicity considerations
- Chemical considerations

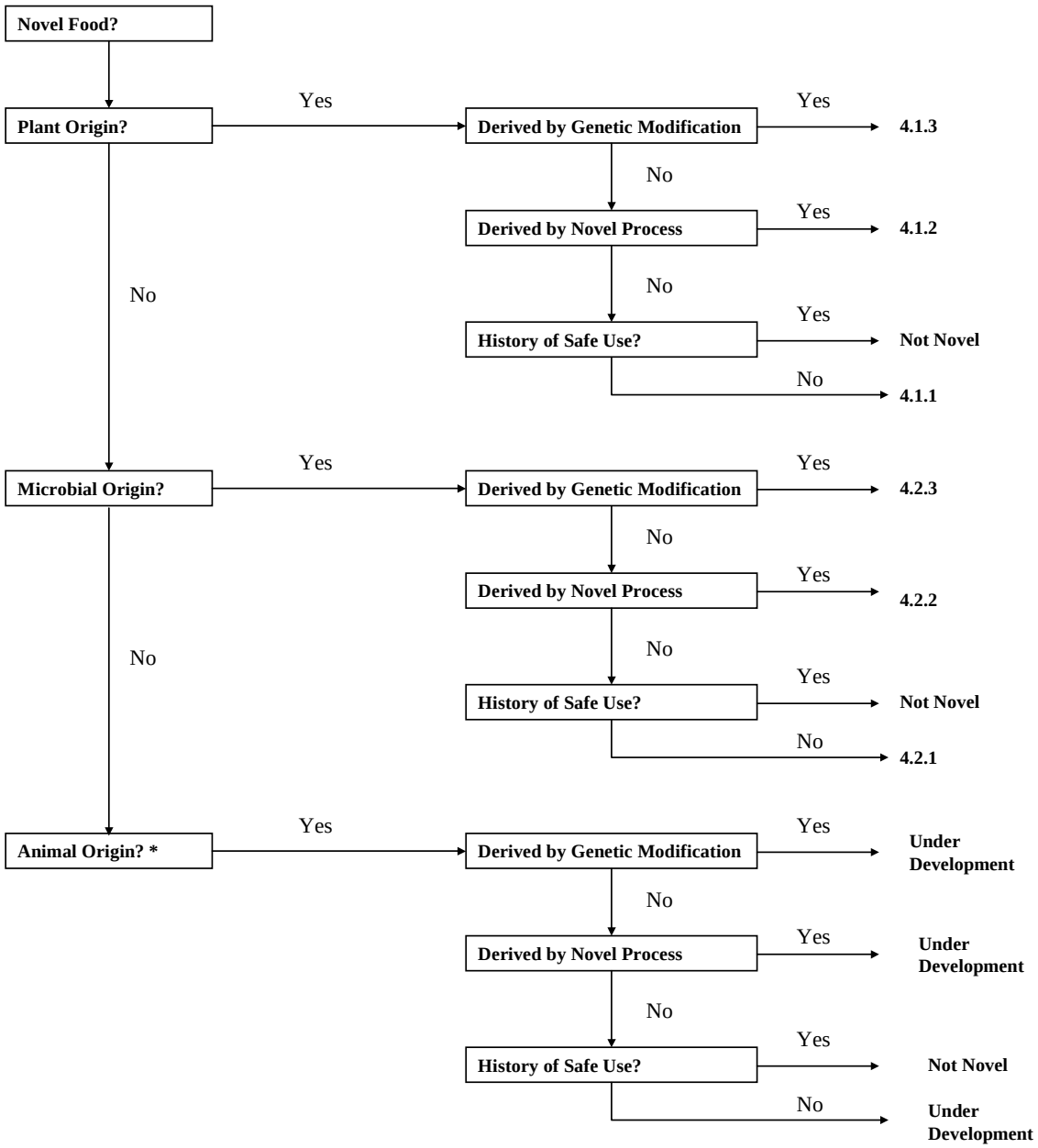
With such a wide range of foods, the amount of information necessary for assessment will also vary widely from one case to another. Therefore, in order to provide guidance for petitioners, this document will highlight the types of information likely to be required for specific types of novel foods. Not all information described may be relevant in every case. The explanations and interpretations indicated in this document are subject to change as additional knowledge and experience are gained in evaluating data and information supplied in novel food submissions.

Experiments intended to generate data to demonstrate the safety of a novel food should be designed and conducted in accordance with sound scientific concepts and principles, as well as, where applicable, Good Laboratory Practice. Primary data should be made available to regulatory authorities upon request. Data should be obtained using sound scientific methods and analysed using appropriate statistical techniques, when applicable. The sensitivity of all analytical methods should be documented and references to analytical methods made available.

The decision tree in Figure 2 provides guidance to petitioners to determine which sections of the guidelines are most appropriate for various novel food categories (genetic modification, novel process, and history of safe use). Petitioners are encouraged to consult with the Novel Foods

Section to clarify which information requirements should be addressed for a particular novel food product prior to making a notification or submission.

Figure 2. Decision tree outlining guideline information requirements for the different categories of novel foods under Division 28 of the Regulations. Following the decision tree will lead to the guideline sections that are pertinent to a particular product.



* Guidelines for the Safety Assessment of Livestock Animals and Fish under development

4.1 Novel Foods Derived From Plants

Plants may be consumed as food or used to produce materials which are used in food or food processing. Novel foods can be derived from plants with no history of safe use as a food source in Canada, manufactured by new processes applied to plant materials, or produced by plants that have been genetically modified by a variety of techniques.

It is recommended that the following information be included for assessing the acceptability of plant-derived foods that are novel for one or more of the above reasons. Note that not all information requirements outlined below may be applicable to all cases.

4.1.1 Substance with No History of Safe Use

4.1.1.1 History of use
4.1.1.2 Dietary exposure
4.1.1.3 Nutritional considerations
4.1.1.4 Toxicology considerations
4.1.1.5 Allergenicity considerations
4.1.1.6 Chemical considerations

4.1.2 Novel Process

4.1.2.1 Detail of novel process
4.1.2.2 Dietary Exposure
4.1.2.3 History of organism
4.1.2.4 Nutritional considerations
4.1.2.5 Toxicology considerations
4.1.2.6 Allergenicity considerations
4.1.2.7 Chemical considerations

4.1.3 Genetic Modification

4.1.3.1 Characterization of derived line
4.1.3.2 Genetic modification considerations
4.1.3.3 History of organism (Host and Donor(s))
4.1.3.4 Dietary exposure
4.1.3.5 Nutritional considerations
4.1.3.6 Toxicology considerations

4.1.3.7 Allergenicity considerations
4.1.3.8 Chemical considerations

4.1.1 Substance with No History of Safe Use

Many traditional foods are considered safe even though the food may contain anti-nutrients, toxins or allergens. Some foods require special preparation or processing to minimize the risks associated with a food. Foods are generally considered safe, provided that appropriate care is taken during development, production, processing, storage, handling and preparation. It is recognized that in many cases the knowledge required to manage the risks associated with traditional foods has been acquired in the course of their long history of use.

Notification is required for foods new to the Canadian marketplace in order to demonstrate that they have a history of safe use. A history of safe use means significant human consumption of a food for which there exists adequate data to provide a reasonable certainty that no harm will result from consumption of the food. In many cases, toxicological and allergenicity data may be required to demonstrate that there are no health concerns related to the food use of a product or ingredient.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.1.1.1 History of use
4.1.1.2 Dietary exposure
4.1.1.3 Nutritional considerations
4.1.1.4 Toxicology considerations
4.1.1.5 Allergenicity considerations
4.1.1.6 Chemical considerations

4.1.1.1 History of Use

A substance may be considered to have a history of safe use as a food if it has been an ongoing part of the diet for a number of generations in a large, genetically diverse human population where it has been used in ways and at levels that are similar to those expected or intended in Canada. The fact that a product has had a history of use according to the above definition in a jurisdiction with a similar food safety system would increase the level of confidence in the evidence presented. The following information would be needed to support a claim that a product has a history of safe use:

- Historical evidence indicating ongoing, frequent consumption by a cross-section of the population where it has been used over several generations (*i.e.* 100 years). This evidence may be derived from various sources including, but not limited to, scientific publications and patents, non-scientific publications and books, cookbooks, books on the history of food culture, and/or affidavits from two or more independent, reputable authorities that include well-documented accounts of the way the food is used and how they know it has the history it does. Limited usage or short term exposure would not be adequate to demonstrate a history of safe use.

- A declaration of any possible adverse effects linked to the food documented in its country of origin and/or a country where there is a high degree of consumption.

- A description of the standard methods of commercial and/or domestic processing and preparation for consumption.

- A description of how the food is cultivated or (if from wild sources) harvested.

- Amounts of the food that people are likely to consume in Canada, including typical serving sizes and expected frequency of consumption, at both average and extreme high consumption levels.

- Analysis of the composition of the food based on randomly selected, statistically valid samples. This analysis should include proximate data as well as amino acid profile, fatty acid profile, mineral and trace mineral composition and vitamin composition, as well as any nutrients, antinutrients and bioactive phytochemicals known to be of particular interest in the product. The analysis should pay special attention to the presence of compounds in the food which may have implications for the health of any groups of the Canadian population (*e.g.* possible toxicants or allergens or unusually high levels of nutrients in the food source or final food product).

- Metabolism and/or gastrointestinal effects in humans.

The submission should include reliable, high quality information and reference sources. Anecdotal evidence will be given less weight than scientifically derived data. Information on the history of human exposure will be particularly important where there are traditional handling or cooking requirements for a food that is novel. This information will need to be made available to consumers in a consistent manner. A current example of this is the advice regarding the necessity for a minimum period of vigorous boiling when cooking various dried beans.

4.1.1.2 Dietary Exposure

In conducting dietary exposure assessments for foods with no history of safe use, the primary issues to be addressed as part of the safety assessment are: the likely role of the food in the diet (*e.g.* a significant protein source, a condiment, *etc.*), the contribution of significant nutrients and endogenous anti-nutrients and toxins to the diet, and the potential for the introduction of novel substances to the food supply.

The introduction of foods with no history of safe use may have nutritional, toxicological or allergenic consequences, and estimation of exposure to components of the food of significance to health should be considered in such cases. For such foods, it may be possible to predict potential consumption patterns based on intakes of similar products routinely consumed as part of the diet. These intake estimates may then be used to calculate the potential dietary exposure to specific components of the novel food that will be the subject of the safety assessment.

4.1.1.3 Nutritional Considerations

General observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the implications of its nutritional characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts of food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods

- excessive intakes of nutrients or other bioactive substances as a result of unusually high levels in the novel food, or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional roles of non-

essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, a wide range of "bioactive" substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These roles are also included in the broad definition of nutritional quality.

Foods with no history of safe use

The main concern with respect to a food with no history of safe use would be to verify that the consumption of the food would not have an adverse effect on the nutritional health of the consumer. Information on nutritional composition and quality is primarily needed to determine how the food could be used in the diet, to establish basic composition information for the food for use in food composition databases, and to permit the validation of nutrient content claims and quantity declarations.

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a food with no history of safe use should be of sufficient quantity and quality to determine its role in the diet and to characterize the average nutritional composition of the food.

- Any studies conducted to evaluate nutritional quality should be performed using the food as it is expected to be consumed by humans.

b. Where published data on nutrient composition of the novel food are inadequate, analytical data may need to be obtained by the petitioner. In this case, an appropriate study design for obtaining data on nutritional composition:

- Considers all major sources of potential variation in nutritional quality, *e.g.* geographic area, season, soil type and fertility, amount of sunlight, temperature, crop management, etc, in designing the study, to ensure these factors are controlled.

- Subjects the novel plant during cultivation to the conditions expected for it in commercial production.

- Locates test plots in several locations where the plant is expected to be grown or collected. Ideally, the conditions under which the plant is grown for collecting data should aim at representing different geographical locations where the plant may be grown as well as different years, rather than relying on data from many replicates at a single field location for only one year.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all potential sources of variation of nutritional quality in the food and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example to account for between year and between plot variation. Ensure sampling is conducted at the appropriate stage of maturity for the respective crop.

- Ensures that the appropriate analyses are performed on all the parts of the plant that may be used as food in Canada. The compositional data should be provided for the raw food, in other words, the edible part of the plant in its unprocessed state as well as for the food prepared for human consumption by recommended and/or expected means to examine the effects, where applicable, of processing, storage and cooking.

- Provides the criteria used for selecting the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed in Nutrient Composition section below.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout. The study samples are analysed within an acceptable time frame from date of collection.

- Uses appropriate and consistent statistical methods chosen in advance based on the study design to analyse and report the results.

c. Nutrient Composition

In the context of the above study guidelines, the following components of novel foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

- proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude carbohydrate
- 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)
- quantitative and qualitative composition of total lipids, *i.e.* saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids
- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin
- qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin and mineral analysis - see Appendix A, "Key Micronutrients"
- presence of naturally occurring or adventitious anti-nutritional factors *e.g.* phytates, trypsin inhibitors, *etc.*
- predictable secondary metabolites, physiologically active (bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutrient bioavailability/Presence of anti-nutrients

In situations where the food with no history of safe use may be a significant component of the Canadian diet, and/or a major supplier of nutrients, animal studies should be conducted to assess nutritional adequacy. This pertains in particular to the evaluation of protein quality, the possibility of unknown anti-nutrients, and questions of nutrient bioavailability.

Information should be provided, if applicable, describing the processing conditions that would be used in the production of the novel food, and the effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- the name of the plant including Latin and common names

- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled.

- a complete description of sample collection and sample preparation;

- a citation and/ or description of the analytical and statistical methods which were used to obtain data for the nutritive and non-nutritive components;

- nutrient and related data expressed as mean ± standard deviation, and as a range;

- results of statistical analyses;

- raw data for all components analysed from all locations used to grow the plant;

- published data if available; and

- intended use of the organism as food in Canada, *i.e.* ingredient type(s), possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing diet-related disease and health promotion.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis.

- A novel food with no history of safe use is not required to meet specific criteria of nutritional quality. The main concern is to document the composition of the food in order to evaluate claims and to determine its potential role in the diet.

4.1.1.4 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that may be introduced to the food supply. For foods that have no history of safe use, it may be difficult to identify individual components which are novel in the context of human consumption in the absence of a traditional counterpart.

Where it is not possible to identify novel components of the food, a case-by-case approach should be used to determine the appropriate toxicological tests to be carried out on the food. The history of the organism from which the food is derived as a source of toxins or antinutrients and a chemical analysis of its components will be considerations in determining requirements for toxicological testing. Depending on these determinations, conventional studies of toxicity, including chronic toxicity, developmental toxicity, genotoxicity or carcinogenicity, may need to be performed on the final food product or its components as appropriate.

It should be noted that the conduct of studies with whole foods presents some challenges due to the potential for inducing nutritional imbalances when the food is incorporated into the diet at high concentrations. In addition, toxicology studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake in the manner used for simple chemicals like food additives.

4.1.1.5 Allergenicity Considerations

The primary consideration in allergenicity assessment of a novel food is the prevention of unexpected and/or unavoidable exposure of susceptible individuals to food allergens. For foods with no history of safe use, the potential exists that one or more component proteins would have the capacity to cross-react with known food allergens or lead to the development of *de novo* hypersensitivity. It should be noted, however, that the vast majority of proteins consumed in the diet are not allergenic.

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of an individual protein or of a whole food. Because existing strategies for the assessment of the allergenic potential of proteins were developed for the evaluation of individual, well-defined proteins (Section 4.1.3.7), they are not easily applied to the entire protein component of a whole food. The protein component of foods with no history of safe use will not be characterized to the extent necessary to apply these assessment strategies.

A preliminary strategy for assessing the allergenic potential of foods with no history of safe use would be to investigate whether plants from the same taxonomic family that are commonly part of the food supply are implicated in the induction of allergic response. The association of a particular family of plants with allergic response might not necessarily preclude the introduction of the novel food from a related species into the marketplace, but risk management measures such as post-market surveillance and labelling where identification of the food item is not obvious will need to be considered. Proteins from an allergenic source should not be added to foods where identity preservation cannot be guaranteed.

4.1.1.6 Chemical Considerations

The identification and levels of chemical contaminants must be reported in a food with no history of safe use. Potential levels and types of contaminants would be specific to the novel food type. It would therefore be necessary to determine the levels and ranges of contaminants which may be present in the food. If possible, a comparison of the levels of chemical contaminants in the novel food with those typically found in similar food products should be made. Examples of potential chemical contaminants are metals (*e.g.* arsenic, cadmium, mercury and lead) and organic contaminants (*e.g.* levels of mycotoxins).

4.1.2 Novel Process

Some processes applied to foods or food ingredients may result in the generation of foods which would be considered novel in relation to traditional counterparts. The application of new processes which cause a food to undergo a major change would trigger the requirement to notify Health Canada. A major change is defined in Division 28 of the Regulations as a change in a food that, based on the manufacturer's experience or generally accepted nutritional or food science theory, places the food outside the accepted limits of natural variations for that food with regard to: the composition, structure, nutritional quality of the food or its generally recognized physiological effects; the manner in which the food is metabolized in the body; or the microbiological safety, the chemical safety or the safe use of the food. Examples of novel processes include: new heat processing techniques; new packaging technologies; and the use of ultraviolet light for reducing the microbial load of a product.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.1.2.1 Details of Novel Process

While the focus of the safety assessment is on the food product, consideration of the process or preparation of the product can guide the safety assessment. Any novel processing or preparation techniques used to produce a novel food should be described in sufficient detail since such processing or preparation techniques may result in potential microbiological, toxicological, allergenicity, or nutritional concerns.

4.1.2.2 Dietary exposure

In conducting dietary exposure assessments for novel foods resulting from the application of a novel process, the primary issues to be addressed as part of the safety assessment are: the potential for alteration of nutrient content of the food, and potential for introduction of novel substances to the food supply.

In cases where the novel process results in the intentional or unintentional alteration of nutrient composition of the food, changes to nutrient intake should be determined for the food itself and in the context of the food as a source of the nutrient in the total diet. Variation of dietary patterns in subgroups in the population (*e.g.* children, infants, elderly, ethnic groups) as well as the potential for change in use and/or exposure to the food compared with the related, traditional food product should be taken into consideration.

Novel processes applied to foods to reduce spoilage due to microbial activity can also increase the availability of exotic foods in the Canadian marketplace. The increased availability may have nutritional, toxicological or allergenic consequences, and estimation of exposure to components of the food of significance to health should be considered in such cases.

If a process applied to a food results in the generation of predictable breakdown products, their amount in the food and the contribution of that food to the diet should be determined.

4.1.2.3 History of Organism(s)

The history of an organism can provide information that is important to the assessment of a novel food. There may be a history of toxin production by certain strains, species or genera and it would be important in such cases to examine the particular variety of the organism being used for the potential to produce such toxins, both under the conditions used in normal manufacturing and also under extreme conditions.

4.1.2.4 Nutritional Considerations

I Unintended nutritional effects

General Observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the implications of its nutritional characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts of food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods

- excessive intakes of nutrients or other bioactive substances as a result of unusually high levels in the novel food, or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional effects of non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, "bioactive"substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These substances are also included in the broad definition of nutritional quality.

Application of novel process to plant foods

The development of novel foods or novel food ingredients through application of a novel process, could result in unintended changes in the composition of the food product which could in turn have an impact on the nutritional value of the food and the nutritional status of the persons consuming it.

Unintended nutritional effects can occur whether the novel process is intended for nutritional or microbiological or other reasons. Evaluation of an intended effect on the nutritional quality of a food is discussed in Part II of this section.

An important step in the safety and nutritional assessment of this type of novel food is a comparison of its composition with its appropriate counterpart(s). To determine whether there are any differences in the nutritional quality of the novel food compared to its appropriate counterpart(s), the major constituents of the food must be analysed, *i.e.* macronutrients and their component parts, as well as individual micronutrients and other bioactive substances selected based on valid criteria. If any nutrients are excluded from the analyses, this should be justified by an acceptable rationale. Also, circumstances may warrant an evaluation of the nutritional "performance" of the new food in its ready-to-eat form, thus either raw or when further processed by traditional/conventional methods used to make the product ready-to-eat. The purpose would be to provide an opportunity to identify major changes that may not have been detected by

compositional analysis, but which could affect, for example, the stability or bioavailability of nutrients in the food or the susceptibility of anti-nutrients to processing that normally destroys them. A performance test could involve re-analysis of a substance following cooking or it could require animal testing for bioavailability.

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a novel food should be of sufficient quantity and quality to allow an assessment of whether any significant unintended effect on the nutritional quality of the food has occurred as a result of the application of the novel process on the food, relative to the food processed using current commercial processes. It should also allow an assessment of the nutritional significance of any change that is detected.

- Data should be provided for the food in its final product state (*i.e.* processed using novel method). Data may also be required for the food prepared for human consumption by conventional means to examine the effects, where applicable, of further processing, storage and cooking to look , for example, at the effectiveness of cooking to destroy anti-nutrients in cases where anti-nutrients normally destroyed by cooking are present.

- Data on the novel food should be compared, at a minimum, to data on two appropriate counterparts, the unprocessed food and the food processed by a currently used equivalent process (see section b, below). It is suggested that the study design include a representation of the various cultivars that are commercially available in the Canadian market; these cultivars should all be subjected to the test and control processes. This would permit assessment with respect to the normal variation expected between cultivars. Literature data (if available) may also be valid for assessing the nutritional relevance of any unintended effect.

b. Where published data on nutrient composition of the novel food are inadequate, analytical data may need to be obtained by the petitioner. In this case, an appropriate study design for obtaining data on nutritional composition:

- Considers all potential sources of variation in nutritional quality, *e.g.* conditions of application (dose, duration, temperature), surface area or volume of plant food, cultivar, consistency of nutrient levels in the starting material, etc, in designing the study, to ensure these factors are controlled.

- Includes in the same study the novel food that is the subject of the notification as well as the appropriate counterparts, *i.e.* the same food in its pre-processed raw state, and the same food subject to a currently used equivalent process. A currently used equivalent process would be a non-novel process that is currently used commercially to achieve the same effect as the novel process (if applicable). In the absence of a currently used equivalent process, the counterpart would be simply the same food in its pre-processed raw state.

- Applies the novel process (test), and currently used equivalent process (control) to a selection of the commercial cultivars available in the current market.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all major sources of variation of nutrient levels in the food and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example, to account for inter-cultivar and between plot variation.

- Ensures processing is conducted at the appropriate stage of maturity for the plant food, and that sampling is conducted at the appropriate stage of processing for the plant food (*i.e.* final product).

- Ensures that the appropriate analyses are performed on all the parts of the plant that may be used as food in Canada.

- Provides the criteria used for selecting the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed in **c. Nutrient Composition** below.

- Ensures samples are analysed within an acceptable time frame from date of collection.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout.

- Uses appropriate and consistent statistical methods chosen in advance, based on the study design, to compare levels of each nutrient in the novel food versus its controls.

c. Nutrient Composition

In the context of the above study guidelines, the following components of foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

- proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude carbohydrate

- 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)

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

- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin

- qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin and mineral analysis - see Appendix A, “Key Micronutrients”

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

- predictable secondary metabolites, physiologically active (bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutritional “Performance” of novel plant food

Consideration should be given to the possible need for the following types of information regarding the novel food:

- Response of known anti-nutrients to processes normally expected to neutralize their activity, measured using compositional analysis.

- Storage stability with regard to nutrient degradation.

- Performance of product in relation to the intended benefit (other than direct health benefits) *e.g.* improved stability of an oil to heating after fatty acid profile modification.

Nutrient bioavailability/Presence of new or altered anti-nutrients

In situations where the novel food may become a significant component of the Canadian diet, and/or a significant supplier of nutrients, animal studies may be needed in assessing nutritional adequacy to determine if there have been changes in the bioavailability of nutrients or if the composition is not comparable to conventional foods.

Information should be provided, if applicable, describing the conditions used in the further processing of the novel food and its derivatives, and the potential effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- a full description of the novel process, the purpose of the process, and the food (s) on which it could be applied, and the food (s) on which it will be applied (for the purpose of the submission);

- the foods on which the test and control processes were applied in the study, and the names and source (*i.e.* where purchased and grown) of all commercial cultivars which were represented in the study);

- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled;

- a complete description of sample collection and sample preparation;

- a citation and/or description of the analytical and statistical methods used to obtain data for the nutritive and non-nutritive components;

- nutrient and related data for test, control, and commercial cultivars (expressed as mean ± standard deviation, and as a range);

- results of statistical analyses;

- raw data for all components analysed;

- published data if available; and

- intended use of the plant as food in Canada, *i.e.* ingredient type(s), possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- "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" (Codex)[1]. If the composition of the novel food is judged not to be nutritionally equivalent to that of its counterparts, *i.e.* significant differences (statistical and biological) exist in the nutrient data, additional nutritional data may be required on a case-by-case basis.

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

- Detection of a major change due to an unintended nutritional effect may not preclude the marketing of the product. However, such changes may require limits on the use of the food in food products or a requirement for labelling that goes beyond basic provisions. See also Part II with respect to safety assessment of high levels of nutrients or bioactive substances.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis and assessment.

- The safety of a major increase in the level of a nutrient or other bioactive component would need to be assessed in a similar way to the safety assessment of an intended nutritional change. For details on this see Part II below.

II Intended nutritional modifications

[1]"Codex Alimentarius Commission", Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology", 3rd Session: Yokohama, Japan 4-8 March 2002: Consideration of Proposed Draft Guideline for the Conduct of Food Safety Assessment of Recombinant-DNA Microorganisms in Food *At Step 4"*; page 13

The term “intended nutritional modification” is taken to include any change or introduced trait intended to improve the nutritional quality or health-related profile of the food, including but not limited to essential nutrients, beneficial bioactive phytochemicals, quantities and nature of the energy-yielding substances, improved nutrient bioavailability, and reduction in anti-nutrient levels.

Evaluation of an intended nutritional change requires steps that are similar to those used in either the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health claims or both. For instance, such a change would trigger questions concerning the intended target group, what level of the targeted nutrient or other substance is expected in the food, what is the expected change in level of exposure to the targeted nutrient or other substance across all age and sex groups and at the upper and lower extremes of intake of the food, and the safety of this level of exposure.

A novel food with an introduced health or nutritional benefit would likely fall into the unofficial category of “functional food”. It is expected that manufacturers will be interested in making health claims for these products. These products would therefore be evaluated in accordance with the criteria being laid out for foods with product-specific health claims. These include attention to the evidence in support of the claim, as well as to product safety and product quality considerations.

Product safety of this type of novel food is intended to be controlled through application of the novel food regulations. The safety evaluation of a food manufactured using a novel process, for the purpose of having an intended nutritional modification should be the same as for other novel foods. With regard to the safety and nutritional evaluation of the intended nutritional modification itself, data requirements are described below.

Product quality assurance refers to ensuring the consistency of the level of biologically active substances in the novel food in delivering the claimed benefits, and to conformance with acceptable procedures in all aspects of product testing. Details about quality assurance are discussed in the Interim Guidance Document on Standards of Evidence, mentioned below.

At this time, regulations for product-specific health claims have not yet been promulgated. Prospective petitioners should refer to the proposed regulatory framework for product-specific health claims which was published in November, 2001, and the Interim Guidance Document on Standards of Evidence which was published in February, 2002. These are both available on the Health Canada web site at:

http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html.

It is important to ascertain to what extent the intended nutritional effect of a novel process remains stable with storage, further processing, and cooking.

The review of unintended nutritional effects in a food manufactured using a novel process for the purpose of having an intended nutritional effect would follow the same steps as for other novel foods.

Nutritional Evaluation of expected or unexpected increased levels of a nutrient or bioactive substance

- Increased levels of a nutrient or other intrinsic bioactive substance in a food need to be evaluated for safety.

- Data needed for this include:

  - the level of the targeted nutrient or other substance expected in the food;

  - intended target group, if applicable, or which group(s) is or are most likely to have high intakes of the food;

  - expected level of exposure to the substance through consumption of the food by the target group, by vulnerable sub-groups and at the upper and lower extremes of intake of the food across all age and sex groups using recent Canadian food consumption data where possible;

  - how the expected level of exposure to the targeted nutrient or other substance differs from the current levels of exposure from all sources;

  - any potential use of the product as a replacement of existing foods; and

  - data in support of the safety of the expected level of exposure.

4.1.2.5 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that are introduced to the food supply. The application of novel processes to foods may result in the generation of novel substances in the resulting food be they intentional or unintentional. Because of the potential wide variety of products generated by the application of novel processes, a determination of the appropriate toxicological testing should be conducted on a case-by-case basis.

Identification of any novel substances generated in the food subjected to a novel process is assisted by the use of the unprocessed food as a comparator. Chemical analysis may provide information on any new substances that have been formed. In addition, information on the nature, duration and intensity of treatment and the chemical composition of the food may be useful in predicting the types of alterations to the food components**.** Depending on these

determinations, conventional studies of toxicity, including assays of metabolism, toxicokinetics, chronic toxicity/carcinogenicity, impact on reproductive function, and teratogenicity, may need to be performed on the final food product or its components as appropriate.

Intentional alteration of the composition of foods by the addition of food components at levels that fall outside the accepted limits for natural variations (*e.g.* "functional" foods) may result in exposures for which there is no history of safe use. Substances that have been traditionally consumed in foods but which have been added to foods at levels outside their normal range will result in consumption of higher amounts of the substance than from a traditional diet. In such cases, the novel aspect of the food is the extent of exposure to the substance, rather than the substance itself, and toxicological testing of the enhanced component will be required to establish an upper limit of tolerability to the substance. The types of studies conducted should be guided by a knowledge of the role of the component in human physiology. Evidence from animal and *in vitro* studies as indicated in the previous paragraph would be required to determine safety. Studies in experimental animals may be of limited usefulness if the commonly used animal model (*e.g.* the rat) differs markedly from humans in the metabolic pathways and chronic conditions that are the basis of the intended functional effect, and it may be necessary to place greater reliance on human response to increased intakes of such food components. Epidemiologic studies may be available for substances that are normally components of foods, and these can provide important information on long-term effects.

4.1.2.6 Allergenicity Considerations

The primary consideration in allergenicity assessment of a novel food is the prevention of unexpected and unavoidable exposure of sensitized individuals to food allergens. In cases where the application of a novel process to a food results in the generation of a novel protein or an alteration of the protein content of a food containing allergenic proteins, a consideration of the allergenic potential of the novel food would be required.

Novel Proteins

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of an individual protein or of a whole food. If the application of a novel process to a food results in the generation of a novel protein that can be isolated and characterized, the assessment strategy that has been developed for foods which are the products of recombinant DNA technology and described in section 4.1.3.7 can be used to assess its potential allergenicity. This strategy involves a weight of evidence approach that relies on the assessment of amino acid sequence homology to known food allergens, and a consideration of the similarity of its properties, in particular, resistance to digestion in the mammalian gastrointestinal tract, to those of known food allergens.

Alteration of endogenous allergen content

If the application of a novel process to a food that contains allergenic proteins results in altered protein content of that food, the potential for increase in the allergenic content should be assessed. While the health impacts of such increases is uncertain, this result would be considered undesirable. Techniques used for assessing the potential for effects on endogenous allergen expression are: the quantitative comparison of protein composition of the edible portion of the modified organism or, where sera from sufficient numbers of individuals with allergies to the food are available, the comparative immunoreactivity to the edible portion of the modified organism can be determined using immunoblotting techniques.

4.1.2.7 Chemical Considerations

The identification and levels of chemical contaminants must be reported. Contaminants could be introduced as a result of the application of the novel process to the food or could be naturally present in the food before application of the process. It would be necessary to provide a comparison of the levels of chemical contaminants in the novel food with those levels typically found in the original food product. Examples of chemical contaminants are metals (*e.g.* arsenic, cadmium, mercury and lead) and organic contaminants (e.g. introduction/or increased levels of mycotoxins).

4.1.3 Genetic Modification

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 unless another major change occurs in the plant.

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.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.1.3.1	Characterization of derived line
4.1.3.2	Genetic modification considerations
4.1.3.3	History of organism
4.1.3.4	Dietary exposure
4.1.3.5	Nutritional considerations
4.1.3.6	Toxicology considerations
4.1.3.7	Allergenicity considerations
4.1.3.8	Chemical considerations

4.1.3.1 Characterization of Derived Line

Where a plant has been modified, whether by conventional breeding, selection and mutagenesis techniques or by recombinant nucleic acid technology, the relationship of the derived variety with the parent varieties should be characterised. The approach of the safety assessment is based on the principle that the safety of novel products is assessed relative to a conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Any significant differences between the novel and the conventional variety are then assessed for potential adverse health effects. Of particular interest to the safety assessment is whether the modification could inadvertently develop or increase the toxicity or allergenicity potential of a new variety or reduce it's nutritional quality.

4.1.3.2 Genetic Modification Considerations

Genetic Modification by Traditional Techniques

Many non-recombinant nucleic acid modification procedures are relatively undefined and poorly characterized in terms of insertion, deletion or rearrangement of genetic material, and the procedures are generally used for transfer of multi-genic traits. Strain selection or conventional breeding techniques can influence the toxin-producing capacity of an organism and may also influence desirable nutritional factors such as vitamin levels or the proportions of unsaturated fatty acids.

It is understood that specific information on the genetic differences between a novel organism such as a plant derived by mutagenesis or traditional breeding methods may not be available. The breeder may have knowledge of the trait selected and the source of that trait which should be provided if available. Agronomic characterization in addition to a consideration of key nutrients (macro and micro nutrients), anti-nutrients, and toxicants will be required to demonstrate the safety of a novel food derived from mutagenesis or traditional breeding techniques. The number of key nutrients, toxicants, and anti-nutrients required for analysis and assessment will be determined on a case-by-case basis and are associated with the organism under consideration. The nutrients and toxicants considered significant for the purposes of establishing the safety of a new food also depends on the potential intake of the food in Canada (dietary exposure considerations).

It is recognized that major food crops have an extensive history of safe use and that the introduction of new varieties of existing crop plants has only rarely resulted in adverse effects in humans. Novel food varieties obtained by outbreeding traditional crop varieties with wild types or exotics could potentially cause nutritional or toxicological concerns. In crosses where parental varieties are well known, toxins may be known and standards of toxin levels may be established. However, where crosses involve wild plants or wild relatives of crop plants, more extensive analysis for toxins in the edible portions of the plant and feeding studies may be necessary. It

should be noted that the extent of backcrossing should be fully described as the process can remove a large percentage of the donor parents genetic material from the progeny selected for food use.

Traditionally developed plants require a multi-disciplinary assessment since details of the modifications may be largely unknown. As experience in the safety assessment of novel foods develops, it may be possible to identify data requirements for particular groups of products more clearly, or to preclude certain products from further detailed evaluation.

Genetic Modification by Modern Techniques

In cases where a plant has been modified using modern genetic techniques, such as recombinant nucleic acid technology, the safety assessment will consider detailed characterization data of a novel organism at the molecular level. The following requirements are based on harmonization efforts with other regulatory authorities and reflects international guidance documents in this area (Codex Alimentarius). In addition to the requirements of previous sections, the following areas should be addressed for these types of products:

i) Description of the genetic modification(s)

Details of all methods and manipulations involved in the modification of an organism must be provided to allow for the identification of all genetic material potentially inserted, deleted, mutated, or rearranged in the host genome. This will provide the necessary information for the analysis of the data supporting the characterization of the modified organism.

The description of the modification process should include:

- information on the method(s) of modification used, *e.g.* Agrobacterium-mediated transformation or direct transformation by methods such as particle bombardment, electroporation, *etc.*;

- description and characterization of all genetic material potentially delivered, if applicable, including the source, identity and expected function in the organism; and

- details of manipulations or modifications to introduced, intermediate and recipient genetic material (*e.g.* change that affects the amino acid sequence of expression product).

Information should be provided on DNA added, inserted, deleted, or modified, including:

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

- the size and identity;

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

- function in the organism.

A summary diagram, outlining the key features of the final construct should be provided. Depending on the nature of the genetic modification, restriction maps and sequence data of the introduced or modified genetic material and adjacent regions, may be required.

ii) Characterization of the genetic modification(s)

In order to provide clear understanding of the impact on the composition and safety of foods derived from genetically modified organisms, a comprehensive molecular and biochemical characterization of the organism should be carried out.

Information should be provided on the DNA insertions into the genome; this should include:

- the characterization and description of all inserted genetic materials;

- the number of insertion sites;

- data to demonstrate if complete or partial copies have inserted into the genome;

- data to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;

- the organization of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and, where appropriate, of surrounding region;

- identification of any open reading frames within the inserted DNA or created by the insertions with contiguous plant genomic DNA including those that could result in fusion proteins;

- in the case of modifications that involve deletions, rearrangements or site-specific, *in vitro* mutagenesis, sequence data of the region before and after modification should be provided.

Information should be provided on any expressed substances in the modified organism; this should include:

- the gene product (*e.g.* a protein or an untranslated RNA);
- the gene product's function;
- the phenotypic description of the new trait(s);
- the level and site of expression of the gene product(s), and the levels of its metabolites;
- 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;
- where genetic manipulations are directed to altered regulation of endogenous genes, the characteristics and level of gene expression should be compared with that of the unmodified host;
- to indicate whether there is any evidence to suggest that one or several endogenous genes in the host plant has been affected by the modification process;
- to confirm the identity and expression pattern of any new fusion proteins;
- to demonstrate the intended effect of the modification has been achieved and that all expressed 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 itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly; and
- to demonstrate that the newly expressed 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.

4.1.3.3 History of Organism(s)

The history of both donor and host organisms can provide information that is important to the assessment of a novel food. There may be a history of toxin production by certain strains, species or genera and it would be important in such cases to examine the particular organism(s) being used in the development of the novel food for the potential to produce such toxins, both under the conditions used in normal manufacturing and also under extreme conditions.

4.1.3.4 Dietary Exposure

In conducting dietary exposure assessments for novel foods produced through genetic modification, the primary issues to be addressed as part of the safety assessment are: the potential for alteration of nutrient content of the food, and the potential for introduction of novel substances to the food supply.
In cases where the nutrient composition of foods has been altered, either intentionally or through genetic modification, changes to nutrient intake should be determined for the food itself and in the context of the food as a source of the nutrient in the total diet. Variation of dietary patterns in subgroups in the population (*e.g.* children, infants, elderly, ethnic groups) as well as the potential for change in use and/or exposure to the food compared with the related, traditional food product should be taken into consideration.

In the case of commodity crops that undergo genetic modification to alter agronomic characteristics, dietary exposure to food or food ingredients derived from the crop is unlikely to be altered. However, if food crops result in the introduction of a novel protein or novel metabolites to the food supply, the content of these substances in the food should be determined and considered together with the toxicological data as part of the safety assessment. The effects of typical food processing procedures on the novel component(s) should be considered in deriving the exposure estimate. In the case of substances covered by existing safety data (*e.g.* permitted agricultural chemicals), documentation of the anticipated increase in exposure to these substances should be provided.

Genetic modification of crops to alter agronomic characteristics such as disease resistance can also increase the availability of exotic foods in the Canadian marketplace. The increased availability may have nutritional, toxicological or allergenic consequences, and estimation of exposure to components of the food of significance to health should be considered in such cases. It may be difficult to predict what increases in exposure to the whole food or food ingredient may occur.

4.1.3.5 Nutritional Considerations

I Unintended nutritional effects

General Observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the implications of its nutritional characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts of food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods

- excessive intakes of nutrients or other bioactive substances as a result of unusually high levels in the novel food, or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional effects of non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, “bioactive” substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These substances are also included in the broad definition of nutritional quality.

Foods from genetically modified plants

The development of novel foods or novel food ingredients through genetic modification, whether by traditional breeding, mutagenesis or recombinant DNA techniques, could result in unintended changes in the composition of the food product which could in turn have an impact on the nutritional value of the food and the nutritional status of the persons consuming it. As more complex or layered genetic modifications are attempted through recombinant DNA techniques, for instance to introduce both improved nutritional traits and agronomic traits into the same organism, these could increase the potential for unintended effects compared to simpler modifications. By the same token, other methods of genetic modification could also introduce multiple changes.

Unintended nutritional effects can occur whether the intended modification is nutritional or agronomic or something else. Evaluation of a modification intended to affect the nutritional quality of a food is discussed in Part II of this section.

An important step in the safety and nutritional assessment of the modified food is a comparison of its composition with its appropriate counterpart. To determine whether there are any significant differences, the major constituents of the food must be analysed, *i.e.* macronutrients and their component parts, as well as individual micronutrients and other bioactive substances selected based on valid criteria. If any nutrients are excluded from the analyses, this should be justified by an acceptable rationale. Also, circumstances may warrant an evaluation of the nutritional "performance" of the new food in its ready-to-eat form, thus either raw or when processed by traditional/conventional methods used to make the product ready-to-eat. The purpose would be to provide an opportunity to identify major changes that may not have been detected by compositional analysis, but which could affect, for example, the stability or bioavailability of nutrients in the food or the susceptibility of anti-nutrients to processing that normally destroys them. A performance test could involve re-analysis of a substance following cooking or it could require animal testing for bioavailability or some other nutritional factor.

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a novel food should be of sufficient quantity and quality to allow an assessment of whether any significant unintended genetic modification affecting the nutritional quality of the food has occurred as a result of the introduction of the novel trait. It should also allow an assessment of the nutritional significance of any change that is detected.

- Data should be provided for the raw food, in other words, the edible part of the plant in its unprocessed state. Data may also be required for the food prepared

for human consumption by conventional means to examine the effects, where applicable, of processing, storage and cooking to look , for example, at the effectiveness of cooking to destroy anti-nutrients in cases where anti-nutrients normally destroyed by cooking are present.

- Data on the novel food should be compared, at a minimum, to data on the near isogenic, non-transgenic parent variety, the most appropriate counterpart, if available, or else a closely related non-transgenic cultivar. Since one or more significant differences could arise, the study design should include crops of the same species from a range of standard cultivars that are in commercial production for the same purposes and grown in the same geographical areas as those typically found on the Canadian market. This would permit assessment with respect to normal variation. Literature data (if available) may also be valid for assessing the nutritional relevance of any unintended effect.

b. Where published data on nutrient composition of the novel food are inadequate, analytical data may need to be obtained by the petitioner. In this case, an appropriate study design for obtaining data on nutritional quality:

- Considers all sources of potential variation in nutritional composition, *e.g.* geographic area, season, soil type and fertility, amount of sunlight, temperature, crop management, etc, in designing the study, to ensure these factors are controlled.

- Subjects the modified plant to the conditions expected for it in commercial production, *i.e.* a plant which is made tolerant to environmental or other stresses (insects, salt, drought, herbicides *etc.*) should be grown under those conditions for the purposes of data collection. The control plants should likewise be grown under conditions appropriate for them.

- Includes in the same study the novel food that is the subject of the notification as well as the appropriate counterpart, *i.e.* the near isogenic parent cultivar, and a selection of the commercial cultivars available in the current market. In the absence of a near isogenic parent cultivar, the most closely related non-transgenic cultivar may be chosen.

- Locates the test plots in several locations which are representative of the major growing areas for the organism. Ideally, the conditions under which the organisms are grown for collecting data should aim at representing different geographical locations where the plant is normally grown as well as different years, rather than relying on data from many replicates at a single field location for only one year.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all major sources of variation of nutrient levels in the food and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example, to account for inter-cultivar and between plot variation.

- Ensures sampling is conducted at the appropriate stage of maturity for the respective crop.

- Ensures that the appropriate analyses are performed on all the parts of the plant that may be used as food in Canada. For example, if the intended uses of a novel corn include the oil and the meal, samples of both corn oil and cornmeal should be analysed for the appropriate nutrients.

- Provides the criteria used for selecting the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed in the Nutrient Composition section below.

- Ensures samples are analysed within an acceptable time frame from date of collection.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout.

- Uses appropriate and consistent statistical methods chosen in advance, based on the study design to compare levels of each nutrient in the novel food versus its controls.

c. Nutrient Composition

In the context of the above study guidelines, the following components of foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

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

- quantitative and qualitative composition of total lipids, *i.e.* saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids
- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin
- qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin and mineral analysis - See Appendix A "Key Micronutrients"
- presence of naturally occurring or adventitious anti-nutritional factors *e.g.* phytates, trypsin inhibitors, *etc.*
- predictable secondary metabolites, physiologically active (bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutritional "Performance" of modified plant

Consideration should be given to the possible need for the following types of information regarding the modified plant:

- Response of known anti-nutrients to processes normally expected to neutralize their activity measured using compositional analysis.

- Storage stability with regard to nutrient degradation.

- Performance of product in relation to the intended benefit (other than direct health benefits) *e.g.* improved stability of an oil to heating after fatty acid profile modification.

Nutrient bioavailability/Presence of new or altered anti-nutrients

In situations where the food from a genetically modified source may become a significant component of the Canadian diet, and/or a significant supplier of nutrients, animal studies may be needed in assessing nutritional adequacy to determine if there have been changes in the bioavailability of nutrients or if the composition is not comparable to conventional foods.

Information should be provided, if applicable, describing the processing conditions used in the production of the novel food and its derivatives, and the potential effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- the names of all the cultivars which were represented in the study;
- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled;
- a complete description of sample collection and sample preparation;
- a citation and/or description of the analytical and statistical methods used to obtain data for the nutritive and non-nutritive components;
- nutrient and related data for test, control, and commercial cultivars (expressed as mean ± standard deviation, and as a range);
- results of statistical analyses;
- raw data for all components analysed from all locations used to grow the plant;
- published data if available; and
- intended use of the plant as food in Canada, *i.e.* ingredient type(s), possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- "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" (Codex)[2]. If the composition of the novel food is judged not to be nutritionally equivalent to that of its parent and commercial varieties, *i.e.*

[2]"Codex Alimentarius Commission", Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology", 3rd Session: Yokohama, Japan 4-8 March 2002: Consideration of Proposed Draft Guideline for the Conduct of Food Safety Assessment of Recombinant-DNA Microorganisms in Food *At Step 4"*; page 13

significant differences (statistical and biological) exist in the nutrient data, additional nutritional data may be required on a case-by-case basis.

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

- Detection of a major change due to an unintended nutritional effect may not preclude the marketing of the product. However, such changes may require limits on the use of the food in food products or a requirement for labelling that goes beyond basic provisions. See also Part II with respect to safety assessment of high levels of nutrients or bioactive substances.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis and assessment.

- The safety of a major increase in the level of a nutrient or other bioactive component would need to be assessed in a similar way to the safety assessment of an intended nutritional change. For details on this see Part II below.

II Intended nutritional modifications

The term “intended nutritional modification” is taken to include any change or introduced trait intended to improve the nutritional quality or health-related profile of the food, including but not limited to essential nutrients, beneficial bioactive phytochemicals, quantities and nature of the energy-yielding substances, improved nutrient bioavailability, and reduction in anti-nutrient levels.

Evaluation of an intended nutritional change requires steps that are similar to those used in either the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health claims or both. For instance, such a change would trigger questions concerning the intended target group, what level of the targeted nutrient or other substance is expected in the food, what is the expected change in level of exposure to the targeted nutrient or other substance across all age and sex groups and at the upper and lower extremes of intake of the food, and the safety of this level of exposure.

A novel food with an introduced health or nutritional benefit would likely fall into the unofficial category of “functional food”. It is expected that manufacturers will be interested in making

health claims for these products. These products would therefore be evaluated in accordance with the criteria being laid out for foods with product-specific health claims. These include attention to the evidence in support of the claim, as well as to product safety and product quality considerations.

Product safety of this type of novel food is intended to be controlled through application of the novel food regulations. The safety evaluation of a novel food genetically modified to have an intended nutritional modification should be the same as for other genetically modified foods. With regard to the safety and nutritional evaluation of the intended nutritional modification, itself, data requirements are described below.

Product quality assurance refers to ensuring the consistency of the level of biologically active substances in the novel food in delivering the claimed benefits, and to conformance with acceptable procedures in all aspects of product testing. Details about quality assurance are discussed in the Interim Guidance Document on Standards of Evidence, mentioned below.

At this time, regulations for product-specific health claims have not yet been promulgated. Prospective petitioners should refer to the proposed regulatory framework for product-specific health claims which was published in November, 2001, and the Interim Guidance Document on Standards of Evidence which was published in February, 2002. These are both available on the Health Canada web site at:

http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html.

Adding a substance through genetic modification differs from adding one through applying it to or mixing it with the food after it is harvested. The decision to proceed with or cease the addition would take place at different stages of production. This could have an effect on the ability to manage the presence of the “added” substance or trait in the food supply if it was later decided that there was a need to control it. Given this potential need, such products should be subject to post-market surveillance to ensure the ability to monitor and control the products. To promote a product that has been altered with the intention of benefiting the consumer, manufacturers themselves would have a requirement for post-market surveillance, in any case, and therefore this should not add any significant additional burden.

It is important to ascertain to what extent the modified nutrient (if the intent was to deliberately modify the level of a nutrient) is bioavailable and remains stable with cultivation, time, processing, storage and cooking.

The review of unintended nutritional effects in a food modified to have an intended nutritional effect would follow the same steps as for other novel foods.

Nutritional Evaluation of expected or unexpected increased levels of a nutrient or bioactive substance

- Increased levels of a nutrient or other intrinsic bioactive substance in a food need to be evaluated for safety.

- Data needed for this include:

  - the level of the targeted nutrient or other substance expected in the food

  - intended target group, if applicable, or which group(s) is or are most likely to have high intakes of the food

  - expected level of exposure to the substance through consumption of the food by the target group, by vulnerable sub-groups and at the upper and lower extremes of intake of the food across all age and sex groups using recent Canadian food consumption data where possible

  - how the expected level of exposure to the targeted nutrient or other substance differs from the current levels of exposure from all sources

  - any potential use of the product as a replacement of existing foods

  - data in support of the safety of the expected level of exposure

4.1.3.6 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that are introduced to the food supply. Novel substances may be introduced to the food supply through recombinant DNA technology, or may be generated by the application of novel processes to foods or [other DNA modification processes]. Introduction of novel substances may be intentional or unintentional.

Genetic modification techniques can result in the production of novel substances by the organism or the intentional or unintentional modification of substances already produced by the organism or their expression.

Novel Substances

In vitro nucleic acid techniques enable the introduction of DNA which can result in the synthesis of new substances in plants. These include the protein expression product and other substances which may be generated as a result of enzymic activity of the protein expression product. The

new substances can be conventional components of plant foods such as proteins, fats, carbohydrates, or vitamins that are novel in the context of that recombinant DNA plant.

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.

Toxicology studies are not considered necessary where the substance or a closely related substance has been consumed safely in food at equivalent intakes or where the new substance is not present in the food. Otherwise, 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 production of the substance from an alternative source, in which case, the material should be shown to be biochemically and functionally equivalent to that produced in the recombinant DNA plant.

For 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. Since proteins that are enzymes have never been shown to be direct-acting carcinogens, mutagens, teratogens or reproductive toxicants (Pariza and Foster 1983) it is generally not necessary to test proteins for these toxicological endpoints when exposure occurs by the oral route. Protein toxins act through acute mechanisms after the administration of a single dose at doses in the nanogram to milligram per kilogram body weight. Therefore, acute oral toxicity studies using gram per kilogram body weight doses of the novel protein are appropriate for assessing the potential toxicity of proteins. A negative result using doses in the gram/kg body weight range together with evidence that the protein is digested to small peptides and amino acids would provide assurance that the protein is not a toxin and is digested to nutrients as are the vast majority of dietary proteins.

Different types of *in vivo* or *in vitro* studies would be needed to assess the toxicity of introduced substances other than proteins. The types of studies are determined on a case-by-case basis and 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.

Unintended Effects

Techniques used in the genetic modification of plants or microorganisms have the potential to induce unintended effects on the genome of the modified organism that could be manifested as

an alteration in the levels of toxicants or antinutrients normally produced by the organism. The intended genetic alteration may also influence the behaviour of the organism with respect to accumulation of contaminants, pesticides, or other substances from the environment that were not anticipated.

Compositional analysis is the method currently used for detection of unintended changes to the genome that result in accumulation of toxic substances either of endogenous or exogenous origin. Because of the influence of environmental stress on production of endogenous components such as toxins and anti-nutrients, data should be collected from a number of different test sites. New, more sensitive technologies that allow the determination of alterations to expression of the organisms' genome are presently under development.

4.1.3.7 Allergenicity Considerations

The primary consideration in allergenicity assessment of a novel food is the prevention of unexpected and unavoidable exposure of sensitized individuals to food allergens. This includes the assessment of the potential for foods containing novel proteins to cross-react with known food allergens or to lead to the development of *de novo* hypersensitivity. In addition, the potential of increasing the allergenic potential of foods already containing allergens as an unintended result of genetic modification should be assessed. The following requirements are based on the Codex guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants.

Section 1 – Introduction

All newly expressed proteins in recombinant-DNA plants that could be present in the final food and are novel in the context of that food, need to be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of a newly expressed protein in humans. Based upon the [best], currently-available scientific information, the recommended approach used takes into account the preponderance of evidence derived from several types of information and data in an integrated, stepwise, case-by-case manner.

Section 2 - Assessment Strategy[3]

[3] This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

The initial steps in assessing possible allergenicity of any newly expressed proteins involve determination of: the allergenicity of the source of the introduced protein; any similarity between the amino acid sequence of the protein and that of known allergens; and certain physicochemical properties, including but not limited to, its susceptibility to enzymatic degradation.

Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

Determination of amino acid sequence homology and physicochemical characteristics will require the isolation of the newly expressed protein from the recombinant-DNA organism, or the synthesis of production of the substance from an alternative source, in which case the material should be shown to be functionally and biochemically equivalent to that produced in the recombinant-DNA organism.

Food proteins that are not allergens and that are altered by mutagenesis techniques need only be assessed for the likelihood that the mutagenized protein is a *de novo* allergen.

The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute toward an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

Section 3 – Initial Assessment

Section 3.1 - Source of the Protein

As part of the data supporting the safety of foods derived from recombinant-DNA organisms, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE-mediated oral, respiratory or contact allergy is available. Specific tools and relevant data that permit confirmation of allergenic potential are available for proteins from some allergenic sources. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; and structural characteristics and amino acid sequence (when available) of known allergenic proteins from that source.

Section 3.2 – Amino Acid Sequence Homology

Amino acid sequence homology comparisons should be used to assess the extent to which a newly expressed protein is similar in structure to known allergens in order to determine whether that protein has allergenic or cross-reactivity potential. Overall structural similarities can be

predicted using sequence homology searches that compare the structure of newly expressed proteins with all known allergens should be conducted using various algorithms such as FASTA or BLASTP. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for the purpose of identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results[4]. Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35% identity in a segment of 80 or more amino acids (FAO/WHO 2001).

Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous IgE-binding epitopes.

A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. This does not preclude further studies where considered necessary (see also section 6). A positive sequence homology result indicates that the newly expressed protein has a high probability of being allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source (see section on Specific Serum Screening).

Section 3.3 – Pepsin Resistance

Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation exists between resistance to digestion by pepsin, and allergenic potential[5]. The resistance of a protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method.

[4] It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segment searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

[5] The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).

Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.

Section 4 – Specific Serum Screening

For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays is required. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test IgE-binding of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals[6]. In addition, the quality of the sera and the assay procedure need to be standardized to produce a valid test result.

In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols.

The identification of a newly expressed protein as an allergen through immunological assays suggests that further development for commercialization of the product be discouraged, unless adequate risk management and risk communication measures could be assured throughout marketing and distribution of the product, since segregation and identity preservation of the new source of this allergen may be difficult or impossible to enforce.

Section 5 – Areas Requiring Further Development

The endpoint of the assessment of the data discussed above is a conclusion as to the likelihood of the protein being a food allergen. The techniques of targeted serum screening (*i.e.* the assessment of binding to IgE in sera of individuals with clinically-validated allergic responses to broadly-related categories of foods) and the use of animal models, once developed and validated, could enhance the weight of evidence used to derive this conclusion. To allow serum screening, steps should be taken to organize an international serum bank. As scientific knowledge and technology evolves, other methods, such as examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens, might also be useful.

Unintended effects on endogenous allergens

Genetic modification techniques have the potential to produce unintended effects on the genome

[6] According to the Joint Report of the FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (22-25 January 2001, Rome, Italy) a minimum of 8 relevant sera is required to achieve a 99% certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

that could lead to an increase in the expression of endogenous allergens. While the potential for health impacts of such increases is uncertain, they are in any case considered undesirable. Techniques used for assessing the potential for effects on endogenous allergen expression are the quantitative comparison of protein composition of the edible portion of the modified organism or, where sera from sufficient numbers of individuals with allergies to the food are available, the comparative immunoreactivity to the edible portion of the modified organism can be determined using immunoblotting techniques.

4.1.3.8 Chemical Considerations

The identification and levels of chemical contaminants must be reported. Potential levels and types of contaminants would, of course, be specific to the food to be modified and, also, the type of process employed to achieve the genetic modification. In this regard, contaminants could be introduced as a result of the modification of the food or could be naturally present in the food before modification. In the latter case, it would be necessary to provide a comparison of the levels of chemical contaminants in the genetically modified food with those levels typically found in the original food product. Consideration should also be given to potential contamination from residues of any chemicals employed to achieve the desired genetic modification. Examples of chemical contaminants are metals (*e.g.* arsenic, cadmium, mercury and lead) and organic contaminants (*e.g.* introduction/or increased levels of mycotoxins).

4.2 Novel Foods Derived from Microorganisms

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. Novel foods or ingredients can be derived from microorganisms not traditionally used as a food source in Canada, manufactured by new processes involving microorganisms, or produced by microorganisms that have been genetically modified by a variety of techniques.

It is recommended that the following information be included for assessing the acceptability of novel 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.

4.2.1 Substance with No History of Safe Use

- 4.2.1.1 History of use
- 4.2.1.2 Dietary exposure
- 4.2.1.3 Nutritional considerations
- 4.2.1.4 Toxicology considerations
- 4.2.1.5 Allergenicity considerations
- 4.2.1.6 Chemical considerations

4.2.2 Novel Process

- 4.2.2.1 Detail of novel process
- 4.2.2.2 Dietary Exposure
- 4.2.2.3 History of organism
- 4.2.2.4 Nutritional considerations
- 4.2.2.5 Toxicology considerations
- 4.2.2.6 Allergenicity considerations
- 4.2.2.7 Chemical considerations

4.2.3 Genetic Modification

- 4.2.3.1 Characterization of derived strain
- 4.2.3.2 Genetic modification considerations
- 4.2.3.3 History of organism (Host and Donor(s))
- 4.2.3.4 Dietary exposure
- 4.2.3.5 Nutritional considerations
- 4.2.3.6 Toxicology considerations
- 4.2.3.7 Allergenicity considerations
- 4.2.3.8 Chemical considerations

4.2.1 Substance with No Safe History of Use

Many traditional foods are considered safe even though the food may contain anti-nutrients, toxins or allergens. Some foods require special preparation or processing to manage the risks associated with a food. Foods are generally considered safe, provided that appropriate care is taken during development, production, processing, storage, handling and preparation. It is recognized that in many cases the knowledge required to manage the risks associated with foods has been acquired in the course of their long history of safe use.

Notification is required for foods new to the Canadian marketplace in order to demonstrate that they have a history of safe use. A history of safe use means significant human consumption for which there exists adequate knowledge to provide a reasonable certainty that no harm will result from the intended use of the food. In many cases, toxicological and allergenicity data may be required to demonstrate that there are no health concerns related to the food use of a product or ingredient.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.2.1.1 History of Use

A substance may be considered to have a history of safe use as a food if it has been an on-going part of the diet for a number of generations in a large, genetically diverse human population where it has been used in ways and at levels that are similar to those expected or intended in Canada. The fact that a product has had a history of use according to the above definition in a jurisdiction with a similar food safety system would increase the level of confidence in the evidence presented. The following information would be needed to support a claim that a product has a history of safe use:

- Historical evidence indicating ongoing, frequent consumption by a cross-section of the population where it has been used over several generations. This evidence may be derived from various sources including, but not limited to, scientific publications and patents, non-scientific publications and books, cookbooks, books on the history of food culture, and/or affidavits from two or more independent,

reputable authorities that include well-documented accounts of the way the food is used and how they know it has the history it does. Limited usage or short term exposure would not be adequate to demonstrate a history of safe use.

- A declaration of any possible adverse effects linked to the food documented in its country of origin and/or a country where there is a high degree of consumption.

- A description of the standard methods of commercial and/or domestic processing and preparation for consumption.

- A description of how the food is produced.

- Amounts of the food that people are likely to consume in Canada, including typical serving sizes and expected frequency of consumption, at both average and extreme high consumption levels.

- Analysis of the composition of the food based on randomly selected, statistically valid samples. This analysis should include proximate data as well as amino acid profile, fatty acid profile, mineral and trace mineral composition and vitamin composition, as well as any nutrients, antinutrients and bioactive phytochemicals known to be of particular interest in the product. The analysis should pay special attention to the presence of compounds in the food which may have implications for the health of any groups of the Canadian population (*e.g.* possible toxicants or allergens or unusually high levels of nutrients in the food source or final food product).

- Metabolism and/or gastrointestinal effects in humans.

The submission should include reliable, high quality information and reference sources. Anecdotal evidence will be given less weight than scientifically derived data. Information on the history of human exposure will be particularly important where there are traditional handling or cooking requirements for a food that is novel. This information will need to be made available to consumers in a consistent manner.

4.2.1.2 Dietary Exposure

In conducting dietary exposure assessments for foods with no history of safe use, the primary issues to be addressed as part of the safety assessment are: the contribution of significant nutrients to the diet, the presence of endogenous anti-nutrients and toxins, and the potential for the introduction of novel substances to the food supply.

The introduction of foods with no history of safe use may have nutritional, toxicological or allergenic consequences, and estimation of exposure to components of the food of significance to

health should be considered in such cases. For such foods, it may be possible to predict potential consumption patterns based on intakes of similar products routinely consumed as part of the diet. These intake estimates may then be used to calculate the potential dietary exposure to specific components of the novel food that will be the subject of the safety assessment.

4.1.2.3 Nutritional Considerations

General observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the implications of its nutritional characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts of food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods

- excessive intakes of nutrients or other bioactive substances as a result of unusually high levels in the novel food, or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional roles of non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, "bioactive" substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These roles are also included in the broad definition of nutritional quality.

Foods with no history of safe use

The main concern with respect to a food with no history of safe use would be to verify that the consumption of the food would not have an adverse effect on the nutritional health of the consumer. Information on nutritional composition and quality is primarily needed to determine how the food could be used in the diet, to establish basic composition information for the food for use in food composition databases, and to permit the validation of nutrient content claims and quantity declarations.

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a food with no history of safe use should be of sufficient quantity and quality to determine its role in the diet and to characterize the average nutritional composition of the food.

- Any studies conducted used to evaluate nutritional quality should have been performed using the food as it is expected to be consumed by humans.

b. Where published data on nutrient composition of the novel food are inadequate, analytical data may need to be obtained by the petitioner. In this case, an appropriate study design for obtaining data on nutritional quality:

- Considers all major sources of potential variation in nutritional composition, *e.g.* composition of the growing medium, fermentation conditions (temperature, pH, stage of growth), etc, in designing the experimental design and sampling methodologies.

- Subjects the novel microorganism or food containing it to the conditions expected for it in commercial production.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all potential sources of variation of nutritional quality in the food and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example to account for intra-strain variation

- Ensures sampling is conducted at the appropriate stage of production.

- Ensures that the appropriate analyses are performed on all products containing the microorganism that are expected to be used as food in Canada.

- Provides the criteria used for selecting of the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed in the Nutrient Composition section below.

- Ensures samples are analysed within an acceptable time frame from date of collection.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout.

- Uses appropriate and consistent statistical methods chosen in advance based on the study design to analyse and report the results.

c. Nutrient Composition

In the context of the above study guidelines, the following components of novel foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

- proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude carbohydrate
- 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)
- quantitative and qualitative composition of total lipids, *i.e.* saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids
- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin
- qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin and mineral analysis - see Appendix A, “Key Micronutrients”
- presence of naturally occurring or adventitious anti-nutritional factors *e.g.* phytates, trypsin inhibitors, *etc.*
- predictable secondary metabolites, physiologically active (bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutrient bioavailability/Presence of anti-nutrients

In situations where the novel microorganism or food containing it may become a significant component of the Canadian diet, and/or a significant supplier of nutrients, animal studies should be conducted to assess nutritional adequacy. This pertains in particular to the evaluation of protein quality, the possibility of unknown anti-nutrients, and nutrient bioavailability.

Information should be provided, if applicable, describing the processing conditions that would be used in the production of the novel food, and the effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- the name of the microorganism including Latin and common names;
- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled;
- a complete description of sample collection and sample preparation;
- a citation and/ or description of the analytical and statistical methods which were used to obtain data for the nutritive and non-nutritive components;
- nutrient and related data expressed as mean ± standard deviation, and as a range;
- results of statistical analyses;
- raw data for all components analysed;
- published data if available; and
- intended use(s) of the microorganism as food in Canada, *i.e.* as food itself or as an ingredient that might modify a food through culture, possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis.

- A novel food with no history of safe use is not required to meet specific criteria of nutritional quality. The main concern is to document the composition of the food in order to evaluate claims and to determine its potential role in the diet.

4.2.1.4 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that may be introduced to the food supply. For foods that have no history of safe use, it may be difficult to identify individual components which are novel in the context of human consumption in the absence of a traditional counterpart.

Where it is not possible to identify novel components of the food, a case-by-case approach should be used to determine the appropriate toxicological tests to be carried out on the food. The history of the organism from which the food is derived as a source of toxins or antinutrients and a chemical analysis of its components will be considerations in determining requirements for toxicological testing. Depending on these determinations, conventional studies of toxicity, including chronic toxicity, developmental toxicity, genotoxicity or carcinogenicity, may need to be performed on the final food product or its components as appropriate.

It should be noted that the conduct of studies with whole foods presents some challenges due to the potential for inducing nutritional imbalances when the food is incorporated into the diet at high concentrations. In addition, toxicology studies on novel foods are used to reach a conclusion as to whether the food is safe to consume under expected consumption patterns, rather than to derive a quantitative limit such as an acceptable daily intake in the manner used for simple chemicals like food additives.

4.2.1.5 Allergenicity Considerations

The primary consideration in allergenicity assessment of a novel food is the prevention of unexpected and/or unavoidable exposure of susceptible individuals to food allergens. For foods with no history of safe use, the potential exists that one or more component proteins would have the capacity to cross-react with known food allergens or lead to the development of *de novo* hypersensitivity. It should be noted, however, that the vast majority of proteins consumed in the diet are not allergenic.

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of an individual protein or of a whole food. Because existing strategies for the assessment of the allergenic potential of proteins were developed for the evaluation of individual, well-defined proteins (Section 4.1.3.7), they are not easily applied to the entire protein component of a whole food. The protein component of foods with no history of safe use will not be characterized to the extent necessary to apply these assessment strategies.

A preliminary strategy for assessing the allergenic potential of foods with no history of safe use would be to investigate whether microorganisms from the same taxonomic family that are commonly part of the food supply are implicated in the induction of allergic response. The association of a particular family of microorganisms with allergic response might not necessarily preclude the introduction of the novel food from a related species into the marketplace, but risk management measures such as post-market surveillance and labelling where identification of the food item is not obvious will need to be considered. Proteins from an allergenic source should not be added to foods where identity preservation cannot be guaranteed.

4.2.1.6 Chemical Considerations

The identification and levels of chemical contaminants must be reported. Potential contamination could occur, for instance, as a result of residues from chemicals (organic or inorganic) employed in processes, such as extraction or purification processes, to produce the desired food product from microorganisms.

4.2.1.7 Microbiological Considerations

For novel microorganisms, petitioners should address the following criteria:

a) Strain Identification

The accurate identification of a strain will provide important information for the safety assessment of microorganisms and/or their products. A microbial strain should have an appropriate taxonomic designation following international codes of nomenclature and

standard taxonomic sources. The taxonomic designation should be provided to a level that distinguishes the microorganism from pathogenic species. In the event the identification is not conclusive, additional data may be required to address the safety of a microorganism.

In general, the methods used to identify an organism should be consistent with methods currently used in microbial taxonomy. A taxonomic designation should be accompanied by a list of the tests used to arrive at the designation, with the results and any other information used to make the designation. A brief description of the type of tests used, or references, should be provided.

b) Pathogenicity

The potential of a viable microorganism in a food product to have adverse effects on human health must be considered. Adverse effects would include, but not be limited to, infection, disease, adverse immunologic reactions and toxicosis. While information from a review of the scientific literature is sufficient to satisfy this information requirement to address these points, petitioners should search various sources for information on the human health effects of a microorganism (databases, regulatory authorities, *etc.*). The search should provide information that would give a complete and thorough overview of any known involvement of a microorganism in an adverse health effect or the lack of any documented adverse health effects caused by a microorganism. In some cases, further testing may be required to address the pathogenic potential of an organism.

c) Antimicrobial Production

Information should be provided on the production of antimicrobial compounds by a microorganism or its close relatives. These include classical antibiotics and other antimicrobials such as bacteriocins. The significance of these compounds in relation to clinically important antimicrobials will be considered. Introduction of microorganisms into the food chain which carry resistance factors to clinically important antibiotics must be avoided.

d) Production/Specifications

Microbial specifications for assuring microbial safety and data demonstrating compliance with these specifications should be provided for a number of production batches. The identification and levels of microbial contaminants must be reported. A food grade fermentation would be expected to yield a pure culture without microbiological contamination prior to down stream processing. However with traditional technologies,

microbial contaminants could be present in the culture and must be identified to demonstrate they are not of safety concern. Certificates of analysis for indicator organisms should be provided to demonstrate microbial safety. Documentation on the quality control of the manufacturing process should be provided, including a description of the manufacturing process and control measures that are applied to ensure quality and prevent microbial contamination.

4.2.2 Novel Process

Some processes applied to foods or food ingredients may result in the generation of foods which would be considered novel in relation to traditional counterparts. The application of new processes which cause a food to undergo a major change would trigger the requirement to notify Health Canada under the *Novel Foods Regulation*. A major change is defined in Division 28 of the Regulations as a change in a food that, based on the manufacturer's experience or generally accepted nutritional or food science theory, places the food outside the accepted limits of natural variations for that food with regard to; the composition, structure or nutritional quality of the food or its generally recognized physiological effects; the manner in which the food is metabolized in the body; or the microbiological safety, the chemical safety or the safe use of the food. Examples of novel processes include: new heat processing techniques; new packaging technologies; the use of ultraviolet light for reducing the microbial load of a product.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.2.2.1	Details of novel process
4.2.2.2	Dietary Exposure
4.2.2.3	History of organism
4.2.2.4	Nutritional considerations
4.2.2.5	Toxicology considerations
4.2.2.6	Allergenicity considerations
4.2.2.7	Chemical considerations

4.2.2.1 Details of Novel Process

While the focus of the safety assessment is on the food product, consideration of the process or preparation of the product can guide the safety assessment. Any novel processing or preparation techniques used to produce a novel food should be described in sufficient detail since such processing or preparation may result in potential microbiological, toxicological, allergenicity, or nutritional concerns.

4.2.2.2 Dietary exposure

In conducting dietary exposure assessments for novel foods resulting from the application of a novel process, the primary issues to be addressed as part of the safety assessment are: the potential for alteration of nutrient content of the food, and the potential for introduction of novel substances to the food supply.

In cases where the novel process results in the intentional or unintentional alteration of nutrient composition of the food, changes to nutrient intake should be determined for the food itself and in the context of the food as a source of the nutrient in the total diet. Variation of dietary patterns in subgroups of the population (*e.g.* children, infants, elderly, ethnic groups) as well as the potential for change in use and/or exposure to the food compared with the related, traditional food product should be taken into consideration.

If a process applied to a food results in the generation of predictable breakdown products, their amount in the food and the contribution of that food to the diet should be determined.

4.2.2.3 History of Organism(s)

The history of an organism can provide information that is important to the assessment of a novel food. There may be a history of toxin production by certain strains, species or genera and it would be important in such cases to examine the particular strain of the organism being used for the potential to produce such toxins, both under the conditions used in normal manufacturing and also under extreme conditions.

4.2.2.4 Nutritional Considerations

I Unintended nutritional effects

General Observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the potential implications of its nutritional quality characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts, or the elderly who consume small amounts. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods

- excessive nutrient intakes as a result of unusually high levels of a given nutrient, or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional effects of non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, "bioactive" substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These intrinsic bioactive substances are also included in the broad definition of nutritional quality.

Application of novel process to microorganisms

Microorganisms constitute a minor component of foods in the Canadian diet. The use of single cell protein is rare. Therefore, it is very unlikely that a change in the microorganisms that are currently in foods would have a direct impact on the nutritional quality of foods and diets. There are two ways, however, that a microorganism in a food could have an impact on the nutritional quality of the food or diet and in turn on the health of the consumer. One way is that microorganisms can have a significant indirect impact on the nutritional quality of foods that they are in. For example, the use of yeast to leaven bread reduces the phytate content which makes the minerals more available for intestinal absorption. The yeast also produces B vitamins in sufficient quantities to significantly affect the content of some of the B vitamins, for example folate, in bread. The other way that a microorganism in a food can have an impact on health is potentially as a "probiotic". Probiotics are thought to be able to populate or alter the population of bacteria in the large intestine and as a result have various beneficial effects on the health of the intestine and the individual.

The development of novel forms of microorganisms through application of a novel process could result in intended or unintended changes in the composition of the food product. This could in turn have an impact on the nutritional value of the food and the nutritional status of the persons consuming it.

Unintended nutritional effects can occur whether the novel process applied to the microorganism is intended for nutritional or functional or other reasons. Evaluation of a microorganism, which was produced using a novel process, intended to affect the nutritional quality of the microorganism or the food of which it is part is discussed in Part II of this section. Thus, discussion of probiotic aspects of microorganisms is limited to that part.

An important step in the safety and nutritional assessment of this type of novel food is a comparison of its composition with its appropriate counterpart. In the case of a novel microorganism (*i.e.* the microorganism which was produced using a novel process), this could apply to the microorganism itself in the event that it constitutes a significant portion of the food mass but it is more likely to apply to the food containing the novel microorganism. To determine whether there are any differences in the nutritional quality of the food containing the novel microorganism compared to its appropriate counterpart, the microorganism should first be subject to laboratory testing of the metabolic products of the microorganism in controlled media. Once into the food production trial phase, the major constituents of the food containing the microorganism must be analysed, *i.e.* macronutrients and their component parts, as well as individual micronutrients selected based on validated criteria. If any nutrients (in the list below) are excluded from the analyses, this should be justified by an acceptable rationale.

Also, circumstances may warrant an evaluation of the nutritional "performance" of the new food in its ready-to-eat form, thus either raw or when processed by traditional/conventional methods used to make the product ready-to-eat. The purpose would be to provide an opportunity to identify major changes that may not have been detected by compositional analysis, but which could affect, for example, the stability or bioavailability of nutrients in the food or the susceptibility of anti-nutrients to further processing that normally destroys them. A performance test could involve re-analysis of a substance following cooking or it could require animal testing for satisfactory growth and nutrient bioavailability .

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a novel microorganism food or for a food containing one should be of sufficient quantity and quality to allow an assessment of whether any significant unintended effect on the nutritional quality of the food has occurred as a result of the introduction of the application of the novel process on the food. It should also allow an assessment of the nutritional significance of any change that is detected.

- Data should be provided for the novel microorganism food or for the food containing one, before further processing. Data may also be required for the food prepared for human consumption by conventional means to examine the effects,

where applicable, of further processing, storage and cooking, for example, to look at the effectiveness of cooking to destroy anti-nutrients in cases where anti-nutrients normally destroyed by cooking are present.

- Data on the novel microorganism food or for the food containing one, should be compared, at a minimum, to data on the most appropriate counterpart (see section b, below). Literature data (if available) may also be valid for assessing the nutritional relevance of any unintended effect.

b. Where published data on nutrient composition of the novel food are adequate, analytical data may need to be obtained by the petitioner. In this case, an appropriate study design for obtaining data on nutritional quality:

- Considers all major sources of potential variation in nutritional quality (*e.g.* composition of the growing medium, production conditions, processing conditions, etc) in designing the study, to ensure these factors are controlled.

- Subjects the novel microorganism or food containing it to the conditions expected for it in commercial production.

- Includes in the same study the novel microorganism that is the subject of the notification as well as the appropriate counterpart, *i.e.* a) the microorganism food/ food containing the microorganism, where the microorganism component was prepared using an equivalent commercial process, (ie. A process which is not novel, and which is currently used to achieve the same or similar effect), if available, or where a) is not applicable, b) the same microorganism food which is commercially available, or the same food which is commercially available, without a microorganism component.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all major sources of variation of nutrient levels and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example, to account for inter-strain variability.

- Ensures processing is conducted at the appropriate stage of production for the microorganism, and that sampling is conducted at the appropriate stage of processing for the novel organism or the food containing the novel organism.

- Ensures that the appropriate analyses are performed on all products containing the microorganism that are expected to be used as food in Canada.

- Provides the criteria used for selection of the nutrients analysed and the rationale

for the exclusion from analysis of any nutrients and other substances listed in the following section entitled “Nutrient Composition”.

- Ensures samples are analysed within an acceptable time frame from date of collection.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout.

- Uses appropriate and consistent statistical methods chosen in advance based on the study design to compare levels of each nutrient in the novel food versus its controls.

c. Nutrient Composition

In the context of the above study guidelines, the following components of foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

- proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude carbohydrate
- 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)
- quantitative and qualitative composition of total lipids, *i.e.* saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids
- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin
- qualitative and quantitative composition of micronutrients, *i.e.* complete vitamin and mineral analysis
- presence of naturally occurring or adventitious anti-nutritional factors *e.g.* phytates, trypsin inhibitors, *etc.*
- predictable secondary metabolites, physiologically active (or bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as

proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutritional "Performance" of novel microorganism

Consideration should be given to the possible need for the following types of information regarding the novel microorganisms or the foods containing them:

- Response of known anti-nutrients to processes normally expected to neutralize their activity measured using compositional analysis.

- Storage stability with regard to nutrient degradation.

- Performance of product in relation to the intended benefit (other than direct health benefits) *e.g.* improved stability of an oil to heating after fatty acid profile modification.

Nutrient bioavailability/Presence of new or altered anti-nutrients

In situations where the novel food may become a significant component of the Canadian diet, and/or a significant supplier of nutrients, animal studies may be needed in assessing nutritional adequacy to determine if there have been changes in the bioavailability of nutrients or if the composition is not comparable to conventional foods.

Information should be provided, if applicable, describing the conditions used in the further processing of the novel food and its derivatives, and the potential effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- a full description of the novel process, the purpose of the process, and the microorganism(s) on which it could be applied, and the microorganism(s) on which it will be applied (for the purpose of the submission);

- the microorganism(s) on which the test and control processes were applied in the study, and the names and sources of all the strains which were represented in the study;

- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled;

- a complete description of sample collection and sample preparation;

- a citation and/ or description of the analytical and statistical methods which were used to obtain data for the nutritive and non-nutritive components;

- nutrient and related data for test, control, and commercial strains (expressed as mean ± standard deviation, and as a range);

- results of statistical analyses;

- raw data for all components analysed;

- published data if available; and

- intended use(s) of the novel microorganism as food in Canada, *i.e.* as food itself or as an ingredient that might modify a food through culture, possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- "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" (Codex)[7]. If the composition of the novel food is judged not to be nutritionally equivalent to that of its counterpart(s), *i.e.* significant differences (statistical and biological) exist in the nutrient data, then additional nutritional data may be required on a case-by-case basis.

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

- Detection of a major change due to an unintended nutritional effect may not

[7]"Codex Alimentarius Commission", Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology", 3rd Session: Yokohama, Japan 4-8 March 2002: Consideration of Proposed Draft Guideline for the Conduct of Food Safety Assessment of Recombinant-DNA Microorganisms in Food *At Step 4"*; page 13

preclude the marketing of the product. However, such changes may require limits on the use of the food in food products or a requirement for labelling that goes beyond basic provisions.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or nutritive substances, the food may need to be subjected to further analysis and assessment.

- The safety of a major increase in the level of a nutrient or other bioactive component would need to be assessed in a similar way to the safety assessment of an intended nutritional change. For details on this see Part II, below.

II Intended nutritional modifications

The term "intended nutritional modification" is taken to include any change or introduced trait intended to improve the nutritional quality or health-related profile of the food, including but not limited to both essential nutrients and beneficial phytochemicals, quantities and nature of the energy-yielding substances, improved nutrient bioavailability, improved probiotic function and reduction in anti-nutrient levels.

Evaluation of an intended nutritional change requires steps that are similar to those used in either the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health claims or both. For instance, such a change would trigger questions concerning the intended target group, what level of the targeted nutrient or other bioactive substance is expected in the food, what is the expected change in level of exposure to the targeted nutrient or other bioactive substance across all age and sex groups and at the upper and lower extremes of intake of the food, and the safety of this level of exposure.

A novel food with an introduced health or nutritional benefit would likely fall into the unofficial category of "functional food". It is expected that manufacturers will be interested in making health claims for these products. These products would therefore be evaluated in accordance with the criteria being laid out for foods with product-specific health claims. These include attention to the evidence in support of the claim, as well as to product safety and product quality considerations.

Product safety of this type of novel food is intended to be controlled through application of the novel food regulations. The safety evaluation of a microorganism or of a food containing a microorganism, where the microorganism was subjected to a novel process, which resulted in the food having an intended nutritional modification (*i.e.* novel food), should cover the same aspects as for other novel foods. With regard to the safety and nutritional evaluation of the intended nutritional modification, itself, data requirements are described below.

At this time, regulations for product-specific health claims have not yet been promulgated. Prospective petitioners should refer to the proposed regulatory framework for product-specific health claims which was published in November, 2001, and the Interim Guidance Document on Standards of Evidence which was published in February, 2002. These are both available on the Health Canada web site at: http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html.

It is important to ascertain to what extent the intended nutritional effect of a novel process remains stable with cultivation, time, further processing, storage and cooking.

The review of unintended nutritional effects in a novel microorganism or a food containing a novel microorganism, *i.e.* where a novel process was applied on the microorganism for the purpose of having an intended nutritional effect would follow the same steps as for other novel foods.

Nutritional Evaluation of expected or unexpected increased levels of a nutrient or bioactive substance

- Increased levels of a nutrient or other bioactive substance (including a microorganism) in a food need to be evaluated for safety.

- Data needed for this include:

  - the level of the targeted nutrient or other bioactive substance expected in the food;

  - intended target group, if applicable, or which group(s) is or are likely to consume the most of the food;

  - expected level of exposure to the substance through consumption of the food by the target group, by vulnerable sub-groups, and at the upper and lower extremes of intake of the food and across all age and sex groups using recent Canadian food consumption data where possible;

  - how the expected level of dietary exposure to the targeted nutrient or other substance differs from the current levels of exposure from all sources;

  - data in support of the safety of the expected level of exposure.

4.2.2.5 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that are introduced to the food supply. The application of novel processes to foods may result in the generation of novel substances in the resulting food be intentional or unintentional. Because of the potential wide variety of products generated by the application novel processes as determination of the appropriate toxicological testing should be conducted on a case-by-case basis.

Identification of any novel substances generated in the food subjected to a novel process is assisted by the use of the unprocessed food as a comparator. Chemical analysis may provide information on any new substances that have been formed. In addition, information on the nature, duration and intensity of treatment and the chemical composition of the food may be useful in predicting the types of alterations to the food components**.** Depending on these determinations, conventional studies of toxicity, including assays of metabolism, toxicokinetics, chronic toxicity/carcinogenicity, impact on reproductive function, and teratogenicity, may need to be performed on the final food product or its components as appropriate.

Intentional alteration of the composition of foods by the addition of food components at levels that fall outside the accepted limits for natural variations (*e.g.* "functional" foods) may result in exposures for which there is no history of safe use. Substances that have been traditionally consumed in foods but which have been added to foods at levels outside their normal range will result in consumption of higher amounts of the substance than from a traditional diet. In such cases, the novel aspect of the food is the extent of exposure to the substance, rather than the substance itself, and toxicological testing of the enhanced component will be required to establish an upper limit of tolerability to the substance. The types of studies conducted should be guided by a knowledge of the role of the component in human physiology. Evidence from animal and *in vitro* studies as indicated in the previous paragraph would be required to determine safety. Studies in experimental animals may be of limited usefulness if the commonly used animal model (*i.e.* the rat) differs markedly from humans in the metabolic pathways and chronic conditions that are the basis of the intended functional effect, and it may be necessary to place greater reliance on human response to increased intakes of such food components. Epidemiologic studies may be available for substances that are normally components of foods, and these can provide important information on long-term effects.

4.2.2.6 Allergenicity Considerations

The primary consideration in allergenicity assessment of a novel food is the prevention of unexpected and unavoidable exposure of sensitized individuals to food allergens. In cases where the application of a novel process to a food results in the generation of a novel protein or an alteration of the protein content of a food containing allergenic proteins, a consideration of the allergenic potential of the novel food would be required.

Novel Proteins

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of an individual protein or of a whole food. If the application of a novel process to a food results in the generation of a novel protein that can be isolated and characterized, the assessment strategy that has been developed for foods which are the products of recombinant DNA technology and described in section 4.1.3.7 can be used to assess its potential allergenicity. This strategy involves a weight of evidence approach that relies on the assessment of amino acid sequence homology to known food allergens, and a consideration of the similarity of its properties, in particular, resistance to digestion in the mammalian gastrointestinal tract, to those of known food allergens.

Alteration of endogenous allergen content

If the application of a novel process to a food that contains allergenic proteins results in altered protein content of that food, the potential for increase in the allergenic content should be assessed. While the health impacts of such increases is uncertain, this result would be considered undesirable. Techniques used for assessing the potential for effects on endogenous allergen expression are: the quantitative comparison of protein composition of the edible portion of the modified organism or, where sera from sufficient numbers of individuals with allergies to the food are available, the comparative immunoreactivity to the edible portion of the modified organism can be determined using immunoblotting techniques.

4.2.2.7 Chemical Considerations

The identification and levels of chemical contaminants must be reported. Potential contamination could occur, for instance, as a result of residues from chemicals (organic or inorganic) employed in processes, such as extraction or purification processes, to produce the desired food product from microorganisms.

4.2.3 Genetic Modification

Microorganisms referred to in this section 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 traditional selection techniques (spontaneous mutation, selective pressures) and 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.

The data to be submitted are to include, but are not necessarily 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.

The safety assessment of novel foods in this category follows a stepwise process of addressing relevant factors that include:

4.2.3.1	Characterization of derived strain
4.2.3.2	Genetic modification considerations
4.2.3.3	History of organism (Host and Donor(s))
4.2.3.4	Dietary exposure
4.2.3.5	Nutritional considerations
4.2.3.6	Toxicology considerations
4.2.3.7	Allergenicity considerations
4.2.3.8	Chemical considerations

4.2.3.1 Characterization of Derived Strain

Where a microorganism has been modified, whether by selection and mutagenesis techniques or by recombinant nucleic acid technology, the relationship of the derived strain with the parent organism(s) should be characterised. The approach of the safety assessment is based on the principle that the safety of novel products is assessed relative to a conventional counterpart having a history of safe use, taking into account both intended and unintended effects. Any significant differences between the novel and the conventional strain are then assessed for potential adverse health effects. Of particular interest to the safety assessment is whether the modification could inadvertently develop or increase the pathogenicity, toxicity, or allergenicity potential of an organism.

4.2.3.2 Genetic Modification Considerations

Genetic Modification by Traditional Techniques

Many non-recombinant nucleic acid modification procedures are relatively undefined and poorly characterized in terms of insertion, deletion or rearrangement of genetic material. Strain selection and mutagenesis techniques can influence the toxin-producing capacity of an organism and may also influence the expression of antimicrobial compounds or other substances not present in food.

For microorganisms derived through classical mutagenesis and selection techniques, information should be provided to fully characterize the novel strain that enables a comparison with the parent organism(s). This characterization will include details of the methods used to modify the organism and a phenotypic and genotypic comparison of the parents and donors, as appropriate. New or altered traits and characteristics acquired and expressed should be described. A comparison of the biological activity, growth and physiological characteristics of the novel microorganism to the parent apart from the intended modification should be performed. In all cases, the degree of exposure to the modified microorganism or its products will be an important factor in determining the extent of the data required for the safety assessment (dietary exposure considerations).

Traditionally modified microorganisms require a multi-disciplinary assessment since details of the modifications may be largely unknown. As experience in the safety assessment of novel foods develops, it may be possible to more clearly identify data requirements for particular groups of products or to preclude certain products from further detailed evaluation.

Genetic Modification by Modern Techniques

In cases where a microorganism has been modified using modern genetic techniques, such as recombinant nucleic acid technology, the safety assessment will consider detailed characterization data of a novel food at the molecular level. The following requirements are based on harmonization efforts with other regulatory authorities and reflects international guidance documents in this area (Codex Alimentarius). In addition to the requirements of previous sections, the following areas should be addressed for these types of products:

i) Description of the genetic modification(s)

Details of all methods and manipulations involved in the modification of an organism must be provided to allow for the identification of all genetic material potentially inserted, deleted, mutated, or rearranged in the host genome. This will provide the necessary information for the analysis of the data supporting the characterization of the

modified organism.

The description of the modification process should include:

- information on the method(s) of modification used, *e.g.* conjugation, electroporation, *etc.*;

- description and characterization of all genetic material potentially delivered, if applicable, including the source, identity, expected function in the organism, and copy number for plasmids; and

- details of manipulations or modifications to introduced, intermediate and recipient genetic material.

Information should be provided on DNA added, inserted, deleted, or modified, including:

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

- the size and identity;

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

- function in the organism.

A summary diagram, outlining the key features of the final construct, should be provided. Depending on the nature of the genetic modification, restriction maps and sequence data of the introduced or modified genetic material and adjacent regions, may be required.

ii) Characterization of the genetic modification(s)

In order to provide clear understanding of the impact on the composition and safety of foods derived from genetically modified microorganisms, a comprehensive molecular and biochemical characterization of the organism should be carried out.

Information should be provided on the DNA insertions into the genome; this should include:

- the characterization and description of all inserted, deleted, or otherwise modified genetic materials;

- the number of insertion sites;

- data to demonstrate if complete or partial copies have inserted into the genome;

- data to demonstrate whether the arrangement of the genetic material used for insertion has been conserved or whether significant rearrangements have occurred upon integration;

- the organization of the inserted genetic material at each insertion site including copy number and sequence data of the inserted material and, where appropriate, of surrounding region;

- identification of any open reading frames within the inserted DNA or created by the insertions with contiguous DNA in the chromosome or in a plasmid, including those that could result in fusion proteins; and

- in the case of modifications that involve deletions, rearrangements or site-specific, *in vitro* mutagenesis, sequence data of the region before and after modification should be provided.

Information should be provided on any expressed substances in the modified organism; this should include:

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

- the gene product's function;

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

- the level and site of expression of the gene product(s), and the levels of its metabolites;

- 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;

- where genetic manipulations are directed to altered regulation of endogenous genes, the characteristics and level of gene expression should be compared with that of the unmodified host;

- to indicate whether there is any evidence to suggest that one or several endogenous genes in the host plant has been affected by the modification process;

- to confirm the identity and expression pattern of any new fusion proteins;

- to demonstrate the intended effect of the modification has been achieved and that all expressed 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 itself or the expression of the corresponding RNA if the phenotypic characteristics cannot be measured directly; and

- to demonstrate that the newly expressed trait(s) are expressed as expected in the appropriate cellular location or is secreted in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene.

4.2.3.3 History of Organism(s)

The history of both donor and host organisms can provide information that is important to the assessment of a novel food. There may be a history of toxin production by certain strains, species or genera and it would be important in such cases to examine the particular strain of the organism being used for the potential to produce such toxins, both under the conditions used in normal manufacturing and also under extreme conditions.

The following detailed information should be provided:

- taxonomic designation of the microorganism to the species level and where applicable, to include subspecies and strains, accompanied by technical data substantiating 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;

- pathogenicity of genus and species;

- evidence pertaining to the potential for production of any toxic compounds and antibiotics; and

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

4.2.3.4 Dietary Exposure

In conducting dietary exposure assessments for novel foods produced through genetic modification, the primary issues to be addressed as part of the safety assessment are: the potential for alteration of nutrient content of the food, and the potential for introduction of novel substances to the food supply.
In cases where the nutrient composition of foods has been altered, either intentionally or through genetic modification, changes to nutrient intake should be determined for the food itself and in the context of the food as a source of the nutrient in the total diet. Variation of dietary patterns in subgroups the population (*e.g.* children, infants, elderly, ethnic groups) as well as the potential for change in use and/or exposure to the food compared with the related, traditional food product should be taken into consideration.

For foods produced from genetically-modified microorganisms, that result in the introduction of a novel protein or novel metabolites to the food supply, their content should be determined and considered together with the toxicological data as part of the safety assessment. The effects of typical food processing procedures on the novel component(s) should be considered in deriving the exposure estimate.

4.2.3.5 Nutritional Considerations

I Unintended nutritional effects

General Observations

The introduction of a novel food into the Canadian food supply requires a determination of nutritional quality of the food and the implications of its nutritional characteristics for the population as a whole and/or for specific subgroups. Population subgroups may be more vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with particular metabolic characteristics, adolescents and others who may consume large amounts of food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in order to ensure that the nutritional status of consumers is not likely to be jeopardized by:

- substitution of foods and food ingredients of significant nutritive value with less nutritious varieties of the same or similar foods;

- excessive intakes of nutrients or other bioactive substances as a result of unusually high levels in the novel food; or

- new or increased levels of anti-nutrients that could adversely affect the nutritional value of the food or the diet.

What is nutritional quality?

Nutritional quality as applied to food is related to the presence of essential nutrients and energy-yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally considered as part of the science of nutrition. These aspects include the nutritional effects of non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient bioavailability and nutrient interactions with other nutrients, with food additives and with natural toxicants. They also include nutrient excesses and the effects (both positive and negative) of food processing on the nutrients and on the organoleptic properties of the food. More recently, "bioactive" substances found principally in plants are being shown to have a possible role to play in improving or protecting human health. These substances are also included in the broad definition of nutritional quality.

Genetically modified microorganisms

Microorganisms constitute a quantitatively minor component of foods in the Canadian diet. The use of single cell protein as a food ingredient is rare. For most foods containing microorganisms, a change in the microorganisms would be unlikely to have a significant direct impact on the nutritional quality of foods and diets. There are two other ways, however, that a microorganism in a food could have an impact on the nutritional quality of the food or diet and in turn on the health of the consumer.

One way is that microorganisms can have a significant indirect impact on the nutritional quality of foods that they are in. For example, the use of yeast to leaven bread reduces the phytate content which makes the minerals more available for intestinal absorption. The yeast also produces B vitamins in sufficient quantities to significantly affect the content of some of the B vitamins, for example folate, in bread. The other way that a microorganism in a food can have an impact on health is potentially as a "probiotic". Probiotics are thought to be able to populate or alter the population of bacteria in the large intestine and as a result have various beneficial effects on the health of the intestine and the individual.

Therefore, the development of novel forms of microorganisms that are used in food through genetic modification, whether by traditional selection methods, mutagenesis or recombinant DNA techniques, could result in intended or unintended changes in the composition of the food product which could in turn have an impact on the nutritional value of the food and the nutritional status of the persons consuming it. As more complex or layered genetic modifications are attempted through rDNA techniques, for instance to introduce both improved nutritional traits and functional traits into the same organism, these could increase the potential for unintended effects compared to simpler modifications. By the same token, other methods of genetic modification could also introduce multiple changes.

Unintended nutritional effects can occur whether the intended modification of the microorganism is nutritional or functional or something else. Evaluation of a modification of a microorganism intended to affect the nutritional quality of the microorganism or the food of which it is part is discussed in Part II of this section. Thus, discussion of probiotic aspects of microorganisms is limited to that part.

An important step in the safety and nutritional assessment of the modified food is a comparison of its composition with its appropriate counterpart. In the case of a modified microorganism, this could apply to the microorganism itself in the event that it constitutes a significant portion of the food mass but it is more likely to apply to the food containing the modified microorganism. To determine whether there are any differences in the nutritional quality of the food containing the modified microorganism compared to its appropriate counterpart, the microorganism should first be subject to laboratory testing of the metabolic products of the microorganism in controlled media. Once into the food production trial phase, the major constituents of the food containing the microorganism must be analysed, *i.e.* macronutrients and their component parts, as well as individual micronutrients selected based on validated criteria. If any nutrients (in the list below) are excluded from the analyses, this should be justified by an acceptable rationale.

Also, circumstances may warrant an evaluation of the nutritional "performance" of the new food in its ready-to-eat form, thus either raw or when processed by traditional/conventional methods used to make the product ready-to-eat. The purpose would be to provide an opportunity to identify major changes that may not have been detected by compositional analysis, but which could affect, for example, the stability or bioavailability of nutrients in the food or the susceptibility of anti-nutrients to processing that normally destroys them. A performance test could involve re-analysis of a substance following cooking or it could require animal testing for satisfactory growth and nutrient bioavailability.

Guidelines for Producing Data for Nutritional Evaluation

a. Function of the data to be submitted

- The information provided for a novel microorganism food or for a food containing one should be of sufficient quantity and quality to allow an assessment of whether any significant unintended genetic modification affecting the nutritional quality of the food has occurred as a result of the introduction of the novel trait. It should also allow an assessment of the nutritional significance of any change that is detected.

- Data should be provided for the novel microorganism food or for the food containing one, before further processing. Data may also be required for the food prepared for human consumption by conventional means to examine the effects, where applicable, of processing, storage and cooking, for example, to look at the

effectiveness of cooking to destroy anti-nutrients in cases where anti-nutrients normally destroyed by cooking are present

.

- Data on the novel food should be compared, at a minimum, to data on the near isogenic, non-transgenic parent strain, *i.e.* the most appropriate counterpart, if available, or else a closely related non-transgenic strain. Since one or more significant differences could arise, the study design should include strains of the same species from a range of standard strains that are used in commercial production for the same purposes and, possibly, at a variety of production plants in Canada. This would permit assessment with respect to normal variation. Literature data (if available) may also be valid for assessing the nutritional relevance of any unintended effect.

b. Where published data on nutrient composition of the novel food are inadequate, analytical data may need to be obtained by the petitioner. In this case, appropriate study design for obtaining data on nutrient composition:

- Considers all major sources of potential variation in nutritional quality (*e.g.* composition of the growing medium, incubation conditions, *etc.*) in designing the study, to ensure these factors are controlled.

- Subjects the modified microorganism or food containing it to the conditions expected for it in commercial production.

- Includes in the same study the novel microorganism that is the subject of the notification as well as the appropriate counterpart, *i.e.* the near isogenic, non-transgenic parent strain, if available, and a selection of the commercial strains available in the current market. In the absence of a near isogenic parent strain, the most closely related non-transgenic strain may be chosen.

- Establishes a sampling plan prior to the commencement of the study. This plan should account for all major sources of variation of nutrient levels and use standard statistical methods for determining numbers of samples to collect and the appropriate method for collecting and compositing, for example to account for inter-strain variation.

- Ensures sampling is conducted at the appropriate stage of incubation.

- Ensures that the appropriate analyses are performed on all products containing the microorganism that are expected to be used as food in Canada.

- Provides the criteria used for selecting of the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed in **c.**

Nutrient Composition below.

- Ensures that analyses for each nutritive or non-nutritive component are conducted for all samples by a single laboratory using internationally approved and validated analytical methods and following consistent and appropriate sample storage and preparation procedures throughout.

- Ensures samples are analysed within an acceptable time frame from date of collection.

- Uses appropriate and consistent statistical methods chosen in advance based on the study design to compare levels of each nutrient in the novel food versus its controls.

c. Nutrient Composition

In the context of the above study guidelines, the following components of foods should be analysed. Where not all are analysed, the petitioner should provide the criteria used to select the nutrients analysed and the rationale for the exclusion from analysis of any nutrients and other substances listed below.

- proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude carbohydrate
- 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)
- quantitative and qualitative composition of total lipids, *i.e.* saponifiable and nonsaponifiable components, complete fatty acid profile, phospholipids, sterols, cyclic fatty acids and known toxic fatty acids
- composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins, non-starch polysaccharides and lignin
- qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin and mineral analysis - see Appendix A, “Key Micronutrients”
- presence of naturally occurring or adventitious anti-nutritional factors *e.g.* phytates, trypsin inhibitors, *etc.*
- predictable secondary metabolites, physiologically active (or bioactive) substances, other detected substances

"Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional analytical methods would be appropriate. When more advanced techniques such as proteomics and metabolomics become available and are validated for use, these should be adopted for this purpose.

d. Nutritional "Performance" of a modified microorganism

Consideration should be given to the possible need for the following types of information regarding the modified microorganism or the foods containing them:

Response of known anti-nutrients to processes normally expected to neutralize their activity measured using compositional analysis.

Storage stability with regard to nutrient degradation.

Performance of product in relation to the intended benefit (other than direct health benefits) *e.g.* improved stability of an oil to heating after fatty acid profile modification.

Nutrient bioavailability/Presence of new or altered anti-nutrients

In situations where the food from a genetically modified source may become a significant component of the Canadian diet, and/or a significant supplier of nutrients, animal studies may be needed in assessing nutritional adequacy to determine if there have been changes in the bioavailability of nutrients or if the composition is not comparable to conventional foods.

Information should be provided, if applicable, describing the processing conditions used in the production of a food, and the potential effects of the processing on nutrient levels and nutrient bioavailability.

e. Information to include in the submission:

- the names of all the strains which were represented in the study;
- a complete description of the experimental design, experimental conditions, and how sources of variation for nutrient levels were controlled;
- a complete description of sample collection and sample preparation;
- a citation and/ or description of the analytical and statistical methods which were used to obtain data for the nutritive and non-nutritive components;
- nutrient and related data for test, control, and commercial strains (expressed as mean ± standard deviation, and as a range);
- results of statistical analyses;

- raw data for all components analysed from all test sites;

- published data if available; and

- intended use(s) of the microorganism as food in Canada, *i.e.* as food itself or as an ingredient that might modify a food through culture, possible end products, level of use if different from current products which it would replace, known patterns of use and consumption of the food and its derivatives.

f. Decision-making process

- "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" (Codex)[8]. If the composition of the novel food is judged not to be nutritionally equivalent to that of its parent and commercial varieties, *i.e.* significant differences (statistical and biological) exist in the nutrient data, then additional nutritional data may be required on a case-by-case basis.

- All aspects of nutritional quality will be evaluated based on modern nutritional principles, standards and guidelines aimed at meeting human nutritional needs. The bases of evaluation include: nutrient intake recommendations, the role of the food in the diet of the population and the role of diet and nutrition in reducing the risk of developing a diet-related disease and health promotion.

- Detection of a major change due to an unintended nutritional effect may not preclude the marketing of the product. However, such changes may require limits on the use of the food in food products or a requirement for labelling that goes beyond basic provisions. See also Part II with respect to safety assessment of high levels of nutrients or bioactive substances.

- The first phase of nutritional evaluation will be based on the nutrient composition data. If there is a finding of unusual or unanticipated components or levels of nutrients or bioactive substances, the food may need to be subjected to further analysis and assessment.

[8]"Codex Alimentarius Commission", Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology", 3rd Session: Yokohama, Japan 4-8 March 2002: Consideration of Proposed Draft Guideline for the Conduct of Food Safety Assessment of Recombinant-DNA Microorganisms in Food *At Step 4"*; page 13

- The safety of a major increase in the level of a nutrient or other bioactive component would need to be assessed in a similar way to the safety assessment of an intended nutritional change. For details on this see Part II, below.

II Intended nutritional modifications

The term "intended nutritional modification" is taken to include any change or introduced trait intended to improve the nutritional quality or health-related profile of the food, including but not limited to essential nutrients, beneficial bioactive phytochemicals, quantities and nature of the energy-yielding substances, improved nutrient bioavailability, and reduction in anti-nutrient levels.

Evaluation of an intended nutritional change requires steps that are similar to those used in either the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health claims or both. For instance, such a change would trigger questions concerning the intended target group, what level of the targeted nutrient or other bioactive substance is expected in the food, what is the expected change in level of exposure to the targeted nutrient or other bioactive substance across all age and sex groups and at the upper and lower extremes of intake of the food, and the safety of this level of exposure.

A novel food with an introduced health or nutritional benefit would likely fall into the unofficial category of "functional food". It is expected that manufacturers will be interested in making health claims for these products. These products would therefore be evaluated in accordance with the criteria being laid out for foods with product-specific health claims. These include attention to the evidence in support of the claim, as well as to product safety and product quality considerations.

Product safety of this type of novel food is intended to be controlled through application of the novel food regulations. The safety evaluation of a novel food genetically modified to have an intended nutritional modification should be the same as for other genetically modified foods. With regard to the safety and nutritional evaluation of the intended nutritional modification itself, data requirements are described below.

At this time, regulations for product-specific health claims have not yet been promulgated. Prospective petitioners should refer to the proposed regulatory framework for product-specific health claims which was published in November, 2001, and the Interim Guidance Document on Standards of Evidence which was published in February, 2002. These are both available on the Health Canada web site at:
http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html.

Adding a substance through genetic modification differs from adding one through applying it to or mixing it with the food after it is harvested. The decision to proceed with or cease the addition would take place at different stages of production. This could have an effect on the ability to

manage the presence of the "added" substance or trait in the food supply if there were later considered to be a need to control it. Given this potential need, such products should be subject to post-market surveillance to ensure the ability to monitor and control the products. To promote a product that has been altered with the intention of benefiting the consumer, manufacturers themselves would have a requirement for post-market surveillance, in any case, and therefore this should not add a significant additional burden.

It is important to ascertain to what extent the nutrient or other targeted substance whose levels have been changed (if the intent was to deliberately modify the level of a nutrient) is bioavailable and remains stable with cultivation, time, processing, storage and cooking.

The review of unintended nutritional effects in a food modified to have an intended nutritional effect would follow the same steps as for other novel foods.

Nutritional Evaluation of expected or unexpected increased levels of a nutrient or bioactive substance

- Increased levels of a nutrient or other bioactive substance (including a microorganism) in a food need to be evaluated for safety.

- Data needed for this include:

  - the level of the targeted nutrient or other bioactive substance expected in the food;

  - expected level of exposure to the targeted nutrient or other bioactive substance through consumption of the food at the upper and lower extremes of intake of the food and across all age and sex groups using recent Canadian food consumption data where possible;

  - intended target group, if applicable, or which group(s) is or are likely to consume the most of the food;

  - how the expected level of dietary exposure to the targeted nutrient or other bioactive substance differs from the current levels in the diet; and

  - data in support of the safety of the expected level of exposure.

4.2.3.6 Toxicology Considerations

Toxicological testing is required for substances of unknown safety that are introduced to the food supply. Novel substances may be introduced to the food supply through recombinant DNA

technology, or may be generated by the application of novel processes to foods or [other DNA modification processes]. Introduction of novel substances may be intentional or unintentional.

Genetic modification techniques can result in the production of novel substances by the organism or the intentional or unintentional modification of substances already produced by the organism or their expression.

Novel Substances

In vitro nucleic acid techniques enable the introduction of DNA which can result in the synthesis of new substances in microorganisms. These include the protein expression product and other substances which may be generated as a result of enzymic activity of the protein expression product. The new substances can be conventional components of genetically modified microorganisms.

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 present in the donor organisms are not transferred to recombinant DNA organisms.

Toxicology studies are not considered necessary where the substance or a closely related substance has been consumed safely in food at equivalent intakes or where the new substance is not present in the food. Otherwise, the use of conventional toxicology studies on the new substance will be necessary. This will require the isolation of the new substance from the recombinant DNA microorganism.

For 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. Since proteins that are enzymes have never been shown to be direct-acting carcinogens, mutagens, teratogens or reproductive toxicants (Pariza and Foster 1983) it is generally not necessary to test proteins for these toxicological endpoints when exposure occurs by the oral route. Protein toxins act through acute mechanisms after the administration of a single dose at doses in the nanogram to milligram per kilogram body weight (bw). Therefore, acute oral toxicity studies using gram/kg bw doses of the novel protein are appropriate for assessing the potential toxicity of proteins. A negative result using doses in the gram/kg bw range together with evidence that the protein is digested to small peptides and amino acids would provide assurance that the protein is not a toxin and is digested to nutrients as are the vast majority of dietary proteins.

Different types of *in vivo* or *in vitro* studies would be needed to assess the toxicity of introduced substances other than proteins. The types of studies are determined on a case-by-case basis and 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.

Unintended Effects

Techniques used in the genetic modification of microorganisms have the potential to induce unintended effects on the genome of the modified organism that could be manifested as an alteration in the levels of toxicants normally produced by the organism. The intended genetic alteration may also influence the behaviour of the organism with respect to accumulation of contaminants, pesticides, or other substances from the environment that were not anticipated.

Compositional analysis is the method currently used for detection of unintended changes to the genome that result in accumulation of toxic substances either of endogenous or exogenous origin. Because of the influence of environmental stress on production of endogenous components such as toxins, data should be collected from a number of different test sites. New, more sensitive technologies that allow the determination of alterations to expression of the organisms' genome are presently under development.

4.2.3.7 Allergenicity Considerations

The primary considerations in allergenicity assessment of a novel food are the prevention of unexpected and unavoidable exposure of sensitized individuals to food allergens. This includes the assessment of the potential for foods containing novel proteins to cross-react with known food allergens or to lead to the development of *de novo* hypersensitivity. In addition, the possibility of increasing the allergenic potential of foods already containing allergens as a result of genetic modification should be assessed.

Section 1 – Introduction

All newly expressed proteins in recombinant-DNA microorganisms that could be present in the final food and are novel in the context of that food, need to be assessed for their potential to cause allergic reactions. This should include consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

At present, there is no definitive test that can be relied upon to measure directly the allergenic potential of a newly expressed protein in humans. Based upon the best, currently-available scientific information, the recommended approach takes into account the preponderance of evidence derived from several types of information and data in an integrated, stepwise, case-by-case manner.

Section 2 - Assessment Strategy[9]

The initial steps in assessing possible allergenicity of any newly expressed proteins involve determination of: the allergenicity of the source of the introduced protein; any similarity between the amino acid sequence of the protein and that of known allergens; and certain physicochemical properties, including but not limited to, its susceptibility to enzymatic degradation.

Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise.

Determination of amino acid sequence homology and physicochemical characteristics will require the isolation of the newly expressed protein from the recombinant-DNA organism, or the production of the substance from an alternative source, in which case the material should be shown to be functionally and biochemically equivalent to that produced in the recombinant-DNA organism.

Food proteins that are not allergens and that are altered by mutagenesis techniques need only be assessed for the likelihood that the mutagenized protein is a *de novo* allergen.

The absolute exposure to the newly expressed protein and the effects of relevant food processing will contribute toward an overall conclusion about the potential for human health risk. In this regard, the nature of the food product intended for consumption should be taken into consideration in determining the types of processing that would be applied and its effects on the presence of the protein in the final food product.

Section 3 – Initial Assessment

Section 3.1 - Source of the Protein

As part of the data supporting the safety of foods derived from recombinant-DNA organisms, information should describe any reports of allergenicity associated with the donor organism. Allergenic sources of genes would be defined as those organisms for which reasonable evidence of IgE-mediated oral, respiratory or contact allergy is available. Specific tools and relevant data that permit confirmation of allergenic potential are available for proteins from some allergenic sources. These include: the availability of sera for screening purposes; documented type, severity and frequency of allergic reactions; and structural characteristics and amino acid sequence (when available) of known allergenic proteins from that source.

[9] This assessment strategy is not applicable for assessing whether newly expressed proteins are capable of inducing gluten-sensitive or other enteropathies. In addition, the strategy is not applicable to the evaluation of foods where gene products are down regulated for hypoallergenic purposes.

Section 3.2 – Amino Acid Sequence Homology

Amino acid sequence homology comparisons should be used to assess the extent to which a newly expressed protein is similar in structure to known allergens in order to determine whether that protein has allergenic or cross-reactivity potential. Overall structural similarities can be predicted using sequence homology searches that compare the structure of newly expressed proteins with all known allergens should be conducted using various algorithms such as FASTA or BLASTP. Strategies such as stepwise contiguous identical amino acid segment searches may also be performed for the purpose of identifying sequences that may represent linear epitopes. The size of the contiguous amino acid search should be based on a scientifically justified rationale in order to minimize the potential for false negative or false positive results[10]. Validated search and evaluation procedures should be used in order to produce biologically meaningful results.

IgE cross-reactivity between the newly expressed protein and a known allergen should be considered a possibility when there is more than 35% identity in a segment of 80 or more amino acids (FAO/WHO 2001).

Sequence homology searches have certain limitations. In particular, comparisons are limited to the sequences of known allergens in publicly available databases and the scientific literature. There are also limitations in the ability of such comparisons to detect non-contiguous IgE-binding epitopes.

A negative sequence homology result indicates that a newly expressed protein is not a known allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of significant sequence homology should be considered along with the other data outlined under this strategy in assessing the allergenic potential of newly expressed proteins. This does not preclude further studies where considered necessary (see also section 6). A positive sequence homology result indicates that the newly expressed protein has a high probability of being allergenic. If the product is to be considered further, it should be assessed using serum from individuals sensitized to the identified allergenic source (see section on Specific Serum Screening).

Section 3.3 – Pepsin Resistance

Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation exists between resistance to digestion by pepsin, and allergenic potential [11]. The resistance of a

[10] It is recognized that the 2001 FAO/WHO consultation suggested moving from 8 to 6 identical amino acid segment searches. The smaller the peptide sequence used in the stepwise comparison, the greater the likelihood of identifying false positives; inversely, the larger the peptide sequence used, the greater the likelihood of false negatives, thereby reducing the utility of the comparison.

[11] The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).

protein to degradation in the presence of pepsin under appropriate conditions indicates that further analysis should be conducted to determine the likelihood of the newly expressed protein being allergenic. The establishment of a consistent and well-validated pepsin degradation protocol may enhance the utility of this method.

Although the pepsin resistance protocol is strongly recommended, it is recognized that other enzyme susceptibility protocols exist. Alternative protocols may be used where adequate justification is provided.

Section 4 – Specific Serum Screening

For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays is required. Sera from individuals with a clinically validated allergy to the source of the protein can be used to test IgE-binding of the protein in *in vitro* assays. A critical issue for testing will be the availability of human sera from sufficient numbers of individuals[12]. In addition, the quality of the sera and the assay procedure need to be standardized to produce a valid test result.

In the case of a newly expressed protein derived from a known allergenic source, a negative result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional testing, such as the possible use of skin test and *ex vivo* protocols.

The identification of a newly expressed protein as an allergen through immunological assays suggests that further development for commercialization of the product be discouraged, unless adequate risk management and risk communication measures could be assured throughout marketing and distribution of the product, since segregation and identity preservation of the new source of this allergen may be difficult or impossible to enforce.

Section 5 – Areas Requiring Further Development

The endpoint of the assessment of the data discussed above is a conclusion as to the likelihood of the protein being a food allergen. The techniques of targeted serum screening (*i.e.* the assessment of binding to IgE in sera of individuals with clinically-validated allergic responses to broadly-related categories of foods) and the use of animal models, once developed and validated, could enhance the weight of evidence used to derive this conclusion. To allow serum screening, steps should be taken to organize an international serum bank. As scientific knowledge and technology evolves, other methods, such as examination of newly expressed proteins for T-cell epitopes and structural motifs associated with allergens, might also be useful.

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[12] According to the Joint Report of the FAO/WHO Expert Consultation on Allergenicity of Foods Derived from Biotechnology (22-25 January 2001, Rome, Italy) a minimum of 8 relevant sera is required to achieve a 99% certainty that the new protein is not an allergen in the case of a major allergen. Similarly, a minimum of 24 relevant sera is required to achieve the same level of certainty in the case of a minor allergen. It is recognized that these quantities of sera may not be available for testing purposes.

Unintended effects on endogenous allergens

Genetic modification techniques have the potential to produce unintended effects on the genome that could lead to an increase in the expression of endogenous allergens. While the potential for health impacts of such increases is uncertain, they are in any case considered undesirable. Techniques used for assessing the potential for effects on endogenous allergen expression are the quantitative comparison of protein composition of the edible portion of the modified organism or, where sera from sufficient numbers of individuals with allergies to the food are available, the comparative immunoreactivity to the edible portion of the modified organism can be determined using immunoblotting techniques.

4.2.3.8 Chemical Considerations

The identification and levels of chemical contaminants must be reported. Levels and types of contaminants would be specific to the food that has been modified. Potential contamination could occur, for instance, as a result of residues from chemicals (organic or inorganic) employed in processes, such as extraction or purification processes, to produce the desired food product from microorganisms.

Appendix A: Nutrition Considerations - Key micronutrients (vitamins and minerals)

Analysis of the most important, or key, micronutrients present in a novel food, along with the analyses of proximate composition and the amino acid and fatty acid profiles, allow compositional comparisons between the novel food and its appropriate comparator that are relevant to assessing the nutritional quality and safety of the food. These compositional comparisons are a major aspect of the safety evaluation process known as "substantial equivalence". The Organisation for Economic Co-operation and Development (OECD) has recognized that uniformity in the application of the concept of substantial equivalence for novel food safety assessments "might be improved through consensus on the appropriate components (*e.g.* key nutrients, key toxicants and antinutritional compounds) on a crop-by-crop basis which should be considered in the comparison". They therefore have begun to develop consensus documents which, they indicate, "should be useful to the development of guidelines, both national and international, and to encourage information sharing among OECD Member countries". It is also noted that, "They are not intended to be a comprehensive description of all issues considered to be necessary for a safety assessment, but a base set for an individual product that supports the comparative approach."[13] The material in the OECD documents, when available for the crop in question, as well as the tables below, should be consulted to determine which components should be analyzed for the purposes of novel food safety assessment. The tables in this Appendix may list nutrients that are in addition to those in a given OECD consensus document. This determination is based on an assessment of the various possible roles for the food in the Canadian diet and the contribution that the food could, therefore, make to the nutrient intakes of those who consume it. This determination is made as follows:

Significance of micronutrients in a given food is determined by identifying those nutrients present in a reasonable daily intake of the food at 5%[14] or more of the Weighted Recommended Nutrient Intake (WRNI).

The reasonable daily intakes for various foods are included in Schedule K in Part D of the Food and Drugs Regulations. The weighted recommended nutrient intakes for vitamins can be found in Part D, Division 1, Table II of the Food and Drugs Regulations. Weighted recommended nutrient intakes for the minerals can be found in Part D, Division 2, Table II of the Food and Drugs Regulations. This method for determining key nutrients is adapted from section 5.2 of the *Codex General Principles for the Addition of Essential Nutrients to Foods*.

The key micronutrients for several plant foods have been determined for common genetically

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[13]OECD Environmental Health and Safety Publications Series on the Safety of Novel Foods and Feeds. 2001: No. 1 - Consensus Document on Key Nutrients and Key Toxicants in Low Erucic Acid Rapeseed (Canola) and No. 2 - Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-Nutrients

[14]This guideline applies to all vitamins and minerals with the exception of vitamin C. Since vitamin C is not present in a wide variety of foods, it would be considered a significant nutrient if it was present at 10% of the reasonable daily intake of the food.

modified plant foods such as rice, soybeans, wheat (hard red spring), corn (using nutrient data for corn and corn flour), tomato, rice bran oil, soybean oil, wheat germ oil, corn oil, canola oil, and cottonseed oil. These are presented in the following pages.

RICE

The vitamins and minerals that are found at significant levels in a reasonable daily intake of rice (135 g) are listed in the tables below. Data for the reference food is taken from the Canadian Nutrient File, food item: Grain, rice, brown, long grain, raw.
A reasonable daily intake of rice of 135 g was determined by multiplying a reference amount or serving size of dry rice (45 g) by 3, since rice is traditionally consumed 3 times daily in certain subpopulations in Canada.

Vitamins

Nutrient	Amount in 135 g rice		WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Vit. A	**0**	RE	870	0	
Vit. D	*****	ug	3	0	
Vit. E	**0.972**	ATE[#]	7	13.9	***
Vit. C	**0**	mg	34	0	
Thiamine	**0.541**	mg	1	54.1	***
Riboflavin	**0.126**	mg	1.2	10.5	***
Niacin	**6.873**	NE	16	43	***
Vit. B6	**0.687**	mg	1	68.7	***
Vit. B12	**0**	ug	1	0	
Folacin	**0.027**	mg	0.195	13.8	***
Pantothenic acid	**2.02**	mg	5	40.3	***

Minerals

Nutrient	Amount in 135 g rice		WRNI (2 yrs +)	% WRNI	*** Key Nutrients
Calcium	**31.05**	mg	780	4	
Phosphorus	**450**	mg	885	50.8	***
Iron	**1.98**	mg	10	19.8	***
Iodide	*	ug	155	0	
Magnesium	**193**	mg	210	91.9	***
Copper	**0.374**	mg	2.0**	18.7	***
Zinc	**2.73**	mg	10	27.3	***
Potassium	**301**	mg	3000**	10	***
Manganese	**5.05**	mg	3.5**	144.4	***

* Data not available
** Average Daily Intake used since there is no RDI
[#]ATE= alpha tocopherol equivalents; 1 mg alpha tocopherol= 1 ATE

Therefore, the key micronutrients in rice are vitamin E, thiamin, riboflavin, niacin, vitamin B6, folacin, pantothenic acid, phosphorus, iron, magnesium, copper, zinc, potassium, and manganese.

Rice Bran Oil
If an intended use is rice bran oil, analyses should include alpha tocopherol, along with the fatty acid profile.

SOYBEANS

The vitamins and minerals that are found at significant levels in a reasonable daily intake of soybeans are listed in the tables below. Schedule K of Part D of the Regulations indicates that a reasonable daily intake of soybeans is 100 g of cooked soybeans which would be equivalent to 50 g dry raw soybeans. Data for the reference food is taken from the Canadian Nutrient File, food code 3400, Soybeans, Dry, Raw.

Vitamins

Nutrient	Amount in 50 g raw soybeans		WRNI (2 yrs +)	% WRNI	*** Key Nutrients
Vit. A	**1**	RE	870	0.1	
Vit. D	*****	ug	3	0.0	
Vit. E	**0.98**	ATE[#]	7	14	***
Vit. C	**3**	mg	34	8.8	
Thiamine	**0.437**	mg	1	43.7	***
Riboflavin	**0.44**	mg	1.2	36.7	***
Niacin	**5.23**	NE	16	32.7	***
Vit. B6	**0.189**	mg	1	18.9	***
Vit. B12	**0**	ug	1	0.0	
Folacin	**0.18755**	mg	0.195	96.2	***
Pantothenic acid	**0.397**	mg	5	7.9	***

Minerals

Nutrient	Amount in 50 g raw soybeans		WRNI (2 yrs +)	% WRNI	*** Key Nutrients
Calcium	**139**	mg	780	17.8	
Phosphorus	**352**	mg	885	39.8	***
Iron	**7.85**	mg	10	78.5	***
Iodide	*	ug	155	0	
Magnesium	**140**	mg	210	66.7	***
Copper	**0.829**	mg	2.0**	41.5	***
Zinc	**2.45**	mg	10	24.5	***
Potassium	**899**	mg	3000**	30	***
Manganese	**1.26**	mg	3.5**	36	***

* Data not available

** Average Daily Intake used since there is no RDI

[#]ATE= alpha tocopherol equivalents; 1 mg alpha tocopherol= 1 ATE

Therefore, the key vitamins and minerals in raw dry soybeans are alpha tocopherol, thiamin, riboflavin, niacin, vitamin B6, folacin, pantothenic acid, phosphorus, iron, magnesium, copper, zinc, potassium, and manganese.

Soybean Oil

If an intended use is soybean oil, analyses should include alpha tocopherol, along with the fatty acid profile.

WHEAT

The vitamins and minerals that are found at significant levels in a reasonable daily intake of wheat (90 g) are listed in the tables below (the reasonable daily intake of wheat = 60% wheat in 5 slices of bread = 0.6 x 150 g = 90 g). Data for the reference food is taken from the Canadian Nutrient File, food code CN4436, Grain, Wheat, Hard Red Spring

Vitamins

Nutrient	Amount in 90 g wheat		WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Vit. A	**0**	RE	870	0	
Vit. D	*****	ug	3		
Vit. E	**1.296**	ATE[#]	7mg	18.5	***
Vit. C	**0**	mg	34	0	
Thiamine	**0.454**	mg	1	45.4	***
Riboflavin	**0.099**	mg	1.2	8.2	***
Niacin	**8.06**	NE	16	50.4	***
Vit. B6	**0.302**	mg	1	30.2	***
Vit. B12	**0**	ug	1	0	
Folacin	**0.039**	mg	0.195	20	***
Pantothenic acid	**0.842**	mg	5	16.8	***

Minerals

Nutrient	Amount in 90 g wheat		WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Calcium	**22.5**	mg	780	2.9	
Phosphorus	**298.8**	mg	885	33.75	***
Iron	**3.24**	mg	10	32.4	***
Iodide	*	ug	155		
Magnesium	**111.6**	mg	210	53.1	***
Copper	**0.369**	mg	2.0**	18.45	***
Zinc	**2.5**	mg	10	25	***
Potassium	**306**	mg	3000**	10.2	***
Manganese	**3.65**	mg	3.5**	104.3	***

*Data not available

** Average Daily Intake used since there is no RDI

[#]ATE= Alpha Tocopherol Equivalents; 1mg alpha tocopherol = 1 ATE

Therefore, the key micronutrients in hard red spring wheat are vitamin E, thiamin, riboflavin, niacin, vitamin B6, folate, pantothenic acid, phosphorus, iron, magnesium, copper, zinc, potassium, manganese.

Wheat germ oil

If an intended use is wheat germ oil, analyses should include alpha tocopherol, along with the fatty acid profile.

CORN- Corn Flour

To determine the key nutrients in corn, the nutrition information for corn flour was used. Corn derivatives, such as the flour, are used as a staple by Hispanic populations; they are used to make products like tacos, tortillas, and corn chips.

The vitamins and minerals that are found at significant levels in corn flour (100 g) are listed in the tables below. Data for the reference food is taken from the Canadian Nutrient File, Grain, Corn Flour (Yellow and White), Whole-Grain. Note that the key nutrients are the same for yellow and white except for vitamin A.

Vitamins

Nutrient	Amount in 100 g **yellow** corn flour	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Vit. A	**47** RE	870	5.4	***
Vit. D	* ug	3	-	
Vit. E	* ATE[#]	7	-	
Vit. C	**0** mg	34	0	
Thiamine	**0.246** mg	1	24.6	***
Riboflavin	**0.08** mg	1.2	6.7	***
Niacin	**2.72** NE	16	17	***
Vit. B6	**0.37** mg	1	37	***
Vit. B12	**0** ug	1	0	
Folacin	**0.025** mg	0.195	12.8	***
Pantothenic acid	**0.658** mg	5	13.2	***

Nutrient	Amount in 100 g **white** corn flour	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Vit. A	**0** RE	870	0	

Minerals

Nutrient	Amount in 100 g yellow corn flour	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Calcium	**7** mg	780	0.9	
Phosphorus	**272** mg	885	30.7	***
Iron	**2.38** mg	10	23.8	***
Iodide	* ug	155	-	
Magnesium	**93** mg	210	44.3	***
Copper	**0.23** mg	2.0**	11.5	***
Zinc	**1.73** mg	10	17.3	***
Potassium	**315** mg	3000**	10.5	***
Manganese	**0.46** mg	3.5**	13.1	***

*Data not available

** Average Daily Intake used since there is no RDI

[#]ATE= Alpha Tocopherol Equivalents; 1mg alpha tocopherol = 1 ATE

Therefore, the key micronutrients in corn flour are vitamin A (yellow corn only), thiamine, riboflavin, niacin, vitamin B6, folacin, pantothenic acid, phosphorus, iron, magnesium, copper, zinc, potassium, and manganese.

Note : For vitamin A, retinol and carotenoids should be declared separately.

Corn Oil

If an intended use is corn oil, analyses should include alpha tocopherol, along with the fatty acid profile.

TOMATO

The vitamins and minerals that are found at significant levels in a reasonable daily intake of tomatoes (100 g) are listed in the tables below. Data for the reference food is taken from the Canadian Nutrient File, food code CN113529, Tomatoes, Red, Ripe, Raw.

Vitamins

Nutrient	Amount in 100 g tomatoes	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Vit. A	**62** RE	870	7.1	***
Vit. D	***** ug	3	0	
Vit. E	***** ATE[#]	7	0	
Vit. C	**19.1** mg	34	56.2	***
Thiamine	**0.059** mg	1	5.9	***
Riboflavin	**0.048** mg	1.2	4	
Niacin	**0.728** NE	16	4.6	
Vit. B6	**0.08** mg	1	8	***
Vit. B12	**0** ug	1	0	
Folacin	**0.015** mg	0.195	7.7	***
Pantothenic acid	**0.247** mg	5	4.9	

Minerals

Nutrient	Amount in 100 g tomatoes	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Calcium	**5** mg	780	0.6	
Phosphorus	**24** mg	885	2.7	
Iron	**0.45** mg	10	4.5	
Iodide	* ug	155	0	
Magnesium	**11** mg	210	5.2	***
Copper	**0.074** mg	2.0**	3.7	
Zinc	**0.09** mg	10	0.9	
Potassium	**222** mg	3000**	7.4	***
Manganese	**0.105** mg	3.5**	3	

* Data not available

** Average Daily Intake used since there is no RDI

[#]ATE= alpha tocopherol equivalents; 1 mg alpha tocopherol= 1 ATE

Therefore, the key micronutrients in tomato are vitamin A, vitamin C, thiamine, vitamin B6, folacin, magnesium and potassium.

Note: For vitamin A, retinol and carotenoids should be declared separately.

Vegetable Oils

Vegetable oils not listed above include cottonseed oil, canola oil, olive oil, sunflower oil, *etc.* Analyses for vegetable oils in general should include alpha tocopherol, along with the fatty acid profile.