

1 **Guidelines for the Safety Assessment of Novel Foods Derived from Plants and**  
2 **Microorganisms (DRAFT)**  
3

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1 **1. Introduction**

2  
3 **1.1 Background**

4  
5 The globalization of the food supply, the demand for more food sources globally, and the rapid  
6 advances in food science and technology have resulted in the introduction of foods not previously  
7 available in the Canadian marketplace. Novel whole foods and food constituents may result from  
8 the importation of new products into Canada, the introduction of a new food source, the use of  
9 new processing techniques, and/or changes in the genetic make-up of the microorganisms, plants  
10 and animals from which foods are derived.

11  
12 Advances in transportation technology and lower transportation costs have increased the variety  
13 of food and food products imported into Canada. Changing consumer food preferences driven by  
14 cultural and ethnic traditions as well as nutritional and health concerns, have also resulted in the  
15 diversification of our food supply. In addition, the increasing global population continues to  
16 drive the introduction of new food sources worldwide. Foods considered non-traditional in  
17 Canada may be widely consumed in other parts of the world. In some cases, adverse effects may  
18 be associated with their consumption or traditional methods may be needed to prepare the food  
19 prior to consumption. In these situations, consumers need to be informed of potential risks and  
20 appropriate preparation techniques. Foods derived from sources not previously used as human  
21 foods must be evaluated for safety as they may contain toxins, contaminants and/or anti-  
22 nutritional factors.

23  
24 On a global level, new techniques for food preservation and processing continue to be developed  
25 to expand the shelf life of foods and food products, to reduce energy requirements for processing,  
26 and for many other purposes. As new processing techniques have the potential to alter the  
27 characteristics of a food, including nutritional and any toxic characteristics, human health  
28 impacts must be considered.

29  
30 Genetic modifications to improve the agronomic, production, processing or nutritional  
31 characteristic of microorganisms, plants and animals may be achieved through traditional  
32 breeding techniques or modern gene technologies. The application of genetic modification  
33 through either traditional breeding or genetic engineering is not considered inherently to increase  
34 or decrease the risk associated with consuming the organism as a food. However, the wide  
35 variety of manipulations possible through genetic modification, and the potential for the  
36 introduction of toxic compounds, unexpected secondary effects and changes in the nutritional  
37 and toxic characteristics of the foodstuff may give rise to safety concerns.

38  
39 Health Canada is responsible for establishing standards and policies governing the safety and  
40 nutritional quality of all food, including novel foods, sold in Canada. The mechanism by which  
41 Health Canada controls the sale of novel foods in Canada is a pre-market notification process  
42 specified under Division 28 of the *Food and Drug Regulations*.

1 The pre-market notification approach used for novel foods entails the submission of information  
2 to Health Canada regarding the product in question so that a determination can be made with  
3 respect to its acceptability as food prior to sale. Thus petitioners of novel foods must submit data  
4 of a sufficiently high calibre to meet the criteria specified by Health Canada.  
5

6 The safety criteria for the assessment of novel foods outlined in the current document were  
7 derived from internationally established scientific principles developed through the Organization  
8 for Economic Cooperation and Development (OECD), Food and Agriculture Organisation  
9 (FAO), World Health Organisation (WHO) and the *Codex Alimentarius Commission*. These  
10 guidelines provide for the flexibility required to determine the need for notification and the safety  
11 assessment of the broad range of food products being developed. This flexibility is needed to  
12 allow novel foods and food products to be assessed on a case-by-case basis and to take into  
13 consideration future scientific advances.  
14  
15

## 16 **1.2 Purpose of Guidelines**

17

18 These guidelines define the criteria and basic information requirements that must be considered  
19 in assessing the safety of novel whole foods and food constituents. They are intended to provide  
20 a basis for dialogue between petitioners and the Health Products and Food Branch (HPFB).  
21 These guidelines are not intended to explicitly define all the data that might be required in the  
22 course of a safety assessment as further data requirements may be identified during the safety  
23 assessment process.  
24  
25

## 26 **1.3 Scope**

27

28 This document encompasses all novel whole foods, novel food products, and novel foods used as  
29 ingredients that are derived from **plant** and **microbial** sources. Safety assessment criteria for  
30 novel foods derived from **animals** are under development and will be available for external  
31 consultation in 2004.  
32

33 Under Section **B.28.001** of the *Food and Drug Regulations*, a “novel food” is defined as follows:  
34  
35

36 "**novel food**" means

- 37 a) a substance, including a microorganism, that does not have a history of safe use as  
38 a food;
- 39 b) a food that has been manufactured, prepared, preserved or packaged by a process  
40 that  
41  
42

- 1 (i) has not been previously applied to that food, and  
2  
3 (ii) causes the food to undergo a major change; and  
4  
5 c) a food that is derived from a plant, animal or microorganism that has been  
6 genetically modified such that  
7  
8 (i) the plant, animal or microorganism exhibits characteristics that were not  
9 previously observed in that plant, animal or microorganism,  
10  
11 (ii) the plant, animal or microorganism no longer exhibits characteristics that were  
12 previously observed in that plant, animal or microorganism, or  
13  
14 (iii) one or more characteristics of the plant, animal or microorganism no longer  
15 fall within the anticipated range for that plant, animal or microorganism.  
16  
17

18 "genetically modify" means to change the heritable traits of a plant, animal or  
19 microorganism by means of intentional manipulation.  
20

21 "major change" means, in respect of a food, a change in the food that, based on the  
22 manufacturer's experience or generally accepted nutritional or food science theory, places  
23 the modified food outside the accepted limits of natural variations for that food with  
24 regard to:  
25

- 26 (a) the composition, structure or nutritional quality of the food or its generally  
27 recognized physiological effects;  
28 (b) the manner in which the food is metabolized in the body; or  
29 (c) the microbiological safety, the chemical safety or the safe use of the food.  
30  
31

## 32 **2. Notification Procedure**

### 33 **2.1 Submission of a Novel Food Notification**

34 Notifying Health Canada regarding the sale or advertisement for sale of a novel food may involve  
35 a one or two step process. In the first step, the manufacturer or importer of the novel food must  
36 notify the HPFB in writing of their intention to sell or advertise a novel food pursuant to section  
37 B.28.002 of the *Food and Drug Regulations*.  
38  
39

40 The notification package (4 copies) should provide the following as indicated in B.28.002 (2):  
41  
42

tion r 1                   ferred to in paragraph (1)(a) shall be signed by the manufacturer or importer, or  
2 a person authorized to sign on behalf of the manufacturer or importer, and shall include the  
3 following information:  
4

- 5           a)       the common name under which the novel food will be sold;
- 6
- 7           b)       the name and address of the principal place of business of the manufacturer and, if  
8 the address is outside Canada, the name and address of the principal place of  
9 business of the importer;
- 10
- 11          c)       a description of the novel food, together with
  - 12                   i) information respecting its development,
  - 13                   ii) details of the method by which it is manufactured, prepared, preserved,  
14                   packaged and stored,
  - 15                   iii) details of the major change, if any,
  - 16                   iv) information respecting its intended use and directions for its  
17                   preparation,
  - 18                   v) information respecting its history of use as a food in a country other  
19                   than Canada, if applicable, and
  - 20                   vi) information relied on to establish that the novel food is safe for  
21                   consumption;
  - 22
- 23          d)       information respecting the estimated levels of consumption by consumers of the  
24          novel food;
- 25
- 26          e)       the text of all labels to be used in connection with the novel food; and
- 27
- 28          f)       the name and title of the person who signed the notification and the date of  
29          signing.
- 30
- 31
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38       Upon receipt of the notification, a letter of acknowledgement in which the file number for the  
39       product is indicated, will be sent to the petitioner. This number, along with pertinent dates,  
40       should be used in all subsequent correspondence.

41  
42       As stated in B.28.003, within 45 days of receiving this notification, HPFB will review the  
43       notification and provide in writing either no objection to the sale of the novel food for

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

3  
4 If additional information is requested, the manufacturer or importer will be required to submit  
5 data for assessment. On the basis of the submitted safety data, HPFB will decide if the novel  
6 food is suitable for consumption. Manufacturers and importers of novel foods are at liberty to  
7 submit the scientific data necessary for the full safety assessment along with the basic  
8 information outlined in the first step of the notification.

9  
10 It is important to note that, under B.28.002 and B.28.003, no person shall sell or advertise for  
11 sale a novel food unless the manufacturer or importer of the novel food has:

12  
13 (a) notified the Director (the Assistant Deputy Minister of Health Products and Food  
14 Branch) of their intention to sell or advertise for sale the novel food; and

15  
16 (b) received a letter indicating that the information submitted in support of the safety of  
17 the novel food for consumption is sufficient to permit the sale of the novel food in  
18 Canada (a letter of no objection).

## 19 20 21 **2.2 Submission of a Safety Assessment Data Package**

22  
23 If the information provided in the notification for a novel food is not considered adequate to  
24 determine the novel food's safety, additional data supporting the safety of the food will be  
25 required. The type of information required to conduct the safety assessment of a novel food will  
26 depend on a number of factors such as the nature of the food, processing methods and the  
27 intended use. The approaches used to assess the safety of novel foods are outlined in these  
28 guidelines. However, the types of studies considered appropriate to demonstrate the safety of a  
29 novel food change with scientific knowledge and development. These guidelines are expected to  
30 be used in conjunction with information available in the scientific literature and from research  
31 and development conducted by the manufacturer.

32  
33 Since novel foods represent a diverse range of products, not all data requirements outlined in this  
34 document will be appropriate for a specific submission. Petitioners should consider the novel  
35 characteristics of the product when addressing the criteria in these guidelines. Consultation with  
36 the Food Directorate in HPFB is encouraged during the development phase of a product to  
37 determine the data necessary to demonstrate the safety of the product. In addition, waiving of  
38 certain data requirements will be considered when accompanied by a sound scientific rationale.

39  
40 The Regulations make it the responsibility of the manufacturer of a novel food to comply with  
41 requirements and to provide a full disclosure of the results of all studies undertaken and  
42 completed to support the safety of the novel food.

1 Within 90 days of receiving the safety assessment package, HPFB will review the data and  
2 provide in writing either a notice of no objection to the sale of the novel food for consumption or  
3 a request for additional scientific data to clarify outstanding issues.  
4  
5

### 6 **2.3 When to apply**

7

8 Written notification should be provided well in advance of the period when the manufacturer  
9 intends to market the product. Health Canada is obligated to respond regarding its acceptability  
10 for sale or whether further information is required for assessment within 45 days of receiving the  
11 notification.  
12  
13

### 14 **2.4 Where to apply**

15

16 Officially, manufacturers and importers are required to notify the Assistant Deputy Minister of  
17 the Health Products and Food Branch (HPFB). However, the Novel Foods Section has been  
18 established in the Food Directorate of HPFB to coordinate the safety evaluation of novel foods  
19 intended for human consumption in Canada. The notification and/or submission package should  
20 be addressed to:  
21

22 Novel Foods Section  
23 Food Directorate  
24 Health Products and Food Branch  
25 Health Canada  
26 4<sup>th</sup> Floor West, Sir Frederick G. Banting Research Centre  
27 Tunney's Pasture, Postal Locator 2204A1  
28 Ottawa, Ontario. K1A 0L2  
29  
30

### 31 **2.5 Standard Operating Procedure**

32

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

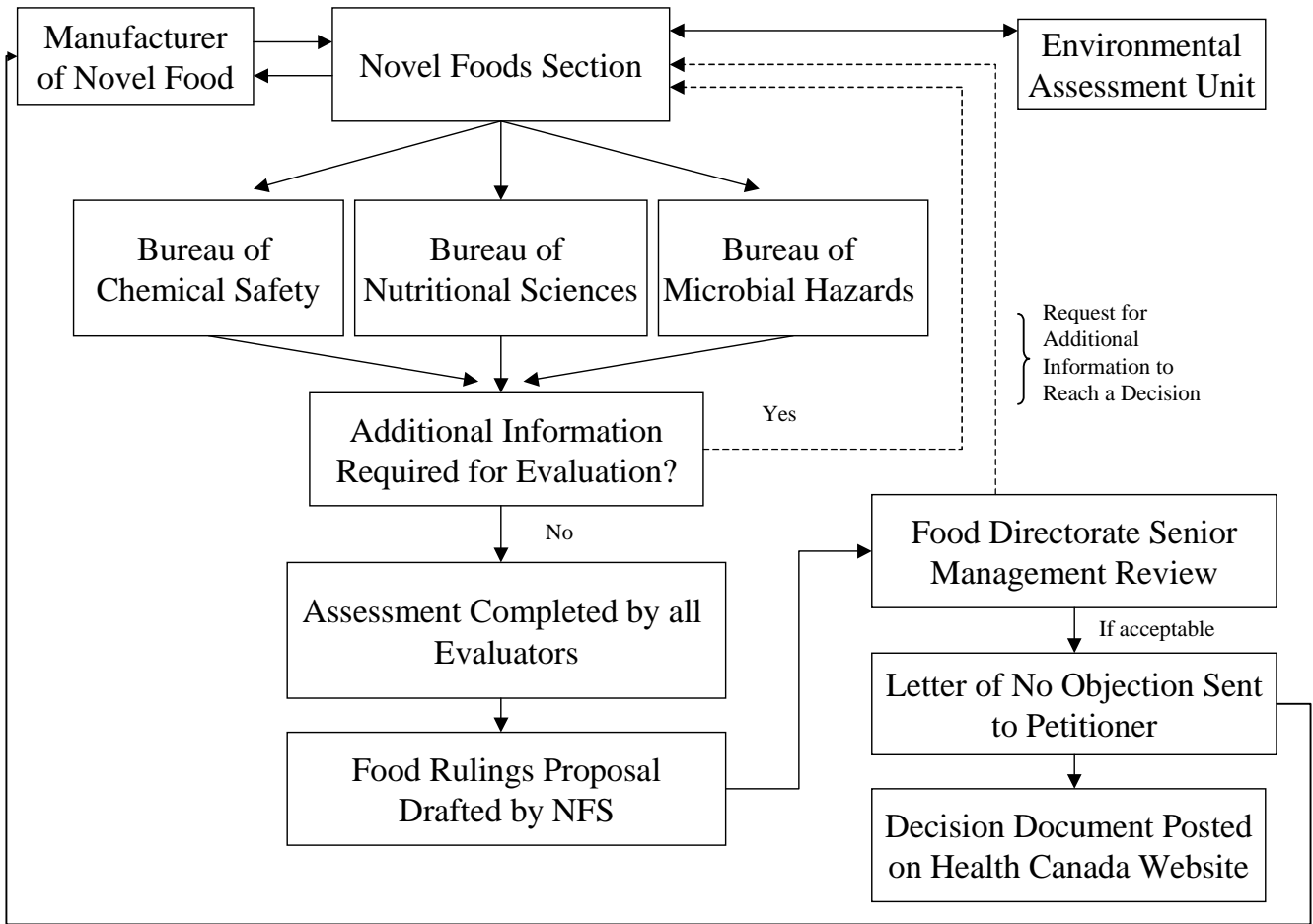


1 assessment of a submission package as outlined in the regulation. All requests for additional  
2 information by evaluators are communicated through the Novel Foods Section which creates a  
3 single window approach to submission reviews. Any request for information resets the 90 day  
4 assessment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

7  
8 At the completion of the safety assessment, if and only if all members of the evaluation team  
9 agree there are no health risks associated with the consumption of the novel food product in  
10 question, a proposal is drafted which contains a summary of the scientific reviews conducted by  
11 the relevant bureaux of the Food Directorate. This proposal is presented to the Food Rulings  
12 Committee consisting of Food Directorate senior management and representatives from other  
13 agencies or departments within the Canadian government. If found acceptable by the Committee,  
14 the petitioner is notified in writing by the Director General of the Food Directorate that, based on  
15 the evaluation of the submitted data, Health Canada has no objection to the sale of the novel food  
16 product as human food in Canada as specified in the letter.

17  
18 Novel food decisions and summary documents are made available on the Health Canada website  
19 for all products for which Health Canada has issued a letter of no objection to the use as food in  
20 Canada (<http://www.hc-sc.gc.ca/food-aliment>).

1 **Figure 1.** Processing a novel food notification/submission in the Food Directorate.  
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 12

1 **3. Other Regulatory Considerations**

2  
3 **3.1 Environmental Impact**

4  
5 Health Canada is in the process of developing Environmental Assessment Regulations for  
6 products regulated under the *Food and Drugs Act*, including novel foods. Until these new  
7 regulations are developed, information on the novel food’s potential environmental and indirect  
8 human health impact will be required pursuant to the New Substances Notification Regulations  
9 under the *Canadian Environmental Protection Act (CEPA)*.

10  
11 Products that are regulated under other federal statutes listed in a CEPA schedule, such as the  
12 *Seeds Act* and the *Feeds Act* are exempted from regulation under CEPA. Therefore, if a novel  
13 food is derived from a plant for which an application has been submitted to the Canadian Food  
14 Inspection Agency (CFIA) for unconfined environmental release or for use as animal feed, this  
15 should be stated in the application to Health Canada.

16  
17 A guidance document on current New Substances Notification Requirements for products  
18 regulated under the *Food and Drugs Act* is available on Health Canada’s website at [www.hc-](http://www.hc-sc.gc.ca/ear-ree)  
19 [sc.gc.ca/ear-ree](http://www.hc-sc.gc.ca/ear-ree) or upon request at 1-888-492-1104.

20  
21  
22 **3.2 Plants with Novel Traits and Novel Feeds**

23  
24 The CFIA is responsible for the regulation of plants with novel traits to be cultivated in Canada.  
25 Under the *Seeds Act*, a new variety of a cultivated species that possesses a novel trait would be  
26 subject to Regulatory Directive Dir94-08 (*Assessment Criteria for Determinating Environmental*  
27 *Safety of Plants with Novel Traits*). More information on the regulations of plants with novel  
28 traits (PNT) is available through the CFIA’s Plant Biosafety Office (phone number) or their  
29 website: (<http://www.inspection.gc.ca/english/plaveg/pbo/pbobbve.shtml>).

30  
31 The Feed Section of the CFIA administers a national livestock feed program, under the authority  
32 of the *Feeds Act* and Regulations, to verify that livestock feeds, including novel feeds,  
33 manufactured or sold in Canada are safe, efficacious and labelled properly. Novel feeds consist  
34 of organisms or parts of products thereof that have not been evaluated and approved for use as  
35 livestock feed in Canada. Novel feeds may be from plant sources, including PNTs, that may be  
36 used as feed must be assessed by the Feed Section prior to their use as a livestock feed. More  
37 information on the regulation of novel feeds from plant sources is available at  
38 [www.inspection.gc.ca/english/anima/feebet/bfeebete.shtml](http://www.inspection.gc.ca/english/anima/feebet/bfeebete.shtml). Please refer to the *Guidelines for*  
39 *the Assessment of Novel Feeds: Plant Sources* for data requirements for a novel feed submission.  
40  
41

1 Livestock feed is an outlet for by-products and residual material of the food processing industry.  
2 By-products of foods derived from novel microorganisms must be assessed by the Feed Section  
3 prior to their incorporation into livestock feed. The draft *Guidelines for the Safety Assessment of*  
4 *Novel Feeds: Microbial Products* can be obtained by contacting the Feed Section of the CFIA.  
5  
6

### 7 **3.3 Harmonization of Regulatory Approvals for Novel Foods and Novel** 8 **Feeds derived from Plants with Novel Traits** 9

10 Health Canada and the CFIA conduct interdepartmental consultations in order to coordinate the  
11 granting of their respective approvals to minimize the potential for unapproved food products to  
12 enter the Canadian marketplace. This approach will continue through a formalized process  
13 which will ensure the approvals of plants with novel traits are granted in a harmonized fashion.  
14

15 Where products are intended for exclusive use as one of either food, feed or molecular farming  
16 (use of plants to produce industrial or therapeutic products), consultations among regulatory  
17 authorities will be required to assess any potential risks associated with release of the product in  
18 an unintended commodity stream. For these products, an identity preservation system or  
19 alternative will be essential to minimize the likelihood of such an event.  
20  
21

### 22 **3.4 Post-Market Information** 23

24 If the Department establishes that there is no objection to the sale of a novel food for human  
25 consumption, it will be permitted to enter the marketplace in the same manner as traditional food  
26 products and therefore subjected to the same post-market standards applicable to all foods in  
27 Canada. It remains the responsibility of a company to ensure that its products are in compliance  
28 with all applicable statutory and regulatory requirements.  
29

30 At the current pace of technological advancement, it is expected that new information on  
31 previously approved products will be identified on occasion. Any post-market information  
32 obtained, which has potential health and safety implications, should be forwarded to Health  
33 Canada for consideration in order to ensure the continued safety and integrity of all novel foods  
34 available in the Canadian marketplace. The sale of a food that poses a hazard to the health of the  
35 consumer contravenes the provisions of the *Food and Drugs Act*.  
36

37 Future novel food products may be composed of significantly different nutrient combinations or  
38 other novel food characteristics not previously encountered in the food supply. These foods may  
39 require post market monitoring to address potential long term health effects. In such cases, post  
40 market information may be a valid approach to include in the assessment of the overall safety of  
41 some products.  
42  
43

#### 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

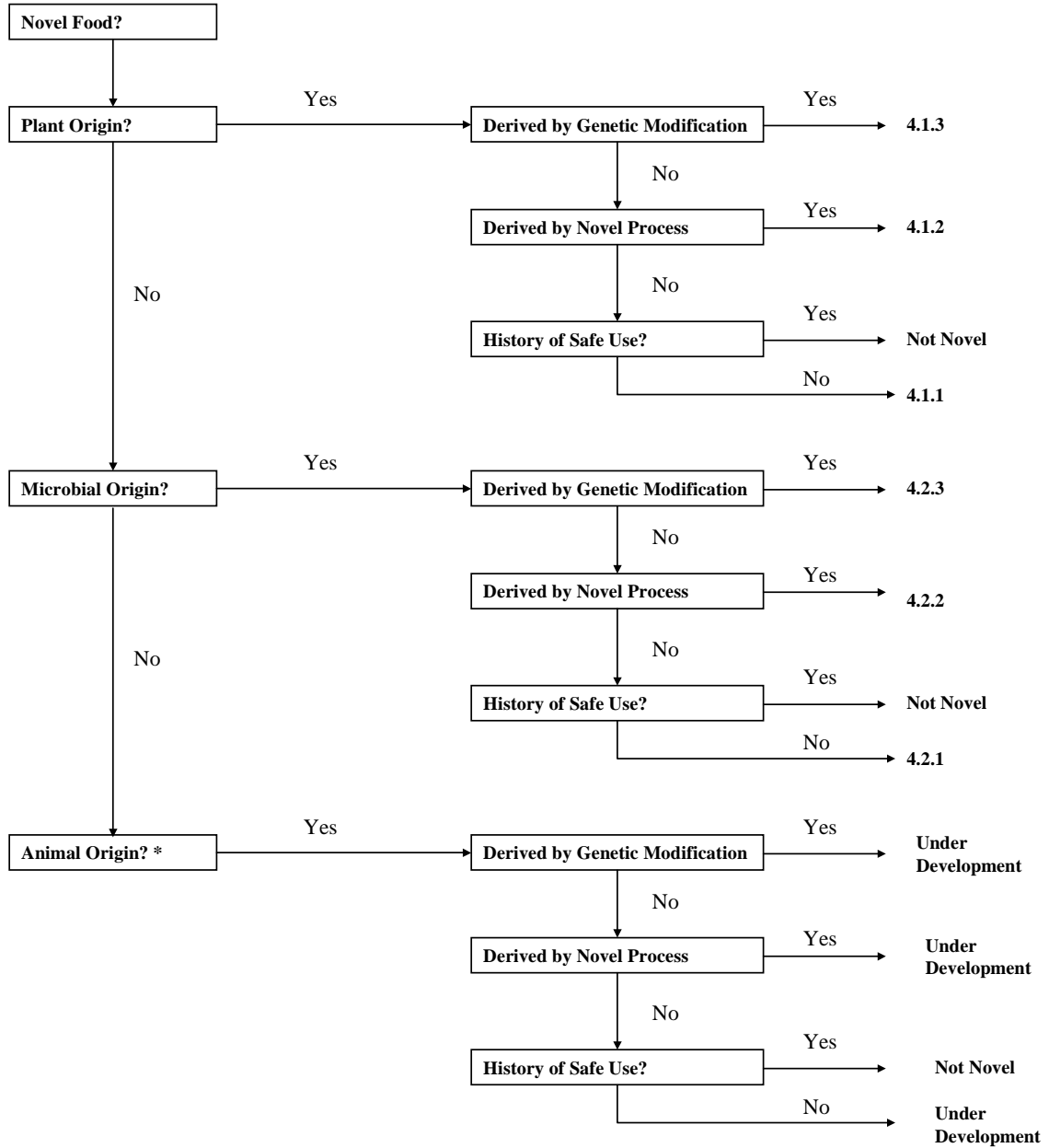
With such a wide range of foods, the amount of information necessary for assessment will also vary widely from one case to another. Therefore, in order to provide guidance for petitioners, this document will highlight the types of information likely to be required for specific types of novel foods. Not 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

- 1 Section to clarify which information requirements should be addressed for a particular novel food
- 2 product prior to making a notification or submission.
- 3
- 4

**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

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## **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



- 1                   4.1.3.7       Allergenicity considerations
- 2                   4.1.3.8       Chemical considerations

### 4.1.1 Substance with No History of Safe Use

3  
4  
5  
6  
7  
8 Many traditional foods are considered safe even though the food may contain anti-nutrients,  
9 toxins or allergens. Some foods require special preparation or processing to minimize the risks  
10 associated with a food. Foods are generally considered safe, provided that appropriate care is  
11 taken during development, production, processing, storage, handling and preparation. It is  
12 recognized that in many cases the knowledge required to manage the risks associated with  
13 traditional foods has been acquired in the course of their long history of use.

14  
15 Notification is required for foods new to the Canadian marketplace in order to demonstrate that  
16 they have a history of safe use. A history of safe use means significant human consumption of a  
17 food for which there exists adequate data to provide a reasonable certainty that no harm will  
18 result from consumption of the food. In many cases, toxicological and allergenicity data may be  
19 required to demonstrate that there are no health concerns related to the food use of a product or  
20 ingredient.

21  
22 The safety assessment of novel foods in this category follows a stepwise process of addressing  
23 relevant factors that include:

- 24
- 25                   4.1.1.1       History of use
- 26                   4.1.1.2       Dietary exposure
- 27                   4.1.1.3       Nutritional considerations
- 28                   4.1.1.4       Toxicology considerations
- 29                   4.1.1.5       Allergenicity considerations
- 30                   4.1.1.6       Chemical considerations

#### 4.1.1.1 History of Use

31  
32  
33  
34  
35 A substance may be considered to have a history of safe use as a food if it has been an ongoing  
36 part of the diet for a number of generations in a large, genetically diverse human population  
37 where it has been used in ways and at levels that are similar to those expected or intended in  
38 Canada. The fact that a product has had a history of use according to the above definition in a  
39 jurisdiction with a similar food safety system would increase the level of confidence in the  
40 evidence presented. The following information would be needed to support a claim that a  
41 product has a history of safe use:  
42

- 1 • Historical evidence indicating ongoing, frequent consumption by a cross-section  
2 of the population where it has been used over several generations (*i.e.* 100 years).  
3 This evidence may be derived from various sources including, but not limited to,  
4 scientific publications and patents, non-scientific publications and books,  
5 cookbooks, books on the history of food culture, and/or affidavits from two or  
6 more independent, reputable authorities that include well-documented accounts of  
7 the way the food is used and how they know it has the history it does. Limited  
8 usage or short term exposure would not be adequate to demonstrate a history of  
9 safe use.
- 10
- 11 • A declaration of any possible adverse effects linked to the food documented in its  
12 country of origin and/or a country where there is a high degree of consumption.
- 13
- 14 • A description of the standard methods of commercial and/or domestic processing  
15 and preparation for consumption.
- 16
- 17 • A description of how the food is cultivated or (if from wild sources) harvested.
- 18
- 19 • Amounts of the food that people are likely to consume in Canada, including  
20 typical serving sizes and expected frequency of consumption, at both average and  
21 extreme high consumption levels.
- 22
- 23 • Analysis of the composition of the food based on randomly selected, statistically  
24 valid samples. This analysis should include proximate data as well as amino acid  
25 profile, fatty acid profile, mineral and trace mineral composition and vitamin  
26 composition, as well as any nutrients, antinutrients and bioactive phytochemicals  
27 known to be of particular interest in the product. The analysis should pay special  
28 attention to the presence of compounds in the food which may have implications  
29 for the health of any groups of the Canadian population (*e.g.* possible toxicants or  
30 allergens or unusually high levels of nutrients in the food source or final food  
31 product).
- 32
- 33 • Metabolism and/or gastrointestinal effects in humans.
- 34

35 The submission should include reliable, high quality information and reference sources.  
36 Anecdotal evidence will be given less weight than scientifically derived data. Information on the  
37 history of human exposure will be particularly important where there are traditional handling or  
38 cooking requirements for a food that is novel. This information will need to be made available to  
39 consumers in a consistent manner. A current example of this is the advice regarding the  
40 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-

1 essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol,  
2 lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient  
3 bioavailability and nutrient interactions with other nutrients, with food additives and with natural  
4 toxicants. They also include nutrient excesses and the effects (both positive and negative) of  
5 food processing on the nutrients and on the organoleptic properties of the food. More recently, a  
6 wide range of “bioactive” substances found principally in plants are being shown to have a  
7 possible role to play in improving or protecting human health. These roles are also included in  
8 the broad definition of nutritional quality.  
9

### 10 **Foods with no history of safe use**

11  
12  
13 The main concern with respect to a food with no history of safe use would be to verify that the  
14 consumption of the food would not have an adverse effect on the nutritional health of the  
15 consumer. Information on nutritional composition and quality is primarily needed to determine  
16 how the food could be used in the diet, to establish basic composition information for the food  
17 for use in food composition databases, and to permit the validation of nutrient content claims and  
18 quantity declarations.  
19

### 20 **Guidelines for Producing Data for Nutritional Evaluation**

#### 21 **a. Function of the data to be submitted**

- 22
- 23
- 24
- 25 • The information provided for a food with no history of safe use should be of  
26 sufficient quantity and quality to determine its role in the diet and to characterize  
27 the average nutritional composition of the food.  
28
- 29 • Any studies conducted to evaluate nutritional quality should be performed using  
30 the food as it is expected to be consumed by humans.  
31

#### 32 **b. Where published data on nutrient composition of the novel food are inadequate, 33 analytical data may need to be obtained by the petitioner. In this case, an 34 appropriate study design for obtaining data on nutritional composition:**

- 35
- 36
- 37 • Considers all major sources of potential variation in nutritional quality, *e.g.*  
38 geographic area, season, soil type and fertility, amount of sunlight, temperature,  
39 crop management, etc, in designing the study, to ensure these factors are  
40 controlled.  
41
- 42 • Subjects the novel plant during cultivation to the conditions expected for it in  
43 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.

- 1 • proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude  
2 carbohydrate
- 3 • content of true protein, non-protein nitrogenous material (*e.g.* nucleic acids and  
4 aminoglycosides), amino acid profile, -- unusual amino acids should be  
5 determined if their presence is suspected (*e.g.* d-amino acids from bacterial  
6 proteins)
- 7 • quantitative and qualitative composition of total lipids, *i.e.* saponifiable and  
8 nonsaponifiable components, complete fatty acid profile, phospholipids, sterols,  
9 cyclic fatty acids and known toxic fatty acids
- 10 • composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins,  
11 non-starch polysaccharides and lignin
- 12 • qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin  
13 and mineral analysis - see Appendix A, "Key Micronutrients"
- 14 • presence of naturally occurring or adventitious anti-nutritional factors *e.g.*  
15 phytates, trypsin inhibitors, *etc.*
- 16 • predictable secondary metabolites, physiologically active (bioactive) substances,  
17 other detected substances  
18

19 "Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional  
20 analytical methods would be appropriate. When more advanced techniques such as  
21 proteomics and metabolomics become available and are validated for use, these should be  
22 adopted for this purpose.  
23

#### 24 **d. Nutrient bioavailability/Presence of anti-nutrients**

25  
26  
27 In situations where the food with no history of safe use may be a significant component of  
28 the Canadian diet, and/or a major supplier of nutrients, animal studies should be  
29 conducted to assess nutritional adequacy. This pertains in particular to the evaluation of  
30 protein quality, the possibility of unknown anti-nutrients, and questions of nutrient  
31 bioavailability.  
32

33 Information should be provided, if applicable, describing the processing conditions that  
34 would be used in the production of the novel food, and the effects of the processing on  
35 nutrient levels and nutrient bioavailability.  
36

#### 37 **e. Information to include in the submission:**

- 38 • the name of the plant including Latin and common names
- 39
- 40 • a complete description of the experimental design, experimental conditions, and  
41 how sources of variation for nutrient levels were controlled.  
42  
43

- 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  $\pm$  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.

1 Where it is not possible to identify novel components of the food, a case-by-case approach  
2 should be used to determine the appropriate toxicological tests to be carried out on the food. The  
3 history of the organism from which the food is derived as a source of toxins or antinutrients and a  
4 chemical analysis of its components will be considerations in determining requirements for  
5 toxicological testing. Depending on these determinations, conventional studies of toxicity,  
6 including chronic toxicity, developmental toxicity, genotoxicity or carcinogenicity, may need to  
7 be performed on the final food product or its components as appropriate.  
8

9 It should be noted that the conduct of studies with whole foods presents some challenges due to  
10 the potential for inducing nutritional imbalances when the food is incorporated into the diet at  
11 high concentrations. In addition, toxicology studies on novel foods are used to reach a  
12 conclusion as to whether the food is safe to consume under expected consumption patterns,  
13 rather than to derive a quantitative limit such as an acceptable daily intake in the manner used for  
14 simple chemicals like food additives.  
15  
16  
17

#### 18 **4.1.1.5 Allergenicity Considerations**

19  
20 The primary consideration in allergenicity assessment of a novel food is the prevention of  
21 unexpected and/or unavoidable exposure of susceptible individuals to food allergens. For foods  
22 with no history of safe use, the potential exists that one or more component proteins would have  
23 the capacity to cross-react with known food allergens or lead to the development of *de novo*  
24 hypersensitivity. It should be noted, however, that the vast majority of proteins consumed in the  
25 diet are not allergenic.  
26

27 At present, there is no definitive test that can be relied upon to measure directly the allergenic  
28 potential of an individual protein or of a whole food. Because existing strategies for the  
29 assessment of the allergenic potential of proteins were developed for the evaluation of individual,  
30 well-defined proteins (Section 4.1.3.7), they are not easily applied to the entire protein  
31 component of a whole food. The protein component of foods with no history of safe use will not  
32 be characterized to the extent necessary to apply these assessment strategies.  
33

34 A preliminary strategy for assessing the allergenic potential of foods with no history of safe use  
35 would be to investigate whether plants from the same taxonomic family that are commonly part  
36 of the food supply are implicated in the induction of allergic response. The association of a  
37 particular family of plants with allergic response might not necessarily preclude the introduction  
38 of the novel food from a related species into the marketplace, but risk management measures  
39 such as post-market surveillance and labelling where identification of the food item is not  
40 obvious will need to be considered. Proteins from an allergenic source should not be added to  
41 foods where identity preservation cannot be guaranteed.  
42  
43





1 **4.1.2 Novel Process**

2  
3 Some processes applied to foods or food ingredients may result in the generation of foods which  
4 would be considered novel in relation to traditional counterparts. The application of new  
5 processes which cause a food to undergo a major change would trigger the requirement to notify  
6 Health Canada. A major change is defined in Division 28 of the Regulations as a change in a  
7 food that, based on the manufacturer’s experience or generally accepted nutritional or food  
8 science theory, places the food outside the accepted limits of natural variations for that food with  
9 regard to: the composition, structure, nutritional quality of the food or its generally recognized  
10 physiological effects; the manner in which the food is metabolized in the body; or the  
11 microbiological safety, the chemical safety or the safe use of the food. Examples of novel  
12 processes include: new heat processing techniques; new packaging technologies; and the use of  
13 ultraviolet light for reducing the microbial load of a product.

14  
15 The safety assessment of novel foods in this category follows a stepwise process of addressing  
16 relevant factors that include:

- 17  
18 4.1.2.1 Details of novel process  
19 4.1.2.2 Dietary Exposure  
20 4.1.2.3 History of organism  
21 4.1.2.4 Nutritional considerations  
22 4.1.2.5 Toxicology considerations  
23 4.1.2.6 Allergenicity considerations  
24 4.1.2.7 Chemical considerations  
25  
26

27 **4.1.2.1 Details of Novel Process**

28  
29 While the focus of the safety assessment is on the food product, consideration of the process or  
30 preparation of the product can guide the safety assessment. Any novel processing or preparation  
31 techniques used to produce a novel food should be described in sufficient detail since such  
32 processing or preparation techniques may result in potential microbiological, toxicological,  
33 allergenicity, or nutritional concerns.  
34  
35

36 **4.1.2.2 Dietary exposure**

37  
38 In conducting dietary exposure assessments for novel foods resulting from the application of a  
39 novel process, the primary issues to be addressed as part of the safety assessment are: the  
40 potential for alteration of nutrient content of the food, and potential for introduction of novel  
41 substances to the food supply.  
42

1 In cases where the novel process results in the intentional or unintentional alteration of nutrient  
2 composition of the food, changes to nutrient intake should be determined for the food itself and  
3 in the context of the food as a source of the nutrient in the total diet. Variation of dietary patterns  
4 in subgroups in the population (*e.g.* children, infants, elderly, ethnic groups) as well as the  
5 potential for change in use and/or exposure to the food compared with the related, traditional  
6 food product should be taken into consideration.  
7

8 Novel processes applied to foods to reduce spoilage due to microbial activity can also increase  
9 the availability of exotic foods in the Canadian marketplace. The increased availability may have  
10 nutritional, toxicological or allergenic consequences, and estimation of exposure to components  
11 of the food of significance to health should be considered in such cases.  
12

13 If a process applied to a food results in the generation of predictable breakdown products, their  
14 amount in the food and the contribution of that food to the diet should be determined.  
15  
16

### 17 **4.1.2.3 History of Organism(s)**

18

19 The history of an organism can provide information that is important to the assessment of a novel  
20 food. There may be a history of toxin production by certain strains, species or genera and it  
21 would be important in such cases to examine the particular variety of the organism being used for  
22 the potential to produce such toxins, both under the conditions used in normal manufacturing and  
23 also under extreme conditions.  
24  
25

### 26 **4.1.2.4 Nutritional Considerations**

27

#### 28 **I Unintended nutritional effects**

29

##### 30 **General Observations**

31

32 The introduction of a novel food into the Canadian food supply requires a determination of  
33 nutritional quality of the food and the implications of its nutritional characteristics for the  
34 population as a whole and/or for specific subgroups. Population subgroups may be more  
35 vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with  
36 particular metabolic characteristics, adolescents and others who may consume large amounts of  
37 food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in  
38 order to ensure that the nutritional status of consumers is not likely to be jeopardized by:  
39

- 40 • substitution of foods and food ingredients of significant nutritive value with less  
41 nutritious varieties of the same or similar foods  
42

- 1 • excessive intakes of nutrients or other bioactive substances as a result of unusually  
2 high levels in the novel food, or
- 3
- 4 • new or increased levels of anti-nutrients that could adversely affect the nutritional  
5 value of the food or the diet.
- 6

## 7 **What is nutritional quality?**

8  
9  
10 Nutritional quality as applied to food is related to the presence of essential nutrients and energy-  
11 yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally  
12 considered as part of the science of nutrition. These aspects include the nutritional effects of  
13 non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre,  
14 cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk),  
15 nutrient bioavailability and nutrient interactions with other nutrients, with food additives and  
16 with natural toxicants. They also include nutrient excesses and the effects (both positive and  
17 negative) of food processing on the nutrients and on the organoleptic properties of the food.  
18 More recently, “bioactive” substances found principally in plants are being shown to have a  
19 possible role to play in improving or protecting human health. These substances are also  
20 included in the broad definition of nutritional quality.

## 21 22 23 **Application of novel process to plant foods**

24  
25 The development of novel foods or novel food ingredients through application of a novel  
26 process, could result in unintended changes in the composition of the food product which could  
27 in turn have an impact on the nutritional value of the food and the nutritional status of the  
28 persons consuming it.

29  
30 Unintended nutritional effects can occur whether the novel process is intended for nutritional or  
31 microbiological or other reasons. Evaluation of an intended effect on the nutritional quality of a  
32 food is discussed in Part II of this section.

33  
34 An important step in the safety and nutritional assessment of this type of novel food is a  
35 comparison of its composition with its appropriate counterpart(s). To determine whether there  
36 are any differences in the nutritional quality of the novel food compared to its appropriate  
37 counterpart(s), the major constituents of the food must be analysed, *i.e.* macronutrients and their  
38 component parts, as well as individual micronutrients and other bioactive substances selected  
39 based on valid criteria. If any nutrients are excluded from the analyses, this should be justified  
40 by an acceptable rationale. Also, circumstances may warrant an evaluation of the nutritional  
41 “performance” of the new food in its ready-to-eat form, thus either raw or when further processed  
42 by traditional/conventional methods used to make the product ready-to-eat. The purpose would  
43 be to provide an opportunity to identify major changes that may not have been detected by

1 compositional analysis, but which could affect, for example, the stability or bioavailability of  
2 nutrients in the food or the susceptibility of anti-nutrients to processing that normally destroys  
3 them. A performance test could involve re-analysis of a substance following cooking or it could  
4 require animal testing for bioavailability.  
5  
6

## 7 **Guidelines for Producing Data for Nutritional Evaluation**

### 8 **a. Function of the data to be submitted**

- 9
- 10 • The information provided for a novel food should be of sufficient quantity and  
11 quality to allow an assessment of whether any significant unintended effect on the  
12 nutritional quality of the food has occurred as a result of the application of the  
13 novel process on the food, relative to the food processed using current commercial  
14 processes. It should also allow an assessment of the nutritional significance of  
15 any change that is detected.  
16
  - 17 • Data should be provided for the food in its final product state (*i.e.* processed  
18 using novel method). Data may also be required for the food prepared for human  
19 consumption by conventional means to examine the effects, where applicable, of  
20 further processing, storage and cooking to look , for example, at the effectiveness  
21 of cooking to destroy anti-nutrients in cases where anti-nutrients normally  
22 destroyed by cooking are present.  
23
  - 24 • Data on the novel food should be compared, at a minimum, to data on two  
25 appropriate counterparts, the unprocessed food and the food processed by a  
26 currently used equivalent process (see section b, below). It is suggested that the  
27 study design include a representation of the various cultivars that are  
28 commercially available in the Canadian market; these cultivars should all be  
29 subjected to the test and control processes. This would permit assessment with  
30 respect to the normal variation expected between cultivars. Literature data (if  
31 available) may also be valid for assessing the nutritional relevance of any  
32 unintended effect.  
33

### 34 **b. Where published data on nutrient composition of the novel food are inadequate, 35 analytical data may need to be obtained by the petitioner. In this case, an 36 appropriate study design for obtaining data on nutritional composition:**

- 37
- 38 • Considers all potential sources of variation in nutritional quality, *e.g.* conditions  
39 of application (dose, duration, temperature), surface area or volume of plant food,  
40 cultivar, consistency of nutrient levels in the starting material, etc, in designing the  
41 study, to ensure these factors are controlled.  
42  
43

- 1 • Includes in the same study the novel food that is the subject of the notification as  
2 well as the appropriate counterparts, *i.e.* the same food in its pre-processed raw  
3 state, and the same food subject to a currently used equivalent process. A  
4 currently used equivalent process would be a non-novel process that is currently  
5 used commercially to achieve the same effect as the novel process (if applicable).  
6 In the absence of a currently used equivalent process, the counterpart would be  
7 simply the same food in its pre-processed raw state.  
8
- 9 • Applies the novel process (test), and currently used equivalent process (control) to  
10 a selection of the commercial cultivars available in the current market.  
11
- 12 • Establishes a sampling plan prior to the commencement of the study. This plan  
13 should account for all major sources of variation of nutrient levels in the food and  
14 use standard statistical methods for determining numbers of samples to collect and  
15 the appropriate method for collecting and compositing, for example, to account  
16 for inter-cultivar and between plot variation.  
17
- 18 • Ensures processing is conducted at the appropriate stage of maturity for the plant  
19 food, and that sampling is conducted at the appropriate stage of processing for the  
20 plant food (*i.e.* final product).  
21
- 22 • Ensures that the appropriate analyses are performed on all the parts of the plant  
23 that may be used as food in Canada.  
24
- 25 • Provides the criteria used for selecting the nutrients analysed and the rationale for  
26 the exclusion from analysis of any nutrients and other substances listed in **c.**  
27 **Nutrient Composition** below.  
28
- 29 • Ensures samples are analysed within an acceptable time frame from date of  
30 collection.  
31
- 32 • Ensures that analyses for each nutritive or non-nutritive component are conducted  
33 for all samples by a single laboratory using internationally approved and validated  
34 analytical methods and following consistent and appropriate sample storage and  
35 preparation procedures throughout.  
36
- 37 • Uses appropriate and consistent statistical methods chosen in advance, based on  
38 the study design, to compare levels of each nutrient in the novel food versus its  
39 controls.  
40

### 41 **c. Nutrient Composition**

42

43

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

- 5
- 6 • proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude  
7 carbohydrate
- 8
- 9 • content of true protein, non-protein nitrogenous material (*e.g.* nucleic acids and  
10 aminoglycosides), amino acid profile, -- unusual amino acids should be  
11 determined if their presence is suspected (*e.g.* d-amino acids from bacterial  
12 proteins)
- 13
- 14 • quantitative and qualitative composition of total lipids, *i.e.* saponifiable and  
15 nonsaponifiable components, complete fatty acid profile, phospholipids, sterols,  
16 cyclic fatty acids and known toxic fatty acids
- 17
- 18 • composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins,  
19 non-starch polysaccharides and lignin
- 20
- 21 • qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin  
22 and mineral analysis - see Appendix A, "Key Micronutrients"
- 23
- 24 • presence of naturally occurring or adventitious anti-nutritional factors *e.g.*  
25 phytates, trypsin inhibitors, *etc.*
- 26
- 27 • predictable secondary metabolites, physiologically active (bioactive) substances,  
28 other detected substances
- 29

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

#### 34 **d. Nutritional "Performance" of novel plant food**

35  
36 Consideration should be given to the possible need for the following types of information  
37 regarding the novel food:

- 38
- 39
- 40 • Response of known anti-nutrients to processes normally expected to neutralize  
41 their activity, measured using compositional analysis.
- 42
- 43 • Storage stability with regard to nutrient degradation.

- 1  
2  
3  
4  
5  
6
- 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.

7  
8

### **Nutrient bioavailability/Presence of new or altered anti-nutrients**

9  
10  
11  
12  
13

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.

14  
15  
16  
17

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.

18  
19

#### **e. Information to include in the submission:**

- 20  
21  
22  
23  
24  
25  
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29  
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31  
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41  
42  
43
- 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  $\pm$  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)<sup>1</sup>. 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**

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<sup>1</sup>“Codex Alimentarius Commission”, Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology”, 3<sup>rd</sup> 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

1  
2 The term “intended nutritional modification” is taken to include any change or introduced trait  
3 intended to improve the nutritional quality or health-related profile of the food, including but not  
4 limited to essential nutrients, beneficial bioactive phytochemicals, quantities and nature of the  
5 energy-yielding substances, improved nutrient bioavailability, and reduction in anti-nutrient  
6 levels.

7  
8 Evaluation of an intended nutritional change requires steps that are similar to those used in either  
9 the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health  
10 claims or both. For instance, such a change would trigger questions concerning the intended  
11 target group, what level of the targeted nutrient or other substance is expected in the food, what is  
12 the expected change in level of exposure to the targeted nutrient or other substance across all age  
13 and sex groups and at the upper and lower extremes of intake of the food, and the safety of this  
14 level of exposure.

15  
16 A novel food with an introduced health or nutritional benefit would likely fall into the unofficial  
17 category of “functional food”. It is expected that manufacturers will be interested in making  
18 health claims for these products. These products would therefore be evaluated in accordance  
19 with the criteria being laid out for foods with product-specific health claims. These include  
20 attention to the evidence in support of the claim, as well as to product safety and product quality  
21 considerations.

22  
23 Product safety of this type of novel food is intended to be controlled through application of the  
24 novel food regulations. The safety evaluation of a food manufactured using a novel process, for  
25 the purpose of having an intended nutritional modification should be the same as for other novel  
26 foods. With regard to the safety and nutritional evaluation of the intended nutritional  
27 modification itself, data requirements are described below.

28  
29 Product quality assurance refers to ensuring the consistency of the level of biologically active  
30 substances in the novel food in delivering the claimed benefits, and to conformance with  
31 acceptable procedures in all aspects of product testing. Details about quality assurance are  
32 discussed in the Interim Guidance Document on Standards of Evidence, mentioned below.

33  
34 At this time, regulations for product-specific health claims have not yet been promulgated.  
35 Prospective petitioners should refer to the proposed regulatory framework for product-specific  
36 health claims which was published in November, 2001, and the Interim Guidance Document on  
37 Standards of Evidence which was published in February, 2002. These are both available on the  
38 Health Canada web site at:

39  
40 [http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health\\_claims-allegations\\_sante/e\\_index.html](http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html).

41  
42 It is important to ascertain to what extent the intended nutritional effect of a novel process  
43 remains stable with storage, further processing, and cooking.

1 The review of unintended nutritional effects in a food manufactured using a novel process for the  
2 purpose of having an intended nutritional effect would follow the same steps as for other novel  
3 foods.  
4

#### 5 **Nutritional Evaluation of expected or unexpected increased levels of a nutrient or** 6 **bioactive substance**

- 7
- 8 • Increased levels of a nutrient or other intrinsic bioactive substance in a food need  
9 to be evaluated for safety.
- 10
- 11 • Data needed for this include:
  - 12
  - 13 – the level of the targeted nutrient or other substance expected in the food;
  - 14
  - 15 – intended target group, if applicable, or which group(s) is or are most likely  
16 to have high intakes of the food;
  - 17
  - 18 – expected level of exposure to the substance through consumption of the  
19 food by the target group, by vulnerable sub-groups and at the upper and  
20 lower extremes of intake of the food across all age and sex groups using  
21 recent Canadian food consumption data where possible;
  - 22
  - 23 – how the expected level of exposure to the targeted nutrient or other  
24 substance differs from the current levels of exposure from all sources;
  - 25
  - 26 – any potential use of the product as a replacement of existing foods; and  
27
  - 28 – data in support of the safety of the expected level of exposure.  
29
- 30

#### 31 **4.1.2.5 Toxicology Considerations**

32  
33 Toxicological testing is required for substances of unknown safety that are introduced to the food  
34 supply. The application of novel processes to foods may result in the generation of novel  
35 substances in the resulting food be they intentional or unintentional. Because of the potential  
36 wide variety of products generated by the application of novel processes, a determination of the  
37 appropriate toxicological testing should be conducted on a case-by-case basis.  
38

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

1 determinations, conventional studies of toxicity, including assays of metabolism, toxicokinetics,  
2 chronic toxicity/carcinogenicity, impact on reproductive function, and teratogenicity, may need to  
3 be performed on the final food product or its components as appropriate.  
4

5 Intentional alteration of the composition of foods by the addition of food components at levels  
6 that fall outside the accepted limits for natural variations (*e.g.* “functional” foods) may result in  
7 exposures for which there is no history of safe use. Substances that have been traditionally  
8 consumed in foods but which have been added to foods at levels outside their normal range will  
9 result in consumption of higher amounts of the substance than from a traditional diet. In such  
10 cases, the novel aspect of the food is the extent of exposure to the substance, rather than the  
11 substance itself, and toxicological testing of the enhanced component will be required to  
12 establish an upper limit of tolerability to the substance. The types of studies conducted should be  
13 guided by a knowledge of the role of the component in human physiology. Evidence from  
14 animal and *in vitro* studies as indicated in the previous paragraph would be required to determine  
15 safety. Studies in experimental animals may be of limited usefulness if the commonly used  
16 animal model (*e.g.* the rat) differs markedly from humans in the metabolic pathways and chronic  
17 conditions that are the basis of the intended functional effect, and it may be necessary to place  
18 greater reliance on human response to increased intakes of such food components.  
19 Epidemiologic studies may be available for substances that are normally components of foods,  
20 and these can provide important information on long-term effects.  
21  
22

#### 23 **4.1.2.6 Allergenicity Considerations**

24  
25 The primary consideration in allergenicity assessment of a novel food is the prevention of  
26 unexpected and unavoidable exposure of sensitized individuals to food allergens. In cases where  
27 the application of a novel process to a food results in the generation of a novel protein or an  
28 alteration of the protein content of a food containing allergenic proteins, a consideration of the  
29 allergenic potential of the novel food would be required.  
30

##### 31 **Novel Proteins**

32 At present, there is no definitive test that can be relied upon to measure directly the allergenic  
33 potential of an individual protein or of a whole food. If the application of a novel process to a  
34 food results in the generation of a novel protein that can be isolated and characterized, the  
35 assessment strategy that has been developed for foods which are the products of recombinant  
36 DNA technology and described in section 4.1.3.7 can be used to assess its potential allergenicity.  
37 This strategy involves a weight of evidence approach that relies on the assessment of amino acid  
38 sequence homology to known food allergens, and a consideration of the similarity of its  
39 properties, in particular, resistance to digestion in the mammalian gastrointestinal tract, to those  
40 of known food allergens.  
41  
42

1 **Alteration of endogenous allergen content**

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

10  
11  
12 **4.1.2.7 Chemical Considerations**

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

1 **4.1.3 Genetic Modification**

2  
3 Plants may be consumed as food or used to produce materials which are used in food or food  
4 processing. The variety of ways by which plants can be modified, and the degree of modification  
5 that can be produced, preclude standardization of the means to assess safety. The methods and  
6 extent of genetic modification, in part, determine both the type and quantity of information  
7 required to make an assessment.

8  
9 The point in the development of the new variety at which data are generated is central to the  
10 assessment of safety. It is expected that for many "novel plants," the final product will be the  
11 result of repeated backcrosses between the initially-modified plant and the host variety. Some  
12 data generated in the initial stages would be accepted for an assessment of the final product. This  
13 would specifically relate to information on the method of modification, the stability of the  
14 transformed plant and molecular biology. The detailed data on the chemical and toxicological  
15 characterization should be generated with genetically stable, converted lines which are  
16 representative of the final food product.

17  
18 It is important to note that not all information requirements outlined below may be appropriate to  
19 all cases. Applicants are encouraged to consult the Food Directorate early in product  
20 development in order to reach agreement on what information is appropriate to the evaluation of  
21 the safety of the product. The following information is recommended for assessing the  
22 acceptability of genetically modified plants and their products intended for use in or as a food.  
23 Once a genetically modified plant is determined to be acceptable, further variety development  
24 using traditional breeding techniques would not result in varieties requiring notification unless  
25 another major change occurs in the plant.

26  
27 Wherever possible, transformation markers which generate safety concerns should not be present  
28 in the final food product. If selectable markers are present in the final food, they will be  
29 evaluated for safety.

30  
31 The safety assessment of novel foods in this category follows a stepwise process of addressing  
32 relevant factors that include:

- 33  
34 4.1.3.1 Characterization of derived line  
35 4.1.3.2 Genetic modification considerations  
36 4.1.3.3 History of organism  
37 4.1.3.4 Dietary exposure  
38 4.1.3.5 Nutritional considerations  
39 4.1.3.6 Toxicology considerations  
40 4.1.3.7 Allergenicity considerations  
41 4.1.3.8 Chemical considerations  
42  
43

### 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 its 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

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

4  
5 Traditionally developed plants require a multi-disciplinary assessment since details of the  
6 modifications may be largely unknown. As experience in the safety assessment of novel foods  
7 develops, it may be possible to identify data requirements for particular groups of products more  
8 clearly, or to preclude certain products from further detailed evaluation.

## 11 **Genetic Modification by Modern Techniques**

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

### 21 **i) Description of the genetic modification(s)**

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

28  
29 The description of the modification process should include:

- 31 • information on the method(s) of modification used, *e.g.* Agrobacterium-mediated  
32 transformation or direct transformation by methods such as particle bombardment,  
33 electroporation, *etc.*;
- 34  
35 • description and characterization of all genetic material potentially delivered, if  
36 applicable, including the source, identity and expected function in the organism;  
37 and
- 38  
39 • details of manipulations or modifications to introduced, intermediate and recipient  
40 genetic material (*e.g.* change that affects the amino acid sequence of expression  
41 product).

42  
43 Information should be provided on DNA added, inserted, deleted, or modified, including:



- 1 • the characterization of all the genetic components including marker genes,  
2 regulatory and other elements affecting the function of the DNA;
- 3
- 4 • the size and identity;
- 5
- 6 • the location and orientation of the sequence in the final vector/construct; and
- 7
- 8 • function in the organism.
- 9

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

## 13

### 14 **ii) Characterization of the genetic modification(s)**

15  
16  
17 In order to provide clear understanding of the impact on the composition and safety of  
18 foods derived from genetically modified organisms, a comprehensive molecular and  
19 biochemical characterization of the organism should be carried out.

20  
21 Information should be provided on the DNA insertions into the genome; this should  
22 include:

- 23
- 24 • the characterization and description of all inserted genetic materials;
- 25
- 26 • the number of insertion sites;
- 27
- 28 • data to demonstrate if complete or partial copies have inserted into the genome;
- 29
- 30 • data to demonstrate whether the arrangement of the genetic material used for  
31 insertion has been conserved or whether significant rearrangements have occurred  
32 upon integration;
- 33
- 34 • the organization of the inserted genetic material at each insertion site including  
35 copy number and sequence data of the inserted material and, where appropriate, of  
36 surrounding region;
- 37
- 38 • identification of any open reading frames within the inserted DNA or created by  
39 the insertions with contiguous plant genomic DNA including those that could  
40 result in fusion proteins;
- 41

- 1 • in the case of modifications that involve deletions, rearrangements or site-specific,  
2 *in vitro* mutagenesis, sequence data of the region before and after modification  
3 should be provided.  
4

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

- 7
- 8 • the gene product (*e.g.* a protein or an untranslated RNA);
  - 9
  - 10 • the gene product's function;
  - 11
  - 12 • the phenotypic description of the new trait(s);
  - 13
  - 14 • the level and site of expression of the gene product(s), and the levels of its  
15 metabolites;
  - 16
  - 17 • to demonstrate whether deliberate modifications made to the amino acid sequence  
18 of the expressed protein result in changes in its post-translational modification or  
19 affect sites critical for its structure or function;
  - 20
  - 21 • where genetic manipulations are directed to altered regulation of endogenous  
22 genes, the characteristics and level of gene expression should be compared with  
23 that of the unmodified host;
  - 24
  - 25 • to indicate whether there is any evidence to suggest that one or several  
26 endogenous genes in the host plant has been affected by the modification process;
  - 27
  - 28 • to confirm the identity and expression pattern of any new fusion proteins;
  - 29
  - 30 • to demonstrate the intended effect of the modification has been achieved and that  
31 all expressed traits are expressed and inherited in a manner that is stable through  
32 several generations consistent with laws of inheritance. It may be necessary to  
33 examine the inheritance of the DNA itself or the expression of the corresponding  
34 RNA if the phenotypic characteristics cannot be measured directly; and
  - 35
  - 36 • to demonstrate that the newly expressed trait(s) are expressed as expected in the  
37 appropriate tissues in a manner and at levels that are consistent with the associated  
38 regulatory sequences driving the expression of the corresponding gene.  
39  
40  
41

### 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

1 for human consumption by conventional means to examine the effects, where  
2 applicable, of processing, storage and cooking to look , for example, at the  
3 effectiveness of cooking to destroy anti-nutrients in cases where anti-nutrients  
4 normally destroyed by cooking are present.  
5

- 6 • Data on the novel food should be compared, at a minimum, to data on the near  
7 isogenic, non-transgenic parent variety, the most appropriate counterpart, if  
8 available, or else a closely related non-transgenic cultivar. Since one or more  
9 significant differences could arise, the study design should include crops of the  
10 same species from a range of standard cultivars that are in commercial production  
11 for the same purposes and grown in the same geographical areas as those typically  
12 found on the Canadian market. This would permit assessment with respect to  
13 normal variation. Literature data (if available) may also be valid for assessing the  
14 nutritional relevance of any unintended effect.  
15

16  
17 **b. Where published data on nutrient composition of the novel food are inadequate,**  
18 **analytical data may need to be obtained by the petitioner. In this case, an**  
19 **appropriate study design for obtaining data on nutritional quality:**  
20

- 21 • Considers all sources of potential variation in nutritional composition, *e.g.*  
22 geographic area, season, soil type and fertility, amount of sunlight, temperature,  
23 crop management, etc, in designing the study, to ensure these factors are  
24 controlled.  
25
- 26 • Subjects the modified plant to the conditions expected for it in commercial  
27 production, *i.e.* a plant which is made tolerant to environmental or other stresses  
28 (insects, salt, drought, herbicides *etc.*) should be grown under those conditions for  
29 the purposes of data collection. The control plants should likewise be grown  
30 under conditions appropriate for them.  
31
- 32 • Includes in the same study the novel food that is the subject of the notification as  
33 well as the appropriate counterpart, *i.e.* the near isogenic parent cultivar, and a  
34 selection of the commercial cultivars available in the current market. In the  
35 absence of a near isogenic parent cultivar, the most closely related non-transgenic  
36 cultivar may be chosen.  
37
- 38 • Locates the test plots in several locations which are representative of the major  
39 growing areas for the organism. Ideally, the conditions under which the  
40 organisms are grown for collecting data should aim at representing different  
41 geographical locations where the plant is normally grown as well as different  
42 years, rather than relying on data from many replicates at a single field location  
43 for only one year.

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32
- 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.

### 33 **c. Nutrient Composition**

34  
35 In the context of the above study guidelines, the following components of foods should be  
36 analysed. Where not all are analysed, the petitioner should provide the criteria used to  
37 select the nutrients analysed and the rationale for the exclusion from analysis of any  
38 nutrients and other substances listed below.

- 39  
40  
41  
42  
43
- 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

1 determined if their presence is suspected (*e.g.* d-amino acids from bacterial  
2 proteins)

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

14  
15 "Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional  
16 analytical methods would be appropriate. When more advanced techniques such as  
17 proteomics and metabolomics become available and are validated for use, these should be  
18 adopted for this purpose.

#### 19 20 **d. Nutritional “Performance” of modified plant**

21  
22 Consideration should be given to the possible need for the following types of information  
23 regarding the modified plant:

- 24  
25 • Response of known anti-nutrients to processes normally expected to neutralize  
26 their activity measured using compositional analysis.
- 27  
28 • Storage stability with regard to nutrient degradation.
- 29  
30 • Performance of product in relation to the intended benefit (other than direct health  
31 benefits) *e.g.* improved stability of an oil to heating after fatty acid profile  
32 modification.

#### 33 34 35 **Nutrient bioavailability/Presence of new or altered anti-nutrients**

36  
37 In situations where the food from a genetically modified source may become a significant  
38 component of the Canadian diet, and/or a significant supplier of nutrients, animal studies  
39 may be needed in assessing nutritional adequacy to determine if there have been changes  
40 in the bioavailability of nutrients or if the composition is not comparable to conventional  
41 foods.



1 Information should be provided, if applicable, describing the processing conditions used  
2 in the production of the novel food and its derivatives, and the potential effects of the  
3 processing on nutrient levels and nutrient bioavailability.  
4

5  
6 **e. Information to include in the submission:**

- 7
- 8 • the names of all the cultivars which were represented in the study;
- 9
- 10 • a complete description of the experimental design, experimental conditions, and  
11 how sources of variation for nutrient levels were controlled;
- 12
- 13 • a complete description of sample collection and sample preparation;
- 14
- 15 • a citation and/or description of the analytical and statistical methods used to  
16 obtain data for the nutritive and non-nutritive components;
- 17
- 18 • nutrient and related data for test, control, and commercial cultivars (expressed as  
19 mean  $\pm$  standard deviation, and as a range);
- 20
- 21 • results of statistical analyses;
- 22
- 23 • raw data for all components analysed from all locations used to grow the plant;
- 24
- 25 • published data if available; and
- 26
- 27 • intended use of the plant as food in Canada, *i.e.* ingredient type(s), possible end  
28 products, level of use if different from current products which it would replace,  
29 known patterns of use and consumption of the food and its derivatives.  
30

31  
32 **f. Decision-making process**

- 33
- 34 • “The statistical significance of any observed differences should be assessed in the  
35 context of the range of natural variations for that parameter to determine its  
36 biological significance” (Codex)<sup>2</sup>. If the composition of the novel food is judged  
37 not to be nutritionally equivalent to that of its parent and commercial varieties, *i.e.*

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<sup>2</sup>“Codex Alimentarius Commission”, Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology”, 3<sup>rd</sup> 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

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

- 3
- 4 • All aspects of nutritional quality will be evaluated based on modern nutritional  
5 principles, standards and guidelines aimed at meeting human nutritional needs.  
6 The bases of evaluation include: nutrient intake recommendations, the role of the  
7 food in the diet of the population and the role of diet and nutrition in reducing the  
8 risk of developing a diet-related disease and health promotion.  
9
- 10 • Detection of a major change due to an unintended nutritional effect may not  
11 preclude the marketing of the product. However, such changes may require limits  
12 on the use of the food in food products or a requirement for labelling that goes  
13 beyond basic provisions. See also Part II with respect to safety assessment of high  
14 levels of nutrients or bioactive substances.  
15
- 16 • The first phase of nutritional evaluation will be based on the nutrient composition  
17 data. If there is a finding of unusual or unanticipated components or levels of  
18 nutrients or nutritive substances, the food may need to be subjected to further  
19 analysis and assessment.  
20
- 21 • The safety of a major increase in the level of a nutrient or other bioactive  
22 component would need to be assessed in a similar way to the safety assessment of  
23 an intended nutritional change. For details on this see Part II below.  
24  
25

## 26 **II Intended nutritional modifications**

27  
28 The term “intended nutritional modification” is taken to include any change or introduced trait  
29 intended to improve the nutritional quality or health-related profile of the food, including but not  
30 limited to essential nutrients, beneficial bioactive phytochemicals, quantities and nature of the  
31 energy-yielding substances, improved nutrient bioavailability, and reduction in anti-nutrient  
32 levels.  
33

34 Evaluation of an intended nutritional change requires steps that are similar to those used in either  
35 the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health  
36 claims or both. For instance, such a change would trigger questions concerning the intended  
37 target group, what level of the targeted nutrient or other substance is expected in the food, what is  
38 the expected change in level of exposure to the targeted nutrient or other substance across all age  
39 and sex groups and at the upper and lower extremes of intake of the food, and the safety of this  
40 level of exposure.  
41

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

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

5  
6 Product safety of this type of novel food is intended to be controlled through application of the  
7 novel food regulations. The safety evaluation of a novel food genetically modified to have an  
8 intended nutritional modification should be the same as for other genetically modified foods.  
9 With regard to the safety and nutritional evaluation of the intended nutritional modification,  
10 itself, data requirements are described below.

11  
12 Product quality assurance refers to ensuring the consistency of the level of biologically active  
13 substances in the novel food in delivering the claimed benefits, and to conformance with  
14 acceptable procedures in all aspects of product testing. Details about quality assurance are  
15 discussed in the Interim Guidance Document on Standards of Evidence, mentioned below.

16  
17 At this time, regulations for product-specific health claims have not yet been promulgated.  
18 Prospective petitioners should refer to the proposed regulatory framework for product-specific  
19 health claims which was published in November, 2001, and the Interim Guidance Document on  
20 Standards of Evidence which was published in February, 2002. These are both available on the  
21 Health Canada web site at:

22  
23 [http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health\\_claims-allegations\\_sante/e\\_index.html](http://www.hc-sc.gc.ca/food-aliment/ns-sc/ne-en/health_claims-allegations_sante/e_index.html).

24  
25 Adding a substance through genetic modification differs from adding one through applying it to  
26 or mixing it with the food after it is harvested. The decision to proceed with or cease the addition  
27 would take place at different stages of production. This could have an effect on the ability to  
28 manage the presence of the “added” substance or trait in the food supply if it was later decided  
29 that there was a need to control it. Given this potential need, such products should be subject to  
30 post-market surveillance to ensure the ability to monitor and control the products. To promote a  
31 product that has been altered with the intention of benefiting the consumer, manufacturers  
32 themselves would have a requirement for post-market surveillance, in any case, and therefore this  
33 should not add any significant additional burden.

34  
35 It is important to ascertain to what extent the modified nutrient (if the intent was to deliberately  
36 modify the level of a nutrient) is bioavailable and remains stable with cultivation, time,  
37 processing, storage and cooking.

38  
39 The review of unintended nutritional effects in a food modified to have an intended nutritional  
40 effect would follow the same steps as for other novel foods.

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

- 3
- 4           •       Increased levels of a nutrient or other intrinsic bioactive substance in a food need  
5           to be evaluated for safety.
  - 6
  - 7           •       Data needed for this include:
    - 8
    - 9           –       the level of the targeted nutrient or other substance expected in the food
    - 10
    - 11           –       intended target group, if applicable, or which group(s) is or are most likely  
12           to have high intakes of the food
    - 13
    - 14           –       expected level of exposure to the substance through consumption of the  
15           food by the target group, by vulnerable sub-groups and at the upper and  
16           lower extremes of intake of the food across all age and sex groups using  
17           recent Canadian food consumption data where possible
    - 18
    - 19           –       how the expected level of exposure to the targeted nutrient or other  
20           substance differs from the current levels of exposure from all sources
    - 21
    - 22           –       any potential use of the product as a replacement of existing foods
    - 23
    - 24           –       data in support of the safety of the expected level of exposure
    - 25
  - 26

27           **4.1.3.6       Toxicology Considerations**

28

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

33

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

37

38           **Novel Substances**

39

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

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

3  
4 The introduced trait should be shown to be unrelated to any characteristics of donor organisms  
5 that could be harmful to human health. Information should be provided to ensure that genes  
6 coding for known toxins or anti-nutrients present in the donor organisms are not transferred to  
7 recombinant DNA plants that do not normally express those toxic or anti-nutritious  
8 characteristics. This assurance is particularly important in cases where a recombinant DNA plant  
9 is processed differently from a donor plant, since traditional processing techniques associated  
10 with the donor organisms may deactivate anti-nutrients or toxicants.

11  
12 Toxicology studies are not considered necessary where the substance or a closely related  
13 substance has been consumed safely in food at equivalent intakes or where the new substance is  
14 not present in the food. Otherwise, the use of conventional toxicology studies on the new  
15 substance will be necessary. This may require the isolation of the new substance from the  
16 recombinant DNA plant, or the production of the substance from an alternative source, in which  
17 case, the material should be shown to be biochemically and functionally equivalent to that  
18 produced in the recombinant DNA plant.

19  
20 For proteins, the assessment of potential toxicity should focus on amino acid sequence similarity  
21 between the protein and known protein toxins and anti-nutrients (*e.g.* protease inhibitors, lectins)  
22 as well as stability to heat or processing and to degradation in appropriate/representative gastric  
23 and intestinal model systems. Since proteins that are enzymes have never been shown to be  
24 direct-acting carcinogens, mutagens, teratogens or reproductive toxicants (Pariza and Foster  
25 1983) it is generally not necessary to test proteins for these toxicological endpoints when  
26 exposure occurs by the oral route. Protein toxins act through acute mechanisms after the  
27 administration of a single dose at doses in the nanogram to milligram per kilogram body weight.  
28 Therefore, acute oral toxicity studies using gram per kilogram body weight doses of the novel  
29 protein are appropriate for assessing the potential toxicity of proteins. A negative result using  
30 doses in the gram/kg body weight range together with evidence that the protein is digested to  
31 small peptides and amino acids would provide assurance that the protein is not a toxin and is  
32 digested to nutrients as are the vast majority of dietary proteins.

33  
34 Different types of *in vivo* or *in vitro* studies would be needed to assess the toxicity of introduced  
35 substances other than proteins. The types of studies are determined on a case-by-case basis and  
36 depend on the original source of the introduced substances and their function. Such studies may  
37 include assays of metabolism, toxicokinetics, chronic toxicity/carcinogenicity, impact on  
38 reproductive function, and teratogenicity.

### 39 40 **Unintended Effects**

41  
42 Techniques used in the genetic modification of plants or microorganisms have the potential to  
43 induce unintended effects on the genome of the modified organism that could be manifested as

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

5  
6 Compositional analysis is the method currently used for detection of unintended changes to the  
7 genome that result in accumulation of toxic substances either of endogenous or exogenous origin.  
8 Because of the influence of environmental stress on production of endogenous components such  
9 as toxins and anti-nutrients, data should be collected from a number of different test sites. New,  
10 more sensitive technologies that allow the determination of alterations to expression of the  
11 organisms' genome are presently under development.

#### 14 **4.1.3.7 Allergenicity Considerations**

15  
16 The primary consideration in allergenicity assessment of a novel food is the prevention of  
17 unexpected and unavoidable exposure of sensitized individuals to food allergens. This includes  
18 the assessment of the potential for foods containing novel proteins to cross-react with known  
19 food allergens or to lead to the development of *de novo* hypersensitivity. In addition, the  
20 potential of increasing the allergenic potential of foods already containing allergens as an  
21 unintended result of genetic modification should be assessed. The following requirements are  
22 based on the Codex guideline for the conduct of food safety assessment of foods derived from  
23 recombinant-DNA plants.

##### 25 **Section 1 – Introduction**

26  
27 All newly expressed proteins in recombinant-DNA plants that could be present in the final food  
28 and are novel in the context of that food, need to be assessed for their potential to cause allergic  
29 reactions. This should include consideration of whether a newly expressed protein is one to  
30 which certain individuals may already be sensitive as well as whether a protein new to the food  
31 supply is likely to induce allergic reactions in some individuals.

32  
33 At present, there is no definitive test that can be relied upon to measure directly the allergenic  
34 potential of a newly expressed protein in humans. Based upon the [best], currently-available  
35 scientific information, the recommended approach used takes into account the preponderance of  
36 evidence derived from several types of information and data in an integrated, stepwise, case-by-  
37 case manner.

##### 38 **Section 2 - Assessment Strategy<sup>3</sup>**

---

<sup>3</sup> 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.

1 The initial steps in assessing possible allergenicity of any newly expressed proteins involve  
2 determination of: the allergenicity of the source of the introduced protein; any similarity between  
3 the amino acid sequence of the protein and that of known allergens; and certain physicochemical  
4 properties, including but not limited to, its susceptibility to enzymatic degradation.  
5

6 Genes derived from known allergenic sources should be assumed to encode an allergen unless  
7 scientific evidence demonstrates otherwise.  
8

9 Determination of amino acid sequence homology and physicochemical characteristics will  
10 require the isolation of the newly expressed protein from the recombinant-DNA organism, or the  
11 synthesis of production of the substance from an alternative source, in which case the material  
12 should be shown to be functionally and biochemically equivalent to that produced in the  
13 recombinant-DNA organism.  
14

15 Food proteins that are not allergens and that are altered by mutagenesis techniques need only be  
16 assessed for the likelihood that the mutagenized protein is a *de novo* allergen.  
17

18 The absolute exposure to the newly expressed protein and the effects of relevant food processing  
19 will contribute toward an overall conclusion about the potential for human health risk. In this  
20 regard, the nature of the food product intended for consumption should be taken into  
21 consideration in determining the types of processing that would be applied and its effects on the  
22 presence of the protein in the final food product.  
23

### 24 **Section 3 – Initial Assessment**

#### 25 **Section 3.1 - Source of the Protein**

26 As part of the data supporting the safety of foods derived from recombinant-DNA organisms,  
27 information should describe any reports of allergenicity associated with the donor organism.  
28 Allergenic sources of genes would be defined as those organisms for which reasonable evidence  
29 of IgE-mediated oral, respiratory or contact allergy is available. Specific tools and relevant data  
30 that permit confirmation of allergenic potential are available for proteins from some allergenic  
31 sources. These include: the availability of sera for screening purposes; documented type, severity  
32 and frequency of allergic reactions; and structural characteristics and amino acid sequence (when  
33 available) of known allergenic proteins from that source.  
34  
35

#### 36 **Section 3.2 – Amino Acid Sequence Homology**

37 Amino acid sequence homology comparisons should be used to assess the extent to which a  
38 newly expressed protein is similar in structure to known allergens in order to determine whether  
39 that protein has allergenic or cross-reactivity potential. Overall structural similarities can be  
40  
41

---

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

9  
10 IgE cross-reactivity between the newly expressed protein and a known allergen should be  
11 considered a possibility when there is more than 35% identity in a segment of 80 or more amino  
12 acids (FAO/WHO 2001).

13  
14 Sequence homology searches have certain limitations. In particular, comparisons are limited to  
15 the sequences of known allergens in publicly available databases and the scientific literature.  
16 There are also limitations in the ability of such comparisons to detect non-contiguous IgE-  
17 binding epitopes.

18  
19 A negative sequence homology result indicates that a newly expressed protein is not a known  
20 allergen and is unlikely to be cross-reactive to known allergens. A result indicating absence of  
21 significant sequence homology should be considered along with the other data outlined under this  
22 strategy in assessing the allergenic potential of newly expressed proteins. This does not preclude  
23 further studies where considered necessary (see also section 6). A positive sequence homology  
24 result indicates that the newly expressed protein has a high probability of being allergenic. If the  
25 product is to be considered further, it should be assessed using serum from individuals sensitized  
26 to the identified allergenic source (see section on Specific Serum Screening).

### 27 28 **Section 3.3 – Pepsin Resistance**

29  
30 Resistance to pepsin digestion has been observed in several food allergens; thus, a correlation  
31 exists between resistance to digestion by pepsin, and allergenic potential<sup>5</sup>. The resistance of a  
32 protein to degradation in the presence of pepsin under appropriate conditions indicates that  
33 further analysis should be conducted to determine the likelihood of the newly expressed protein  
34 being allergenic. The establishment of a consistent and well-validated pepsin degradation  
35 protocol may enhance the utility of this method.

---

<sup>4</sup> 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.

<sup>5</sup> The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).



1 Although the pepsin resistance protocol is strongly recommended, it is recognized that other  
2 enzyme susceptibility protocols exist. Alternative protocols may be used where adequate  
3 justification is provided.  
4

#### 5 6 **Section 4 – Specific Serum Screening**

7  
8 For those proteins that originate from a source known to be allergenic, or have sequence  
9 homology with a known allergen, testing in immunological assays is required. Sera from  
10 individuals with a clinically validated allergy to the source of the protein can be used to test IgE-  
11 binding of the protein in *in vitro* assays. A critical issue for testing will be the availability of  
12 human sera from sufficient numbers of individuals<sup>6</sup>. In addition, the quality of the sera and the  
13 assay procedure need to be standardized to produce a valid test result.  
14

15 In the case of a newly expressed protein derived from a known allergenic source, a negative  
16 result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional  
17 testing, such as the possible use of skin test and *ex vivo* protocols.  
18

19 The identification of a newly expressed protein as an allergen through immunological assays  
20 suggests that further development for commercialization of the product be discouraged, unless  
21 adequate risk management and risk communication measures could be assured throughout  
22 marketing and distribution of the product, since segregation and identity preservation of the new  
23 source of this allergen may be difficult or impossible to enforce.  
24

#### 25 **Section 5 – Areas Requiring Further Development**

26  
27 The endpoint of the assessment of the data discussed above is a conclusion as to the likelihood of  
28 the protein being a food allergen. The techniques of targeted serum screening (*i.e.* the  
29 assessment of binding to IgE in sera of individuals with clinically-validated allergic responses to  
30 broadly-related categories of foods) and the use of animal models, once developed and validated,  
31 could enhance the weight of evidence used to derive this conclusion. To allow serum screening,  
32 steps should be taken to organize an international serum bank. As scientific knowledge and  
33 technology evolves, other methods, such as examination of newly expressed proteins for T-cell  
34 epitopes and structural motifs associated with allergens, might also be useful.  
35

#### 36 **Unintended effects on endogenous allergens**

37  
38 Genetic modification techniques have the potential to produce unintended effects on the genome

---

<sup>6</sup> 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.

1 that could lead to an increase in the expression of endogenous allergens. While the potential for  
2 health impacts of such increases is uncertain, they are in any case considered undesirable.  
3 Techniques used for assessing the potential for effects on endogenous allergen expression are the  
4 quantitative comparison of protein composition of the edible portion of the modified organism  
5 or, where sera from sufficient numbers of individuals with allergies to the food are available, the  
6 comparative immunoreactivity to the edible portion of the modified organism can be determined  
7 using immunoblotting techniques.  
8  
9

#### 10 **4.1.3.8 Chemical Considerations**

11  
12 The identification and levels of chemical contaminants must be reported. Potential levels and  
13 types of contaminants would, of course, be specific to the food to be modified and, also, the type  
14 of process employed to achieve the genetic modification. In this regard, contaminants could be  
15 introduced as a result of the modification of the food or could be naturally present in the food  
16 before modification. In the latter case, it would be necessary to provide a comparison of the  
17 levels of chemical contaminants in the genetically modified food with those levels typically  
18 found in the original food product. Consideration should also be given to potential  
19 contamination from residues of any chemicals employed to achieve the desired genetic  
20 modification. Examples of chemical contaminants are metals (*e.g.* arsenic, cadmium, mercury  
21 and lead) and organic contaminants (*e.g.* introduction/or increased levels of mycotoxins).  
22  
23  
24  
25  
26

1 **4.2 Novel Foods Derived from Microorganisms**  
2

3 Microorganisms have been an important component of food for millennia. They may be  
4 consumed as inocula in fermented milk, meat or vegetable products or their metabolites may be  
5 used in food and in food processing. More recently, microorganisms have also been consumed  
6 directly as food in the form of single cell protein. Novel foods or ingredients can be derived  
7 from microorganisms not traditionally used as a food source in Canada, manufactured by new  
8 processes involving microorganisms, or produced by microorganisms that have been genetically  
9 modified by a variety of techniques.  
10

11 It is recommended that the following information be included for assessing the acceptability of  
12 novel microorganisms and their products that are intended for use in or as a food. It is important  
13 to note that not all information requirements outlined below may be appropriate to all cases.  
14

15 **4.2.1 Substance with No History of Safe Use**

- 16 4.2.1.1 History of use
- 17 4.2.1.2 Dietary exposure
- 18 4.2.1.3 Nutritional considerations
- 19 4.2.1.4 Toxicology considerations
- 20 4.2.1.5 Allergenicity considerations
- 21 4.2.1.6 Chemical considerations  
22

23 **4.2.2 Novel Process**

- 24 4.2.2.1 Detail of novel process
- 25 4.2.2.2 Dietary Exposure
- 26 4.2.2.3 History of organism
- 27 4.2.2.4 Nutritional considerations
- 28 4.2.2.5 Toxicology considerations
- 29 4.2.2.6 Allergenicity considerations
- 30 4.2.2.7 Chemical considerations  
31

32 **4.2.3 Genetic Modification**

- 33 4.2.3.1 Characterization of derived strain
- 34 4.2.3.2 Genetic modification considerations
- 35 4.2.3.3 History of organism (Host and Donor(s))
- 36 4.2.3.4 Dietary exposure
- 37 4.2.3.5 Nutritional considerations
- 38 4.2.3.6 Toxicology considerations
- 39 4.2.3.7 Allergenicity considerations
- 40 4.2.3.8 Chemical considerations  
41  
42

1 **4.2.1 Substance with No Safe History of Use**  
2

3 Many traditional foods are considered safe even though the food may contain anti-nutrients,  
4 toxins or allergens. Some foods require special preparation or processing to manage the risks  
5 associated with a food. Foods are generally considered safe, provided that appropriate care is  
6 taken during development, production, processing, storage, handling and preparation. It is  
7 recognized that in many cases the knowledge required to manage the risks associated with foods  
8 has been acquired in the course of their long history of safe use.  
9

10 Notification is required for foods new to the Canadian marketplace in order to demonstrate that  
11 they have a history of safe use. A history of safe use means significant human consumption for  
12 which there exists adequate knowledge to provide a reasonable certainty that no harm will result  
13 from the intended use of the food. In many cases, toxicological and allergenicity data may be  
14 required to demonstrate that there are no health concerns related to the food use of a product or  
15 ingredient.  
16

17 The safety assessment of novel foods in this category follows a stepwise process of addressing  
18 relevant factors that include:  
19

- |    |         |                                |
|----|---------|--------------------------------|
| 20 | 4.2.1.1 | History of use                 |
| 21 | 4.2.1.2 | Dietary exposure               |
| 22 | 4.2.1.3 | Nutritional considerations     |
| 23 | 4.2.1.4 | Toxicology considerations      |
| 24 | 4.2.1.5 | Allergenicity considerations   |
| 25 | 4.2.1.6 | Chemical considerations        |
| 26 | 4.2.1.7 | Microbiological considerations |
| 27 |         |                                |
| 28 |         |                                |

29 **4.2.1.1 History of Use**  
30

31 A substance may be considered to have a history of safe use as a food if it has been an on-going  
32 part of the diet for a number of generations in a large, genetically diverse human population  
33 where it has been used in ways and at levels that are similar to those expected or intended in  
34 Canada. The fact that a product has had a history of use according to the above definition in a  
35 jurisdiction with a similar food safety system would increase the level of confidence in the  
36 evidence presented. The following information would be needed to support a claim that a  
37 product has a history of safe use:  
38

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

1 reputable authorities that include well-documented accounts of the way the food is  
2 used and how they know it has the history it does. Limited usage or short term  
3 exposure would not be adequate to demonstrate a history of safe use.  
4

- 5 • A declaration of any possible adverse effects linked to the food documented in its  
6 country of origin and/or a country where there is a high degree of consumption.  
7
- 8 • A description of the standard methods of commercial and/or domestic processing  
9 and preparation for consumption.  
10
- 11 • A description of how the food is produced.  
12
- 13 • Amounts of the food that people are likely to consume in Canada, including  
14 typical serving sizes and expected frequency of consumption, at both average and  
15 extreme high consumption levels.  
16
- 17 • Analysis of the composition of the food based on randomly selected, statistically  
18 valid samples. This analysis should include proximate data as well as amino acid  
19 profile, fatty acid profile, mineral and trace mineral composition and vitamin  
20 composition, as well as any nutrients, antinutrients and bioactive phytochemicals  
21 known to be of particular interest in the product. The analysis should pay special  
22 attention to the presence of compounds in the food which may have implications  
23 for the health of any groups of the Canadian population (*e.g.* possible toxicants or  
24 allergens or unusually high levels of nutrients in the food source or final food  
25 product).  
26
- 27 • Metabolism and/or gastrointestinal effects in humans.  
28

29 The submission should include reliable, high quality information and reference sources.  
30 Anecdotal evidence will be given less weight than scientifically derived data. Information on the  
31 history of human exposure will be particularly important where there are traditional handling or  
32 cooking requirements for a food that is novel. This information will need to be made available to  
33 consumers in a consistent manner.  
34

#### 35 **4.2.1.2 Dietary Exposure**

36  
37 In conducting dietary exposure assessments for foods with no history of safe use, the primary  
38 issues to be addressed as part of the safety assessment are: the contribution of significant  
39 nutrients to the diet, the presence of endogenous anti-nutrients and toxins, and the potential for  
40 the introduction of novel substances to the food supply.  
41

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

1 health should be considered in such cases. For such foods, it may be possible to predict potential  
2 consumption patterns based on intakes of similar products routinely consumed as part of the diet.  
3 These intake estimates may then be used to calculate the potential dietary exposure to specific  
4 components of the novel food that will be the subject of the safety assessment.  
5  
6

### 7 **4.1.2.3 Nutritional Considerations**

#### 8 **General observations**

9  
10  
11 The introduction of a novel food into the Canadian food supply requires a determination of  
12 nutritional quality of the food and the implications of its nutritional characteristics for the  
13 population as a whole and/or for specific subgroups. Population subgroups may be more  
14 vulnerable for different reasons: *e.g.* young children, pregnant and lactating women, those with  
15 particular metabolic characteristics, adolescents and others who may consume large amounts of  
16 food, or the elderly who consume small amounts of food. A nutrition evaluation is needed in  
17 order to ensure that the nutritional status of consumers is not likely to be jeopardized by:  
18

- 19 • substitution of foods and food ingredients of significant nutritive value with less  
20 nutritious varieties of the same or similar foods
- 21
- 22 • excessive intakes of nutrients or other bioactive substances as a result of unusually  
23 high levels in the novel food, or
- 24
- 25 • new or increased levels of anti-nutrients that could adversely affect the nutritional  
26 value of the food or the diet.  
27

#### 28 **What is nutritional quality?**

29  
30 Nutritional quality as applied to food is related to the presence of essential nutrients and energy-  
31 yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally  
32 considered as part of the science of nutrition. These aspects include the nutritional roles of non-  
33 essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre, cholesterol,  
34 lipotropic substances, other components of specific foods (*e.g.* human milk), nutrient  
35 bioavailability and nutrient interactions with other nutrients, with food additives and with natural  
36 toxicants. They also include nutrient excesses and the effects (both positive and negative) of  
37 food processing on the nutrients and on the organoleptic properties of the food. More recently,  
38 “bioactive” substances found principally in plants are being shown to have a possible role to play  
39 in improving or protecting human health. These roles are also included in the broad definition of  
40 nutritional quality.  
41  
42

1 **Foods with no history of safe use**

2  
3 The main concern with respect to a food with no history of safe use would be to verify that the  
4 consumption of the food would not have an adverse effect on the nutritional health of the  
5 consumer. Information on nutritional composition and quality is primarily needed to determine  
6 how the food could be used in the diet, to establish basic composition information for the food  
7 for use in food composition databases, and to permit the validation of nutrient content claims and  
8 quantity declarations.  
9

10 **Guidelines for Producing Data for Nutritional Evaluation**

11  
12 **a. Function of the data to be submitted**

- 13  
14 • The information provided for a food with no history of safe use should be of  
15 sufficient quantity and quality to determine its role in the diet and to characterize  
16 the average nutritional composition of the food.  
17  
18 • Any studies conducted used to evaluate nutritional quality should have been  
19 performed using the food as it is expected to be consumed by humans.  
20  
21

22 **b. Where published data on nutrient composition of the novel food are inadequate,**  
23 **analytical data may need to be obtained by the petitioner. In this case, an**  
24 **appropriate study design for obtaining data on nutritional quality:**  
25

- 26 • Considers all major sources of potential variation in nutritional composition, *e.g.*  
27 composition of the growing medium, fermentation conditions (temperature, pH,  
28 stage of growth), etc, in designing the experimental design and sampling  
29 methodologies.  
30  
31 • Subjects the novel microorganism or food containing it to the conditions expected  
32 for it in commercial production.  
33  
34 • Establishes a sampling plan prior to the commencement of the study. This plan  
35 should account for all potential sources of variation of nutritional quality in the  
36 food and use standard statistical methods for determining numbers of samples to  
37 collect and the appropriate method for collecting and compositing, for example to  
38 account for intra-strain variation  
39  
40 • Ensures sampling is conducted at the appropriate stage of production.  
41  
42 • Ensures that the appropriate analyses are performed on all products containing the  
43 microorganism that are expected to be used as food in Canada.

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- 17
- 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.

18 **c. Nutrient Composition**

19

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

- 24
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- 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



1 "Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional  
2 analytical methods would be appropriate. When more advanced techniques such as  
3 proteomics and metabolomics become available and are validated for use, these should be  
4 adopted for this purpose.  
5

#### 6 **d. Nutrient bioavailability/Presence of anti-nutrients**

7  
8 In situations where the novel microorganism or food containing it may become a  
9 significant component of the Canadian diet, and/or a significant supplier of nutrients,  
10 animal studies should be conducted to assess nutritional adequacy. This pertains in  
11 particular to the evaluation of protein quality, the possibility of unknown anti-nutrients,  
12 and nutrient bioavailability.  
13

14 Information should be provided, if applicable, describing the processing conditions that  
15 would be used in the production of the novel food, and the effects of the processing on  
16 nutrient levels and nutrient bioavailability.  
17

#### 18 **e. Information to include in the submission:**

- 19
- 20 • the name of the microorganism including Latin and common names;
  - 21
  - 22 • a complete description of the experimental design, experimental conditions, and  
23 how sources of variation for nutrient levels were controlled;
  - 24
  - 25 • a complete description of sample collection and sample preparation;
  - 26
  - 27 • a citation and/ or description of the analytical and statistical methods which were  
28 used to obtain data for the nutritive and non-nutritive components;
  - 29
  - 30 • nutrient and related data expressed as mean  $\pm$  standard deviation, and as a range;
  - 31
  - 32 • results of statistical analyses;
  - 33
  - 34 • raw data for all components analysed;
  - 35
  - 36 • published data if available; and
  - 37
  - 38 • intended use(s) of the microorganism as food in Canada, *i.e.* as food itself or as  
39 an ingredient that might modify a food through culture, possible end products,  
40 level of use if different from current products which it would replace, known  
41 patterns of use and consumption of the food and its derivatives.  
42  
43

1           **f. Decision-making process**  
2

- 3           • All aspects of nutritional quality will be evaluated based on modern nutritional  
4           principles, standards and guidelines aimed at meeting human nutritional needs.  
5           The bases of evaluation include: nutrient intake recommendations, the role of the  
6           food in the diet of the population and the role of diet and nutrition in reducing the  
7           risk of developing a diet-related disease and health promotion.  
8  
9           • The first phase of nutritional evaluation will be based on the nutrient composition  
10          data. If there is a finding of unusual or unanticipated components or levels of  
11          nutrients or nutritive substances, the food may need to be subjected to further  
12          analysis.  
13  
14          • A novel food with no history of safe use is not required to meet specific criteria of  
15          nutritional quality. The main concern is to document the composition of the food  
16          in order to evaluate claims and to determine its potential role in the diet.  
17  
18

19           **4.2.1.4 Toxicology Considerations**  
20

21           Toxicological testing is required for substances of unknown safety that may be introduced to the  
22           food supply. For foods that have no history of safe use, it may be difficult to identify individual  
23           components which are novel in the context of human consumption in the absence of a traditional  
24           counterpart.  
25

26           Where it is not possible to identify novel components of the food, a case-by-case approach  
27           should be used to determine the appropriate toxicological tests to be carried out on the food. The  
28           history of the organism from which the food is derived as a source of toxins or antinutrients and a  
29           chemical analysis of its components will be considerations in determining requirements for  
30           toxicological testing. Depending on these determinations, conventional studies of toxicity,  
31           including chronic toxicity, developmental toxicity, genotoxicity or carcinogenicity, may need to  
32           be performed on the final food product or its components as appropriate.  
33

34           It should be noted that the conduct of studies with whole foods presents some challenges due to  
35           the potential for inducing nutritional imbalances when the food is incorporated into the diet at  
36           high concentrations. In addition, toxicology studies on novel foods are used to reach a  
37           conclusion as to whether the food is safe to consume under expected consumption patterns,  
38           rather than to derive a quantitative limit such as an acceptable daily intake in the manner used for  
39           simple chemicals like food additives.  
40  
41  
42

#### 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

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

5  
6 In general, the methods used to identify an organism should be consistent with methods  
7 currently used in microbial taxonomy. A taxonomic designation should be accompanied  
8 by a list of the tests used to arrive at the designation, with the results and any other  
9 information used to make the designation. A brief description of the type of tests used, or  
10 references, should be provided.

#### 11 **b) Pathogenicity**

12  
13 The potential of a viable microorganism in a food product to have adverse effects on  
14 human health must be considered. Adverse effects would include, but not be limited to,  
15 infection, disease, adverse immunologic reactions and toxicosis. While information  
16 from a review of the scientific literature is sufficient to satisfy this information  
17 requirement to address these points, petitioners should search various sources for  
18 information on the human health effects of a microorganism (databases, regulatory  
19 authorities, *etc.*). The search should provide information that would give a complete and  
20 thorough overview of any known involvement of a microorganism in an adverse health  
21 effect or the lack of any documented adverse health effects caused by a microorganism.  
22 In some cases, further testing may be required to address the pathogenic potential of an  
23 organism.  
24

#### 25 **c) Antimicrobial Production**

26  
27 Information should be provided on the production of antimicrobial compounds by a  
28 microorganism or its close relatives. These include classical antibiotics and other  
29 antimicrobials such as bacteriocins. The significance of these compounds in relation to  
30 clinically important antimicrobials will be considered. Introduction of microorganisms  
31 into the food chain which carry resistance factors to clinically important antibiotics must  
32 be avoided.  
33  
34

#### 35 **d) Production/Specifications**

36  
37 Microbial specifications for assuring microbial safety and data demonstrating compliance  
38 with these specifications should be provided for a number of production batches. The  
39 identification and levels of microbial contaminants must be reported. A food grade  
40 fermentation would be expected to yield a pure culture without microbiological  
41 contamination prior to down stream processing. However with traditional technologies,  
42  
43

1 microbial contaminants could be present in the culture and must be identified to  
2 demonstrate they are not of safety concern. Certificates of analysis for indicator  
3 organisms should be provided to demonstrate microbial safety. Documentation on the  
4 quality control of the manufacturing process should be provided, including a description  
5 of the manufacturing process and control measures that are applied to ensure quality and  
6 prevent microbial contamination.  
7  
8

## 9 **4.2.2 Novel Process**

10  
11 Some processes applied to foods or food ingredients may result in the generation of foods which  
12 would be considered novel in relation to traditional counterparts. The application of new  
13 processes which cause a food to undergo a major change would trigger the requirement to notify  
14 Health Canada under the *Novel Foods Regulation*. A major change is defined in Division 28 of  
15 the Regulations as a change in a food that, based on the manufacturer's experience or generally  
16 accepted nutritional or food science theory, places the food outside the accepted limits of natural  
17 variations for that food with regard to; the composition, structure or nutritional quality of the  
18 food or its generally recognized physiological effects; the manner in which the food is  
19 metabolized in the body; or the microbiological safety, the chemical safety or the safe use of the  
20 food. Examples of novel processes include: new heat processing techniques; new packaging  
21 technologies; the use of ultraviolet light for reducing the microbial load of a product.  
22

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

- 25
- 26 4.2.2.1 Details of novel process
- 27 4.2.2.2 Dietary Exposure
- 28 4.2.2.3 History of organism
- 29 4.2.2.4 Nutritional considerations
- 30 4.2.2.5 Toxicology considerations
- 31 4.2.2.6 Allergenicity considerations
- 32 4.2.2.7 Chemical considerations
- 33
- 34

### 35 **4.2.2.1 Details of Novel Process**

36  
37 While the focus of the safety assessment is on the food product, consideration of the process or  
38 preparation of the product can guide the safety assessment. Any novel processing or preparation  
39 techniques used to produce a novel food should be described in sufficient detail since such  
40 processing or preparation may result in potential microbiological, toxicological, allergenicity, or  
41 nutritional concerns.  
42  
43  
44

## 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.

1 Unintended nutritional effects can occur whether the novel process applied to the microorganism  
2 is intended for nutritional or functional or other reasons. Evaluation of a microorganism, which  
3 was produced using a novel process, intended to affect the nutritional quality of the  
4 microorganism or the food of which it is part is discussed in Part II of this section. Thus,  
5 discussion of probiotic aspects of microorganisms is limited to that part.  
6

7 An important step in the safety and nutritional assessment of this type of novel food is a  
8 comparison of its composition with its appropriate counterpart. In the case of a novel  
9 microorganism (*i.e.* the microorganism which was produced using a novel process), this could  
10 apply to the microorganism itself in the event that it constitutes a significant portion of the food  
11 mass but it is more likely to apply to the food containing the novel microorganism. To determine  
12 whether there are any differences in the nutritional quality of the food containing the novel  
13 microorganism compared to its appropriate counterpart, the microorganism should first be  
14 subject to laboratory testing of the metabolic products of the microorganism in controlled media.  
15 Once into the food production trial phase, the major constituents of the food containing the  
16 microorganism must be analysed, *i.e.* macronutrients and their component parts, as well as  
17 individual micronutrients selected based on validated criteria. If any nutrients (in the list below)  
18 are excluded from the analyses, this should be justified by an acceptable rationale.  
19

20 Also, circumstances may warrant an evaluation of the nutritional “performance” of the new food  
21 in its ready-to-eat form, thus either raw or when processed by traditional/conventional methods  
22 used to make the product ready-to-eat. The purpose would be to provide an opportunity to  
23 identify major changes that may not have been detected by compositional analysis, but which  
24 could affect, for example, the stability or bioavailability of nutrients in the food or the  
25 susceptibility of anti-nutrients to further processing that normally destroys them. A performance  
26 test could involve re-analysis of a substance following cooking or it could require animal testing  
27 for satisfactory growth and nutrient bioavailability .  
28  
29

## 30 **Guidelines for Producing Data for Nutritional Evaluation**

### 31 **a. Function of the data to be submitted**

- 34 • The information provided for a novel microorganism food or for a food containing  
35 one should be of sufficient quantity and quality to allow an assessment of whether  
36 any significant unintended effect on the nutritional quality of the food has  
37 occurred as a result of the introduction of the application of the novel process on  
38 the food. It should also allow an assessment of the nutritional significance of any  
39 change that is detected.
- 40 • Data should be provided for the novel microorganism food or for the food  
41 containing one, before further processing. Data may also be required for the food  
42 prepared for human consumption by conventional means to examine the effects,  
43



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

- 4  
5 • Data on the novel microorganism food or for the food containing one, should be  
6 compared, at a minimum, to data on the most appropriate counterpart (see section  
7 b, below). Literature data (if available) may also be valid for assessing the  
8 nutritional relevance of any unintended effect.

9  
10 **b. Where published data on nutrient composition of the novel food are adequate,**  
11 **analytical data may need to be obtained by the petitioner. In this case, an**  
12 **appropriate study design for obtaining data on nutritional quality:**

- 13  
14 • Considers all major sources of potential variation in nutritional quality (*e.g.*  
15 composition of the growing medium, production conditions, processing  
16 conditions, etc) in designing the study, to ensure these factors are controlled.
- 17  
18 • Subjects the novel microorganism or food containing it to the conditions expected  
19 for it in commercial production.
- 20  
21 • Includes in the same study the novel microorganism that is the subject of the  
22 notification as well as the appropriate counterpart, *i.e.* a) the microorganism food/  
23 food containing the microorganism, where the microorganism component was  
24 prepared using an equivalent commercial process, (*ie.* A process which is not  
25 novel, and which is currently used to achieve the same or similar effect), if  
26 available, or where a) is not applicable, b) the same microorganism food which is  
27 commercially available, or the same food which is commercially available,  
28 without a microorganism component.
- 29  
30 • Establishes a sampling plan prior to the commencement of the study. This plan  
31 should account for all major sources of variation of nutrient levels and use  
32 standard statistical methods for determining numbers of samples to collect and the  
33 appropriate method for collecting and compositing, for example, to account for  
34 inter-strain variability.
- 35  
36 • Ensures processing is conducted at the appropriate stage of production for the  
37 microorganism, and that sampling is conducted at the appropriate stage of  
38 processing for the novel organism or the food containing the novel organism.
- 39  
40 • Ensures that the appropriate analyses are performed on all products containing the  
41 microorganism that are expected to be used as food in Canada.
- 42  
43 • Provides the criteria used for selection of the nutrients analysed and the rationale

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

- 3
- 4 • Ensures samples are analysed within an acceptable time frame from date of  
5 collection.
- 6
- 7 • Ensures that analyses for each nutritive or non-nutritive component are conducted  
8 for all samples by a single laboratory using internationally approved and validated  
9 analytical methods and following consistent and appropriate sample storage and  
10 preparation procedures throughout.
- 11
- 12 • Uses appropriate and consistent statistical methods chosen in advance based on  
13 the study design to compare levels of each nutrient in the novel food versus its  
14 controls.
- 15
- 16

### 17 **c. Nutrient Composition**

18  
19 In the context of the above study guidelines, the following components of foods should be  
20 analysed. Where not all are analysed, the petitioner should provide the criteria used to  
21 select the nutrients analysed and the rationale for the exclusion from analysis of any  
22 nutrients and other substances listed below.

- 23
- 24 • proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude  
25 carbohydrate
- 26 • content of true protein, non-protein nitrogenous material (*e.g.* nucleic acids and  
27 aminoglycosides), amino acid profile, -- unusual amino acids should be  
28 determined if their presence is suspected (*e.g.* d-amino acids from bacterial  
29 proteins)
- 30 • quantitative and qualitative composition of total lipids, *i.e.* saponifiable and  
31 nonsaponifiable components, complete fatty acid profile, phospholipids, sterols,  
32 cyclic fatty acids and known toxic fatty acids
- 33 • composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins,  
34 non-starch polysaccharides and lignin
- 35 • qualitative and quantitative composition of micronutrients, *i.e.* complete vitamin  
36 and mineral analysis
- 37 • presence of naturally occurring or adventitious anti-nutritional factors *e.g.*  
38 phytates, trypsin inhibitors, *etc.*
- 39 • predictable secondary metabolites, physiologically active (or bioactive)  
40 substances, other detected substances
- 41

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

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

3  
4  
5 **d. Nutritional “Performance” of novel microorganism**

6  
7 Consideration should be given to the possible need for the following types of information  
8 regarding the novel microorganisms or the foods containing them:

- 9
- 10 • Response of known anti-nutrients to processes normally expected to neutralize  
11 their activity measured using compositional analysis.
  - 12 • Storage stability with regard to nutrient degradation.
  - 13 • Performance of product in relation to the intended benefit (other than direct health  
14 benefits) *e.g.* improved stability of an oil to heating after fatty acid profile  
15 modification.  
16  
17

18  
19  
20 **Nutrient bioavailability/Presence of new or altered anti-nutrients**

21  
22 In situations where the novel food may become a significant component of the Canadian  
23 diet, and/or a significant supplier of nutrients, animal studies may be needed in assessing  
24 nutritional adequacy to determine if there have been changes in the bioavailability of  
25 nutrients or if the composition is not comparable to conventional foods.

26  
27 Information should be provided, if applicable, describing the conditions used in the  
28 further processing of the novel food and its derivatives, and the potential effects of the  
29 processing on nutrient levels and nutrient bioavailability.

30  
31  
32 **e. Information to include in the submission:**

- 33
- 34 • a full description of the novel process, the purpose of the process, and the  
35 microorganism(s) on which it could be applied, and the microorganism(s) on  
36 which it will be applied (for the purpose of the submission);
  - 37 • the microorganism(s) on which the test and control processes were applied in the  
38 study, and the names and sources of all the strains which were represented in the  
39 study;
  - 40 • a complete description of the experimental design, experimental conditions, and  
41 how sources of variation for nutrient levels were controlled;  
42  
43

- 1
- 2 • a complete description of sample collection and sample preparation;
- 3
- 4 • a citation and/ or description of the analytical and statistical methods which were
- 5 used to obtain data for the nutritive and non-nutritive components;
- 6
- 7 • nutrient and related data for test, control, and commercial strains (expressed as
- 8 mean  $\pm$  standard deviation, and as a range);
- 9
- 10 • results of statistical analyses;
- 11
- 12 • raw data for all components analysed;
- 13
- 14 • published data if available; and
- 15
- 16 • intended use(s) of the novel microorganism as food in Canada, *i.e.* as food itself
- 17 or as an ingredient that might modify a food through culture, possible end
- 18 products, level of use if different from current products which it would replace,
- 19 known patterns of use and consumption of the food and its derivatives.
- 20
- 21

22 **f. Decision-making process**

23

- 24 • “The statistical significance of any observed differences should be assessed in the
- 25 context of the range of natural variations for that parameter to determine its
- 26 biological significance” (Codex)<sup>7</sup>. If the composition of the novel food is judged
- 27 not to be nutritionally equivalent to that of its counterpart(s), *i.e.* significant
- 28 differences (statistical and biological) exist in the nutrient data, then additional
- 29 nutritional data may be required on a case-by-case basis.
- 30
- 31 • All aspects of nutritional quality will be evaluated based on modern nutritional
- 32 principles, standards and guidelines aimed at meeting human nutritional needs.
- 33 The bases of evaluation include: nutrient intake recommendations, the role of the
- 34 food in the diet of the population and the role of diet and nutrition in reducing the
- 35 risk of developing a diet-related disease and health promotion.
- 36
- 37 • Detection of a major change due to an unintended nutritional effect may not

---

<sup>7</sup>“Codex Alimentarius Commission”, Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology”, 3<sup>rd</sup> 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

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

- 4  
5 • The first phase of nutritional evaluation will be based on the nutrient composition  
6 data. If there is a finding of unusual or unanticipated components or levels of  
7 nutrients or nutritive substances, the food may need to be subjected to further  
8 analysis and assessment.
- 9  
10 • The safety of a major increase in the level of a nutrient or other bioactive  
11 component would need to be assessed in a similar way to the safety assessment of  
12 an intended nutritional change. For details on this see Part II, below.

## 13 14 15 **II Intended nutritional modifications**

16  
17 The term “intended nutritional modification” is taken to include any change or introduced trait  
18 intended to improve the nutritional quality or health-related profile of the food, including but not  
19 limited to both essential nutrients and beneficial phytochemicals, quantities and nature of the  
20 energy-yielding substances, improved nutrient bioavailability, improved probiotic function and  
21 reduction in anti-nutrient levels.

22  
23 Evaluation of an intended nutritional change requires steps that are similar to those used in either  
24 the addition of a vitamin or mineral nutrient to a food or the evaluation of foods with health  
25 claims or both. For instance, such a change would trigger questions concerning the intended  
26 target group, what level of the targeted nutrient or other bioactive substance is expected in the  
27 food, what is the expected change in level of exposure to the targeted nutrient or other bioactive  
28 substance across all age and sex groups and at the upper and lower extremes of intake of the  
29 food, and the safety of this level of exposure.

30  
31 A novel food with an introduced health or nutritional benefit would likely fall into the unofficial  
32 category of “functional food”. It is expected that manufacturers will be interested in making  
33 health claims for these products. These products would therefore be evaluated in accordance  
34 with the criteria being laid out for foods with product-specific health claims. These include  
35 attention to the evidence in support of the claim, as well as to product safety and product quality  
36 considerations.

37  
38 Product safety of this type of novel food is intended to be controlled through application of the  
39 novel food regulations. The safety evaluation of a microorganism or of a food containing a  
40 microorganism, where the microorganism was subjected to a novel process, which resulted in the  
41 food having an intended nutritional modification (*i.e.* novel food), should cover the same aspects  
42 as for other novel foods. With regard to the safety and nutritional evaluation of the intended  
43 nutritional modification, itself, data requirements are described below.

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

7  
8 It is important to ascertain to what extent the intended nutritional effect of a novel process  
9 remains stable with cultivation, time, further processing, storage and cooking.

10  
11 The review of unintended nutritional effects in a novel microorganism or a food containing a  
12 novel microorganism, *i.e.* where a novel process was applied on the microorganism for the  
13 purpose of having an intended nutritional effect would follow the same steps as for other novel  
14 foods.

15  
16 **Nutritional Evaluation of expected or unexpected increased levels of a nutrient or**  
17 **bioactive substance**

- 18  
19 • Increased levels of a nutrient or other bioactive substance (including a  
20 microorganism) in a food need to be evaluated for safety.
  - 21  
22 • Data needed for this include:
    - 23  
24 – the level of the targeted nutrient or other bioactive substance expected in  
25 the food;
    - 26  
27 – intended target group, if applicable, or which group(s) is or are likely to  
28 consume the most of the food;
    - 29  
30 – expected level of exposure to the substance through consumption of the  
31 food by the target group, by vulnerable sub-groups, and at the upper and  
32 lower extremes of intake of the food and across all age and sex groups  
33 using recent Canadian food consumption data where possible;
    - 34  
35 – how the expected level of dietary exposure to the targeted nutrient or other  
36 substance differs from the current levels of exposure from all sources;
    - 37  
38 – data in support of the safety of the expected level of exposure.
- 39  
40  
41  
42

#### 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.

1 **Novel Proteins**

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

11  
12 **Alteration of endogenous allergen content**

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

21  
22  
23 **4.2.2.7 Chemical Considerations**

24 The identification and levels of chemical contaminants must be reported. Potential  
25 contamination could occur, for instance, as a result of residues from chemicals (organic or  
26 inorganic) employed in processes, such as extraction or purification processes, to produce the  
27 desired food product from microorganisms.  
28  
29  
30  
31  
32



### 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

1 modified organism.

2  
3 The description of the modification process should include:

- 4
- 5 • information on the method(s) of modification used, *e.g.* conjugation,  
6 electroporation, *etc.*;
  - 7
  - 8 • description and characterization of all genetic material potentially delivered, if  
9 applicable, including the source, identity, expected function in the organism, and  
10 copy number for plasmids; and
  - 11
  - 12 • details of manipulations or modifications to introduced, intermediate and recipient  
13 genetic material.
  - 14

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

- 16
- 17 • the characterization of all the genetic components including marker genes, vector  
18 genes, regulatory and other elements affecting the function of the DNA;
  - 19
  - 20 • the size and identity;
  - 21
  - 22 • the location and orientation of the sequence in the final vector/construct; and
  - 23
  - 24 • function in the organism.
  - 25

26 A summary diagram, outlining the key features of the final construct, should be provided.  
27 Depending on the nature of the genetic modification, restriction maps and sequence data  
28 of the introduced or modified genetic material and adjacent regions, may be required.  
29

30  
31 **ii) Characterization of the genetic modification(s)**

32  
33 In order to provide clear understanding of the impact on the composition and safety of  
34 foods derived from genetically modified microorganisms, a comprehensive molecular and  
35 biochemical characterization of the organism should be carried out.

36  
37 Information should be provided on the DNA insertions into the genome; this should  
38 include:

- 39
- 40 • the characterization and description of all inserted, deleted, or otherwise modified  
41 genetic materials;
  - 42
  - 43 • the number of insertion sites;

- 1
- 2 • data to demonstrate if complete or partial copies have inserted into the genome;
- 3
- 4 • data to demonstrate whether the arrangement of the genetic material used for
- 5 insertion has been conserved or whether significant rearrangements have occurred
- 6 upon integration;
- 7
- 8 • the organization of the inserted genetic material at each insertion site including
- 9 copy number and sequence data of the inserted material and, where appropriate, of
- 10 surrounding region;
- 11
- 12 • identification of any open reading frames within the inserted DNA or created by
- 13 the insertions with contiguous DNA in the chromosome or in a plasmid, including
- 14 those that could result in fusion proteins; and
- 15
- 16 • in the case of modifications that involve deletions, rearrangements or site-specific,
- 17 *in vitro* mutagenesis, sequence data of the region before and after modification
- 18 should be provided.
- 19

20 Information should be provided on any expressed substances in the modified organism;

21 this should include:

22

- 23 • the gene product (*e.g.* a protein or an untranslated RNA);
- 24
- 25 • the gene product's function;
- 26
- 27 • the phenotypic description of the new trait(s);
- 28
- 29 • the level and site of expression of the gene product(s), and the levels of its
- 30 metabolites;
- 31
- 32 • to demonstrate whether deliberate modifications made to the amino acid sequence
- 33 of the expressed protein result in changes in its post-translational modification or
- 34 affect sites critical for its structure or function;
- 35
- 36 • where genetic manipulations are directed to altered regulation of endogenous
- 37 genes, the characteristics and level of gene expression should be compared with
- 38 that of the unmodified host;
- 39
- 40 • to indicate whether there is any evidence to suggest that one or several
- 41 endogenous genes in the host plant has been affected by the modification process;
- 42
- 43 • to confirm the identity and expression pattern of any new fusion proteins;

- 1
- 2
- 3
- 4
- 5
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- 7
- 8
- 9
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- 11
- 12
- 13
- 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.

#### 14 **4.2.3.3 History of Organism(s)**

15

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

21

22 The following detailed information should be provided:

- 23
- 24
- 25
- 26
- 27
- 28
- 29
- 30
- 31
- 32
- 33
- 34
- 35
- 36
- 37
- 38
- 39
- 40
- 41
- 42
- 43
- 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.

1 **What is nutritional quality?**

2  
3 Nutritional quality as applied to food is related to the presence of essential nutrients and energy-  
4 yielding substances (in appropriate quantity and quality) and to other aspects of food traditionally  
5 considered as part of the science of nutrition. These aspects include the nutritional effects of  
6 non-essential amino acids, specific types of fatty acids and carbohydrates, dietary fibre,  
7 cholesterol, lipotropic substances, other components of specific foods (*e.g.* human milk),  
8 nutrient bioavailability and nutrient interactions with other nutrients, with food additives and  
9 with natural toxicants. They also include nutrient excesses and the effects (both positive and  
10 negative) of food processing on the nutrients and on the organoleptic properties of the food.  
11 More recently, “bioactive” substances found principally in plants are being shown to have a  
12 possible role to play in improving or protecting human health. These substances are also  
13 included in the broad definition of nutritional quality.

14  
15  
16 **Genetically modified microorganisms**

17  
18 Microorganisms constitute a quantitatively minor component of foods in the Canadian diet. The  
19 use of single cell protein as a food ingredient is rare. For most foods containing microorganisms,  
20 a change in the microorganisms would be unlikely to have a significant direct impact on the  
21 nutritional quality of foods and diets. There are two other ways, however, that a microorganism  
22 in a food could have an impact on the nutritional quality of the food or diet and in turn on the  
23 health of the consumer.

24  
25 One way is that microorganisms can have a significant indirect impact on the nutritional quality  
26 of foods that they are in. For example, the use of yeast to leaven bread reduces the phytate  
27 content which makes the minerals more available for intestinal absorption. The yeast also  
28 produces B vitamins in sufficient quantities to significantly affect the content of some of the B  
29 vitamins, for example folate, in bread. The other way that a microorganism in a food can have an  
30 impact on health is potentially as a “probiotic”. Probiotics are thought to be able to populate or  
31 alter the population of bacteria in the large intestine and as a result have various beneficial effects  
32 on the health of the intestine and the individual.

33  
34 Therefore, the development of novel forms of microorganisms that are used in food through  
35 genetic modification, whether by traditional selection methods, mutagenesis or recombinant  
36 DNA techniques, could result in intended or unintended changes in the composition of the food  
37 product which could in turn have an impact on the nutritional value of the food and the  
38 nutritional status of the persons consuming it. As more complex or layered genetic modifications  
39 are attempted through rDNA techniques, for instance to introduce both improved nutritional  
40 traits and functional traits into the same organism, these could increase the potential for  
41 unintended effects compared to simpler modifications. By the same token, other methods of  
42 genetic modification could also introduce multiple changes.

1 Unintended nutritional effects can occur whether the intended modification of the microorganism  
2 is nutritional or functional or something else. Evaluation of a modification of a microorganism  
3 intended to affect the nutritional quality of the microorganism or the food of which it is part is  
4 discussed in Part II of this section. Thus, discussion of probiotic aspects of microorganisms is  
5 limited to that part.  
6

7 An important step in the safety and nutritional assessment of the modified food is a comparison  
8 of its composition with its appropriate counterpart. In the case of a modified microorganism, this  
9 could apply to the microorganism itself in the event that it constitutes a significant portion of the  
10 food mass but it is more likely to apply to the food containing the modified microorganism. To  
11 determine whether there are any differences in the nutritional quality of the food containing the  
12 modified microorganism compared to its appropriate counterpart, the microorganism should first  
13 be subject to laboratory testing of the metabolic products of the microorganism in controlled  
14 media. Once into the food production trial phase, the major constituents of the food containing  
15 the microorganism must be analysed, *i.e.* macronutrients and their component parts, as well as  
16 individual micronutrients selected based on validated criteria. If any nutrients (in the list below)  
17 are excluded from the analyses, this should be justified by an acceptable rationale.  
18

19 Also, circumstances may warrant an evaluation of the nutritional “performance” of the new food  
20 in its ready-to-eat form, thus either raw or when processed by traditional/conventional methods  
21 used to make the product ready-to-eat. The purpose would be to provide an opportunity to  
22 identify major changes that may not have been detected by compositional analysis, but which  
23 could affect, for example, the stability or bioavailability of nutrients in the food or the  
24 susceptibility of anti-nutrients to processing that normally destroys them. A performance test  
25 could involve re-analysis of a substance following cooking or it could require animal testing for  
26 satisfactory growth and nutrient bioavailability.  
27  
28

## 29 **Guidelines for Producing Data for Nutritional Evaluation**

### 30 **a. Function of the data to be submitted**

- 31 • The information provided for a novel microorganism food or for a food containing  
32 one should be of sufficient quantity and quality to allow an assessment of whether  
33 any significant unintended genetic modification affecting the nutritional quality of  
34 the food has occurred as a result of the introduction of the novel trait. It should  
35 also allow an assessment of the nutritional significance of any change that is  
36 detected.  
37
- 38 • Data should be provided for the novel microorganism food or for the food  
39 containing one, before further processing. Data may also be required for the food  
40 prepared for human consumption by conventional means to examine the effects,  
41 where applicable, of processing, storage and cooking, for example, to look at the  
42  
43



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

- 3
- 4 • Data on the novel food should be compared, at a minimum, to data on the near  
5 isogenic, non-transgenic parent strain, *i.e.* the most appropriate counterpart, if  
6 available, or else a closely related non-transgenic strain. Since one or more  
7 significant differences could arise, the study design should include strains of the  
8 same species from a range of standard strains that are used in commercial  
9 production for the same purposes and, possibly, at a variety of production plants  
10 in Canada. This would permit assessment with respect to normal variation.  
11 Literature data (if available) may also be valid for assessing the nutritional  
12 relevance of any unintended effect.

13

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

- 17
- 18 • Considers all major sources of potential variation in nutritional quality (*e.g.*  
19 composition of the growing medium, incubation conditions, *etc.*) in designing the  
20 study, to ensure these factors are controlled.
  - 21
  - 22 • Subjects the modified microorganism or food containing it to the conditions  
23 expected for it in commercial production.
  - 24
  - 25 • Includes in the same study the novel microorganism that is the subject of the  
26 notification as well as the appropriate counterpart, *i.e.* the near isogenic, non-  
27 transgenic parent strain, if available, and a selection of the commercial strains  
28 available in the current market. In the absence of a near isogenic parent strain, the  
29 most closely related non-transgenic strain may be chosen.
  - 30
  - 31 • Establishes a sampling plan prior to the commencement of the study. This plan  
32 should account for all major sources of variation of nutrient levels and use  
33 standard statistical methods for determining numbers of samples to collect and the  
34 appropriate method for collecting and compositing, for example to account for  
35 inter-strain variation.
  - 36
  - 37 • Ensures sampling is conducted at the appropriate stage of incubation.
  - 38
  - 39 • Ensures that the appropriate analyses are performed on all products containing the  
40 microorganism that are expected to be used as food in Canada.
  - 41
  - 42 • Provides the criteria used for selecting of the nutrients analysed and the rationale  
43 for the exclusion from analysis of any nutrients and other substances listed in c.

1                   **Nutrient Composition** below.

- 2
- 3                   • Ensures that analyses for each nutritive or non-nutritive component are conducted
- 4                   for all samples by a single laboratory using internationally approved and validated
- 5                   analytical methods and following consistent and appropriate sample storage and
- 6                   preparation procedures throughout.
- 7
- 8                   • Ensures samples are analysed within an acceptable time frame from date of
- 9                   collection.
- 10
- 11                  • Uses appropriate and consistent statistical methods chosen in advance based on
- 12                  the study design to compare levels of each nutrient in the novel food versus its
- 13                  controls.
- 14
- 15

16                  **c. Nutrient Composition**

17

18                  In the context of the above study guidelines, the following components of foods should be

19                  analysed. Where not all are analysed, the petitioner should provide the criteria used to

20                  select the nutrients analysed and the rationale for the exclusion from analysis of any

21                  nutrients and other substances listed below.

22

- 23                  • proximate composition *e.g.* ash, moisture content, crude protein, crude fat, crude
- 24                  carbohydrate
- 25                  • content of true protein, non-protein nitrogenous material (*e.g.* nucleic acids and
- 26                  aminoglycosides), amino acid profile, -- unusual amino acids should be
- 27                  determined if their presence is suspected (*e.g.* d-amino acids from bacterial
- 28                  proteins)
- 29                  • quantitative and qualitative composition of total lipids, *i.e.* saponifiable and
- 30                  nonsaponifiable components, complete fatty acid profile, phospholipids, sterols,
- 31                  cyclic fatty acids and known toxic fatty acids
- 32                  • composition of the carbohydrate fraction *e.g.* sugars, starches, chitin, tannins,
- 33                  non-starch polysaccharides and lignin
- 34                  • qualitative and quantitative composition of micronutrients, *i.e.* significant vitamin
- 35                  and mineral analysis - see Appendix A, "Key Micronutrients"
- 36                  • presence of naturally occurring or adventitious anti-nutritional factors *e.g.*
- 37                  phytates, trypsin inhibitors, *etc.*
- 38                  • predictable secondary metabolites, physiologically active (or bioactive)
- 39                  substances, other detected substances
- 40

41                  "Fingerprinting" of the product by such techniques as HPLC, GC-MS, and conventional

42                  analytical methods would be appropriate. When more advanced techniques such as

43                  proteomics and metabolomics become available and are validated for use, these should be

44                  adopted for this purpose.

1  
2 **d. Nutritional “Performance” of a modified microorganism**  
3

4 Consideration should be given to the possible need for the following types of information  
5 regarding the modified microorganism or the foods containing them:  
6

7 Response of known anti-nutrients to processes normally expected to neutralize their  
8 activity measured using compositional analysis.  
9

10 Storage stability with regard to nutrient degradation.  
11

12 Performance of product in relation to the intended benefit (other than direct health  
13 benefits) *e.g.* improved stability of an oil to heating after fatty acid profile modification.  
14

15 **Nutrient bioavailability/Presence of new or altered anti-nutrients**  
16

17 In situations where the food from a genetically modified source may become a significant  
18 component of the Canadian diet, and/or a significant supplier of nutrients, animal studies  
19 may be needed in assessing nutritional adequacy to determine if there have been changes  
20 in the bioavailability of nutrients or if the composition is not comparable to conventional  
21 foods.  
22

23 Information should be provided, if applicable, describing the processing conditions used  
24 in the production of a food, and the potential effects of the processing on nutrient levels  
25 and nutrient bioavailability.  
26  
27

28 **e. Information to include in the submission:**  
29

- 30 • the names of all the strains which were represented in the study;  
31  
32 • a complete description of the experimental design, experimental conditions, and  
33 how sources of variation for nutrient levels were controlled;  
34  
35 • a complete description of sample collection and sample preparation;  
36  
37 • a citation and/ or description of the analytical and statistical methods which were  
38 used to obtain data for the nutritive and non-nutritive components;  
39  
40 • nutrient and related data for test, control, and commercial strains (expressed as  
41 mean  $\pm$  standard deviation, and as a range);  
42  
43 • results of statistical analyses;  
44

- 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)<sup>8</sup>. 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.

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<sup>8</sup>“Codex Alimentarius Commission”, Joint FAO/WHO Food Standard Programme; Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology”, 3<sup>rd</sup> 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](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

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

7  
8 It is important to ascertain to what extent the nutrient or other targeted substance whose levels  
9 have been changed (if the intent was to deliberately modify the level of a nutrient) is bioavailable  
10 and remains stable with cultivation, time, processing, storage and cooking.

11  
12 The review of unintended nutritional effects in a food modified to have an intended nutritional  
13 effect would follow the same steps as for other novel foods.

#### 14 15 **Nutritional Evaluation of expected or unexpected increased levels of a nutrient or** 16 **bioactive substance**

- 17  
18 • Increased levels of a nutrient or other bioactive substance (including a  
19 microorganism) in a food need to be evaluated for safety.
- 20  
21 • Data needed for this include:
  - 22  
23 – the level of the targeted nutrient or other bioactive substance expected in  
24 the food;
  - 25  
26 – expected level of exposure to the targeted nutrient or other bioactive  
27 substance through consumption of the food at the upper and lower  
28 extremes of intake of the food and across all age and sex groups using  
29 recent Canadian food consumption data where possible;
  - 30  
31 – intended target group, if applicable, or which group(s) is or are likely to  
32 consume the most of the food;
  - 33  
34 – how the expected level of dietary exposure to the targeted nutrient or other  
35 bioactive substance differs from the current levels in the diet; and
  - 36  
37 – data in support of the safety of the expected level of exposure.

#### 38 39 40 **4.2.3.6 Toxicology Considerations**

41  
42 Toxicological testing is required for substances of unknown safety that are introduced to the food  
43 supply. Novel substances may be introduced to the food supply through recombinant DNA

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

3  
4 Genetic modification techniques can result in the production of novel substances by the organism  
5 or the intentional or unintentional modification of substances already produced by the organism  
6 or their expression.

## 7 8 **Novel Substances**

9  
10 *In vitro* nucleic acid techniques enable the introduction of DNA which can result in the synthesis  
11 of new substances in microorganisms. These include the protein expression product and other  
12 substances which may be generated as a result of enzymic activity of the protein expression  
13 product. The new substances can be conventional components of genetically modified  
14 microorganisms.

15  
16 The introduced trait should be shown to be unrelated to any characteristics of donor organisms  
17 that could be harmful to human health. Information should be provided to ensure that genes  
18 coding for known toxins present in the donor organisms are not transferred to recombinant DNA  
19 organisms.

20  
21 Toxicology studies are not considered necessary where the substance or a closely related  
22 substance has been consumed safely in food at equivalent intakes or where the new substance is  
23 not present in the food. Otherwise, the use of conventional toxicology studies on the new  
24 substance will be necessary. This will require the isolation of the new substance from the  
25 recombinant DNA microorganism.

26  
27 For proteins, the assessment of potential toxicity should focus on amino acid sequence similarity  
28 between the protein and known protein toxins and anti-nutrients (*e.g.* protease inhibitors, lectins)  
29 as well as stability to heat or processing and to degradation in appropriate/representative gastric  
30 and intestinal model systems. Since proteins that are enzymes have never been shown to be  
31 direct-acting carcinogens, mutagens, teratogens or reproductive toxicants (Pariza and Foster  
32 1983) it is generally not necessary to test proteins for these toxicological endpoints when  
33 exposure occurs by the oral route. Protein toxins act through acute mechanisms after the  
34 administration of a single dose at doses in the nanogram to milligram per kilogram body weight  
35 (bw). Therefore, acute oral toxicity studies using gram/kg bw doses of the novel protein are  
36 appropriate for assessing the potential toxicity of proteins. A negative result using doses in the  
37 gram/kg bw range together with evidence that the protein is digested to small peptides and amino  
38 acids would provide assurance that the protein is not a toxin and is digested to nutrients as are the  
39 vast majority of dietary proteins.

40  
41 Different types of *in vivo* or *in vitro* studies would be needed to assess the toxicity of introduced  
42 substances other than proteins. The types of studies are determined on a case-by-case basis and  
43 depend on the original source of the introduced substances and their function. Such studies may

1 include assays of metabolism, toxicokinetics, chronic toxicity/carcinogenicity, impact on  
2 reproductive function, and teratogenicity.

### 3 4 **Unintended Effects**

5  
6 Techniques used in the genetic modification of microorganisms have the potential to induce  
7 unintended effects on the genome of the modified organism that could be manifested as an  
8 alteration in the levels of toxicants normally produced by the organism. The intended genetic  
9 alteration may also influence the behaviour of the organism with respect to accumulation of  
10 contaminants, pesticides, or other substances from the environment that were not anticipated.

11  
12 Compositional analysis is the method currently used for detection of unintended changes to the  
13 genome that result in accumulation of toxic substances either of endogenous or exogenous origin.  
14 Because of the influence of environmental stress on production of endogenous components such  
15 as toxins, data should be collected from a number of different test sites. New, more sensitive  
16 technologies that allow the determination of alterations to expression of the organisms' genome  
17 are presently under development.

### 18 19 20 **4.2.3.7 Allergenicity Considerations**

21  
22 The primary considerations in allergenicity assessment of a novel food are the prevention of  
23 unexpected and unavoidable exposure of sensitized individuals to food allergens. This includes  
24 the assessment of the potential for foods containing novel proteins to cross-react with known  
25 food allergens or to lead to the development of *de novo* hypersensitivity. In addition, the  
26 possibility of increasing the allergenic potential of foods already containing allergens as a result  
27 of genetic modification should be assessed.

### 28 29 **Section 1 – Introduction**

30  
31 All newly expressed proteins in recombinant-DNA microorganisms that could be present in the  
32 final food and are novel in the context of that food, need to be assessed for their potential to  
33 cause allergic reactions. This should include consideration of whether a newly expressed protein  
34 is one to which certain individuals may already be sensitive as well as whether a protein new to  
35 the food supply is likely to induce allergic reactions in some individuals.

36  
37 At present, there is no definitive test that can be relied upon to measure directly the allergenic  
38 potential of a newly expressed protein in humans. Based upon the best, currently-available  
39 scientific information, the recommended approach takes into account the preponderance of  
40 evidence derived from several types of information and data in an integrated, stepwise, case-by-  
41 case manner.



## 1 **Section 2 - Assessment Strategy**<sup>9</sup>

2  
3 The initial steps in assessing possible allergenicity of any newly expressed proteins involve  
4 determination of: the allergenicity of the source of the introduced protein; any similarity between  
5 the amino acid sequence of the protein and that of known allergens; and certain physicochemical  
6 properties, including but not limited to, its susceptibility to enzymatic degradation.

7  
8 Genes derived from known allergenic sources should be assumed to encode an allergen unless  
9 scientific evidence demonstrates otherwise.

10  
11 Determination of amino acid sequence homology and physicochemical characteristics will  
12 require the isolation of the newly expressed protein from the recombinant-DNA organism, or the  
13 production of the substance from an alternative source, in which case the material should be  
14 shown to be functionally and biochemically equivalent to that produced in the recombinant-DNA  
15 organism.

16  
17 Food proteins that are not allergens and that are altered by mutagenesis techniques need only be  
18 assessed for the likelihood that the mutagenized protein is a *de novo* allergen.

19  
20 The absolute exposure to the newly expressed protein and the effects of relevant food processing  
21 will contribute toward an overall conclusion about the potential for human health risk. In this  
22 regard, the nature of the food product intended for consumption should be taken into  
23 consideration in determining the types of processing that would be applied and its effects on the  
24 presence of the protein in the final food product.

## 25 26 **Section 3 – Initial Assessment**

### 27 28 **Section 3.1 - Source of the Protein**

29  
30 As part of the data supporting the safety of foods derived from recombinant-DNA organisms,  
31 information should describe any reports of allergenicity associated with the donor organism.  
32 Allergenic sources of genes would be defined as those organisms for which reasonable evidence  
33 of IgE-mediated oral, respiratory or contact allergy is available. Specific tools and relevant data  
34 that permit confirmation of allergenic potential are available for proteins from some allergenic  
35 sources. These include: the availability of sera for screening purposes; documented type, severity  
36 and frequency of allergic reactions; and structural characteristics and amino acid sequence (when  
37 available) of known allergenic proteins from that source.

---

38  
<sup>9</sup> 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<sup>10</sup>. 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<sup>11</sup>. The resistance of a

---

<sup>10</sup> 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.

<sup>11</sup> The method outlined in the U.S. Pharmacopoeia (1995) was used in the establishment of the correlation (Astwood *et al.* 1996).

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

5  
6 Although the pepsin resistance protocol is strongly recommended, it is recognized that other  
7 enzyme susceptibility protocols exist. Alternative protocols may be used where adequate  
8 justification is provided.

#### 9 10 **Section 4 – Specific Serum Screening**

11  
12 For those proteins that originate from a source known to be allergenic, or have sequence  
13 homology with a known allergen, testing in immunological assays is required. Sera from  
14 individuals with a clinically validated allergy to the source of the protein can be used to test IgE-  
15 binding of the protein in *in vitro* assays. A critical issue for testing will be the availability of  
16 human sera from sufficient numbers of individuals<sup>12</sup>. In addition, the quality of the sera and the  
17 assay procedure need to be standardized to produce a valid test result.

18  
19 In the case of a newly expressed protein derived from a known allergenic source, a negative  
20 result in *in vitro* immunoassays may not be considered sufficient, but should prompt additional  
21 testing, such as the possible use of skin test and *ex vivo* protocols.

22  
23 The identification of a newly expressed protein as an allergen through immunological assays  
24 suggests that further development for commercialization of the product be discouraged, unless  
25 adequate risk management and risk communication measures could be assured throughout  
26 marketing and distribution of the product, since segregation and identity preservation of the new  
27 source of this allergen may be difficult or impossible to enforce.

#### 28 29 **Section 5 – Areas Requiring Further Development**

30  
31 The endpoint of the assessment of the data discussed above is a conclusion as to the likelihood of  
32 the protein being a food allergen. The techniques of targeted serum screening (*i.e.* the  
33 assessment of binding to IgE in sera of individuals with clinically-validated allergic responses to  
34 broadly-related categories of foods) and the use of animal models, once developed and validated,  
35 could enhance the weight of evidence used to derive this conclusion. To allow serum screening,  
36 steps should be taken to organize an international serum bank. As scientific knowledge and  
37 technology evolves, other methods, such as examination of newly expressed proteins for T-cell  
38 epitopes and structural motifs associated with allergens, might also be useful.

---

<sup>12</sup> 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.

1     **Unintended effects on endogenous allergens**  
2

3     Genetic modification techniques have the potential to produce unintended effects on the genome  
4     that could lead to an increase in the expression of endogenous allergens. While the potential for  
5     health impacts of such increases is uncertain, they are in any case considered undesirable.

6     Techniques used for assessing the potential for effects on endogenous allergen expression are the  
7     quantitative comparison of protein composition of the edible portion of the modified organism  
8     or, where sera from sufficient numbers of individuals with allergies to the food are available, the  
9     comparative immunoreactivity to the edible portion of the modified organism can be determined  
10    using immunoblotting techniques.  
11

12  
13    **4.2.3.8        Chemical Considerations**  
14

15    The identification and levels of chemical contaminants must be reported. Levels and types of  
16    contaminants would be specific to the food that has been modified. Potential contamination  
17    could occur, for instance, as a result of residues from chemicals (organic or inorganic) employed  
18    in processes, such as extraction or purification processes, to produce the desired food product  
19    from microorganisms.  
20

## **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.”<sup>13</sup> 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%<sup>14</sup> 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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<sup>13</sup>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

<sup>14</sup>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	<b>0</b> RE	870	0	
Vit. D	* ug	3	0	
Vit. E	<b>0.972</b> ATE <sup>#</sup>	7	13.9	***
Vit. C	<b>0</b> mg	34	0	
Thiamine	<b>0.541</b> mg	1	54.1	***
Riboflavin	<b>0.126</b> mg	1.2	10.5	***
Niacin	<b>6.873</b> NE	16	43	***
Vit. B6	<b>0.687</b> mg	1	68.7	***
Vit. B12	<b>0</b> ug	1	0	
Folacin	<b>0.027</b> mg	0.195	13.8	***
Pantothenic acid	<b>2.02</b> mg	5	40.3	***

### Minerals

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

\* Data not available

\*\* Average Daily Intake used since there is no RDI

<sup>#</sup>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	<b>1</b> RE	870	0.1	
Vit. D	* ug	3	0.0	
Vit. E	<b>0.98</b> ATE <sup>#</sup>	7	14	***
Vit. C	<b>3</b> mg	34	8.8	
Thiamine	<b>0.437</b> mg	1	43.7	***
Riboflavin	<b>0.44</b> mg	1.2	36.7	***
Niacin	<b>5.23</b> NE	16	32.7	***
Vit. B6	<b>0.189</b> mg	1	18.9	***
Vit. B12	<b>0</b> ug	1	0.0	
Folacin	<b>0.18755</b> mg	0.195	96.2	***
Pantothenic acid	<b>0.397</b> mg	5	7.9	***

### Minerals

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

\* Data not available

\*\* Average Daily Intake used since there is no RDI

<sup>#</sup>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 <sup>#</sup>	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

<sup>#</sup>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	<b>47</b> RE	870	5.4	***
Vit. D	* ug	3	-	
Vit. E	* ATE <sup>#</sup>	7	-	
Vit. C	<b>0</b> mg	34	0	
Thiamine	<b>0.246</b> mg	1	24.6	***
Riboflavin	<b>0.08</b> mg	1.2	6.7	***
Niacin	<b>2.72</b> NE	16	17	***
Vit. B6	<b>0.37</b> mg	1	37	***
Vit. B12	<b>0</b> ug	1	0	
Folacin	<b>0.025</b> mg	0.195	12.8	***
Pantothenic acid	<b>0.658</b> mg	5	13.2	***

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

### Minerals

Nutrient	Amount in 100 g yellow corn flour	WRNI (2 yrs +)	% WRNI	*** (Key Nutrients)
Calcium	<b>7</b> mg	780	0.9	
Phosphorus	<b>272</b> mg	885	30.7	***
Iron	<b>2.38</b> mg	10	23.8	***
Iodide	* ug	155	-	
Magnesium	<b>93</b> mg	210	44.3	***
Copper	<b>0.23</b> mg	2.0**	11.5	***
Zinc	<b>1.73</b> mg	10	17.3	***
Potassium	<b>315</b> mg	3000**	10.5	***
Manganese	<b>0.46</b> 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	<b>62</b> RE	870	7.1	***
Vit. D	* ug	3	0	
Vit. E	* ATE <sup>#</sup>	7	0	
Vit. C	<b>19.1</b> mg	34	56.2	***
Thiamine	<b>0.059</b> mg	1	5.9	***
Riboflavin	<b>0.048</b> mg	1.2	4	
Niacin	<b>0.728</b> NE	16	4.6	
Vit. B6	<b>0.08</b> mg	1	8	***
Vit. B12	<b>0</b> ug	1	0	
Folacin	<b>0.015</b> mg	0.195	7.7	***
Pantothenic acid	<b>0.247</b> mg	5	4.9	

### Minerals

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

\* Data not available

\*\* Average Daily Intake used since there is no RDI

<sup>#</sup>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.