



A risk-based classification scheme for genetically modified foods I: Conceptual development

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ABSTRACT

The predominant paradigm for the premarket assessment of genetically modified (GM) foods reflects heightened public concern by focusing on foods modified by recombinant deoxyribonucleic acid (rDNA) techniques, while foods modified by other methods of genetic modification are generally not assessed for safety. To determine whether a GM product requires less or more regulatory oversight and testing, we developed and evaluated a risk-based classification scheme (RBCS) for crop-derived GM foods. The results of this research are presented in three papers. This paper describes the conceptual development of the proposed RBCS that focuses on two categories of adverse health effects: (1) toxic and antinutritional effects, and (2) allergenic effects. The factors that may affect the level of potential health risks of GM foods are identified. For each factor identified, criteria for differentiating health risk potential are developed. The extent to which a GM food satisfies applicable criteria for each factor is rated separately. A concern level for each category of health effects is then determined by aggregating the ratings for the factors using predetermined aggregation rules. An overview of the proposed scheme is presented, as well as the application of the scheme to a hypothetical GM food.

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1. Introduction

The predominant paradigm currently in place in many jurisdictions for the premarket assessment of genetically modified (GM) foods focuses on foods derived from crops bioengineered by recombinant deoxyribonucleic acid (rDNA) techniques. Extensive work supported the development of guidelines in the safety assessment of GM foods at the international and national levels (e.g., FAO/WHO, 2001; Codex Alimentarius, 2003; Cellini et al., 2004; EFSA, 2004; NRC, 2004). This paradigm reflects heightened public concern about the potential for this category of GM foods to contain undesirable or unintentionally altered constituents. However, foods derived from other methods of genetic modification, such as induced mutation and wide-cross hybridization, are also subject to similar alterations, but they are generally not assessed for safety. One exception is the premarket assessment approach adopted by Health Canada since the late 1990s with a focus on novelty (Health Canada, 2006). There is the need to implement a risk-based ap-

proach to the assessment of GM foods. This paper describes a risk-based classification scheme (RBCS) for the testing of foods derived from GM crops, regardless of the method of genetic modification. A risk-based approach offers the potential for more efficient testing by focusing less on lower concern GM foods, and more on those foods of higher concern.

This paper is the first in a series of three papers on the development and evaluation of the proposed RBCS. It describes the conceptual development of the RBCS, the rationale underlying the factors and criteria included in the scheme, and their aggregation in the assignment of concern levels. An overview of the proposed scheme is presented with an example illustrating how the scheme may be applied to a hypothetical GM food. The companion papers will report on the evaluation of selected functional aspects of the proposed framework and its implications on the graded testing of GM foods.

In assessing the potential risks of GM foods, it is important to consider both adverse health effects that may arise from substances that are intentionally introduced or modified in food crops, and adverse effects that may be produced unexpectedly as a result of the genetic modification process. In the proposed RBCS, an introduced or modified gene product and the GM food itself are assessed separately.

In order to classify GM foods into different concern levels, the factors that may affect the level of risk associated with the consumption of GM foods were identified through a literature review.

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Topics reviewed included a comparison of the molecular and biochemical basis of unintended changes in GM crops that have the potential for generating new toxic compounds, or modifying the expression of endogenous toxic metabolite (Chao, 2007).

Scientific judgment of separate multidisciplinary groups of experts was elicited through self-administered questionnaires in order to: (1) confirm the scope and importance of the factors and the appropriateness of the criteria identified from the literature review; (2) test the feasibility of differentiating GM foods into a manageable number of concern levels using defined factors and criteria and verify the scientific and logical soundness of proposed concern levels; and (3) simplify the structure of the RBCS and pilot test its use. In total, 27 experts affiliated with academic, governmental, and public research institutions from ten countries in North America, Europe, and the Asia-Pacific region participated in three consultations (Appendix A).

2. Factors affecting potential health risks of GM foods

In this section, we summarize the most important factors affecting potential health risks and levels of concern about GM foods in the RBCS. We also present criteria by which these factors can be evaluated, and rules for aggregating across risk factors. This aggregation is done separately for toxic and antinutritional effects due to intended changes (Part A), similar effects due to unintended changes (Part B), and allergenic effects from both intended and unintended changes (Part C).

Regardless of the method of genetic modification, substances present in GM foods can be broadly divided into two categories based on the types of adverse health effects they may produce: protein and non-protein substances. Protein substances are generally the direct products of introduced or modified genes and are known as primary gene products. Non-protein substances in GM foods are produced in metabolic pathways catalyzed by the introduced or modified enzymatic proteins. Non-protein substances are known as secondary gene products and belong to a greater diversity of chemical classes.

Adverse health effects of these two major categories of substances in GM foods include: toxic, antinutritional, and allergenic effects. Toxic and antinutritional effects can be produced by protein and non-protein substances and are grouped together as one major category of health effects for the purposes of testing, while allergenic effects are attributable primarily to protein substances and are considered in the RBCS as a separate category of health effects that require different considerations for testing.

In GM crops, potential adverse health effects can result due to intentional and unintentional changes in the nature or the level of these substances. Intended changes include both the direct and indirect effects of primary and secondary gene products. Unintended changes can be predictable or unpredictable. Predictable effects are unintended changes that go beyond the expected changes from introducing the target gene(s), but that may be explicable in terms of our current knowledge of plant biology and metabolic pathway integration and interactions. Unpredictable effects are those changes that fall outside our present level of understanding of gene regulation and gene interactions (Kuiper et al., 2001; Cellini et al., 2004). To differentiate the sources of uncertainty, intended and unintended changes are classified separately for potential toxic and antinutritional effects in the RBCS. In contrast, no distinction is made in classifying intended and unintended changes in the allergenic potential of introduced and endogenous proteins in GM foods because of the role of food matrix and food processing in influencing protein structure and allergenic properties (Teuber 2002; Wal, 2003; van Wijk et al., 2005).

2.1. Factors

2.1.1. Part A. Potential for toxic and antinutritional effects due to intended changes

In the context of the proposed RBCS, the risks posed by a dietary substance of interest are primarily determined by: the level of intake and hazard characteristics of the substance. Eight factors related to (1) exposure, (2) the nature of the substance that is intentionally introduced or modified in a GM crop, and (3) other factors can be grouped as follows (Table 1). The criteria used to evaluate the factors considered here are discussed in Section 2.2.

2.1.1.1. Dietary exposure to gene products (factors A.1–A.3). It is expected that many substances intentionally introduced or modified in GM foods will be similar to those already present in the food supply. Substances that have a prior history of consumption from foods will require less scrutiny than those that do not. Genetic modification may alter the food sources of these substances and their exposure levels. For example, β -carotene may be added to GM rice, which is not a conventional source of this nutrient. Further, depending on the strategy of the genetic modification, the extent of dietary exposure may be limited by the tissue of expression in the modified crop. For example, gene expression in the non-edible part of the crop would have limited impact on altering dietary exposure.

2.1.1.2. Nature of gene products (factors A.4–A.7). For some substances with a dietary history, their inherent toxic or antinutritional properties can be based on prior knowledge. For substances introduced to a heterologous crop, the demonstration of structural similarity and functional equivalence with substances with a prior history of known or presumed safe food use provides a reasonable level of relative safety. In genetic modification, a novel gene product may also be a protein that is encoded by DNA sequences that have been substantially modified, or is engineered for specific functional properties. Structure–activity characterization of the gene product provides screening level information that may signal the need for further testing.

Consideration is given to the metabolic fate in the modified plant of a gene product that is new to the modified plant, or an endogenous gene product that is modified to be expressed at substantially higher levels. The proteolytic profile or metabolic fate of a gene product following ingestion would also be relevant if a protein substance is resistant to digestion, or if a non-protein substance is in a structural class for which there is less knowledge of its metabolism, pharmacology and toxicology.

The potential for adverse health effects from synergistic and interactive effects among intentionally altered substances and endogenous food constituents is another important factor in the RBCS. In the context of foods as complex mixtures, the effects of the food matrix, bioavailability, and processing are also relevant in considering the safety of a substance present in a new food source.

Excluded from general consideration as core factors in the RBCS are factors related to gene transfer and viral recombination. One major concern about gene transfer is related to the use of antibiotic resistance genes as selectable markers. This type of concern can be addressed by employing best practices in the selection of marker genes encoding traits that pose little relevance in human and veterinary medicine. For example, the use of antibiotic resistance marker genes such as *npt II* under the control of an eukaryotic promoter in a transgenic plant poses a low risk for expression in bacteria because a bacterial promoter is required. In addition, *npt II* genes are widely distributed in the environment (van den Eede et al., 2004). Similarly, concerns about viral recombination in the generation of new viral organisms with respect to plant health

Table 1

Factors and criteria for determining potential for toxic and antinutritional effects due to intended changes (Part A of the RBCS)

Factors	Criteria ^a
<i>(1) Dietary exposure to gene products</i>	
A.1. Expression of gene product(s) in edible part(s) of the modified crop	A.1.1. Substances with NO nutritional role <i>and</i> NOT altered in the modified crop due to intentional gene silencing, <i>or</i> <i>Non-protein substances only</i> A.1.2. Total dietary intake of gene product(s) is below the threshold of toxicological concern for the chemical class where applicable, <i>or</i> A.1.3. Gene products NOT expressed, detected or changed in the edible part(s) of the modified crop (a) Tissue-specific expression NOT involving the edible part(s) of the modified crop, <i>or</i> (b) The edible part is used as a food ingredient in a limited form as a highly refined and well-defined chemical entity (e.g., refined oil from oilseed rape and cottonseed by hot solvent extraction).
A.2. Prior history of consumption from foods	(a) Naturally occurring food constituents, <i>or</i> (b) Other substances that have a history of consumption from foods without reported adverse health effects
A.3. Expected change in total dietary exposure (based on theoretical estimates)	<i>Substances with known toxicity concerns</i> (a) Total dietary intake below established upper limit (ADI, tolerance level, or equivalent) with an adequate margin of exposure <i>Other substances</i> (b) Negligible change or changes within normal variations in dietary intakes
<i>(2) Nature of gene products</i>	
A.4. Prior knowledge of safety/mode of action	
A.4.1. Food constituents and substances with a prior history of consumption from foods (with dietary history)	<i>Substances with known toxicity concerns</i> (a) Established upper limit (ADI, tolerance level, or equivalent) <i>Other substances</i> (b) Well-established biological function or specificity of mode of action with NO potential for mammalian toxicity, antinutritional, and adverse metabolic effects under normal conditions of consumption, <i>or</i> (c) Limited empirically based toxicity profile <i>and</i> simple chemical structure or predictable metabolism suggestive of low order of oral toxicity
A.4.2. Substantially modified or novel substances (without dietary history).	<i>Pesticidal and enzymatic proteins</i> (a) NO structural or functional similarity to known proteins with toxic or antinutritional properties relevant to mammals <i>and</i> at low expression level <i>Non-protein (low molecular weight) substances</i> (b) WITHOUT structural similarity to known mutagen or carcinogen <i>and</i> <0.5 ppm in the total diet^b <i>Hormonal protein and non-protein complex substances</i> (c) WITHOUT structural similarity to known mutagen or carcinogen <i>and</i> <0.1% diet^c
A.5. Structure–activity characterization	<i>Proteins</i> (a) NO amino acid sequence identity when compared with known mammalian toxins^d, rapid and complete pepsin digestion using standard testing conditions, <i>and</i> structurally similar and functionally identical to comparator/source protein <i>Non-proteins</i> (b) Structurally similar to natural food constituents
A.6. Digestion and degradation products	<i>In edible parts of modified crop</i> (a) NO potentially toxic metabolites expected from catabolism of an introduced/modified substance or of an exogenous chemical (e.g., detoxification of a herbicide or mycotoxin), <i>and</i> <i>In mammalian metabolism from consumption of the gene product</i> (b) NO products of digestion or bioactivation at levels of immunological, pharmacological, or toxicological significance
A.7. Synergism and interactions	
A.7.1. Between gene products introduced/modified	(a) NO synergistic toxic effects expected
A.7.2. Between gene products introduced/modified and food constituents	(a) NO negative effect on bioavailability of food constituents expected
<i>(3) Other factors</i>	
A.8. Prior experience with gene products introduced/modified	(a) Comparable in expression levels, gene source, transfer method, and host plant, <i>and</i> (b) No expected increase in total dietary exposure and approved by at least one national competent authority

^a A 'yes' rating to a criterion means that the gene product is potentially non-toxic and nutritionally adequate due to *intended* changes to the genome with respect to that specific factor.

^b At levels <0.5 ppm, chronic toxicity and carcinogenicity testing is generally not required for substances without structural similarity to known mutagen or carcinogen (equivalent to structure category B for food additive assessment), unless indicated by findings in shorter term testing (FDA, 2003).

^c The level of <0.1% of diet was chosen as a reference point for complex substances because substances at concentrations > 0.1% cannot be evaluated in animal testing using the approach based on an uncertainty factor of 100. It is assumed that tests conducted using a substance at a dietary concentration > 10% in the diet of experimental animals will likely lead to results that are not meaningful, and may require alternative types of testing (LSRO, 1999).

^d The level of amino acid sequence identity with known mammalian toxins as indicator of potential toxicity of novel proteins has not been the subject of deliberation in the scientific literature. The criterion included here is provisional for discussion purposes.

are also best addressed by sound practices such as avoiding the use of complete viral genes (without the presence of replication origins or gene or sequences that might enhance recombination). How-

ever, transgenes from less commonly used microbial sources may warrant a case-by-case evaluation as part of considerations for testing.

2.1.1.3. *Other factors (factor A.8).* Prior experience with gene products may reduce the level of scrutiny required if the necessary criteria are met. Additional factors may also be included in the scheme in the future as new genetic modification technologies evolve and new factors are identified, or as new tests are developed.

2.1.2. Part B. Potential for toxic and antinutritional effects due to unintended changes

Factors affecting the likelihood of occurrence of unintended changes with the potential for adverse health effects are grouped into four categories in Part B of the RBCS: (1) genetic sources of toxic or antinutritional substances, (2) the factors affecting their expression, (3) indicators of their expression, and (4) instability of the modified trait. Among the six factors in Part B of the RBCS listed in Table 2, factors marked with an asterisk are of secondary importance. Failure to meet the criteria for any one of the secondary factors will trigger the need to justify the scope of other metabolites or other relevant parameters to be analyzed.

2.1.2.1. *Genetic sources (factors B.1–B.2).* As noted earlier, two main concerns of unintended changes in GM foods with the potential for adverse health effects are: (1) increased expression of endogenous toxic substances (or metabolites) in different tissues or at different levels, and (2) generation of new toxic compounds. Consequently, the safety of the host plant is an important consideration. The safety of the donor organism is also important in genetic modification techniques other than rDNA. For example, poorly characterized gene modification, such as occurs with wide-cross hybridization and modification using mutagenesis, may increase the need for scrutiny of the donor organism.

The genetic potential of secondary metabolism is never fully expressed as it is highly influenced by complex interactions with environmental factors (Hadacek, 2002). This has given rise to concerns that critical toxicants, inherent in the plant but hitherto unexpressed, may be unintentionally induced by genetic modification (Facchini et al., 2004). Also of concern is that the introduction of new pathways may not cause metabolic problems or identifiable phenotypic changes (Tattersall et al., 2001; Kristensen et al., 2005).

Table 2

Factors and criteria for determining potential for toxic and antinutritional effects due to unintended changes (Part B of the RBCS)

Factors	Criteria ^a
<i>(1) Genetic sources</i>	
B.1. Safety of donor organism (gene source).	(a) NOT a source of known toxicant, antinutrient, or pathogenicity/infectivity <i>Characterized gene modification</i> (a) Absence of transfer of undesirable characteristics to the host plant is supported by experimental evidence <i>Poorly characterized gene modification</i> (b) Genetically related organisms (in the same genus) are NOT sources of known toxicant or antinutrient, or (c) Extent of backcrossing is sufficient to remove large percentage of genetic material from the donor organism
B.2. Safety of host plant.	(a) NOT a source of known/significant toxicant or antinutrient
<i>(2) Factors affecting unexpected expression of known or novel substances</i>	
B.3. Potential for novel/alterd patterns of metabolites.	
B.3.1. Knowledge of gene product function	(a) Protein gene product(s) have well-characterized function in the modified plant.
B.3.2. Impact of gene product function in the modified plant	(a) Expression product of the introduced/modified gene does NOT have a metabolic, regulatory, or signalling function in the modified plant, or (b) Expression of the introduced/modified gene is NOT expected to have a systemic effect on the metabolism of the modified plant
B.3.3. Substrate specificity of gene product	(a) Narrow substrate specificity (i.e., unlikely to interact with endogenous substrates to form novel compounds)
B.4. Potential for disrupting coding or regulatory DNA sequences in the modified plant	
B.4.1. In the inserted/modified DNA	(a) NO novel open reading frames (ORFs), disruptions of existing ORFs, or functional expression
*B.4.2. In genomic sequences bordering the site of insertion/modification	(a) NO novel open reading frames (ORFs), disruptions of existing ORFs, or functional expression
<i>(3) Indicators of unexpected expression of known or novel substances</i>	
B.5. Chemical analysis of food derived from the modified plant	
B.5.1. Key nutrients	(a) Within acceptable ranges of natural variation, or (b) NOT expected to have adverse nutritional, metabolic, or toxicological effects
B.5.2. Known toxicants/antinutrients	(a) Within acceptable ranges of natural variation, or (b) NOT expected to have adverse nutritional, metabolic, or toxicological effects.
B.5.3. Other metabolites/parameters	(a) Reasonably justified to be sufficient in scope, and (b) NO unknown compounds or significantly altered levels of endogenous metabolites outside the range of natural variation, or (c) If changes occur, the likelihood of negative health effects can reasonably be justified to be low
<i>(4) Instability of the modified trait</i>	
B.6. Health consequences of instability of the modified trait.	<i>Applicable if the modified trait is intended to provide nutrition or health benefits</i> (a) Adequate quality control should be in place to ensure consistency in lot-to-lot expression of the targeted gene product

* Factors marked with an asterisk (*) are secondary factors. Failure to meet the criteria for any one of the secondary factors will trigger the need to justify the scope of metabolites or other relevant parameters to be analyzed.

^a A 'yes' rating to a criterion means that the GM food is potentially non-toxic and nutritionally adequate due to *unintended* changes to the genome with respect to that specific factor.

For food crops with a history of traditional breeding, available information tends to support the premise that unintended effects of genetic modification, when they occur, would most likely result in a change in the level of expression of already recognized toxicants in the plant. This is deduced from observations that (1) the ability to synthesize particular classes of secondary metabolites is generally restricted to selected plant groups (Cheeke, 1998, p. 87; Osbourn et al., 2003); (2) certain classes of plant chemicals with potent toxic effects (such as alkaloids) require highly substrate-specific enzymes and are highly compartmentalized in their biosynthesis (Croteau et al., 2000; Jørgensen et al., 2005); (3) as genes encoding enzymes of specific pathways in eukaryotes are not organized and regulated as a unit as is the case for prokaryotes, there is the requirement for a higher level of coordination of multiple metabolic alterations in effecting the synthesis of potentially toxic metabolites (Hadacek, 2002); (4) the production of secondary metabolites with potent biological activity is likely a rare property of any one molecule despite the vast diversity and metabolic flexibility of plant metabolism (Jones and Firn, 1991; Firn and Jones, 2003); (5) only 21 food-born toxic substances have been identified to be of plant origin (<0.1% of the total number of food constituents identified) and only 9 out of the 21 toxicants are biosynthesized by plants, with the remaining being pass-through contaminants in honey and milk (IFBC, 1990). On balance, the likelihood of occurrence of novel substances or naturally occurring constituents in a GM plant with previously unknown acute toxicity would be considered low (WHO, 1995).

2.1.2.2. Factors affecting unexpected expression of known or novel substances (factors B.3–B.4). Unintended changes in GM crops can theoretically result from alterations (1) at the genomic level from structural changes to DNA sequences or to chromosomal organization; (2) at the transcription level from changes to gene expression resulting from position effects, the disruption, modification or silencing of active genes, or the activation of silent genes; and (3) at the metabolic level from interactions between novel gene products and endogenous enzymes and metabolites, as well as alterations in flow through of metabolites in biochemical pathways (Conner and Jacobs, 1999; Kuiper et al., 2001; Cellini et al., 2004).

At the metabolic level, factors affecting unexpected effects on known or novel substances can be related to the function of the gene product, its likelihood for systemic effects in the modified plant, and its substrate specificity, if the protein has enzymatic activity (factor B.3). For example, based on current knowledge, it is generally assumed that recombinant pesticidal proteins that are introduced into food crops to date probably have minimal interactions with the plant's metabolic pathways and are unlikely to induce broad, unexpected metabolic effects (Ellis, 2001; ILSI, 2004). Limited publicly available data appear to support this view in that the extent of metabolic interconnectivity may be predicted in part by gene function (Le Gall et al., 2003; Defernez et al., 2004; El Ouakfaoui and Miki, 2005). Unintended effects at the metabolic level are likely to receive increased attention as the engineering of metabolic pathways plays a greater role in achieving complex alterations of quality traits in the next generation of GM crops.

At the genomic level, the extent and the likelihood of unintended changes can in part be related to the interruption of coding or regulatory sequences of the modified plant (factor B.4). At present, concerns about genomic disruptions resulting from plant genetic engineering are addressed by identifying the presence and plausible expression of unknown DNA sequences (open reading frames) in the inserted gene and flanking regions. In contrast, few molecular analytic techniques have been applied to the risk assessment of crops modified by conventional methods.

Recombination and induced mutation represent two strategies of introducing novelty into a modified crop. Recombination intro-

duces new allelic combinations from a genetically related donor organism or completely new genes from a more distantly related donor organism. Wide-crosses or somatic hybridization between plant species by protoplast fusion is an example of conventional method of recombination between distantly related plant species or genera, while rDNA techniques represent more modern methods of recombination capable of introducing completely new genes. Modification by physical and chemical mutagenesis, as well as somaclonal variation produced by tissue culture, are examples of induced mutation using conventional techniques³. The available data indicate no major qualitative differences in DNA disruptions between recombination and induced mutation (Chao, 2007; Appendix B).

Past experience with the process used in commercializing crops modified by conventional breeding methods has resulted in very few documented cases of unacceptably high levels of known toxicants in modified foods. It has been suggested that foods derived from conventionally bred crops could provide a reference point by which the safety of crops modified by rDNA techniques can be compared. Comparisons can be made at the molecular, metabolic, and phenotypic or morphologic levels. The level on which emphasis is placed can vary, depending on the method of genetic modification. For example, molecular characterization methods (factor B.4) typically used in bioengineered crops are generally not amenable to assessing changes in plants modified by non-rDNA conventional methods. In applying factors B.3 and B.4, knowledge of the intended gene expression products is required. While recent advances in molecular profiling techniques have allowed development of methods for characterizing the plant genome, these types of information and methods are not currently applied to the assessment of crops modified by non-rDNA techniques. Consequently, another factor (B.5) would assume greater importance in practice in assessing foods derived from crops modified by conventional methods.

2.1.2.3. Indicators of unexpected expression of known or novel substances (factor B.5). For the reasons given above, identifying differences in expressed proteins or metabolites (factor B.5) assumes greater weight in crops modified using conventional methods than characterizing changes at the molecular level. The assessment of phenotypic alterations is another important part of screening unintended changes in a plant breeding program regardless of the genetic modification method. While phenotypic screening is not a factor in the RBCS, it can be included as part of the justification for the scope of the metabolites to be analyzed.

2.1.2.4. Instability of the modified trait (factor B.6). Although data to support genetic stability of the modified trait are generally required as part of premarket assessment, the duration of maintenance of genetic stability is uncertain (FIFRA SAP, 2004). Factors affecting genetic stability are also not well-characterized. This question is prompted by observations that biotic and abiotic stresses are known to affect not only the expression of genetic traits, but also the regulation of DNA repair-related genes (Molinier et al., 2005). For instance, it is unknown whether interaction of the modified plant genome with environmental factors such as pollution can affect the stability of its specific genetic trait. For food safety assessment and risk management, priority will be given to those health-related traits that are intended to reduce toxic or allergenic substances in crops for human consumption.

³ For a detailed description of conventional methods of genetic modification, several reviews and texts are available (Potrykus, 1991; Birch, 1997; Jain et al., 1998; van Harten, 1998; Mohan Jain, 2001; Predieri, 2001; Zhong, 2001; Chahal and Gosal, 2002; Davey et al., 2005; Liu et al., 2005).

2.1.3. Part C. Potential for allergenic effects

An allergenic reaction is an interaction between an individual with a genetic (atopic) background and an allergen (primarily protein in nature) that is recognized by an immunoglobulin E (IgE). Ideally, the assessment of potential for allergenic effects in GM foods should consist of two elements: (1) allergenicity (the likelihood of inducing *de novo* sensitization in a non-allergic individual), and (2) assessment of allergenic activity (the propensity of a substance to elicit allergic reactions) (Spök et al., 2005). These two elements correspond to two stages in the development of allergic reactions: (1) sensitization, and (2) elicitation of a reaction. In current practice, however, assessment focuses on the elicitation process because of our limited knowledge on the sensitization process and available methods to measure individual allergen-specific susceptibility.

While *in vitro* or *in vivo* tests (e.g., specific serum screening for IgE binding, skin prick test, or food challenge) would be more reliable in assessing the allergenic potential of proteins than tests of their structural and physical–chemical characteristics, the latter properties have become the cornerstone of screening for the allergenic activity of proteins in the safety assessment of GM foods for practical and ethical reasons. Five factors in Part C of the RBCS (Table 3) focus on three aspects critical to the assessment of the allergenic potential of GM foods: allergenicity of the gene source, nature of the protein gene product, and allergenicity of the gene recipient.

2.1.3.1. Allergenicity of the gene source (factor C.1)

At the present, a protein from an allergenic source would be considered to be highly probable of being allergenic unless proven otherwise (FAO/WHO, 2001; Codex Alimentarius, 2003).

2.1.3.2. Nature of protein gene products (factors C.2–C.4).

(1) *Potential to cross-react with known allergens (factor C.2).* The lack of validated methods for testing the allergenicity (i.e., *de novo* sensitization) of novel proteins in animal models or humans increases our reliance on assessing the allergenic activity (i.e., propensity to elicit a reaction to previously exposed allergens) of proteins in the GM food product. Identifying the presence of individual epitopes (minimal peptide units within proteins that can be recognized by the immune system) on the protein of interest that shares sequence identity with known allergens remains the scientific principle by which the allergenic potential of a novel protein is assessed. Thus, *in silico* methods for predicting cross-reactivity with known allergens based on amino acid sequence identity are the principal tools currently being used in assessing the allergenic potential of transgenic proteins (Björklund et al., 2005). However, the predictive value of the proposed bioinformatics algorithms has not been verified given the relatively low incidence of allergy. Despite this challenge, it is now generally accepted that matches of > 35% identity over 80 or more amino acids are reasonable indicators of cross-reactivity of proteins that would act as allergens if consumed by an individual allergic to the sequence matched allergen (Codex Alimentarius, 2003).

(2) *Stability during pepsin digestion (factor C.3).* At present, protein stability appears to be a major characteristic currently being used to differentiate known food allergens from normal dietary proteins in safety assessment of GM foods (Helm, 2001). However, the property of digestive stability is not shared by all common major protein allergens and cannot be used alone in detecting or defining a potential allergen (Mendelsohn et al., 2003). There are also concerns that, for individuals with impaired

Table 3

Factors and criteria for determining potential for allergenic effects due to intended and unintended changes (Part C of the RBCS)

Factors	Criteria ^a
(1) <i>Allergenicity of the gene source</i>	
C.1. Allergenicity of donor organism (gene source)	(a) NOT a known source of allergenicity or gluten-sensitive enteropathy
(2) <i>Nature of protein gene products</i>	
C.2. Potential to cross-react with known allergens	(a) Comparison of amino acid sequence identity with known allergens: NO sequence identity of $\geq 35\%$ over 80 or more amino acids based on a sequential search ^b
C.3. Stability measured by pepsin digestion (under standard testing conditions)	<i>Novel or substantially modified proteins</i> (without dietary history) (a) Rapid and complete digestion with NO stable fragments (<10 min ^c). <i>Other proteins</i> (with dietary history) (b) Rapid or moderate digestion with NO stable fragments (<15 min ³)
C.4. Secondary factors ^d	
C.4.1. Structural/functional characteristics	(a) NO post-translational modification involving glycosylation with primarily N-linked, specific xylose or fucose substitutions, or (b) NO structural or functional relationship to specific protein families or functional categories commonly associated with allergenicity
C.4.2. Heat stability	(a) Heat labile (NOT applicable to lipid transfer proteins)
C.4.3. Abundance	<i>Pesticidal and enzymatic proteins</i> (a) <0.1% protein ^e <i>Other proteins</i> (b) <1% total protein ^e
(3) <i>Allergenicity of the gene recipient</i>	
C.5. Allergenicity of host plant	(a) NOT a common source of allergenicity or gluten-sensitive enteropathy, or <i>Host plant is a common source of allergenicity or gluten-sensitive enteropathy</i> (b) Rating for factors B.5.1, B.5.2, and B.5.3 is “yes” or “not applicable”

^a A ‘yes’ rating to a criterion means that the GM food is potentially non-allergenic due to *intended and unintended* changes to the genome with respect to that specific factor.

^b The Codex Alimentarius Task Force on Foods Derived from Biotechnology (Codex Alimentarius, 2003) accepted 35% or higher in amino acid sequence identity to an allergen, in a segment of 80 or more amino acids, as a reasonable criterion for considering a protein likely to be allergenic and therefore requiring further testing. However, contrary view exists that cross-reactivity is rare below 50% identity. In most situations cross-reactivity requires more than 70% identity (Aalberse, 2000). We have excluded the criterion for “NO identical matches in amino acids in a short sequence (e.g., 6–8 contiguous amino acids” currently required in several international and national guidelines as recent evidence does not support its use (Goodman and Hefle, 2005).

^c Listed here are provisional cut-off values. Guidelines for interpreting digestion assay results remain to be established (Bannon et al., 2002).

^d Secondary factors are triggered only when the evidence on the primary factors is equivocal.

^e While not all abundant food proteins are significant allergens, a number of potent food allergens (e.g., peanut allergens) are abundant in the allergenic source, often representing more than 1% of the total protein in the allergenic food (Metcalf et al., 1996). This contrasts certain novel proteins in GM foods that are typically expressed at very low levels (<0.1% protein) and are unlikely to become novel allergens.

gastric digestion, current *in vitro* methods for assessing protein digestibility may not be adequate in identifying the potential for adverse health effects of novel protein substances (Jensen-Jarolim and Untersmayr, 2006). Impaired gastric digestion due to hypoacidity is a condition not uncommon in the elderly, in early childhood, in chronic atrophic gastritis, and in different treatments where stomach acid neutralization or inhibition is an important therapeutic goal. In addition, certain anti-acids are increasingly being marketed and purchased as over-the-counter drugs without the need for prescription. As well, *in vitro* digestibility test cannot be used to assess those properties of potential allergens that can elicit reactions before the food reaches the stomach for pepsin digestion.

(3) *Secondary factors (factor C.4)*. Given the overlap in the characteristics of allergenic and non-allergenic proteins, no single factor or test can fully predict the allergenic potential of any new protein. A weight of evidence approach based on a combination of factors is increasingly favoured over a strict sequential decision tree application of testing strategies (FAO/WHO, 2001; Codex Alimentarius, 2003; König et al., 2004; Poulsen, 2004). We proposed the addition of three secondary factors described below that may add weight in assessing whether a protein could be allergenic. These secondary factors are triggered only when the evidence on the primary factors (C.2 and C.3) is equivocal. This approach recognizes the limitations of these factors in independently determining the allergenic potential of proteins with limited dietary history or exposure (Goodman et al., 2008).

- (a) *Structural/functional characteristics*. Post-translational glycosylation, and specifically carbohydrate structures containing an $\alpha(1,3)$ -fucosyl residue or $\beta(1,2)$ -xylose, may be capable of binding to IgE from individuals sensitized to unrelated sequences (van Ree et al., 2000). There is some overlap between this secondary factor and the potential to cross-react with known allergens (factor C.2). However, given the limitations in identifying potential cross-reactivity of a protein with known allergens based on linear sequence alone, and that the level of sequence homology is not always correlated with the conservation of allergenicity (Mills et al., 2003), the inclusion of additional structural characteristics as secondary factors is justified.
- (b) *Heat stability*. While not all allergenic proteins demonstrate heat stability, many food allergens are heat stable. Notable exceptions include lipid transfer proteins, such as Bet v 1-related proteins, and profilins, which are allergens that are heat labile.
- (c) *Abundance*. Highly allergenic proteins are often expressed at relatively high levels (FAO/WHO, 2001). At this time, however, it is not possible to define a level of expression below which a protein can be considered safe from an allergenicity point of view, although the concept of threshold doses for allergenic foods has been proposed (Taylor et al., 2002). Protein abundance is related to both the probability of sensitization and elicitation; however, threshold levels for sensitization which depend on dose, exposure duration, and the number of repeated exposures, would be difficult to determine (Taylor and Hefle, 2001; Haslberger, 2003; Goodman et al., 2005). The induction of allergic sensitization in a previously naïve individual almost invariably requires exposure to concentrations of the allergen that are greater than those necessary to elicit a response in a sensitized individual (IPCS, 1999).

2.1.3.3. Allergenicity of the gene recipient (factor C.5). For an allergenic host plant, unintended changes in the level or potency of

endogenous allergenic proteins would increase the level of concern. However, current guidelines on the safety assessment of GM foods have not addressed the level of change that would be acceptable or unacceptable. Little is known about the natural variability in allergenicity of available food crops and the clinical significance of this variability (Goodman et al., 2008). In addition, practical considerations for testing may restrict the application of this factor to common food allergens, as further testing, typically involving serum screening, requires the use of sera from a sufficient number of patients known to be sensitized to the protein or the food source (FAO/WHO, 2001). The health impact of an unintended change in an endogenous allergenic protein in a common allergenic food may be relatively small, as the offending food would likely be avoided by individuals who have been positively diagnosed. However, it is less clear if and to what extent these changes would sensitize or elicit reactions in previously clinically asymptomatic individuals. If this were to occur, there would be a higher likelihood that more people would be affected by common allergens than by less common allergens in foods. These arguments suggest that a higher priority should be given to assessing unintended changes in allergenic proteins in common allergenic foods.

2.2. Criteria

The concepts of familiarity and novelty underlie the criteria in the proposed RBCS (Tables 1–3) used in determining the potential health risks of GM foods. The concept of familiarity is based on the fact that most GM crops are developed from plants whose biology is well understood (European Commission, 2003). In contrast, novelty represents limited prior knowledge, conferring greater uncertainty in the assessment of potential risk (Madsen et al., 2002). As described in previous sections, limitations exist in the current state of knowledge in assessing unintended effects of genetic modification and the allergenic potential of GM foods. Several criteria in parts B and C of the RBCS may be considered provisional, subject to refinement as new knowledge and testing methods evolve.

In assessing intended effects, the criteria vary for some factors, depending on the nature of the substance. In assessing unintended changes, the criteria depend on the method of genetic modification due to differences in analytical capability to characterize molecular changes.

In assigning a concern level for a GM food, the criteria developed are used to rate each factor in the RBCS. A 'yes' rating to a criterion means that the criterion has been met. An 'uncertain' rating is also available where information is insufficient or equivocal and further evaluation is required. An option for 'not applicable' is also provided.

2.3. Aggregation

An aggregated concern level is assigned separately for each category of health effects based on the ratings for individual factors in each category using the criteria specified for each part of the RBCS (Tables 1–3). The rules for aggregation are summarized in Tables 4–6 (Parts A, B, and C of the RBCS, respectively). There are three concern levels for Part A, and two concern levels for each of Parts B and C.

In all three parts of the RBCS, concern Level I is assigned only when the criteria for all applicable critical factors are met (i.e., rated 'yes' according to the criteria in Tables 1–3). For Part A, failure to meet the criteria (i.e., rated 'no') for only one critical factor would lead to Level II; failure to meet the criteria for more than one critical factor, or any of the remaining factors, would lead to Level III. For Parts B and C, failure to meet the criteria for any one critical factor would result in Level II. For Part B, one critical factor (B.5.3 – other metabolites or parameters) would be triggered if the criteria for any

Table 4

Aggregation of critical factors in determining concern levels for toxic and antinutritional effects due to intended changes (Part A of the RBCS)

Factors ¹	Levels ^{2,3}						
	Ia	Ib	Ic	IIa	IIb	IIc	III
A.1. Expression of gene product(s) in edible part(s) of the modified crop		✓					
A.2. Prior history of consumption from foods	✓			✓	✓ or ?	X	X ^a
A.3. Expected change in total dietary exposure (theoretical estimates)	✓			? ^a	✓		X ^a
A.4. Prior knowledge of safety/mode of action	✓			? ^a	✓		X ^a
A.5. Structure–activity characterization	✓			? ^{a,b}	? or X	✓	X ^a
A.6. Digestion and degradation products	✓			✓	✓	✓	X
A.7. Synergism and interactions	✓			✓	✓	✓	X
A.8. Prior experience with gene products introduced/modified			✓				

¹ The criteria for evaluating these factors are listed in Table 1.² Level I, substances with prior dietary history, negligible exposure, or prior experience (Ia, substances with prior dietary history. Ib, substances with negligible exposure. Ic, substances produced by well-characterized GM process based on prior experience). Level II, substances with limited and defined concerns with respect to either expected exposure or toxicity profile (IIa, substances with dietary history. IIb, substances with questionable dietary history, i.e., dietary substances with modified structures. IIc, substances without dietary history, i.e., novel substances with limited/defined structural concerns). Level III, substances not meeting criteria for Level I or II (substances with or without dietary history posing concerns with respect to expected exposure and toxicity profile, or other factors related to metabolism or interactions, or any combination of more than one of the factors).³Legend:✓, criteria for the factors indicated must be met (i.e., rated 'yes' according to the criteria in Table 1), or not applicable.

?, limited or equivocal information.

?^a, limited or equivocal information on only one of the factors indicated (A.3, A.4, or A.5).?^b, for factor A.5, uncertainty is limited to status of pepsin digestion test only.

X, for any one of the factors indicated.

X^a, for more than one of the factors indicated.**Table 5**

Aggregation of critical factors in determining concern levels for toxic antinutritional effects due to unintended changes (Part B of the RBCS)

Factors ¹	Levels ^{2,3}			
	Ia	Ib	Ic	II
B.1. Safety of donor organism <i>Applicable if donor organism and host plant are different.</i>	✓	✓		X
B.2. Safety of host plant				
B.3. Potential for novel/alterd patterns of metabolites				
B.4. Potential for disrupting coding or regulatory DNA sequences in modified plant				
B.4.1. In the inserted/modified DNA	✓ ^a			
B.4.2. In genomic sequences bordering site of insertion/modification				
B.5. Biochemical analysis of edible part of modified crop				
B.5.1. Key nutrients	✓	✓		X
B.5.2. Known toxicants and antinutrients	✓	✓		X
B.5.3. Other metabolites/parameters				
<i>Applicable if criteria for factors B.2, B.3, or B.4.2 are not met.</i>	✓ ^b	✓		X
B.6. Health consequences of instability of the modified trait <i>Applicable if the modified trait is intended to provide nutrition or health benefits.</i>	✓			X
Criteria A.1.3 (Part A of RBCS) ⁴			✓	

¹ The criteria for evaluating these factors are listed in Table 2.² Level I, GM foods that meet baseline requirements for testing for unintended effects (Ia, GM foods derived from crop transformed by characterized gene modification based on nucleic acid techniques. Ib, GM foods derived from crop transformed by poorly characterized gene modification based on undirected mutagenesis, somaclonal variation, or wide-cross hybridization. Ic, GM foods include the following three subgroups: GM foods derived from crops transformed using tissue-specific modification not involving the edible parts of the modified crop; GM foods with limited ingredient use of the edible part and the ingredient is highly refined; and GM foods derived from crops transformed by genetic modification process based on prior experience). Level II, GM foods that require further assessment beyond baseline testing for unintended effects.³Legend:✓, criteria for the factors indicated must be met (i.e., rated 'yes' according to the criteria in Table 2), or not applicable.✓^a, criteria must be met if information for factor B.1 is limited or equivocal for characterized gene modification.✓^b, criteria must be met if information for factors B.2, B.3, or B.4.2 is limited or equivocal.

X, for any one of the factors indicated.

⁴ Criteria 1.3 of Part A: gene products not expressed, detected, or changed in the edible part(s) of the modified crop, including: (a) tissue-specific expression not involving the edible part(s), or (b) the edible part is used as a food ingredient in a limited form as a highly refined and well-defined chemical entity.

one of the secondary factors are not met. For Part C, failure to meet the criteria for one critical factor (C.3 – pepsin digestion) would trigger the need to meet the criteria for all secondary factors.

The approach to aggregation is developed with the following considerations in mind.

- (1) All GM foods should be classifiable.
- (2) The assignment of concern levels should be mutually exclusive, so that a GM food would be assigned into only one level for each category of health effects.
- (3) In aggregating the factors, the RBCS gives more weight to factors that pose a greater potential for concern or are subject to less certainty. For example, more weight is placed

on assessing metabolic changes (factor B.5) in the GM food compared to the potential for novel or altered patterns of metabolites (factor B.3) because of the less certain nature of the latter factor in contributing to adverse health effects, in contrast to changes in the levels of metabolites that reflect directly changes in components in foods that are eaten by consumers (FIFRA SAP, 2004).

- (4) There should be flexibility in matching the characteristics of the product and the availability of analytical methods to the criteria to be met. For example, for foods derived from crops modified using non-nucleic acid-based techniques, the need to justify the scope of metabolites or other analyzed parameters is more important than the need to meet the criteria

Table 6
Aggregation of critical factors in determining concern levels for allergenic effects due to intended and unintended changes (Part C of the RBCS)

Factors ¹	Levels ^{2,3}			
	Ia	Ib	IIa	IIb ⁴
C.1. Allergenicity of donor organism <i>Applicable where donor organism and host plant are different.</i>	✓		X	X
C.2. Potential for protein gene product to cross-react with known allergens	✓		X	
C.3. Pepsin digestion	✓			
C.4. Secondary factors <i>Applicable if criteria for factor C.3 are not met.</i>				
C.4.1. Structural/functional characteristics	✓ ^a		X ^a	
C.4.2. Heat stability	✓ ^a		X ^a	
C.4.3. Abundance	✓ ^a		X ^a	
C.5. Allergenicity of host plant	✓		X	X
Criteria A.1.3 (Part A of RBCS) ⁵		✓		

¹ The criteria for evaluating these factors are listed in Table 3.
² Level I, protein gene products and GM foods that meet baseline requirements for testing for allergenic activity (Ia, expression in edible parts; Ib, negligible expression in edible parts and in derived food ingredient). Level II, protein gene products and GM foods that require further assessment beyond baseline testing for allergenic activity (IIa, characterized protein and gene modification. IIb, uncharacterized protein and gene modification, such as in somatic hybrid).
³ Legend: ✓, criteria for the factors indicated must be met (i.e., rated 'yes' according to the criteria in Table 3), or not applicable.
✓^a, criteria for the factors indicated must be met if information for factor C.3 is limited or equivocal.
X, for more than one of the factors indicated.
X^a, for any one of the factors listed if factor C.4 is applicable. Factor C.4 is applicable when information for factor C.3 is limited or equivocal.
⁴ Information on protein expression products (factors C.2–C.4) may be limited in poorly characterized gene modification.
⁵ Criteria A.1.3 of Part A: gene products not expressed, detected, or changed in the edible part(s) of the modified crop, including: (a) tissue-specific expression not involving the edible part(s), or (b) the edible part is used as a food ingredient in a limited form as a highly refined and well-defined chemical entity.

for molecular characterization, simply due to differences or difficulties that may be encountered in available molecular methods for characterizing gene modifications using rDNA versus other techniques.

3. Interpretation of concern levels in the RBCS

To facilitate application of the RBCS and the assignment of concern levels for specific GM foods, we developed a combined RBCS-product rating form (not shown here). This section explains how the concern levels are applied by providing an overview of their organization in the proposed RBCS and the characteristics of GM foods in the different concern levels. As summarized in Fig. 1, the RBCS comprises a total of seven concern levels in three categories of potential health effects, with 2 or 3 levels in each of the three categories. This aggregation pattern reflects the extent and nature of further assessment and testing required for each concern level. A product in Level I requires limited further testing, whereas a product in Levels II and III requires progressively rigorous additional

testing (Chao and Krewski, 2008). The level of additional testing increases in proportion to the need to reduce uncertainty. However, the increased need for testing does not necessarily mean that a product is more risky.

In Part A, subcategories of Level I are distinguished based on exposure to and prior experience with the introduced or modified gene product (Table 4). Subcategory Ia represents the principal group of gene products in Level I. Subcategories Ib and Ic are special categories for which the application of the RBCS in its entirety would not be necessary. If the human food use of a crop is restricted only to the production of highly refined ingredients of well-defined chemical composition, verification in the extracted fractions of the absence of an intended primary gene product that does not have a nutritional role would be sufficient when available standards for the testing of food ingredients are applied. An example is oilseed rape for which the only food use is the highly refined oil fraction and the composition of the oil is not intentionally altered. Similarly, in tissue-specific expression of introduced or modified genes that do not affect the edible parts of a food crop, verification of the absence of intended gene products in the rele-

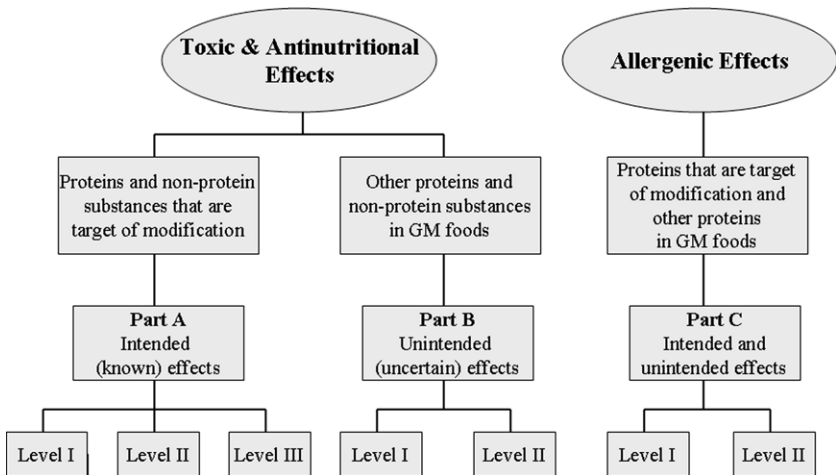


Fig. 1. Schematic representation of the risk-based classification scheme for GM foods.

vant plant parts would be sufficient when typical postharvest processing procedures are followed. However, in both of these cases for subcategory Ib, if a crop is modified by intentional silencing of gene expression, unintended effects will need to be assessed, in addition to intended changes. Subcategory Ic applies to substances produced by well-characterized GM process based on prior experience.

Three subcategories of Level II substances may be distinguished based on dietary history. If the introduced substance has a dietary history (i.e., a constituent naturally occurring in foods), it may be classified as Level II, provided that uncertainty is limited only to either exposure or toxicity. If the introduced substance has a questionable dietary history due to substantial modification, it may be classified as Level II, provided that it does not have structural–functional similarity to substances with toxicity concerns. A substance that does not meet the criteria for Levels I or II is classified as Level III.

In Part B, applicable criteria vary depending on whether the gene modification can be characterized by molecular methods (Table 5). Poorly characterized gene modifications include undirected mutagenesis, somaclonal variation, or wide-cross hybridization. Even for bioengineered crops, comprehensive characterization based on comparative sequence analysis may not always be possible due to limited genomic databases for the crop species in question at the present (EFSA, 2004). In cases where information on the potential for interruption of genomic sequences bordering the site of insertion or modification is limited, failure to justify the scope of the metabolites analyzed would result in a Level II classification. Level II also applies where the process of gene modification is poorly characterized and the safety of the donor plant is uncertain with equivocal evidence in supporting that a large percentage of genetic material from the donor organism has been removed by backcrossing.

In Part C, concern Level I is assigned when all the criteria for the three critical factors are met (Table 6). The three critical factors are: (1) allergenicity of the gene source, (2) likelihood of the protein gene product to cross-react with known allergens, and (3) protein digestibility. Where information on pepsin digestibility is equivocal for eligibility for Level I, the criteria for all secondary factors will have to be met for Level I classification. Failure to meet criteria for Level I will result in classification into Level II.

4. Example

4.1. General description of product

To illustrate the use of the RBSC and the application of concern levels above Level I, consider a hypothetical example, a field corn to which a mutant gene from barley encoding chymotrypsin inhibitor-2 (CI-2) was introduced. The DNA sequence transferred was engineered to confer multiple lysine substitutions as a means to generate a lysine-enriched protein for the purpose of enhancing the nutritional quality of field corn for feed use. Field corn is also consumed by humans, mainly in the form of food ingredients.

4.1.1. Information for assessing potential toxic and antinutritional effects from intended changes (Part A)

Limited data are available on the level of CI-2 type protein from grains and on current intakes. Dietary exposure to the recombinant CI-2 protein is expected to vary widely depending on the role of maize in the human diet. For population groups who consume corn products primarily through refined ingredients (such as starches and sweeteners), nutritionally significant increase in the intake of lysine from bioengineered CI-2 protein in modified maize is unlikely. However, maize consumption can be high in some population groups for whom maize in various processed forms is a staple food.

Assessment of the introduced protein will focus on the safety implications of its altered structure.

4.1.2. Information for assessing potential toxic and antinutritional effects from unintended changes (Part B)

Both the gene source (barley) and the modified plant (maize) are cereal grains with a long history of breeding and safe food use. Both crops are consumed by humans primarily in processed form. Their inherent constituents are not known to be toxic or antinutritional under common conditions of consumption. The introduction of the modified CI-2 protein will affect amino acid metabolism in the modified crop. However, the modified protein is not expected to have other direct interactions with the metabolic pathways in the modified crop. Targeted biochemical characterization focusing on key nutrients (including amino acids), known toxicants, and antinutrients, as suggested in the OECD consensus document for the food use of maize (OECD, 2002), would be considered sufficient for the assignment of concern levels.

4.1.3. Information for assessing potential allergenic effects from intended and unintended changes (Part C)

Both the gene source and the modified crop are sources of known allergens. Barley is also a source of proteins associated with gluten-sensitive enteropathy. The engineered CI-2 protein also demonstrated resistance in the pepsin digestion assay.

4.2. Application of RBSC

4.2.1. Part A

Assignment of concern Level III is determined by insufficient or equivocal information provided for more than one critical factor (A.4 and A.5). In addition, criteria for other factors (A.6 and A.7.2) are also not met.

4.2.1.1. Factor A.4—prior knowledge of safety/mode of action. The protein (barley-derived CI-2 protein) was extensively engineered with multiple lysine substitutions prior to transfer to the modified maize. While the safety of the native protein is not in question, the same conclusion cannot be made for the engineered protein based on the information provided.

4.2.1.2. Factor A.5—structure–activity characterization. The modified CI-2 protein was resistant to pepsin digestion. When compared to the native protein, differences in several aspects of structure–activity characterization (thermodynamic stability and inhibitory activity against proteases) were observed.

4.2.1.3. Factors A.6 and A.7.2. The protein's resistance to digestion also raises questions about its metabolic fate in mammalian metabolism, its bioavailability, and potential effect on the bioavailability of other food constituents.

4.2.2. Part B

Concern Level I is assigned because the criteria for a combination of critical factors are met: a history of safe food use of the gene source (B.1); absence of unknown DNA sequences in the post-transformation insert (B.4.1); and absence of biologically significant differences in the levels of key nutrients and known toxicants when compared with unmodified maize (B.5.1 and B.5.2). There is no concern about the human health consequences of instability of the modified trait (B.6), as the nutritional modification is intended primarily for animal feed. Meeting the criterion for factor B.5.3 is not considered essential, because criteria are met for all secondary factors (B.2, B.3, and B.4.2) related to the safety of the host plant, potential for novel or altered patterns of metabolites (as indicated by the function of the gene product) and the absence of unknown

DNA sequences in the regions flanking the transgene insertion site.

4.2.3. Part C

Failure to meet two critical factors (C.1 and C.3) contributes to the assignment of concern level II for the CI-2 protein. In addition, the protein is expressed at a high level, equivalent to 10% of total seed protein (C.4.3). However, the potential for alterations in the allergenicity of the modified maize would be considered to be low based on the assessment in Part B.

4.2.3.1. C.1—allergenicity of donor organism. Barley, the gene source of CI-2 protein, is a known and common source of hordeins, which are responsible for inducing gluten-sensitive enteropathy (C.1). It is also a source of other allergenic proteins.

4.2.3.2. C.3—pepsin digestion. The recombinant protein is resistant to pepsin digestion.

4.2.4. Further testing

Further testing is guided by those factors for which criteria are not met in each part of the RBCS. With respect to toxic and antinutritional effects due to intended changes (Part A), the assignment of concern Level III (due to gaps in information on the safety or mode of action of the modified protein, its resistance to pepsin digestion and unknown metabolic fate and bioavailability) points to the need for toxicity testing. For a pesticidal protein not likely to have chronic effects (such as those mediated by hormonal activity) and expressed at low level in the GM crop, further testing required would include a 28-day toxicity study in a rodent species as a minimum. The assignment of concern Level I for toxic and antinutritional effects due to unintended changes (Part B) indicates no further testing. For a protein substance in a GM food classified as concern Level II with respect to allergenic effects due to both intended and unintended changes (Part C), specific serum screening for IgE binding is required. Considering that the protein was derived from a known allergenic source and was resistant to pepsin digestion, IgE binding of an extract of the GM food and of the pepsin digested fractions would be indicated (Akkerdaas et al., 2005). A comparison of the digestibility profiles between the native and recombinant proteins is also suggested, although this is not required or accepted as part of premarket assessment of GM foods at present (Chao and Krewski, 2008).

5. Discussion and conclusion

While the RBCS described in this paper is consistent with the stepwise approach implicit in current practice in the premarket assessment of foods derived from crops modified by rDNA technology, it has other characteristics that warrant elaboration.

5.1. Characteristics and intended uses of the proposed RBCS

The modular structure of the proposed RBCS allows each component of the scheme to be modified independently of the other components. This flexible structure also allows new factors to be added in the future as genetic modification technology evolves and criteria to be adjusted as new testing methods are developed. The explicit criteria and the types of evidence on which the criteria are based provide clarity and transparency in determining concern levels. This proposed RBCS is equally applicable to genetic modification involving multiple or stacked genes. For example, synergism and interactions among gene products (factor A.7) and prior experience with the gene products introduced or modified (factor A.8) are among the factors included in the RBCS. The highest concern

level assigned to the expression product of each of the introduced or modified genes and to their potential interactive effects, where applicable, will determine the extent of further testing required.

It is unreasonable to expect that the classification scheme proposed here will be able to anticipate all future possible approaches to genetic modification and their associated intended and unintended effects. However, in its present form, the proposed RBCS can address unusual cases under “other considerations”, as discussed further in a companion paper (Chao and Krewski, 2008). Unusual cases include foods derived from crops that are modified by uncommon techniques or using uncommon source organisms, or that are intended for use by vulnerable population groups (e.g., as a major ingredient in infant formula). The provision for “other considerations” ensures that special cases that require more detailed assessment are not overlooked.

While the proposed RBCS is intended to provide guidance to biotechnology industry and regulatory authorities on the extent of testing for GM crops of different levels of concern, it can also be used to guide decision-making regarding the acceptability for field testing of GM crops from a health risk perspective. The RBCS may also be useful in decisions regarding the continuation or termination of the development of a GM crop by researchers and plant breeders. The conceptual framework underlying the proposed RBCS may inform risk communication regarding GM foods. Effective communication is essential in facilitating public understanding of how the potential risks of a new technology used in food production are assessed and managed.

It is important to recognize that the proposed RBCS does not predict the level of health risk of a GM food. A higher level of concern indicated by the RBCS reflects a higher level of uncertainty about the GM food or information gaps that need to be filled. As a tool for differentiating those GM foods that require more testing from those that do not, the proposed RBCS has been developed to anticipate and identify GM foods that would require testing beyond Level I, even though at present few GM foods at the time of submission for formal regulatory approval would be expected to present significant concerns requiring the type of testing suggested for Level II or III.

5.2. Potential limitations

Several limitations of the proposed RBCS are recognized. Although other factors such as indirect or non-dietary routes of exposure to gene products via geobiochemical pathways or dermal and respiratory routes could have been considered in the RBCS, only the most important factors are included. The development of the RBCS is limited by available information, current testing methods, and assumptions about the function of gene products and their potential for inducing unintended effects (Chao and Krewski, 2008). There are also methodological limitations, including the potential for selection bias in consultations with experts during the course of development of the scheme. However, the responses received were diverse and indicative of what might be expected based on the literature. Risk is defined narrowly here in scientific terms, without considering the broader social, cultural, and political dimensions of risk. The proposed scheme does not address ecological risks and genetic contamination of plant species that are not directly related to health.

5.3. Implications

Risks to human health from GM foods should be placed in the proper context. In this regard, Brock et al. (2003) state the following

"Of all the risks to human health arising from the environment, none presents a greater challenge to our understanding than those associated with the consumption of foods. This sweeping conclusion is based on the adoption of the broadest possible view of health risks, taken to encompass the total burden of morbidity and mortality that can be related to food consumption."

Genetic modification of food crops is among a wide range of technology-driven changes to our food supply from production to processing, including the use of food additives and food irradiation. Concerns about unintended changes of genetic modification of food crops using rDNA techniques highlight areas for improvement in the management of food safety issues. First is the apparent lack of available methodologies, for example, animal models for allergenicity testing and whole-food testing methods, as well as research incentives in improving our knowledge regarding the potential health effects of a multitude of non-nutritive biologically active substances in the food supply (with the exception of exploiting their potential for therapeutic uses). Second is the highly uneven approach to the testing of substances intentionally introduced to foods compared to natural constituents (NRC, 1996; Gold et al., 2001; Brock et al., 2003). As a class, the impact of natural non-nutritive food constituents on human health—both adverse and beneficial—can be substantial in comparison with low molecular weight substances added to foods (NRC, 1996; Brock et al., 2003). Third, unintended changes are not unique to genetically engineered crops (Kleter and Peijnenburg, 2003; Finely et al., 2005).

Compared to intentional changes at the food processing level, such as the addition of direct food additives, changes to food composition induced by genetic modification at the level of crop production may be less predictable, but are not necessarily more risky in terms of health effects. In addition, food constituents can also be extracted from modified crops and added back to foods, often at higher levels. From a public health perspective, a holistic approach to prioritizing the relative importance of the vast number or classes of natural food constituents in a total diet context is needed.

Interest in proposing process-based distinctions among GM crops (e.g., cisgenesis)⁴ as criteria in determining the need for regulatory oversight (Schouten et al., 2006) and in conceptual discourse (Madsen et al., 2002; Nielsen, 2003) continues, despite the questionable practice of requiring premarket assessment of crops and derived foods based exclusively on a particular method of genetic modification (Stirn and Beusmann, 2003; NRC, 2004; Cellini et al., 2004; Wilson et al., 2004). While risk-based approaches to ecological risk assessment have been published (e.g., FAO, 2001; Traynor et al., 2001; Hancock, 2003; Koivisto et al., 2002; Strauss, 2003), no systematic approach has been developed to review or revise this process-based policy for assessing human health risks of GM foods. By delineating the sources of intended and unintended changes, the proposed RBCS provides a starting point for developing a scientifically justifiable approach to the assessment of GM foods that could be more widely adopted than the process-based approach currently in place in many jurisdictions. The proposed RBCS differentiates the sources of uncertainty in potential health risks of GM foods. This should serve to anchor more focused deliberations on the relative importance of intended vs. unintended effects. It is also hoped that more focused deliberations will stimulate support for the research necessary to test the assumptions underlying the RBCS, and in bridging information gaps that fuel debates about potential adverse

health effects of GM foods. The expected increase in complexity in metabolically engineering GM crops for food use would argue for the need to review the applicability of the assumptions underlying the current approaches to assessing the first generation of GM crops to future GM crops.

In conclusion, a RBCS can provide a more efficient approach to the assessment and testing of GM foods in proportion to their potential for health risks. Such an approach makes it explicit that more evaluation resources are to be devoted to GM foods that are more likely to pose health risks than those that are less likely to do so. Adoption of an RBCS for GM foods will benefit not only regulatory agencies, but will also facilitate decision-making in the development of GM crops for field-testing and commercialization. The proposed RBCS may also make an important contribution in promoting safety considerations in product design during the early stages of GM crop development. However, realization of the full benefits of a risk-based approach requires the development and validation of more sensitive testing methods than those currently available, an issue addressed in a companion paper in this series.

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Appendix A. Expert Consultation on the RBCS

During the course of developing the RBCS for GM foods presented in this paper, we sought comments from 27 individuals over three consultations on the conceptual basis for the proposed scheme. In the first consultation, there was general agreement about the importance of specific factors related to intended effects and the need for comprehensiveness in order to accommodate the types and range of intended and unintended effects. Expert opinions were varied regarding the weighting that should be put on assessing human health implications of unintended effects. However, there was acknowledgement that factors related to unintended effects of nucleic acid-based genetic modification should be separate from those related to conventional methods of genetic modification in the RBCS.

Results of the second consultation indicated support for the general direction of the scheme. There was general acceptance of the use of core information with discriminating value in classifying GM foods. Core information is a subset of all the information that is typically required in premarket assessment. Comments on other aspects of the draft RBCS indicated need for improvement, including: how factors are aggregated into concern levels, the number of concern levels, the need for clearer definition of dietary exposure, and the need for more specific criteria and analytical data in differentiating among concern levels for other factors.

A revised RBCS based on these comments were subjected to a third review by two separate groups of experts. One group focussed on the scientific and logical soundness of the conceptual basis of the RBCS and the appropriateness of the factors and criteria

⁴ Cisgenesis is the genetic modification of a recipient plant with a natural gene from a crossable—sexually compatible—plant. Such a gene includes its introns and is flanked by its native promoter and terminator in the normal-sense orientation. Cisgenic plants can harbour one or more cisgenes, but they do not contain any transgenes (Schouten et al., 2006).

in determining concern levels for further testing, which is the subject of this paper. The other expert group focussed on the proposed testing options, which is the subject of another paper (Chao and Krewski, 2008). Major comments about the conceptual basis of the proposed scheme centered on: the complexity of the structure of the scheme, the abstract nature of some criteria, and the need to separate the placement of the rules for determining concern levels from the factors and criteria themselves. Several reviewers also commented positively on the comprehensiveness, flexibility, and transparency of the proposed scheme in identifying uncertainties associated with GM food assessment. In response to the suggestions for improvement, the scheme was simplified and pilot-tested by seven experts, five of whom participated in earlier consultations. This paper reports on the scheme that was the result of this iterative process of development and refinement.

Appendix B. DNA disruptions due to recombination and induced mutation

The predominate mechanism of genetic recombination in plants is non-homologous end joining (NHEJ), which is more error prone than homologous recombination, common in other eukaryotes (Schnable et al., 1998; Cellini et al., 2004). This mechanism applies in plants regardless of the method of recombination, whether based on rDNA techniques, conventional methods, or natural processes that occur in plants (Hanin and Paszkowski, 2003; Cellini et al., 2004). Data also suggest that hot spots involving gene-rich regions are preferred in both natural recombination and in transgene integration (Schnable et al., 1998; Cellini et al., 2004). Within conventional methods of recombination, larger linkage blocks can be expected to result from intergeneric hybridization (such as those obtained by cell fusion) compared to intra- or inter-specific hybridization involving similar genes that share a common ancestry (NRC, 2000, p. 61).

At the genome level, the effects of chemical and physical mutagens are difficult to generalize in terms of the size or number of structural changes, rearrangements, or duplications. It is also not possible to determine with confidence whether there are major material differences between different types of induced mutation (e.g., somaclonal variation vs. mutation induced by chemical or radiation (Larkin, 1998)). Genetic alterations associated with induced mutation employed in plant breeding include: alterations in DNA methylation patterns, variability in the copy number of DNA sequences, random point mutations, position effects, transposition effects, chromosome mutations, and chromosome rearrangements (Lee and Phillips, 1988; Phillips et al., 1994; Brar and Jain, 1998; Gupta, 1998; Kaeppler et al., 1998; Mohan Jain, 2001; The Royal Society, 2003). Many of these changes are also observed in GM crops modified by rDNA techniques.

The available data do not suggest major differences in the type of random effects on genomic and chromosomal structure of the modified plant between recombination and induced mutation, as well as among conventional and nucleic acid-based methods of recombination. However, the extent of modification of genomic structure and the frequency of occurrence of a specific type of effect may vary among methods. Many factors ultimately determine whether a change at the genome level will become expressed in a plant, including the rate of DNA damage and repair.

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