Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants

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I. INTRODUCTION

Modern biotechnology, involving the use of recombinant-DNA (r-DNA) technologies, also known as genetic engineering, has emerged as a powerful tool with many potential applications in agriculture and healthcare. New plant varieties developed using r-DNA techniques, commonly referred to as genetically modified (GM), genetically engineered (GE) or transgenic plants, have been and are being developed with the aim of: enhancing productivity; decreasing dependence on the use of agricultural chemicals; modifying the inherent properties of crops; and improving the nutritional value of foods and livestock feeds. As more GE plants are released and the resultant food products are commercially available and are traded across various countries, concerns have been expressed about their safety for human and animal health and the environment. With this increased awareness, the concept of food safety assurance (i.e., that a food is safe for human consumption according to its intended use) has assumed importance as with any method of genetic manipulation, including genetic engineering of plants, there is a possibility of introducing unintended changes along with the intended changes, which may in turn have an impact on the nutritional status or health of the consumer.

To address the human health safety of foods derived from GE plants, there is a need to adopt a systematic and structured approach to their risk analysis. Risk analysis is a science based process comprised of risk assessment, risk management and risk communication and is an analytical tool to systematically evaluate safety concerns addressing human health safety of GE foods within a framework for decision making. It also provides further basis for reviewing the safety evaluation parameters as and when further information becomes available.

In Bangladesh, the manufacture, import, use, research and release of genetically modified organisms (GMOs) as well as products made by the use of such organisms are governed by the Biosafety Guidelines of Bangladesh, gazetted in 2008. The Biosafety Guidelines describe the institutional arrangements for risk assessment–the National Committee on Biosafety (NCB), the Biosafety Core Committee (BCC), and Institutional Biosafety Committees (IBCs)–as well as the general provisions for risk assessment and risk management. Under the Biosafety Guidelines, imported GE food products are required to have documentation from the exporting country that the items are fit for human consumption and an Annex to the Guidelines briefly describes the information required for imported living modified organisms (LMOs) intended for direct use in food or feed.

However, the Biosafety Guidelines mainly address the environmental impacts of LMOs and there is a need for comprehensive guidance for the safety assessment of foods derived from GE plants, particularly with respect to impact on human health. This has assumed importance in view of the GE food crops under field trial in Bangladesh, as well

as increased global trade in foods derived from GE crops approved for cultivation in other countries.

The Bangladesh Agricultural Research Council (BARC), in collaboration with the Bangladesh Standards and Testing Institution (BSTI), the Department of Environment DoE), the Institute of Public Health (IPH), the Directorate General of Food, and other relevant stakeholders, has taken the initiative to develop these guidelines to establish the safety assessment procedures for foods derived from GE plants, also taking into consideration the international *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants*[1]. As the apex body for the National Agricultural Research System BARC has much of the expertise necessary for both the development and safety assessment of agricultural products of biotechnology.

II. SCOPE AND OBJECTIVE

These guidelines are not intended to describe the GE food regulatory institutions, administrative or decision-making procedures within Bangladesh's regulatory framework, but only to provide technical guidance on the safety assessment process for whole foods, food products, and foods used as ingredients, that are derived from GE plant sources.

This document is intended to provide guidance to both applicants and reviewers for regulatory purposes. No attempt is made to explicitly define all of the data that might be required during a safety assessment, as this may vary case-by-case, and generally, data and information requirements are applicable only to those plant parts used as a food source.

The objective of the guidelines is to provide a system to ensure that foods derived from GE plants are as safe as existing foods in Bangladesh.

III. DEFINITIONS

Antinutrient means a substance that interferes with the utilisation of one or more nutrients by the body.

Conventional counterpart means a related plant variety, its components and/or products for which there is experience of established safety based on common use as food.

Donor organism means the organism from which genetic material is obtained for transfer to the recipient organism.

[1] Codex Alimentarius Commission, 2003.

Genetically engineered food (GE food) means both the food and food ingredients composed of or containing genetically engineered organisms/plants obtained through modern biotechnology.

Genetically engineered plant (GE plant) means a plant in which the genetic material has been changed through *in vitro* nucleic acid techniques, including recombinant-deoxyribonucleic acid (r-DNA) and direct injection of nucleic acid into cells or organelles. For the purposes of these guidelines, synonyms include genetically modified (GM), r-DNA, transgenic, or bioengineered plants.

Hazard means a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect subject to exposure.

Modern biotechnology means the application of:

i. *In vitro* nucleic acid techniques, including recombinant-deoxyribonucleic acid (r-DNA) and direct injection of nucleic acid into cells or organelles, of plants/crops **OR**

ii. Fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombinant barriers and that are not techniques used in traditional breeding and selection of plants/crops.

Risk, in relation to any article of food, means the probability of an adverse effect on the health of consumers of such food and the severity of that effect, consequential to a food hazard.

Risk analysis, in relation to an article of food, means a process consisting of three components, i.e., risk assessment, risk management and risk communication.

Risk assessment means a scientifically-based process consisting of the following steps: i) hazard identification; ii) hazard characterisation; iii) exposure assessment; and iv) risk characterisation.

Transgenic plant means a plant in which a transgene has been integrated into its genome.

Transformation means the unique DNA recombination event that took place through the integration of a transgene(s) in one plant cell for genetic modification, which was then used to generate entire transgenic plants.

IV. OVERARCHING PRINCIPLES

IV.1 CONCEPT OF SAFETY ASSESSMENT

Detecting any potential adverse effects and relating these conclusively to an individual characteristic can be extremely difficult in the safety assessment process. In practice, very few foods consumed today are subjected to any systematic safety

assessment process, yet they are generally accepted as safe to eat. In view of the difficulties of applying traditional toxicological testing and risk assessment procedures to food as a whole, an alternative approach has been adopted in developing the framework for the safety assessment of GE foods. This approach acknowledges that the goal of the assessment is not establishing absolute safety, but to consider whether the GE food is as safe as its traditional counterpart, where such a counterpart exists.

This comparative approach, embodied in the concept of substantial equivalence, is not a safety assessment in itself. Substantial equivalence represents the starting point which is used to structure the safety assessment of a new food relative to its counterpart. This concept has been described in international consensus documents, such as the *Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants* on which these guidelines have been based, and is used to identify similarities and differences between the new food and its conventional counterpart. This is considered to be the most appropriate strategy to date for safety assessment of foods derived from GE plants.

Accordingly the safety assessment of foods derived from GE plants in these guidelines is based on the evaluation of these foods relative to their conventional counterparts that have a history of safe use. This takes into account both intended and unintended effects. In practical terms, the starting point is the identification of differences between the GE plant and its conventional counterpart, considering various factors such as the genetic modification, the toxicology or allergenicity of any expressed proteins or any differences in the composition or agronomic characteristics. Any differences identified are then subjected to a risk analysis to determine if they pose any greater risks to human and animal health than the conventional counterpart.

While the objective of the assessment is to determine if the GE food presents any new or greater risks in comparison with its traditional counterpart, or whether it can be used interchangeably with its traditional counterpart without affecting the health or nutritional status of consumers, the inherent objective is to establish the relative safety of the new product such that there is a reasonable certainty that no harm will result from intended uses under the anticipated conditions of processing and consumption. If a new or altered hazard, nutritional or other food safety concern is identified by the safety assessment, it is further evaluated to determine its relevance to human health. Following the safety assessment and, if necessary, further risk analysis, the food or component of food may be subjected to risk management options before it is considered for commercial distribution. Where no suitable counterpart exists for comparison, the safety of a GE food must be evaluated from data derived directly from historical experience with broadly similar products or experimental studies with the food.

IV.2 FRAMEWORK FOR SAFETY ASSESSMENT

Safety assessment is designed to identify whether a hazard, nutritional or other safety concern is present and if present, to collect and analyze information on its nature and severity following a structured and integrated approach performed on a case-by-case basis. The safety assessment of foods derived from GE plants follows a stepwise process aided by a series of structured questions. Factors taken into account in the safety assessment include:

- Identity;
- Source;
- Composition;
- Effects of processing/cooking
- Transformation process;
- The recombinant-DNA (e.g., stability of insertion, potential for gene transfer);
- Protein expression product of the novel DNA;
    - Effects on function;
    - Potential toxicity;
    - Potential allergenicity;
- Possible secondary effects from gene expression or the disruption of the host DNA or metabolic pathways, including composition of critical macro-, micro-nutrients, anti-nutrients, endogenous toxicants, allergens, and physiologically active substances; and,
- Potential intake and dietary impact of the introduction of the GE food.

With a wide range of foods available, the amount of information necessary for assessment may vary from case to case. Therefore, in order to provide guidance for applicants, these guidelines describe the types of information generally required to assess the safety of foods derived from GE plants. All requirements may not be relevant in every case and the explanations and interpretations are also subject to change as new knowledge and experience are gained.

It is the responsibility of the developer to make all the pertinent scientific data available for review. In addition to the scientific data generated through experiments, the same needs to be supplemented from a variety of sources such as scientific literature, general technical information, independent scientists, regulatory agencies, or international bodies. Data should be evaluated using appropriate science-based risk assessment methods.

Experiments intended to generate data to demonstrate the safety of foods derived from GE plants need to be designed and conducted in accordance with sound scientific concepts and principles, as well as, where applicable, Good Laboratory Practices (GLP).

Primary data should be made available to regulatory authorities upon request. Data need to be obtained using sound scientific methods and analyzed using appropriate statistical techniques, where applicable. The sensitivity of all analytical methods should be documented and references to analytical methods made available.

V. CORE INFORMATION

V.1 DESCRIPTION OF THE GE PLANT

A description of the GE plant must be provided. This description should identify the crop, the transformation event(s) to be reviewed, a pedigree map of each transformation event, and the type and purpose of the modification, sufficient to aid in understanding the food being submitted for safety assessment.

V.2 DESCRIPTION OF THE UNMODIFIED HOST PLANT AND ITS USE AS FOOD

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

(a) Common or usual name; botanical name; and taxonomic classification;

(b) Centre of origin, history of cultivation and development through breeding, in particular identifying traits that may adversely impact on human health;

(c) Information on the host plant's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and

(d) History of safe use for consumption as food.

V.2.1 History of Safe Use and Dietary Exposure

A food may be considered to have a history of safe use if it has been commonly used in the diet for a number of generations in a large (at least two), genetically diverse human population where it has been used in ways and at levels that are similar to those expected or intended in Bangladesh. The fact that a product has had a history of use according to the above definition in a jurisdiction with a similar food safety system would increase the level of confidence in the evidence presented.

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

The submission needs to include reliable information from referenced sources. Anecdotal evidence (based on unsubstantiated or hearsay reports) will be given less weight than scientifically derived data. Information on the history of human exposure

will be particularly important where there is traditional handling, storing or cooking requirements for processing the food.

V.3 DESCRIPTION OF THE DONOR ORGANISM(S)

Information must be provided on any donor organism of the introduced DNA and, when appropriate, on other related species. It is particularly important to determine if the donor organism(s) or other closely related members of the family naturally exhibit characteristics of human pathogenicity or toxin production, or have other traits that affect human or animal health (e.g., presence of allergens). The description of the donor organism(s) should include:

(a) Common name;
(b) Scientific name;
(c) Taxonomic classification;
(d) Information about the natural history of the organism as concerns human health;
(e) Information on naturally occurring toxins, anti-nutrients and allergens; for micro-organisms, additional information on human pathogenicity and the relationship to known human pathogens; and
(f) Information on the past and present use, if any, in the food supply and exposure route(s) other than intended food use (e.g. possible presence as contaminants).

V.4 DESCRIPTION OF THE GENETIC MODIFICATION

Detailed information is required on the genetic modification to allow for the identification of all genetic material potentially delivered to the host plant and to provide all relevant information required for the analysis of the data supporting the characterisation of the DNA inserted in the plant.

V.4.1 Method of Genetic Modification

(a) A description, including references, is required for the method used to effect the genetic modification (e.g., *Agrobacterium*-mediated transformation or direct transformation by methods such as particle bombardment).
(b) If applicable, for direct transformation methods, a description of the nature and source of any carrier DNA used should be provided, including how the transforming DNA was isolated and purified (e.g., if the transforming DNA was a plasmid vector-derived restriction fragment).
(c) Manipulations or modifications to introduced DNA sequences should be detailed (e.g., re-synthesis of genes to incorporate plant-preferred codons; introduction or

deletion of post-translational modification sites; any changes that would affect the amino acid sequence of the expressed product).

V.4.2 Potentially Introduced Genetic Material

The submission must include a detailed description of all of the genetic elements contained on the potentially introduced genetic material, including both coding and non-coding regions of known function. For each genetic element, this should include:

(a) Name of the gene sequence or regulatory element;

(b) The portion and size of the sequence;

(c) The location, order, and orientation of the sequence in the vector or transforming DNA;

(d) The function in the plant;

(e) Provide references from the scientific literature, including, if applicable, sequence accession numbers from nucleotide sequence databases;

(f) The source (scientific and common name of the donor organism);

(g) Whether the genetic component is responsible for disease or injury to plants or other organisms, or if it encodes a known toxicant, allergen, pathogenicity factor or irritant;

(h) Whether the donor organism is a known source of significant toxicants, allergens, or irritants;

(i) Whether there is any history of safe use of the donor organism, or components thereof, including whether the introduced genetic element is present in other genetically engineered plants authorised for use in food, feed, or processing.

A detailed map of the plasmid vector or transforming DNA should be provided, with the location and orientation of all the sequences described above. The map should also indicate the cleavage sites of any restriction endonucleases used in subsequent analyses of the inserted DNA, including any regions used as hybridisation probes. The nucleotide sequence of the entire potentially introduced DNA should be provided.

V.5 MOLECULAR CHARACTERISATION OF THE GE PLANT

The molecular-genetic characterisation of the modified plant should be sufficient to demonstrate that the introduced DNA has been stably incorporated into the plant's genetic material (whether the nuclear genome or a plastid genome) and that the introduced DNA (or trait) is inherited over multiple generations in a predictable manner. Methods of demonstrating this may include, but not be limited to:

(a) Southern blot hybridisation of genomic plant DNA digested with one, or more, restriction endonucleases and probed with DNA sequences complementary to different genetic elements contained on the transforming DNA;

(b) Polymerase chain reaction (PCR) analysis using primers designed to amplify different regions of the introduced DNA;

(c) The use of DNA-based methods (e.g., Southern hybridisation, PCR analysis), protein-based methods [e.g., enzyme linked immunosorbent assay (ELISA), western immunoblotting], or biological assay to demonstrate stable inheritance of the introduced DNA (or trait) over multiple generations;

(d) The use of methods, such as those described above, to demonstrate segregation of the introduced DNA (or trait) within a segregating generation.

On a case-by-case basis, and if warranted by observations of biologically significant unintended phenotypic characteristics, other more elaborate methods of molecular characterisation may be required to explain these phenomena.

For any introduced sequences intended to result in the expression of a new protein product, information should be provided on:

(a) The level of expression of the protein in relevant plant tissues that may be used in food or for animal feed (e.g., seed or grain; above ground vegetative tissue);

(b) The levels of affected plant metabolites in cases where the protein is intended, or anticipated, to affect plant metabolic pathways or alter the levels of plant metabolites;

(c) The molecular size of the protein (e.g., via western immunoblotting) to confirm that it is as expected (in the case of any significant deviations from the anticipated size, additional data explaining the discrepancy may be required);

(d) In cases where deliberate changes were introduced into the amino acid sequence (e.g., changes affecting post-translational modification or affecting sites critical for structure or function), data should be provided to demonstrate the effectiveness of these changes;

(e) If protein expression is inducible, either in response to a stage of plant development, a biotic or abiotic stress, or some external agent, then levels of expression in relevant plant tissues before and after induction should be reported; and

(f) If the protein is intended to alter endogenous gene expression (e.g., transcription factor) then levels of gene expression should be compared with that of the unmodified host plant.

In cases where the genetic modification is not intended to result in the expression of a new protein (e.g., expression of a non-translatable mRNA, truncated sense constructs,

antisense constructs, small interfering RNAs, or ribozymes), data should be provided to demonstrate that the intended effect has been achieved.

In any case where the intent of the genetic modification is to alter the regulation of endogenous genes, the characteristics and level of gene expression should be compared with that of the unmodified host.

VI.SAFETY ASSESSMENT

VI.1 ASSESSMENT OF POSSIBLE TOXICITY

Toxicological testing is required for substances of unknown safety that are introduced into the food supply. *In vitro* nucleic acid techniques enable the introduction of DNA that can result in the synthesis of new substances in plants. These include the protein expression product and other substances, which may be generated as a result of enzymatic activity of the protein expression product. The new substances can be conventional components of plant foods such as proteins, fats, carbohydrates, vitamins, which are novel in the context of that GE plant.

The safety assessment has to take into account the following:

(a) The chemical nature and function of the newly expressed substance;

(b) The concentration of the substance in the edible parts of the GE plant, including variations and mean values;

(c) Current dietary exposure and possible effects on population sub-groups, if applicable;

(d) Information, if any, that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to GE plants that do not normally express those toxin or anti-nutrient characteristics. This assurance is particularly important in cases where the GE plant is processed differently from a donor plant, since conventional food processing techniques associated with the donor organisms may deactivate, degrade or eliminate anti-nutrients or toxicants.

In cases where the intended genetic modification results in the expression of a substance that has, or is closely related to a substance that has, a history of safe (dietary) exposure to humans and animals, further toxicological testing is not necessary. For example, if the intended genetic modification results in the expression of a plant virus coat protein for the purpose of conferring resistance to infection and disease caused by the virus, toxicity testing on the expressed virus coat protein is not required as there is a history of safe consumption (and exposure) by humans and animals eating virus-infected plant material. Otherwise, the use of conventional toxicology studies on the new substance is necessary. Where possible, these studies should be performed on the new substance as expressed in the GE plant, however, where this is not feasible because of the amounts required, alternative sources may be used. In this case, studies demonstrating

that the material isolated from the alternative source is biochemically and functionally equivalent to the plant-expressed form are required.

For proteins, the toxicological assessment is based on a weight-of-evidence that considers the following parameters:

(a) Amino acid sequence similarity between the protein and known protein toxins. An accepted threshold for significant sequence similarity is >35% sequence identity over an 80-amino acid window.

(b) Digestibility, as commonly assessed using an *in vitro* pepsin digestion assay.

(c) Stability to heat or processing, where this can be measured (e.g., in the case of proteins with some enzymatic or measurable biological activity).

(d) Acute oral toxicity testing. Proteins exhibiting toxicity generally exert their effect at low dosages (e.g., nanogram to microgram per kg body weight) and in a short time frame. Acute toxicity tests at higher dosages (e.g., 0.1-1 g/kg body weight) are therefore considered adequate for evaluating potential toxicity. When a protein demonstrates no acute oral toxicity in high-dose testing using a standard laboratory mammalian test species (e.g., mouse), this supports the determination that the protein will be non-toxic to humans and other mammals, and will not present a hazard under any realistic exposure scenario, including long-term exposure.

Proteins that are enzymes have never been shown to be direct-acting carcinogens, mutagens, teratogens or reproductive toxicants (Pariza and Foster, 1983). Hence, it is generally not necessary to test enzymes for these toxicological endpoints when exposure occurs by the oral route.

Potential toxicity of non-protein substances that have not been safely consumed in food should be assessed on a case-by-case basis depending on the identity and biological function in the plant of the substance and dietary exposure. The type of studies to be performed may include studies on metabolism, toxicokinetics, sub-chronic toxicity, chronic toxicity/carcinogenicity, reproduction and development toxicity according to the traditional toxicological approach.

VI.2 ASSESSMENT OF POSSIBLE ALLERGENICITY

The primary consideration in allergenicity assessment of a newly expressed novel protein in a food derived from a genetically engineered (GE) plant is the prevention of unexpected exposure of sensitized individuals to food allergens. All newly expressed proteins in GE plants that could be present in the final food need to be assessed for their potential to cause allergic reactions. This requires consideration of whether a newly expressed protein is one to which certain individuals may already be sensitive as well as

whether a protein new to the food supply is likely to induce allergic reactions in some individuals.

At present, there is no single definitive test that can be relied upon to predict allergic response in humans to a new protein in the diet, hence a weight of evidence approach is recommended that considers: the source of the introduced protein; the structural properties of the protein, including thermal stability and susceptibility to enzymatic digestion; amino acid sequence similarity with known allergens; and serum screens using documented sera from allergic individuals if the protein is similar to known allergens or comes from an allergenic source. Evidence from all of these studies is taken into account in coming to a conclusion on the potential allergenicity of the newly expressed novel protein.

The following types of information are considered:

(a) **The source of the introduced gene**. Genes derived from known allergenic sources should be assumed to encode an allergen unless scientific evidence demonstrates otherwise. Allergenic sources would be defined as those organisms for which reasonable evidence of IgE-mediated oral, respiratory or dermal allergy is available. Information should be provided on any substantiated reports of allergenicity associated with the donor organism.

(b) **Amino acid sequence similarity with known allergens**. Sequence comparisons should be conducted against peer-reviewed allergen databases using appropriate search algorithms (e.g., sliding 80-mer FASTA searches). Significant sequence similarity with a known allergen can be considered when there is >35% sequence identity in a segment of 80, or more, amino acids. Sequence matches less than this threshold are not considered "significant" and, if the source of the gene is not a common allergen, there is consequently no justification for serum IgE tests. In these cases, an affirmative statement should be made indicating a lack of evidence to require serum testing.

All numerically "significant" matches of the introduced protein must be interpreted. There is a clear gradient of probable immunological cross-reactivity based on the extent of sequence similarity. A match of 38% identity over 80 amino acids is not very likely to be cross-reactive, while one that is > 80% identity is highly likely to be cross-reactive. Further, there may be very little (if any) published data demonstrating the allergenicity of a given protein, and when available, such reports should be carefully reviewed by someone familiar with clinical allergy to verify the "significance" of the finding.

(c) **Pepsin resistance**. Typically, most food allergens tend to be stable to the peptic and acidic conditions of the digestive system in order to reach and pass through the intestinal mucosa to elicit an allergic response. *In vitro* digestibility studies of proteins in the presence of pepsin at acid pH (pH 1.2 – pH 2.0) have

demonstrated a good correlation between resistance to degradation and allergenic potential. Investigation of proteins that have been tested, suggest a strong positive predictive value that food allergens causing systemic reactions are relatively stable in the assay, while non-allergenic food proteins are typically digested relatively quickly. Although the pepsin resistance protocol is strongly recommended, it is recognised that other digestibility protocols exist and alternative protocols may be used where adequate justification is provided.

VI.2.1 Serum Testing

Only if there is evidence that the source of the gene causes allergies frequently enough to suspect some individuals may already be sensitized to the protein (if it is also expressed in a source material of expected human exposure) or in cases where the newly expressed protein exhibits significant sequence similarity to a known allergen, should an assessment be made of the feasibility of conducting a serum IgE study. If a sufficient number of subjects (5 minimum, preferably more than 10 with proven allergy to the source) allergic to the source are found by contacting recognized allergists, and informed consent is found, then serum testing with individual sera should be undertaken using the source, pure novel protein, and the GE product as test materials.

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

VI.2.2 Other Considerations

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

As scientific knowledge and technology evolves, other methods and tools may be considered in assessing the allergenicity potential of newly expressed proteins as part of the assessment strategy. Currently, however, the use of animal models or the analysis of protein structure for T-cell epitopes or motifs associated with allergens, have not been validated for regulatory purposes.

VI.3 COMPOSITIONAL ANALYSIS

For GE plants without purposefully altered nutritional properties, the compositional analysis is part of the weight-of-evidence approach for evaluating whether there were any

unanticipated consequences of the genetic modification. Data should be provided on the levels of key nutrients and antinutrients present in the edible portions of the plant (e.g., seed or grain), including other plant parts (e.g., forage) that may be used as feed for livestock. The compounds chosen for testing should be those recognised as key nutrients and antinutrients for the plant species (e.g., those identified in international consensus documents on nutrient properties, where applicable).

Material subject to compositional analysis should be obtained from confined field trials conducted in a range of environmental conditions representative of the intended area of commercial cultivation. Considerations for field trial sites include:

(a) The location of trial sites needs to be representative of the range of environmental conditions under which the plant varieties would be expected to be grown.

(b) The number of trial sites need to be sufficient to allow accurate assessment of compositional characteristics over this range. Trials have to be conducted over a sufficient number of generations to allow adequate exposure to the variety of conditions met in nature.

(c) Each trial site is required to be replicated to minimise environmental effects, and to reduce any effect from naturally occurring genotypic variation within a crop variety.

(d) Sampling of adequate number of plants and the methods of analysis need to be sufficiently sensitive and specific to detect variations in key components.

Comparisons should be made between the GE plant and an appropriate counterpart (e.g., near-isogenic line or parental line), and considering the normal range of variation for the nutrient in other cultivated varieties of the plant (e.g., comparisons with data from the published scientific literature or nutrient databases). The focus should be on identifying and discussing any biologically significant differences in nutrient composition.

Consideration should also be given to whether the introduced trait is likely to result in changes in consumption patterns for the crop, and whether there may be differential impacts on subgroups of the population (e.g., children, infants, elderly, ethnic groups, etc) due to varying exposure.

Compositional analyses should normally include the following (the applicant may provide valid scientific rationale to exclude items or include additional items):

(a) Proximates (i.e., moisture, protein, fat, carbohydrate, ash, fibre)

(b) Amino acids

(c) Fatty acids

(d) Vitamins

(e) Minerals

(f) Naturally occurring antinutrients (e.g., phytates, trypsin inhibitors, lectins, alpha-galactosides, etc)

(g) Predictable secondary metabolites or other physiologically active substances normally associated with the plant species

Detection of a major compositional change due to an unintended 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.

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, the food may need to be subjected to further analysis and assessment. Additional *in vitro* or *in vivo* studies may be needed on a case-by-case basis to assess the toxicity of expressed substances, taking into account the potential accumulation of any substances or toxic metabolites that might result from the genetic modification.

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.

VI.4 INTENDED NUTRITIONAL MODIFICATIONS

Foods derived from GE plants that have undergone modification to intentionally alter nutritional quality or functionality need to be subjected to additional nutritional assessment to assess the consequences of the changes and whether the nutrient intakes are likely to be altered by the introduction of such foods into the food supply.

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

The use of plant breeding, including *in vitro* nucleic acid techniques, to change nutrient levels in crops can result in broad changes to the nutrient profile. The intended

modification in plant constituents could change the overall nutrient profile of the plant product and this change could affect the nutritional status of individuals consuming the food. Unexpected alterations in nutrients could have the same effect. Although the GE plant components may be individually assessed as safe, the impact of the change on the overall nutrient profile needs to be determined.

When the modification results in a food product, such as vegetable oil, with a composition that is significantly different from its unmodified counterpart, it may be appropriate to use additional foods or food components whose nutritional composition is closer to that of the food derived from the GE plant as the appropriate comparator.

Nutritional changes to a specific food may have a greater impact in some geographical areas or in some cultural population than in others due to variations in food consumption patterns. The nutrient and the populations affected need to be identified.

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

VI.5 UNINTENDED EFFECTS

Unintended effects can result from the random insertion of DNA sequences into the plant genome which may cause disruption or silencing of existing genes, activation of silent genes, or modifications in the expression of existing genes. Unintended effects may also result in the formation of new or changed patterns of metabolites. The assessment for unintended effects takes into account the agronomic/phenotypic characteristics of the plant that are typically observed by breeders in selecting new varieties for commercialisation.

VII. SUPPORTING REFERENCES

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Annex 2: Proforma summary application for GE food safety assessment

VIII. PROFORMA SUMMARY APPLICATION FOR THE SAFETY ASSESSMENT OF A FOOD DERIVED FROM GENETICALLY ENGINEERED PLANT MATERIAL

This proforma does not replace the full submission dossier, including supporting studies, that contain the complete set of data required for the safety assessment.

A.1 APPLICANT	
Name: _____ Organisation: _____ Address: _____ _____ _____ _____ Telephone: _____ Fax: _____ E-mail: _____ _____	Submission Date: _____ **NO INFORMATION CONTAINED HEREIN SHALL BE TREATED AS CONFIDENTIAL BUSINESS INFORMATION**

A.2-1 Name of GE plant event	A.2-2 Unique identifier of the regulated article
A.2-3 Country of origin	A.2-4 Planned date of first importation
A.2-5 Has the regulated article received authorisation for cultivation and use in food and/or livestock feed in the country of origin?	Yes No (If Yes, list all relevant permit and/or authorisation numbers and relevant competent national authority(ies))

Type of Authorisation	Competent National Authority	Date of Authorisation	Permit or Authorisation No.	Official Authorisation Documentation Attached	
				Yes No	
				Yes No	

A.2-6 Has the regulated article received authorisation for cultivation and use in food and/or livestock feed in other countries?

Yes
No
(If Yes, list below)

Country	Type of Authorisation	Competent National Authority	Date of Authorisation	Permit or Authorisation No.	

A.2-7 Has the regulated article undergone a safety assessment in the country of origin consistent with the "Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants" published by the CODEX Alimentarius Commission?	Yes No If Yes, has a copy of this safety assessment report or equivalent decision summary published by the relevant competent authority(ies) of the country of origin been attached to this application? Yes No

DETECTION AND IDENTIFICATION REQUIREMENTS

A.3-1 Briefly describe the event-specific detection method for the genetically engineered plant event

GENERAL DESCRIPTION OF THE REGULATED ARTICLE

A.4-1 Name of the recipient or parental plant and the intended function of the genetic modification

A.4-2 Intended use of any derived products and types of users

A.4-3 Describe any specific instructions and/or recommendations for use, storage and handling
A.4-4 Describe any proposed packaging and labelling requirements
A.4-5 If applicable, geographical areas within Bangladesh to which distribution of the product is intended to be limited, including the identification of any geographical areas for which use and distribution of the product is unsuited

INFORMATION RELATING TO THE RECIPIENT OR PARENTAL PLANTS	
B.1 Scientific name:	B.2 Common name:
B.2 Is the recipient plant known to be a significant source of toxicants or antinutrients?	Yes No (If Yes, identify the compounds, the levels that induce toxicity)
B.3 Is the recipient plant known to be a significant source of allergens?	Yes No (If Yes, identify the allergenic protein(s))
B.4 Is the recipient plant a significant dietary source of particular macro- or micro-nutrients, either for the population generally or particular population subgroups?	Yes No (If Yes, describe below)
B.5 Is any special food processing technique or procedure important in reducing the effects of naturally occurring toxicants or antinutrients?	Yes No (If Yes, describe below)

INFORMATION RELATING TO THE GENETIC MODIFICATION

METHOD OF MODIFICATION

C.1-1 Describe and provide references for the method used to effect the genetic modification

AT–*Agrobacterium* mediated transformation
PF–protoplast fusion
BT–Biolistic and particle gun transformation
OO–other (describe below)

C.1-2 Describe any manipulations or modifications to introduced DNA sequences

POTENTIALLY INTRODUCED GENETIC MATERIAL

C.2-1 Provide a detailed description of all of the genetic elements contained on the potentially introduced genetic material, including both coding and non-coding regions of known function. For each element, the description should include the intended function in the plant, the source (donor organism), whether the genetic element is responsible for disease or injury to plants or other organisms, and whether the donor organism has a history of safe use or whether it is a known source of significant allergens, toxicants or irritants.

Starting Pos (bp)	Ending Pos (bp)	Size (kb)	Name	Donor Organism	Donor Organism Source of Toxins or Allergens (Yes/No)	Protein Expressed (Yes/No)	Function in the Plant	

C.2-2 Provide a detailed map of the plasmid vector or transforming DNA with the location and orientation of all the sequences described above.

MOLECULAR CHARACTERISATION OF THE GE PLANT

C.3-1 Describe the chromosomal location(s) of the inserted DNA (i.e., nucleus, chloroplasts, mitochondria, or maintained in a non-integrated fashion), and how this was determined

Nuclear genome
Chloroplast genome
Mitochondrial genome
Transposable element
Extra chromosomal plasmid
Viral vector
Other (describe below)

C.3-2 Describe how genetic stability of the introduced trait over multiple generations was demonstrated

C.3-3 Describe how segregation of the introduced trait within a generation was demonstrated

C.3-4 For any introduced sequences intended to result in the expression of a new protein product, provide information on the level of expression of the protein in relevant plant tissues. Also indicate if protein expression is inducible, and if so how, and whether there is a likelihood of affecting plant metabolic pathways.

Protein	Plant Tissue	Concentration (µg/g fresh weight)

C.3-5 Is the genetic modification intended to alter the expression (regulation) of endogenous genes? Yes No (If Yes, specify below)

POTENTIAL MAMMALIAN TOXICITY AND ALLERGENICITY

D.1 Describe the safety studies undertaken to demonstrate lack of potential toxicity of any newly expressed proteins in the GE plant that do not have a history of safe consumption

Protein	Amino acid sequence similarity with known toxins (>35% identity over 80 amino acids)	Rapidly digested via in vitro pepsin digestibility assay	Activity is stable to heat or processing	Acute oral toxicity testing
	Yes □ No □	Yes □ No □	Yes □ No □	Toxicity observed Yes □ No □ Dose tested: _____ mg/kg BW

Summarize results of toxicity testing, below:

D.2 Describe the safety assessment performed to predict lack of potential allergenicity of any newly expressed proteins in the GE plant that do not have a history of safe consumption

Protein	Donor organism a known source of significant allergens	Amino acid sequence similarity with known allergens (>35% identity over 80 amino acids)	Rapidly digested via in vitro pepsin digestibility assay	Stable to heat or processing
	Yes □ No □	Yes □ No □	Yes □ No □	Yes □ No □

Provide additional details as necessary, below:

NUTRITIONAL ANALYSIS

E.1 Describe the results of compositional analyses. Data should be provided on the levels of key nutrients and antinutrients present in the edible portions of the plant (e.g., seed or grain), including other plant parts (e.g., forage) that may be used as animal feed

APPLICANT VERIFICATION

This application is submitted in accordance with requirements specified by the Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants.

Signature of applicant Date

By my signature, above, I attest that the information contained herein is accurate and complete to the best of my knowledge and belief, and that this application includes all relevant data and information upon which to base a decision, including all data and information that are unfavourable to the application.

IX. DRAFTING COMMITTEE

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