NATIONAL BIOSAFETY AUTHORITY

GUIDELINES FOR THE SAFETY ASSESSMENT OF FOODS DERIVED FROM GENETICALLY MODIFIED CROPS IN KENYA

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FOREWORD

The National Biosafety Authority (NBA) was established vide Biosafety Act of 2009 to exercise general supervision and control over the transfer, handling and use of genetically modified organisms (GMOs) in Kenya with the aim of ensuring safety of human and animal health, and provision of an adequate level of protection to the environment. The Authority regulates all activities involving GMOs in food, feed, research, industry, cultivation, trade, import, export and transboundary movements.

NBA is the National Focal Point for the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) and is mandated to implement the provisions of the Cartagena Protocol on all biosafety matters pertaining to GMOs.

Since its establishment, the Authority has made great strides in establishing a strong Biosafety framework in Kenya by developing and publishing the implementing Biosafety Regulations namely; Biosafety (Contained use) Regulations, 2011, Biosafety (Environmental Release) Regulations, 2011, Biosafety (Import, Export and Transit) Regulations, 2011; and the Biosafety (Labeling Regulations), 2012. These regulations laid down clear procedures on handling GMOs whether crops, animals or microorganisms.

To support and elaborate the Regulations, the Authority has for developed a number of manuals, guidelines and standard operating procedures on various regulatory processes. These documents have been developed based on the International Organization for Standardization (ISO) standards. It provides a detailed stepwise process of assessing potential adverse effects of GM crops to human and animal health. The Guideline also provides a detailed checklist on data requirements on safety that will guide applicants and developers during GM crop development stages and submission of dossiers for consideration by the Authority.

This guideline was prepared through a series of consultative meetings to gather experts and public views. We are grateful for the active participation and cooperation demonstrated by the regulatory agencies and other stakeholders during the process of developing this guideline. We sincerely thank our development partners for the support in development of this guideline which will go a long way in improving the biosafety systems in Kenya in regards to safety of GM foods.

Finally, I hope that applicants, developers, expert reviewers, Regulatory Agencies and other stakeholders will make use of the valuable information provided by this guideline.

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ABBREVIATIONS AND ACRONYMS

DNA	:	Deoxyribonucleic Acid
DPH	:	Department of Public Health
DVS	:	Department of Veterinary Services
GLP	:	Good Laboratory Practices
GM	:	Genetically Modified
GMO	:	Genetically Modified Organism
KEBS	:	Kenya Bureau of Standards
KEPHIS	:	Kenya Plant Health Inspectorate Service
KIPI	:	Kenya Industrial Property Institute
KWS	:	Kenya Wildlife Service
NBA	:	National Biosafety Authority
NEMA	:	National Environment Management Authority
OECD	:	Organization for Economic Co-operation and Development
PCPB	:	Pest Control Products Board
rDNA	:	recombinant Deoxyribonucleic Acid
SOP	:	Standard Operating Procedure

DEFINITION OF TERMS

Allergen is a type of antigen that produces an abnormally vigorous immune response in which the immune system fights off a perceived threat that would otherwise be harmless to the body. Such reactions are called allergies.

Allergenicity means the capacity of GM food to cause an allergic reaction upon consumption.

Anti-nutrient means a substance that interferes with the absorption and utilisation of one or more nutrients by the body.

Compositional Analysis is the process of determining if there are any significant changes in nutrient composition in a GM crop in comparison to its traditional counterpart and to assess the safety of the intended or unintended changes

Conventional counterpart means the equivalent non -genetically modified crop variety or a near- isogenic line, 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.

Genetically Modified food (GM food) means food derived from GM crops obtained through modern biotechnology. Where the word "food" is used, it also implies "feed".

Genetically Modified crop means a crop 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 r-DNA or transgenic crops.

Hazard means a biological, chemical or physical agent in food (or condition of food) that has an inherent potential to cause an adverse health effect.

Modern biotechnology means the application of:

- *In vitro* nucleic acid techniques including the use of recombinant-deoxyribonucleic acid (r-DNA) and direct injection of nucleic acid into cells or organelles **OR,**

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

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 is equal to ***f*** (Hazard x Exposure).

Risk assessment is a science-based process to systematically evaluate safety concerns, in this case addressing human and animal health safety of GM foods within a framework for decision making.

Substantial equivalence is a concept that guides food safety assessment in determining whether GM food is as safe as its conventional counterpart.

Transgenic crop a crop in which a one or more foreign gene has been integrated into its genome.

Transformation means the unique DNA recombination that took place through the integration of a gene in one crop cell for genetic modification, which was then used to generate entire transgenic crops.

Toxin is a poisonous substance, such as a protein or a metabolite that is produced by living cells or organisms and is capable of causing adverse human health when introduced into the body tissues but is often also capable of inducing neutralizing antibodies or antitoxins.

Toxicity means the capacity of a food to be poisonous which is dependent on the amount and concentration of a toxin.

CHAPTER ONE

BACKGROUND OF NBA

1.1 Background

The National Biosafety Authority (NBA) is a state corporation in Kenya mandated to ensure safety of human and animal health and provide adequate protection of the environment from harmful effects that may result from genetically modified organisms (GMOs).

The Authority was established pursuant to the provisions of the Biosafety Act, 2009 to regulate all activities involving GMOs in food, feed, research, industry, trade and environmental release and it fulfills its mandate by ensuring and assuring safe development, transfer, handling and use of GMOs in Kenya.

NBA has made great strides in establishing strong Biosafety framework in Kenya by developing and publishing the implementing Biosafety Regulations. These regulations laid down a clear procedure on handling GMOs whether crops, animals or microorganisms. NBA is the National Focal Point for the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD) and is mandated to implement the provisions of the Cartagena Protocol on all Biosafety matters pertaining to GMOs.

1.2 Vision Statement

A World-class Biosafety Agency

1.3 Mission Statement

To ensure and assure safe development, transfer, handling and use of genetically modified organisms (GMOs) in Kenya.

1.4 NBA Core Values

a) Good governance and integrity

b) Professionalism

c) Customer Focus

d) Inclusiveness.

1.5 Objectives of the Biosafety Act

a) To facilitate responsible research and minimize risks that may be posed by genetically modified organisms;

b) To ensure adequate level of protection in the development, transfer, handling and use of genetically modified organisms that may have an adverse effect on the health of the people and the environment; and

c) To establish a transparent, science-based and predictable process for reviewing and making decisions on the development, transfer, handling and use of genetically modified organisms and related activities.

1.6 NBA Core Functions

The Biosafety Act No.2 of 2009, Section 7(2) lists the functions of NBA as follows:

a) Consider and determine applications for approval for the development, transfer, handling and use of genetically modified organisms, and related activities in accordance with the provisions of the Biosafety Act;
b) Co-ordinate, monitor and assess activities relating to the safe development, transfer, handling and use of genetically modified organisms in order to ensure that such activities do not have adverse effect on human health and the environment;
c) Co-ordinate research and surveys in matters relating to the safe development, transfer, handling and use of genetically modified organisms, and to collect, collate and disseminate information about the findings of such research, investigation or survey;
d) Identify national requirements for manpower development and capacity building in biosafety;
e) Advise the Government on legislative and other measures relating to the safe development, transfer, handling and use of genetically modified organisms;
f) Promote awareness and education among the general public in matters relating to biosafety; and
g) Establish and maintain a Biosafety clearing house (BCH) to serve as a means through which information is made available to facilitate exchange of scientific, technical, environmental and legal information on, and experience with, living modified organisms;
h) To exercise and perform all other functions and powers conferred on by the Act.

CHAPTER TWO

INTRODUCTION

2.1 Introduction to food safety assessment

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 among others. New crop varieties developed using r-DNA techniques, commonly referred to as genetically modified (GM), genetically engineered (GE) or transgenic crops, have been and are being developed with traits intended to provide benefit to farmers, consumers, and industry. These traits include stress resistance, disease resistance, herbicide resistance, pest resistance, improved nutrition, improved shelf life, and the production of useful by-products among others. Globally, the majority of genetically modified crops consist of commodity crops such as soybean, maize, cotton and rapeseed. In Kenya, insect resistant cotton has been approved for commercialization, insect resistant maize has gone through national performance trials, virus resistant cassava has been approved to undergo national performance trials and other crops are at different stages of research and development.

As more GM crops are released and the resultant food products are commercially available and traded across various countries, concerns have been expressed about their safety for human and animal health and the environment. The concept of food safety assurance (i.e., that a food is safe for human consumption according to its intended use) is of utmost importance. As with any method of genetic manipulation, including genetic modification of crops, there is a possibility of introducing unintended changes along with the intended changes, which may in turn have an adverse impact on the nutritional composition of the crop or health of the consumer.

To address the safety of foods derived from GM crops, there is a need to adopt a systematic and structured approach to the assessment of potential risk. The framework for decision making is provided through the use of problem formulation, a methodology that allows risk assessors to establish the key questions that need to be addressed; identify existing information that is relevant to address those questions; and identify missing information needed to characterise the risk and facilitate the decision-making process. The Codex Alimentarius Commission, established by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) in 1963 developed harmonised international food standards, guidelines and codes of practice to protect the health of the consumers and ensure fair practices in the food trade. The Codex Alimentarius covers all types of foods, and also provides principles for the risk assessment of foods derived from modern biotechnology.
Risk in the context of food safety includes two elements: i) **hazard**, an intrinsic factor and ii) the **probability** or chance that the event will occur.

Risk assessment is a scientifically based process consisting of the following steps: i) hazard identification; ii) hazard characterization; iii) exposure assessment; and iv) risk

characterization. **Risk management** is the process of weighing policy alternatives in consultation with all interested parties, considering risk assessment and other factors relevant for the protection of human health and for the promotion of fair trade practices as well as, if necessary, selecting appropriate prevention and control options. **Risk communication** is the interactive exchange of information and opinions among assessors, risk managers, consumers, industry, the academic community and other interested parties throughout the risk analysis process. The information exchange concerns risk-related factors and risk perceptions, including the explanation of risk assessment findings and the basis of risk management decisions.

It is vitally important that risk communication with the public comes from credible and trusted sources. A science-based approach for communicating effectively in situations of high stress, high concern and controversy is especially important to help individuals understand the process of risk assessment and management, to form scientifically valid perceptions of the likely hazards and to participate in making decisions about how risks should be managed. Risk communication can be done orally, in written form or through visual statements. Risk management requires incorporation of risk communication as an integral part of risk process.

In Kenya, National Biosafety Authority (NBA) being the National Focal Point on biosafety matters collaborates with a number of regulatory agencies as specified in the First Schedule of Biosafety Act.

These include:

i). State Department of Public Health (DPH)
ii). Directorate of Veterinary Services (DVS)
iii). Kenya Bureau of Standards (KEBS)
iv). Kenya Plant Health Inspectorate Services (KEPHIS)
v). Kenya Industrial Property Institute (KIPI)
vi). Kenya Wildlife Service (KWS)
vii). Pest Control Products Board (PCPB)
viii). National Environment Management Authority (NEMA)

The Authority, in collaboration with the above regulatory agencies, has taken the initiative to develop these guidelines to establish the safety assessment procedures for foods derived from GM crops, also taking into consideration the international "*Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA plants*"[1].

2.2. Objectives of the guidelines

2.2.1. Overall objective

The objective of these guidelines is to provide general guidance on how food and feed safety assessment of GM crops will be conducted in Kenya.

2.2.2. Specific Objectives

i. To guide applicants, expert reviewers, risk assessors and decision makers on the requirements for food/feed assessment data required in GMO applications for environmental release;
ii. To provide a detailed stepwise process of assessing safety of foods and feeds derived from GM crops; and
iii. To provide clarity on specific parameters analyzed in determination of food/feed safety of GM crops.

2.3. Scope

These guidelines are applicable to foods/feeds derived from GM crops meant for environmental release both for single and stacked gene events. They do not apply to GM animals, GM microorganism and pharmaceuticals for human use. For stacked gene events, safety assessment shall be guided by the general framework provided in the Environmental Risk Assessment Guidelines.

NB: These guidelines are generic and are not specific to any particular crop. Food/feed safety assessment will be customized depending on the crop being evaluated.

CHAPTER THREE

PRINCIPLES OF FOOD SAFETY ASSESSMENT

3.1. Overarching Principles

Modern biotechnology has made available new products through genetic recombination. Such include food derived from recombinant DNA crops otherwise known as GM foods. There is need to ensure that the GM food is as safe as the conventional counterpart. Internationally accepted approaches to assessing safety of the GM foods have been developed. These are articulated in two important documents published in 2003 by the CAC/GL; *"Principles for the risk analysis of foods derived from modern biotechnology"* otherwise known as codex principles and "G*uideline for the conduct of food safety assessment of foods derived from recombinant DNA plants"* otherwise known as codex guidelines. Most Kenyan standards are adopted from international standards setting organizations such as Codex Alimentarius Commission and OECD while taking into account national laws. The Codex food safety assessment standards have been adopted in Kenya (KSCAC/GL) in line with international Food Safety Management Systems (ISO 22000).

3.1.1 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, an alternative approach has been adopted in developing the framework for the safety assessment of GM foods. This approach acknowledges that the goal of the assessment is not establishing absolute safety, but whether the GM food is as safe as its conventional counterpart, where such a counterpart exists. This gives confidence that no harm (nutrition, allergenicity, or toxicity) will result from intended uses under the anticipated conditions of consumption.

3.1.1.1 Concept of Substantial Equivalence

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 conventional 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 GM crops.

Accordingly, the safety assessment of foods derived from GM crops 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 GM crop 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 GM 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 GM food must be evaluated from data derived directly from historical experience with broadly similar products or experimental studies with the food.

3.1.2 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 analyse 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 GM crops follows a stepwise process aided by a series of structured questions. Factors considered in the safety assessment include:

i. Identity of the GM crop - Source of the gene, the recombinant-DNA (e.g. stability of insert, potential for gene transfer), transformation process, protein expression product of the novel DNA, effects of function
ii. Potential toxicity
iii. Potential allergenicity
iv. Possible secondary effects from gene expression or the disruption of host DNA or metabolic pathways including composition of critical macro-, micro-nutrients, anti-nutrients, endogenous toxicants, allergens, and physiologically active substances.
v. Nutritional composition - Effects of processing/cooking, potential intake and dietary impact of the introduction of the GM 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

GM crops. 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 GM crops need to be designed and conducted in accordance with sound scientific concepts and principles, as well as, where applicable, Good Laboratory Practices (GLP). Data need to be analysed using appropriate statistical techniques, where applicable. The sensitivity of all analytical methods should be documented and references to analytical methods made available. Relevant data should be made available to regulatory authorities upon request.

3.2. Core Information

3.2.1 Description of the GM Crop

This description should identify the crop, the transformation event(s) to be reviewed, a genetic 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.

3.2.2 Description of the Unmodified Host Crop and its Use as Food

A comprehensive description of the unmodified host crop 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 crop's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and
(d) History of safe use for consumption as food.

3.2.2.1 History of Safe Use and Dietary Exposure

A food may be considered to have a history of safe use if it has been used in ways and at levels that are similar to those expected or intended in Kenya. 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 crop is sourced, cultivated, transported and stored, whether special processing is required to make the crop safe to eat, and the crop's normal role in the diet (e.g., which part of the crop is used as a food source, whether its consumption is important in the population, what important macro- or micro-nutrients it contributes to the diet).

The submission needs to include reliable information from referenced sources. Information on the history of human exposure will be particularly important where there is traditional handling, storing or cooking requirements for processing the food.

3.2.3 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 and animal 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).

3.2.4 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 crop and to provide all relevant information required for the analysis of the data supporting the characterisation of the DNA inserted in the crop.

3.2.4.1 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 crop-preferred codons; introduction or deletion of post-translational modification sites; any changes that would affect the amino acid sequence of the expressed product).

3.2.4.2 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 crop;
(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 crops 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 Modified crops authorised for use in food, feed, or processing.

A detailed map of the plasmid vector or transforming DNA and any modification therein 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.

3.2.5 Molecular Characterization of the GM Crop

The molecular-genetic characterisation of the modified crop should be sufficient to demonstrate that the introduced DNA has been stably incorporated into the crop's genetic material (whether the nuclear genome or cytoplasmic 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 limited to:

(a) The use of DNA-based methods (e.g., Southern hybridisation, PCR analysis, DNA sequencing),
(b) 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;
(c) The use of methods, such as those described above, to demonstrate segregation of the introduced DNA (or trait) within a segregating population or to demonstrate stability through clonal 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 crop tissues that may be used in food or for animal feed (e.g., seed or grain; above ground vegetative tissue) and how detected;
(b) The function of the expressed protein;
(c) The levels of affected crop metabolites in cases where the protein is intended, or anticipated, to affect crop metabolic pathways or alter the levels of crop metabolites;
(d) The molecular size of the protein if detectable (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);
(e) If protein expression is inducible, either in response to a stage of crop development, a biotic or abiotic stress, or some external agent, then levels of expression in relevant crop tissues after induction should be reported;
(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 crop; and
(g) The identity of any novel metabolites known to result from the production of the protein.

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.

3.3. Safety Assessment

3.3.1 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 crops. 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 crop foods such as proteins, fats, carbohydrates, vitamins, which are novel in the context of that GM crop.

The safety assessment has to consider 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 GM crop, including variations and mean values;
(c) Current dietary exposure and possible effects on the population, if applicable;

(d) Information, if any, that genes coding for known toxins or anti-nutrients present in the donor organisms are not transferred to GM crops that do not normally express those toxin or anti-nutrient characteristics. This assurance is particularly important in cases where the GM crop is processed differently from a donor crop, 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 may not be necessary provided its accumulation is similar.
For proteins, the toxicological assessment is based on a weight-of-evidence that considers the following parameters:

(a) The concentration of the substance in the GM crop.
(b) Amino acid sequence similarity between the protein and known protein toxins. Above 40% sequence identity, two proteins can be considered homologous if they share common ancestry
(c) Digestibility, as commonly assessed using an *in vitro* pepsin digestion assay.
(d) Stability to heat or processing, where this can be measured (e.g., in the case of proteins with some enzymatic or measurable biological activity).
(e) 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 or rats), 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.

It is generally not necessary to test enzymes for toxicological endpoints when exposure occurs by the oral route because enzymes have never been shown to act directly as carcinogens, mutagens, teratogens or reproductive toxicants.

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 crop 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.

3.3.2 Assessment of Possible Allergenicity

The primary consideration in allergenicity assessment of a newly expressed novel protein in a food derived from a genetically modified crop is the prevention of unexpected exposure of sensitized individuals to food allergens. All newly expressed proteins in GM crops 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 protein.

The following types of information are considered first when assessing the allergenicity potential of a GM crop:

(a) **The source of the introduced gene**. Genes derived from known allergenic sources should be carefully assessed for their allergenicity potential unless scientific evidence demonstrates otherwise. Allergenic sources would be defined as those organisms for which reasonable evidence of Immunoglobulin E (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) **Whether the gene encodes a protein that is known to be an allergen, or whether the protein is sufficiently similar to an allergen to expect cross reactions.** This is determined by comparing the amino acid sequence of the novel protein with that of known allergenic proteins. 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) **Whether the protein is likely to sensitize and become an allergen.** This is evaluated by conducting a Pepsin digestion resistance assay and heat stability testing. 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.

3.3.2.1 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 GM product as test materials.

In the case of a newly expressed protein with allergenicity potential, 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.

3.3.2.2 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.

3.3.3 Compositional Analysis

For GM crops without purposefully altered nutritional properties, the compositional analysis is part of the weight-of-evidence approach for evaluating whether there were any unintended changes resulting from the genetic modification. Data should be provided on the levels of key nutrients and anti-nutrients present in the edible portions of the crop (e.g., seed or grain), including other crop 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 anti-nutrients for the crop species (e.g., those identified in international consensus documents on nutrient properties, where applicable).

Where there's no existing data, compositional analysis should be carried out from at least one confined field trial for three seasons; or from three CFTs for at least one season. Where there's already existing data generated in other countries under similar CFT conditions, this data may be considered as adequate. 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 crop varieties would be expected to be grown.
(b) 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.
(c) An adequate number of plants should be sampled and the methods of analysis need to be sufficiently sensitive and specific to detect variations in key components.

Comparisons should be made between the GM crop and its conventional counterpart (e.g., near-isogenic line), and considering the normal range of variation for the nutrient in other cultivated varieties of the crop (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., ash, carbohydrate, crude fat, crude protein, moisture)
(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 crop species.

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.

3.3.4 Intended Nutritional Modifications

Foods derived from GM crops 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 GM crop. 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 crop 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 crop constituents could change the overall nutrient profile of the crop 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 GM crop 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 GM crop 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 GM crops 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.

CHAPTER FOUR

4.0 REFERENCES

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APPENDICES

APPENDIX I: DOSSIER PREPARATION CHECKLISTS

NB: *The following checklists are provided to guide applicants on required information and prepare their environmental release dossiers for submission to NBA in respective sections in the application form. The checklists are an aid and are not a replacement for the full submission dossier, including supporting studies that contain the complete set of data required for the safety assessment.*

Checklist 1: Description of the GE Crop

- ☐ Identification of the crop
- ☐ Name of the transformation event(s)
- ☐ Pedigree map for each transformation event
- ☐ Purpose of the modification, sufficient to aid in understanding the nature of the food being submitted for safety assessment.

Checklist 2: Description of the Non-Transgenic Host Crop and its Use as Food

- ☐ Common or usual name; botanical name; and, taxonomic classification;
- ☐ History of cultivation and development through breeding, in particular identifying traits that may adversely impact on human health;
- ☐ Information on the host crop's genotype and phenotype relevant to its safety, including any known toxicity or allergenicity; and
- ☐ History of safe use for consumption as food.

Checklist 3: History of Safe Use and Dietary Exposure

- ☐ Information on how the crop is typically cultivated, transported and stored
- ☐ Information on special processing required to make the crop safe to eat
- ☐ The crop's normal role in the diet
- ☐ Part of the crop is used as a food source
- ☐ Is consumption of the crop important in particular subgroups of the population?
- ☐ What important macro- or micro-nutrients does the food contribute to the diet?

Checklist 4: Description of the Donor Organisms

For each donor organism, provide the following:

- ☐ Common name
- ☐ Scientific name
- ☐ Taxonomic classification
- ☐ Information about the natural history of the organism as concerns human health
- ☐ Information on naturally occurring toxins, anti-nutrients and allergens

- For donor microorganisms, additional information on human pathogenicity and the relationship to known human pathogens
- 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).

Checklist 5: Description of the Genetic Modification(s)

Provide:

- Information on the specific method used for the modification
- Description and characterization of all genetic material used to modify the crop, including the source (e.g., crop, microbial, viral, synthetic), identity and expected function in the crop
- Details of modifications introduced, intermediate and recipient genetic material (e.g., changes in amino acid sequence that may affect expression of the expressed protein)

Provide a summary diagram of all genetic components of the vector, including coding regions, and non-coding sequences of known function and for each genetic component include:

- A citation where these functional sequences are characterized.
- Indicate the portion and size of the sequence inserted.
- Indicate the location, order, and orientation in the vector.
- Indicate the function in the crop.
- Indicate the source (common and scientific and/or trade name, of the donor organism).
- Indicate if the genetic component is responsible for disease or injury to crops or other organisms and is a known toxicant, allergen, pathogenicity factor, or irritant.
- Indicate if the donor organism is responsible for any disease or injury to crops or other organisms, produces toxicants, allergens or irritants or whether closely related to organisms that do.
- Indicate if there is a history of safe use of the donor organism or components thereof, if available.

Checklist 6: Characterization of the Genetic Modification(s)

Information about the DNA insertion(s) into the crop genome is required, including:

- Characterization and description of the inserted genetic material.
- Number of insertion sites.
- Organisation of the inserted genetic material at each insertion site including copy number and data to demonstrate if complete or partial copies were inserted, and if the arrangement of the genetic material was conserved or if significant rearrangements have occurred upon integration.
- Sequence data of the inserted material and of the flanking regions bordering the site of insertion.
- Identification of any open reading frames within the inserted DNA or created by the insertions with contiguous crop genomic DNA including those that could result in fusion proteins.

For any expressed substances in the GE crop provide:

- The gene product(s) (e.g. a protein or an untranslated RNA);

- The gene product(s)' function;
- The phenotypic description of the new trait(s);
- The level and site of expression of the expressed gene product(s) in the crop, and the levels of its metabolites in the edible portions; and
- The amount of the target gene product(s), where possible, if the function of the expressed sequence(s)/gene(s) is to alter the accumulation of a specific endogenous mRNA or protein.

Information is required to demonstrate each of the following (where applicable):

- Deliberate modifications made to the amino acid sequence of the expressed protein result in changes in its post-translational modification or affect sites critical for its structure or function.
- The intended effect of the modification has been achieved and that all expressed traits are expressed and inherited in a manner that is stable through several generations consistent with laws of inheritance.
- The newly expressed trait(s) are expressed as expected in the appropriate tissues in a manner and at levels that are consistent with the associated regulatory sequences driving the expression of the corresponding gene.
- Evidence to suggest that one or several genes in the host crop has been affected by the transformation process.
- Confirm the identity and expression pattern of any new fusion proteins.

Checklist 7: Assessment of Possible Toxicity

- Indicate if the donor organism(s) is a known source of toxins.
- Amino acid sequence homology comparison of the newly expressed protein and known protein toxins and anti-nutrients.
- Demonstrate the susceptibility of each newly expressed protein to pepsin digestion.
- Where a host other than the transgenic crop is used to produce sufficient quantities of the newly expressed protein for toxicological analyses, demonstrate the structural, functional and biochemical equivalence of the non-crop expressed protein with the crop expressed protein.
- Results of the acute oral toxicity study completed for newly expressed proteins (that don't have, or are not closely related to proteins with a history of safe use).

Checklist 8: Assessment of Possible Allergenicity (Proteins)

- Indicate if the donor organism(s) is a known source of allergens (defined as those organisms for which reasonable evidence of IgE mediated oral, respiratory or contact allergy is available).
- Amino acid sequence homology comparison of the newly expressed protein and known allergens.
- Demonstrate the susceptibility of each newly expressed protein to pepsin digestion.
- Where a host other than the transgenic crop is used to produce sufficient quantities of the newly expressed protein for allergenistic analyses, demonstrate the use of non-

allergenic genes the structural, functional and biochemical equivalence of the non-crop expressed protein with the crop expressed protein.

- ☐ For those proteins that originate from a source known to be allergenic, or have sequence homology with a known allergen, testing in immunological assays is to be performed where sera are available.

Checklist 9: Compositional Analyses of Key Components

For all parts of the GE crop and its conventional counterpart that may be used as food in Kenya, provide the following:

- ☐ Proximates (i.e., ash, carbohydrate, crude fat, crude protein, moisture)
- ☐ Amino acids
- ☐ Fatty acids
- ☐ Vitamins
- ☐ Minerals
- ☐ Naturally occurring antinutrients (e.g., phytates, trypsin inhibitors, lectins, alpha-galactosides, etc)
- ☐ Predictable secondary metabolites or other physiologically active substances normally associated with the crop species