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NBMA

FEDERAL REPUBLIC
OF NIGERIA

NATIONAL BIOSAFETY GUIDELINES FOR RISK ASSESSMENT AND RISK MANAGEMENT OF GENETICALLY MODIFIED PLANTS WITH STACKED GENES

August, 2022

National Biosafety Management Agency

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ARRANGEMENT OF GUIDELINES

INTRODUCTION

Gene Stacking is the combination of two or more genes of interest into an organism. The combined traits resulting from this process is referred to as stacked traits. Gene stacking can be achieved either through conventional breeding or molecular technique. Conventional breeding techniques can be employed to combine traits from two or more GM events. These events from the use of conventional techniques are sometimes referred to as "Breeding Stacks". It does not involve insertion of new DNA sequence into the genome and may not be regarded as a new GM Event. However, an individual event may contain two or more transgenes stacked through molecular techniques, and these events are referred to as "Molecular Stacks".

GM plants containing stacked genes, are increasingly being adopted due to their ability to offer multiple benefits in a single product. For example, they have provided improved seeds with combinations of insect resistance and herbicide tolerance genes. These stacks can also be referred to as "pyramids" when they are characterized by multiple genes controlling a trait such as pest resistance.

Nigeria's biosafety regulatory process in line with section 31(1) of the National Biosafety Management Agency Act 2015 (as Amended) ("the Act"), states that:

> *"Every applicant seeking approval for any genetically modified organism under this Act shall prior to the submission of the application, carry out a mandatory risk assessment of the potential risk the genetically modified organisms pose to human health, animal, plant or the environment in Nigeria".*

Furthermore, section 33 states that:

> *"Every person, institution or body that carries out any activity relating to genetically modified organism shall develop and maintain a risk management plan and strategy in accordance with the provisions of the Fourth Schedule to this Act".*

Risk assessment is to identify risks from plausible sets of circumstances that may result in harm to human health, animal, plant or the environment, and estimating the level of risk on the basis of the seriousness and likelihood of harm. Risk management on the other hand, evaluates, selects and implements plans or actions to ensure risks are appropriately managed.

The NBMA Act, 2015 (as Amended) defines Genetically Modified Organisms (GMO) as any organism, living or non-living that possesses a novel combination of genetic material obtained through the use of modern biotechnology. In view of this, a GM Event is a GMO resulting from the insertion of recombinant DNA into the plant genome as a result of a single transformation process, whereas a "Molecular Stack" is a GMO containing two or more transgenes resulting from its transformation process. Finally, a "Breeding Stack" is a GMO containing combined traits from two or more GMOs resulting from conventional breeding techniques.

Therefore, Molecular Stacks and Breeding Stacks are GMOs which under this Act, require risk assessment and risk management plan. However, unlike Molecular Stacks which will result in new events, GM stacked products produced via conventional breeding do not result in a new event(s). In other words, events that are stacked through conventional or traditional breeding typically have undergone regulatory assessments by regulators either locally or globally. Thus, in consideration of the processes leading to the development of these stacks, biosafety regulations for risk assessment and risk management will be applied on a case-by-case basis.

Considering that Molecular Stacks result in new events which have to undergo the regulatory process as a new GMO, these guidelines will emphasize the following possible scenarios involving GM Breeding Stacks:

i. Stacking of GM Events through conventional/ traditional breeding where the single events **have been** approved by NBMA,
ii. Stacking of GM Events through conventional/ traditional breeding where one or more of the single events have not been approved by NBMA,
iii. Stacking of GM Events through conventional/ traditional breeding where **none** of the single events have been approved by NBMA.

Accordingly, these guidelines are for applicants wishing to carry out GMO stacking and the release of Stacked GMOs in Nigeria.

This document, which stems from the above consideration, was developed in consultation with line Ministries, Departments and Agencies in Nigeria, as well as with other relevant institutions and individual experts.

In exercise of the powers conferred on it by section 41 (3) (b) of the National Biosafety Management Agency Act, 2015 (as amended) ("the Act") and of all other powers enabling it in that behalf, the National Biosafety Management Agency ("the Agency") provides the following guidelines:

PART I - OBJECTIVE

1. Objective:

The objective of these guidelines is to provide guidance and information on the risk assessment and risk management requirements and procedures for plants with Genetically Modified (GM) stacked events through traditional breeding or molecular

techniques.

2. Application and Scope:
These Guidelines apply to every person, institution or body wishing to carry out GM stacking or release of GM stacked events within Nigeria ranging from containment, confined field trials, multi-locational trials and general release, except for pharmaceutical processes and products, which are not covered by the Act. Imports intended for direct use as food, feed, or for processing will be covered under existing guidelines and regulations.

PART II - DETERMINATION OF THE RISK ASSESSMENT PROCESS FOR GM PLANTS WITH STACKED EVENTS

A. Breeding Stacks

3. Stacking of GM events through conventional breeding where the events have been approved by the NBMA

(1) Where GM events are stacked using conventional/ traditional breeding, and all the single events that make up the higher order stacks are already approved events in Nigeria, the risk assessment **shall** be limited to:
   a. an assessment of the intactness and stability of inserted genetic elements;
   b. an assessment of potential interactions between combined events and the resulting phenotypic traits in the case that the potential for interactions cannot be excluded based on the individual modes of action of the respective GM Events;
   c. an assessment of potential interactions of the stacked events on the conservation and sustainable use of biological diversity in the likely potential receiving environment, also taking into account risks to human health, depending on the scope of the application and in case the potential for interactions cannot be excluded based on the individual modes of action of the respective GM Events.

(2) An applicant under this provision shall make a simple notification to the NBMA with all relevant information for review and decision making.
(3) Other additional and key considerations for various categories of breeding stacks are summarized in the Table provided in Annexure II of these Guidelines.

4. Stacking of GM events through conventional breeding where one or more of the single events have not been approved by the NBMA

(1) Where GM events are stacked using conventional or traditional breeding, and any of the single events constituting the stack do not have biosafety approval, the applicant shall conduct a risk assessment on all the unapproved single events.
(2) The applicant under this provision shall submit a complete dossier for the unapproved events in relevant application formats to the NBMA for risk assessment review.
(3) A simultaneous review process will be adopted for the unapproved single event and the higher order stack (final stacked product) to ensure seamless decision making.
(4) The outcome of the risk assessment of the higher order stack will also cover the intermediates (or sub-stacks) and their singles, where applicable. Consequently, where the higher order stacks and the singles are found to be safe following a risk assessment review, the intermediates will also be considered to be safe.
(5) In these Guidelines, risk analysis process shall be as provided in -
   a) 'a', 'b' and 'c' of Guideline 3(1), and;
   b) risk assessment, risk management and risk communication in Part VIII of the Act; and Annexure I of these Guidelines.
(6) Other additional and key considerations for various categories of Breeding Stacks are summarized in the Table provided in Annexure II of these Guidelines

5. Stacking of GM events through conventional breeding where none of the single events have been approved by the NBMA

(1) Where GM events are stacked using conventional or traditional breeding, and all the single events constituting this stack do not have biosafety approval, the application will be treated as new, requiring a risk assessment on all the single GM events included in the higher order breeding stack.
(2) Under this provision, the applicant shall be required to submit a complete dossier for the single events included in the higher order breeding stack in relevant application formats to the NBMA for risk assessment review.
(3) A simultaneous review process will be adopted for the single events and the higher order stack to ensure seamless decision making.
(4) The outcome of the risk assessment of the higher order stack will also cover the intermediates and their singles, where applicable. Consequently, where the higher order stacks and the singles are found to be safe following a risk assessment review, the intermediates will also be considered to be safe.
(5) In these Guidelines, full risk analysis process shall be as provided in -
   a) a', 'b' and 'c' of Guideline 3(1), and
   b) risk assessment, risk management and risk communication as provided in Part VIII the Act; and Annexure I of these Guidelines).
(6) Other additional and key considerations for various categories of Breeding Stacks are summarized in the Table provided in Annexure II of these Guidelines.

B. Molecular Stacks

6. Molecular Stacks

The development of stacked events using molecular techniques could be carried out through re-transformation, co-transformation or using multi-gene cassettes. Whichever method is used, the following parameters need to be assessed prior to release-

(a) Assessment of the Intactness of the Inserted Loci and Genotypic Stability

The following should be provided by prospective applicants:

(i) information on the genetic stability of the transgenic locus (loci); phenotypic stability and inheritance pattern of the introduced genes
(ii) information showing that the integrity of the genes is retained in the GMO (plant),
(iii) source of the genetic material, the sampling design and the number of plants used for the analysis. When analyzing the inheritance pattern, appropriate statistical methods should be applied, and
(iv) a comparison between the structure of the inserts in stacked traits and that of the insert present in the corresponding single events.

(b) Assessment of Potential Interactions

Where GM plants contain stacked events for food, feed, import or processing, the main objective of the analysis is to assess the potential for any interactions between the events which may raise safety concerns (if there is a potential for gene or gene product interactions specific to the modes of action of the associated single GM event, or depending on the outcome of an individual risk assessment). If newly expressed proteins are present in the GM plant, their levels should be compared, in the same field trials, with the levels in any set of GM plants that have been previously assessed by the NBMA. Applicants will therefore be required to provide the information on the function of the nucleic acid region intended for insertion. This information should comprise the following elements:

(i) complete sequence of the nucleic acid intended to be inserted, including information on the donor organism and any deliberate alteration to the corresponding sequence in the donor organism,
(ii) history of safe use of the gene product arising from the regions intended for insertion, and
(iii) data on the possible relationship of the gene product with known toxins, anti-nutrients and allergens.

(c) Assessment of Effects of Stacked Transgenic Events on the Conservation and Sustainable Use of Biological Diversity

For instances of deliberate release into the environment, the objective of the analysis is to assess the potential interactions of the stacked events on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking into account risks to human health. An Applicant is therefore to provide information on the following:

(i) phenotypic characteristics, including the modified traits, compared to the parent GMOs and to relevant non-modified recipient organisms (plants),
(ii) compositional analysis, in accordance with the OECD guidelines and guideline 5 of the NBMA (National Biosafety Guidelines on Genetically Modified Food and Feed, 2021),
(iii) additional information depending on the nature of the combined traits. For example, further toxicological analysis of the stacked event may be required to address any synergistic effects arising from the stacking of two or more traits that result in a broadened target range or increased toxicity, and
(iv) the level of expression of any introduced genes or modified traits, compared to the parent GMOs.

(d) Compositional Assessment Using Substantial Equivalence

The provisions of Guideline 5 of the National Biosafety Guidelines for Genetically Modified Food and Feed, 2021, shall apply.

C. GM Stacks for use as Food, Feed or for Processing

GM stacked events imported as part of a consignment for direct use as food, feed or for processing (and not for cultivation) would be regulated in accordance with existing Guidelines for the Importation of Genetically Modified Organisms for Food, Feed or for Processing.

D. Decision making on GM events stacked through conventional/traditional breeding

Once the single events and highest order stacks are approved by NBMA, any other sub-combinations of those events will require a notification to the NBMA for approval for use as a parental line or for commercialization with the exception of sub-combinations that develop through natural segregation in cultivated fields of approved higher order stacks.

PART III - FOOD AND FEED SAFETY ASSESSMENT

7. Case-by-case assessment

(1) The general provisions of assessment shall comply with Guideline 5 of the National Biosafety Guidelines on Genetically Modified Food and Feed, 2021.
(2) A case-by-case assessment of the stacked events shall be carried out to ascertain the nutritional composition as well as food and feed safety, for

possible toxicity and allergenicity.

PART IV - ENVIRONMENTAL RISK ASSESSMENT

8. Environmental Risk Assessment

(1) Environmental Risk Assessment (ERA) shall be carried out, case-by-case in line with the provisions of Part VIII (Third Schedule) of the Act.

(2) The ERA shall consider the evaluation of the individual events and where applicable, additional data from molecular characterization and comparative compositional analysis of the stacked events when determining potential interactions between genes or between gene products.

(3) The ERA shall evaluate any interactions between the stacked events which could result in modified environmental effects of the GM plant. The combination of transgenes may result in changes in expression levels which may lead to a significant biological impact that may need to be assessed. However, it should be noted that expression levels may vary significantly also in the individual events.

(4) In addition to the provisions of Part VIII (Third Schedule) of the NBMA Act, 2015 (as amended), the following guidelines shall apply-

(a) Invasiveness and selective advantage or disadvantage

Comparison between plants containing the stacked events and the most appropriate comparators during one growing season and multiple geographical locations representative of the various environments in which the GM plants will be cultivated are necessary. Additional field data may be required if changes are observed in behavior, fitness, reproduction, dissemination, and others.

(b) Interactions between the stacked events and target organisms

To identify and evaluate possible altered efficacy of biocidal gene products to target organisms in the stacked events as compared to the individual events, the potential impact on target organisms shall be assessed in two seasons multi-locational field trials initially. If biologically relevant changes are observed, additional studies might be required.

(c) Potential impact and interactions between the stacked events and non-target organisms

Stacked biocidal events may have different effects on non-target organisms when compared with the individual events. Therefore, there is a need to focus on changes in sensitivity of non-target organisms and specificity of biocidal gene products. To test the hypothesis that such combined events do not interact, a tiered approach would be adopted starting with an assessment of the individual modes of action. Where relevant, confined

studies are conducted with minimum data from two seasons field trials.

PART V - IMPACTS OF SPECIFIC CULTIVATION, MANAGEMENT AND HARVESTING TECHNIQUES

9. Evaluation of differences in the specific cultivation, management and harvesting techniques

The specific cultivation, management and harvesting techniques of plants containing the stacked events, and any environmental impacts, should be evaluated and, where appropriate, supported by relevant data.

PART VI - ENVIRONMENTAL MONITORING PLAN

10. General Surveillance

(1) Applicants are required to put in place a plan for General Surveillance that would be designed to identify the aspects of the environment that need to be protected from harm.

(2) Applicants may adopt different approaches for surveillance which includes but not limited to -

(a) monitoring of stacked events, the process, and their cultivation sites by using appropriate survey instruments as determined by the Agency; and

(b) utilization of data collected by existing monitoring networks

(3) Applicants should carry out monitoring and evaluation of risk for a specified period equivalent to the life cycle of the relevant species.

PART VII - MISCELLANEOUS

11. DEFINITION OF TERMS

In these guidelines –

"Act" means the National Biosafety Management Agency (NBMA) Act, 2015 (as Amended).

"Applicant" means any person, institution, body or their authorized representative who applies for a Biosafety Permit under these guidelines.

"Biocides": a chemical substance or micro-organism intended to destroy, deter, render harmless or exert a controlling effect on any harmful organism by chemical or biological means.

"Biocidal Product": Any substance or mixture, in the form in which it was supplied to the user, consisting of, containing or generating one or more active substances, with the intention of destroying, deterring, rendering harmless or otherwise exerting a controlling effect on, any harmful organisms by any means other than mere physical

or mechanical action.

"Breeding Stacks": means a GMO containing combined traits from two or more GMOs resulting from conventional breeding techniques.

"Conventional Breeding" involves identifying parent plants with desirable characteristics to create favorable combinations in the next generation.

"Co-transformation" means transformation with two or more independent transgenes.

"DNA": means deoxyribonucleic acid.

"Event" means a genotype produced from the transformation of a specie using a specific genetic construct.

"Gene Pyramiding": can be defined as the occurrence of two or more genes that may be different in sequence but direct the synthesis of the same protein.

"Gene Stacking": means the combination of two or more genes of interest in the genome of a single plant.

"Higher Order Stack": refers to the desired final product that will eventually go to the end users.

"Intermediary": means a combination of two or more GM events within a higher order stack.

"Molecular Stacks": means a plant transformed by using molecular methods, where two or more traits are simultaneously (Co-Transformation) or sequentially (Re-transformation) introduced into a host plant by standard delivery systems such as Agrobacterium-mediated and biolistic methods.

"Parental line": means a plant of a desirable trait that is crossed with another plant.

"Parental event": means individual GM events with prior regulatory approval.

"Stacking": means processes that involve combining genes (at least two novel genes) of interest into a single plant line through conventional breeding or molecular techniques.

"Stacked Traits": means multiple traits in a single variety of crop.

"Stacked Events": The creation of a genetically modified organism (GMO) with more than one genetic modification. This can be done by (a) cross-breeding two GMOs with each having one or more pre-existing modifications (b) carrying out a second genetic modification in an existing GMO or (c) introducing multiple genes or traits at once.

"Sub-Stack": means a combination of two or more GM events within a higher order stack.

"Synergistic Effect": means an interaction of elements that when combined produce a total effect that is greater than the sum of the effect of the individual elements.

"Transformation": the specific process where exogenous genetic material is directly taken up and incorporated by a cell through its cell membrane.

"Re-transformation": means the use of modern biotechnology to produce a GMO where the recipient plant is already a GMO i.e. a plant harboring a transgene is transformed with other transgenes.

Annexure I Guidelines 4 (5) (b), 5(5) (b)

PART A Assessment of the intactness and stability of inserted genetic elements

Rationale

It is important to confirm the presence and structure of the transgenes in the stacked events and their inheritance, to appropriately assess possible adverse effects on the conservation and sustainable use of biological diversity in the likely potential receiving environment and of potential adverse effects on human health.

The requirement is to establish that each of the events stacked in the plant has the same molecular properties and characteristics as in the individual events separately. Comparisons between the insert structures in the original events and the GM stacks should be carried out on materials representative of those designed for commercial production that will enter the environment and the food or feed chain.

To assess intactness, applicants are required to use appropriate molecular approaches such as Southern Blot and Polymerase Chain Reaction (PCR) analyses. The applicant should also ensure that probe-restriction enzyme combinations used are sufficient to prove intactness and stability of the insert as well as the flanking regions.

PART B Assessment of potential interactions between combined events and the resulting phenotypic effects

Rationale

The combination of two or more transgene events in one GMO may impact the expression level of individual transgenes and promote interactions between expressed products of the different transgenes.

Thus, in addition to information about the characteristics of the single transgenic events, specific information about the potential for interactions between the stacked genes or modified traits in stacked GMO shall be considered. For example, it should be determined whether the different transgenes affect the same biochemical pathways or physiological processes, or are expected to or may have any combinatorial, antagonistic or synergistic effects that may result in potential for new or increased adverse effects relative to the parent GMOs.

The requirement is to determine the potential for any interactions between the stacked events which could impact on human or animal health and the environment. At the DNA level this would include, for example, assessing possibilities for gene silencing. Analysis of the levels of newly expressed proteins in GM stacks is also required. Stability of protein expression and phenotype should be assessed on materials representative of those designed for commercial production.

PART C Assessment of effects of stacked transgenic genes on the conservation and sustainable use of biological diversity

Rationale

Assessment of interactions is based on the environmental risk assessment data for the stacked GMO. The assessment will be in comparison with the closely related non-modified recipient species and the parent GMOs in the likely receiving environment, taking into consideration the results of the genotypic and phenotypic assessments. If by interaction a new adverse effect is identified, additional supporting data may be required which may include but not limited to:

i. Phenotypic characteristics, including the modified traits, compared to the parent GMOs and to relevant non-modified recipient organisms (plants)
ii. Compositional analysis, including the levels of toxins, allergens or anti-nutritional factors known to be present in the parent GMOs or non-modified recipients
iii. Additional information depending on the nature of the combined traits. For example, further toxicological analysis of the stacked GMO may be required to address any synergistic effects arising from the stacking of two or more traits that result in a broadened target range or increased toxicity; and
iv. Level of expression of any introduced genes or modified traits, compared to the parent

PART D History of safe use of the final stacked product

Previous approvals on the products and the countries of approval should be stated. History of safe use of the products in those countries should be indicated.

Annexure II Guidelines 3 (3) 4(6), 5(6)

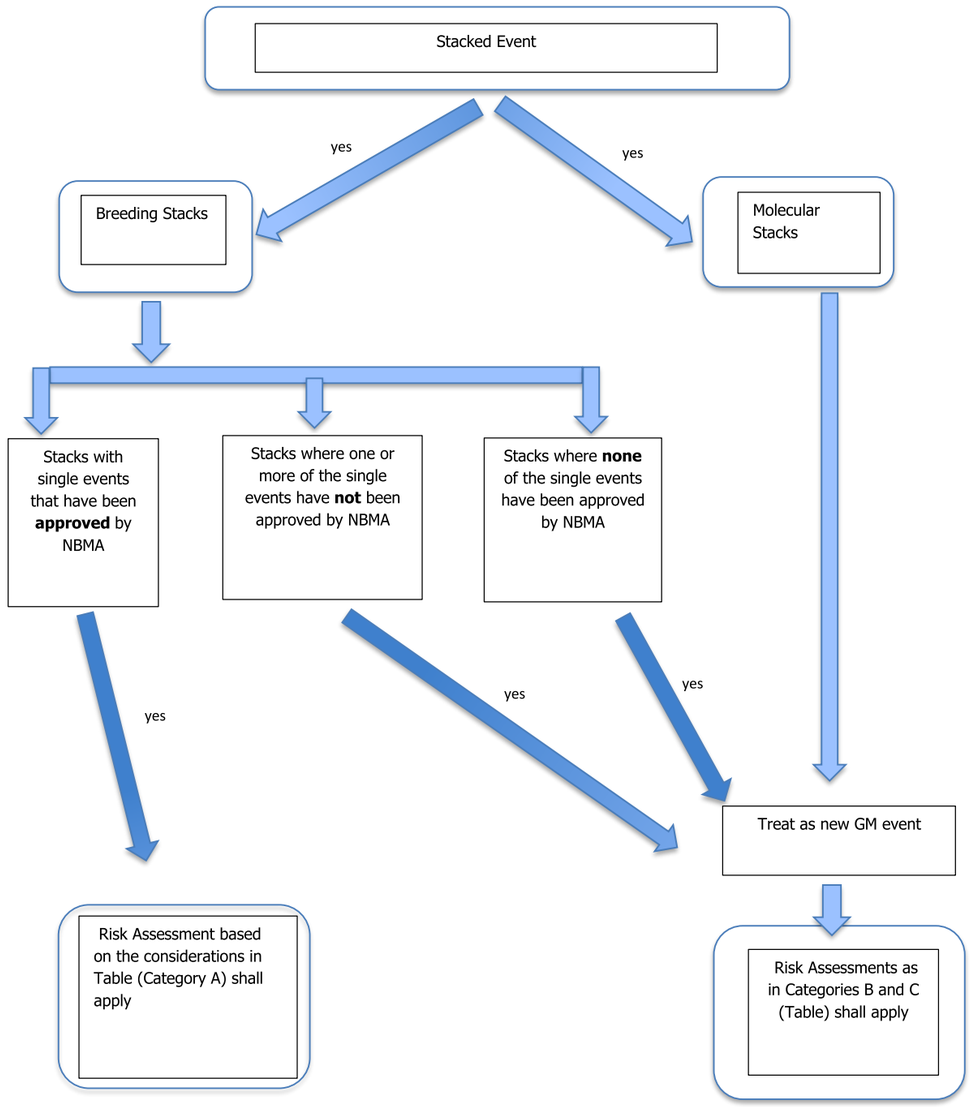
Additional considerations are summarized in the Table below.

TABLE: Key Considerations for various categories of Breeding Stacks

Category		Considerations
A	Stacking of GM Events through conventional breeding where the candidate single events have been approved by the NBMA	o Applicant to ensure notification in writing to the NBMA with information including: o Assessment of the intactness and stability of inserted genetic elements o Depending on the scope of the application, and in case the potential for interactions cannot be excluded based on the individual modes of action of the respective GM events, an assessment of potential interactions between combined events, the resulting phenotypic traits, and effects of stacked transgenic traits on the conservation and sustainable use of biological diversity in the potential receiving environment, taking into account risks to human health, shall be conducted. o Risk assessment conducted on the higher order GM events stacked through conventional breeding to also cover intermediates or sub-stacks
B	Stacking of GM Events through conventional breeding where one or more of the candidate single events have not been approved by the NBMA	Complete dossier to be submitted for the unapproved single events and the higher order stack in the prescribed format to the NBMA for risk assessment review Risk Assessment to be conducted on all unapproved single events Assessment of the intactness and stability of inserted gene Depending on the scope of the application, and in case the potential for interactions cannot be excluded based on the individual modes of action of the respective GM events, an assessment of potential interactions between combined events, the resulting phenotypic traits, and effects of stacked transgenic traits on the conservation and sustainable use of biological diversity in the potential receiving environment, taking into account risks to human health, shall be conducted. A simultaneous review process to be adopted for unapproved single events and the higher order stack to ensure seamless decision making The outcome of risk assessment conducted on the higher order GM event stacked through conventional breeding to also cover intermediates (sub-stacks) and singles where applicable for purposes of decision making CFT data will only be required for the combination product(s) that the applicant intends to make commercially available to end users and in accordance with existing guidelines. Where the applicant intends to commercialize any sub-combination other than the ones approved, supplemental efficacy data may be requested, as appropriate.
C	Stacking of GM Events through conventional breeding where none of the candidate single events have been approved by the NBMA	A new application in the prescribed format Complete dossier to be submitted for all single events and the higher order stack in the prescribed format to the NBMA for risk assessment Risk assessment review to be conducted on all single events and the higher order stack Assessment of the intactness and stability of inserted gene Depending on the scope of the application, and in case the potential for interactions cannot be excluded based on the individual modes of action of the respective GM events, an assessment of potential interactions between combined events, the resulting phenotypic traits, and effects of stacked transgenic traits on the conservation and sustainable use of biological diversity in the potential receiving environment, taking into account risks to human health, shall be conducted. A simultaneous review process to be adopted for unapproved single events and the higher order stack to ensure seamless decision making The outcome of risks assessment conducted on higher order GM event stacked through conventional breeding to also cover intermediates or sub-stacks and singles, where applicable, for purposes of decision making CFT data will only be required for the combination product(s) that the applicant intends to make commercially available to end users and in accordance with existing guidelines. Where required, modalities for supplemental using data transportability or bridging may be explored.

Annexure III

Stacked Event Flow Chart.



EXPLANATORY NOTE

(This note does not form part of these Guidelines, but it is intended to explain its purport)

These guidelines seek to guide an applicant wishing to carry out GMO stacking and the release of Stacked GMOs in Nigeria.

NOTE