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Principles and practice of risk Assessment in Biosafety

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Safety and Risk

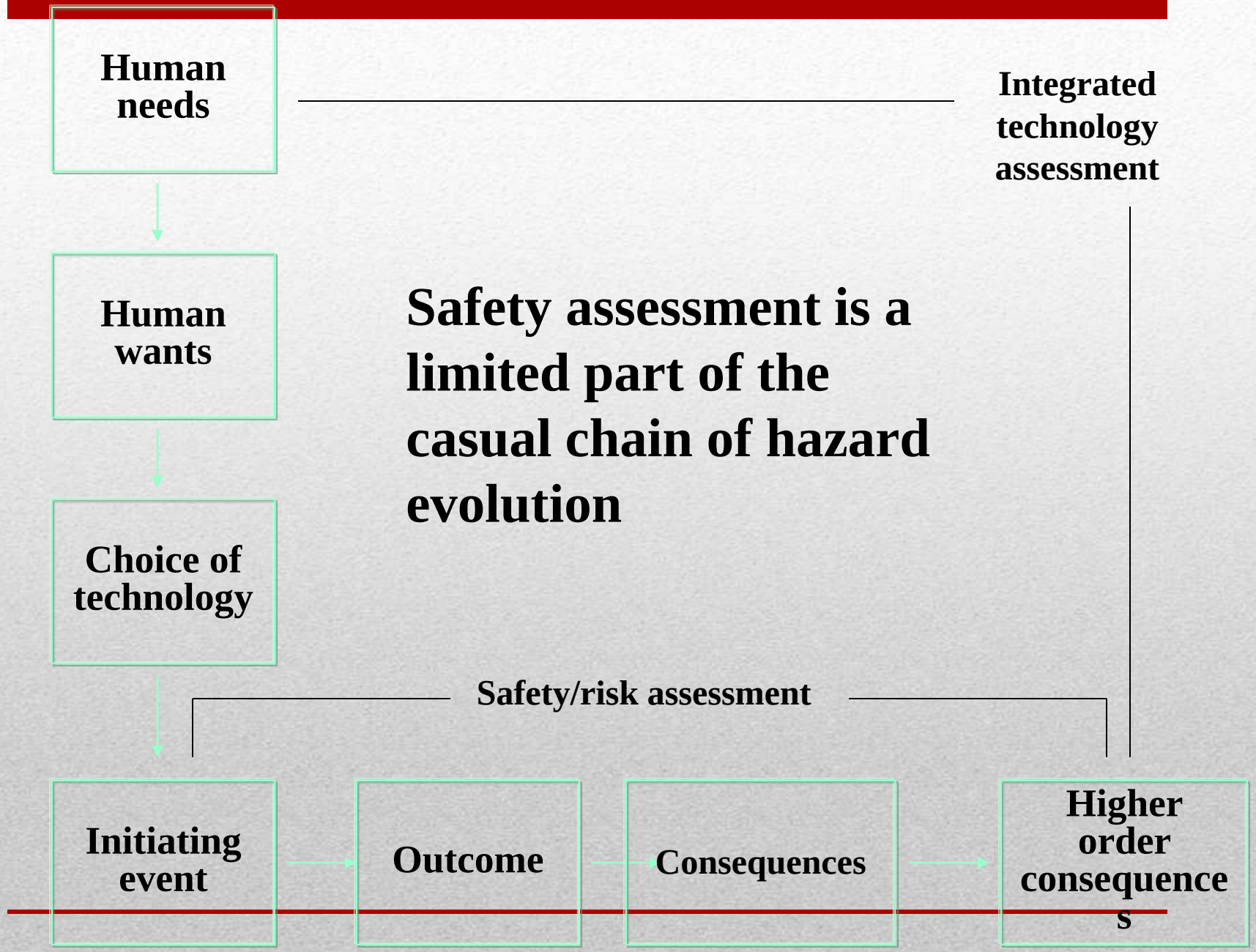
- "**Safe**" and "**Safety**" are ideal concepts which, while desirable, are unattainable in absolute terms.
- Planning for safety
- Practical planning for safety is difficult as safety cannot be measured directly.
- Practical planning for safety is performed by evaluating its opposite, namely, **risk**.

What is Safety?

Safety is an exercise in recognizing what the risks are and then introducing

- procedures,
- practices,
- equipment, and
- facilities

to control the identified risks or reduce them to acceptable levels.


Human needs
Human wants
Choice of technology
Initiating event
Outcome
Consequences
Higher order consequences
Integrated technology assessment
Safety/risk assessment
Safety assessment is a limited part of the casual chain of hazard evolution

Biological risks

Basic Concepts

Hazards and Risks

- **Hazard** is a characteristic that, in particular circumstances, could lead to harm.
  - The negative or undesired impact of an event is commonly referred to as ’**hazard**‘.
- **Risk** is the chance, in quantitative terms, of harm occurring from a defined hazard,
  - the expression "risk of (a specific event or set of events)" must refer to the hazard.

Hazards and Risks

- **Harm** occurs when a hazard is realized and damage is done to a human being (or other organisms or to a population or to the environment).
- **Damage** is the loss of inherent quality suffered by an entity (physical or biological).

Risk Analysis

A process consisting of three components

Risk assessment,
Risk management and
Risk communication.

Risk analysis

- risk analysis involves a scientific process to estimate the risks to human life and health, as well as the impact on the environment, associated with the use of a particular GMO or its products.
- The prevention, reduction or elimination of these risks requires methods of risk management that are normally implemented as actions conforming to particular regulations. Risk assessment and risk management have to be implemented along with risk communication,

Risk Assessment

A scientifically based process for decision making consisting of :

- (i) hazard identification,
- (ii) hazard characterization,
- (iii) exposure assessment,
- (iv) risk characterization.

The identification of biological, chemical, and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Hazard Identification

The qualitative and/or quantitative evaluation of the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food.

Hazard Characterization

The determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response).

Dose-Response Assessment

The qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via exposures from any relevant source

Exposure Assessment

Risk Characterization

The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population ***based on hazard identification, hazard characterization and exposure assessment***

Risk can be defined thus: Risk = f (Hazard, Exposure)

The process of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options.

Risk Management

The interactive exchange of information and opinions throughout the risk analysis process concerning *hazards and risks, risk-related factors and risk perceptions,* among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions.

Risk Communication

Vulnerability

Weakness in the information system

- Makes it possible for a hazard to occur.
- Increases probability of a hazard.
- Increases damage.

From Hazard to Risk

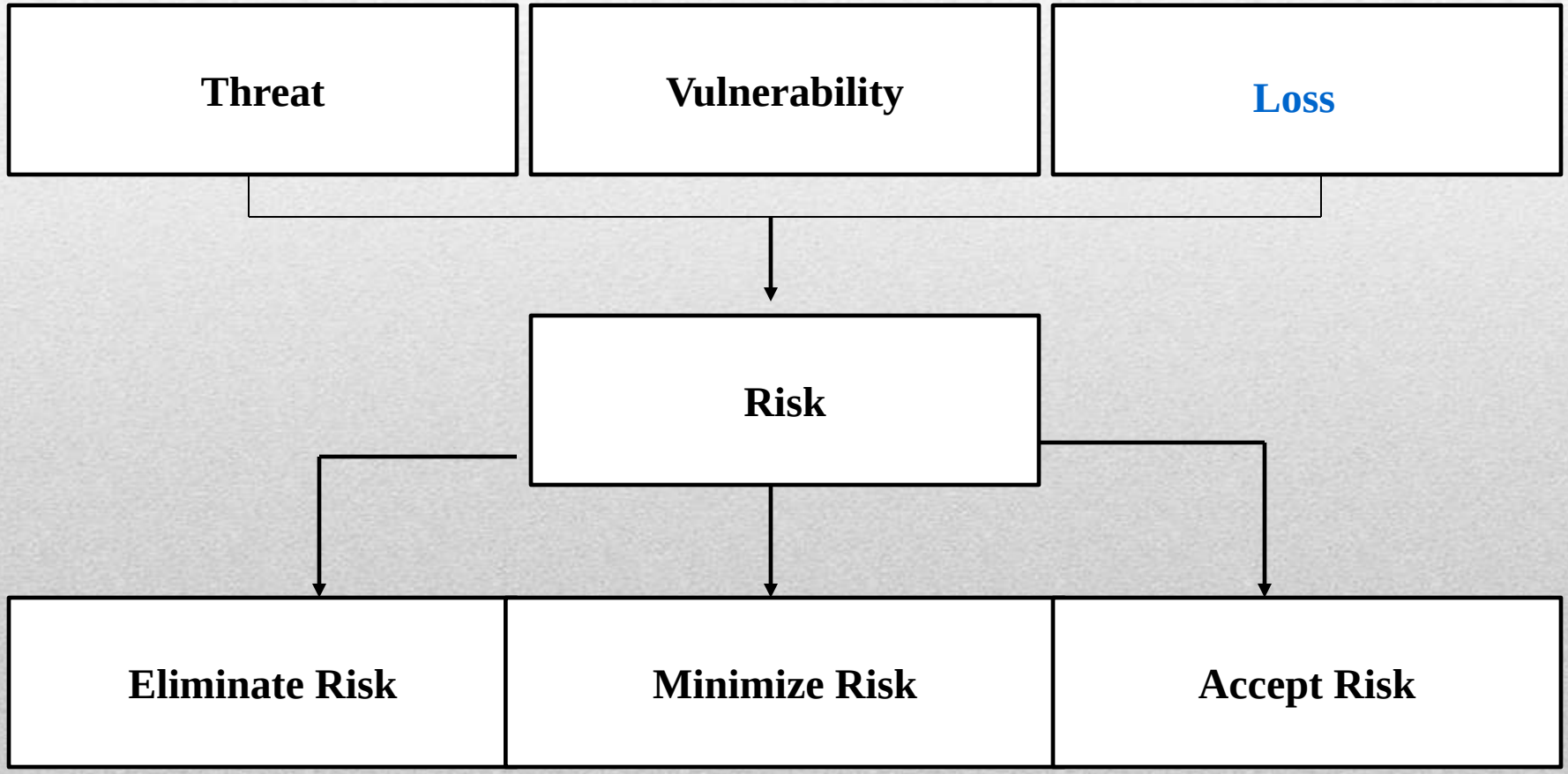


Table 1. Indicative direct and indirect risks arising from the GM phenotype, the introduced trait or changes in agricultural practice (after Tzotzos *et al.*, 2009)

Risk source	Potential risk	Mechanism
GM phenotype	Evolution of increased weediness (direct)	Sexual transfer of crop alleles to wild relatives; seed dispersal
GM phenotype	Loss of biodiversity in the wild (indirect)	Extinction by hybridization. Indirectly, from the intensification of agriculture
GM trait	Harm to non-target organisms (direct)	Toxicity. Starvation through reduction of food resources
GM trait	Evolution of resistance in the targeted pathogen, pest or weed population (direct)	Selection pressure from transgene products (e.g. Bt toxin) or application of agricultural input (e.g. herbicide)
Change in agricultural practice	Loss of agricultural biodiversity (indirect)	Increased use of chemical inputs

Risk hypothesis

RA of GMO- basic principles

- RA - To assess if the introduction of a GMO into the environment would have adverse effects on human and animal health or the environment.
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- RA of a GMO involves generating, collecting and assessing information on the GMO to determine its potential adverse impact relative to its non-GM counterpart or comparator

RA of GMO- basic principles

- RA should be carried out in a scientifically sound and transparent manner.
- It should include any relevant data (e.g. research data, scientific publications, monitoring reports) obtained prior to and/or during the risk assessment process. and should include the use of models
- The final risk evaluation should result in qualitative and if possible quantitative conclusions on the risks
- Any uncertainties associated with the identified risks should also be outlined.

RA of GMO- basic principles

- Should be on a case-by-case basis as required information may vary:
- according to the species of GM plants concerned,
- the introduced genes,
- their intended use/s
- the potential receiving environment/s,
- specific cultivation requirements
- the presence of other GM plants in the vicinity

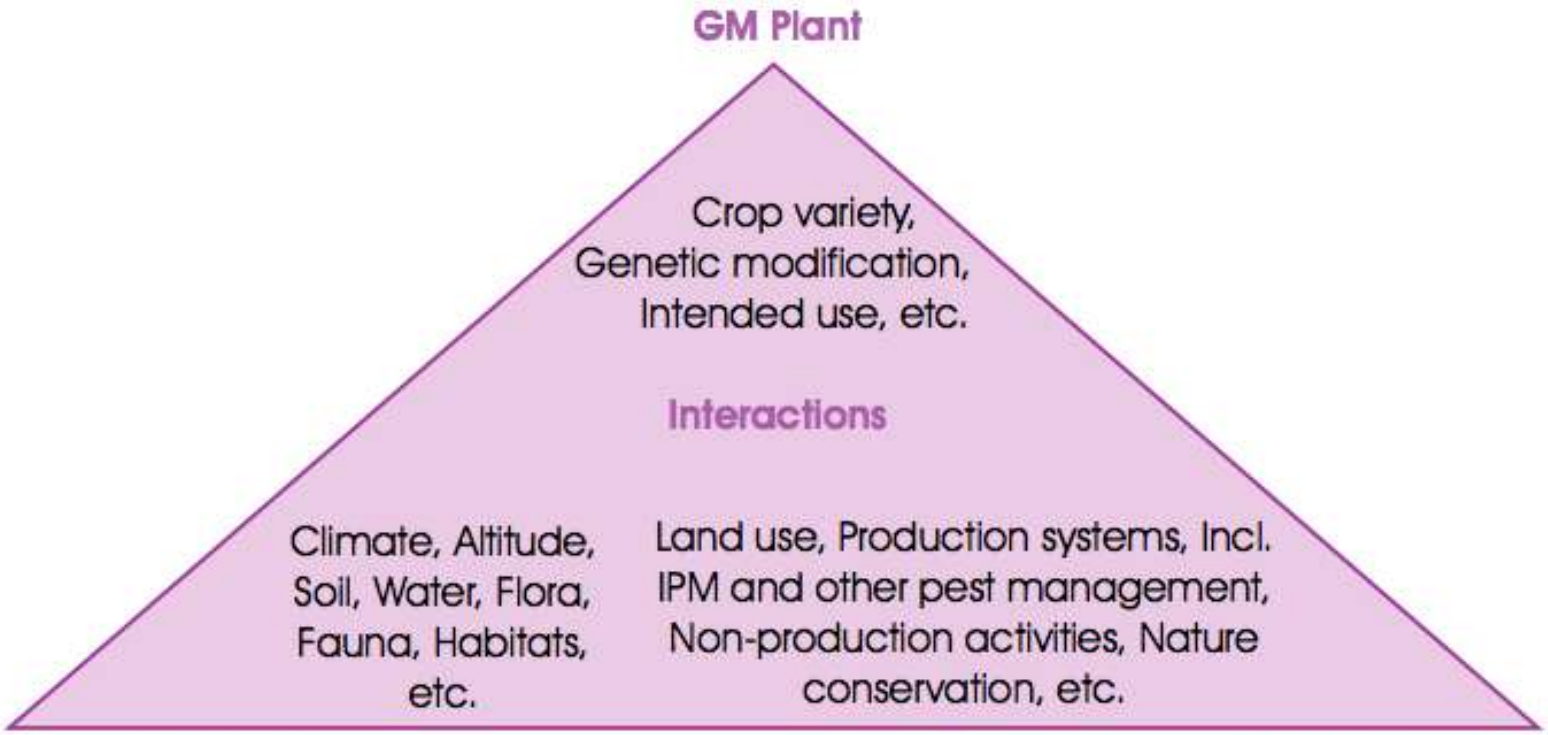
- (1) To identify characteristics of the GM plant and, where appropriate, the associated production and management systems capable of causing potential adverse effects to the environment;
- (2) To identify the potential adverse effects linked to those harmful characteristics;
- (3) To identify exposure pathways through which the GM plant may adversely affect the environment;
- (4) To define assessment endpoints
- (5)To define measurement endpoints that can be used to assess the potential harm to the assessment endpoints defined;
- (6) To formulate testable hypotheses that are clearly phrased and easily transferable to data to be generated or evaluated;
- (7) To set the limits of concern for each measurement endpoint

RA process for GMO

Intended vs unintended

- Intended effects are those that are designed to occur and which fulfill the original objectives of the genetic modification. Alterations to the phenotype may be identified through a comparative analysis of growth performance, yield, pest and disease resistance, *etc*.

- Unintended effects of genetic modification are those which are consistent (non-transient) differences between the GM plant and its appropriate comparator, which go beyond the primary intended effect/s of introducing the transgene



Source: EFSA (2010)

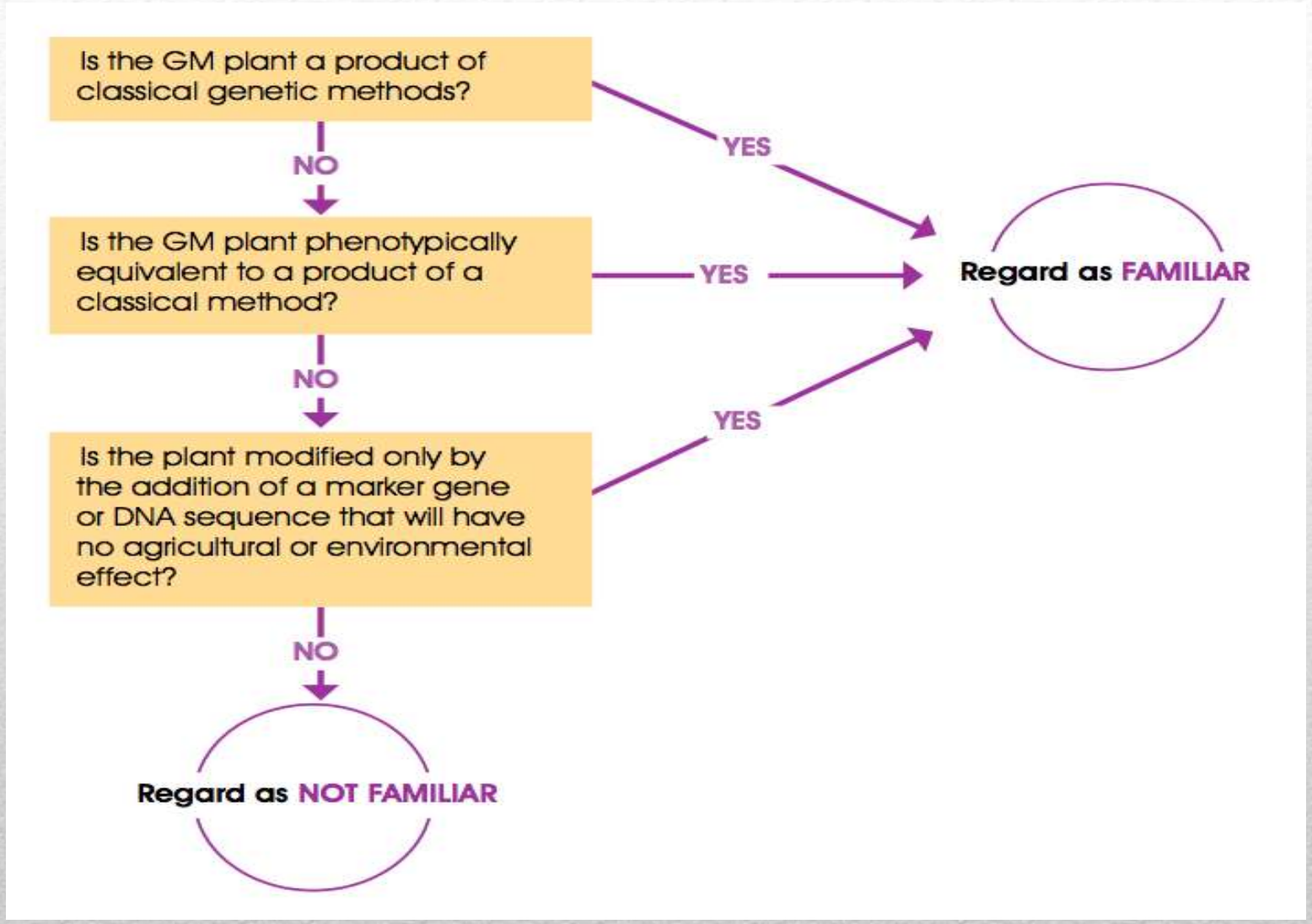
Figure 4. *The receiving environment/s made up of three components that can interact*

Receiving environment

Scenarios for RA

- .

- Invasiveness
- Plant to Plant
- Plant to microorganism
- GM to target organism
- GM to Non target organism
- the environmental impact of the specific management and production systems (*e.g*. agriculture, forest tree or others) associated with the GM plant, includ


Is the GM plant a product of classical genetic methods?
YES
NO
Is the GM plant phenotypically equivalent to a product of a classical method?
YES
NO
Is the plant modified only by the addition of a marker gene or DNA sequence that will have no agricultural or environmental effect?
YES
NO
Regard as FAMILIAR
Regard as NOT FAMILIAR

the environmental impact of the specific management and production systems (*e.g.* agriculture, forest tree or others) associated with the GM plant,

- The production system is defined by the specific use of the GM plant, the context in which the GM plant is grown, its cultivation (including crop rotation), management and harvesting, and the crop type in which the transgenic trait/s has been introduced.

- *For example, grain maize, forage maize and sweet corn have different production systems with different environmental impacts in similar receiving environment/s.*
- *All may receive the same GM event but the subsequent changes in management and production systems, and, consequently, the resulting environmental impacts may differ.*

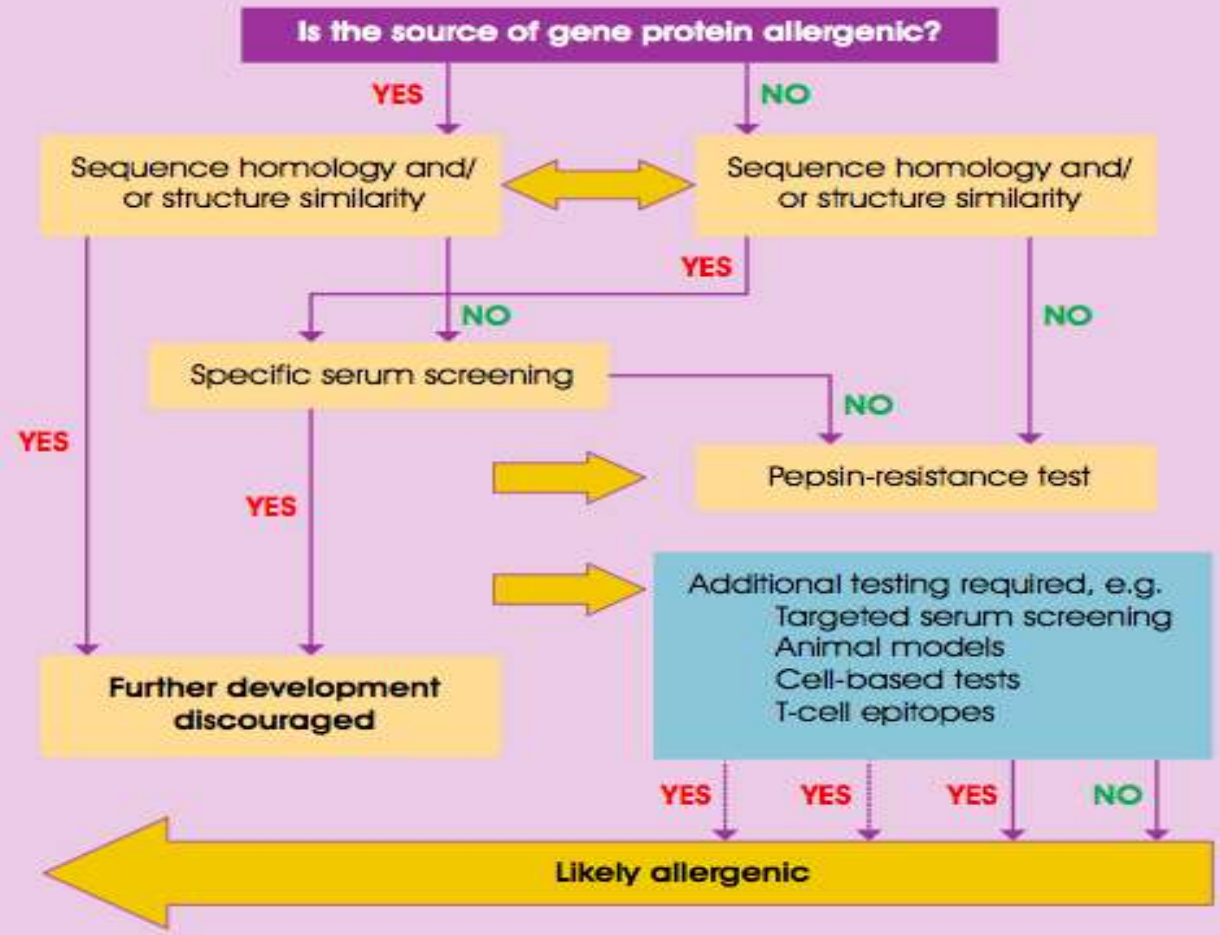
Production systems

RA on human & animal health

- GM technology is now widely used in the production of such crops as corn, soybean, rice, canola, tomato, brinjal and papaya used as human food, or as animal feed
- In the framework of GMO risk assessment, an assessment is required to determine whether the GM plant and its products present a new hazard for human and animal health.

- the nature of the introduced protein/s and its potential effects on humans and animals, and
- whether the phenotype of the GM plant has been significantly altered

Figure 8. Flow chart for assessing allergenicity of a novel protein in a GM plant.



Note: **YES** *indicates potential allergenicity, with fainter shades of red indicate less potential (after Davies, 2005)*

Information required

- Risk management plan
- Emergency response plan
- Post market surveillance and monitoring
- Data handling and record keeping
- Reporting of unexpected events

After RA

Summary of RA process for GMO

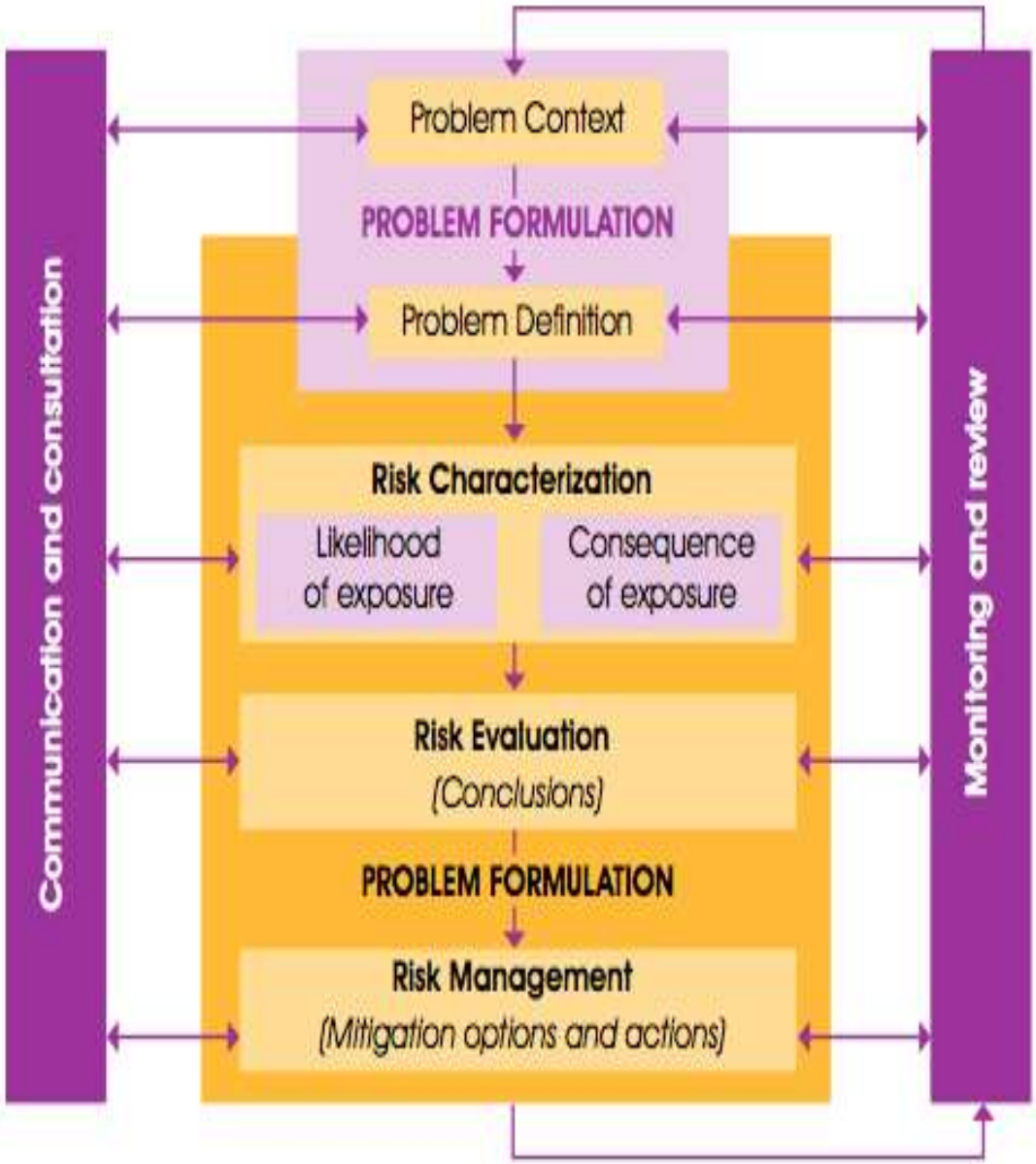
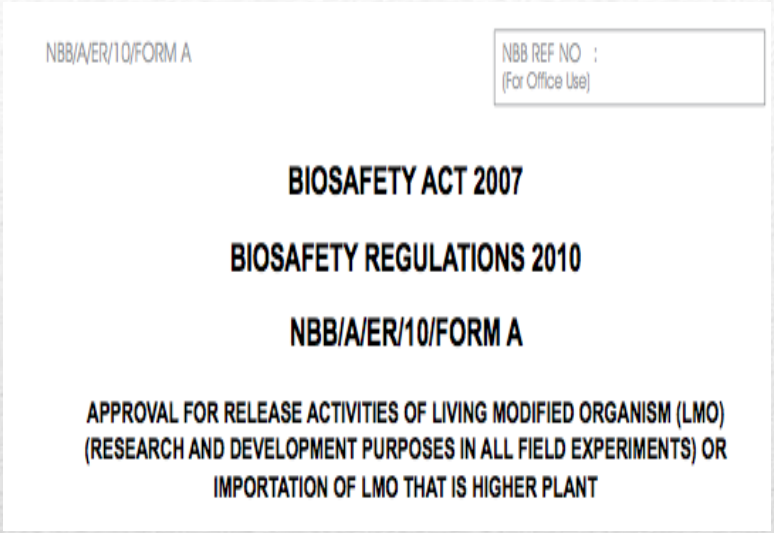


Figure 9. Process flow for risk assessment (after Wolf et al., 2010)

RA in the National Biosafety Act 2007



NBB/A/ER/10/FORM A

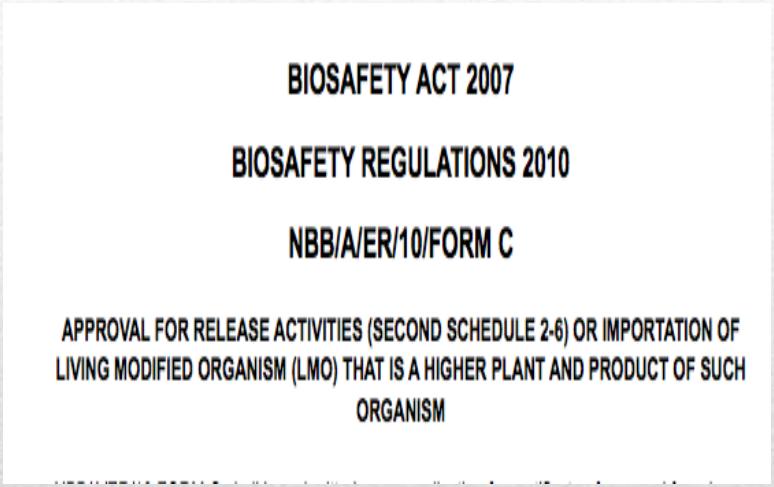
NBB REF NO :
(For Office Use)

BIOSAFETY ACT 2007

BIOSAFETY REGULATIONS 2010

NBB/A/ER/10/FORM A

APPROVAL FOR RELEASE ACTIVITIES OF LIVING MODIFIED ORGANISM (LMO) (RESEARCH AND DEVELOPMENT PURPOSES IN ALL FIELD EXPERIMENTS) OR IMPORTATION OF LMO THAT IS HIGHER PLANT



BIOSAFETY ACT 2007

BIOSAFETY REGULATIONS 2010

NBB/A/ER/10/FORM C

APPROVAL FOR RELEASE ACTIVITIES (SECOND SCHEDULE 2-6) OR IMPORTATION OF LIVING MODIFIED ORGANISM (LMO) THAT IS A HIGHER PLANT AND PRODUCT OF SUCH ORGANISM

RA is required for release activities

What information is required by the biosafety regulations?

Environmental risk assessment for GMOs

Characterization of the Recipient Organism

- Scientific name, common, strain and cultivar names
- Taxonomy, phenotypic and genetic markers
- Description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts
- Potential for genetic transfer and exchange with other organisms

Characterization of the Donor Sequence (s)

- Scientific name, common or trade name
- If the genetic component transferred is responsible for disease or injury to plants or other organisms, and is a known toxicant, allergen, pathogenicity factor, or irritant
- If the donor sequence is responsible for any disease or injury to plants or other organisms, produces toxicants, allergens or irritants or is related to sequences that do.

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Characterization of the Donor Sequence(s)

- If there is a safe history of safe use of the source sequence or components thereof
- If there is a significant modification that affects the amino acid sequence of genes designed to be expressed in the plant, provide the citation.
  - If the modified amino acid sequence has been published a relevant citation is sufficient.
  - If not, the complete sequence highlighting the modifications should be submitted.
  - Modifications that affect only a few amino acids can simply be stated without providing the complete sequence.

Transformation Method

- **Description of the transformation method**
  - Describe and provide references for the transformation method, e.g. *Agrobacterium* mediated transformation or direct transformation by methods such as particle bombardment, electroporation, PEG transformation of protoplasts, etc.

Transformation Method

- **Description of the transformation method**
  - For direct transformation methods, describe the nature and source of any carrier DNA used.
  - For *Agrobacterium*-mediated transformation, provide the strain designation of the *Agrobacterium* used during the transformation process, and indicate how the Ti plasmid based vector was disarmed, and whether *Agrobacterium was* cleared from the transformed tissue.

Transformation Method

- For transformation systems other than *Agrobacterium*, provide the following information:
  - Does the system utilize a pathogenic organism or nucleic acid sequences from a pathogen?
  - How were any pathogenesis-related sequences removed prior to transformation?
  - Did the transformation process involve the use of helper plasmids or a mixture of plasmids? If so, describe these in detail.

Transformation Vector

- Nature and source of the vector used
- Full characterization of the genetic construct. Submitted information should include description of coding regions, and non-coding sequences of known function as well as citations where these functional sequences were described, isolated, and characterized (publicly available database citations may be used)

Transformation Vector

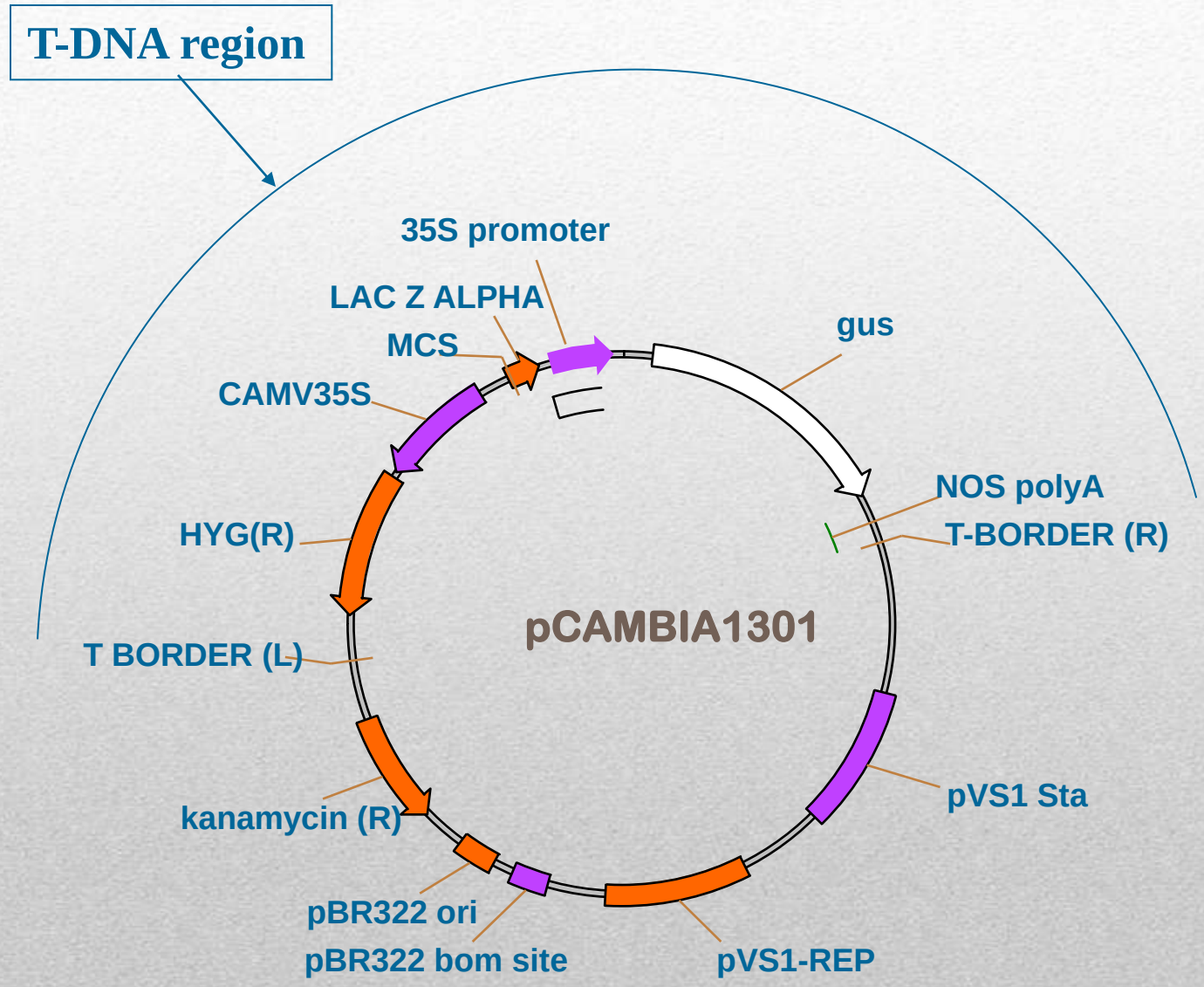
- **Coding regions: information requirements for risk assessment**
  - Coding regions may include truncated sense constructs, sequences engineered to be nontranslatable, antisense constructs, and constructs containing ribozymes, regardless of whether or not the coding region is designed or expected to be expressed in the transgenic plant.
  - Information may also be required indicating the number of copies which have been inserted, including integration of partial copies; and for allopolyploid plants, information indicating into which parental genome insertion has occurred.

Transformation Vector

- **Non-coding regions: information requirments for risk assessment**
  - DNA analysis may be necessary for introns, leader sequences, terminators, and enhancers of plant-expressible cassettes.
  - DNA analysis may be necessary for promoters and other regulatory regions associated with bacteria-expressible cassettes.
  - For noncoding regions which have no known plant function and are not associated with expression of coding regions:
  - DNA analysis maybe required for some sequences of known function (e.g., *ori V* and *ori322*, *bom*, T-DNA borders of *Agrobacterium*, and bacterial transposable elements).
  - DNA analysis is not required for any remaining sequences of the plasmid backbone.

Transformation Vector

- Detailed map of the vector with the location of all introduced sequences. The map is required for the analysis of data supporting the characterization of the DNA, including as appropriate the location of restriction sites and/or primers used for PCR and regions used as probes.
- The degree to which the vector contains only DNA sequences required to perform the intended function


T-DNA region
35S promoter
LAC Z ALPHA
MCS
gus
CAMV35S
NOS polyA
T-BORDER (R)
HYG(R)
pCAMBIA1301
T BORDER (L)
pVS1 Sta
kanamycin (R)
pBR322 ori
pBR322 bom site
pVS1-REP

Characterization of the Genetically Modified Organism

- Description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed
- Structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism
- Stability of the organism in terms of genetic traits.
  - In the case of plants which are infertile or vegetatively propagated, there should be data demonstrating that the transgene trait is stably maintained and expressed during vegetative propagation over a number of cycles appropriate to the crop.

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Characterization of the Genetically Modified Organism

- Activity of the expressed protein(s).
- Description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector.
- Sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.
- History of previous releases or uses of the GMO.

health considerations:

- (i) toxic or allergenic effects of the non-viable GMOs and/or their metabolic products.
- (ii) product hazards.
- iii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity.
- (iv) capacity for colonization.

Characterization of the Genetically Modified Organism

if the organism is pathogenic to humans who are immuno-competent:

- **diseases caused and mechanism of pathogenicity including invasiveness.**
- **Communicability.**
- **infective dose.**
- **host range, possibility of alteration.**

Characterization of the Genetically Modified Organism

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- possibility of survival outside of human host.
- presence of vectors or means of dissemination.
- biological stability.
- antibiotic-resistance patterns.
- Allergenicity.
- availability of appropriate therapies.

Characterization of the Genetically Modified Organism

Conditions of Release and Receiving Environment

- **General information on the release**
  - Description of the purpose of the proposed field trial, including type of data to be collected.
  - Location description.
  - Dates of field trials.
  - Description of seeding, spraying (if any), monitoring and harvesting practices.
  - Post-release treatment of the site.
  - Techniques foreseen for elimination or inactivation of the GMOs and any resulting seeds at the end of the trial.
  - Description of any previous releases of the GMOs, especially at different scales and in different ecosystems.

Conditions of Release and Receiving Environment

- **General information on the environment (both on the site and in the wider environment)**
  - Geographical location of the site(s).
  - Physical or biological proximity to natural ecosystems or protected areas.
  - Proximity of related wild and cultivated plant species.
  - Climatic characteristics of the region(s) likely to be affected .
  - Existence of endangered species near the trial site.
  - Description of local livestock and migratory species.
  - Description of target and non-target organisms likely to be affected and effects on these organisms.

Interactions Between GMOs and the Environment

- **General characteristics affecting survival, multiplication and dissemination**
  - Biological features which affect survival, multiplication and dispersal.
  - Known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.).
  - Sensitivity to specific agents.

Interactions Between GMOs and the Environment

- **General interactions with the environment:**
- predicted habitat of the GMOs
- studies of the behavior and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses
- genetic transfer capability
  - (a) post-release transfer of genetic material from GMOs into organisms in affected ecosystems
  - (b) post-release transfer of genetic material from indigenous organisms to the GMOs

Interactions Between GMOs and the Environment

- **General interactions with the environment:**
- likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism
- measures employed to ensure and to verify genetic stability
  - description of genetic traits which may prevent or minimize dispersal of genetic material
  - methods to verify genetic stability
- routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.
- description of ecosystems to which the GMOs could be disseminated

Interactions Between GMOs and the Environment

- **Potential environmental impact:**
  - potential for excessive population increase in the environment
  - competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s)
  - identification and description of the target organisms
  - anticipated mechanism and result of interaction between the released GMOs and the target organism
  - identification and description of non-target organisms which may be affected unwittingly

Interactions Between GMOs and the Environment

- **Potential environmental impact:**
  - likelihood of post-release shifts in biological interactions or in host range
  - known or predicted effects on non-target organisms in the environment, impact on population levels of competitors: prey, hosts, symbionts, predators, parasites and pathogens
  - known or predicted involvement in biogeochemical processes
  - other potentially significant interactions with the environment
  - changes in farming practices

Monitoring, Control, Waste Treatment and Emergency Response Plans

- **A. Monitoring techniques:**
  - Methods for tracing the GMOs, and for monitoring their effects.
  - Specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.
  - Techniques for detecting transfer of the donated genetic material to other.
  - Duration and frequency of the monitoring .

Monitoring, Control, Waste Treatment and Emergency Response Plans

- **B. Control of the release:**
  - Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of release or the designated area for use.
  - Methods and procedures to protect the site from intrusion by unauthorized individuals.
  - Methods and procedures to prevent other organisms from entering the site.

C. Waste treatment:

- Type of waste generated
- Expected amount of waste
- Possible risks
- Description of treatment envisaged

Monitoring, Control, Waste Treatment and Emergency Response Plans

- **D. Emergency response plans:**
  - Methods and procedures for controlling the GMOs in case of unexpected spread.
  - M
  - ethods for decontamination of the areas affected, e.g. eradication of the GMOs.
  - Methods for disposal or sanitation of plants, soils, etc. that were exposed during or after the spread.
  - Methods for the isolation of the area affected by the spread.
  - Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.
  - Plans for delaying or managing the development of resistance in susceptible pests to pesticides or herbicides.

Monitoring, Control, Waste Treatment and Emergency Response Plans

Goals of Biosafety Procedures – GMO context

- Define regulatory authority to prevent the development and/or importation of potentially dangerous GMOs
- Anticipate detrimental effects that might follow the release of a GMO during experimentation or commercialization.
- Design monitoring systems for the early detection of adverse outcomes.
- Plan intervention strategies to avert and, if necessary, remediate adverse environmental or health effects,
- Encourage continued development of effective biosafety principles and procedures,
- Provide public information about biosafety, including such information become part of school curricula

Thank you