CBD

UNEP

Convention on Biological Diversity

Distr.
GENERAL

UNEP/CBD/BS/AHTEG-RA&RM/4/2
25 April 2012

ENGLISH ONLY

AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT UNDER THE CARTAGENA PROTOCOL ON BIOSAFETY
Fourth meeting
Montreal, 4 - 8 June 2011

# GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

*(Revised on 2 April 2012)*

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## PREFACE

In accordance with the precautionary approach[1] the objective of the Protocol is "to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, specifically focusing on transboundary movements".[2] For this purpose, Parties shall ensure that *risk assessments* are carried out to assist in the process of making informed decisions regarding living modified organisms (LMOs).

According to Article 15 of the Protocol, risk assessments shall be carried out in a scientifically sound manner and be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of LMOs on the conservation and sustainable use of biological diversity, taking also into account risks to human health.[3]

Four general principles of risk assessment are specified in Annex III of the Protocol:

- "Risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations".
- "Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk".
- "Risks associated with living modified organisms or products thereof should be considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment".
- "Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the LMO concerned, its intended use and the likely potential receiving environment".

This document was developed by the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management, with input from the Open-ended Online Expert Forum, in accordance with terms of reference set out by the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety (COP-MOP) in its decisions BS-IV/11 and BS-V/12 in response to an identified need for further guidance on risk assessment of LMOs.[4] It is intended to be a "living document" that will be updated and improved as appropriate and when mandated by the Parties to the Cartagena Protocol on Biosafety.

[1] "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation" (Principle 15 of the Rio Declaration on Environment and Development) at: (http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=78&ArticleID=1163), and in line with Articles 10.6 and 11.8 of the Protocol.

[2] http://bch.cbd.int/protocol/text/article.shtml?a=cpb-01.

[3] Article 15, paragraph 1.

[4] The Open-ended Online Expert Forum and the AHTEG on Risk Assessment and Risk Management were established by the COP-MOP in decision BS-IV/11. These groups were extended by the COP-MOP in decision BS-V/12. The terms of reference for these groups may be found in the annexes to decisions BS-IV/11 and BS-V/12 (http://bch.cbd.int/protocol/decisions/decision.shtml?decisionID=11690, http://bch.cbd.int/protocol/decisions/decision.shtml?decisionID=12325).

## OBJECTIVE AND SCOPE OF THIS GUIDANCE

The objective of this Guidance is "to provide a reference that may assist Parties and other Governments in implementing the provisions of the Protocol with regards to risk assessment, in particular its Annex III and, as such, this Guidance is not prescriptive and does not impose any obligations upon the Parties".[5]

This Guidance consists of two parts. In Part I, the Roadmap for Risk Assessment of LMOs is presented. In Part II, specific guidance is provided on the risk assessment of specific types of LMOs and traits. The topics contained in Part II were identified and prioritized by the Open-ended Online Expert Forum and the AHTEG in accordance with the terms of reference in decisions BS-IV/11 and BS-V/12, taking into account the need of Parties for additional guidance.

# PART I:
# ROADMAP FOR RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

## BACKGROUND

This "Roadmap" provides guidance on assessing environmental risks of living modified organisms (LMOs),[6] taking into account risks to human health, consistent with the Cartagena Protocol on Biosafety (hereinafter "the Protocol") and in particular with its Article 15 and Annex III.[7] Accordingly, this Roadmap complements Annex III and national biosafety policies and legislations. Specifically, the Roadmap facilitates and enhances the effective use of Annex III by elaborating on the steps and points to consider in environmental risk assessment and by directing users to relevant background materials. The Roadmap may be useful as a reference for risk assessors when conducting or reviewing risk assessments and as a training tool in capacity-building activities.

This Roadmap provides information that is broadly relevant to the risk assessment of all types of LMOs and their intended uses within the scope and objective of the Protocol. However, it has been developed based largely on living modified (LM) crop plants because the experience to date with environmental risk assessments of LMOs has been mainly gained from these organisms.[8]

The Roadmap may be applied to all types of environmental releases of LMOs, including those of limited duration and scale as well as large-scale releases. Nevertheless, the amount and type of information available and needed to support risk assessments of the different types of intentional release into the environment may vary from case to case.

## INTRODUCTION

According to the Protocol, risk assessment of LMOs is a structured process conducted in a scientifically sound and transparent manner, and on a *case-by-case* basis in relation to the likely potential receiving environment. Its purpose is to identify and evaluate the potential adverse effects of LMOs, and their *likelihood* and *consequences* as well as to make a recommendation as to whether or not the risks are acceptable or manageable. Risk assessments serve as an input for decision-making regarding LMOs. This Roadmap describes an integrated risk assessment process in three sub-sections: "Overarching Issues in the Risk Assessment Process", "Planning Phase of the Risk Assessment", and "Conducting the Risk Assessment".

The potential effects caused by an LMO may vary depending on the characteristics of the LMO, on how the LMO is used, and on the environment exposed to the LMO. The effects may be intended or

[5] Decision BS-V/12.

[6] Including products thereof, as described in paragraph 5 of Annex III to the Protocol.

[7] Article 15 (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-15) and Annex III (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-43).

[8] Decisions on LMOs may be found, *inter alia*, in the BCH (http://bch.cbd.int) and links to national and intergovernmental websites relevant for this purpose.

*unintended*, and may be considered beneficial, neutral or adverse depending on the impact on a *protection goal*.

What is considered an adverse effect as well as an "acceptable risk" depends on protection goals and *assessment endpoints*. The choice of protection goals may be informed by the Party`s national policies and legislation as well Annex 1 of the Convention on Biological Diversity as relevant to the Party responsible for conducting the risk assessment.

The Roadmap includes five steps drawn from Annex III that describe a tiered process in which the results of one step are relevant to other steps. Importantly, the steps of a risk assessment may need to be conducted in an iterative manner, where certain steps may be repeated or re-examined when new information arises or a change in circumstances has occurred that could change its conclusions. Similarly, issues included in the 'Establishing the context and scope' section below may be taken into consideration while conducting the risk assessment and again at the end of the risk assessment process to determine whether the objectives and criteria set out at the beginning of the risk assessment have been addressed.

Ultimately, the concluding recommendations derived from the risk assessment are taken into account in the decision-making process for an LMO. In the decision-making process, in accordance with the country's policies and protection goals, other Articles of the Protocol or other relevant issues may also be taken into account and are listed in the last paragraph of this Roadmap: 'Related Issues'.

The risk assessment process according to the Roadmap is illustrated in Figure 1.

*›› See references relevant to "Introduction":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#introduction

## OVERARCHING ISSUES IN THE RISK ASSESSMENT PROCESS

This section gives guidance on issues that are relevant to all the steps of the risk assessment. It focuses on provisions related to the quality and relevance of information to be considered in the risk assessment, as well as the means to identify and describe uncertainties that may arise.

### Quality and relevance of information

An important question in a risk assessment is whether the information presented is of sufficient quality and relevance to characterize the risk posed by the LMO.

A number of issues should be considered to ensure the quality and relevance of the information used as well as the outcome of the risk assessment. For example:

- Criteria for the quality of scientific information.
    - Data of acceptable scientific quality should be used in the risk assessment. Data quality should be consistent with the accepted practices of scientific evidence-gathering and reporting and may include independent review of the methods and designs of studies.
    - Appropriate statistical methods should be used to strengthen the scientific conclusions of a risk assessment and, where appropriate, be described in the risk assessment report. Risk assessments frequently use data generated from multiple scientific fields, which may be divergent or even contradictory;
    - Reporting of data and methods should be sufficiently detailed and transparent to allow independent verification and reproduction. This would include ensuring the accessibility of data used by the risk assessors (e.g., the availability of relevant data or information and, if requested and as appropriate, sample material), taking into account the provisions of Article 21 of the Protocol on the confidentiality of information;

- The relevance of information for the risk assessment
    - Data may be considered relevant if they are linked to protection goals or assessment endpoints, contribute to the identification and evaluation of the potential adverse effects of the LMO, or if they can affect the outcome of the risk assessment or the decision.
    - Relevant data may be derived from a variety of sources such as new experimental data, data from relevant peer reviewed scientific literature, as well as data and experience from previous risk assessments, regarded as of acceptable scientific quality, in particular for the same or similar LMOs introduced in similar receiving environments.[9]
    - Information from national and international standards and guidelines may be used in the risk assessment, as well as knowledge and experience of farmers, growers, scientists, regulatory officials, and indigenous and local communities depending on the type of LMO;
    - The process of risk assessment may give rise to the need for further relevant information about specific subjects, which may be identified and requested during the assessment process, while on the other hand information on other subjects may not be relevant in some instances.[10]
    - The information that is relevant to perform a risk assessment will vary from case to case depending on the nature of the modification of the LMO, on its intended use, and on the scale and duration of the environmental introduction. In cases of environmental releases whose objective is to generate information for further risk assessments and where *exposure* of the environment to the LMO is limited, such as for some early-stage experimental releases and trials, less information may be available or required when performing the risk assessment. The uncertainty resulting from the limited information available in such cases may be addressed by risk management and monitoring measures.
    - To the extent possible, impartial experts with relevant background in the different scientific disciplines should be involved in conducting or providing inputs to risk assessments**.** Experts should not be biased or improperly impaired by interests that could be affected by the assessment in which they participate.

## Identification and consideration of uncertainty

Uncertainty is an inherent and integral element of scientific analysis and risk assessment. According to the Protocol, "where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies or monitoring the living modified organism in the receiving environment".[11] The Protocol also states that "lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the living modified organism in question (…), in order to avoid or minimize such potential adverse effects".[12] Whether and to what extent there is scientific uncertainty is therefore critical in the context of precautionary action. There is no internationally agreed definition of "scientific uncertainty", nor are there internationally agreed general rules or guidelines to determine its occurrence. The issue of uncertainty is

[9] Risk assessments can be found, *inter alia*, in the BCH (http://bch.cbd.int) and ICGEB (http://rasm.icgeb.org).

[10] Annex III, paragraphs 6 and 7.

[11] Annex III, paragraph 8 (f).

[12] Article 10, paragraph 6.

dealt with – sometimes differently – in each international instrument incorporating precautionary measures.[13]

Considerations of uncertainty strengthen the scientific validity of a risk assessment. An analysis of uncertainty includes considerations of its source and nature, and focuses on uncertainties that can have a significant impact on the conclusions of the risk assessment.

For each identified uncertainty, the *nature* of the uncertainty may be described as arising from: (i) lack of information, (ii) incomplete knowledge, and (iii) biological or experimental variability, for example, due to inherent heterogeneity in the population being studied or to variations in the analytical assays. Uncertainty resulting from lack of information includes, for example, information that is missing and data that is imprecise or inaccurate (e.g., due to study designs, model systems and analytical methods used to generate, evaluate and analyze the information).

In some cases more information will not necessarily contribute to a better understanding of potential adverse effects, therefore risk assessors should look to ensure that any further information requested will contribute to better evaluations of the risk(s). Although uncertainties originating from lack of information may be reduced by further research, uncertainties arising from incomplete knowledge or from inherent variability may be irreducible. In such cases, instead of reducing uncertainty, the provision of additional information may actually give rise to new uncertainties.

As such, the various forms of uncertainty should be considered and described in each step of the risk assessment. In addition, when communicating the results of a risk assessment, it is important to describe, quantitatively or qualitatively, what impact uncertainty may have on the estimated level of risk and on the conclusions and recommendations of the risk assessment.

In cases where the nature of the uncertainty implies that it cannot be addressed through the provision of more data during the risk assessment, where necessary, it may be dealt with by *risk management* and/or monitoring in accordance with paragraphs 8(e) and 8(f) of Annex III (see step 5).

*›› See references relevant to "Identification and consideration of uncertainty":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#uncertainty

## PLANNING PHASE OF THE RISK ASSESSMENT

### Establishing the context and scope

Risk assessments are carried out on a case-by-case basis, in relation to the likely potential receiving environment. Each risk assessment starts by establishing its context and scope in a way that is consistent with the country's protection goals, assessment endpoints, *risk thresholds*, *management strategies* and policies.

Establishing the context and scope for a risk assessment in line with the country's policies and regulations may involve an information-sharing and consultation process with risk assessors, decision-makers and various stakeholders prior to conducting the actual risk assessment, to identify protection goals, assessment endpoints and risk thresholds relevant to the assessment. It may also involve identifying questions to be asked that are relevant to the case being considered. The risk assessors should, at the outset of the process, have knowledge of national requirements for risk assessment and criteria for acceptability of risks. They may also use questions or checklists designed for the case under consideration to assist in the subsequent steps.

Several points may be taken into consideration, as appropriate, that are specific to the Party involved[14] and to the particular risk assessment. These include:

[13] *An Explanatory Guide to the Cartagena Protocol on Biosafety*, paragraphs 52-66 (http://data.iucn.org/dbtw-wpd/edocs/EPLP-046.pdf).

[14] See Protocol provisions with regard to whose responsibility it is to ensure that risk assessments are carried out.

- Existing environmental and health policies and strategies based on, for instance:
  - (i) Regulations and international obligations of the Party involved;
  - (ii) Guidelines or regulatory frameworks that the Party has adopted; and
  - (iii) Protection goals, assessment endpoints, risk thresholds and management strategies as laid down, for instance, in relevant legislation of the Party;
- Intended handling and use of the LMO, including practices related to the use of the LMO, taking into account user practices and habits;
- The nature and level of detail of the information that is needed (see above), which may, among other things, depend on the biology/ecology of the recipient organism, the intended use of the LMO and its likely *potential receiving environment*, and the scale and duration of the environmental exposure (e.g., whether it is for import only, field testing or for commercial use). For small-scale releases, especially at early experimental stages, the nature and detail of the information that is required or available may differ compared to the information required or available for large scale or commercial environmental release.
- Identification of methodological and analytical requirements, including requirements for review mechanisms, that must be met to achieve the objective of the risk assessment as specified, for instance, in guidelines published or adopted by the Party that is responsible for conducting the risk assessment (i.e. typically the Party of import according to the Protocol);
- Experience and history of use of the non-modified recipient organism, taking into account its *ecological function*.
- Approaches for describing the potential adverse effects of the LMO and its transfer, handling and use,
- Use of terms for describing the likelihood (step 2), the magnitude of consequences (step 3) and risks (step 4), and the acceptability or manageability of risks (step 5).

Some risk assessment frameworks combine the process of setting the context and scope of the risk assessment with the identification of potential adverse effects associated with the modifications of the LMO into a single step called "Problem formulation" (see step 1).

*›› See references relevant to "Setting the context and scope":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#context

### The choice of comparators

Risk assessments can be conducted in a comparative manner where risks associated with an LMO are considered in the context of the risks posed by the non-modified recipients or parental organisms in the likely potential receiving environment.[15, 16]

The comparative approach aims at identifying changes between an LMO and its comparator(s) that may lead to adverse effects. The choice of comparators can have large effects on the relevance, interpretation and conclusions drawn from the risk assessment process. Therefore, the one or more comparators that are chosen should be selected on the basis of their capacity to generate information that is consistent and relevant for the risk assessment.

To account for variation due to interaction with the environment, the LMO and its comparator(s) should ideally be evaluated at the same time and location, and under the same environmental conditions.

[15] Annex III, paragraph 5.

[16] A comparator is used as an element to establish the baseline for a comparative risk assessment in accordance with Annex III.

Some risk assessment frameworks use a non-modified genotype with a genetic background as close as possible to the LMO being assessed, e.g., a *(near-)isogenic line* as the primary choice of comparator.[17] In such risk assessment frameworks where the use of a (near-)isogenic non-modified recipient organism as the comparator is required, additional comparators may prove useful depending on the biology of the organism and types of modified traits under assessment. In practice, the (near-)isogenic non-modified organism is used in step 1 and throughout the risk assessment. When the likelihood and potential consequences of adverse effects are evaluated, broader knowledge and experience with additional comparators such as defined non-modified reference lines may also be taken into consideration, as appropriate, along with the non-modified recipient organism. Results from experimental field trials or other environmental information and experience with the same or similar LMOs in the same or similar receiving environments may also be taken into account.

In other risk assessment frameworks, the choice of an appropriate comparator will depend on the specific LMO being considered, the step in the risk assessment and on the questions that are being asked.

In some cases, the non-modified recipient organisms or the parental organisms alone may not be sufficient to establish an adequate basis for a comparative risk assessment, such as for the risk assessment of certain LM plants tolerant to abiotic stress, stacked LMOs, LM mosquitoes, and pharmaceutical producing LMOs. In such cases additional comparators may be necessary (for more guidance on some of these examples, please refer to Part II of this Guidance).

## CONDUCTING THE RISK ASSESSMENT

To fulfil its objective under Annex III, as well as other relevant Articles of the Protocol, risk assessment as described in Annex III is conducted in steps in an integrated process and iterative manner, as appropriate. Paragraph 8 of Annex III describes the key steps of the risk assessment process. Paragraph 9 of Annex III lists and describes points to consider in the process for risk assessment of LMOs depending on the particular case.

The steps of risk assessment under the Protocol are similar to those used in other risk assessment frameworks. Although the terminology may differ between the various approaches, in general terms, risk assessment is defined as a science-based process that includes at least the following common components (corresponding to the steps 1 to 4 respectively): "*hazard identification*", "*exposure assessment*", "*hazard characterization*", and "*risk characterization*".[18]

In this section, the steps indicated in paragraph 8(a)-(e) of Annex III are described in further detail and points to consider are provided for each step. Some points to consider are taken from paragraph 9 of Annex III. Additional points to consider were added on the basis of commonly used methodologies of LMO risk assessment and risk management insofar as they were in line with the principles of Annex III. The relevance of each point to consider will depend on the case being assessed. The guidance provided below on the steps in risk assessment is not exhaustive, thus additional guidance and points to consider may be relevant, as appropriate. Lists of background documents relevant to each section are provided through the links.

*›› See references relevant to "Conducting the Risk Assessment":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#riskassessment

[17] EFSA (2011) Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed. Available at http://www.efsa.europa.eu/en/efsajournal/doc/2149.pdf.

[18] See WHO (2004) IPCS risk assessment terminology. Available at http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf.

**Step 1: "An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health."** [19]

*Rationale:*

The purpose of this step is to identify changes in the LMO that could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. The potential adverse effects may be direct or indirect, immediate or delayed.[20]

The question that risk assessors ask in this step is what adverse effects could occur, why and how. This step is very important in the risk assessment process as the questions raised will determine what risk scenarios are considered in all subsequent steps. This step may also be referred to as "hazard identification" – the difference between the concepts of "*hazard*" and "*risk*" is important and must be understood by the risk assessor. In many cases, this step is performed as part of a problem formulation process when setting the context and scope of the risk assessment. In that case, this step is not limited to the identification of hazards, but also takes into account protection goals and appropriate assessment endpoints.

In this step, risk assessors identify scientifically plausible scenarios and risk hypotheses to predict if the LMO could have an adverse effect on the assessment endpoints. In doing so, risk assessors analyse what novel characteristics of the LMO, as well as its transfer, handling and use, could give rise to adverse effects in an interaction with the likely potential receiving environment. For example, if the protection goal is maintenance of biodiversity, a risk hypothesis could assess what novel characteristics of the LMO might affect specific "targets" such as a component of the food web or the population size of certain species in the likely potential receiving environment. The targets are called assessment endpoints, and their unambiguous specification is crucial to focus the risk assessment.

It is important to define a causal link or pathway between a characteristic of the LMO and a possible adverse effect, otherwise the risk assessment may generate information that will not be useful for decision-making (see also steps 2 and 3). Depending on the LMO, its intended use and the likely potential receiving environment, possible concerns that could lead to adverse effects include, but are not limited to, the potential of the LMO to: (i) affect non-target organisms, (ii) cause unintended effects on target organisms, (iii) become persistent or invasive or develop a fitness advantage in ecosystems with limited or no management, (iv) transfer genes to other organisms/populations, and (v) become genotypically or phenotypically instable.

In this step, a comparison of the LMO may be carried out with the non-modified recipient or parental organisms in the likely potential receiving environment, taking into consideration the new trait(s) of the LMO (see 'The choice of comparators' in the chapter on 'Planning Phase').

The novel characteristics of the LMO to be considered can be described in *genotypic* and *phenotypic* terms. These include any changes in the LMO, ranging from the nucleic acid (including any deletions), to gene expression level to morphological changes. The novel characteristics of the LMO may cause adverse effects which may be intended or unintended, direct or indirect, immediate or delayed, combinatorial or cumulative, as well as predicted or unpredicted. For example, an adverse effect may also be caused by changes in the expression levels of endogenous genes as a result of the genetic modification or by *combinatorial effects* of two or more genes, gene products or physiological pathways.

[19] The bold printed headings of each step are direct quotes from Annex III of the Protocol.

[20] See also article 2, paragraph 2(b) of the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress (http://bch.cbd.int/protocol/NKL_text.shtml).

*Points to consider regarding characterization of the LMO:*

(a) Relevant characteristics of the non-modified recipient organism, such as:

(i) its biological characteristics, in particular those that, if changed or upon interaction with the new *gene products* or traits of the LMO, could lead to changes that may cause adverse effects;

(ii) its taxonomic relationships;

(iii) its origin, centres of origin and centres of genetic diversity;

(iv) ecological function; and

(v) whether it is a component of biological diversity that is important for the conservation and sustainable use of biological diversity in the context of Article 7(a) and Annex I of the Convention;

(b) Characteristics related to the transformation method, including the characteristics of the *vector* such as its identity, source or origin and host range, and information on whether the transformation method results in the presence of (parts of) the vector in the LMO, including any marker genes;

(c) Relevant characteristics of the genes and of other functional sequences, such as promoters, that have been inserted into the LMO (e.g., functions of the gene and its gene product in the donor organism with particular attention to characteristics in the recipient organism that could cause adverse effects);

(d) Molecular characteristics of the LMO related to the modification, such as characteristics of the modified genetic elements; insertion site(s) and copy number of the inserts; stability, integrity and genomic organization in the recipient organism; specificity of the genetic elements (e.g., transcription factors); levels of gene expression and intended and *unintended gene products*;

(e) Genotypic (see point (d) above) and phenotypic changes in the LMO, either intended or unintended, in comparison with the non-modified recipient, considering those changes that could cause adverse effects. These may include changes in native/endogenous gene expression and regulation at the transcriptional, translational and post-translational levels due to the insert itself or to genomic changes that have occurred due to transformation or recombination.

Points to consider regarding the intended use and the likely potential receiving environment:

(f) Protection goals and assessment endpoints relevant to the likely potential receiving environment (see Planning phase, Setting the context and scope);

(g) Availability of sufficient data to establish a meaningful *baseline* for the likely receiving environment which will serve as a basis for the risk assessment;

(h) The intended spatial scale, duration and level of confinement (such as biological confinement) of the environmental release, taking into account user practices and habits;

(i) Characteristics of the likely potential receiving environment including relevant ecosystem functions and services, in particular its attributes that are relevant to potential interactions of the

LMO that could lead to adverse effects (see also paragraph (k) below),[21] taking into account the characteristics of the components of biological diversity, particularly in centres of origin and centres of genetic diversity;

Points to consider regarding the potential adverse effects resulting from the interaction between the LMO and the likely potential receiving environment:

(j) Characteristics of the LMO in relation to the likely potential receiving environment (e.g., information on phenotypic traits that are relevant for its survival, or its potential adverse effects – see also paragraph (e) above);

(k) Considerations for *unmanaged and managed ecosystems* concerning the use of an LMO that are relevant for the likely potential receiving environment. These include potential adverse effects resulting from the use of an LMO, such as changes in farm management practices; dispersal of the LMO through mechanisms such as seed dispersal or *outcrossing* within or between species, or through transfer into habitats where the LMO may persist or proliferate; as well as effects on species distribution, food webs and changes in bio-geochemical characteristics;

(l) Potential for outcrossing and transfer of *transgenes*, via *vertical gene transfer*, from an LMO to other sexually compatible species that could lead to *introgression* of the transgene(s) into populations of sexually compatible species, and whether these would lead to adverse effects;

(m) Whether *horizontal gene transfer* of transgenic sequences from the LMO to other organisms in the likely potential receiving environment could occur and whether this would result in potential adverse effects. With regard to horizontal gene transfer to micro-organisms (including viruses), particular attention may be given to cases where the LMO is also a micro-organism;

(n) Potential adverse effects on target organisms such as pests and weeds developing resistance to the target trait (e.g., pesticides and herbicides);

(o) Potential adverse effects on non-target organisms such as toxicity, allergenicity and *multi-trophic effects* which can affect the survival, development, or behaviour of these organisms;

(p) Potential adverse effects of the incidental exposure of humans to (parts of) the LMO (e.g., exposure to modified gene products in pollen), and the toxic or allergenic effects that may ensue taking into account the agricultural practices that may be used with the LMO, such as type of irrigation, number and amount of herbicide applications, methods for harvesting and waste disposal, etc;

(q) *Cumulative effects* with any other LMO present in the environment.

*›› See references relevant to "Step 1":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step1

[21] Examples of relevant attributes of the receiving environment include, among others: (i) ecosystem type (e.g., agroecosystem, horticultural or forest ecosystems, soil or aquatic ecosystems, urban or rural environments); (ii) extension of dimension (small, medium, large or mixed scale); (iii) previous use/history (intensive or extensive use for agronomic purposes, natural ecosystem, or no prior managed use in the ecosystem); (iv) the geographical zone(s) in which the release is intended, including climatic and geographic conditions and the properties of soil, water and/or sediment; (v) specific characteristics of the prevailing faunal, floral and microbial communities including information on sexually compatible wild or cultivated species; and (vi) biodiversity status, including the status as centre of origin and diversity of the recipient organism and the occurrence of rare, endangered, protected species and/or species of cultural value.

**Step 2: "An evaluation of the likelihood of adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism."**

Rationale:

In order to determine and characterize the overall risk of an LMO (step 4), risk assessors evaluate the likelihood that each of the potential adverse effects identified in step 1 will occur. The evaluation of likelihood may be undertaken at the same time as the evaluation of the consequences should the adverse effects be realized (step 3) or in an inverse order.

This step may be referred to as "exposure assessment" where plausible pathways of a hazard leading to adverse effects are identified. It aims to determine whether the receiving environment will be exposed to an LMO that has the potential to cause adverse effects, taking into consideration the intended transfer, handling and use of the LMO, and the expression level, dose and environmental fate of transgene products

For each of the risk hypotheses or scenarios identified in step 1, the route of exposure to the LMO being assessed (or its products) should be determined. Furthermore, when possible the causal link between the LMO and the potential adverse effect should be established. This can be achieved by building conceptual models describing relationships between the LMO, pathways of exposure and potential adverse effects in the environment. For example, for an LMO producing a potentially toxic gene product, oral, respiratory or dermal exposure pathways could be relevant.

Experimental studies and models may be used for an assessment of the potential level and type of exposure, combined with the use of statistical tools relevant for each case. Past experience with similar situations (e.g., same recipient organism, LMO, trait, receiving environment, etc), if available, may also be used in assessing the level and type of exposure, taking into account user practices and habits.

In some circumstances, particularly when there is a high level of uncertainty, it may be difficult to assess the likelihood of adverse effects being realized. In such cases, the "worst-case scenario" may be considered by assigning a likelihood of 100% that an adverse effect will occur and concentrating on the evaluation of its consequences.

Likelihood may be expressed quantitatively or qualitatively. For example, qualitative terms could include 'highly likely', 'likely', 'unlikely', and 'highly unlikely'. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.

*Points to consider:*

(a) The relevant characteristics of the likely potential receiving environment that may be a factor in the occurrence of the potential adverse effects (see also step 1 (f), (g) and (i)), taking into account the variability of the environmental conditions and long-term adverse effects related to the exposure to the LMO.

(b) Levels of expression in the LMO and persistence and accumulation in the environment (e.g., in the food chain) of substances with potentially adverse effects newly produced by the LMO, such as toxins, allergens and some insecticidal proteins. In the case of field trials, the level of persistence and accumulation in the receiving environment may be low depending on the scale of the release, its temporary nature and the implementation of management measures;

(c) Information on the location of the release and the receiving environment (such as geographic and biogeographic information, including, as appropriate, geographic coordinates);

(d) Factors that may affect spread of the LMO, such as its ecological range and ability to move (e.g., LM insects, birds and fish may be particularly mobile); its reproductive ability (e.g., numbers of offspring, time to seeding, abundance of seed and vegetative propagules, dormancy, pollen viability); and its ability to spread using natural means (e.g., wind, water) or

anthropogenic mechanisms (e.g., rearing or cultivation practices, seed saving and exchange, etc);

(e) Factors that affect presence or persistence of the LMO that may lead to its establishment in the environment, such as, in the case of LM plants, lifespan, seed dormancy, ability of LM seedlings to establish among existing wild or cultivated vegetation and to reach reproductive stage, or the ability to propagate vegetatively;

(f) When assessing the likelihood of outcrossing from the LMO to sexually compatible species, the following issues are relevant:

(i) the biology of the sexually compatible species;

(ii) the potential environment where the sexually compatible species may be located;

(iii) Introgression of the transgene into the sexually compatible species;

(iv) Persistence of the transgene in the ecosystem; and

(g) Expected type and level of exposure of the environment where the LMO is released, and mechanisms by which incidental exposure could occur at that location or elsewhere (e.g., *gene flow*, incidental exposure due to losses during transport and handling, intentional spread by people, or unintentional spread by people via machinery, mixed produce or other means).

*›› See references relevant to "Step 2":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step2

**Step 3: "An evaluation of the consequences should these adverse effects be realized."**

Rationale:

This step, which may also be referred to as "hazard characterization", describes an evaluation of the magnitude of the consequences of the possible adverse effects, based on the risk scenarios established in step 1, paying special attention to protected areas and centres of origin and centres of genetic diversity, and taking into account protection goals and endpoints of the country where the risk assessment is being carried out. As discussed in the previous step, the evaluation of consequences of adverse effects may be undertaken at the same time as the evaluation of likelihood (step 2) or in an inverse order.

In this step, results of tests conducted under different conditions, such as laboratory experiments or experimental releases, may be considered. The scale and duration of the intended use (e.g., small or large) may influence the severity of potential consequences and should therefore be taken into account.

The evaluation of consequences of adverse effects can be comparative and considered in the context of the adverse effects caused by the non-modified recipients or parental organisms in the likely potential receiving environment, (see Planning Phase of the Risk Assessment). The evaluation of consequences may also consider the adverse effects associated with the existing practices or with practices that will be introduced along with the LMO (such as various agronomic practices, for example, for pest or weed management).

It is important to also assess in this step the duration of the potential adverse effect (i.e., short or long term), the scale (i.e., are implications local, national or regional), the mechanisms of effect (direct or indirect), the reversibility (or lack thereof) of effects, and the expected ecological scale (i.e., individual organisms – for example of a protected species – or populations).

The evaluation of the consequence of adverse effects may be expressed qualitatively or quantitatively. For instance, qualitative terms such as 'major', 'intermediate', 'minor' or 'marginal' may be used. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.

*Points to consider:*

(a) Relevant knowledge and experience with the non-modified recipient or parental organisms, or current agricultural practices with the organism that the LMO would replace, in the likely potential receiving environment. This may include the effects of:

(i) agricultural practices on the level of inter- and intra-species gene flow; dissemination of the recipient; abundance of volunteers in crop rotation; change in abundance of pests, beneficial and other organisms such as pollinators, decomposers, organisms involved in biological control or soil microorganisms involved in nutrient cycling;

(ii) pest management affecting non-target organisms through pesticide applications or other management approaches while following accepted agronomic practices;

(iii) the behaviour of populations of unmodified animal or insect species, including interactions between predators and prey, their role in food webs and other ecological functions, disease transmission, allergies and interaction with humans or other animal species;

(b) Consequences resulting from combinatorial and cumulative effects in the likely potential receiving environment;[22]

(c) Relevant knowledge and experience with the LMO in similar receiving environments;

(d) Results from laboratory experiments examining, as appropriate, dose-response relationships or particular effect levels (e.g., *EC50*, *LD50*) for acute, chronic or sub-chronic effects including immunogenic effects;

(e) Results from field trials evaluating, for instance, potential invasiveness; and

(f) Possible consequences of transgene introgression resulting from outcrossing to sexually compatible species.

*›› See references relevant to "Step 3":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step3

**Step 4: "An estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized."**

*Rationale:*

The purpose of this step, which may also be referred to as "risk characterization", is to determine and characterize the overall risk of the LMO. This can be achieved by characterising individual risks on the basis of an analysis of the potential adverse effects completed in step 1, their likelihood (step 2) and consequences (step 3), and combining them into an overall risk, taking into consideration any relevant uncertainty that was identified in each of the preceding steps and how it could affect the estimation of the overall risk of the LMO (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).

To date, there is no universally accepted approach for estimating the overall risk but rather a number of approaches are available for this purpose. For example, the characterization of the overall risk often derives a best estimate of risk from multiple lines of evidence. These lines of evidence may be quantitatively or qualitatively weighted and combined. Risk matrixes, risk indices or models may be used for this purpose.[23]

[22] See "Use of terms" section.

[23] See references in the list of background materials [to be added].

A description of the risk characterization may be expressed qualitatively or quantitatively. Qualitative terms such as 'high', 'medium', 'low', 'negligible' or 'indeterminate' (e.g., due to uncertainty or lack of knowledge) have been used to characterize the overall risk of an LMO. Parties could consider describing these terms and their uses in risk assessment guidelines published or adopted by them.

The outcome of this step should include a description explaining how the estimation of the overall risk was performed.

Points to consider:

(a) The identified potential adverse effects (step 1);

(b) The assessments of likelihood (step 2);

(c) The evaluation of the consequences should the adverse effects be realized (step 3);

(d) Risk management strategies (see step 5) that may affect risk estimates if implemented;

(e) Any interaction, such as *synergism*, between the identified individual risks; and

(f) Broader ecosystem and landscape considerations, including cumulative effects due to the presence of various LMOs in the receiving environment.

*›› See references relevant to "Step 4":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step4

**Step 5: "A recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks"**

*Rationale:*

In step 5, risk assessors prepare a report summarizing the risk assessment process and the identified risks, and provide recommendation(s) as to whether or not the risks are acceptable or manageable and, if needed, recommendation(s) for risk management options that could be implemented to manage the risks associated with the LMO. The recommendation is made in the context of criteria for the acceptability of risk that were identified in the planning phase of the risk assessment, taking into account established protection goals, assessment endpoints and risk thresholds, as well as risks posed by the non-modified recipient organism and its use.

This step is an interface between the process of risk assessment and the process of decision-making. Importantly, while the risk assessor provides a recommendation as to whether or not the risks are acceptable or manageable, the ultimate decision about whether or not to approve the LMO is up to the decision maker. Moreover, the "acceptability" of risks is typically decided at a policy level and may vary from country to country.

In evaluating the acceptability of the overall risk of the LMO, it is important to consider whether risk management options can be identified that could reduce the identified risks and uncertainties. The need, feasibility and efficacy of the management options, including the capacity to enact them, should be considered on a case-by-case basis. If such measures are identified, the preceding steps of the risk assessment may need to be revisited in order to evaluate how the application of the proposed risk management measures would change the outcome of the steps.

The recommendation on the acceptability of risk(s) should take into account any available scientific analysis of potential benefits for the environment, biodiversity, and human health (e.g., change in the use of crop protection products, reduction of infections in the case of mosquitoes), and should also take into account risks associated with other existing user practices and habits.

Further, the sources and nature of uncertainty that could not be addressed during the preceding steps of the risk assessment should be described in relation to how they could affect the conclusions of the risk

assessment. For assessments where uncertainties could not be addressed, it is imperative that the difficulties encountered during the risk assessment be made transparent to the decision makers. In such cases, it may also be useful to provide an analysis of alternative options to assist the decision makers.

Some uncertainties may be dealt with by monitoring (e.g., checking the validity of assumptions about the effects of the LMO on components of the ecosystem and environment), requests for more information, or implementing the appropriate risk management options.

Monitoring can be applied as a tool to detect unexpected and long-term adverse effects. Monitoring can also be a means to reduce uncertainty, to address assumptions made during the risk assessment, to validate conclusions of the assessment on a wider (e.g., commercial) level of application, and to establish a causal link or pathway between LMOs and adverse effects. Monitoring may also be used to evaluate whether risk management strategies are being implemented effectively, including whether those strategies are able to detect potential adverse effects before the consequences are realized.

The issues mentioned in the 'Setting the context and scope' section may be taken into consideration again at the end of the risk assessment process to evaluate whether the objectives that were set out at the beginning of the risk assessment have been met.

The recommendation(s) are submitted, typically as part of a risk assessment report, for consideration in the decision-making process.

*Points to consider related to the risk management strategies:*

(a) Existing management practices, if applicable, that are in use for the non-modified recipient organism or for other organisms that require comparable risk management and that might be appropriate for the LMO being assessed (e.g., physical containment, separation from breeding partners, isolation distances to reduce outcrossing potential of the LMO, modifications in herbicide or pesticide management, crop rotation, soil tillage);

(b) Methods to detect and identify the LMO, and their specificity, sensitivity and reliability in the context of environmental monitoring (e.g., monitoring for short- and long-term, immediate and delayed effects; specific monitoring on the basis of scientific hypotheses and supposed cause/effect relationship as well as general monitoring), including plans for appropriate contingency measures to be applied if warranted based on monitoring results;

(c) Management options in the context of the intended use (e.g., isolation distances to prevent outcrossing, and the use of refuge areas to minimize the development of resistance to insecticidal proteins); and

(d) Methods for evaluating the proposed risk management and monitoring strategies for feasibility, efficacy and effectiveness.

*Points to consider related to the acceptability of risks:*

(e) Established criteria and thresholds for determining risk acceptability, including those set out in national legislation or guidelines;

(f) Protection goals of the Party, as identified when setting the context and scope for a risk assessment;

(g) Any relevant experience with the non-modified recipient organism(s) or other reference line(s) (including practices associated with their use in the likely potential receiving environment) which were used to establish the ***baseline*** for the risk assessment;

(h) Scientific analyses of potential benefits of the LMO, carried out using similar principles of sound science as those used throughout the risk assessment;

(i) Ability to identify, evaluate and confine adverse effects in the event that the LMO is released into the environment, as well as to take appropriate response measures.

*›› See references relevant to "Step 5":*
http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step5

**RELATED ISSUES**

Risk assessment is one input to decision-making regarding LMOs. Other issues that may be part of the decision-making process, as appropriate, and that are mentioned in other articles of the Protocol, include:

- Risk Management (Article 16);
- Capacity-building (Article 22);
- Public Awareness and Participation (Article 23);
- Socio-economic Considerations (Article 26);
- Liability and Redress (Article 27).

A number of other issues, which are not mentioned in the Protocol (e.g., co-existence, ethical issues), may also be taken into account in the decision-making process regarding an LMO in accordance with a country's policies and regulations.

# ANNEX:
# FLOWCHART FOR THE RISK ASSESSMENT PROCESS

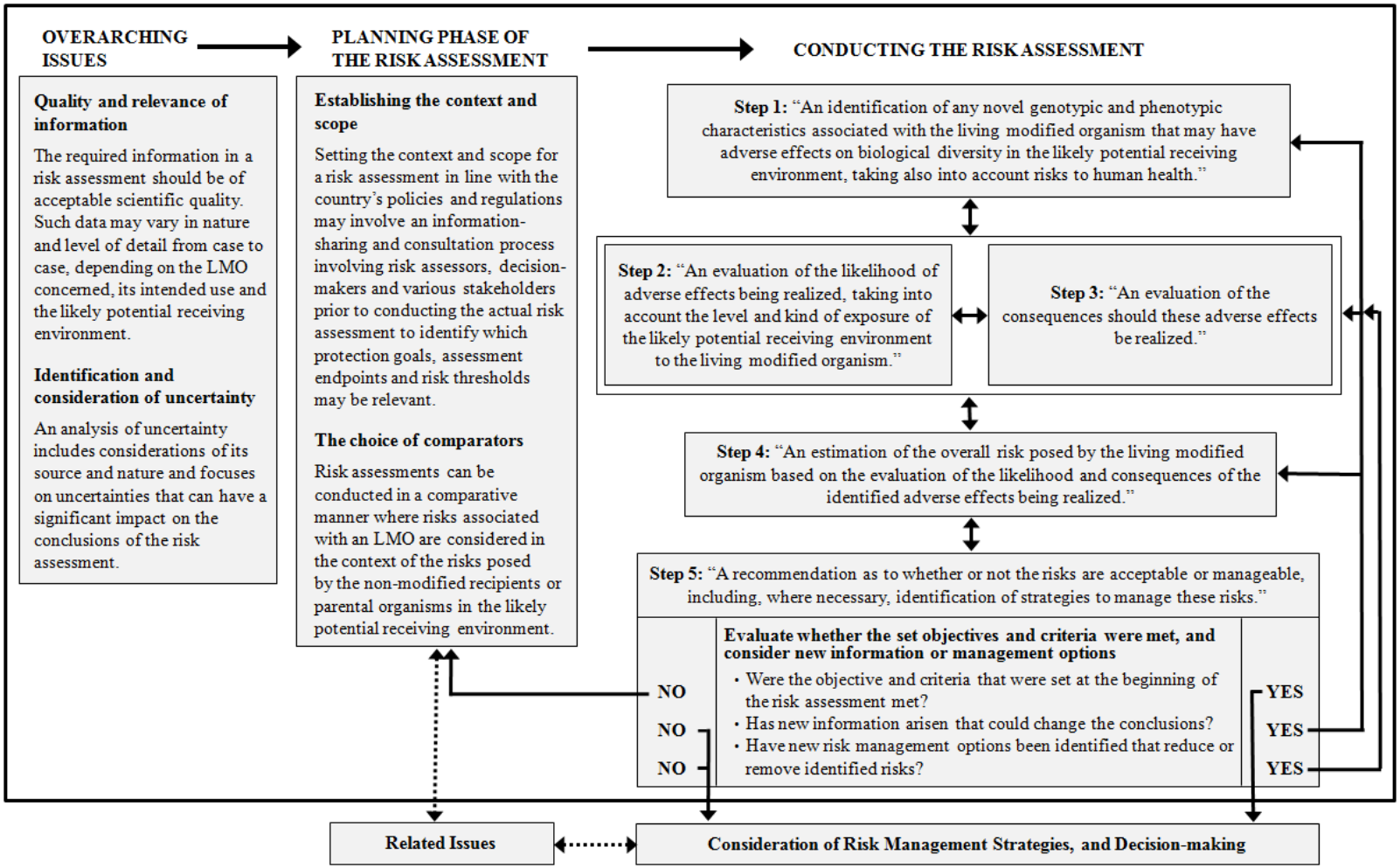


**Figure 1. The Roadmap for Risk Assessment.** The flowchart represents the risk assessment process, which includes "Overarching issues", "Planning phase of the risk assessment" and "Conducting the risk assessment", to *identify* and *evaluate* the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. As results are gathered at each step and new information arises, risk assessments may need to be conducted in an iterative manner, where certain steps may be repeated or re-examined as shown by the solid and double-headed arrows. The box around steps 2 and 3 shows that these steps may sometimes be considered simultaneously or in reverse order. Dotted arrows indicate the flow to and from issues outside the risk assessment process.

# PART II:
# SPECIFIC TYPES OF LMOS AND TRAITS

The guidance contained in this section, Part II, should be considered in the context of the Cartagena Protocol on Biosafety. The elements of Article 15 and Annex III of the Protocol apply to these specific types of LMOs and traits. Accordingly, the methodology and points to consider contained in Annex III[24] are also applicable to these types of LMOs and traits. The guidance in the sub-sections below complements the Roadmap for Risk Assessment of LMOs, giving emphasis to issues that may be particularly relevant when assessing the risks of the respective types of LMOs and traits.

## A. RISK ASSESSMENT OF LIVING MODIFIED PLANTS WITH STACKED GENES OR TRAITS

### INTRODUCTION

Worldwide, a growing number of LMOs with stacked transgenic traits, particularly LM plants, are being developed for commercial uses. As a result, the number of stacked genes in a single LMO and the number of LMOs with two or more transgenic traits is growing.

Stacked LMOs can be produced through different approaches. In addition to the cross-breeding of two LMOs, multiple traits can be achieved by transformation with a multi-gene *transformation cassette*, retransformation of an LMO or simultaneous transformation with different transformation cassettes or vectors.

### OBJECTIVE AND SCOPE

This guidance complements the Roadmap for Risk Assessment of LMOs, giving emphasis to issues that are of particular relevance to the risk assessment of LM plants with stacked traits generated through cross breeding. As such, risk assessments of this type of LM plant follow the general principles outlined in the Roadmap, but also take into account the specific issues outlined in this section of the present document.

For the purpose of this document, a stacked event is an LMO generated through *conventional* cross-breeding involving two or more LMOs that are either single *transformation events* or already stacked events. Accordingly, the cassettes containing the transgenes and other genetic elements that were inserted in the original transformation events may be physically unlinked (i.e. located separately in the genome) and can segregate independently.

It is assumed that the individual transformation events making up the stacked event have either been assessed previously or are being assessed concomitantly to the stacked event in accordance with Annex III of the Cartagena Protocol on Biosafety and as described in the Roadmap.[25]

LM plants that contain multiple genetically-modified traits or genes but that are the result of a single transformation event, e.g., through *re-transformation*, *co-transformation* or transformation with a multi-gene transformation cassette, are not covered in this part of the guidance document.

This guidance also includes considerations for unintentional stacked events as the result of natural crossings between stacked events and other LMOs or compatible relatives in the receiving environment.

[24] Paragraphs 8 and 9 of Annex III.

[25] While stacked events are also considered to be LMOs in accordance with Article 3 of the Protocol, the biosafety legislation of different countries may vary regarding the extent to which these types of LMOs are regulated.

/…

## PLANNING PHASE OF THE RISK ASSESSMENT

**The choice of comparators** *(see "Planning Phase of the Risk Assessment", "The choice of comparators" in the Roadmap)*

*Rationale:*

As for any other type of LMO, the risk assessment of a stacked LM plant can be conducted in a comparative manner. In the case of stacked LM plants, in addition to using non-modified recipient organisms as seen under "The choice of comparators" section of the Roadmap, the LMOs that were involved in the cross-breeding process leading to the stacked LM plant under consideration may also be used as comparators, as appropriate and according to national regulations.

In cases where parental LMOs have highly *heterozygous genomes* or significantly differ from each other, the resulting stacked LMOs will display high variability and a vast range of phenotypes. This variability should be taken into account during the establishment of a baseline for a comparative risk assessment.

For example, stacked LM plants may be the result of multiple rounds of cross-breeding among many different genotypes and possibly involve several stacked events. In such cases, choosing the appropriate comparators among the single transformation LMOs and the intermediate stacked events that gave rise to the stacked LM plant under assessment may not be a straight forward action and the choice of comparator should be justified.

(Near-)isogenic lines to be used as comparators may be lacking, which may present challenges for data interpretation when establishing the baseline for the risk assessment of a stacked LM plant. Therefore, in risk assessment frameworks that rely on the (near-)isogenic non-modified recipient organism as the primary comparator, it may be useful to also use the closest available non-modified genotype as a comparator.

*Points to consider:*

(a) Level of heterozygosity between the non-modified recipient organisms used to produce the parental LMOs;

(b) Phenotypic variability among non-modified hybrids produced through crosses between the non-modified recipient organisms;

(c) Number of crossings and the use of intermediate stacked LMOs as additional comparators.

## CONDUCTING THE RISK ASSESSMENT

**Sequence characteristics at the insertion sites, genotypic stability and genomic organization** *(see "Step 1", "Point to consider (d)" and "Step 5" in the Roadmap)*

*Rationale:*

Plant breeding results in changes (mutations/recombinations) within a plant's genome and this may also occur at the insertion site(s) in the LM plant. During cross-breeding, changes may occur to the molecular characteristics of the inserted genes/genetic elements at the insertion site(s) as a result of recombination, mutation and rearrangements. The potential for an identified change in the transgenes and/or genetic elements to lead to an adverse effect should be assessed.

Transgenes with similar genetic sequences may undergo recombination, since homologous recombination acts on genomic regions that have identical or highly similar sequence. Complex inserts with multiple repeats may be less stable and could be more likely to undergo rearrangements during cross-breeding. In many cases, such changes may result in the loss of the intended phenotype.

As with single event LMOs, molecular characterization of the stacked LM plant may be carried out in accordance with step 1 of the Roadmap, point to consider (d). If differences in relation to the parental LMOs are found, intended and unintended possible adverse effects need to be assessed. In addition, changes to the molecular characteristics of the transgenes and other genetic elements may influence the ability to detect the LMO, which may be needed in the context of risk management measures (see below as well as step 5 of the Roadmap).The extent to which a molecular characterization of the stacked LMO is needed may vary case by case and should take into account the results of the risk assessments of the parental LMOs.

*Points to consider:*

(a) Whether methods to carry out molecular characterization are available, for example PCR-based methods, and whether they are specific and sensitive enough for the characterization of the stacked LM plant;

(b) Genome stability and whether methods to detect the stacked LM plant would remain reliable after introduction into the environment, particularly in the context of risk management measures;

(c) Phenotypic changes that may indicate underlying changes to any of the transgenes and genetic elements present in the stacked LM plant (e.g., loss of a trait present in the parental LMOs);

**Potential interactions between combined genes and their resulting phenotypic changes and effects on the environment** *(see "Step 1", "Point to consider (e)" in the Roadmap)*

*Rationale:*

It is possible that the crossing of two or more LMOs resulting in stacked events may influence the expression level of the transgenes or of endogenous genes through *trans-regulation*. For example, changes in gene expression in stacked events are more likely to occur if the transgenes or their regulatory elements in the parental LMOs share similar genetic elements with each other or with an endogenous sequences (e.g., same binding sites for transcriptional factors), and if they are localized in the same intracellular compartment (e.g., nucleus, chloroplast).

There may also be interactions between the expressed products of two or more transgenes and endogenous genes. This is most likely to occur if the gene products belong to the same metabolic pathway or physiological process. Some of the interactions may lead to changes that can be detected during the phenotypic characterization of the stacked LM plant, whereas other interactions may not be detectable through a typical phenotypic characterization. Previous risk assessments of the parental LMOs provide useful information on the mode of action and molecular characteristics of the individual genes as a starting point to assess the potential for interactions. In addition to information about the characteristics of the parental LMOs, specific information on potential for interactions between the altered or inserted genes and DNA elements (e.g., promoters and other regulatory elements), proteins, metabolites or modified traits and endogenous genes and their products in the stacked LM plant should be considered and assessed. For example, it would be appropriate to consider whether the different transgenes belong to the same biochemical pathways or physiological processes.

*Points to consider:*

(a) Effects of the parental LMOs on the environment;

(b) Information on transcriptional and post-transcriptional regulation of genes and their products that may be predictive of interactions between the novel and endogenous genes and/or DNA elements in the stacked LM plant;

(c) Whether transgenes with similar functions or belonging to the same metabolic pathways were stacked.

(d) Levels of expression of the transgenes and their products compared to the parental LMOs and to the non-modified recipient organisms.

**Combinatorial and cumulative effects** *(see "Step 1", "Point to consider (d) and (o)", "Step 2", "Point to consider (d)" and "Step 3", "Point to consider (b)" in the Roadmap)*

*Rationale:*

Assessment of combinatorial and cumulative effects[26] is based on the environmental risk assessment data for the stacked event LM plant in comparison to the closely related non-modified recipient organism(s) and the parental LMOs in the likely receiving environment, taking into consideration the results of the genotypic and phenotypic assessments outlined above.

Proteins and metabolites produced due to the presence of multiple transgenes in the same stacked LM plant may interact with each other as well as with endogenous genes and metabolic pathways. These interactions could lead to unpredicted combinatorial effects. For example, the impact on non-target organisms could be broader than the sum of the individual parental LMOs, or the evolution of resistance in target organisms (e.g., insect pests) could happen faster than in the case of single event LMOs.

Possible interactions at the DNA- or RNA-level, or between proteins and metabolites, can be investigated including associated potential adverse effects. An assessment of potential combinatorial and cumulative effects may be performed, for instance, by conducting a phenotypic characterization, compositional analyses, toxicity tests on non-target organisms and any other analysis that integrates these multiple and interacting factors to predict potential adverse effects. Also, indirect effects due to changed agricultural management procedures, combined with the use of the transgenic stacked event LMOs, may occur and should be evaluated.

If potential new or increased adverse effects on the conservation and sustainable use of biological diversity or on human health are identified in relation to the stacked event through the above analysis of possible interactions, additional supporting data on the stacked event may be required.

*Points to consider:*

(a) Effects of the use of pesticides, other chemicals or agricultural practices commonly used in the cultivation of the parental LMOs;

(b) Phenotypic characteristics compared to the parent LMOs and to the non-modified recipient organisms;

(c) Interactions between the stacked transgenes or their products, or interactions among the physiological pathways in which the transgenes are involved, taking into account the possibility that these interactions could result in potentially harmful substances (e.g., anti-nutritional factors) some of which may persist or accumulate (e.g., via the food chain) in the environment;

(d) Combinatorial and cumulative effects arising from the presence of two or more modified traits in the environment that could result in a broadened target range or increased toxicity.

**Crossing and segregation of transgenes** *(see "Step 1", "Point to consider (k)", "Step 2", "Point to consider (g)", "Step 3", "Point to consider (d)" in the Roadmap)*

*Rationale:*

A set of new stacked LMOs may arise in the environment through crossings between the stacked event LMOs and other LM plants or sexually-compatible non-modified relatives in the receiving environment. These crossings can be controlled (i.e. mediated by man) or uncontrolled (i.e. natural outcrossings

[26] See definition of combinatorial and cumulative effects in the "Use of Terms" section.

through pollination) and, depending on the number of stacked genes and traits and on their segregation patterns, the new stacked LMOs could contain new and/or different combinations of transgenes and DNA fragments. These new LMOs could result in cumulative and/or combinatorial effects.

In cases where a large number of different sexually-compatible stacked LMOs are cultivated in the same environment, there are more possible variations of new stacked events arising which contain different combinations of transgenes and DNA fragments, and probability of new stacking occurring is higher. This should be taken into account when establishing risk scenarios or risk hypotheses

*Points to consider:*

(a) Presence of sexually-compatible non-modified relatives and their ecological function, for example if the non-modified plant plays an important role in the ecosystem of the receiving environment;

(b) Presence of other single-event and stacked LMOs of the same species;

(c) Possible new combinations of transgenes and/or DNA fragments should the stacked event under consideration cross, intentionally or unintentionally, with other LM plants, stacked or not, or with non-modified relatives;

(d) Possible impacts of the new stacked events on non-target organisms or a change in the range of non-target organisms;

(e) Scientifically plausible risk scenarios or risk hypotheses involving the stacked events with different combinations of transgenes and DNA fragments.

**Methods for distinguishing the combined transgenes in a stacked event from the parental LMOs** *(see "Step 5", "Point to consider (f)" in the Roadmap)*

*Rationale:*

In the context of paragraphs 8(f) and 9(f) of Annex III of the Protocol, some of the risk management strategies for stacked events may require methods for the detection and identification of these LM plants in the context of environmental monitoring. Currently, many detection methods for LMOs rely on DNA-based techniques, such as polymerase chain reaction (PCR) or protein-based ELISA tests.

Several of the current PCR-based detection methods are designed to be specific for a single transformation event. While these methods may be used to detect and identify single transformation events, when the analysis is carried out in bulk (i.e. mixing material collected from various test individuals), these methods are not sensitive or specific enough to differentiate between single transformation events and a stacked event arising from a cross between these single transformation events. For example, although some software may help predict the presence of stacked LM seeds in a bulk sample,[27] it is not possible to unequivocally distinguish a sample containing material from different single transformation events from another sample containing one or more stacked LM events.

PCR-based detection methods that are specific to a single transformation event often rely on the amplification of DNA sequences that flank the insertion sites and that are unique for a single transformation event. In the future, it may become a challenge to detect single transformation events produced through site-specific insertions because the flanking sequences could be the same among different LMOs. This could become challenging particularly in cases where the stacked event contains multiple transformation cassettes with similar DNA sequences.

Based on the considerations above, the detection of each and all individual transgenes in a stacked event, if needed or required, may become a challenge and may need special consideration.

[27] See, for example, SeedcalcStack9 software at www.seedtest.org.

*Points to consider:*

(a) Level of similarity/difference between different transformation constructs in the stacked LM plant;

(b) Availability and specificity of detection methods;

(c) Whether environmental monitoring strategies will be recommended at the end of the risk assessment.

## BIBLIOGRAPHIC REFERENCES

See references relevant to "*Risk Assessment of LM Plants with Stacked Genes or Traits*": http://bch.cbd.int/onlineconferences/stackedref_ahteg_ra.shtml

## B. RISK ASSESSMENT OF LIVING MODIFIED PLANTS WITH TOLERANCE TO ABIOTIC STRESS

### INTRODUCTION

While the same general principles used in the risk assessments of other types of LMOs also apply to LM plants with increased tolerance to abiotic stress,[28] there are a number of specific issues that may be of particular importance when assessing the risks of LM plants tolerant to abiotic stresses.

As outlined in the section on "Context and scope" and in step 1 of the Roadmap, identifying protection goals, assessment endpoints and establishing scientifically plausible risk scenarios are some of the first actions to be taken during a risk assessment.

An important consideration in performing a comparative risk assessment of an LM plant with tolerance to abiotic stress is the multiple interactions between the new trait and the receiving environment, and the associated need to design aproperly controlled field experiment.

In plants, any gene (or gene product) or gene combinations providing increased tolerance to abiotic stress may have *pleiotropic effects* on the stress physiology of the plant. For example, drought, temperature and salt stress are interconnected by common metabolic and signal transductor pathways. Such pleiotropic effects may be classified as "unintended predicted effects" (see the Roadmap, step 1) and may be evaluated during the risk assessment by considering the crosstalk mechanisms between different stress responses of the plant, and by evaluating whether the identified changes may cause adverse effects. Disciplines such as plant physiology, plant pathology and entomology may provide useful context based on non-modified crops to clarify cross-talk mechanisms among abiotic stress responses and how these responses may affect susceptibility to biotic stresses (e.g., predators, pests and pathogens) in an LM crop that is tolerant to abiotic stresses.

The stress tolerance of the LM plant should be assessed with respect to an appropriate range of potential environmental conditions that reflect the potential conditions to which the LMO is likely be exposed, including for example variation in the duration and periodicity of the stressor (e.g., drought, flood, suboptimal temperatures, salt or other toxic ions). These variations pose difficulties for (i) controlling and measuring conditions in field experiments and (ii) characterizing the phenotype of the LM plant itself, which in many cases may be subject to the interaction between external and physiological parameters.

Some of the issues that could arise from the introduction of LM plants tolerant to abiotic stress into the environment and which may lead to adverse effects include, for example: a) increased selective advantage(s), other than the intended tolerance trait, which may lead to potential adverse effects (e.g., resulting from the introduction of a transcription factor affecting more than one trait); b) increased persistence in agricultural areas and increased invasiveness in natural habitats; c) adverse effects on organisms exposed to the LM plant; and d) adverse consequences of potential gene flow to wild or conventional relatives. While these potential adverse effects may exist regardless of whether the tolerant plant is a product of modern biotechnology or conventional breeding, some specific issues may be more relevant in the case of abiotic stress tolerant LM plants.

In this context, questions that may be relevant to the risk assessment of LM plants with tolerance to abiotic stress in connection with the intended use and the receiving environment include:

- Does the tolerance trait have the potential to affect other tolerance and/or resistance mechanisms of the LM plant, for example, via pleiotropism?

[28] For the purpose of this guidance, "abiotic stresses" are non-living environmental factors which are detrimental to or inhibit the growth, development and/or reproduction of a living organism. Types of abiotic stresses include, for example, drought, salinity, cold, heat, acidic or basic soils, soil pollution and air pollution (e.g., nitrous oxides, ozone, high $CO_2$ concentration). Increased tolerance to abiotic stress has long been a target of plant breeders working towards improved crops that would be able to cope with the stress. In the context of this document, herbicides are not considered a type of abiotic stress.

- Does the tolerance trait have the potential to provoke an increase of the invasiveness, persistence or weediness of the LM plant that could cause adverse effects to other organisms, food webs or habitats?
- Does an LM plant arising from outcrossing with the abiotic stress tolerant LM plant have the potential to change or colonize a habitat or ecosystem beyond the targeted receiving environment?
- Does an LM plant expressing tolerance to a particular abiotic stress have other advantages in the targeted receiving environment that could cause adverse effects?
- What are the adverse impacts in regions that have not been exposed to commercial agriculture but may become exposed to stress tolerant LM plants?

The following sections elaborate on specific issues that may be taken into account, on a case-by-case basis, when assessing the risks of LM plants tolerant to abiotic stress and the potential adverse effects to conservation and sustainable use of biodiversity, taking also into account risks to human health.

## PLANNING PHASE OF THE RISK ASSESSMENT

**The choice of comparators** *(see "Planning Phase of the Risk Assessment", "The choice of comparators" in the Roadmap)*

*Rationale:*

As outlined in the Roadmap, the first step in the risk assessment process involves the characterization of genotypic or phenotypic changes, either intended and unintended, associated with the abiotic stress-tolerant LM plant, that may have adverse effects on biodiversity in the likely receiving environment, taking into account risks to human health.

The identification of genotypic and phenotypic changes in the abiotic stress tolerant LM plant, either intended or unintended, is typically carried out in comparison with the non-modified recipient organism. The non-modified comparator provides the baseline information for comparison during trials when it is grown at the same time and location as the LM plant. Comparisons should also be made, as appropriate, in a range of environments with different stressor intensities and durations.

While the comparative approach should be used to assess whether the LM plants with tolerance to abiotic stress have increased fitness advantages under non-stress conditions, additional approaches (and comparators) for risk assessment need to be implemented for assessing potential adverse effects under abiotic stress.

LM plants with tolerance to abiotic stress may present specific challenges in the experimental design to generate data for the risk assessment. In some cases, for instance, an approach uses different reference plant lines, which typically include a range of genotypes representative of the natural variation in the plant species. Another important consideration is whether the experimental design is properly controlled for the effect of the abiotic stress trait. In the extreme case, when the non-modified plant cannot be grown in the range of conditions of the receiving environment because the abiotic stress conditions prevent or severely affect the growth of the non-modified plant, a comparative approach between the LM plant and the non-modified plant will need to be adjusted. In such cases, non-modified varieties or distant relatives that are tolerant to abiotic stress may become useful comparators. It is noted however that, in situations where the non-modified recipient organism, or (near-)isogenic or closely related lines cannot be used for a comparative risk assessment, the use of non-isogenic lines or distant relatives as comparators can make it more difficult to identify statistically meaningful differences.

In situations where a suitable comparator is not available, the characterization of the abiotic stress tolerant LM plant may be similar to that carried out for alien species, where the whole plant is considered a novel genotype in the receiving environment. On a case by case basis, information available from *"omics"*

*technologies*, for example, "transcriptomics" and "metabolomics", as it becomes available, may help to detect phenotypic and compositional changes (e.g., the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison with field grown plants under suboptimal conditions.

Where non-modified organisms are unsuitable as comparators, insight may be gained by comparing LM individuals grown under stress to individuals grown under normal conditions.

*Points to consider:*

(a) Characteristics of the LM plant with and without the influence of the abiotic stress or other stresses, if applicable; and

(b) Whether comparators that can generate meaningful data are available and can be used in appropriately designed experiments.

## CONDUCTING THE RISK ASSESSMENT

### Unintended characteristics including crosstalk between stress responses *(see "Step 1" in the Roadmap)*

*Rationale:*

The abiotic-stress-tolerant LM plant may have characteristics such as tolerance to other types of biotic and abiotic stresses (i.e. crosstalk in biochemical signalling), which could lead to a selective advantage of these plants under stress conditions other than that related to the modified trait. For instance, plants modified to become tolerant to drought or salinity may be able to compete better than their counterparts at lower or higher growing temperatures. The characteristics of an LM plant with increased tolerance to an abiotic stress may affect its general biology (e.g., if the genes alter multiple characteristics of the plant) or its distribution range in the likely potential receiving environment, which may cause adverse effects. Other changes could influence seed dormancy, viability, and/or germination rates under other types of stresses. Particularly in cases where genes involved in abiotic stress are also involved in crucial aspects of physiology, modifications involving these genes may have pleiotropic effects. If the stress tolerance trait leads to an increased physiological fitness, introgression of the transgenes for stress tolerance may occur at higher frequencies than observed among non-modified plants.

The response mechanisms to abiotic and biotic stresses in plants may have interactions and cross-talk mechanisms. For that reason, an LM plant modified to acquire drought or salinity tolerance may, for example, also acquire modified tolerance to biotic stresses, which could result in changes in interactions with its herbivores, parasitoids and pathogens. Such crosstalk between the different types of stress-response mechanisms could, therefore, have both direct and indirect effects on organisms that interact with them.

*Points to consider:*

(a) Any intended or unintended change that may lead to selective advantage or disadvantage acquired by the LM plant under other abiotic or biotic stress conditions that could cause adverse effects;

(b) Any change in the resistance to biotic stresses and how these could affect the population of organisms interacting with the LM plant; and

(c) A change in the substances (e.g., toxin, allergen, or nutrient profile) of the LM plant that could cause adverse effects.

**Testing the LM plant in representative environments** *(see "Step 1" in the Roadmap)*

*Rationale:*

LM plants with tolerance to abiotic stress are intended to be cultivated under abiotic stress conditions. Therefore, in accordance with the general principles of Annex III to the Protocol that risk assessments should be carried out on a case-by-case basis, it is of particular importance that the assessment of potential adverse effects of LM plants with tolerance to abiotic stress be conducted in relation to the 'likely potential receiving environment' of the LM plant under consideration.

Regionally variation in receiving environments that may influence the characteristics and the behaviour of the LM plant as well as its interactions with the environment should be taken into account during the risk assessment. Regions and locations where data are collected or field trials are conducted should represent the range of agricultural, plant health and environmental conditions the LM plant is expected to encounter

Different environments may be distinguished, for example, by differences in flora and fauna, soil property/chemistry, agricultural practices, climatic and geographic conditions, etc. Relevant characteristics of a specific region such as agricultural practice, climatic and geographic conditions should be determined at the start of the risk assessment as these characteristics may lead to differences in potential adverse environmental effects which only become evident if assessed on a regional level.

*Points to consider:*

(a) The likely potential receiving environment where exposure to the LM plant may occur and its characteristics such as information on geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin and centres of genetic diversity;

(b) Regional differences that may influence the characteristics and the behaviour of the LM plant with tolerance to abiotic stress including, for example, agricultural practices and agronomic structures (e.g., input of nitrogen fertilizers), cultivation systems (e.g., low-tillage farming), crop rotation practices, climatic conditions, occurrence of non-target organisms, as well as other abiotic and biotic conditions;

(c) Locations where field trials have been conducted to generate data for the risk assessment, if applicable, and how the conditions of the field trials represent the range of conditions expected in the likely potential receiving environment(s) in different regions;

(d) Relatives which can crossbreed with the LM plant in the likely receiving environment and the possible consequences of introgressing the abiotic stress tolerance traits into these species;

(e) How the LM plant behaves when the tolerance trait is not expressed because of the absence of the stressor, e.g., drought tolerance under normal water regimes.

**Persistence in agricultural areas and invasiveness of natural habitats** *(see "Step 1", "Step 2", "Step 3" and "Step 5" in the Roadmap)*

*Rationale:*

Climate conditions, water availability and soil salinity are examples of factors that limit the growth, productivity, spread or persistence of a plant species. Expression of the genes for abiotic stress tolerance could result in increased persistence of the modified plant in agricultural areas. Expression of these genes may also alter the capacity of LM plants to establish in climatic and geographic zones beyond those initially considered as the likely potential receiving environments.

In the event where the modified gene is a transcription factor conferring tolerance to abiotic stress, the transcription factor may also affect the response mechanisms to other forms of abiotic stress. For example, the seeds of a plant modified for drought or salinity tolerance may acquire in addition tolerance to cold resulting in an increased winter survivability of the seeds. Therefore, an abiotic stress-tolerant LM plant may acquire the potential to persist better than its non-modified counterpart and other species under different abiotic-stress conditions.

Most tolerance traits can be expected to have a "metabolic cost" associated with them – usually energy cost which may impact the potential for the plant to persist under conditions of low selection pressure (i.e. low abiotic stress). The metabolic cost can have a significant impact on the potential of the LM plant to survive and persist in an environment over time and should be taken into account when assessing the potential of the LM plant to persist in agricultural areas and natural habitats.

*Points to consider:*

(a) Consequences of any increased potential for persistence of the modified plant in agricultural habitats, and invasiveness and persistence in natural habitats;

(b) Need for and feasibility of control measures if the abiotic stress-tolerant LM plant shows a higher potential for persistence in agricultural or natural habitats, that could cause adverse effects;

(c) Characteristics that are generally associated with weediness such as prolonged seed dormancy, long persistence of seeds in the soil, germination under a broad range of environmental conditions, rapid vegetative growth, short lifecycle, very high seed output, high seed dispersal and long-distance seed dispersal; and

(d) Effects of climate change that could change the habitat range of the LM plant in the likely potential receiving environment;

(e) Implications of modified agricultural practices associated with use of the LM plant expressing tolerance to abiotic stress.

**Effects on the abiotic environment and ecosystem** *(see "Step 3" in the Roadmap)*

*Rationale:*

The cultivation of LMOs may lead to changes in the abiotic characteristics of the receiving environment, such as climate, abiotic soil fractions. Changes to the abiotic environment resulting from the use of LMOs will depend largely on the introduced trait, and may be relevant for LMOs with altered tolerance of certain environmental conditions.

The development of LM plants with tolerance to abiotic stress(es) may allow for an expansion of arable lands and cultivation areas of these plants in natural environments. The increase in the area of land for food production may be harmful to the natural environment and the consequences to biodiversity should be assessed.

The cultivation of LM plants with tolerance to abiotic stress may also lead to changes at ecosystem-level, for example by allowing certain accompanying pests to breed in ecosystems where they were not previously present.

*Points to consider:*

(a) Changes in the geography and extension of arable lands;

(b) Agricultural practices related to the LM plant and how these may alter the abiotic environment and ecosystem;

(c) Modelling tools, if available, to predict how the changes in agricultural practices due to the LM plant may affect the abiotic environment.

## BIBLIOGRAPHIC REFERENCES

See references relevant to "*Risk Assessment of LM plants with Tolerance to Abiotic Stress*": http://bch.cbd.int/onlineconferences/abioticref_ahteg_ra.shtml

## C. RISK ASSESSMENT OF LIVING MODIFIED MOSQUITOES

### INTRODUCTION

Living modified (LM) mosquitoes are being developed through modern biotechnology to reduce transmission of vector-borne human pathogens, particularly those that cause malaria, dengue and chikungunya. Control and reduction of such diseases is a recognized public health goal. The impacts of such diseases on human health are staggering. For instance, in 2008, there were 247 million cases of malaria and nearly one million deaths.[29] Therefore, specific and comprehensive considerations should be undertaken with regard to the potential benefits and adverse effects of LM mosquitoes.

The biology and ecology of mosquitoes, on the one hand, and their impact on public health as vectors of human and animal diseases, on the other hand, pose specific considerations and challenges during the risk assessment process.

Two strategies of modern biotechnology, namely self-limiting and self-propagating strategies, are being developed to produce LM mosquitoes to control vector-borne diseases.

Self-limiting strategies are being developed to control mosquito vectors by suppressing their population or reducing their competence by developing LM mosquitoes that are unable to produce viable offspring. This can be achieved, for instance, by interrupting larval development of the offspring. As such, LM mosquitoes developed under self-limiting strategies are not expected to pass the modified trait to subsequent generations. Modern biotechnology techniques for the development of self-limiting LM mosquitoes populations (e.g., "Release of Insects carrying a Dominant Lethal" or RIDL) are different from those based on the use of irradiation to induce male sterility because they aim to produce populations that are *behaviourally sterile* Other self-limiting strategies target metabolic processes of the mosquito vectors and aim at lowering their fitness and thereby reducing their populations.

Self-propagating strategies, also known as self-sustaining, rely on *gene-drive systems* that promote the spread and persistence of the transgene through populations of the same mosquito species. As opposed to the self-limiting strategy, the modifications in the LM mosquitoes produced through self-propagating strategies are intended to be heritable and to spread through the target population and, thus, to persist in the ecosystem at least in the medium term. The objective of the self-propagating strategies is, hence, population replacement of the non-modified mosquitoes by the LM mosquitoes that have been modified to render them less capable of transmitting a disease. In a related approach, gene-drive systems may be used to promote the spread of a gene that confers a fitness load or a male bias in the offspring ratio. In this way, gene-drive systems may be used to suppress vector population sizes or induce a cascade of population crashes. An example of such a system is an X-shredding homing endonuclease gene (HEG) which can be driven into a population at the same time as biasing the offspring ratio towards males and hence potentially inducing an all-male population crash.

Another strategy, the so-called paratransgenesis, is under development to control, reduce or eliminate the capacity of the mosquitoes to transmit pathogens – mainly, but not exclusively, by blocking the development of the pathogen in the vector. Paratransgenesis focuses on utilizing symbionts of insects, which may be genetically modified to express molecules within the vector that are deleterious to the pathogens they transmit. So, rather than genetically modifying the mosquitoes, the focus of paratransgenesis is on the genetic modification of microorganisms that inhabit the mosquito midgut. Such microorganisms may have a specific, symbiotic relationship with the mosquito, or may be commonly associated with the mosquito but not have an obligate relationship. Paratransgenesis can be used as a self-limiting strategy for population suppression or as a limited self-propagating strategy for population replacement (see above). In the case of paratransgenesis the mosquito itself will not be genetically modified, but the symbionts or parasites will most likely be the product of modern biotechnology, and therefore this type of strategy is also being mentioned here.

[29] WHO (2010) Malaria fact sheet. Available at http://www.who.int/mediacentre/factsheets/fs094/en/.

The mosquitoes developed through the different strategies will differ, for example, in their ability to persist in the environment and to spread the inserted transgenes into the local mosquito population, or even into other organisms. Therefore, the risk assessment needs and criteria will depend on the specific characteristics of the LMO and the strategy used.

Since this guidance is not focused on one particular type of technology or genetic mechanism, additional and more specific guidance may be necessary when conducting the risk assessment of a particular LM mosquito depending, among other things, on the strategy used. The risk assessment of LM mosquitoes performed on a case-by-case basis may also benefit from a broader approach using laboratory and confined field tests together with mathematical modelling.

## OBJECTIVE AND SCOPE

The objective of this document is to give additional guidance on the risk assessment of LM mosquitoes in accordance with Annex III to the Cartagena Protocol on Biosafety.[30] Accordingly, it complements the Roadmap for Risk Assessment of LMOs, giving emphasis to specific issues that may need special consideration for the environmental release of LM mosquitoes.

This document focuses on the risk assessment of LM mosquitoes of the family *Culicidae*, developed through self-limiting and self-propagating strategies to be used in the control of human and zoonotic diseases such as malaria, dengue, chikungunya, yellow fever and West Nile. Paratransgenesis is not in the scope of this guidance.

## PLANNING PHASE OF THE RISK ASSESSMENT

Specific and comprehensive considerations are warranted regarding the potential adverse effects of a particular LM mosquito, taking into account the species of the mosquito, the LM trait, the intended and unintended receiving environment, and the objective and scale of the intended release. These considerations should focus on, for instance: (a) the kinds of possible adverse effects for which there are scientifically plausible scenarios; (b) the species as well as ecological and epidemiological processes that could be affected by the introduction of the LM mosquitoes; (c) the protection goals of the country where the LM mosquitoes will be introduced; and (d) a conceptual link between the identified protection goals and the introduction of the LM mosquito into the environment.

The biology and, to some extent, the ecology of the mosquito species that transmit malaria and dengue are rather well known in many regions of the world. However, in certain regions and in the environment where LM mosquitoes are likely to be introduced, more information may be needed depending on the nature and scale of the LM strategy to be deployed. In many of these environments few studies have been conducted to examine gene flow among vectors, their mating behaviour, the interactions among vectors sharing one habitat, how pathogens respond to the introduction of new vectors, etc. Such information may be needed to establish a baseline in order to successfully assess the risks of LM mosquitoes. Additionally, methods for the identification of specific ecological or environmental hazards are also needed.

Identification of the likely potential receiving environment of an LM mosquito will depend on several factors, including whether specific release sites have been planned and whether natural or artificial barriers are present that could limit the dispersal of the LM mosquito. In some cases, risk assessors may need to consider the entire national territory or even neighbouring countries as the likely potential receiving environment (see also "Unintentional Transboundary Movement" below).

[30] The Parties to the Cartagena Protocol on Biosafety have mandated the AHTEG to 'develop a "roadmap", such as a flowchart, on the necessary steps to conduct a risk assessment in accordance with Annex III to the Protocol and, for each of these steps, provide examples of relevant guidance documents'. The Roadmap is meant to provide reasoned guidance on how, in practice, to apply the necessary steps for environmental risk assessment as set out in Annex III of the Protocol. The Roadmap also demonstrates how these steps are interlinked.

**The choice of a comparator** *(see "Planning Phase of the Risk Assessment", "The choice of comparators" in the Roadmap)*

*Rationale:*

The line/strain used as a recipient organism for transformation may serve as a comparator for the risk assessment of LM mosquitoes. The approach of using a (near-)isogenic line may be a challenge. Where successive passages are used to develop a strain of the LM mosquito, the parental LM strain may be used as an additional comparator.

## CONDUCTING THE RISK ASSESSMENT

**Characterization of the LM mosquito** *(See "Step 1" in the Roadmap)*

*Rationale:*

Description of the mosquito species should include its sub-species and strains, including their bio-geographical distribution, ecological niche, and capacity to transmit the pathogen, and may include the use of reliable molecular markers.

*Points to consider:*

(a) Description of the genetic modification, and the molecular characterization associated with the relevant technologies with particular attention to sequences which might influence the mobility of the insert in the mosquito (such as transposable elements);

(b) *Stability of the transgene* and the likelihood of mutations in the transgene(s) and changes in the insertion site(s) (in the case of mobile DNAs) in response to selection in the receiving environment.

**Effects on biological diversity (species, habitats, ecosystems, and ecosystem function and services)** (*See "Step 2" and "Step 3" in the Roadmap*)

*Rationale:*

The role of mosquitoes in natural ecosystems should be assessed, as the release of LM mosquitoes may have a negative impact on the target vector and pathogen[31] and other non-target species. Potential adverse effects will vary from case to case and may include:

*New or more vigorous pests, especially those that have adverse effects on human health:*

(a) The released LM mosquitoes may not function as expected, for example due to gene silencing or undetected failures in the development of self-limiting LM mosquitoes, which could result in the release of sexually competent mosquitoes and thus increase the vector population or disease transmission.

(b) Mosquito species are currently able to transmit several pathogens from viruses to filaria to human beings and animals. An LM mosquito, in which the capacity of transmission of one of these pathogens has been modified, may have a positive effect on the transmission of other pathogens.

(c) Suppression of the target mosquito might cause the population of another vector species to increase, resulting in higher levels of the target disease or the development of a new disease in humans and/or animals. These other vector species may include other mosquito vectors of other diseases.

[31] For the purpose of this guidance, the term "target vector" refers to the mosquito that transmits the disease and "target pathogen" is the disease causing agent transmitted by the target mosquito.

(d) The released LM mosquitoes may become pests.

(e) The released LM mosquitoes may cause other pests to become more serious, including agricultural pests and other pests that affect human activities. For example, the replacement of *Aedes aegypti* by *Aedes albopictus* could occur as the result of a release. Such risks should be monitored through time and at the appropriate geographical scale.

*Harm to or loss of other species:* The released LM mosquitoes might cause other species (for instance, birds, bats or fish that rely seasonally on mosquitoes for food) to become less abundant. These include species of ecological, economic, cultural and/or social importance such as wild food, endangered, keystone, iconic and other relevant wildlife species. Ecological effects might result from competitive release if the target mosquito population is reduced, or from trophic consequences of species that rely on mosquitoes for food at specific times of the year. Effects may also occur if (i) the target mosquitoes transmit a disease to animal species, (ii) the released LM mosquitoes transmit a disease to animal species more efficiently, (iii) another vector of an animal disease was released from control when the target mosquito population was reduced, or (iv) the target pathogen's abundance is reduced or eliminated, leading to effects on other organisms that interact with it, for example, by altering the population of another animal that hosts the pathogen.

Mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that will not allow interspecific gene flow. However, if interspecific mating between released LM mosquitoes and other mosquito species occurs, it could disrupt the population dynamics of these other species. Moreover, cessation of transmission of pathogens to other animals (e.g., West Nile virus to birds, Rift Valley fever virus to African mammals) might alter the population dynamics of those species, favouring increases in their numbers.

*Disruption of ecological communities and ecosystem processes:* The ecological communities in the ephemeral, small aquatic habitats occupied by the non-LM mosquitoes are unlikely to be disrupted beyond the possibilities already addressed above under "harm to or loss of other species." However, if the released LM mosquitoes were to inhabit natural habitats (e.g., tree-holes), disruption of the associated community is a possibility.

The introduction of LM mosquitoes may have adverse effects on valued ecosystem processes, often referred to as "ecosystem services", such as pollination, or on processes that support normal ecosystem functioning. The adult male and female mosquitoes feed on nectar of flowers and participate in the pollination of plants in a similar way as butterflies, Hymenoptera and other Diptera. In cases where mosquito species are significant pollinators, mosquito control of any kind may reduce the rate of pollination of some plant species or cause a shift to different kinds of pollinators.

Moreover, mosquitoes, both adults as well as larvae, are a food source for many predators (e.g., insects, lizards and even birds), and are responsible for the transfer of large amounts of biomass from aquatic to terrestrial ecosystems. As such, habitats in which mosquitoes are the dominant insect fauna (e.g., high Arctic tundra) could be affected if mosquitoes were eliminated. However, common target vector species are usually associated with human activity and therefore not as closely tied to ecosystem services.

*Points to consider:*

(a) The natural dispersal range and seasonality of the host mosquito;

(b) Impacts on the target mosquitoes and pathogens resulting from the management and use of the strategy under consideration;

(c) Whether the LM mosquitoes have the potential to cause adverse effects on other species which will result in the other species becoming agricultural, aquacultural, public health or environmental pests, or becoming a nuisance or a health hazard;

(d) The effect of the transgene on the fitness of the LM mosquito in the receiving environment, including the areas to which the LM mosquito may spread, in particular if a self-sustaining technology is implemented;

(e) Whether the target mosquito species is native or exotic to a given area;

(f) The normal and potential habitat range of the target mosquito species and whether the habitat range is likely to be affected by climate change;

(g) Whether the LM mosquitoes would be more susceptible to infection by other vector-borne disease pathogens;

(h) Whether the mosquito is a member of a species complex in which inter-specific mating occurs;

(i) Whether the introduction of LM mosquitoes is likely to affect other mosquito species that are pollinators or otherwise known to be beneficial to ecosystem processes;

(j) The consequences of likely mutations resulting from the mosquito's interactions with other organisms in the environment, and any potential changes in its response to abiotic stresses;

(k) Whether the LM mosquitoes are likely to affect other organisms with which they interact (e.g., predators of mosquitoes), and whether that could lead to an adverse effect (e.g., on the food chain);

(l) Whether, in the absence of the target mosquito, niche displacement by other disease vector species may occur, and if so, whether that can result in an increased incidence of the target disease or other diseases in humans or animals;

(m) Whether the LM mosquito has potential for natural long-distance transboundary dispersal or transport by anthropogenic mechanisms (e.g., used tires, aircraft, ships);

(n) Whether changes in land management in the receiving environment (e.g., wetland drainage, irrigation practices) would occur as a result of the introduction of LM mosquitoes, and what consequences these changes could have on biodiversity.

**Vertical gene transfer** (*See "Step 2" and "Step 3" in the Roadmap*)

*Rationale:*

For self-propagating LM mosquitoes, gene-drive systems for moving genes into wild populations may be the initial focus when assessing the risks of vertical gene transfer from LM mosquitoes to non-LM mosquitoes through cross-fertilization. The risk of vertical gene transfer in self-limiting LM mosquitoes is likely to be lower than for self-propagating LM mosquitoes, but should nevertheless be assessed on a case-by-case basis (see below). Various factors may influence gene flow and any associated adverse effects, such as the strategy used in the development of the LM mosquito, characteristics of the transgenes, characteristics of the gene-drive system, the stability of the trait(s) carried by the mosquito over generations, and characteristics of the receiving environment.

Some LM mosquitoes are being developed to spread the introduced trait rapidly through the target mosquito population. For instance, when introduced into *Anopheles gambiae*, the trait may be expected to spread throughout the *A. gambiae* species complex. Other LM mosquito technologies are designed to be self-limiting and, in such cases, spread of the transgenes or genetic elements in the target mosquito population is not intended or expected. For the self-limiting technologies, the potential for an unexpected spread of the introduced trait should be considered by focusing on the assumption that any management strategy to limit the spread could fail. The likelihood and consequences of this hazard can be gauged by assessing the fitness of the transgene should the self-limiting mechanism fail to prevent spread of the transgene.

Gene flow between different species should be considered for all of the LM mosquito technologies in spite of the fact that mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that will not allow interspecific gene flow. Identifying the key reproductive isolating mechanisms and possible conditions that could lead to the breakdown of such mechanisms is of particular importance in the risk assessment of LM mosquitoes with this trait. In addition, the fitness (dis)advantage conferred by the introduced trait to the LM mosquito and frequency of the introduction of the LM mosquito into the environment will affect its population size as well as the likelihood and rate of spread of the transgenes or genetic elements.

For self-sustaining strategies, the initial numbers of LM mosquitoes released may be small, however their persistence in the environment will provide continuing opportunities for novel interactions and mutations that may not be detected in limited trials. Although sexual sterility (cytoplasmic incompatibility) may prevent the transfer of the microorganism to some species, the risks due to rare exceptions to the normal mating pattern should be considered.

*Points to consider:*

(a) Whether LM mosquitoes have the potential to transfer the modified traits to wild mosquito populations (when it is not an intended strategy), and if so, the occurrence of any potential undesirable consequences;

(b) Whether LM mosquitoes have the potential to induce undesirable characteristics, functions or behaviour within the target mosquito species or a sexually compatible species complex.

**Horizontal gene transfer**

*Rationale:*

LM mosquitoes may be associated with symbionts and/or parasites such as microorganisms. In particular, potential adverse effects as a result of the interaction between LM mosquitoes and *Wolbachia* could warrant attention because mosquitoes are currently infested by these bacteria. Empirical evidence suggests that horizontal gene transfer between mosquitoes and *Wolbachia* may occur. Since *Wolbachia* seems to reduce host fitness and to hamper virus transmission, such as for the Dengue viruses, potential adverse effects to the *Wolbachia* could change the capacity of the mosquitoes to transmit diseases.

*Points to consider:*

(a) Presence of symbionts and parasites in the LM mosquitoes and whether there may be exchange of genetic information between the host and the microorganism;

(b) Whether LM mosquitoes have the potential to induce undesirable characteristics, functions, or behaviour in other organisms, particularly in bacteria living in symbiosis;

(c) Nucleic acid sequences in the LM mosquito which might influence the mobility of the insert and transgenes (such as mobile elements) and that share homology with sequences in the microorganism.

**Persistence of the transgene in the ecosystem** (*See "Step 2", "Point to consider (f)" and "Step 3", "Point to consider (a)(iii)" and "Point to consider (b)" in the Roadmap*)

*Rationale:*

Some of the transgenes in LM mosquitoes are designed not to persist whereas others are expected to spread rapidly and/or persist in wild populations. In cases where LM mosquitoes have been found through the risk assessment process to have the potential to cause adverse effects to biological diversity, taking

also into account human health, methods to reduce the persistence of the transgene in the ecosystem need to be considered.

*Point to consider:*

(a) Any undesirable consequence should the transgene persist in the ecosystem;

(b) Methods to reduce the persistence of the transgene.

**Evolutionary responses (especially in target mosquito vectors or pathogens of humans and animals)** (*See "Step 1" in the Roadmap*)

*Rationale:*

Any strong ecological effect also exerts an evolutionary selection pressure on the human and animal pathogens and the mosquito vectors. The main evolutionary effects of concern are those that could result in a breakdown in the effectiveness of the technology and the resumption of previous disease levels. Some LM mosquito strategies aim at modifying the mosquito vector's ability to transmit diseases by altering its physiological mechanisms. An evolutionary effect resulting in the development of resistance to modified physiological mechanisms in the targeted pathogen might occur when modifying mosquito vector competence. This might harm the effectiveness of the strategy used and result in a population of pathogens that may be transmitted more easily by additional vectors.

Other evolutionary effects could be hypothesized, including effects resulting from climate change, but they would first imply the occurrence of some adverse effect on a species, community or ecosystem.

*Points to consider:*

(a) Whether the target mosquito vector has the potential to evolve and avoid population suppression, regain vector competence or acquire new or enhanced competence against another disease agent, and if so, the occurrence of any possible undesirable consequences;

(b) Whether the trait has the potential to evolve and thus lose its effectiveness, or the pathogen to evolve and overcome the limitation posed by the genetic modification, and if so, the occurrence of any possible undesirable consequences.

**Unintentional transboundary movements**[32]

*Rationale:*

Mosquitoes, being LM or not, have very broad geographical distribution. Individual mosquitoes however within their lifetime have dispersal distances commonly of less than 5 km and for some urban species, as short as 200 meters. Confinement will therefore be highly dependent upon the species and the strategy used to develop the LM mosquito. Self-limiting sterile male types of technologies are expected to be highly confined temporally and spatially. On the other extreme, confinement of self-propagating LM mosquitoes to a particular receiving environment or to a country is unlikely and may result in transboundary movement between countries.

The risk of dispersal due to anthropogenic activities, such as transport and trade of potential sources of breeding sites such as tyres or lucky bamboos should be considered. The consequences of water management practices, such as irrigation or sewage water treatment, on the introduced LM mosquito strains should also be taken into account.

In cases where LM mosquitoes are modified with gene-drive systems, confinement may not be possible even when efforts are made to reduce long-distance dispersal due to anthropogenic activities.

[32] See Article 17 (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-17).

*Points to consider:*

(a) The type of strategy used in the development of the LM mosquito (i.e., self-limiting or self-propagating with gene-drive systems);

(b) Presence of natural or artificial barriers that could limit the spread and unintentional transboundary movement of the LM mosquito.

**Risk management strategies** (*See "Step 5" in the Roadmap*)

*Rationale:*

Risk assessors should consider risk management strategies such as monitoring the LM mosquitoes to ensure that the technology is functioning as intended and to identify unintended adverse effects. Strategies for halting release or recalling the LM mosquitoes, as well as mitigation methods if an unanticipated effect occurs, should be considered. Careful implementation of the technology including the planning of mitigation measures (such as an alternative set of control measures should a problem occur) and the integration of other population control methods should also be taken into account. In some circumstances methods to reduce the persistence of the transgene in the environment or to mitigate adverse effects resulting from the expression of the transgene might be needed. Monitoring during and after the environmental release of the LM mosquitoes to enable prompt detection of unexpected adverse effects may also be considered.

In the development of LM mosquitoes, male and female mosquitoes are commonly segregated at the pupal stage, according to the size of pupae. Some self-limiting strategies rely on releasing male LM mosquitoes only and require that no female LM mosquitoes are released. Understanding and measuring the reliability and failure rate of this segregation process and having quality control measures in place will be important in such cases.

*Points to consider:*

(a) Availability of monitoring methods to:

(i) Measure the efficacy and effectiveness of LM mosquito technology, including gene-drive systems and segregation of male LM mosquitoes;

(ii) Detect the transgene and other markers that distinguish the LM mosquito from non-LM mosquitoes in the receiving environment;

(iii) Detect the spread of the transgenes into mosquito strains other than the target strain, for example by using reliable molecular markers to distinguish the strains;

(iv) Assess the potential evolutionary long-term effects of the LM mosquito technology (monitoring for transgene stability and proper function over time);

(v) Determine the level to which the identified adverse effects may be realized, including detection of unexpected and undesirable spread of the transgenic trait (e.g., monitor for undesirable functions or behaviours within target species and other wild related species);

(b) Availability and feasibility of mechanisms to recall or confine the LM mosquitoes and transgenes in case they spread unexpectedly (e.g., mass release of wild-type mosquitoes above a certain threshold, alternative control methods including genetic control);

(c) Effectiveness and availability of conventional methods of mosquito control (e.g., insecticides, larval site destruction, trapping) to control LM mosquito strains as compared to the non-modified strain;

(d) Availability of methods for managing the dispersal of the LM mosquitoes and ensuring that they do not establish themselves beyond the intended receiving environment (e.g., vegetation-free zones, traps, high threshold gene-drive systems);

(e) Availability of methods to manage potential development of resistance (e.g., in the target vector or pathogen);

(f) Whether the release of an LM mosquito would affect pest control activities, such as the use of personal protection and insecticides that control other vectors.

## RELATED ISSUES

There are other issues that may be taken into consideration in the decision for environmental releases of LM mosquitoes which are not covered by Annex III of the Protocol. They encompass, *inter alia*, social, economic, cultural and health issues associated with the use of LM mosquitoes. LM mosquitoes will require broader considerations of how target-disease risk affects human behaviour, veterinary medicine, public health practices and national health priorities.

## BIBLIOGRAPHIC REFERENCES

See references relevant to "*Risk Assessment of LM Mosquitoes*":

http://bch.cbd.int/onlineconferences/mosquitoesref_ahteg_ra.shtml

*Annex*

## USE OF TERMS

This section provides a working glossary of key terms used in this document. An attempt was made to adapt definitions that are used in internationally accepted risk assessment guidance to the context of this document.

**Assessment endpoint –** In the context of environmental risk assessment: An explicit expression of the environmental value that is to be protected, operationally defined as an entity (such as salmon or honeybees, soil quality) and its attributes (such as their abundance, distribution or mortality) (adapted from IPCS, 2001, Integrated Risk Assessment, http://www.who.int/ipcs/publications/new_issues/ira/en/). [back to the text]

**Baseline** – A measurement of the existing conditions of an environment or its components without the LMO under consideration and taking into account different practices in use (e.g., agricultural practices). The baseline measurement provides quantitative (e.g., number of organisms, variability of abundance) and/or qualitative information about the receiving environment as a reference for estimating effects of the LMO or its use including, if applicable, information on the assessment endpoints. [back to the text]

**Behavioural sterility** – A type of sterility that is caused by changes in behaviour rather than to physiological changes. [back to the text]

**Case-by-case** – An assessment approach where each LMO release is considered relative to the environment in which the introduction is to occur and to the intended use of the LMO in question (IUCN, 2003, An Explanatory Guide to the Cartagena Protocol on Biosafety, http://bch.cbd.int/database/record-v4.shtml?documentid=41476). [back to the text]

**Combinatorial effects –** Effects that arise from the interactions between two (or more) genes in one organism, including epistatic interactions. The effects may occur at the level of gene expression, or through interactions between RNA, or among gene products. The effects may be analysed as qualitative or quantitative; quantitative effects are often referred to as resulting in antagonistic, additive or synergistic effects (see also "Cumulative effects" for distinction). [back to the text]

**Consequence (of the adverse effect)** – The outcome, extent and severity of an adverse effect associated with exposure to an LMO, its handing and use, or its products (in the context of Annex III paragraph 5). [back to the text]

**Conventional** – Resulting from traditional breeding and selection and not involving the use of modern biotechnology as defined in Article 3 of the Cartagena Protocol on Biosafety. [back to the text]

**Co-transformation –** Techniques of modern biotechnology using two or more transformation vectors to produce an LMO. [back to the text]

**Cumulative effects** – Effects that occur due to the presence of multiple LMOs or their products in the receiving environment (see also "Combinatorial effects" for distinction). [back to the text]

**EC50 (median effective concentration)** – A concentration that is statistically or graphically estimated to cause a specified effect in 50% of a group of test organisms under specified experimental conditions (IPCS, 2001, Integrated Risk Assessment, www.who.int/ipcs/publications/new_issues/ira/en/). [back to the text]

**Ecological function (or "ecological services")** – the role of an organism in ecological processes. The relevance of specific ecological functions in the risk assessment will depend on the protection goals. For example, organisms may be part of the decomposer network playing an important role in nutrient cycling in soils, or may be important as a pollen source for pollinators and pollen feeders. [back to the text]

**Exposure** – The route and level of contact of an LMO or its products to the likely potential receiving environment, including, for instance, the co-occurrence of the LMO or its products and target- or non target-organisms. (adapted from IPCS, 2001, Integrated Risk Assessment, www.who.int/ipcs/publications/new_issues/ira/en/). [back to the text]

**Gene-drive system –** Method for introducing and spreading a desired gene into populations, e.g., mosquito (adapted from Hood E, 2008, Selfish DNA versus Vector-Borne Disease, Environmental Health Perspectives 116: A69; www.ncbi.nlm.nih.gov/pmc/articles/PMC2235231/pdf/ehp0116-a00066.pdf). [back to the text]

**Gene flow –** For the use of this term in the context of this Guidance, see "Vertical gene transfer" and "Horizontal gene transfer". [back to the text]

**Gene product** – The RNA or protein that results from the expression of a gene. [back to the text]

**Genotypic (characteristics)** – Relating to "genotype" as all or part of the genetic constitution of an organism. [back to the text]

**Hazard** – The potential of an organism to cause harm to human health and/or the environment (UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

**Hazard characterization** – The qualitative and/or quantitative evaluation of the nature of the adverse effects associated with an LMO (adapted from CODEX, 2001, Definitions of Risk Analysis Terms Related to Food Safety, http://www.fao.org/DOCREP/005/Y2200E/y2200e00.htm). [back to the text]

**Hazard identification** – The identification of the type and nature of adverse effects that an LMO has an inherent capacity to cause in an organism, system or (sub)population. (adapted from WHO, 2004, IPCS Risk Assessment Terminology, http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf). [back to the text]

**Heterozygous (genomes) –** Having different alleles at the corresponding chromosomal loci. [back to the text]

**Horizontal gene transfer** –The transfer of genetic information from one organism to another through means other than from parent to offspring (i.e. vertical) inheritance. Also referred to as "horizontal gene flow" or "lateral gene transfer". [back to the text]

**Introgression –** Introduction of genetic elements from an organism into the genetic pool of organisms of another species, sub-species or population eventually resulting in some fertile offspring. [back to the text]

**Isogenic line, (near-)** – In the case of a LM plant, its isogenic line is the non-LM line from which the LM plant is derived. Thus, the only difference between the isogenic line and the derived LM plant is the presence of the recombinant DNA. Near-isogenic lines are lines genetically identical to the LM plant except for some loci (adapted from EFSA, 2011, Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed, http://www.efsa.europa.eu/en/efsajournal/doc/2149.pdf). [back to the text]

**LD50 (median lethal dose)** – A statistically or graphically estimated dose that is expected to be lethal to 50% of a group of organisms under specified conditions. [back to the text]

**Likelihood (of the adverse effect)** – Probability or possibility of the adverse effect occurring, taking into account the level and kind of exposure of the likely potential receiving environment to the LMO. [back to the text]

**Management strategies** – See "Risk management". [back to the text]

**Multi-trophic (effects) –** Involving more than two trophic levels in a food web. [back to the text]

**"Omics" technologies** – A collection of - usually high-throughput - techniques to study an organism or group of organisms at the level of the genome, gene transcripts, proteins or metabolites, which depending on the level are specifically called "genomics", "transcriptomics", "proteomics" and "metabolomics", respectively. [back to the text]

**Outbreeding –** Breeding of individuals or populations that would typically not reproduce without human intervention, for instance, if the individuals are not closely related. [back to the text]

**Outcrossing** – The transmission of genetic elements from one group of individuals (e.g., population, crop variety) to another. In plants, outcrossing most commonly results from cross-pollination (adapted from GMO Compass, www.gmo-compass.org/eng/glossary. See also "Vertical gene transfer"). [back to the text]

**Potential receiving environment** – The range of environments (ecosystem or habitat, including other organisms) which is likely to come in contact with a released organism due to the conditions of the release or the specific ecological behaviour of the organism (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

**Phenotypic (characteristics)** – Relating to "phenotype" as the observable physical or biochemical characteristics of an organism, as determined by both genetic and environmental factors. [back to the text]

**Pleiotropic effects** – Effects of a single gene on multiple phenotypic traits. [back to the text]

**Protection goal** – A defined goal set out by a country that relates to desired environmental outcomes, and that guides the formulation of strategies for the management of human activities that may affect the environment. [back to the text]

**Re-transformation –** Use of modern biotechnology, as defined in the Protocol, to produce an LMO where the recipient organism is already an LMO. [back to the text]

**Risk** – The combination of the magnitude of the consequences of a hazard and the likelihood that the consequences will occur (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

**Risk assessment** – The process of estimating risks that may be associated with an LMO on the basis of what adverse effects may be caused, how likely the adverse effects are to occur, and the consequences should they occur (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). Risk assessment is often considered as part of a broader process called 'risk analysis' which may also include considerations such as risk management and risk communication. [back to the text]

**Risk characterization –** The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of potential adverse effects based on hazard identification, hazard characterization and exposure assessment (adapted from CODEX, 2001, Definitions of Risk Analysis Terms Related to Food Safety, http://www.fao.org/DOCREP/005/Y2200E/y2200e00.htm). [back to the text]

**Risk management** – The measures to ensure that risks identified in the risk assessment are reduced, controlled, or eliminated (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

**Risk threshold** – The level of tolerance to a certain risk or the level of change in a particular variable beyond which a risk is considered unacceptable. [back to the text]

**Stability (of the transgene) –** Permanence of the transgene in a defined genomic context and without changes to its structure or to its products. [back to the text]

**Synergism –** A interaction of elements that when combined produce a total effect that is greater than the sum of the individual elements. [back to the text]

**Transformation cassette –** A transformation cassette comprises a group of DNA sequences (e.g., parts of a vector and one or more of the following: a promoter, the coding sequence of a gene, a terminator, other regulatory sequences), which are physically linked and often originated from different donor organisms. The transformation cassette is integrated into the genome of a recipient organism through methods of modern biotechnology to produce an LMO. A transformation cassette may also be called "expression cassette" (mainly when a specific expression pattern is aimed at), "DNA cassette" or "gene construct". [back to the text]

**Transformation event** – An LMO, typically an LM plant, with a specific modification that is the result of the use of modern biotechnology applying *in vitro* nucleic acid techniques according to Article 3 (i) (a) of the Protocol. [back to the text]

**Transgene** – A nucleic acid sequence in an LMO that results from the application of modern biotechnology as described in Article 3 (i) (a) of the Protocol. [back to the text]

***Trans*-regulation –** Transcriptional regulation of gene expression by regulatory elements that were themselves transcribed in a different region of the genome. For example, a transcriptional factor transcribed in one chromosome may regulate the expression of a gene located in another chromosome. On the other hand, "*cis*-regulatory elements" are those that are physically and operationally linked to the genes that they regulate, e.g., promoters. [back to the text]

**Unintended effects** – Effects that appear in addition to or, in some cases, instead of the intended effects. Some unintended effects may be foreseen while others are unanticipated. [back to the text]

**Unintended gene product** – Gene products that occur, for example, when the inserted gene construct changes during the modification process (such as deletions, duplications, etc.) that give rise to gene products (e.g., proteins or metabolites) which are different from those intended originally, as well as when new open-reading frames are created through the fusion of (parts of) the transgenes to endogenous sequences forming chimeric gene products. [back to the text]

**Unmanaged and managed ecosystems –** An "unmanaged ecosystem" is an ecosystem that is free from significant human intervention, such as wetlands and nature preserves, as opposed to a "managed ecosystem", which is an ecosystem affected by varying degrees of human activities, such as farm lands, plantations, aquaculture sites and urban parks. [back to the text]

**Vector –** In the context of genetic modification, a vector is an organism (e.g., virus) or a DNA molecule (e.g., plasmid) used to assist the transfer of genetic material from a donor organism to a recipient organism (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). In the context of epidemiology, a vector is an organism, often an arthropod (e.g., mosquito), that transmits a pathogen (e.g., plasmodium) to a host (e.g., humans). [back to the text]

**Vertical gene transfer** – Transfer of genetic information from one organism to direct descendants via asexual division, crossing or sexual recombination. Also referred to as "vertical gene flow". [back to the text]

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