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Item 3.1 of the provisional agenda*

CHAIR'S DRAFT FOR A REVISION OF THE "GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS"

Note by the Executive Secretary

1. In accordance with the terms of reference provided in decision BS-V/12, the Open-ended Online Expert Forum and the ad hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management are to develop a revised version of the "Guidance on Risk Assessment of Living Modified Organisms" as one of the expected outcomes of their work to be presented to the sixth meeting of the Parties to the Protocol.

2. Following a scientific review process[1] of the first version of the Guidance and a round of online discussions under the Open-ended Online Forum[2] as set out in decision BS-V/12, the Chair of the AHTEG, in consultation with the AHTEG Bureau and the Secretariat, incorporated the views submitted by Parties, other Governments and relevant organizations into a Chair's draft.

3. The Chair of the AHTEG is circulating the attached draft to the Group as the basis of its work under item 3.1 of the provisional agenda.

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* UNEP/CBD/BS/AHTEG-RA&RM/3/1.

1 All submissions received through the scientific review are available in the Biosafety-Clearing House (BCH) at http://bch.cbd.int/onlineconferences/guidance_ra/review.shtml.

2 All contributions received through the online discussion groups are available in the BCH at http://bch.cbd.int/onlineconferences/discussiongroups_ra.shtml.

Annex

GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

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 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]

Annex III of the Protocol states that "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. (…) 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".[4]

This document was developed by the Open-ended Online Expert Forum and the Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management in accordance with its 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.[5] It is intended to be a "living document" that will be modified and improved over time as new experience becomes available and new developments in the field of applications of LMOs occur, as and when mandated by the Parties to the Cartagena Protocol on Biosafety.

This Guidance consists of two parts. In part I the Roadmap for Risk Assessment of LMOs is presented. The Roadmap provides an overview of the risk assessment process. In part II specific guidance is provided on the risk assessment of specific types of LMOs and traits.

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[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] Annex III, paragraphs 3, 4 and 6.

[5] 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).

PART I:

ROADMAP FOR RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

BACKGROUND

This "Roadmap" provides an overview of the process of environmental risk assessment for a living modified organism (LMO) in accordance with Annex III[6] to the Cartagena Protocol on Biosafety (hereinafter "the Protocol") and all other articles related to risk assessment. Accordingly, this Roadmap is a guidance document and does not replace Annex III. The overall aim of the Roadmap is facilitating and enhancing the effective use of Annex III by elaborating the technical and scientific process of how to apply the steps and points to consider in the process of risk assessment.

The purpose of this Roadmap is to provide further guidance on using Annex III with additional background material and links to useful references relevant to risk assessment. The Roadmap may be useful as a reference for risk assessors when conducting or reviewing risk assessments and in capacity-building activities.

This Roadmap aims at providing a set of information that would be broadly relevant in the risk assessment of LMOs belonging to different taxa[7] and their intended uses within the scope and objective of the Protocol, and in accordance with Annex III. However, it has been developed based largely on living modified (LM) crop plants because of the extensive experience to date with environmental risk assessments for these organisms.

The Roadmap applies to all types of environmental releases of LMOs, including those of limited duration and scale (e.g., field trials of LM crops) as well as long-term, large scale releases (e.g., for commercial production), taking into account that the amount and type of information available and needed to support risk assessments of the different types of environmental releases may vary from case to case.

INTRODUCTION

The novel combination of genetic material in an LMO may have several effects which may vary depending on the LMO itself, the environment exposed to the LMO and how the LMO is used. The effects may be intended or unintended, taking into account that some *unintended effects* may be predictable.

According to the Annex III, the objective of risk assessment under the Protocol is "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".[8]

In this context, risk assessment of LMOs is a structured process performed on a *case-by-case* basis to identify and evaluate the potential adverse effects, and their *likelihood* and *consequences* as well as to identify possible ways for managing the identified risks.

What is considered an adverse effect as well as an "acceptable risk" depends on *protection goals* and *assessment endpoints* taken into consideration when scoping the risk assessment. The choice of protection goals by the Party, as laid down in its relevant legislation implementing the Convention on Biological Diversity, could be informed by Annex 1 of the Convention. Societal and economic considerations may be taken into account, in accordance with Article 26 of the Protocol, in addition to the environmental

[6] http://www.cbd.int/biosafety/articles.shtml?a=cpb-43.

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

[8] Annex III, paragraph 1.

considerations that are taken into consideration in the environmental risk assessment that is the subject of this guidance.

Paragraph 8 of Annex III provides a description of the key steps of the risk assessment process to identify and evaluate the potential adverse effects and to identify strategies to manage risks. The steps of risk assessment under the Protocol are similar to those used in other risk assessment frameworks. Although the terminology varies among the various approaches to risk assessment, in general terms, they comprise actions for "hazard identification", "hazard characterization", "exposure assessment", and "risk characterization".

Paragraph 9 of Annex III describes, depending on the case, points to consider in the process for LMO risk assessment.

In drawing from Annex III, the Roadmap includes five steps that describe an integrated process whereby the results of one step may be relevant to other steps. Also, risk assessment may need to be conducted in an iterative manner, where certain steps may be repeated or re-examined to increase or re-evaluate the confidence in the conclusions of the risk assessment (see Flowchart). When new information arises that could change its conclusions, the risk assessment may need to be re-examined accordingly. Similarly, the issues mentioned in the 'Setting the context and scope' section below can be taken into consideration again at the end of the risk assessment process to determine whether the objectives and criteria that were set out at the beginning of the risk assessment have been met.

The concluding recommendations derived from the risk assessment in step 5 are required to be taken into account in the decision-making process on an LMO. In the decision-making process, other Articles of the Protocol or other relevant issues may also be taken into account and are addressed in the last paragraph of this Roadmap: 'Related Issues'.

A flowchart illustrating the risk assessment process according to this Roadmap is annexed hereto.

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

PLANNING PHASE

Overarching issues in the risk assessment process

An LMO risk assessment may be preceded by a design/planning phase during which some overarching issues may be considered to ensure the quality and relevance of the information used. Although there are no internationally accepted standards for data gathering, some considerations at the planning phase may be useful. These entail, for example:

- Setting criteria for relevancy of the data in the context of a risk assessment – e.g. data may be considered relevant if they are linked to protections goals or assessment endpoints, contribute to the identification and evaluation of the potential adverse effects of the LMO, or can affect the outcome of the risk assessment.
- Establishment of scientifically robust criteria for the inclusion of scientific information.
    - It is crucial that only data of acceptable scientific quality are used in the risk assessment process. 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. Data may be derived from a variety of sources, e.g. new experimental data as well as data from relevant peer reviewed scientific literature. Sound statistical tests should be used in the risk assessment and be fully described in the risk

assessment report. Also, it is important to have expertise in multiple fields even when this leads to diverging or contradictory views;

- Sound science is based on transparency, verifiability, and reproducibility (e.g. reporting of methods and data in sufficient detail, so that the resulting data and information could be confirmed independently), and on the accessibility of data by the risk assessors (e.g. the availability of relevant, required data or information or, if requested and as appropriate, of sample material), taking into account the provisions of Article 21 of the Protocol on the confidentiality of information. The provisions of sound science serve to ensure and verify that the risk assessment is carried out in a scientifically sound and transparent manner;
- Useful information can also be gained from international standards and guidelines and, in the case of LM crop plants, also from the experience of farmers and growers.

- Identification and consideration of uncertainty.

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".[9]

Uncertainty is inherent in the concept of risk. To date, "there is no internationally agreed definition of 'scientific uncertainty', nor are there internationally agreed general rules or guidelines to determine its occurrence. Those matters are thus dealt with – sometimes differently – in each international instrument incorporating precautionary measures".[10, 11]

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.

The *source(s)* of uncertainty may stem from the data/information itself or from the choice of study design including the methods used, and the analysis of the information.

For each identified source of uncertainty, the *nature* of the uncertainty may be described as arising from: (i) lack of information, (ii) incomplete knowledge, and (iii) inherent variability, for example, due to heterogeneity in the population being studied.

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

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

[10] *An Explanatory Guide to the Cartagena Protocol on Biosafety*, paragraph 57 (http://data.iucn.org/dbtw-wpd/edocs/EPLP-046.pdf).

[11] Article 10, paragraph 6, of the Protocol: "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."

In cases where the nature of the uncertainty implies that it cannot be addressed through the provision of more data during the risk assessment, it may need to be dealt with by monitoring or possibly *risk management* (see step 5).

Uncertainty is an inherent and integral element of a risk assessment. As such, it is important to conduct a systematic analysis of the various forms of uncertainty that can arise when identifying potential adverse effects (step1), their likelihood (step 2) and consequences (step 3), and during the evaluation of the overall risk of an LMO (step 4). 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 conclusions and recommendations of the risk assessment.

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

Setting the context and scope

A risk assessment carried out on a case-by-case basis starts by setting its context and scope in a way that is consistent with the country's protection goals, assessment endpoints, *risk thresholds* and *management strategies*.

Setting the context and scope for a risk assessment in line with the country's policies and regulations may involve an information and consultation process of risk assessors, decision-makers and various stakeholders prior to conducting the actual risk assessment to identify which protection goals, assessment endpoints and risk thresholds may be relevant. It may also involve framing the risk assessment process and identifying questions to be asked that are relevant to the case being considered.

A number of aspects may be taken into consideration, as appropriate, that are specific to the Party involved and to the specific case of risk assessment. These aspects include:

- Existing environmental and health policies and strategies based on, for instance:
  - (i) Regulations and the 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 done, for instance, in the relevant legislation of the Party;
- Intended (and as well as potential, unintended) conditions of handling and use of the LMO, taking into account customary practices and habits that could affect the protection goals or assessment endpoints;
- Identification of methodological and analytical requirements, including any reviewing mechanisms, that is required to achieve the objective of the risk assessment as laid down, 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);
- The nature and level of detail of the information that is required, which may, amongst 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 cultivation. For small scale field releases, especially at early experimental stages, less information may be necessary as compared to the information for large scale environmental release, and for commercial scale planting;

- Experience and history of use of the non-modified recipient, taking into account its *ecological function*; and
- Criteria for describing the level of the (potential) environmental adverse effects of LMOs, as well as criteria for the terms that are used to describe the levels of likelihood (step 2), the magnitude of consequences (step 3) and risks (step 4) and the acceptability or manageability of risks (step 5; see risk assessment steps below).

Some risk assessment approaches 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 assessment is done in a comparative manner, meaning that risks associated with LMOs should be considered in the context of the risks posed by the non-modified recipient organism in the likely potential receiving environment.[12]

The choice of the appropriate comparator depends on the nature and the scope of the risk assessment and on the questions that are being asked. In choosing the appropriate comparator and establishing a *baseline*, it is important to determine the validity and relevance of the information for the risk assessment. Moreover, it is important that any practice associated with the use of the comparator(s), for example, agricultural management systems, also be taken into account when establishing the baseline for a comparative risk assessment. Other issues may also be taken into account when choosing a comparator, for instance, that the behavior of a *transgene*, as that of any other gene, may vary because it depends on the genetic and physiological background of the recipient as well as on the ecological characteristics of the environment that the LMO is introduced into.

Ideally, the non-modified comparator that is going to provide the baseline for comparison is one that is grown at the same time and location as the LMO under consideration. Nevertheless, establishing a good baseline for a comparative risk assessment may prove difficult in certain cases, such as for the risk assessment of LM plants tolerant to abiotic stress and LM mosquitoes (please refer to Part II of this Guidance).

Experience and knowledge with similar organisms may be taken into consideration, as appropriate, along with the non-modified recipient organism in the risk assessment of an LMO. For instance, (near-)isogenic lines or closely related non-modified organisms should be used in step 1 of the risk assessment (see below) where the novel genotypic or *phenotypic characteristics* associated with the LMO are identified.

When the potential consequences of adverse effects are evaluated, broader experience, such as mentioned in step 3 (a) may also be taken into account when establishing a baseline. Results from experimental field trials or other environmental information and experience with the same LMO may be taken into account as information elements in a new risk assessment for that LMO.

Moreover, the experience of some countries in applying the concepts of "*familiarity*" and "*substantial equivalence*" in their risk assessment frameworks for comparisons with non-modified agricultural

[12] Annex III, paragraph 5.

varieties or similar LMOs and their interaction with the environment may also be taken into consideration.

THE RISK ASSESSMENT

To fulfill its objective under Annex III, as well as other relevant Articles of the Protocol, risk assessment is performed in five steps in an integrated process and iterative manner, as appropriate. These five steps are indicated in Paragraph 8 (a)-(e) of Annex III and also detailed below. Their titles have been taken directly from the paragraphs 8 (a)-(e) of Annex III.

For each step a rationale and points to consider are provided. Some points to consider are taken from paragraph 9 of Annex III, whereas others have been added based on generally accepted methodology of LMO risk assessment and risk management. The relevance of each point to consider will depend on the case being analyzed.

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

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." [13]

Rationale:

The purpose of this step is to identify adverse effects resulting from changes due to the genetic modification(s), including any deletions, compared to the non-modified organism, and identify what, if any, changes could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

The question that is asked in this step is "what could go wrong". The step is similar to the 'hazard identification step' in other risk assessment guidance. In some other risk assessment approaches, this step is performed together with the context and scoping phase in the so-called "Problem formulation" step, which is not limited to the identification of hazards, but also take into account the operationalisation of protection goals and the identification of appropriate assessment endpoints.

In performing this step of the risk assessment, the difference in the concepts of "*risk*" and "*hazard*" has to be taken into account (see Use of Terms).

In this step, scientifically plausible scenarios are identified in which novel characteristics of the LMO could give rise to adverse effects in an interaction with the likely potential receiving environment. In this regard, it is important to define, as far as possible, a causal link or pathway between a characteristic of the LMO and a possible adverse effect otherwise the next steps of the risk assessment may generate information that will not contribute to reaching a recommendation that will be useful for the decision-making process.

The comparison of the LMO carried out in step 1 is performed with the non-modified recipient, or a (near-)isogenic line or, as appropriate, with a non-modified organism of the same species, 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 genotypic or phenotypic. These include any changes in the LMO, ranging from changes at the transcriptional or translational level to visual changes.

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

The novel characteristics of the LMO that may cause adverse effects may be intended or unintended, predicted or unpredicted, taking into account that an adverse effect may also be caused by, for example, changes in the expression levels of endogenous genes as a result of the genetic modification or by *combinatorial effects* of two or more genes. The points to consider below provide information elements on which hazard identification can be built.

The type and level of detail of the information required in this step may 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 release. For example, the information needed to conduct the risk assessment for an LMO to be released into the environment will differ from the information needed for an LMO to be imported for direct use as food, feed or for processing. Alternatively, less information may be available in the case of releases whose objective is to generate information for further risk assessments, such as field trials, especially at early experimental stages. Likewise, in cases where the exposure of the environments to the LMO is limited, such as for some early-stage field trials, less information may be needed in performing this step of the risk assessment. The resulting uncertainty in such cases may be addressed by risk management measures (see step 5).

Points to consider regarding the characterization of the LMO:

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

(i) its biological characteristics, in particular those that, if changed or interacting 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, centers of origin and centers of genetic diversity;

(iv) ecological function; and

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

(b) 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 that could cause adverse effects in the recipient);

(c) 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 or integrity within the genome of the recipient organism, levels of gene expression and intended and *unintended gene products*;

(d) 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;

(e) Consideration of genotypic (see point to consider (c) 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 at the transcriptional and translational level and may be due to the insert itself or to genomic changes due to the transformation or recombination processes.

Point to consider regarding the receiving environment:

(f) Characteristics of the likely potential receiving environment, in particular its attributes that are relevant to potential interactions of the LMO that could lead to adverse effects (see also paragraph (h) below),[14] taking into account the characteristics that are components of biological diversity particularly in centers of origin and genetic diversification;

(g) The intended scale and duration of the environmental release.

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

(h) Protection goals or assessment endpoints (see Introduction, Setting the context and scope);

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

(j) Considerations for *unmanaged* (such as wetlands and nature preserves) and *managed ecosystems* (such as agricultural, forest and aquaculture systems) concerning the use of an LMO and that are relevant for the likely potential receiving environment. These include the potential effects resulting from the use of an LMO including, for instance, changes in farm management practices, dispersal of the LMO through ways 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;

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

(l) Effects on target and non-target organisms;

(m) Effects of the incidental exposure of humans to (parts of) the LMO (e.g. exposure to pollen), and the toxic or allergenic effects that may ensue; and

(n) Potential adverse effects as a consequence of *horizontal gene transfer* of transgenic sequences from the LMO to any other organism in the likely receiving environment. 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;

(o) *Cumulative effects* with any other LMO present in the environment; and

(p) A consideration of uncertainty arising in step 1 that may significantly impact the identification of hazards in this step (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" in the Introduction).

›› See references relevant to "Step 1":

[14] 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.

http://bch.cbd.int/onlineconferences/roadmapref_ahteg_ra.shtml#step1

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 in Step 4, the likelihood that each of the adverse effects identified in Step 1 will occur has to be assessed and evaluated.

One aspect to be considered is whether the receiving environment will be exposed to the LMO in such a way that the identified adverse effects may actually occur, e.g. taking into consideration the intended use of the LMO, and the expression level, dose and environmental fate of transgene products as well as plausible pathways of a hazard leading to adverse effects. For determining the route of exposure to the LMO being assessed or its products, it is needed to establish causality between the LMO and the potential adverse effect. This can be done by building conceptual models describing relationships between the LMO, and pathways of exposure and potential effects in the environment. For example, concerning an LMO producing a potentially toxic gene product, oral or dermal exposure would be relevant.

Conceptual models, validated through experimental studies, may be used for an assessment of the potential exposure, combined with the use of statistical tools relevant for each case.

Examples of issues to be considered in this step include (i) the potential of the LMO (or its derivatives resulting from outcrossing) to spread and establish beyond the receiving environment (in particular into protected areas and centers of origin and genetic diversity), and whether that could result in adverse effects; and (ii) the possibility of occurrence of adverse (e.g. toxic) effects on organisms (or on organisms other than the 'target organism' for some types of LMOs).

The levels of likelihood may be expressed, for example, by the terms 'highly likely', 'likely', 'unlikely', 'highly unlikely'. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.

Points to consider:

(a) Information relating to the type and intended use of the LMO, including the scale and duration of the release, bearing in mind, as appropriate, user habits, patterns and agronomic practices. For example, in the case of field trials, the level of exposure in the receiving environment may be minimized due to the scale of the release, its temporary nature and the implementation of management measures;

(b) The relevant characteristics of the likely potential receiving environment that may experience or may be a factor in the occurrence of the potential adverse effects (see also step 1 (e), (f) and (g)), taking into account the variability of the environmental conditions and any long-term adverse effects. 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 insecticidal proteins, toxins and allergens;

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

(d) Factors that may affect spread of the LMO, such as its reproductive ability (e.g. time to seeding, number of seed and vegetative propagules, dormancy, pollen viability), its spread by natural means (e.g. birds, wild animals, wind, water, etc) and its spread by people (e.g. deliberate spread, accidental spread by machinery, mixed produce, etc);

(e) Factors that affect persistence of the LMO, such as the ability of seedlings to establish amongst existing vegetation;

(f) When assessing the likelihood of outcrossing and outbreeding 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) the chance of introgression of the transgene into the sexually compatible species;

(g) Expected exposure of the environment where the LMO is released and means by which incidental exposure could occur at that location or elsewhere (e.g. through gene flow or incidental exposure due to losses during transport and handling);

(h) A consideration of uncertainty arising in step 2 (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).

›› 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 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 genetic diversification, and taking into account protection goals and endpoints of the country where the risk assessment is being carried out.

In this step, results of tests done under different conditions, such as laboratory experiments or experimental field releases, are considered. The scale of the intended use (e.g. trial or commercial) should be taken into account. The evaluation is comparative and should be considered in the context of the adverse effects caused by the non-modified recipient or, if more appropriate, by a near-isogenic line, other non-modified organisms of the same species or other comparators (see Introduction). The evaluation may also be considered in the context of the adverse effects that occur in the environment and which are associated with existing practices or the introduced management system related to the LMO (such as various agronomic practices, for example, for pest or weed management) if such information is available and relevant.

It is important to also assess in this step whether the consequence of an adverse effect is of short or long term, direct or indirect, or either reversible or irreversible.

The evaluation of the consequence of adverse effects may be expressed qualitatively or quantitatively. For instance, 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 experience with existing practices with the non-modified recipient or, if more appropriate, with a non-modified organism of the same species in the likely potential receiving environment, may be useful in order to establish baselines to evaluate, for example, the effects of:

(i) agricultural practices on the level of inter- and intra-species gene flow, dissemination of the recipient, abundance of volunteer plants in crop rotation, abundance of pests, abundance of beneficial organisms such as pollinators and pest predators;

(ii) pest management on non-target organisms through pesticide applications while following accepted agronomic practices;

(iii) in relevant cases: the behaviour of relevant wild-type populations of unmodified animal or insect species, including interactions between predators and prey, disease transmission and interaction with humans or animal species;

(b) Combinatorial and cumulative effects;[15]

(c) Results from laboratory experiments examining, *inter alia*, dose-response relationships (e.g., *EC50*, *LD50*) in the context of determining effects on non-target organisms, and from field trials evaluating, for instance, potential invasiveness;

(d) For the case of outcrossing to sexually compatible species, the possible adverse effects that may occur, after introgression, due to the expression of the transgenes in the sexually compatible species; and

(e) A consideration of uncertainty arising in step 3 that may significantly impact the evaluation of consequences should the adverse effects be realized (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).

›› 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 is to determine and characterize the level of the overall risk based on the individual risks that were identified on the basis of the identification of scientifically plausible scenarios and an analysis of the potential adverse effects in step 1, their likelihood (step 2) and consequences (step 3), and also taking into consideration any relevant uncertainty that emerged in the preceding steps.

A consideration of whether the adverse effects are reversible or irreversible is an important task during this step.

To date, there is no universally accepted method to estimate the overall risk but rather a number of methods 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 weighted and combined. Risk matrixes are often used for this purpose.

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

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

[15] See "Use of terms" section.

Points to consider:

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

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

(c) The evaluation of the consequences (step 3);

(d) Risk management options, if identified in step 5;

(e) Any interaction, such as addition or synergism, between the identified individual risks;

(f) Any cumulative effect due to the presence of various LMOs in the receiving environment; and

(g) A consideration of uncertainty arising in this and the previous steps (see "Identification and consideration of uncertainty" under "Overarching issues in the risk assessment process" above).

›› 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.

This step is an interface between the process of risk assessment and the process of decision-making. It is noted that, while the risk assessors provide recommendations based on their scientific findings, it is the decision-makers who decide whether or not the risks identified are acceptable and whether or not to approve the LMO. Deciding whether and which risk management measures should be implemented is also part of the decision-making process.

The "acceptability" of risks varies from country to country. As seen in the "Setting the context and scope" section in the Introduction, during the planning phase of a risk assessment, risk assessors identify what are the criteria for the acceptability of risks within their own countries. On this basis, a recommendation as to whether the overall risk posed by the LMO is acceptable or not is made in relation to established protection goals, assessment endpoints and risk thresholds, also taking into account risks posed by the non-modified recipient and its use.

In evaluating the acceptability of the overall risk of the LMO, a question arises as to whether risk management options can be identified that could reduce the identified risks and uncertainties. If such measures are identified, the preceding steps of the risk assessment may need to be revisited.

In the process of the formulation of risk management options, the effect of the proposed options on the identified risks should be explained. The appropriate steps of the risk assessment should then be reiterated by taking into account the implementation of the risk management options to estimate the new levels of likelihood, consequence and risk and to assess if the risk management measures are appropriate and sufficient.

The recommendation on the acceptability of risk(s) should also acknowledge the previously identified uncertainties. For assessments associated with uncertainties, 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 management 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 is a helpful tool to detect unexpected adverse effects and a means to reduce uncertainty, address assumptions made during the risk assessment and to validate its conclusions on a wider (e.g. commercial) level of application. Monitoring may also be used as an early warning instrument for risk management. On the other hand, it is worth noting that monitoring is not an appropriate risk management tool to reduce risks.

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

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

Points to consider related to the acceptability of risks:

(a) Established criteria and thresholds for the acceptable/unacceptable levels of risk, including those set out in national legislation or guidelines, as well as the protection goals of the Party, as identified when setting the context and scope for a risk assessment;

(b) Any relevant experience with the use of the non-modified recipient(s) used to establish baselines for the risk assessment, and practices associated with its use in the potential receiving environment;

Points to consider related to the risk management strategies:

(c) 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. isolation distances to reduce outcrossing potential of the LMO, modifications in herbicide or pesticide management, crop rotation, soil tillage, etc.;

(d) 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 in case the results from monitoring call for them;

(e) 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 against these proteins); and

(f) The feasibility of the implementation of the proposed risk management or monitoring strategies and methods for measuring their efficacy and effectiveness.

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

RELATED ISSUES

Issues that may be part of the decision making process, as appropriate, and that are mentioned in other articles of the Protocol, or are emerging in ongoing discussions on the decision making process include, *inter alia*:

- Risk management (Article 16, i.e. implementation of risk management as part of the decision-making process);
- Capacity-building (Article 22);
- Public awareness and participation (Article 23);
- Socio-economic considerations (Article 26);
- Liability and redress (Article 27);
- Co-existence;
- Ethical issues.

Annex

FLOWCHART FOR RISK ASSESSMENT

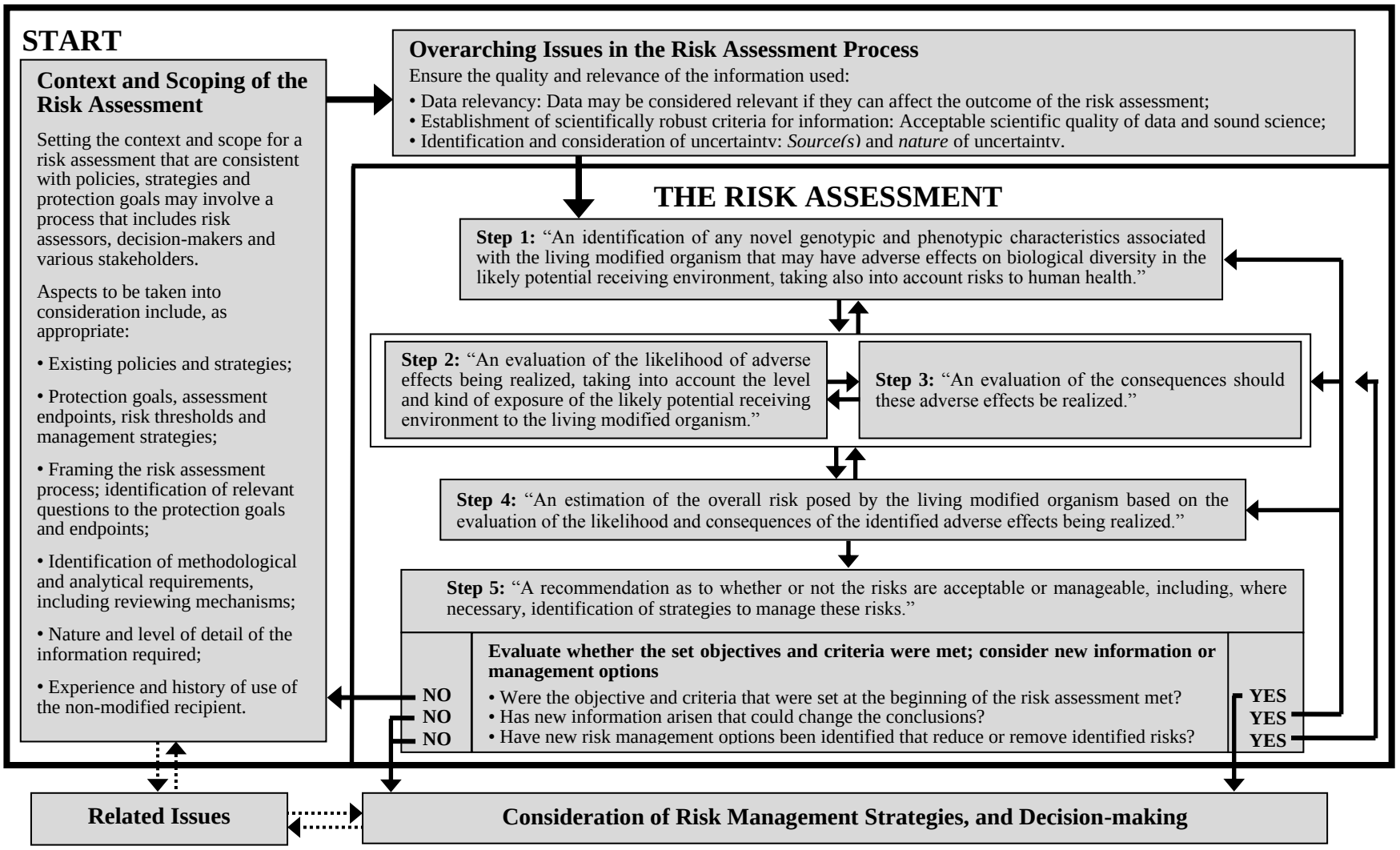

Figure 1. The Roadmap for Risk Assessment. The flowchart represents the steps to *identify* and *evaluate* the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. Risk assessments may need to be conducted in an iterative manner, where certain steps may be repeated or re-examined as shown by the flow of solid 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

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 transgene cassettes (i.e., co-transformation).

This guidance focuses on stacked transgenic traits that have been produced through cross-breeding involving two or more LM plants.

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.

OBJECTIVE

The objective of this section is to give additional guidance on the risk assessment of LM plants with stacked genes or traits generated through conventional crossing of single or multiple event LM plants.

It aims at complementing the Roadmap for Risk Assessment of LMOs while 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 plants also follow the general principles outlined in the Roadmap, but take into account the specific issues outlined in this section of the present document.

SCOPE

This guidance focuses on stacked events resulting from conventional crossings between single transformation events or already stacked events as parental lines so that the resulting LM plant contains two or more transgenic traits and/or transformation cassettes. It is understood that the individual transformation events making up the stacked event have been assessed previously in accordance with Annex III of the Cartagena Protocol on Biosafety and as described in the Roadmap.

LM plants with multiple transgenic traits or genes resulting from re-transformation, co-transformation or transformation with a multi-gene transformation cassette are outside the scope of this section and should be assessed according to the Roadmap.

Likewise, the scope of this section is restricted to those LM plants generated through the methods of modern biotechnology as defined in Art. 3(i)(a) of the Protocol. LM plants derived from fusion of cells are not covered in this guidance.

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

THE RISK ASSESSMENT

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

Rationale:

As for any other type of LMO, the risk assessment of a stacked LM plant is done 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.

It is noted that the lack of isogenic lines to be used as comparators may present challenges to the interpretation of data when establishing the baseline for the risk assessment of a stacked LM plant. In the case of recipient organisms that are highly heterozygous, the resulting hybrids will display high variability and a vast range of phenotypes. This variability should be taken into account during the establishment of a baseline for the comparative risk assessment.

Moreover, many of the stacked LM plants produced are the result of multiple rounds of cross-breeding involving 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 should be carefully considered.

Points to consider:

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

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

(c) Level of stacking and the use of intermediate stacked LMOs as additional comparators.

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

Rationale:

During cross-breeding, changes may occur to the molecular characteristics of the inserted genes/genetic elements at the insertion site as a result of recombination, mutation and rearrangements.

Although recombination, mutation and rearrangements are not limited to LMOs, transgenes with similar genetic sequences may be more likely to undergo recombination, since homologous recombination acts on genomic regions that have identical or highly similar sequence. Alternatively, complex inserts with multiple repeats are known to be less stable and could also be more likely to undergo rearrangements during cross-breeding.

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).

A molecular characterization of the stacked LM plant may be carried out to confirm the intactness of the insertion sites, transgenes and other genetic elements of the transformation cassette as compared to the parental LMOs. If changes are found, they may also be an additional basis for assessing any intended or unintended possibly adverse effects. 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 assessment of the parental LMOs.

Points to consider:

(a) Availability, specificity and sensitivity of methods to carry out a molecular characterization of the stacked LM plant;

(b) Consequences for reliability of detection methods

(c) Phenotypic changes that may suggest 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);

(d) Whether an identified change in the sequence of the transgenes and/or genetic elements could lead to an adverse effect.

Potential interactions between combined genes and the resulting phenotypic changes *(see "Step 1", "Point to consider (d)" in the Roadmap)*

Rationale:

It is possible that the combination of two or more LMOs in a stacked event may influence the expression level of the transgenes or of endogenous genes through *trans-regulation*.

Changes in gene expression that may be specifically attributable to stacked events are most likely to occur if the transgenes or regulatory elements from the two parental LMOs bear similar genetic elements among themselves or to an endogenous sequence (e.g. same binding sites for transcriptional factors) and 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. Therefore, 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 should be assessed whether the different transgenes belong to the same biochemical pathways or physiological processes.

Points to consider:

(a) 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;

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

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

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

Rationale:

Assessment of combinatorial and cumulative effects[16] is based on the environmental risk assessment data for the stacked event LMO 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 through the insertion of multiple genes in the same stacked LM plant can have unpredicted synergistic effects also on endogenous genes and metabolic pathways. 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 on DNA- or RNA-level and/or between proteins and metabolites should be investigated and the potential adverse effects arising from them should be thoroughly assessed. An assessment of potential combinatorial and cumulative effects may be performed, for instance, by conducting phenotypic and compositional analyses, toxicity tests on non-target organisms and any other study that integrate these multiple and interacting factors to predict the adverse effects. Also, indirect effects due to changed agricultural management procedures, combined with the use of the transgenic stacked event LMO, may be taken into consideration.

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 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) Compositional analysis of the stacked event to determine the amounts of substances with potentially harmful effects (e.g. allergens, anti-nutritional factors, etc.) taking into account the possibility of persistence and accumulation of these substances in the environment, such as in the food chain, and if the amounts differ from those produced by the parental LMOs or non-modified recipient organisms;

(d) Additional information depending on the nature of the combined traits. For example, further toxicological analysis of the stacked event may be required to address any combinatorial effects arising from the stacking of two or more insecticidal traits that result in a broadened target range or increased toxicity.

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

Crossing and segregation of transgenes *(see "Step 1", "Point to consider (k)", "Step 2", "Point to consider (f)", "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 intentional (i.e. mediated by man) or unintentional (i.e. natural outcrossings) and, depending on the segregation patterns, the new stacked LMOs could contain new and/or different combinations of transgenes and DNA fragments.

The higher the number of different sexually-compatible stacked LMOs being cultivated in the same environment, the more possible variations of new stacked events arising which contain different combinations of transgenes and DNA fragments, and the higher the probability of new unintentional stacking occurring.

Stacked events may have altered environmental impacts as a result of cumulative and combinatorial effects of the stacked traits prevalent in different LMOs of the same species in the receiving environment.

A risk assessment should address the possible adverse effects by all such stacked events with different combinations of transgenes and DNA fragments.

Points to consider:

(a) Presence of sexually-compatible non-modified relatives and their ecological function;

(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 LMOs, 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 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 (d)" in the Roadmap)*

Rationale:

Some of the risk management strategies for stacked events may involve 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 detection analysis is done 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. As such, in a bulk analysis of seeds, for example, it is not possible to tell apart a sample containing material from different transformation LMOs from another sample containing one or more stacked LM plants.

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 are unique for a single transformation event. It may become a challenge, however, in the future, when to detect single transformation events LMOs produced through site-specific insertions since the flanking sequences would be the same for different single transformation LMOs. This could become a problem particularly in the cases where the stacked event contains multiple transgenes and transformation cassettes with similar DNA sequences.

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

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 LMOs 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

The aim of this document is to provide further guidance for the risk assessment of living modified (LM) plants with improved tolerance to abiotic stress.

This guidance should be considered in the context of the Cartagena Protocol on Biosafety. The elements of Article 15 and Annex III of the Protocol also apply to LM plants with tolerance to abiotic stress. Accordingly, the methodology and points to consider[17] contained in Annex III are also applicable to this type of LMO.

The potential environmental adverse effects of an LM plant with abiotic stress tolerance depend on (i) the receiving environment; (ii) the plant species that was modified; (iii) phenotypic and genotypic changes made to the plant; and (iv) its intended use. A risk assessment would be performed on a case-by-case basis in accordance with Annex III of the Protocol and following the general principles of the Roadmap.

The considerations in this guidance complement the Roadmap for Risk Assessment of LMOs and aim at providing a general overview of issues that may be relevant when assessing the risks of LM plants with tolerance to abiotic stress(es). More specific and detailed guidance, as well as relevant background documents and risk assessment tools, are necessary for assessing the various types of modifications conferring abiotic stress tolerance to LM plants and impacting a range of key features of the plant, such as reproduction, composition and interaction with other species.

For the purpose of this guidance, “abiotic stresses” are deviations from physiologically normal conditions as reactions to non-living environmental factors and are detrimental or suboptimal to the growth, development and/or reproduction of a living organism. Types of abiotic stresses include, for example, drought, salinity, cold, heat, soil pollution and air pollution (e.g., nitrous oxides, ozone).

THE RISK ASSESSMENT

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, 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.

A major difficulty in performing a risk assessment of an LM plant with tolerance to abiotic stress via a comparative approach is the multiple interactions between the new trait and the receiving environment.

In plants, any gene or gene combinations providing increased tolerance to some abiotic stress is expected to have *pleiotropic effects* on the stress physiology of the plant, e.g. drought, temperature and salt stress are interconnected and plant responses to these stresses share multiple components and genes. Such pleiotropic effects may be classified as "unintended predicted effects" (see the Roadmap, step 1) and may be inferred during the risk assessment by examining the cross talk between different stress responses of the plant and assessing if the identified changes may cause adverse effects.

The stress tolerance of the LM plant should be assessed with respect to a set of environmental conditions, characterized by different intensities, durations and rates of installation and withdrawal of the stressor

[17] Paragraphs 8 and 9 of Annex III, respectively.

(e.g. drought, flood, suboptimal temperatures, salt or other toxic ions, etc.). This poses two difficulties (i) of controlling/measuring these conditions in field experiments to analyze the phenotype of the LM plant and generate data for the risk assessment, and (ii) of defining the phenotype of the LM plant itself, which in many cases may not be an unequivocal attribute of the LM plant but a complex relationship between external and physiological parameters.

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 receiving environment include:

- Would the tolerance trait have the potential to affect other tolerance and/or resistance mechanisms of the LM plant, for example, via pleiotropic effects?
- Would the tolerance trait have the potential to increase the invasiveness, persistence or weediness of the LM plant that causes adverse effects to other organisms, food webs or habitats?
- Would any 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?
- Would a LM plant expressing tolerance to a particular abiotic stress have other advantages in the targeted receiving environment that could cause adverse effects?

Some of the potential adverse effects to be evaluated in the risk assessment, from the introduction of LM plants tolerant to abiotic stress into the environment include, for example: a) increased selective advantage(s) other than the intended tolerance 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) consequences of potential gene flow to wild or conventional relatives. While these 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.

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 biodiversity.

The choice of comparators *(see "Planning Phase", "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, biological, intended and unintended changes 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. This step is the 'hazard identification step' in other risk assessment guidance.

The identification of genotypic and phenotypic changes in the abiotic stress tolerant LM plant, either intended or unintended, is typically done in comparison with the non-modified recipient organism. The non-modified comparator provides the baseline information for comparison of trials when it is grown at the same time and location as the LM plant. Comparisons with the observed range of changes in the non-modified plant in different environments, also provides baseline information.

The complexity of choosing the appropriate comparator(s) is increased for LM plants tolerant to abiotic stress due to the need to evaluate the expression of the new trait(s) in a range of environmental conditions 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 any 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.

Challenges with respect to experimental design: LM plants with tolerance to abiotic stress may present unique challenges in the experimental designing 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. In such conditions, choosing appropriate comparators could be a challenge and there are several proposals on whether and how the comparative approach can be used to characterize LM plants tolerant to abiotic stress in these likely receiving environments.

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 some situations where a suitable comparator is not available to allow for a meaningful comparison to be carried out, a characterization of the abiotic stress tolerant LM plant as a novel genotype in the receiving environment may be conducted. In the future, information available from *"omics" technologies*, for example, "transcriptomics" and "metabolomics", if available, may help to detect phenotypes and compositional changes (e.g., the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison between field grown plants at a suboptimal condition.

Points to consider:

(a) Characteristics of the LM plant under the abiotic stress and non-stress conditions and under different stresses, if applicable; and

(b) Whether one or more suitable comparators are available and the possibility of their use in the appropriate experimental design.

Unintended characteristics *(see "Step 1" in the Roadmap)*

Rationale:

Both intended and unintended changes to the LM plant which are directly or indirectly associated with the abiotic stress tolerance that may have adverse effects should be investigated. These include changes to the biology of the plant species (e.g. if the genes alter multiple characteristics of the plant) or to its distribution range in relation to the potential receiving environment (e.g. if the plant can grow where it has not grown before) that may cause adverse effects.

The abiotic-stress-tolerant LM plant may have unintended characteristics such as tolerances to other types of biotic and abiotic stresses, 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 and higher growing temperatures.

It is also possible the LM plants with enhanced tolerance to an abiotic stress could have changes in 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 steps in 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 have interactions and cross-talk. For that reason, a LM plant modified to acquire drought or salinity tolerance may, for example, also acquire a changed tolerance to biotic stresses, which could result in changes in interactions with their herbivores, parasitoids and pathogens. Such cross-talk 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:

Since LM plants with tolerance to abiotic stress are intended to be cultivated under abiotic stress conditions, it is important to consider the importance of regional aspects for the evaluation of specific characteristics and the environmental behaviour of this type of LMO as well as of its interactions with the environment. 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.

Hence, regionally differing factors 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 procedure. Regions and locations selected to collect data or conduct field trials should represent the range of agricultural, plant health and environmental conditions the LM plant is expected to encounter if and when a decision is taken to allow its commercial cultivation.

Different environments may be defined, for example, by the differences in flora and fauna, agricultural practices, climatic and geographic conditions, etc. Such relevant factors of a specific region or location should be determined at the start of the risk assessment, and calls for a broad and integrative concept. This is important as these factors 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 the location, its geographical, climatic and ecological characteristics, including relevant information on biological diversity and centres of origin;

(b) Regionally differing factors that may influence the characteristics and the behaviour of the LM plant with tolerance to abiotic stress including, for example, differences in occurrence or in the number of generations of target organisms, different agricultural practices and agronomic structures (e.g. input of nitrogen fertilizers), different cultivation systems (e.g. low-tillage farming), different crop rotation practices, different climatic conditions, different 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 regionally differing factors of the likely potential receiving environment(s);

(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.

Increased persistence in agricultural areas and invasiveness of natural habitats *(see "Step 1", "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 spread to and establish in climatic and geographic zones beyond those initially considered as the likely or potential receiving environments.

The gene(s) inserted for tolerance to, for instance, drought and salinity might also affect molecular response mechanisms to other forms of abiotic stress, such as cold temperatures. For example, when the genetic modification affects genes that also regulate key processes in seeds, such as abscisic acid (ABA) metabolism, physiological characteristics such as dormancy and accumulation of storage lipids may also be changed. In such cases, the seeds of a tolerant 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 under different abiotic-stress conditions.

Most tolerance traits can be expected to have a "metabolic cost" associated with them – usually an 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 the increased potential for persistence of the modified plant in agricultural habitats and consequences of increased potential for invasiveness and persistence in natural habitats;

(b) Need for and the 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 on agriculture and biodiversity and how this could change the habitat range of the LM plant in comparison to the non modified plant.

(e) If the LM plant expressing tolerance, would have a change in its agriculture practices;

(f) The "metabolic cost" of the modified traits.

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 or gases. Changes of the abiotic environment by 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 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 in the ecosystem, for example, by allowing certain accompanying pests to breed in different ecosystems than before.

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) Availability of modelling tools 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, including eradication of such diseases, is a recognized public health goal.

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 new considerations and challenges during the risk assessment process, which have dealt mainly with LM crop plants thus far.

Two strategies of modern biotechnology, namely self-limiting and self-propagating strategies, are being used (or are under development) to develop 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 genetically modified to be sterile. Being sterile, the LM mosquitoes that are developed under self-limiting strategies are unable to pass the modified trait on indefinitely through subsequent generations. Modern biotechnology techniques for developing sterile LM mosquitoes (e.g. "Released Insect with a Dominant Lethal" or RIDL) are different from those based on the use of irradiation to induce male sterility, but experience may be drawn also from such applications developed for other arthropod species.

Self-propagating strategies, also known as self-sustaining, rely on *gene-drive systems* that promote the spread of the transgenic traits through populations of the same or sexually compatible species. As opposed to the self-limiting strategy, LM mosquitoes produced through self-propagating strategies contain heritable modifications intended to spread through the target population.

A third strategy, the so-called paratransgenesis, is under development to control mosquitoes and other arthropod species. Paratransgenesis focuses on utilizing genetically modified insect symbionts to express molecules within the vector that are deleterious to pathogens they transmit. So rather than genetically modifying mosquitoes, the focus is on the genetic modification of bacteria that inhabit the mosquito midgut. Paratransgenesis can be used as self-limiting strategy (see above) either as population suppression or population replacement strategies. The microorganism may have a specific, symbiotic relationship with the insect, or it may be commonly associated with the insect but does not have an obligate relationship. It is noted that although in the case of paratransgenesis the mosquito itself will not be genetically modified, the symbionts or parasites will most likely be the product of modern biotechnology, and therefore this type of strategy is also being mentioned here.

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

This guidance document aims at helping to conduct risk assessments for environmental releases of LM mosquitoes.

It complements the Roadmap for Risk Assessment of LMOs and focuses on specific issues that may need special consideration on the risk assessment for environmental releases of LM mosquitoes. 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, of the strategy used. Moreover, 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.

The main emphasis of this guidance document is the assessment of potential risks to biodiversity. Nevertheless, the potential adverse effects to human health arising from environmental releases of LM mosquitoes should also be considered. Although the focus of this guidance is on LM mosquitoes, in principle, it may also be useful for the risk assessment of similar non-LM mosquito strategies.

OBJECTIVE

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.[18] Accordingly, it aims at complementing the Roadmap for Risk Assessment on specific issues that may need special consideration for the environmental release of LM mosquitoes.

SCOPE

This document focuses on the specifics aspects of risk assessment of LM mosquitoes developed to be used in the control of human and zoonotic diseases such as malaria, dengue, chikungunya, yellow fever and West Nile.

THE RISK ASSESSMENT

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

Rationale:

Specific and comprehensive considerations should be undertaken with respect to the potential adverse effects of a particular LM mosquito, taking into account the species of the mosquito, the LM trait, the intended receiving environment, and the objective and scale of the intended release. These considerations should focus on, for instance: (a) description of the genetic modification, with particular attention to sequences which might influence the mobility of the insert in the insect (such as transposable elements); (b) the kinds of possible adverse effects for which there are scientifically plausible scenarios; (c) the species and ecological processes that could be affected by the introduction of the LM mosquitoes; (d) the protection goals of the country where the LM mosquitoes will be introduced; and (e) a conceptual link between the identified protection goals and the introduction of the LM mosquito into the environment.

With regard to the taxonomy, defining a species is too vague for mosquitoes since they have worldwide distribution with many subspecies or strains, which have different properties, including ecological niche and capacity of pathogen transfer. For this reason, one of the first actions in the risk assessment should consist in the complete taxonomic characterisation of the strain used, including the use of reliable molecular markers and its bio-geographic origins and distribution.

The general approach of using a near isogenic line as a comparator will be a challenge for the risk assessment of LM mosquitoes. The line/strain used as recipient organism for transformation may serve as control and comparator in this case.

The biology and, to some extent, the ecology of the mosquito species that transmit malaria and dengue are well known in many regions of the world. However, in certain regions and in the environment where the LM mosquito is likely to be released, more information may be needed depending on the nature and scale

[18] 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.

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 between vectors sharing one habitat, how pathogens respond to the introduction of new vectors, etc.

An assessment of the current management measures to control the diseases transmitted by the mosquitoes, e.g. with pesticides, physical mitigation (e.g. mosquito nets) or medication taken by the host, may also be carried out. 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.

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

Rationale:

The role of mosquitoes in natural communities should be assessed, as the release of LM mosquitoes may have a negative impact on the target vector and pathogen[19] and other species. While addressing the interaction between LM mosquitoes and any other species, emphasis should be made to evaluation of the fitness of the LM mosquito and particularly its competitive capacity with the native strains and with other species of the same guild sharing the same kind of environment. This should be done for the aquatic larvae as for the adults.

New or more vigorous pests, especially those that have adverse effects on human health: (i) the released LM mosquitoes may not function as expected, for example gene silencing or undetected production failures could result in the release of non-sterile or competent mosquitoes and thus increase the vector population or disease transmission; (ii) 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. This point should also be taken into consideration; (iii) suppression of the target mosquito might result in the population of another vector species to increase and result 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; (iv) the released LM mosquitoes might become pests; (v) the released LM mosquitoes might cause other pests to become more serious, including agricultural pests and other pests that affect human activities. For example, the replacement of *A. aegypti* by *Ae. albopictus* could happen as the result of a release. Such risks should be monitored through time and at an 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 and this may affect other organisms that interact with it, for example, by altering the population of another animal that hosts the pathogen.

[19] 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.

Although mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that will not allow interspecific gene flow, if sterile interspecific mating between released LM mosquitoes and other mosquito species should occur, 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 released LM mosquitoes might degrade some valued ecosystem process. This might include processes such as pollination or support of normal ecosystem functioning. These processes are often referred to as "ecosystem services". However, the valued ecosystem processes may also be culturally or socially specific. Under some circumstances, mosquito species are significant pollinators. In those cases, mosquito control of any kind might reduce the rate of pollination of some plant species or cause a shift to different kinds of pollinators. Habitats in which mosquitoes are the dominant insect fauna (e.g., high Arctic tundra, tree holes) would be changed if mosquitoes were eliminated; however, the 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 of the host mosquito;

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

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

(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 invasive 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) Any other species (e.g. animal hosts, larval pathogens or predators of mosquitoes) in addition to the pathogen, that typically interact with the LM mosquito in the likely receiving environment;

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

(i) Genetic markers that are available to identify the transgene and which may distinguish genotypes of the released LM mosquitoes from those in the receiving environment;

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

(k) The likelihood of transgene mutation and target insertion site alteration (in the case of mobile DNAs) in response to selection in the receiving environment;

(l) The consequences of likely mutations for the mosquito interactions with other organisms in the environment and changes in its response to abiotic stresses;

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

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

(o) Whether the transgenic mosquito has potential for natural long-distance transboundary dispersal or transport by anthropogenic activities (used tires, aircraft, ships);

(p) Whether land management changes (wetland drainage, irrigation practices) would be likely to result from releasing the LMO and the consequences of these changes on biodiversity;

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

(r) Role of density dependence with regard to the release;

(s) Role of seasonality.

Vertical gene flow (*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 flow 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 smaller 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, the transgenes, the gene-drive system and the stability of the trait(s) carried by the mosquito over generations, as well as the receiving environment, etc.

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. Paratransgenic mosquitoes may mate occasionally with other species, so the effect on those species must be estimated. 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 and paratransgenic 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 and paratransgenic mosquitoes have the potential to induce undesirable characteristics, functions or behaviour within the target mosquito species or sexually compatible species complex.

Horizontal gene transfer

Rationale:

The risk of horizontal gene transfer among microorganisms increases with the use of paratransgenesis and should be assessed on a case-by-case basis.

Potential adverse effects as a result of the interaction between LM mosquitoes and *Wolbachia* could be given particular attention because mosquitoes are currently infested by these bacteria. Horizontal gene transfer between those species appears to occur and *Wolbachia* appears to reduce host fitness and to hamper virus transmission, such as for the Dengue viruses.

Points to consider:

(a) Sequences which might influence the mobility of the insert and transgenes (such as transposable elements);

(b) Presence of symbionts and parasites in the LM mosquitoes and whether there may be exchange of genetic information between the host and the microorganism (in the case of transgenic mosquitoes) or between the transgenic microorganism and other microorganisms in the host (in the case of paratransgenesis);

(c) In the case of paratransgenic mosquitoes, whether the microorganism specifically associated with the mosquito may be involved in horizontal gene transfer and, if not, possible effects that the transgenic microorganism will have in other hosts;

(d) Whether paratransgenic and LM mosquitoes have the potential to induce undesirable characteristics, functions, or behaviour to other organisms, in particular of bacteria living in symbiosis.

Persistence of the transgene in the environment (*See "Step 2", "Point to consider (e)" 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 through wild populations. In cases where the LM mosquitoes have been found through the risk assessment process to have the potential to cause adverse effects to the biological diversity, taking also into account human health, methods to reduce the persistence of the transgene in the environment need to be considered.

Point to consider:

(a) Any undesirable consequence should the transgene persist in the environment.

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 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 through changes in its physiological mechanisms. An evolutionary effect resulting in the development of resistance to 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 require the occurrence of some adverse effect on a species, community or ecosystem effect. Therefore, consideration of secondary evolutionary effects can be postponed until such effects are identified and found to be significant.

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 to 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 movement

Rationale:

Mosquitoes, being LM or not, have very broad dissemination spectra and geographical distribution. Therefore, it will be unlikely (if not impossible) to ensure the containment of the LM mosquitoes to a particular receiving environment and to a country. In other words, it is likely that the release of LM mosquitoes will result in unintentionally transboundary movements between countries.

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

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

Rationale:

Risk assessors may want to consider risk management strategies such as the quality control of the released LM mosquitoes and monitoring them and the environment for potential unintended adverse effects. There

should also be strategies in place for halting the release and application of mitigation methods if an unanticipated effect occurs. Careful implementation of the technology including the availability of mitigation measures (such as an alternative set of control measures should a problem occur) and the integration of other population control methods should be considered. 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 so as to address prompt detection of unexpected adverse effects may also be considered.

Points to consider:

(a) Availability of monitoring methods to:

(i) Measure the efficacy and effectiveness of LM mosquito technology;

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

(iii) Assess the potential evolutionary breakdown of the LM mosquito technology (monitoring for transgene stability and proper function over time);

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

(b) Availability of mechanisms to recall or contain 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 and paratransgenic 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.

OTHER ISSUES

There are other factors 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 application and acceptance of the technology. LM mosquitoes will require consideration of 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 guidances to the context of this document.

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

Baseline – A baseline consists of a measurement of the existing conditions of the environment and ecosystems prior to the introduction of the LMO under consideration and serves as a starting point for the risk assessment and as a basis to which all following measurements are compared. [back to the text]

Case-by-case – A case-by-case approach is one where each release of an LMO is considered relative to the environment in which the release is to occur, and/or 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 may arise from the interactions between two (or more) genes, 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 qualitative or quantitative; quantitative effects are often referred to as resulting in antagonistic, additive or synergistic effects (see also "Cumulative effects"). [back to the text]

Consequence (of the adverse effect) – Severity of adverse effects associated with exposure to an LMO or its products. [back to the text]

Conventional – Not involving the use of modern biotechnology. [back to the text]

Cumulative effects – Effects that occur due to the presence of multiple LMOs in the receiving environment (see also "Combinatorial effects"). [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") – Refers to the role of an organism in ecological processes. Which ecological functions or services are taken into account here will be dependent on the protection goals set for the risk assessment. For example, organisms may be part of the decomposer network playing an important role in nutrient cycling in soils or be important as a pollen source for pollinators and pollen feeders. [back to the text]

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

Familiarity –The concept of familiarity allows the risk assessor to draw on previous knowledge and experience with the introduction of the LMO under consideration into the environment. As familiarity depends also on the knowledge about the environment and its interactions with introduced organisms, the risk assessment in one country may not be applicable to another country (OECD, 2006, Safety Assessment of Transgenic Organisms, vol 1). [back to the text]

Gene-drive system – Method for introducing a desired gene into a mosquito population (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 product – The RNA or protein that results from the expression of a gene. [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]

Horizontal gene transfer – Movement of genetic information from one organism to another through means other than sexual transmission. [back to the text]

Introgression – Introduction of genetic elements from an organism into the genetic pool of organism of another species, sub-species or population occurring when mating between the two produce fertile hybrids. [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, possibility or chance of the adverse effect to occur. [back to the text]

Management strategies – Appropriate mechanisms and measures to regulate, manage and control risks identified in the risk assessment. [back to the text]

"Omics" technologies – A collection of 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]

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). [back to the text]

Potential receiving environment – An ecosystem or habitat, including humans and animals, which is likely to come in contact with a released organism (UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

Phenotypic characteristics – The observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influence. [back to the text]

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

Protection goal – A 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]

Risk – The combination of the magnitude of the consequences of a hazard, if it occurs, 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 measures to estimate what risks may be associated with an LMO and what adverse effects may be caused, how likely the adverse effects are to occur, and what would the consequences be should they occur (adapted from UNEP, 1995, International Technical Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf). [back to the text]

Risk management – The measures to ensure that risks involved in the production and handling of an LMO are reduced (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. Risk thresholds may be defined in the national legislation or in the decision-making process of each country. [back to the text]

Substantial equivalence – Substantial equivalence is a concept used in food safety as described in the OECD publication "Safety Evaluation of Food Derived by Modern Biotechnology" in 1993 (http://bch.cbd.int/database/record-v4.shtml?documentid=48488). [back to the text]

Transformation cassette – A transformation cassette comprises a group of genetic elements (e.g. parts of a vector and one or more of the following: a promoter, the coding sequence of a gene and a terminator), 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. In some cases, a transformation cassette may also be called "expression cassette", "DNA cassette" or "gene construct". [back to the text]

Transformation event – An LMO resulting from 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 genetic element or 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 –** Type of transcriptional regulation that is done by trans-regulatory elements which modify the expression of genes distant from the gene that was originally transcribed to create them. 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 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. Unintended effects can be divided into two categories: those that can be foreseen and those that are genuinely unanticipated. [back to the text]

Unintended gene product – Gene products that occur, for example, when the inserted gene construct suffers 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. [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]

Vertical gene flow – Transfer of genetic information from one organism to another organism via crossing or sexual recombination. [back to the text]