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AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT UNDER THE CARTAGENA PROTOCOL ON BIOSAFETY
Brasilia, 16-20 November 2015

DRAFT REVISED GUIDANCE ON RISK ASSESSMENT FOR LIVING MODIFIED ORGANISMS

1. The document herein attached is the outcome of the work of the Subgroup of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management (AHTEG), in consultation with the Secretariat and taking into account the input from the "Open-ended Online Forum" and AHTEG, in response to decision BS-VII/12.

2. This document is being presented for the consideration of the AHTEG at its face-to-face meeting to be held from 16 to 20 November 2015 in Brasilia.

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GUIDELINES FOR RISK ASSESSMENT AND MONITORING OF LIVING MODIFIED ORGANISMS

Comment [A1]: Comments G35 and G238

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TABLE OF CONTENTS

PREFACE

In accordance with the precautionary approach,[1] the objective of the Cartagena Protocol on Biosafety (hereinafter "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).

Comment [A2]: Comment R54

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

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

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

[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 (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-10) and 11.8 (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-11) of the Protocol.

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

[3] Article 15, paragraph 1(http://bch.cbd.int/protocol/text/article.shtml?a=cpb-15).

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

OBJECTIVE AND SCOPE OF THIS GUIDANCE

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

This Guidance addresses LMOs that result from the application of modern biotechnology as described in Article 3(i)(a) of the Protocol.

This Guidance consists of three parts: Part I containing a Roadmap for Risk Assessment of LMOs, Part II containing guidelines for the risk assessment of specific types of LMOs or traits, and Part III containing guidelines for monitoring of LMOs released into the environment. The topics contained in Parts II and III were identified and prioritized by the Open-ended Online Expert Forum and the AHTEG in accordance with the terms of reference in decisions BS-IV/11 and BS-V/12, taking into account the need of Parties for additional guidance.

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

[5] Decision BS-V/12.

PART I:

ROADMAP FOR RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS

BACKGROUND

This "Roadmap" provides guidance on identifying and evaluating the potential adverse effects of living modified organisms (LMOs)[6] on the conservation and sustainable use of biological diversity in the likely potential receiving environment taking into account risks to human health, consistent with the Cartagena Protocol on Biosafety (hereinafter "the Protocol") and in particular with its Article 15 and Annex III (hereinafter "Annex III").[8] Accordingly, this Roadmap supplements Annex III and may also supplement national biosafety policies and legislations. Specifically, the Roadmap is intended to facilitate and enhance the effective use of Annex III by elaborating on the steps and points to consider in identifying and evaluating the potential adverse effects and by pointing users to relevant background materials. The Roadmap may be useful as a reference for designing and planning risk assessment approaches and identifying the need to develop further guidance as well as reviewing newly developed or adapted guidance. It may also be useful for risk assessors when conducting or reviewing risk assessments and as a tool for training.

The Roadmap introduces basic concepts of risk assessment rather than providing detailed guidance for individual case-specific risk assessments. In particular, the "elements for consideration" listed in the Roadmap may need to be complemented by other guidance during an actual risk assessment.

This Roadmap provides information that is relevant to the risk assessment of all types of LMOs and their intended uses within the scope and objective of the Protocol. However, it has been developed based largely on living modified (LM) crop plants because most of the available knowledge has been gained from these organisms.[9]

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

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

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

[9] Decisions on LMOs may be found, *inter alia*, in the BCH (http://bch.cbd.int) and links to national and intergovernmental websites relevant for this purpose. In accordance with BCH records, XX LM crop plants, XX LM trees, XX LM animals and XX LM microorganisms have been released into the environment to date.

INTRODUCTION

According to the Protocol, risk assessment of LMOs is a structured process conducted in a scientifically sound and transparent manner, and on a *case-by-case* basis in the context of the risks posed by the non-modified recipients or parental organisms in the likely *potential receiving environment*. Its purpose is to identify and evaluate the potential adverse effects of LMOs, and their *likelihood* and *consequences* as well as to make a recommendation as to whether or not the estimated overall risk is acceptable and/or manageable, taking into consideration any relevant uncertainty. Risk assessments serve as a basis for decision-making regarding LMOs. This Roadmap describes an integrated risk assessment process in three sub-sections:

- Overarching Issues in the Risk Assessment Process
- Planning Phase of the Risk Assessment
- Conducting the Risk Assessment

The potential effects caused by an LMO may vary depending on the characteristics of the LMO, on how the LMO is used, and on the environment exposed to the LMO. The effects may be intended or *unintended*, and may be considered beneficial, neutral or adverse depending on the impact on a *protection goal*.

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

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Protection goals, assessment endpoints and measurement endpoints

Protection goals are broadly defined and valued environmental outcomes (e.g. biodiversity or ecological functions), sometimes called general protection goals or generic endpoints.

Examples of protection goals include...

'Assessment endpoints' and 'measurement endpoints' are important concepts and understanding the difference between these two terms is key to understanding risk assessment.

'Assessment endpoints' define, in operational terms, the environmental values that are to be protected. An assessment endpoint must include an entity (e.g. such as salmon, honeybees, or soil quality) and a specific attribute of that entity (e.g. such as their abundance, distribution or mortality. Assessment endpoints are sometimes called specific protection goals or operational protection goals. Assessment endpoints may serve as starting point for the "problem formulation" step of the risk assessment.

'Measurement endpoints'…"

Protection goals and endpoints are aimed at defining and targeting the processes in the risk assessment by helping frame the questions at the beginning of the assessment, for example during the problem formulation phase. The choice of relevant protection goals and assessment endpoints may change after an objective analysis of the characteristics of the LMO or as the risk assessment progresses and new information emerges.

The Roadmap describes the risk assessment process as a sequence of five steps, in which the results of one step are relevant to the others. This stepwise structure is drawn from paragraph 8 of Annex III of the Protocol:

- Step 1 Hazard identification: "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;

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- Step 2 Exposure assessment: “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”;
- Step 3: Hazard characterization: “An evaluation of the consequences should these adverse effects be realized”;
- Step 4: Risk characterization: “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”;
- 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”.

Comment [A18]: Comment R122

Importantly, the steps of a risk assessment may be revisited when new information arises or a change in circumstances has occurred that could change its conclusions. Similarly, issues included in the ‘Establishing the context and scope’ section below may be taken into consideration while conducting the risk assessment and again at the end of the risk assessment process to determine whether the objectives and criteria set out at the beginning of the risk assessment have been addressed.

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

The risk assessment process according to this Roadmap is illustrated in page XX as a flowchart, which may also serve as a checklist.

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In addition to the approach described in the Roadmap, other approaches to risk assessment exist and may be used, as appropriate, e.g. worst case scenarios and event tree analysis.

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» See references relevant to “Introduction”:

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

OVERARCHING ISSUES IN THE RISK ASSESSMENT PROCESS

This section provides guidance on matters that are relevant to all the steps of the risk assessment. It focuses on provisions related to the quality and relevance of information to be considered in the risk

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assessment, as well as means to identify and describe the degree of uncertainty that may arise during the risk assessment.

The need for further relevant information about specific subjects may arise during the risk assessment process in which case additional information may be requested from the LMO notifier or developer. Consultative meetings between regulators and the developers of the LMO may be helpful in the planning phase of the risk assessment and allow for discussions regarding the approaches that may be taken in the assessment. Discussions may also take place during the assessment to facilitate a common understanding among the different players, and completion of the assessment.

Independent experts with a background in relevant scientific disciplines can serve in an advisory capacity during the risk assessment process or perform the risk assessment themselves.

Quality and relevance of information[10]

An important question in a risk assessment is whether the available information that will be used to characterize the risk posed by the LMO is relevant and scientifically sound.

A number of points are typically considered to ensure the quality and relevance of the information used as well as the outcome of the risk assessment. For example:

- Criteria for the quality of scientific information:
  - The information used in the risk assessment should be of acceptable scientific quality and consistent with best practices of scientific evidence-gathering and reporting. An independent review of the design and methods of studies used in the risk assessment, and of the quality of reporting may be conducted to ensure appropriate data quality.
  - Appropriate statistical methods should be used where appropriate, to strengthen the scientific conclusions of a risk assessment and be described in the risk assessment report. Risk assessments frequently use data generated from multiple scientific fields;
  - The reporting of the information, including its source and methods used, should be sufficiently detailed and transparent to allow independent verification and reproduction. This would include ensuring that relevant information and/or sample and reference materials are available and accessible to risk assessors, as appropriate.

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[10] The term "information" is being used in a broad sense and includes, for example, experimental data, both raw and analysed.

taking into account the provisions of Article 21 of the Protocol on the confidentiality of information.

- The relevance of information for the risk assessment:
  - Information is considered relevant if it is linked to protection goals or assessment endpoints, or if it contributes to the identification and evaluation of potential adverse effects of the LMO, outcome of the risk assessment or decision-making;
  - The information that is relevant to perform a risk assessment will vary from case to case depending on the nature of the modification of the LMO, on its intended use, and on the scale and duration of the environmental introduction, as well as on the risk assessors' level of familiarity with the trait or organism being assessed;
  - Relevant information may be derived from a variety of sources such as new experiments, peer-reviewed scientific literature, as well as from previous risk assessments, in particular for the same or similar LMOs introduced in similar receiving environments;[12]
  - Information from national and international standards and guidelines may be used in the risk assessment, as well as knowledge and experience of, for example, farmers, growers, scientists, regulatory officials, and indigenous peoples and local communities;

Information requirements in the case of field trials or experimental releases

For small-scale releases, especially at early experimental stages or in the early steps of environmental releases of LMOs that are conducted in a step-wise manner, the nature and detail of the information that is required or available may differ compared to the information required or available for large scale or commercial environmental releases. Typically, less information is required, or even available, for risk assessments where the *exposure* of the environment to the LMO is limited, for example, in field trials and small-scale experimental releases, as the objective of such environmental releases is to generate information for further risk assessments. In such cases, the uncertainty resulting from the limited available information may be addressed by risk management and monitoring measures.

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

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Deleted: <#>Relevant information may be derived from a variety of sources such as new experimental data, data from relevant peer reviewed scientific literature, as well as data, experience and outcomes from previous risk assessments if regarded as of acceptable scientific quality, in particular for the same or similar LMOs introduced in similar receiving environments;[11]¶
<#>Information from national and international standards and guidelines may be used in the risk assessment, as well as knowledge and experience of, for example, farmers, growers, scientists, regulatory officials, and indigenous and local communities depending on the type of LMO, its intended use and the likely potential receiving environment. ;¶

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Therefore, some of the information identified throughout the Roadmap may not be known or be only partly relevant in the context of a release for field trial or other experimental purposes where the environment would have limited exposure to the LMO.

Identification and consideration of uncertainty

Uncertainty is an inherent element of scientific analysis and risk assessment. Risk assessments do not provide definitive answers regarding safety or risk as there is always some degree of uncertainty. The more knowledgeable and experienced the risk assessors are, the more they may be able they to identify and deal with uncertainty.

The consideration of uncertainty and its importance to effective decision making are subject to much discussion. The importance assigned to uncertainty and the determination of its occurrence, are dealt with differently, depending on the extent to which a regulatory framework incorporates precautionary measures.

According to paragraph 8(f) of annex III 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". Furthermore, paragraph 6 of article 10 of the Protocol states that, "Lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision […] in order to avoid or minimize such potential adverse effects".

Considerations of uncertainty may strengthen the scientific validity of a risk assessment and provide transparency in the decision making process. Relevant considerations include the source and nature of uncertainties, focusing on uncertainties that can have a significant impact on the conclusions of the risk assessment.

For each identified uncertainty, the *nature* of the uncertainty may be described as arising from: (i) lack of information, (ii) incomplete knowledge, and (iii) biological or experimental variability, for example, due to inherent heterogeneity in the population being studied or to variations in the analytical assays. Uncertainty resulting from lack of information includes, for example, information

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Comment [A33]: Comments R175 and R305: (these two points were moved to the beginning of this section).

Comment [A34]: Comments R156, R165, R314 (add examples).

Deleted: <#>Additional considerations with regard to scientific further information: and availability of scientific expertise:¶

<#>The process of risk assessment may give rise to the need for further relevant information about specific subjects, which may be identified and requested during the assessment process; ¶

<#>Independent experts with the relevant background in the different scientific disciplines can serve in an advisory function during the risk assessment Whether independent experts with the relevant background in the different [various] scientific disciplines are available to conduct risk assessments or to provide input into the risk assessment process or even perform the risk assessment themselves..

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Comment [A36]: Comments R156 and R266.

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that is missing and data that is imprecise or inaccurate (e.g., due to study designs, model systems and analytical methods used to generate, evaluate and analyze the information).

In some cases more information will not necessarily contribute to a better understanding of potential adverse effects, therefore risk assessors should look to ensure that any further information requested will contribute to better evaluations of the risk(s). For example, uncertainties originating from lack of information may be reduced by further testing or by requesting additional information from the developers of the LMO. However, in cases of incomplete knowledge or inherent variability, the provision of additional information will not necessarily reduce the uncertainty.

In cases where uncertainty cannot be addressed through the provision of more information, where appropriate, it may be dealt with by the implementation of risk management and/or monitoring in accordance with paragraphs 8(e) and 8(f) of Annex III to the Protocol (see step 5 and Part III).

The various forms of uncertainty are considered and described for each identified risk and under the estimation of the overall risk. In addition, when communicating the results of a risk assessment, it is important to describe, either quantitatively or qualitatively, those uncertainties that may have an impact on the overall risk, as well as on the conclusions and recommendations of the risk assessment in a way that is relevant for decision-making. Furthermore, uncertainties associated with specific potential adverse effects may not allow the completion of a specific assessment or the drawing of any conclusions regarding the level of overall risk.

Practical guidance on how to deal with uncertainty can be found among the references below.

» See references relevant to "Identification and consideration of uncertainty":
http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

PLANNING PHASE OF THE RISK ASSESSMENT

Establishing the context and scope

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

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

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

(i) Regulations and international obligations of the Party involved;

(ii) Environmental and health policies and strategies;

(iii) Guidelines and regulatory frameworks that the Party has adopted;

(iv) Protection goals, ecosystems functions and services, assessment endpoints, risk thresholds and management strategies derived from (i) to (iii) above;

(v) Intended handling and use of the LMO, including practices related to the use of the LMO, taking into account user practices and traditional knowledge;

(vi) Availability of baseline information for the likely potential receiving environment;

(vii) The nature and level of detail of the information that is needed (see above), which may, among other things, depend on the biology/ecology of the recipient organism, the intended use of the LMO and its likely potential receiving environment, and the scale and duration of the environmental exposure (e.g., whether it is for import only, field testing or for commercial use);

(viii) Identification of methodological and analytical requirements, including requirements for review mechanisms, that must be met to achieve the objective of the risk assessment as specified, for instance, in guidelines published or adopted by the Party that is responsible for conducting the risk assessment (i.e., typically the Party of import according to the Protocol);

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

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(ix) Experience and history of use of the non-modified recipient or parental organism, taking into account its *ecological function*;

(x) Information from previous risk assessments of the same or similar LMOs, including the use of related surrogate systems, modified traits in other organisms;

(xi) Criteria to characterize the likelihood (step 2) and magnitude of consequences (step 3) of individual risks, and for combining them into the overall risk (step 4), and the acceptability or manageability of risks (step 5);

(xii) Proposed limits and controls to restrict the spread and persistence of the LMO (particularly relevant for field trials).

Some risk assessment frameworks combine the process of establishing 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).

Problem formulation

Problem formulation is an approach to structuring a risk assessment. It usually starts by identifying protection goals and defining assessment endpoints. This is followed by the identification of hazards of the LMO and its use. After identifying the hazards and potential adverse effects, conceptual models are developed to describe the hypothesized relationship between the adverse effects and the assessment endpoints. This means describing and modelling scenarios and pathways on how the LMO may cause harm to a protection goal. Finally, an analysis plan is developed for obtaining the needed data and how to test these hypothetical scenarios and pathways.

» See references relevant to "Establishing the context and scope":
http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

The choice of comparators

In a comparative risk assessment, risks posed by an LMO are considered in the context of the risks posed by the non-modified recipients or parental organisms, in the likely potential receiving environment, including wild type/native genotypes, if relevant.[15]

In practice, a comparative approach aims at identifying, in relation to the appropriate *comparator(s)*, the phenotypic and genotypic changes of an LMO that may lead to adverse effects, and changes in

[15] Annex III, paragraph 5.

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the nature and levels of risk of the LMO. The choice of comparators can have large effects on the relevance, interpretation and conclusions drawn from the risk assessment process. Therefore, the one or more comparators that are chosen should be selected on the basis of their capacity to generate information that is consistent and relevant for the risk assessment.

To account for variation due to interaction with the environment, the LMO and its comparator(s) should ideally be evaluated at the same time and location, and under similar environmental and management conditions. Moreover, risks regarding potential adverse effects to beneficial organisms may be compared between the LMO (e.g. a Bt crop) and the non-modified recipient under different environmental conditions (e.g. different pesticide types/application regimes) if these are appropriate to differences in standard management practices that are expected to apply under normal production conditions.

Choosing the appropriate comparator(s) may, in some cases, be difficult or challenging. On the one hand, some risk assessment approaches require the use a non-modified genotype with a genetic background as close as possible to the LMO being assessed, e.g. a *(near-)isogenic line*, as the primary comparator, with additional comparators, such as defined non-modified reference lines, being used depending on the biology of the organism and types of modified traits under assessment. In these risk assessment approaches, the (near-)isogenic non-modified organism is used in step 1 and throughout the risk assessment, whereas broader knowledge and experience with additional comparators is used, along with the non-modified recipient organism, when assessing the likelihood and potential consequences of adverse effects. Results from experimental field trials or other environmental information and experience with the same or similar LMOs in the same or similar receiving environments may also be taken into account.

On the other hand, in some risk assessment approaches, the choice of an appropriate comparator will depend on the specific LMO being considered, the step in the risk assessment and on the questions that are being asked. These risk assessment approaches do not require that a non-modified (near-)isogenic line be used as comparator throughout the assessment, and, in some circumstances, may use another LMO as a comparator (e.g. when assessing an LM cotton in environments where LM cotton is already the standard cultivated form of cotton). The impact of using additional comparators that are not (near-)isogenic lines on the level of analytical rigour of the comparative assessment may be taken into consideration when deciding on appropriate comparators.

Comment [A55]: Comment R352.

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Comment [A56]: Comment R179.

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Comment [A58]: Comment R277.

Comment [A59]: Comment R399.

In some cases, the non-modified recipient organisms or the parental organisms alone may not be sufficient to establish an adequate basis for a comparative assessment. In such cases, additional and/or alternative approaches and/or comparators may be necessary (for concrete examples and more guidance, please refer to Part II, Section B, of this Guidance). For example, for some indicators such as the levels of endogenous toxins, the range of values in cultivated varieties may provide more relevant information than a single (near-)isogenic line would. In another example, many LMOs are developed by backcrossing the original LMO into elite varieties. In such cases, the original non-modified recipient organism is not cultivated and is, therefore, not sufficient as the non-modified comparator.

Comment [A60]: Comment R307.

Comment [A61]: Comment R139.

Furthermore, an alternative to the comparative approach may be necessary when dealing with LMOs whose recipient organism is, for example, a non-domesticated species, or when dealing with organisms produced from synthetic biology where appropriate comparators do not exist.

Comment [A62]: Comment R60

CONDUCTING THE RISK ASSESSMENT

To fulfil the objective under Annex III of the Protocol, as well as provisions under other relevant articles, a risk assessment is conducted in a stepwise process and in an iterative manner, where steps can be repeated to incrementally build on previous findings, for example, when new data is obtained or new issues need to be considered, as appropriate.

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Comment [A63]: Comment R192 (add reference to G.W. Suter II).

Paragraph 8 of Annex III describes the key steps of the risk assessment process. Paragraph 9 of Annex III lists and describes points to consider in the process for risk assessment of LMOs depending on the particular case.

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

Comment [A64]: Comment R278.

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In this section, the steps indicated in paragraph 8(a)-(e) of Annex III are described in further detail and elements for consideration are provided for each step. Some elements for consideration were taken from paragraph 9 of Annex III, while others were added on the basis of commonly used methodologies of LMO risk assessment and risk management insofar as they were in line with the principles of Annex III. The relevance of each point to consider will depend on the case being

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assessed. The guidance provided below on the steps in risk assessment is not exhaustive, thus additional guidance and elements for consideration may be relevant, as appropriate. Lists of background documents relevant to each section are provided through the links.

» See references relevant to "Conducting the Risk Assessment":

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

Step 1 – Hazard identification: "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." [16]

Rationale:

The purpose of this step is to identify changes in the LMO, resulting from the use of modern biotechnology, that could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.[17]

The question that risk assessors ask in this step is "what could go wrong, why and how?". This step is very important in the risk assessment process as the answers to this question will determine what risk scenarios are considered in all subsequent steps.

In many cases, this step is performed as part of a problem formulation process when establishing the context and scope of the risk assessment (see above). Whether step 1 and "establishing the context and scope" are done in parallel or in sequence, together these actions are among the most important in a risk assessment as they form the basis for the subsequent steps.

In this step, risk assessors identify scientifically plausible risk scenarios and risk hypotheses to predict if the LMO could have an adverse effect on the assessment endpoints. In doing so, risk assessors analyse what novel characteristics of the LMO, as well as its transfer, handling and use, could give rise to adverse effects in an interaction with the likely potential receiving environment. For example, if the protection goal is maintenance of biodiversity, a risk hypothesis could assess what novel characteristics of the LMO might affect specific assessment endpoints, such as a

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Comment [A65]: Comment R489

Comment [A66]: Comment R88.

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Comment [A67]: Comment R23.

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Comment [A68]: Comment R176 (Add "scenario" to the use of terms section, see definition in Suter II).

Deleted: This step may also be referred to as "hazard identification" – the difference between the concepts of "*hazard*" and "*risk*" is important and must be understood by the risk assessor.

Comment [A69]: Comments R279, R90 and R308.

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Comment [A70]: Comment R281

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

[17] See also article 2, paragraph 2(b) of the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress (http://bch.cbd.int/protocol/nkl/article2/).

component of the food web or the population size of certain species in the likely potential receiving environment. The targets are called assessment endpoints, and their unambiguous specification is crucial to focus the risk assessment.

It is important to define direct or indirect links or pathways between the LMO and possible adverse effects, otherwise the risk assessment may generate information that will not be useful for decision-making (see also steps 2 and 3). Potential adverse effects could arise, for example, from changes in the potential of the LMO to: (i) affect non-target organisms, (ii) cause unintended effects on target organisms, (iii) become persistent or invasive or develop a fitness advantage in ecosystems with limited or no management, (iv) transfer genes to other organisms/populations, and (v) become genotypically or phenotypically unstable.

In this step, a comparison of the LMO should be considered in the context of the non-modified recipient or parental organisms in the likely potential receiving environment and the baseline environmental conditions prior to the release of the LMO. Choosing appropriate comparators is particularly relevant for this step in order to enable the consideration of the new trait(s) of the LMO, and any associated changes in management practices (see 'The choice of comparators' in the chapter entitled 'Planning Phase of the Risk Assessment').

The novel characteristics of the LMO to be considered can include any changes in the LMO, ranging from the nucleic acid (including any deletions), to gene expression level to morphological and behavioural changes.

The LMO may cause adverse effects which may be direct or indirect, immediate or delayed, combinatorial or cumulative, as well as predicted or unpredicted. For example, an adverse effect may also be caused by changes in the expression levels of endogenous genes as a result of the genetic modification or by *combinatorial effects* of two or more genes, gene products or physiological pathways.

Elements for consideration regarding characterization of the LMO:

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

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

(ii) Its taxonomic relationships;

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Comment [A71]: Comments R178 and 164.

Comment [A72]: Comment R62.

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Comment [A73]: Comment R116 (add "non-target organisms" to the "use of terms").

Comment [A74]: Comment R342.

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Comment [A75]: Comment R180

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Comment [A76]: Comment R36 and others.

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(iii) Its provenance, centre(s) of origin and centre(s) of genetic diversity;

(iv) Its ecological function; and

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

(b) Relevant characteristics of the donor organism(s), such as:

(i) its taxonomic status and common name;

(ii) its provenance;

(iii) relevant biological characteristics;

(iv) Relevant characteristics of the genes and of other functional sequences, such as promoters, that have been inserted into the LMO, including functions of the genes and their gene products in the donor organism with particular attention to characteristics in the recipient organism that could cause adverse effects;

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

(d) Molecular characteristics of the LMO related to the modification, such as characteristics of the modified genetic elements; insertion site(s) and copy number of the inserts; stability, integrity and genomic organization in the recipient organism; specificity of the genetic elements (e.g., transcription factors); levels and specificity of gene expression and intended and *unintended gene products*, such as novel proteins being encoded by sequences put together at the insertion sites or elongation of the intended protein due to faulty or lacking terminator sequences;

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

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Comment [A79]: Comments R344, R361, R362 and R367.

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

Comment [A80]: Comment R64.

Elements for consideration regarding the intended use and the likely potential receiving environment:

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

(g) Availability of data on the likely receiving environment which may serve as a basis for the risk assessment;

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

(i) Characteristics of the likely potential receiving environment including relevant ecosystem functions and services, in particular its attributes that are relevant to potential interactions of the LMO that could lead to adverse effects (see also paragraph (k) below), taking into account the characteristics of the components of biological diversity, particularly in centres of origin and centres of genetic diversity;

Attributes of the receiving environment

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.

(j) Potential indirect adverse effects to biodiversity as a result of pests or pathogens developing resistance to the target trait (e.g. insect or disease resistance trait) and weeds developing resistance to the herbicide.

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Comment [A81]: Comments R65, R106, R141, R404

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Comment [A82]: Comment R218.

Comment [A83]: Comment R284.

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Elements for consideration regarding the potential adverse effects resulting from the interaction between the LMO and the likely potential receiving environment:

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

(l) Considerations for *unmanaged and managed ecosystems*, concerning the use of an LMO, that are relevant for the likely potential receiving environment;

(m) Potential adverse effects resulting from the use of an LMO, such as changes in farm management practices;

(n) Dispersal of the LMO through mechanisms such as seed dispersal or *outcrossing* within or between species, or through transfer into habitats where the LMO may persist or proliferate; as well as effects on species distribution, food webs and changes in bio-geochemical characteristics;

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

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

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

(r) Potential adverse effects of the incidental exposure of humans to (parts of) the LMO (e.g., exposure to modified gene products in pollen);

(s) Potential adverse effects of changes in agricultural practices, such as type of irrigation, number and amount of herbicide applications, methods for harvesting and waste disposal, that were induced by use of the LMO. Where use of other regulated products or practices are changed, interplay with the respective risk assessments and regulations needs to be considered;

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

» See references relevant to "Step 1":

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Comment [A84]: Comment R285

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Comment [A85]: Comments R66 and R67.

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

Step 2 – Exposure assessment: "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 this step the risk assessors evaluate the likelihood that each of the potential adverse effects identified in step 1 will occur. The evaluation of likelihood may be undertaken at the same time as the evaluation of the consequences should the adverse effects be realized (step 3). While steps 2 and 3 are independent of each other, in some frameworks they are carried out in a reverse order.

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

For each of the risk scenarios and risk hypotheses identified in step 1, the pathway of exposure to the LMO being assessed (or its products) should be determined. Furthermore, it is important to define a causal link between the LMO and the potential adverse effect by building conceptual models describing relationships between the LMO, pathways of exposure and potential adverse effects in the environment, taking also into account risks to human health. For example, for an LMO producing a potentially toxic gene product, oral, respiratory or dermal pathways of exposure could be relevant.

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

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

Comment [A86]: Comment R489

Comment [A87]: Comment R68.

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Comment [A88]: Add the term "pathway of exposure" to the use of terms

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In some circumstances, particularly when there is a high level of uncertainty in assessing the likelihood, it may be difficult to assess the likelihood of adverse effects being realized. In such cases, it may be useful to to reverse order of Steps 2 and 3 (see above and Fig 1).

Elements for consideration:

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

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

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

(d) Factors that may affect spread of the LMO, such as its ecological range and ability to move; its reproductive ability (e.g., numbers of offspring, time to set seed, abundance of seed and vegetative propagules, dormancy, pollen viability); and its ability to spread using natural means (e.g., wind, water) or through human activities (e.g., rearing or cultivation practices, seed saving and exchange, etc);

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

(f) When assessing the likelihood of outcrossing from the LMO to sexually compatible species as a step in the pathway to an adverse effect, 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) Persistence of the LMO in the environment;

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Comment [A90]: Comments R69, R142 and R253.

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Points to consider

Comment [A91]: Comment R233.

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Comment [A92]: Comment R287.

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

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

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

» *See references relevant to "Step 2":*
http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

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

Comment [A93]: Comment R489

Rationale:

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

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

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

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It is important to also assess in this step the duration of the potential adverse effect (i.e., short or long term), the scale (i.e., are implications local, national or regional), the mechanisms of effect (direct or indirect), the potential for redress (or lack thereof) in the event an adverse/unintentional effect, and the expected ecological scale (i.e., individual organisms – for example of a protected species – or populations), taking into account the attributes of the potential receiving environments (see Step 1, footnote 16) and potential changes resulting from human activities.

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

Points that may be considered:

(a) Relevant knowledge and experience with the non-modified recipient or parental organisms, or current use of the organism, in the likely potential receiving environment, and their interactions with other species, including sexually compatible species. This may include the effects of:

(i) Agricultural practices on the level of inter- and intra-species gene flow;

(ii) Dissemination of the recipient organism;

(iii) Abundance of volunteers in crop rotation;

(iv) Changes in the abundance of pests, beneficial organisms such as pollinators, decomposers, organisms involved in biological control or soil microorganisms involved in nutrient cycling;

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

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

(b) Potential adverse effects resulting from combinatorial and cumulative effects in the likely potential receiving environment;

(c) Relevant knowledge and experience with the LMO and non-modified organisms with similar phenotypic characteristics in similar receiving environments;

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Comment [A96]: Comment R183.

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

(e) Results from field trials containing information about the potential for invasiveness and impacts in the environment; and

(f) Potential adverse effects resulting from outcrossing/interbreeding to sexually compatible species and introgression of the transgene(s).

» See references relevant to "Step 3":

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

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

Rationale:

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

To date, there is no universally accepted approach for estimating the overall risk but rather a number of approaches are available for this purpose. As indicated in paragraph 8(d) of Annex III of the Protocol, the estimation of the overall risk is '*based on the evaluation of the likelihood and consequences of the identified adverse effects being realized*'. For example, the characterization of overall risk is often the best estimate which is derived from the combination of the identified individual risks. By combining evidence from each identified risk, the overall risk may be supported

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by multiple lines of evidence. These lines of evidence may be quantitatively or qualitatively weighted and combined. Risk matrixes, risk indices or models may be used for this purpose.[20]

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

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

Elements for consideration:

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

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

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

(d) Individual risks and any interaction among them, such as *synergism* or *antagonism*;

(e) Any risk management strategies (see step 5) that may affect risk estimates if implemented;

(f) Broader ecosystem and landscape considerations, including cumulative effects due to the presence of various LMOs in the receiving environment, taking into account potential environmental changes caused by human activities.

» See references relevant to "Step 4":

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

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, identified individual risks and the estimated overall risk, and provide recommendation(s) as to whether or not

[20] See references in the list of background materials.

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Comment [A100]: Comments R196, R293, R356, R452 (add examples and/or schematic explanation)

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Comment [A101]: Comment R257 (Attempt to better structure this rationale by using boxes, schematic explanations, etc)

the risks are acceptable or manageable and, if needed, recommendation(s) for risk management options that could be implemented to manage the risks associated with the LMO. The recommendation is made in the context of criteria for the acceptability of risk that were identified in the planning phase of the risk assessment, taking into account established protection goals, assessment endpoints and risk thresholds, as well as risks posed by the non-modified recipient organism and its use.

This step is an interface between the process of risk assessment and the process of decision-making. Importantly, while the risk assessor provides a recommendation as to whether or not the risks are acceptable or manageable, the ultimate decision about whether or not to approve the LMO notification is a prerogative of the decision maker. Moreover, the "acceptability" of risks is typically decided at a policy level and may vary from country to country, for instance, some countries may choose to accept a 'negligible' risk associated with the development of a certain technology while others may not.

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

The recommendation on the acceptability of risk(s) may take into account any available scientific analysis of potential benefits for the environment, biodiversity, and human health (e.g., change in the use of crop protection products, reduction of infections in the case of mosquitoes), and may also take into account risks associated with other existing user practices and habits. However, balancing risk acceptability with potential benefits is not laid out in the provisions of the Protocol.

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Comment [A102]: Comment R215.

Further, the sources and nature of uncertainty that could not be addressed during the preceding steps of the risk assessment can be described in relation to how they could affect the conclusions of the risk assessment. For assessments where uncertainties could not be addressed, difficulties encountered during the risk assessment may be made transparent to the decision makers. In such cases, it may also be useful to provide an analysis of alternative options to assist the decision makers.

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In accordance with Annex III paragraph 8(f) "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 and/or monitoring the living modified organism in the receiving environment".

Environmental monitoring (see Part III) can be a means to reduce uncertainty, to address assumptions made during the risk assessment, to validate conclusions of the assessment on a wider (e.g., commercial) level of application, and to establish a causal link or pathway between LMOs and adverse effects. Monitoring may also be used to evaluate whether risk management strategies are being implemented effectively, including whether those strategies are able to detect potential adverse effects before the consequences are realized. Monitoring can also be applied as a tool to detect effects that were not anticipated in the risk assessment and long-term adverse effects.

Comment [A103]: Comments R148 and R50.

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

The recommendation(s) are submitted, typically as part of a risk assessment report, including monitoring plans to reduce uncertainty, where appropriate, for consideration in the decision-making process.

Elements for consideration related to the risk management strategies:

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

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

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

(d) Methods for evaluating the proposed risk management and monitoring strategies for feasibility, efficacy and effectiveness, and whether or not a proposed risk management measure may introduce additional risks or increased level of identified risks.

Comment [A104]: Comment R149

Elements for consideration related to the acceptability of risks:

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

(f) Protection goals and assessment endpoints as identified when establishing the context and scope for a risk assessment;

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

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

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

» See references relevant to "Step 5":

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

RELATED ISSUES

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

- Risk Management (Article 16);
- Capacity-building (Article 22);

Comment [A105]: Comment R55 (add links to the Protocol's articles)

- Public Awareness and Participation (Article 23);
- Socio-economic Considerations (Article 26);
- Liability and Redress (Article 27).

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

ANNEX: FLOWCHART FOR THE RISK ASSESSMENT PROCESS

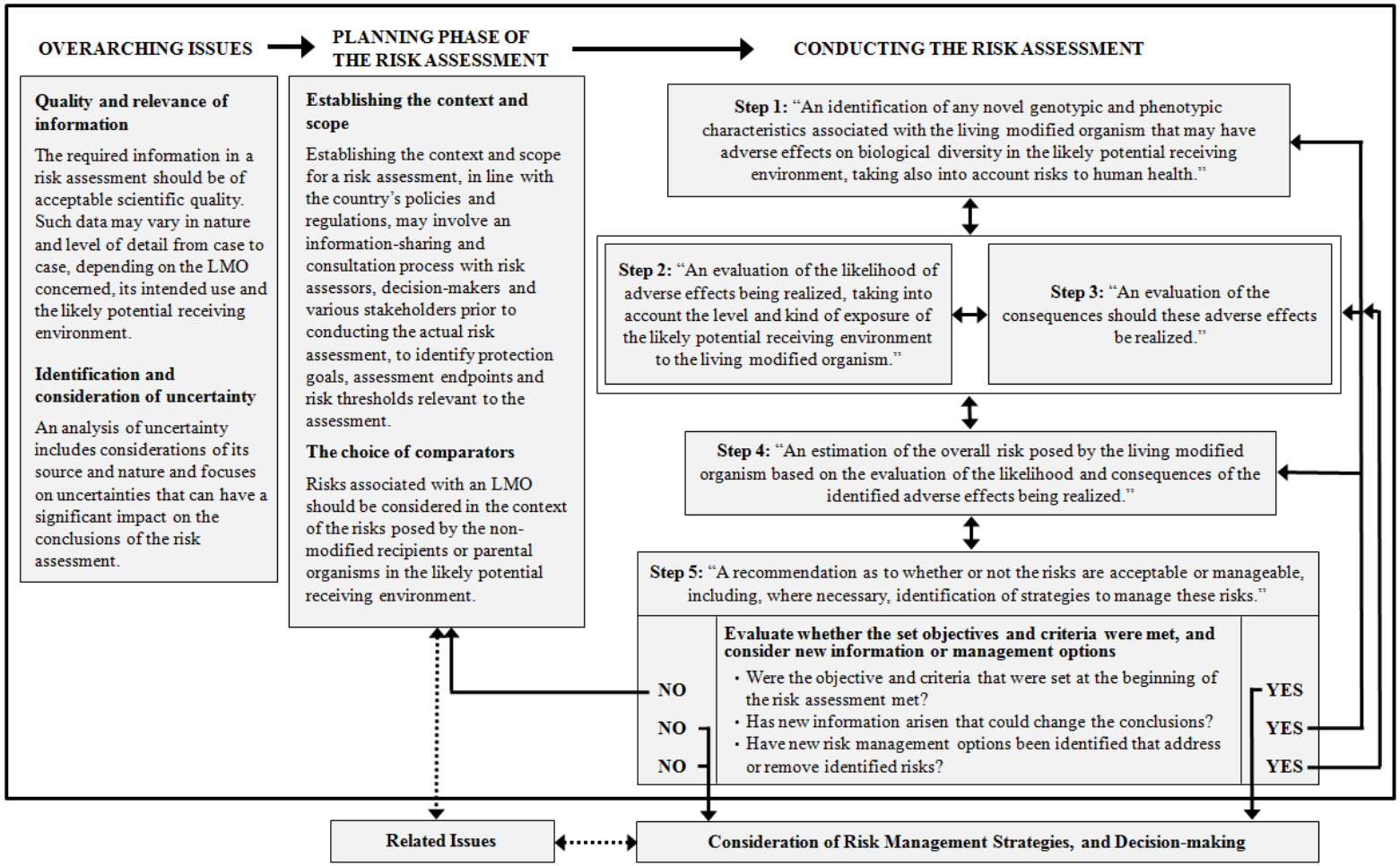

Figure 1. The Roadmap for Risk Assessment. The flowchart illustrates the risk assessment process, which includes "Overarching issues", "Planning phase of the risk assessment" and "Conducting the risk assessment", to *identify* and *evaluate* the potential adverse effects of LMOs on the conservation and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

Comment [A106]: Comment R76 (Place the flowchart at the beginning of the Roadmap to provide an overview to assist the user)

/…

sometimes be considered simultaneously or in reverse order. Dotted arrows indicate the flow to and from issues outside the risk assessment process.

PART II:
SPECIFIC TYPES OF LMOS AND TRAITS

The guidance contained in this section, Part II, should be considered in the context of the Cartagena Protocol on Biosafety. The elements of Article 15 and Annex III of the Protocol apply to these specific types of LMOs and traits. Accordingly, the methodology and points to consider contained in Annex III[21] are also applicable to these types of LMOs and traits. The guidance in the sub-sections below complements the Roadmap for Risk Assessment of LMOs, giving emphasis to issues that may be particularly relevant when assessing the risks of the respective types of LMOs and traits.

Only those considerations that may be particularly relevant to the specific types of LMOs or traits dealt with in Part II are further developed below. Considerations that may be more broadly applicable to different types of LMOs were described in the Roadmap and will not be repeated in this section.

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. As a result, the number of stacked genes in a single LM plant and the number of LM plants with two or more transgenic traits is growing.

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

This guidance complements the Roadmap for Risk Assessment of LMOs, with emphasis on issues that are of particular relevance to the risk assessment of LM plants with stacked traits generated through cross-breeding. Some issues already covered in the Roadmap are further elaborated on this section in an attempt to emphasize points that may need particular consideration when assessing risks which may result from the combination of genetic elements

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from two or more parental LM plants. As such, risk assessments of this type of LM plant follow the general principles outlined in Annex III and the Roadmap, but also take into account the specific issues outlined in this section of the present document.

The scope of this document is on stacked LM plants generated through *conventional breeding* of two or more parental LM plants that are either single *transformation events* or already stacked events. Accordingly, the cassettes containing the transgenes and other genetic elements that were inserted in the original transformation events may be physically unlinked (i.e., located separately in the genome) and can segregate independently.

It is assumed that the individual transformation events making up the stacked event have either been assessed previously or are being assessed concomitantly to the stacked event in accordance with Annex III of the Cartagena Protocol on Biosafety and as described in the Roadmap.[22]

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

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

PLANNING PHASE OF THE RISK ASSESSMENT

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

Rationale:

As seen in the Roadmap, choosing the appropriate comparator(s) is a crucial step for conducting a comparative assessment. In the case of stacked LM plants, in addition to using non-modified recipient organisms as comparators (see "The choice of comparators" in the Roadmap), the LM plants 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.

Where parental organisms have highly *heterozygous genomes* or significantly differ from each other, the resulting offspring may display high variability and a vast range of phenotypes. In the case of stacked LM plants, this variability should be taken into account when establishing a basis for a comparative assessment.

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

(Near-)isogenic lines to be used as comparators may be lacking, and this may present challenges for data interpretation when conducting the risk assessment of a stacked LM plant. Therefore, in risk assessment approaches that rely on the (near-)isogenic non-modified recipient organism as the primary comparator, it may be useful to also use the closest available non-modified genotype as a comparator. Information on the genetic diversity of the recipient or parental organisms may be helpful in identifying the best available comparator for a risk assessment when (near-)isogenic lines are not available.

Comment [A107]: Comment S4

Elements for consideration:

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(a) Level of heterozygosity among the non-modified recipient organisms used to produce the parental LM plants;

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

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

CONDUCTING THE RISK ASSESSMENT

Sequence characteristics at the insertion sites, genotypic stability and genomic organization

(see "Step 1", "Point to consider (d)" and "Step 5" in the Roadmap)

Rationale:

During cross-breeding, changes may occur to the molecular characteristics of the inserted genes/genetic elements at the insertion site(s) as a result of recombination, mutation and rearrangements. Transgenes with similar genetic sequences may undergo recombination, since homologous recombination acts on genomic regions that have identical or highly similar sequence. Multiple inserts with highly similar sequences may be less stable and could be more likely to undergo rearrangements during cross-breeding. In many cases, such changes may result in the loss of the intended phenotype, which in some cases may be relevant for the assessment of risks.

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

Elements for consideration:

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(a) Whether or not methods to carry out molecular characterization are available, for example PCR-based methods, and if they are specific and sensitive enough for the characterization of the stacked LM plant;

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

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

Rationale:

The expression level of transgenes or endogenous genes in a stacked LM plant may be changed as compared to the parental LM plant due to *trans-regulation*. Such changes are more likely to occur if the parental LM plants contain transgenes or regulatory elements that share similarities among them or with endogenous sequences (e.g., same binding sites for transcriptional factors).

The products of transgenes and endogenous genes may also interact. This is most likely to occur if the gene products belong to the same metabolic pathway or physiological process. Some of the interactions may lead to changes that can be detected during the phenotypic characterization of the stacked LM plant, whereas other interactions may not be detectable through a typical phenotypic characterization. Previous risk assessments of the parental LM plants provide useful information on the mode of action and molecular characteristics of the individual genes as a starting point to assess the potential for interactions.

In addition to information about the characteristics of the parental LM plant, specific information on potential for interactions among transgenes and other genetic 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, paying particular attention to transgenes that belong to the same biochemical pathways or physiological processes.

Elements for consideration:

Deleted: *Points to consider*

(a) Effects of the parental LM plants on the environment;

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

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

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

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

Rationale:

An assessment of the risks of a stacked LM plant to cause combinatorial and cumulative effects[23] should be considered in the context of the closely related non-modified recipient organism(s) and the parental LM plants in the likely potential receiving environment, taking into account the results of the genotypic and phenotypic assessments outlined above.

Combinatorial effects may occur due to interactions among the proteins and metabolites produced by the transgenes or endogenous genes of a stacked LM plant. For example, the stacking of various insecticidal proteins in an LM plant could have a synergistic effect on non-target organisms that could be broader than the sum of the effects of the individual parental LM plants. Likewise, the evolution of resistance in target organisms (e.g., insect pests) to such stacked LM plants could happen faster than the development of resistance to the parental LM plants.

The risks of multiple stacked LM plants being cultivated in the same environment to cause cumulative adverse effects (e.g., due to changes in agricultural practices) may also be considered.

An assessment of potential combinatorial and cumulative effects may be performed, for instance, by conducting specific tests with the stacked LM plant(s) such as compositional analyses and toxicity tests on target and non-target organisms. Where appropriate, in-depth genotypic and phenotypic characterization of the stacked LM plant may be conducted.

Elements for consideration:

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

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

[23] See definitions in the "Use of Terms" section.

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

(d) Combinatorial and cumulative effects arising from the presence of two or more insecticidal proteins that could result in increased toxicity to non-target organisms or faster development of resistance in the target organisms.

Crossing and segregation of transgenes *(see "Step 1", "Element for consideration (l)" and "(m)", "Step 2", "Element for consideration (f)", "Step 3", "Element for consideration (f)" in the Roadmap)*

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Rationale:

Due to genetic recombination, the offspring of a crossing will have combinations of genes that differ from those found in either parent. In the case of stacked events, the number of new combinations of transgenes that may result from a cross will depend on the number transgenes involved in a crossing, their location in the genome and their distance from each other.

Comment [A108]: Comment S37.

As a result, a set of new stacked LM plants may arise in the environment through crossings between a stacked LM plant and other LM plants. Successive crossings with non-modified sexually-compatible relatives in the receiving environment may also result in the stacking of genes and traits. These crossings can either be mediated by man or occur naturally through pollination and may result in a range of new stacked LM plants containing new and/or different combinations of transgenes and other genetic elements.

The larger the number of different sexually-compatible LM plants, stacked or not, being cultivated in the same environment, the more variations and complexity of new stacked LM plants may occur. The presence of sexually-compatible LM plants being cultivated in the likely potential receiving environment of the stacked LM plant under consideration is to be taken into account when establishing risk scenarios or hypotheses during step 1 of the risk assessment.

Elements for consideration:

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(a) Presence of other single-event and stacked LM plants of the same species;

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

(c) Potential adverse effects of the new stacked LM plants, including enhanced fitness as compared to the non-modified recipient or parental organisms, invasiveness, effects on non-target organisms, allergenicity and toxicity to humans;

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Comment [A109]: Comment S21

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

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

Rationale:

In the context of paragraphs 8(f) and 9(f) of Annex III of the Protocol, some of the risk management strategies for stacked events may require methods for the detection and identification of these LM plants in the context of environmental monitoring. Currently, many detection methods for LM plants 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 to a single transformation event. While these methods may be used to detect and identify single transformation events, when the analysis is carried out in bulk (i.e., mixing material collected from various test individuals), these methods are not sensitive or specific enough to differentiate between single transformation events and a stacked event arising from a cross between these single transformation events. For example, although some software may help predict the presence of stacked LM seeds in a bulk sample, it is not possible to unequivocally distinguish a sample containing material from different single transformation events from another sample containing one or more stacked LM events.

PCR-based detection methods that are specific to a single transformation event often rely on the amplification of DNA sequences that flank the insertion sites and that are unique to a single transformation event. In the future, it may become a challenge to detect single transformation events produced through site-specific insertions because the flanking sequences could be the

same among different LMOs. This could become challenging particularly in cases where the stacked event contains multiple transformation cassettes with similar DNA sequences.

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

Elements for consideration:

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

(b) Availability, specificity and reliability of methods to detect stacked LM plants in the context of risk management strategies.

BIBLIOGRAPHIC REFERENCES

See references relevant to "*Risk Assessment of Living Modified Plants with Stacked Genes or Traits*":

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

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

INTRODUCTION

While the same general principles used in the risk assessments of other types of LMOs also apply to LM plants with increased tolerance to abiotic stress,[24] 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 “Establishing the context and scope” and in step 1 of the Roadmap, identifying protection goals, assessment endpoints and establishing scientifically plausible risk scenarios are some of the first actions to be taken during a risk assessment.

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

In plants, any gene (or gene product) or gene combinations providing increased tolerance to abiotic stress may have *pleiotropic effects* on the stress physiology of the plant. For example, drought, temperature and salt stress are interconnected by common metabolic and signal transduction pathways. Such pleiotropic effects may be classified as "unintended predicted effects" (see the Roadmap, step 1) and may be evaluated during the risk assessment by considering the *cross-talk* mechanisms between different stress responses of the plant, and by evaluating whether or not the identified changes may cause adverse effects. Disciplines such as plant physiology, plant pathology and entomology may provide useful context based on non-modified crops to clarify cross-talk mechanisms among abiotic stress responses and how these responses may change susceptibility to biotic stresses (e.g., predators, pests and pathogens) in an LM plant that is tolerant to abiotic stresses.

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

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

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

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

- Does the tolerance trait have the potential to affect other tolerance and/or resistance mechanisms of the LM plant, for example, via pleiotropism?
- Does the tolerance trait have the potential to cause an increase of the invasiveness, persistence or weediness of the LM plant that could cause adverse effects to other organisms, food webs or habitats?
- Does an LM plant arising from outcrossing with the abiotic stress tolerant LM plant have the potential to change or colonize a habitat or ecosystem beyond the intended receiving environment?
- Does an LM plant expressing tolerance to a particular abiotic stress have other advantages in the targeted receiving environment that could cause adverse effects?
- What are the adverse effects in regions that have not been exposed to commercial

agriculture but may become exposed to stress tolerant LM plants?

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

PLANNING PHASE OF THE RISK ASSESSMENT

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

Rationale:

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

The identification of genotypic and phenotypic changes in the abiotic stress tolerant LM plant, either intended or unintended, is typically carried out in comparison with the non-modified recipient organism and/or plants which are not LMOs but exhibit a similar abiotic stress tolerance. The non-modified comparator provides the baseline information for comparison during trials when it is grown at the same time and location as the LM plant. Comparisons should also be made, as appropriate, in a range of environments with different stressor intensities and durations.

While the comparative approach should be used to assess whether or not the LM plants with tolerance to abiotic stress have increased fitness advantages under non-stress conditions, additional approaches (and comparators) for risk assessment need to be implemented for assessing potential adverse effects under abiotic stress.

LM plants with tolerance to abiotic stress may present specific challenges in the experimental design to generate data for the risk assessment. In some cases, for instance, an approach uses different reference plant lines, which typically include a range of genotypes representative of the natural variation in the plant species. Another important consideration is whether the

experimental design is properly controlled for the effect of the abiotic stress trait. In the extreme case, when the non-modified plant cannot be grown in the range of conditions of the receiving environment because the abiotic stress conditions prevent or severely affect the growth of the non-modified plant, a comparative approach between the LM plant and the non-modified plant will need to be adjusted. In such cases, non-modified varieties or distant relatives that are tolerant to abiotic stress may become useful comparators. It is noted however that, in situations where the non-modified recipient organism, or (near-)isogenic or closely related lines cannot be used for a comparative risk assessment, the use of non-isogenic lines or distant relatives as comparators can make it more difficult to identify statistically meaningful differences.

In situations where a suitable comparator is not available, the characterization of the abiotic stress tolerant LM plant may be similar to that carried out for alien species, where the whole plant is considered a novel genotype in the receiving environment. On a case by case basis, available information from *"omics" technologies*, for example, "transcriptomics" and "metabolomics", may help to detect phenotypic and compositional changes (e.g., the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison with field grown plants under suboptimal conditions.

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

Elements for consideration:

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

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

CONDUCTING THE RISK ASSESSMENT

Unintended characteristics including cross-talk between stress responses *(see "Step 1" in the Roadmap)*

Rationale:

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

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

Elements for consideration:

Deleted: *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 living modified plant in representative environments *(see "Step 1" in the Roadmap)*

Rationale:

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

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

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

Elements for consideration:

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

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

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

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

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

Persistence in agricultural areas and invasiveness of natural habitats *(see "Step 1", "Step 2", "Elements for consideration (b), (f) and (g)", and "Step 4", "Element for consideration (e)" in the Roadmap)*

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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 an unwanted increased persistence of the LM plant in agricultural areas. Expression of these genes may also change the capacity of LM plants to establish in climatic and geographic zones beyond those initially considered as the likely potential receiving environments.

In the event where the modified gene is a transcription factor conferring tolerance to abiotic stress, the transcription factor may also affect the response mechanisms to other forms of abiotic stress. For example, the seeds of a plant modified for drought or salinity tolerance may acquire in addition tolerance to cold resulting in an increased winter survivability of the seeds. Therefore,

an abiotic stress-tolerant LM plant may acquire the potential to persist better than its non-modified counterpart and other species under different abiotic-stress conditions.

Most tolerance traits can be expected to have a "metabolic cost" associated with them – usually 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.

Elements for consideration:

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

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

(c) Characteristics, 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;

(d) Effects of climate change that could change the ecological range of the LM plant; and

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

Effects on the abiotic environment and ecosystem *(see "Step 3", "Elements for consideration (a) and (e)" in the Roadmap)*

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Rationale:

Changes to the abiotic environment resulting from the use of LM plants will depend largely on the introduced trait, and may be relevant for LM plants with modified tolerance to certain environmental conditions.

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

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

Elements for consideration:

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

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

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

BIBLIOGRAPHIC REFERENCES

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

C. RISK ASSESSMENT OF LIVING MODIFIED TREES

BACKGROUND

During its eighth and ninth meetings, the Conference of the Parties to the CBD recognized "the uncertainties related to the potential environmental and socio-economic impacts, including long-term and transboundary impacts, of genetically modified trees on global forest biological diversity", recommended "Parties to take a precautionary approach when addressing the issue of genetically modified trees", and urged Parties to undertake a number of actions with regard to LM trees, such as "to develop risk-assessment criteria specifically for genetically modified trees".[25] Moreover, forest biodiversity is one of the seven thematic programmes of work under the Convention on Biological Diversity (CBD).

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Comment [A110]: Comment T 45.

SCOPE

According to the Food and Agriculture Organisation of the United Nations (FAO), a tree is: "a woody perennial with a single main stem, or, in the case of coppice, with several stems, having a more or less definite crown".[26] This guidance focuses on true botanical trees and does not cover any additional species such as palms, bamboos and shrubs.

Comment [A111]: Comment T20.

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INTRODUCTION[28]

Tree species belong to many different taxonomic orders and families of angiosperms (flowering plants; e.g., mahogany, poplar, apple) and gymnosperms ("naked seed" plants; e.g., pine, spruce, cedar). Trees differ from other plants, such as annual crops, due to characteristics such as size, perennial growth habit with a long lifespan, and delayed onset of reproductive maturity.

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High fecundity together with seed dormancy, many pathways for dispersal of propagules, and high seed viability are important aspects of the reproductive capacity of many, although not all, tree species. Moreover, the potential for vegetative propagation in certain trees raises the possibility that new individuals can be established from branches or roots.

Comment [A112]: Comment T18.

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[25] See COP decisions VIII/19 paragraphs 2 and 3 (http://www.cbd.int/decision/cop/?id=11033) and IX/5 paragraphs 1(s)-(z) (http://www.cbd.int/decision/cop/?id=11648).

[26] "Training manual on inventory of trees outside forests (TOF)" available at ftp://ftp.fao.org/docrep/fao/006/AC840E/AC840E.pdf.

[28] The biology of trees is relevant for risk assessment. Not all aspects of trees biology or use are unique to them or shared by all trees but are discussed here to focus the risk assessment of LM trees.

Because of their perennial growth and, in many cases, long lifespan and large size, trees, develop complex, direct, indirect and multi-level ecological interactions with other organisms ranging from decomposers to birds and from insect pollinators to large wild animals. Those interactions may span over several generations of the other species if they have shorter lifespans. Moreover, the root systems of trees can be extensive and are often associated with microorganisms and fungi, such as mycorrhizae (symbiotic associations).

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Regarding reproductive maturity and breeding systems, many tree species undergo a distinct juvenile phase which may last from several years to more than a decade before the onset of reproductive maturity. As a result, some tree species have gone through only a limited number of breeding cycles by the time they are planted for commercial purposes. Additionally, some tree species are dioecious (i.e., plants that are either male or female) and cannot undergo selfing (i.e., common practice for increasing homogeneity of many crops), leading to the increased use of methods for vegetative propagation to ensure uniformity of the propagated trees for plantation use. By using cuttings from some tree species, in particular some fruit trees, a desirable selected genotype may be grafted onto a rootstock of a different genotype. For many forest and fruit tree species, clonal multiplication of identical individuals can be achieved through regeneration of entire trees from vegetative propagules such as cuttings or somatic embryos.

Tree species and genotypes are highly diverse and exhibit a wide range of distribution and complex associations with other organisms, as well as significant ecological, economic, environmental, climatic and socio-economic values. Fruit, ornamental, and forest tree species of economic interest grow in various regions of the world from temperate to tropical climates. Thirty one per cent of the total global land area or more than 4 billion ha, is covered by forests. Minimally managed forest habitats and non-managed forests like tropical rainforests or boreal forests are of high conservation value. Accordingly, many countries regard trees as important components of biodiversity and have protection goals to ensure their conservation. Such protection goals should be taken into account when assessing the possible adverse effects of LM trees and emphasis should be given to the precautionary approach.

A number of LM trees have been developed through the use of modern biotechnology and introduced into the environment.[29] The majority of these LM trees are species of economic interest used in managed orchards, forests and plantations. The modified traits include herbicide

[29] See the LMO registry in the BCH (http://bch.cbd.int/database/organisms/) and background documents for this section.

tolerance, wood composition (e.g., lignin), growth rate and phenology (including flowering and fruiting), resistance to pests and diseases, and abiotic stress tolerance.

PLANNING PHASE OF THE RISK ASSESSMENT

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

Rationale:

As with the risk assessments of any other type of LMO, a comprehensive planning phase is needed to define, among other things, how a comparative approach can be carried out in the risk assessment of an LM tree.

In instances where LM tree species have a long lifespan and high potential for dispersal, outcrossing and establishment beyond the intended receiving environment (e.g., into natural or less managed ecosystems) should be taken into account.

In forestry, the use of well adapted provenances (i.e., trees that have evolved or been bred within the region where they will be grown commercially)[30] is of great importance because they may show better adaptive capabilities and consequently better performance than unselected germplasm. [31] These regional provenances, whether naturally occurring, domesticated or introduced but locally bred and adapted, may provide appropriate comparators for LM trees in accordance with national protection goals and good forest management practices.

For those LM tree species for which there is little or no information with regard to their ecological functions and interactions in the likely potential receiving environment, the comparative approach may be challenging. In such cases, the assessment of the overall risk of the LM tree may involve a high degree of uncertainty which must be described in the conclusions of the risk assessment and communicated to decision makers.

Comment [A114]: Comment T7.

Elements for consideration:

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[30] A comparable concept for crop plants would be regionally adapted crop varieties.

[31] For example the Ministerial Conference on the Protection of Forests in Europe recommended "Native species and local provenances should be preferred where appropriate. The use of species, provenances, varieties or ecotypes outside their natural range should be discouraged where their introduction would endanger important/valuable indigenous ecosystems, flora and fauna".

(a) Availability of information and knowledge of the biology and ecological interactions of the species and/or genotype (including regional provenances or ecotypes as appropriate) that can be used as a comparator;

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

(c) Design of field trials in relation to established methodologies for the non-modified trees, including for example the length of the period before flowering, the length/age of trials, testing in different environments and exposure to multiple biotic and abiotic stresses.

CONDUCTING THE RISK ASSESSMENT

The information provided in this section aims at covering different tree species and management practices and may be taken into account on a case-by-case basis.

Presence of genetic elements and propagation methods *(see "Step 1", "Point to consider (b)" in the Roadmap)*

Rationale:

The transformation method used may lead to the presence of modified genetic elements in an LM tree that could be linked to potential adverse effects (e.g., some antibiotic resistance genes). The cross-breeding process (including back-crossing) is an option to reduce the presence of such genetic elements.

Many tree species have a long juvenile period and, for the purposes of forestry and plantations, their multiplication is typically achieved through clonal and vegetative propagation. In such cases, the removal of undesirable genetic elements in LM trees through cross-breeding would not be feasible.

Elements for consideration:

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(a) Transformation methods used which may possibly lead to the presence of genetic elements that may have an adverse effect;

(b) Propagation method(s) used – cross-breeding (including the degree of back-crossing, if possible, in that species) and/or vegetative propagation.

Long lifespan, genetic and phenotypic characterisation and stability of the modified genetic elements *(see "Step 1", "Point to consider (d) and (e)" in the Roadmap)*

Rationale:

In unmanaged ecosystems, the lifespan of some trees can range from several decades to several centuries or longer. Such trees can tolerate and adapt to the different biotic and abiotic conditions they encounter during their lives. The phenotypic characterization of an LM tree should consider its developmental stage and a range of environmental conditions. To the extent possible, it may also be important to consider whether and how management practices, that could affect the characterization of the LM tree, would change over time.

Taking into account the long lifespan of some trees, transgene instability, including those causing gene silencing and variable expression levels, should be considered in the context of its possible relevance for risk assessment. Similarly, genetic/environmental interactions, that may play a role in the expression level of the transgenes, should be duly considered. Consequently, an assessment of the stability of the transgenes and their levels of expression at different points during the lifespan of the LM tree may be important considerations, in particular where transgenic approaches are used for containment strategies (e.g., male sterility or ablation of floral organs).

Due to the large size and long lifespan of many tree species, data obtained from glasshouse experiments may be limited with regard to, for example, the number of generations and experimental replications that can be observed. This may present a challenge when the risk assessment of an LM tree calls for data to reflect the changing characteristics of the LM tree and the likely potential receiving environment over time. The risk assessment of LM trees may benefit from a broader approach using mathematical modelling.

Comment [A115]: Comment T3.

Elements for consideration:

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(a) Changes in the interactions with other organisms, and changes in the ability to maintain role and function in ecosystems;

(b) Phenotypic changes over time in response to different stressors and different developmental stages;

(c) Potential for variability in transgene expression levels, including gene silencing over time;

(d) Availability of data from glasshouse experimentation (including exposure to biotic and abiotic stresses).

Dispersal mechanisms *(see "Step 1", and "Step 2", "Elements for consideration (d), (e) and (h)" in the Roadmap)*

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Rationale:

Forest trees, like other plants, have developed a variety of ways to reproduce and disseminate via seeds, pollen and/or vegetative propagules. Trees often produce large amounts of pollen and seed per individual and propagules may be designed to spread over long distances (e.g., by wind, water, or animals including insects). The potential for vegetative propagation in certain trees raises the possibility of establishing new individuals from branches or root parts.

Seeds inside fruits may travel as commodities around the globe and be released at the place of consumption such as road margins, railways or touristic areas, as well as in farmers' fields and local gardens.

Many trees are capable of vegetative propagation which increases the exposure of the environment, both in terms of time and space, particularly in the case of large trees with a long lifespan. Therefore, the potential for and means of vegetative propagation are relevant considerations during the risk assessment of LM trees.

Elements for consideration:

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(a) Available information on the dispersal mechanisms and viability of pollen and seed for the non-modified and LM tree species;

(b) Potential for and mechanisms of vegetative propagation in the non-modified and LM tree species;

(c) Climatic conditions, or management practices that affect reproductive biology;

(d) Potential for dispersal mechanisms from anthropogenic activities (e.g., trade and consumption of fruits);

(e) Expansion of the distribution area of an LM tree due to dispersal mechanisms throughout its lifespan.

The likely potential receiving environment(s) *(see "Step 1", "Elements for consideration (f) and (g)", "Step 2", "Elements for consideration (b), (d), (f) and (h)", "Step 3", "Elements for consideration (a) and (e)" in the Roadmap)*

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Rationale:

The identification and characterisation of likely potential receiving environment(s) may be dependent on the LM tree in question, its habitats, the traits and modified characteristics and its mechanisms for dispersal. With some trees the intensity of management in the likely potential receiving environment may be less than for some annual plants. The domestication level of some forest trees may be low and trees can often survive without human intervention. Therefore, the potential for dispersal of propagative material into environments other than the intended receiving environment is an important consideration during the risk assessment.

Many tree species (e.g., poplars and eucalyptus) can propagate through vegetative means. When characterizing the likely potential receiving environment during the risk assessment of such an LM tree, the movement of seeds as well as the movement of vegetative propagules should be taken into account. Issues related to unintentional transboundary movements may also be taken into account in cases where LM trees could cross national boundaries through, for example, pollen or seed dispersal by physical and biological vectors, including the international trade of fruits with seeds.

Elements for consideration:

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(a) Environments and their degree of management which offer the potential for seeds and/or vegetative propagules to establish;

(b) Presence and proximity of species in the receiving environment with which the LM tree may hybridize;

(c) Proximity of protected areas, centres of origin and genetic diversity or ecologically sensitive regions;

(d) Ecosystem functions and services of the potential receiving environment (e.g., relevant components of food webs);

(e) Change in landscape patterns and sensitivity of the receiving environment to human activities.

Exposure of the ecosystem to living modified trees and potential consequences *(see "Step 2" and "Step 3" in the Roadmap)*

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Rationale:

Some trees remain relatively undisturbed for much of their life cycle and may engage in a variety of ecological interactions, such as providing habitat for other organisms and functioning as part of complex and elaborate food webs. In determining the likelihood of an adverse effect of an LM tree, an assessment of the exposure to the LM tree should take into account the expected duration of the trees' presence in the receiving environment, the nature of the transgenic traits, the intended use of the LM tree (e.g., processing, trade routes), as well as dispersal mechanisms. Given the late onset of reproductive maturity of a number of tree species, pollen and seed production may not occur during field trials.

The expansion of tree cultivation areas for bioenergy may also increase the diversity of environments exposed to LM trees including those modified to mitigate potential invasiveness.

Elements for consideration:

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(a) Duration of the presence of the LM trees in the likely potential receiving environment;

(b) Persistence and potential long-term adverse effects of the LM trees in the environment including potential for the non-modified recipient organism to be invasive;

(c) Consequences of the modified trait on invasive characteristics;

(d) Long-term interactions that could lead to adverse effects to other organisms including via food web interactions;

(e) Consequences on ecosystem functions and biodiversity arising from the changes in land use for the cultivation of LM trees.

Deleted: of the cultivation of LM trees

Comment [A116]: Comment T45

Risk management strategies *(see "Step 4", "Point to consider (e)" and "Step 5" in the Roadmap)*

Rationale:

The need for risk management strategies designed for LM trees will depend on the results of risk assessment, and may vary depending on the LM tree and the conditions under which it is grown. When the recommendations of the risk assessment include measures for limiting or preventing dispersal of forest or plantation LM trees, strategies that may be used include delaying or preventing flowering (e.g., fast-growing trees for pulp or biomass/bioenergy production being cut before reaching the reproductive phase) and biological confinement (e.g., induction of male sterility or flower ablation). While complete flower ablation is not desirable for many fruit or horticultural tree species, male sterility may be appropriate in some species (e.g., apples) where pollen from a different variety (which could be non-modified) is usually required. However, male sterility approaches will not prevent the production of seeds by LM trees fertilized by fertile trees. Where applications involve genetic modification of only the rootstock in grafted trees, dispersal may be managed by ensuring that the rootstocks do not produce shoots or flowers.

Elements for consideration:

Deleted: *Points to consider*

(a) Type and intended use of the LM tree;

(b) Degree and type of management (e.g., grafting of fruit trees, rotation period of forest trees);

(c) Specific effects and risks of any containment strategy achieved through the use of modern biotechnology.

BIBLIOGRAPHIC REFERENCES

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

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

D. RISK ASSESSMENT OF LIVING MODIFIED MOSQUITOES

INTRODUCTION

Living modified (LM) mosquitoes are being developed through modern biotechnology to reduce transmission of vector-borne human pathogens, particularly those that cause malaria, dengue and chikungunya. Control and reduction of such diseases is a recognized public health goal. The impacts of such diseases on human health are staggering. For instance, in 2008, there were 247 million cases of malaria and nearly one million deaths.[32] Therefore, specific and comprehensive considerations should be undertaken with regard to the potential benefits and adverse effects of LM mosquitoes.

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

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

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

Self-propagating strategies, also known as self-sustaining strategies, rely on *gene-drive systems* that promote the spread and persistence of the transgene through populations of the same mosquito species. As opposed to the self-limiting strategy, the modifications in LM mosquitoes produced through self-propagating strategies are intended to be heritable and to spread through the target population and, thus, to persist in the ecosystem at least for the medium term. Hence,

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

the objective of self-propagating strategies is the replacement of the non-modified mosquito population by the LM mosquitoes that have been modified to render them less capable of transmitting a disease. In a related approach, gene-drive systems may be used to promote the spread of a gene that confers a fitness load or a male bias in the offspring ratio. In this way, gene-drive systems may be used to suppress vector population sizes or induce a cascade of population crashes. An example of such a system is an X-shredding homing endonuclease gene (HEG) which can be driven into a population at the same time as biasing the offspring ratio towards males and hence potentially inducing an all-male population crash.

Another strategy, the so-called paratransgenesis, is under development to control, reduce or eliminate the capacity of vectors to transmit pathogens mainly, but not exclusively, by blocking the development of the pathogen in the vector. Paratransgenesis focuses on utilizing symbionts of insects to express molecules, within a vector, that are deleterious to the pathogens transmitted by the vector. In the case of paratransgenesis for the control of diseases transmitted by mosquitoes, the mosquito itself will not be genetically modified, but the microorganism that inhabits the mosquito (e.g. in its mid-gut) will be the product of modern biotechnology. Such microorganisms may have a specific, symbiotic relationship with the mosquito, or may be commonly associated with the mosquito but not have an obligate relationship. Paratransgenesis can be used as a self-limiting strategy for population suppression or as a limited self-propagating strategy for population replacement (see above).

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

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

OBJECTIVE AND SCOPE

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

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This section focuses on the risk assessment of LM mosquitoes of the family *Culicidae*, developed through self-limiting and self-propagating strategies to be used in the control of human and zoonotic diseases such as malaria, dengue, chikungunya, yellow fever and West Nile.

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This section does not consider the potential adverse effects of LM microorganisms released into the environment. Thus, paratransgenesis is not in the scope of this guidance.

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PLANNING PHASE OF THE RISK ASSESSMENT

In addition to the considerations raised in the Roadmap, the risk assessment of LM mosquitoes focuses on ecological and epidemiological processes that may be adversely affected by the introduction of the LM mosquito, taking into account the species of the mosquito, the LM trait, the intended and unintended receiving environment, and the objective and scale of the intended release. The biology and, to some extent, the ecology of the mosquito species that transmit malaria and dengue are rather well known in many regions of the world. However, in certain regions and in the environment where LM mosquitoes are likely to be introduced, more information may be needed depending on the nature and scale of the LM strategy to be deployed. In many of these environments few studies have been conducted to examine gene flow among disease-transmitting vectors, their mating behaviour, the interactions among vectors sharing one habitat, how pathogens respond to the introduction of new vectors, etc. Such information may be needed to establish a baseline in order to assess the risks of LM mosquitoes. Additionally, methods for the identification of specific ecological or environmental hazards are also needed.

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

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

Rationale:

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

CONDUCTING THE RISK ASSESSMENT

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

Rationale:

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

Elements for consideration:

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

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

Containment of the living modified mosquito

Rational:

Different strategies for the containment of LM mosquitoes can be applied, including physical, biological and chemical containment. In cases where there are uncertainties with regard to the potential adverse effects of a widespread release of LM mosquitoes into the environment, a release limited to in a particular geographic zone may be desirable. Any containment measures

used as a means of limiting the release of the LM mosquito, either in location or in duration, must be taken into account in each of the steps of the risk assessment.

Elements for consideration:

(a) The containment strategy (physical, biological and chemical) and its effectiveness;

(b) Success rate of separating sexes or induction of sterility in cases of biological containment, as appropriate;

(c) Potential for spread of the genes responsible for the biological containment.

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

Rationale:

The role of mosquitoes in natural ecosystems should be assessed, as the release of LM mosquitoes may have unintended effects on the target vector and pathogen[33] and other non-target species which may lead to adverse effects. Potential unintended effects will vary from case to case and may include:

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

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

  Mosquito species are currently able to transmit several pathogens, such as viruses and filaria, to human beings and animals. An LM mosquito, in which the capacity of transmission of one of these pathogens has been modified, may enhance the transmission of other pathogens.

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

[33] 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.

The released LM mosquito may become a more vigorous pest by, for example, becoming a host to a broader range of pathogens.

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

- *Harm to or loss of other species:*

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

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

- *Disruption of ecological communities and ecosystem processes:*

The ecological communities in the ephemeral, small aquatic habitats occupied by the non-LM mosquitoes are unlikely to be disrupted beyond the possibilities already addressed above under "harm to or loss of other species." However, if the released LM mosquitoes were to

inhabit natural habitats (e.g., tree-holes), disruption of the associated community is a possibility.

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

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

Elements for consideration:

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(a) The natural dispersal range and seasonality of the host mosquito in relation to the likely potential receiving environment where the LM mosquito may be released;

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Rationale:

For self-propagating LM mosquitoes, gene-drive systems for moving genes into wild populations may be the initial focus when assessing the likelihood of vertical gene transfer from LM mosquitoes to non-LM mosquitoes through cross-fertilization. The likelihood of vertical gene transfer in self-limiting LM mosquitoes is likely to be lower than for self-propagating LM mosquitoes, but should be assessed on a case-by-case basis (see below). Various factors may influence gene flow and any associated adverse effects, such as the strategy used in the

development of the LM mosquito, characteristics of the transgenes, characteristics of the gene-drive system, the stability of the trait(s) carried by the mosquito over generations, and characteristics of the receiving environment.

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

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

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

Elements for consideration:

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

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

Horizontal gene transfer

Rationale:

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

Elements for consideration:

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

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

(c) Nucleic acid sequences in the LM mosquito which might influence the mobility of the insert and transgenes (such as mobile elements) through recombination with genes in the microorganisms.

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

Rationale:

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

adverse effects to biological diversity, taking into account human health, methods to reduce the persistence of the transgene in the ecosystem need to be considered.

Point to consider:

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

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

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

Rationale:

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

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

Elements for consideration:

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

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

Unintentional transboundary movements[34]

Rationale:

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

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

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

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

Elements for consideration:

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

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

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

Rationale:

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

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

Elements for consideration:

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(a) Availability of monitoring methods to:

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

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

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

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

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

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

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

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

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

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

RELATED ISSUES

There are other issues that may be taken into consideration in the decision for environmental releases of LM mosquitoes which are not covered by Annex III of the Protocol. They encompass, *inter alia*, the potential social, economic, cultural and health benefits associated with the use of LM mosquitoes to control wild-type mosquitoes that are vectors of human and animal pathogens

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and parasites or, alternatively, the use of chemical pesticides or other means to achieve the same result. The use of LM mosquitoes will require broader considerations of how target-disease risk affects human behaviour, veterinary medicine, public health practices and national health priorities in order to address the risks to human and animal health caused by the exposure to wild-type mosquitoes that are vectors of pathogens and parasites.

BIBLIOGRAPHIC REFERENCES

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

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

Comment [A120]: Comment Mq40: Add publications regarding the case of risk assessment of LM mosquitoes in Brazil.

PART III:
MONITORING OF LIVING MODIFIED ORGANISMS RELEASED INTO THE ENVIRONMENT

In accordance with the terms of reference for the AHTEG, this document provides guidance on monitoring of living modified organisms released in the environment,[35] and complements the Roadmap for Risk Assessment of Living Modified Organisms (LMOs).

INTRODUCTION

Monitoring of LMOs released into the environment may allow for the identification of adverse effects in a timely manner and as early as possible. Monitoring may also inform on the need for appropriate response measures such as changes to risk management strategies, emergency response measures, a new risk assessment, or re-evaluation of prior decisions.

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Paragraph 8(f) of Annex III to the Protocol states that "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 and/or monitoring the living modified organism in the receiving environment". Article 16 of the Protocol and, in particular, paragraphs 2 and 4 may also be relevant with respect to the implementation of monitoring. The Convention on Biological Diversity (CBD) covers monitoring in its article 7, "Identification and Monitoring".[36]

OBJECTIVE AND SCOPE

This document aims at offering science-based practical guidance for monitoring adverse effects of LMOs released into the environment that could affect the conservation and sustainable use of biological diversity, taking into account risks to human health. In this guidance, monitoring of LMOs refers to the systematic observation, collection, and analysis of data undertaken based on the risk assessment and following the release of an LMO into the environment, and in

[35] Decision BS-IV/11 of the Conference of the Parties serving as the meeting of the Parties to the Protocol (http://bch.cbd.int/protocol/decisions/decision.shtml?decisionID=11690).

[36] See CBD article 7(a) to (d) (http://www.cbd.int/convention/articles/?a=cbd-07).

accordance with the objective of the Protocol.[37] This guidance may be applicable to all types of LMOs, and scales of release into the environment (i.e., small- and large-scale releases).

Although monitoring of potential adverse effects to human health is within the context of the Cartagena Protocol, it is not the focus of this section of the Guidance. Literature relevant to monitoring in the context of human health can be found among the background documents for this section (see below).

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This document does not address decisions as to whether or not monitoring should be implemented, or who bears the responsibility and costs for implementation.

MONITORING AND ITS PURPOSES

For the purposes of this document, monitoring is categorized as "case-specific monitoring", or "general monitoring".[38]

Case-specific monitoring may be conducted to address uncertainty in the level of risk for effects anticipated in the risk assessment. The purpose of case-specific monitoring may vary, depending on the type, duration (e.g., short- or long-term) and scale (e.g., small- and large-scale) of the release, as well as on uncertainties regarding the level of risk or its management:

- *Monitoring during experimental, short-term and/or small-scale environmental releases*

  Monitoring can generate data during experimental, short-term and small-scale releases in order to provide supporting information (e.g., to test specific risk scenarios) for future risks assessments that may involve a larger scale of release of the same LMO. When environmental releases of an LMO are conducted in a step-wise manner, monitoring at smaller scales may increase the scientific strength or certainty of risk assessments for subsequent larger scale releases.

- *Monitoring during long-term and/or large-scale environmental releases*

  During long-term and large-scale releases of an LMO (e.g., for commercial purposes), monitoring may be conducted in order to gather further information to address uncertainties regarding the level of risk, or to confirm that conclusions of the risk assessment are accurate once the environmental release has taken place. In some cases, effects may be identifiable

[37] See Article 1 of the Protocol (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-1).

[38] Some experts in the Open-ended Online Forum and AHTEG are of the view that "general monitoring" should not be part of this Guidance.

but difficult to estimate or address in the framework of a risk assessment (e.g., these may include long-term, multi-trophic, or cumulative effects, as well as changes to management practices and effects on human health). Using broader approaches to monitoring may be useful in such cases (see considerations on general monitoring below).

• *Monitoring to evaluate the efficacy of specific risk management strategies*

In cases where risk management strategies are implemented along with an environmental release, monitoring may be used to evaluate the effectiveness of these risk management strategies.

General monitoring is used in some approaches to account for effects that were not anticipated in the risk assessment. General monitoring starts with general observations of changes in indicators and parameters, such as assessment endpoints, which are often defined within national protection goals or are related to the conservation and sustainable use of biological diversity, taking into account risks to human health.

General monitoring may utilize existing monitoring networks, including monitoring networks that are not focused on biosafety, for the surveillance of broader protection goals and assessment endpoints wherever possible. In case changes that could lead to an adverse effect are detected through general monitoring, possible causes for the observed changes are examined and, where appropriate, a more specific hypothesis is developed and tested to establish whether or not a causal relationship exists between LMO(s) and the adverse effect, and be followed up by case-specific monitoring or further research.

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DEVELOPMENT OF A MONITORING PLAN

A monitoring plan is developed when the recommendation of a risk assessment and/or the national biosafety policy calls for monitoring activities to be carried out in conjunction with the environmental release of the LMO. In such cases, the competent authority(ies) or the entity responsible for the risk assessment may outline the requirements of a monitoring plan (including the reporting of monitoring data). The monitoring plan should be transparent, of scientific quality in the context of well constructed hypotheses, and in sufficient detail so that the relevance of the data can be appraised.[39]

[39] See Roadmap "Overarching issues in the risk assessment process", "Quality and relevance of information".

If a monitoring plan is to be developed by the notifier, it may be evaluated by the competent national authority and may be subject to modification before a decision for release is granted. Importantly, the proposed activities for case-specific monitoring should be relevant to the identified uncertainties regarding the level of risk posed by the LMO under consideration.[40] Information relevant for developing the monitoring plan may be available from the risk assessment and, if applicable, from previous monitoring activities, including those from other countries. For example, the choice of protection goals and assessment endpoints (which may include the selection of indicators and parameters) may often be derived from the context and scoping phase of the risk assessment (See Roadmap, "Establishing the context and scope"). The scientific and technical details of the specific LMO, including detection methods, would in many cases be available from the information required for conducting the risk assessment as outlined in Annex III of the Protocol.[41]

When developing (or evaluating) a monitoring plan, the following may be considered:

1. Choice of indicators and parameters for monitoring ("what to monitor?");
2. Monitoring methods, baselines including reference points, and duration of monitoring ("how to monitor?");
3. Monitoring sites and regions ("where to monitor?");
4. Reporting of monitoring results ("how to communicate?").

The sections below address these issues in terms of rationales and elements for consideration.

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[40] See Roadmap "Overarching issues in the risk assessment process", "Identification and consideration of uncertainty".

[41] See paragraph 9 of Annex III to the Protocol (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-43).

1. Choice of indicators and parameters for monitoring ("what to monitor?")

Rationale:

Monitoring for potential adverse effects of an LMO involves the observation of changes to *indicators* (e.g., species, populations, soil, environmental processes, etc.) and/or *parameters* (i.e., a component to be measured in the observation of an indicator, such as species abundance or soil organic matter).

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Results obtained from monitoring may assist in evaluating the estimates of environmental exposure which were made during the risk assessment (see step 2 in the Roadmap). Therefore, monitoring the exposure of the environment to LMOs may be a highly relevant element of an overall monitoring approach].

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The selection of indicators and parameters to be monitored will vary from case to case, depending on the LMO, characteristics of the likely potential receiving environment, specific risk scenarios established during the risk assessment, (see the Roadmap), and on the protection goals and biosafety legislation or policies of each country

Elements for consideration:

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(a) The potential of the indicators and parameters to signal changes related to adverse effects as early as possible and/or before the consequences are realized;

(b) Characteristics of the indicators and their level of exposure to the LMO, as well as parameters for the distribution and abundance of those indicators that are organisms;

(c) Quantitative and qualitative variability of the indicators and parameters to be observed and how this variability could affect the ability of these indicators and parameters to signal changes that may lead to potential adverse effects;

(d) The usefulness of the candidate indicators and parameters to establish relevant baselines, including reference points;

(e) The importance of the candidate indicators and parameters to relevant key ecological processes and functions or to the identified protection goals;

(f) Whether sampling and analysis would be easy or difficult and how these would affect the choice of indicators and parameter.

2. Monitoring methods, baselines including reference points, and duration of monitoring ("how to monitor?")

i. Selecting monitoring methods

Rationale:

Monitoring methods are largely dependent on the indicators and parameters chosen in the preceding step, as well as the ability of these indicators and parameters to address uncertainty regarding the level of risk and to signal changes that could lead to an adverse effect. The selection of monitoring methods should also take into account the level of sensitivity and specificity needed to detect changes in the indicators and parameters.

The description of the monitoring methodology includes the means for sampling and observing indicators and parameters, and for the analysis of the resulting data. Appropriate methods for collecting monitoring data may include observations, descriptive studies and questionnaires addressed to those who are exposed to or are handling to the LMO. For ecological issues, or effects occurring outside of the receiving environment, additional knowledge and tools may be required to gather relevant data.

The best available science should always be used for monitoring. In some cases, the harmonization of methods, data formats, and analytical approaches facilitates the comparison of results from monitoring in different environments. When the use of existing surveillance programs is to be considered, the monitoring plan should guide the choice and use of these programs.

Elements for consideration:

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(a) Relevance of the monitoring methodology to generate the necessary information to address uncertainty related to the level of risk;

(b) The nature of the effect to be monitored (e.g., whether short- or long-term, delayed or indirect, cumulative, etc.);

(c) Relevance, suitability and adaptability of existing surveillance programs, as well as the accessibility to those data, in the context of broader environmental monitoring;

(d) The specification of the range or magnitude of changes in a parameter or indicator to signal changes that could lead to an adverse effect;

(e) The scientific quality of the sampling, analytical and statistical methods to be employed;[42]

(f) The availability of relevant standardized methods, and whether and how these could be taken into account;

(g) Whether methods are adequate to meet the objectives of the proposed monitoring plan;

(h) The availability and use of descriptive studies or questionnaires, taking into account their replicability and verifiability;

(i) Findings from ongoing and/or other monitoring activities, if relevant;

(j) Relevant local, regional and international monitoring practices.

ii. Establishing baselines, including reference points

Rationale:

The establishment of relevant baselines, including reference points is necessary for observing and analysing changes during monitoring. A baseline is a measurement or description of the existing conditions of the likely potential receiving environment, and/or comparable reference environment, including the relevant indicators and parameters. Therefore, the methodology by which the baseline is derived should be described in the monitoring plan in order to verify that it will provide useful information in relation to the environment where the LMO may be released. Natural and human induced variation that may occur in baseline data should be taken into account when analysing monitoring data.

[42] See also considerations on "Quality and relevance of information" in the Roadmap.

Elements for consideration:

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(a) The scientific quality of methods used for generating baseline data including reference points;

(b) The appropriate spatial scale of the baseline including reference points to be established;

(c) Effects of temporal and spatial variation (i.e., human induced or natural variation in the physical environment);

(d) The scale of the likely potential spread of the LMO.

iii. Establishing the duration and frequency of monitoring

Rationale:

The duration of the monitoring, including the frequency at which observations or measurements need to be made, is determined on a case-by-case basis and will depend on the type of changes that may lead to adverse effects that are to be monitored (e.g., immediate or delayed, short- or long-term), the type of LMO (e.g., short or long life cycles,[43] transgenic traits introduced), and the duration of the proposed environmental release. Where general monitoring is used, the type of changes to be monitored may be broader to account for unanticipated effects. The duration or frequency of monitoring may be adjusted, if appropriate, on the basis of the results of on-going monitoring activities.

Elements for consideration:

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(a) How long it would take for changes in a parameter to likely become apparent;

(b) Characteristics of the indicators to be measured or described (e.g., persistence, life-cycle and generation time of species when used as indicators);

(c) Life-cycle and generation time of the LMO as it is being used in the environment;

(d) Whether variability in the monitored parameters over time could affect the results and conclusions of monitoring;

[43] See article 16.4 of the Protocol (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-16).

(e) Potential for environmental changes, both biotic and abiotic.

3. Choice of monitoring sites ("where to monitor?")

Rationale:

Monitoring sites are selected on a case-by-case basis depending on the geographical location of the release in the likely potential receiving environment, the parameters and indicators that will be used in the monitoring, as well as the intended use of the LMO, and taking into account the associated management practices.

The choice of monitoring site may include areas beyond the intended receiving environment where the LMO may be introduced.

Relevant information regarding the sites to be monitored includes, for example, specific locations, their size and relevant environmental characteristics. In this context location registries (e.g., national and regional databases) may be a useful information tool for LMO-monitoring and the selection of relevant monitoring sites or regions.

Elements for consideration:

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(a) Dissemination and establishment of the LMO in the likely potential receiving environment;

(b) The type of LMO as well as indicators and parameters to be monitored and, in case of indicators that are species, their biological or ecological characteristics and life cycles;

(c) Appraisal of suitable, relevant reference sites where the LMO is not present for comparison over the duration of the monitoring, if applicable;

(d) Pathways through which the environment is likely to be exposed to the LMO(s);

(e) The distribution patterns, including seasonal distribution (e.g., migration), of the selected indicators that are species, in the likely potential receiving environment for consistent detection and observation;

(f) Appraisal of protected areas and centres of origin and genetic diversity or ecologically sensitive regions, particularly in the context of monitoring the presence of LMOs;

(g) The appropriate number of monitoring sites and the statistical power of the conclusions that can be drawn;

(h) The continued availability of the monitoring sites throughout the duration of monitoring;

(i) Current management practices and possible changes to those practices over the duration of monitoring.

(j) Sites that were previously used for field trials or experimental releases.

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4. Reporting of monitoring results ("how to communicate?")

Rationale:

Reporting of monitoring results serves four main objectives: i) to inform competent authorities of any changes that can be related to adverse effects; ii) to allow verification of the quality and relevancy of data derived from monitoring to ensure the activities have been carried out in a manner that meets the intended objectives set out in the monitoring plan; iii) to indicate, if appropriate, the need for changes to the monitoring plan and/or other risk management strategies (or for follow-up studies or risk assessments); and iv) to recommend, if appropriate, the re-evaluation of a decision and the necessity of any emergency measures.

The report of monitoring activities may be communicated in different forms, for example, depending on the target audience. From the report, the regulatory authority should be able to interpret the results and decide whether or not a specific action is required.

Elements for consideration:

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(a) Reporting requirements set out by the competent authority(ies) or in national biosafety regulations, if available;

(b) The completeness of the report, including transparency in presentation of methods, data and analytical tools used to draw conclusions;

(c) Accessibility to raw data accrued during the monitoring activities, taking into account information that may be confidential.[44]

[44] See article 21 of the Protocol (http://bch.cbd.int/protocol/text/article.shtml?a=cpb-21).

USE OF TERMS This section provides a working glossary of key terms used in this document. An attempt was made to adapt definitions that are used in internationally accepted risk assessment guidance to the context of environmental risk assessment conducted under the Cartagena Protocol.

Comment [A129]: Comments R264 and R427 (add "adverse effect" to the use of terms, see e.g. EFSA Guidance Glossary)

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Antagonism – An interaction of elements that when combined produce a total effect that is less than the sum of the effect of the individual elements. [back to the text]

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

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

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

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

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

Comparator – Non-modified recipients or parental organisms of the LMO. A comparator is used as an element to establish the basis for a comparative assessment in accordance with Annex III. [back to the text]

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

Conventional breeding – Not involving the use of modern biotechnology as defined in Article 3 of the Cartagena Protocol on Biosafety. [back to the text]

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

Cross-talk – Instances in which one or more components of a signal transduction pathway affect a different pathway. [back to the text]

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

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

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

Exposure – The route and level of contact between the likely potential receiving environment and the LMO or its products. [back to the text]

Exposure assessment – Evaluation of the exposure of the environment, including organisms, to an LMO or products thereof. (Adapted from WHO, 2004, IPCS Risk Assessment Terminology, http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf) [back to the text]

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

Gene flow – The transfer of genetic material from one organism to another by vertical or horizontal gene transfer; or the movement of an organism from one environment to another. [back to the text]

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

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

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

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

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

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

Horizontal gene transfer – The transfer of genetic material from one organism to another through means other than inheritance from parent to offspring (i.e., vertical). [back to the text]

Introgression – Movement of a gene or genetic element from one species into the gene pool of another species or population, which may result in a stable incorporation or some fertile offspring. [back to the text]

Isogenic line, (Near-) – Isogenic lines: two or more lines differing from each other genetically at one locus only; near-isogenic lines are two or more lines differing from each other genetically at several loci [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 of the adverse effect occurring, taking into account the level and kind of exposure of the likely potential receiving environment to the LMO. [back to the text]

Monitoring – Systematic observation of a process over a period of time. In the context of this document, 'Case-specific monitoring' is used to describe measures intended to observe changes in indicators and parameters that are related to the released an LMO under consideration and its modified trait(s). 'General monitoring' is used for general observation of the changes in indicators and parameters that may affect the natural ecosystem, taking into account human health.

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Comment [A130]: Comment Mn82.

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

No-observed-effect level (NOEL) – Greatest concentration or amount of a substance, found by experiment or observation, that causes no alterations of morphology, functional capacity, growth, development, or life span of target organisms distinguishable from those observed in normal (control) organisms of the same species and strain under the same defined conditions of exposure. (IUPAC, 2007, Glossary of Terms Used in Toxicology, 2nd edition, Pure Appl. Chem. 79: 1153-1344, http://sis.nlm.nih.gov/enviro/iupacglossary/frontmatter.html) [back to the text]

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

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/. See also "Vertical gene transfer") [back to the text]

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

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

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

Protection goal –Defined and valued environmental outcomes that guide the formulation of strategies for the management of activities that may affect the environment [and consider the social and economic aspects of each country or region].. [back to the text]

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

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

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

Risk characterization – The qualitative and/or quantitative estimation, including attendant uncertainties, of the overall risk. (Adapted from CODEX, 2001, Definitions of Risk Analysis Terms Related to Food Safety, http://www.fao.org/DOCREP/005/Y2200E/y2200e00.htm) [back to the text]

Risk management – The measures to ensure that risks identified in the risk assessment are reduced, controlled, or eliminated. (Adapted from UNEP, 1995, International Technical

Guidelines for Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf) [back to the text]

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

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

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

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

Transformation event – An LMO with a specific modification that is the result of the use of modern biotechnology according to Article 3 (i) (a) of the Protocol. [back to the text]

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

***Trans*-regulation –** Transcriptional regulation of gene expression by regulatory elements that were themselves transcribed in a different region of the genome. For example, a transcriptional factor transcribed in one chromosome may regulate the expression of a gene located in another chromosome. [back to the text]

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

Unintended gene product – Gene products (e.g., RNA, proteins), which are different from those originally intended. [back to the text]

Unmanaged and managed ecosystems – An "unmanaged ecosystem" is an ecosystem that is free from significant human intervention. As opposed to a "managed ecosystem" which is an ecosystem affected by varying degrees of human activities. [back to the text]

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

Vertical gene transfer – Transfer of genetic material from one organism to its offspring via asexual, parasexual or sexual reproduction. Also referred to as "vertical gene flow". [back to the text]
