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AD HOC TECHNICAL EXPERT GROUP ON RISK ASSESSMENT AND RISK MANAGEMENT UNDER THE CARTAGENA PROTOCOL ON BIOSAFETY
Second meeting
Ljubljana, 19-23 April 2010
Item 3.1 of the provisional agenda*

DRAFT GUIDANCE DOCUMENTS ON RISK ASSESMENT

Draft texts for further deliberations

Note by the Executive Secretary

1. At its fourth meeting, the Conference of the Parties serving as the meeting of the Parties to the Protocol, in its decision BS-IV/11, established an Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management and an open-ended online forum on specific aspects on risk assessment through the Biosafety Clearing-House in accordance with the terms of reference annexed to that decision.

2. The AHTEG was mandated to meet twice prior to the fifth meeting of the Parties, to be held in Nagoya, Japan, from 11 to 15 October 2010.

3. At its first meeting, held in Montreal from 20 to 24 April 2009, the Group considered the need for further guidance on specific aspects of risk assessment, prioritized topics for subsequent development of guidance documents, and established four sub-working groups to focus on each of the topics.

4. The following draft guidance documents, which were developed by the AHTEG sub-working groups on the basis of the discussions within the AHTEG and the Open-Ended Online Expert Forum on Risk Assessment and Risk Management,[1] are annexed hereto, namely: Roadmap for Risk Assessment (annex I); Risk Assessment and Risk Management of Living Modified Crops with Tolerance to Abiotic Stress (annex II); Risk Assessment and Risk Management of Living Modified Mosquitoes (annex III); and Risk Assessment and Risk Management of Living Modified Organisms with Stacked Genes or Traits (annex IV).

5. The AHTEG is invited to further consider these drafts as working documents for deliberations at its second meeting.

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* UNEP/CBD/BS/AHTEG-RA&RM/2/1.

[1] Available at http://bch.cbd.int/onlineconferences/forum_RA.shtml .

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Annex I

DRAFT ROADMAP FOR RISK ASSESSMENT

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DRAFT ROADMAP FOR RISK ASSESSMENT

Prepared by the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management

Version of 21 March 2010

This "roadmap" provides an overview of the process of environmental risk assessment for a living modified organism (LMO) in accordance with Annex III[1] to the Cartagena Protocol on Biosafety (hereinafter "the Protocol") and the other articles related to risk assessment. This Roadmap was developed in response to decision BS-IV/11[2] of the Conference of the Parties serving as the meeting of the Parties to the Protocol (COP-MOP). Annex III is the basis of the Roadmap. Accordingly, this Roadmap is a guidance document and does not replace Annex III. The overall aim of the Roadmap is clarifying and enhancing the usability of Annex III by elaborating the technical and scientific process of how to apply the steps and points to consider in the process of risk assessment.

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

The Roadmap applies to all types of LMOs and their intended uses within the scope of the Protocol, and in accordance with Annex III. However, it has been developed based largely on living modified crop plants because of the extensive experience to date with environmental risk assessments for these organisms. It is intended to be a "living document" that will be modified and improved on over time as and when mandated by COP-MOP, and in the light of new experience, information and developments in the field of applications of LMOs, e.g. when other types of LMOs have been evaluated more extensively in environmental risk assessments.

INTRODUCTION

General introduction

Background

In accordance with the precautionary approach,[3] the objective of the Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of LMOs 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.[4]

For this purpose, Parties shall ensure that risk assessments are carried out when making informed decisions regarding LMOs.

The objective of risk assessment is to *identify* and *evaluate* the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment,

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

2 http://www.cbd.int/biosafety/cop-mop/results/?id=11690 .

3 Principle 15 of the Rio Declaration on Environment and Development (1992), http://www.unep.org/Documents.Multilingual/Default.asp?DocumentID=78&ArticleID=1163 .

4 http://www.cbd.int/biosafety/articles.shtml?a=cpb-01 .

taking also into account risks to human health.[5] An LMO may have several environmental effects, intended or unintended. The environmental effects may be adverse, and it is this potential for an LMO to cause adverse effects that is taken into consideration, on a case-by-case basis, in an environmental risk assessment. Adverse effects are taken into account in an environmental risk assessment, on a case-by-case basis. What is considered an adverse effect depends on protection goals and risk assessment end-points as chosen by the Party and set out in existing policies and strategies when scoping the risk assessment.

According to the general principles of Annex III of the Protocol, risk assessments shall be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of LMOs on the conservation and sustainable use of biological diversity, taking also into account risks to human health.[6]

Annex III states[7] that 'risk assessment should be carried out in a scientifically sound and transparent manner, and can take into account expert advice of, and guidelines developed by, relevant international organizations. Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or an acceptable risk.' 'Risk assessment should be carried out on a case-by-case basis. The required information may vary in nature and level of detail from case to case, depending on the LMO concerned, its intended use and the likely potential receiving environment.'

The risk assessment process

Risk assessment is a structured process. Paragraph 8 of Annex III provides a description of the key steps of the risk assessment process to identify, evaluate and manage potential risks. Paragraph 9 describes, depending on the case, points to consider in this process. The steps describe an integrated process whereby the results of one step may be relevant to other steps. Also, risk assessment may need to be conducted in an iterative (i.e. repetitive) manner, where certain steps may be repeated or reexamined to increase the confidence in the conclusions of the risk assessment. When new information arises that could change its conclusions, the risk assessment may need to be re-examined accordingly. Similarly, the issues mentioned in the 'overarching issues' section below can be taken into consideration again at the end of the risk assessment process to determine whether the objectives and criteria that were set out at the beginning of the risk assessment have been met.

Risk assessment is done in a comparative manner, meaning that 'risks associated with living modified organisms should be considered in the context of the risks posed by the non-modified recipient organism in the likely potential receiving environment.'[8] Additionally, experience with an LMO with the same, or, as appropriate, similar, genotypic and phenotypic characteristics may also be taken into consideration in the risk assessment of an LMO. For instance, the comparison with the isogenic non-modified recipient is used in Step 1 of the risk assessment (see below) where the novel genotypic or phenotypic characteristics associated with the LMO are identified. But when the potential consequences of adverse effects are evaluated, broader experience, such as mentioned in Step 3 (a), may be taken into account, as a baseline. Results from experimental field trials or other environmental information and experience with the same LMO may be taken into account as information elements in a new risk assessment for that LMO. In all cases where information, including baseline data, is derived from other sources, it is important to establish the validity of the information for the risk assessment. For instance, it should be taken into account that the behavior of a transgene in an LMO may vary, because it may depend on the genetic and physiological

[5] Annex III, 1.
[6] Article 15, 1.
[7] Annex III, 3, 4 and 6.
[8] Annex III, 5.

background of the recipient as well as on the ecological characteristics of the environment that the LMO is introduced into.

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

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

Overarching issues in the design/planning phase of the risk assessment process

There are some overarching issues to consider in the design/planning phase of the risk assessment process to ensure the quality and relevance of the information used. These entail, among others:

- Setting criteria for relevancy in the context of a risk assessment – e.g. data may be considered relevant if they can affect the outcome of the risk assessment.

- Establishment of scientifically robust criteria for the inclusion of scientific information.

    - Data should be of an acceptable scientific quality. Data quality should be consistent with the accepted practices of scientific evidence-gathering and reporting and may include independent review of the methods and designs of studies. Data may be derived from a variety of sources, e.g. new experimental data as well as data from relevant peer reviewed scientific literature.

    - The principles of transparency, verifiability, and reproducibility (e.g. reporting of methods and data in sufficient detail, so that a reconstruction can be done of how experimental data were obtained); and the principle of accessibility of data (e.g. the availability of relevant, required data or information or, if requested and as appropriate, of sample material), are necessary to ensure and verify that the risk assessment is carried out in a scientifically sound and transparent manner.

- Identification of the types and sources of uncertainty.

  "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."[9]

  Uncertainty is inherent in the concept of risk. In communicating the results of risk assessment and risk management it is therefore important to consider and analyze the various forms of uncertainty that may exist at each step of the risk assessment and in combination at step 4 of the risk assessment. Current literature is replete with discussions on uncertainty analysis in risk assessment and in decision-making. To date, however, there is no universally accepted approach for addressing uncertainty in the risk assessment of LMOs. (*See references relevant to "Identification of the types and sources of uncertainty"*).

  In risk assessment uncertainty can arise at each of the steps of the process. Types of uncertainty can differ, for instance, at: 1) the level at which uncertainty is generated, - e.g. statistical uncertainties, or uncertainties with respect to the relevance of risk scenarios, recognized lack of

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

knowledge, etc.; 2) the source of uncertainty, - e.g. what is the context of the risk assessment, which data are used as inputs, study/model design, choice of parameters, which conclusions are drawn from results, etc.; and 3) the nature of uncertainty, - e.g. due to naturally occurring variations or due to the way that the information is obtained in scientific experiments. Each of these types of uncertainty may be analyzed to characterize the specific uncertainties present in the risk assessment.

Where these considerations result in uncertainty regarding the level of risk, this may be addressed by requesting further information on the specific issues of concern. It should be kept in mind that there is always uncertainty in a scientific process, and uncertainty cannot always be reduced by providing additional information. For example, new uncertainties may arise as a result of the provision of additional information. Uncertainties may also be addressed in decision-making by requiring the implementation of appropriate risk management strategies or of targeted monitoring of the LMO in the receiving environment.

Uncertainty, due to, for instance, lack or ambiguity of scientific data or ignorance, may be addressed by applying the precautionary approach,[10] taking into account that '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'. [11] (*See references relevant to "Precautionary approach"*).

Context and scoping of the risk assessment

In setting the context and scope for a risk assessment, a number of aspects should be taken into consideration, as appropriate, that are specific to the Party involved and to the specific case of risk assessment. These aspects include:

- (i) Existing policies and strategies based on, for instance, regulations and the international obligations of the Party involved; (ii) Guidelines or regulatory frameworks that the Party has adopted; and (iii) Protection goals, end-points and management strategies that the Party has adopted. Setting the context and scope for a risk assessment that are consistent with these policies, strategies and protection goals may involve a process that includes risk assessors, decision-makers and various stakeholders prior to conducting the actual risk assessment;

- (i) Framing the risk assessment process; (ii) Taking into account the expected (potential) conditions of handling and use of the LMO; (iii) Taking into account customary practices and habits that could affect the protection goals or end-points; identification of relevant questions to be asked for that purpose;

- Identification of methodological and analytical requirements, including any reviewing mechanisms, that is required to achieve the objective of the risk assessment as laid down, for instance, in guidelines published or adopted by the Party that is responsible for conducting the risk assessment (i.e. typically the Party of import according to the Protocol);

- The nature and level of detail of the information required may depend on the intended use of the LMO and the likely potential receiving environment. For small scale field releases, especially at

[10] As stated in Article 1 of the Protocol; see Principle 15 of the Rio Declaration on Environment and Development (1992).

[11] Annex III, paragraph 4.

early experimental stages, less information may be available compared to the information available for large scale environmental release, and for commercial scale planting;

- Experience and history of use of the non-modified recipient, taking into account its ecological function;[12] and

- Establishing criteria for describing the level of the (potential) environmental adverse effects of LMOs, as well as criteria for the terms that are used to describe the levels of likelihood (Step 2), the magnitude of consequences (Step 3) and risks (Step 4) and the manageability of risks (Step 5; see risk assessment steps below).

(*See references relevant to "Context and scoping"*).

THE RISK ASSESSMENT

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

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

(*See references relevant to "Risk Assessment in general"*).

Step 1: "An identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health." [13]

Rationale:

The purpose of this step is to identify biological changes resulting from the genetic modification(s), including any deletions, compared to the non-modified organism, and identify what, if any, changes could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. This step is similar to the 'hazard identification step' in other risk assessment guidance. The comparison of the LMO with the non-modified recipient or, as appropriate, with a non-modified organism of the same species, serves this purpose.

In this step, scientifically plausible scenarios are identified in which novel characteristics of the LMO could give rise to adverse effects in an interaction with the likely potential receiving environment. The novel characteristics of the LMO to be considered can be genotypic, phenotypic and biological, intended and unintended. The points to consider below provide information elements on which hazard identification can be built.

[12] The term 'ecological function' (or: 'ecological services)' provided by an organism refers to the role of the organism in ecological processes. Which ecological functions or services are taken into account here will be dependent on the protection goals set for the risk assessment. For example, organisms may be part of the decomposer network playing an important role in nutrient cycling in soils or be important as a pollen source for pollinators and pollen feeders.

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

The type and level of detail of the information required in this step may vary from case to case depending on the nature of the modification of the LMO and on the scale of the intended use of the LMO. For small scale field releases, especially at early experimental stages, less information may be available and the resulting uncertainty may typically be addressed by risk management measures.

Points to consider regarding the characterization of the LMO:

(a) Relevant characteristics of the non-modified recipient (e.g. (i) its biological characteristics, in particular those that, if changed, or interacting with the new gene products or traits of the LMO, could cause changes in the behavior of the non-modified recipient in the environment in a way that may cause adverse effects; (ii) its taxonomic relationships, (iii) its origin, centers of origin and centers of genetic diversity); (*See references relevant to "Step 1 – Point to consider (a)"*).

(b) Relevant characteristics of the genes and of other functional sequences, such as promoters, that have been inserted into the LMO (e.g. functions of the gene and its gene product in the donor organism with particular attention to characteristics that could cause adverse effects in the recipient); (*See references relevant to "Step 1 – Point to consider (b)"*).

(c) Molecular characteristics of the LMO related to the modification (e.g. (a) characteristics of the insert(s) including (i) gene products (intended and unintended), (ii) levels of expression, (iii) functions, (iv) insertion site in the genome of the recipient, (v) stability or integrity within the genome of the recipient; (b) (i) the transformation method, (ii) the characteristics of the vector if and, as far as it is present in the LMO, including its identity, source or origin and host range) with particular attention paid to any characteristics that are related to potential adverse effects. The availability and relevance of this information may vary according to the type of application. Characteristics related to adverse effects may also result from changed expression levels of endogenous genes due to effects of a transgene (e.g. due to insertional disruption of a gene, chimeric genes that have arisen by linking endogenous genes to inserted genes or to regulatory effects). Adverse effects may also result from combinatorial effects (the effects of combinations of genes) such as cumulative, synergistic, or antagonistic effects, of the transgene product with endogenous genes or products of other transgenes present in the LMO; (*See references relevant to "Step 1 – Point to consider (c)"*).

(d) Identification of genotypic and phenotypic, biological changes in the LMO, either intended or unintended, in comparison with the non-modified recipient, considering those changes that could cause adverse effects. These may include changes at the transcriptional and translational level and may be due to the insert itself or to genomic changes due to the transformation or recombination processes.

Point to consider regarding the receiving environment:

(e) Characteristics of the likely potential receiving environment, in particular its attributes that are relevant to potential interactions of the LMO that could lead to adverse effects (see also paragraph (f) below);[14]

[14] Examples of relevant attributes of the receiving environment include, among others: (i) 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 ecosystem type(s) or 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,

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

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

(g) Considerations for unmanaged and managed ecosystems (such as agricultural, forest and aquaculture systems) that are relevant for the likely potential receiving environment. These include the potential for dispersal of the LMO through, for instance, seed dispersal or outcrossing within or between species, or through transfer into habitats where the LMO may persist or proliferate;

(h) Unintentional outcrossing and flow of transgenes from an LMO to other sexually compatible species may occur, which could lead to introgression of the transgene(s) into the population of the sexually compatible species; and

(i) Adverse effects as a consequence of horizontal gene transfer (HGT) of transgenic sequences from the LMO to any other organism in the likely receiving environment. Concerning HGT to micro-organisms (including viruses), particular attention needs to be given to transgenic sequences present in the LMO that have been derived from micro-organisms as well as in cases where the LMO itself is a micro-organism.

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

Rationale:

The potential adverse effects identified in Step 1 may result in risks, but this depends on the likelihood and the consequence of the effects. In order to characterize the overall risk (in Step 4), the likelihood of each adverse effect being realized has to be assessed and evaluated beforehand. One aspect to be considered is whether the receiving environment will be exposed to the LMO in such a way that the identified adverse effects may actually occur, e.g. taking into consideration the intended use of the LMO, and the expression level, dose and environmental fate of transgene products as well as plausible pathways leading to adverse effects. Other aspects to be considered here are (i) the potential of the LMO or its derivatives (e.g. sexually compatible organisms in which transgenes could introgress) to spread and establish beyond the receiving environment, and whether that could result in the possibility to affect or displace the same or other species; and (ii) the possibility of occurrence of adverse (e.g. toxic) effects on organisms (or on organisms other than the 'target organism' for some types of LMOs). The levels of likelihood may be expressed, for example, by the terms 'highly likely', 'likely', 'unlikely', 'highly unlikely'. It is recommended that these terms and their uses be described, for instance, in the risk assessment guidelines published or adopted by the Party.

Points to consider:

(a) Information relating to the type and intended use, including proposed risk management measures if applicable, of the LMO as well as the scale of release;

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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.

(b) The relevant characteristics of the likely potential receiving environment that may experience or may be a factor in the occurrence of the potential adverse effects (see also Step 1, (e), (f) and (g));

(c) Levels of expression in the LMO and persistence and accumulation in the environment (e.g. in the food chain) of potentially harmful substances newly produced by the LMO such as insecticidal proteins; effects of the transgene on the levels of expression of endogenous toxins or allergens;

(d) Available information on the location of the release and the receiving environment (such as geographic and biogeographic information, including, as appropriate, coordinates, information on the sexually compatible species and whether they are co-localized with the LMO and whether flowering occurs at the same time, or in general, interbreeding can occur);

(e) For the case of outcrossing from an LMO to sexually compatible species, considerations should include: (i) the biology of the sexually compatible species, (ii) the potential environment where the sexually compatible species may be located, (iii) the chance of introgression of the transgene into the sexually compatible species; and

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

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

Rationale:

This step describes an evaluation of the magnitude of the consequences in the likely potential receiving environment, taking into account, among others, results of tests done under different conditions such as laboratory experiments or experimental field releases. The results from these tests may be used, for instance, to assess potential invasiveness, the potential to cause harm to non-target organisms, and also unintended effects. The evaluation should be considered in the context of the adverse effects caused by the non-modified recipient or, if more appropriate, by a non-modified organism of the same species. It should also be considered in the context of the adverse effects that occur in the environment due to comparable existing practices such as agronomic practices for pest or weed management if such information is available or relevant. The evaluation of the consequence of adverse effects may be expressed as, for instance, 'major', 'intermediate', 'minor' or 'marginal'. It is recommended that these terms and their uses be described, for instance, in the risk assessment guidelines published or adopted by the Party. (*See references relevant to "Step 3"*).

Points to consider:

(a) Relevant experience with the consequences of existing practices with the non-modified recipient or, if more appropriate, with a non-modified organism of the same species in the likely potential receiving environment, may be useful in order to establish baselines to evaluate, for example, the consequences of (i) agricultural practices, such as the level of inter- and intra-species gene flow, dissemination of the recipient, abundance of volunteer plants in crop rotation; or (ii) pest management, including effects on non-target organisms in pesticide applications while following accepted agronomic practices; (*See references relevant to "Step 3 – Point to consider (a)"*);

(b) Direct and indirect, immediate and delayed effects as well as combinatorial effects, such as dominant/recessive effects, effects of gene silencing, and cumulative, synergistic or antagonistic effects, leading to adverse consequences. (*See references relevant to "Step 3 – Point to consider (b)"*);

(c) Results from field trials evaluating, for instance, potential invasiveness, and laboratory experiments examining dose-response relationships (e.g., EC 50s, LD 50s); and

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

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

Rationale:

The purpose of this step is to determine and characterize the cumulative level of risk posed by the LMO on the biological diversity, taking also into account human health, based on an analysis of the potential adverse effects identified in Step 1, their likelihood (Step 2) and consequences (Step 3), and also taking into consideration any relevant uncertainty that emerged in the preceding steps.

It should then be determined whether the identified risks meet the criteria for acceptability relative to assessment endpoints and thresholds, as established in relevant statutes or regulations. 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 LMO in the receiving environment (see also Step 5). The estimation of the 'overall risk' in this step does not take into account the potential benefits of the LMO under the conditions of use.[15] Description of the risk characterization may be expressed as, for instance, 'negligible', 'low', 'medium', 'high' or 'indeterminate due to uncertainty or lack of knowledge'. It is recommended that these terms and their uses be described, for instance, in the risk assessment guidelines published or adopted by the Party. (*See references relevant to "Step 4"*).

Points to consider:

(a) The assessments of likelihood (Step 2);

(b) The evaluation of the consequences (Step 3);

(c) Potential cumulative adverse effects due to the presence of multiple LMOs in the receiving environment and synergistic/combinatorial potential adverse effects due to the presence of multiple transgenes or DNA sequences in the LMO and traits that may interact; and

(d) Analysis of the combined uncertainty analyses conducted in this and the previous steps to characterize and address uncertainties (including variability) inherent in the scientific information used in the risk assessment.

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:

If the evaluation of the overall risk conducted in the previous step leads to the conclusion that the identified risks are not negligible, the question arises whether those risks are acceptable and whether risk management options can be identified that have the potential to remove the identified risks or reduce their magnitude. The acceptability of risks can relate to, among other things, risks posed by the non-modified

[15] Consideration of risks versus (environmental) benefits may be performed during the process of decision making.

recipient and its use. In the process of the formulation of risk management options, the effect of the proposed options on the identified risks should be explained. The risk assessment should then be reiterated by taking into account the implementation of the risk management options to estimate the new levels of likelihood, consequence and risk. In this way, Step 5 provides an interface between the process of risk assessment and the process of determining whether risk management measures are necessary and, if so, which measures could be implemented to manage the risks associated with the LMO.
The recommendation of acceptability of risk(s) should acknowledge the previously identified uncertainties. Some uncertainties may be addressed by monitoring (e.g. checking the validity of assumptions about the ecological effects of the LMO), requests for more information, or implementing the appropriate risk management options.

The recommendation(s) as to whether or not the risks are acceptable or manageable and recommendations for risk management options are submitted for consideration in the decision-making process. (*See references relevant to "Step 5"*).

Points to consider related to the acceptability of risks:

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

(b) Relevant risks posed by the use of the non-modified recipient, and practices associated with its use in the potential receiving environment, providing a baseline for the comparison with the LMO.

Points to consider related to the RM strategies:

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

(d) Methods to detect and identify the LMO and their specificity, sensitivity and reliability in the context of environmental monitoring (e.g. monitoring for short- and long-term, immediate and delayed effects; specific monitoring on the basis of scientific hypotheses and supposed cause/effect relationship as well as general monitoring) including plans for appropriate contingency measures to be applied in case the results from monitoring call for them; (*See references relevant to "Step 5 – Point to consider (d)"*).

(e) Management options in the context of the intended use (e.g. mitigating the effect of an LMO producing insecticidal proteins by the use of refuge areas to minimize the development of resistance against these proteins).

ISSUES RELATED TO RISK ASSESSMENT AND DECISION-MAKING ON LMOs

The environmental risk assessment of an LMO is part of the decision-making process in which other issues may also be taken into account. In risk assessment and decision making, a number of articles of the Protocol are relevant:

- **Article 14:** Bilateral, Regional and Multilateral Agreements and Arrangements
- **Article 16:** Risk Management

- **Article 17:** Unintentional Transboundary Movements and Emergency Measures
- **Article 22:** Capacity-building
- **Article 23:** Public Awareness and Participation
- **Article 26:** Socio-economic Considerations
- **Article 27:** Liability and Redress

Some further issues that are frequently mentioned in relation to risk assessment and decision-making on LMOs, but that are not within the scope of Annex III of the Protocol, include:

- Ethical issues;
- Effects on human health specifically related to food or feed safety;
- Consumers practices, patterns and habits;
- Coexistence.

ANNEX – FLOWCHART FOR RISK ASSESSMENT

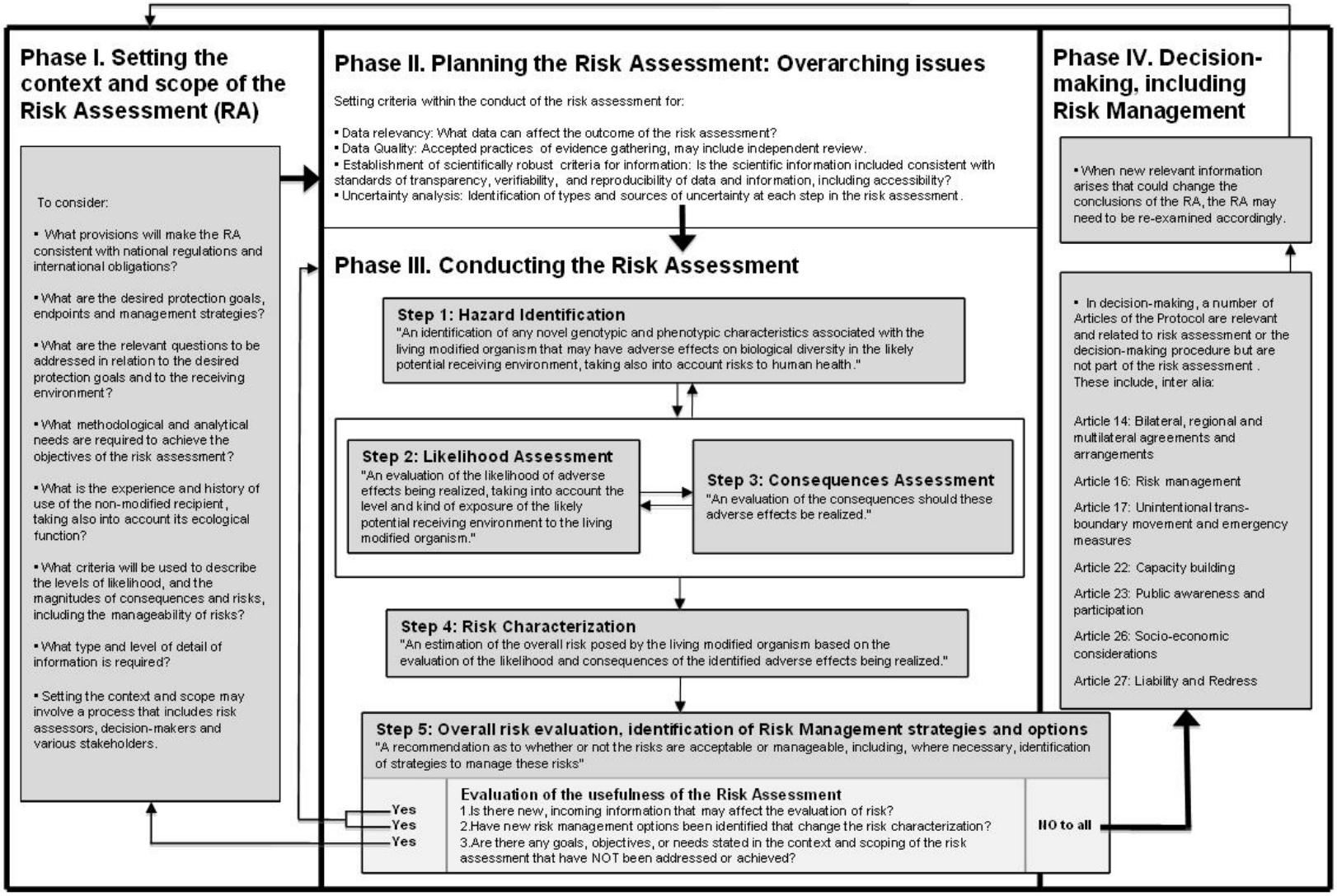

Figure 1. The Roadmap for Risk Assessment. The flowchart comprises the process described in the Roadmap to identify, evaluate, and manage the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. This includes the following phases: I. Setting the context and scope of the Risk Assessment; II. Planning the Risk Assessment; III. Conducting the Risk Assessment, and is further linked to IV. Decision-making, including Risk Management through the process of identifying and recommending Risk Management strategies and options to be considered for implementation in decision-making.

Annex II

**DRAFT GUIDANCE ON
RISK ASSESSMENT AND RISK MANAGEMENT OF
LIVING MODIFIED CROPS TOLERANT TO ABIOTIC STRESS**

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DRAFT GUIDANCE DOCUMENT ON RISK ASSESSMENT OF LIVING MODIFIED CROPS WITH TOLERANCE TO ABIOTIC STRESS

Prepared by the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management

Version of 21 March 2010

GENERAL CONSIDERATIONS

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

This guidance document should be considered in the context of the Cartagena Protocol on Biosafety. The elements of Articles 15 Annex III of the Protocol also apply to LM crops with tolerance to abiotic stress. Accordingly, the methodology and points to consider[1] contained in Annex III are also applicable to this type of LMO.

Because the potential environmental adverse effects of an LM crop with abiotic stress tolerance will depend on (i) the receiving environment; (ii) the modified crop, (iii) phenotypic changes resulting from the genotypic changes made to the plant and (iv) its intended use; their risk assessment must be performed on a case-by-case basis in accordance with the General Principle 6 of Annex III of the Protocol.

This guidance document complements the Roadmap for Risk Assessment developed by the AHTEG on Risk Assessment and Risk Management, and focuses on issues that are of particular relevance to the risk assessment of LM crops tolerant to abiotic stress.

USE OF TERMS

Abiotic stresses are environmental conditions caused by non-living factors that are detrimental or suboptimal to the growth, development and/or reproduction of a living organism. Types of primary abiotic stresses include, for example, drought, salinity, cold, heat, air pollution (e.g., nitrous oxides, ozone), etc.

RISK ASSESSMENT

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

Questions that may be particularly relevant to the risk assessment of LM crops with tolerance to abiotic stress in connection with the intended use and receiving environment include:

- Would the tolerance trait have the potential to increase the invasiveness or weediness in the LM crop or to cause adverse effects to other organisms?
- Would a LM plant expressing tolerance to a particular abiotic stress have other advantages in the targeted receiving environment that cause adverse effects?
- Would the abiotic stress tolerant crop, or LMOs derived by outcrossing, have the potential to

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

colonize an ecosystem beyond the targeted receiving environment?

- Would the abiotic stress tolerance trait have the potential to affect pest and disease resistance mechanisms of the LM crop?

Some of the potential adverse effects to be evaluated in the risk assessment, from the introduction of crops tolerant to abiotic stress into the environment include, for example: a) increased selective advantage(s) other than the intended tolerance trait; b) increased persistence in agricultural areas and increase invasiveness of natural habitats; c) adverse effects on organisms exposed to the crop; and d) consequences of potential increased gene flow to wild or conventional relatives. While these adverse effects may exist regardless of whether the tolerant crop is a product of modern biotechnology or conventional breeding, some specific issues may be more relevant in the case of stress tolerant LM crops.

Characterization of the LM crop with tolerance to abiotic stress in comparison with its non-modified crop *(see Step 1 of the Roadmap for Risk Assessment)*

Rationale:

The first step in the risk assessment process involves the characterization of any novel genotypic and phenotypic changes associated with the abiotic stress tolerant LM crop that may have adverse effects on biodiversity in the likely receiving environment, taking into account risks to human health. This step is the 'hazard identification step' in other risk assessment guidance.

The identification of genotypic and phenotypic changes in the LMO, either intended or unintended, is typically done in comparison with the non-modified organism (see "step 1" of the Roadmap). The non-modified comparator provides the baseline information for comparison between trials when it is grown at the same time and location as the LM crop. Comparisons with the observed range of changes in the non-modified crop in different environments, also provides baseline information.

However, in the case of LM crops that are tolerant to abiotic stress, a straight forward comparative approach between the LM crop and the non-modified crop may be limited when the non-modified crop has never have been grown in the range of conditions of the receiving environment because the stress conditions prevent or severely affect the growth of the non-modified crop. In such conditions, choosing good comparators could be a challenge and there are several proposals on whether and how the comparative approach can be used to characterize LM crops tolerant to abiotic stress in these likely receiving environments.

In some cases, for instance, an approach using different reference lines, typically including a range of genotypes that represent the natural variation in the crop species, and/or commercial or adapted varieties, may be useful. However, the use of non-isogenic reference lines can make it more difficult to identify statistically meaningful differences. In some situations when a comparator may not be available to carry out a meaningful comparison, some propose to characterize the tolerant LM crop as a novel genotype in the receiving environment. To this end, information available from "omics" technologies, for example, "transcriptomics" and "metabolomics" may be used. These techniques may help to detect phenotypes (eg, the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison between field grown plants. However other approaches emphasize the importance of testing the phenotype of the LM crop in the environment, rather than characterizing the genotype (e.g., sequences, insertion sites, etc) because much of the genotypic information is not predictive of the resultant phenotype.

Points to consider:

(a) Phenotypic characteristics of the LM crop in the likely potential receiving environment;

(b) Phenotypic characteristics of the LM crop under stressed and non-stress conditions;

(c) Phenotypic characteristic of the LM crop under different stresses, if applicable;

(d) Effects on the frequency or likelihood of gene flow to wild or domestic relatives;

(e) Whether one or more suitable comparators are available; and

(f) Genotypic and phenotypic analyses that may inform the characterization the LM crop in the receiving environment.

Unintended or unanticipated traits *(see Step 1 of the Roadmap for Risk Assessment)*

Rationale:

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

The genetic modification or transgene products may confer other unintended or unanticipated traits such as tolerances to other types of biotic and abiotic stresses, which could lead to a selective advantage of these crop plants under conditions other than that related to the modified trait. For instance, crops modified to become tolerant to drought or salinity may be able to compete better than their counterparts at lower and higher growing temperatures.

It is also possible the LM crops with enhanced tolerance to an abiotic stress could have changes in seed dormancy, viability, and/or germination rates under other types of stresses. Particularly if genes involved in abiotic stress are involved in crucial steps in physiology, modifications involving these genes may therefore be expected to have pleiotropic effects. Such LM crops may also transfer genes for stress tolerance at higher frequencies than observed in non-modified crops.

A potential mechanism for interactions between abiotic and biotic stresses may exist in plants. For example, drought or salinity-tolerant LM crops may acquire a changed tolerance to biotic stresses, which could result in changed interactions with their predators, parasitoids and pathogens, and, therefore, have both direct and indirect effects on organisms that interact with them.

Points to consider:

(a) Any unintended change that may lead to selective advantage or disadvantage acquired by the LM crop 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 crop; and

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

Increased persistency in agricultural areas and invasiveness of natural habitats *(see Steps 1, 3 and 5 of the Roadmap for Risk Assessment)*

Rationale:

In environments where water depletion or elevated salt content are the main factors limiting the growth, productivity, spread or persistence of a crop, expression of the genes for drought and salinity tolerance, respectively, could result in increased persistence of the modified crop in agricultural areas.

Climate changes and their potential ecological consequences may also alter the capacity of LM crops tolerant to abiotic stress, to spread to and establish in climatic and geographic zones beyond those initially considered as the likely or potential receiving environments.

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

Points to consider:

(a) Consequences of the increased potential for persistency of the modified crop in agricultural habitats and consequences of increased potential for invasiveness in natural habitats;

(b) Need for control measures if the stress-tolerant crop shows a higher potential for persistency in agricultural or natural habitats, that could cause adverse effects;

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

(d) Effects of climate change on agriculture and biodiversity and how this could change the habitat range of the LM crop in comparison to the non modified crop.

(e) If the LM crop expressing tolerance, would have a change in its inputs requirements, e.g. fertilizers?

BIBLIOGRAPHIC REFERENCES

See references relevant to the "Guidance Document on Risk Assessment and Risk Management of LM Crops with Resistance or Tolerance to Abiotic Stress".

Annex III

DRAFT GUIDANCE ON
RISK ASSESSMENT AND RISK MANAGEMENT OF LIVING MODIFIED MOSQUITOES

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DRAFT GUIDANCE DOCUMENT ON RISK ASSESSMENT AND RISK MANAGEMENT OF LIVING MODIFIED MOSQUITOES

Prepared by the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management

Version of 21 March 2010

OBJECTIVE

The Ad Hoc Technical Expert Group (AHTEG) on Risk Assessment and Risk Management has developed a Roadmap for Risk Assessment which sets out the necessary steps to conduct a risk assessment in accordance with Annex III to the Cartagena Protocol on Biosafety.[1]

The present document aims at complementing the Roadmap on specific issues that may need special consideration for the environmental releases of LM mosquitoes. It focuses mainly on Paragraphs 8 (a) and (e) of Annex III.

The present Guidance Document also provides additional information that may contribute to better understanding of the issue and help regulators to conduct risk assessment in the particular case of the environmental release of LM mosquitoes.

Reference will be made, for each topic of this document, to which step of Annex III it refers to. Suggestions for supporting bibliographies are also provided through links to web pages in the Biosafety Clearing House.

This is intended to be a "living document" that will be shaped and improved with time as new experience becomes available and new developments in the field of applications of LMOs occur, as and when mandated by the Parties to the Protocol**.**

INTRODUCTION

LM mosquitoes are being developed to control populations of vectors in order to reduce transmission of vector borne human pathogens, particularly those that cause malaria, dengue and chikungunya.

Various genetic strategies are being developed to control mosquito vectors by suppressing their population or reducing their vector competence. These strategies can be subcategorized according to the technology involved and the method of implementation. Some are intended to develop LM mosquitoes that are sterile or self-limiting (i.e, unable to pass the modification on indefinitely through subsequent generations), and thus depend on continued releases of male mosquitoes. Others aim at LM mosquito populations that are self-sustaining or self-propagating (i.e., heritable modifications intended to spread through the target population) and would depend on small and infrequent releases. The strategy under consideration is an

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

important factor in the risk assessment and risk management process, as it is recognized that there may be different sets of challenges to address the specific strategies.

The biology and ecology of mosquitoes, and their importance to public health as vectors of human disease and morbidity, pose new considerations and challenges to the risk assessment and risk management of LMOs, which have mainly dealt with LM crop plants.

SCOPE

This document focuses on the risk assessment and risk management of LM mosquitoes developed for use in vector control of human diseases such as malaria, dengue, chikungunya and yellow fever.

POTENCIAL ADVERSE EFFECTS

(see Step 1 of the Roadmap for Risk Assessment)

A specific and comprehensive list should be provided of potential adverse effects of a particular LM mosquito, taking into account the species of the mosquito, the LM trait, the molecular mechanisms of genetic modification, the intended receiving environment, and the objective and scale of the intended release. This list should consider, for instance: (a) the kinds of possible adverse effects for which there is solid scientific evidence from established natural phenomena; (b) the protection goals of the country where the LM mosquitoes will be introduced; (c) the species and ecological processes that could be affected by the introduction of the LM mosquitoes; and (d) a conceptual link between the identified protection goals and the introduction of the LM mosquito into the environment.

Effects on biological diversity (species, habitats, ecosystems, and ecosystem services)

Rationale:

The release of LM mosquitoes may have a negative impact on the target and other species, such as:

New or more vigorous pests, especially those that have adverse effects on human health: (i) The released LM mosquitoes may not function as expected. Gene silencing or production failures could result in the release of non-sterile or competent mosquitoes and thus increase the vector population or disease transmission. (ii) The released LM mosquitoes could transmit another disease more efficiently. Such diseases might include yellow fever, chikungunya, etc. (iii) Suppression of the target mosquito might enable another vector species to increase and result in higher levels of the target disease or a new disease in humans. These include other mosquitoes vectors of other diseases. (iv) The released LM mosquitoes might become nuisance pests. (v) The released LM mosquitoes might cause other pest problems to become more serious, including agricultural pests and other pests that affect other valued human activities.

Harm to or loss of other species. The released LM mosquitoes might cause other valued non-pest species (for instance fishes that rely on mosquitoes for food during some specific time of the year) to become less abundant. These include species of economic, cultural, and/or social importance such as wild foods, iconic species and endangered species. Ecological effects might result from competitive release if the target mosquito is reduced or from trophic consequences of species that rely on mosquitoes for food during some specific time of the year. Effects might also occur if (i) the target mosquito was also transmitting a disease to another animal species, (ii) the released LM mosquitoes transmit a disease of another animal species more efficiently, or (iii) a vector of an animal disease was released from ecological control by the reduction of the target mosquito. Sterile interspecific matings between released LM mosquitoes and other mosquito species could disrupt the population dynamics of these other species leading to harm or loss of

valued ecological species. However, more subtly, cessation of transmission of pathogens to other animals (e.g., West Nile virus to birds, Rift Valley fever virus to African mammals) might alter the population dynamics of those species, favoring increases in their numbers.

Disruption of ecological communities and ecosystem processes. The ecological communities in the ephemeral, small aquatic habitats occupied by the vector mosquitoes targeted with LM mosquitoes are unlikely to be greatly disrupted beyond the possibilities already addressed above under "harm to or loss of other species." However, if the released LM mosquitoes were to inhabit more natural habitats, such as tree-holes, disruption of the associated community is a possibility. The released LM mosquitoes might degrade some valued ecosystem process. This might include processes such as pollination or support of normal ecosystem functioning. These processes are often referred to as ecosystem services. However, the valued processes may be culturally or socially specific. Under some circumstances, mosquitoes are significant pollinators; therefore mosquito control of any kind might either reduce pollination of some species of plants or cause a shift to different kinds of pollinators. Habitats in which mosquitoes are the dominant insect fauna (e.g., high Arctic tundra, tree holes) would be changed if mosquitoes were eliminated; however, the common target vector species are usually associated with human activity and therefore not as closely tied to ecosystem services.

Points to consider:

(a) What is the impact of the strategy under consideration on the target mosquitoes?

(b) May the LM mosquitoes have an adverse effect on other species becoming agricultural, aquacultural, public health, or environmental pests or produce nuisances or health hazards?

(c) What is the habitat range of the target species? Is the habitat range expected to be affected by climate change?

(d) Is the target species native / invasive in a given area?

(e) Will the release affect mosquito species that are pollinators or otherwise are known to participate in valued ecosystem processes?

(f) What species do the target mosquitoes typically interact with in the environment?

(g) May the LM mosquitoes have an adverse effect on other interacting organisms, for instance, predators?

(h) May species replacement by other vector species occur, and if so, can it result in higher levels of the target disease or a new disease in humans or animals?

(i) Are adequate monitoring methods available prior to, during and after the trials to determine the level to which the identified effects might be realised?

Gene Flow

Rationale:

Gene flow in regard to biosafety refers to the transfer of transgenes or modified genetic elements from the LMO to non-modified organisms. It can occur via cross-hybridization or independent movement of the transgenes or genetic elements. Whether gene flow occurs and what adverse effects it might have depend on various factors such as the LM technology used, the construct and transgenes used, including promoters, the drive system and its stability over generations, the trait or traits carried by the mosquitoes, the receiving environment, etc.

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

Gene flow through cross-hybridization: Some LM mosquitoes are being designed to spread a trait rapidly through the target mosquito population. For instance, for *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, thus, spread of the transgenes or genetic elements in the target mosquito population is not expected. For the self limiting technologies, the potential for an unexpected spread of the transgenic trait should be considered by focusing on the ways that any management strategy to limit the spread could fail. Gene flow between different species should be considered for all of the LM mosquito technologies. Mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that will not allow interspecific gene flow. Identifying the key reproductive isolating mechanisms and the conditions leading to their breakdown could be a focus of this assessment. In addition, the fitness conferred by the transgenic trait and the size and frequency of the introduction of the LM mosquito into the environment will also determine the likelihood and rate of spread of the transgenes or genetic elements.

Independent movement of the transgenes or genetic elements: This is commonly referred to as "horizontal gene flow", which is the movement of genetic information from one organism to another through means other than sexual transmission. Gene drive systems for moving genes into wild populations should be one of the initial foci of the risk assessment. The risk of horizontal gene flow in LM mosquitoes that do not contain a gene drive system is likely be smaller but should nevertheless be assessed on a case by case basis.

Points to consider:

(a) Does the release of the LM mosquitoes have the potential to pass their modified traits to wild populations and to non-related organisms? If so, what may be the undesirable consequences?

(b) Will the LM mosquitoes induce undesirable functions or behaviors within target species, other wild related species or non-related organisms?

(c) What mechanisms are available to recall a trait which has spread unexpectedly (for example, mass release of wild-type mosquitoes above a certain threshold, alternative control methods, including genetic control)?

Persistence of the transgene in the environment

Rationale:

Inserted transgene(s) may spread and persist in natural populations. Some of the transgenes in LM mosquitoes are designed not to persist whereas others are expected to spread rapidly through wild population. In cases where the LM mosquitoes have been found through the risk assessment process to have the potential to cause adverse effects to the biological diversity, taking also into account human health, methods to reduce the persistence of the transgene in the environment or to mitigate the expression

of the transgene may be needed. Monitoring during and after the environmental release of the LM mosquitoes to address prompt detection of unexpected adverse effects may be recommended.

Points to consider:

(a) Are monitoring methods available to:

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

(ii) Assess the potential evolutionary breakdown of the mosquito technology (monitoring for transgene intactness and proper function over time)?

(b) Are methods available for managing the dispersal and to prevent that the LM mosquitoes do not establish themselves beyond the intended receiving environment (example: vegetation-free zones, traps)?

(c) Are alternative control measures available, should a problem occur?

Evolutionary responses (especially in vector or pathogen)

Rationale:

Any strong ecological effect also exerts an evolutionary selection pressure. The main evolutionary effects are those that could result in a breakdown in the technology and the resumption of previous disease levels. An evolutionary effect resulting in the development of resistance to physiological mechanism in the targeted pathogen could also be observed when modifying vector competence. Such resistance would harm to the effectiveness of the technology, and might result in a population of pathogens that will be transmitted more easily by all populations of its vector. For example, *Anopheles* mosquitoes limit the population of *Plasmodium* parasites in an individual mosquito through a cytokine-nitric oxide pathway. Conceivably, genetic modification could enhance this pathway to create pathogen-incompetent vectors. However, if the pathogen develops resistance to this pathway, it could presumably be transmitted more frequently by all populations of the vector, whether or not the LM version.

Other evolutionary effects could be hypothesized, including, for example, effects resulting from climate change, but they would first require the occurrence of some adverse effect on a species, community or ecosystem effect. Therefore, consideration of secondary evolutionary effects can be postponed until such effects are identified and found to be significant.

Points to consider:

(a) Does the mosquito vector have the potential to evolve to avoid population suppression, regain vector competency or acquire new or enhanced competency of another disease agent? If so, what may be the undesirable consequences?

(b) Does the trait have the potential to evolve to lose effectiveness or the pathogen to overcome the limitation posed by the genetic modification? If so, what may be the undesirable consequences?

(c) Are monitoring methods available to:

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

(ii) Assess the potential evolutionary breakdown of the mosquito technology (monitoring for transgene intactness and proper function over time);

(iii) Detect unexpected and undesirable spread of the transgenic trait (monitor for undesirable functions or behaviors within target species and other wild related species). Are methods available to manage the development of resistance?

RISK MANAGEMENT STRATEGIES

(see Step 5 of the Roadmap for Risk Assessment)

Risk assessors may want to consider the adoption of operational management processes following the design criteria for implementation of the risk management strategies laid out in the risk assessment. A set of risk management strategies could be, for instance, control quality of the released LM mosquito population and monitoring for potential unintended effects, followed by halting the release and application of mitigation methods when an unanticipated effect occurs. Careful implementation of the technology including insurance of the availability of mitigations (such as an alternative set of control measures should a problem occur) and, in most cases, integration with other population control methods is recommended.

OTHER ISSUES

There are other dimensions that should be taken into consideration in the decision for environmental releases of LM mosquitoes which are not governed by Annex III of the Protocol. They encompass among others: economic, health and social trade-offs associated with the technology application as well as social and cultural issues that are expected to influence the acceptance of these methods.

BIBLIOGRAPHIC REFERENCES

See references relevant to the "*Guidance Document on Risk Assessment and Risk Management of LM Mosquitoes*".

Annex IV

**DRAFT GUIDANCE ON
RISK ASSESSMENT AND RISK MANAGEMENT OF LIVING MODIFIED ORGANISMS
WITH STACKED GENES OR TRAITS**

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DRAFT GUIDANCE DOCUMENT ON RISK ASSESSMENT AND RISK MANAGEMENT OF LIVING MODIFIED ORGANISMS WITH STACKED GENES OR TRAITS

Prepared by the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management

Version of 19 March 2010

OBJECTIVE

The objective of this document is to give additional guidance on the risk assessment (RA) and risk management (RM) of LMOs with stacked events generated through conventional crossing of single event LMOs. Accordingly, it is meant to complement the Roadmap for Risk Assessment [1] and address special aspects of LMOs with stacked transgenes/traits resulting from the conventional crossing of first-level transformation eventsFor the time being it will be restricted to plant LMOs.

INTRODUCTION

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

Stacked transgenic traits can be produced through different approaches. In addition to the cross-hybridising of two LMOs, multiple trait characters can be achieved by transformation with a multigene cassette, retransformation of a single trait transformation event with a second construct or simultaneous transformation with different transgene cassettes (i.e., cotransformation).

This guidance document focuses on stacked transgenic traits that have been produced through cross breeding of two or more LMOs. LMOs with multiple transgenic traits resulting from re-transformation, co-transformation or transformation with a multigene cassette should be assessed according to the Roadmap taking into specific account interactions between the multiple transgenic traits as addressed in this additional guidance.

The Roadmap provides the basis for the risk assessment and the present guidance document is meant to complement it in those aspects which may need special consideration due to the stacking of events through cross breeding.

This is intended to be a "living document" that will be shaped and improved with time as new experience becomes available and new developments in the field of applications of LMOs occur, as and when mandated by the Parties to the Protocol.

SCOPE

This guidance document focuses on stacked events (StaEv; see "Use of terms") resulting from conventional crossings between two or more single transformation events (TraEv; see Use of terms) as parental lines so that the resulting LMO contains two or more transgenic traits. It is understood that the

[1] In accordance with a mandate from the Parties to the Cartagena Protocol on Biosafety (the Protocol), the AHTEG has developed 'a "roadmap", such as a flowchart, on the necessary steps to conduct a risk assessment in accordance with Annex III to the Protocol and, for each of these steps,' has provided 'examples of relevant guidance documents'. The Roadmap is presented, together with the present document, to the Parties of the Protocol on the occasion of the fifth meeeting of the Conference of the Parties serving as the meeting of the Parties.

individual TraEvs making up the StaEv have been assessed previously in accordance with Annex III of the Cartagena Protocol on Biosafety and as described in the Roadmap.

POINTS TO CONSIDER

Assessment of the intactness of the inserted loci and genotypic stability *(see Step 1, Point to consider (c) of the Roadmap for Risk Assessment)*

Rationale:

The combination of transgenic traits via cross breeding may change the molecular characteristics of the inserted genes/gene fragments at the insertion site and/or influence the detection and regulation of the expression of the transgenes. It is necessary to confirm the presence and structure of the TraEvs in the StaEv LMO, and their inheritance, in order to appropriately assess possible adverse effects on the conservation and sustainable use of biological diversity in the likely potential receiving environment and of potential adverse effects on human health.

Assessment of potential interactions between combined events and the resulting phenotypic effects *(see Step 1, Point to consider (d) of the Roadmap for Risk Assessment)*

Rationale:

The combination of two or more transgene events (TraEvs) in one LMO (ie. a StaEv LMO) may influence the expression level of each of the transgenes and there may be interaction between the expressed products of the different transgenes. In addition, the stacked transgenes may alter the expression of endogenous genes.

Therefore, in addition to information about the characteristics of the parental single-TraEv LMOs, specific information about the potential for interactions between the stacked proteins or modified traits in the StaEv LMO should be considered. For example, it should be determined whether the different transgenes affect the same biochemical pathways or physiological processes, or are expected to or may have any combinatorial, e.g. antagonistic, additive or synergistic effects that may result in potential for new or increased adverse effects relative to the parent LMOs

Assessment of cumulative and combinatorial synergistic, additive or antagonistic adverse effects of stacked transgenic traits on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account potential adverse effects to human health *(see Step 1, Point to consider (c) and Step 2, Point to consider (c) of the Roadmap for Risk Assessment)*

Rationale:

Assessment of cumulative and combinatorial synergistic, additive or antagonistic adverse effects is based on the environmental risk assessment data for the StaEv LMO in comparison to the closely related non-modified recipient species and the parent LMOs in the likely receiving environment, taking into consideration the results of the genotypic and phenotypic assessments outlined above.

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

(i) Phenotypic characteristics, including the levels of expression of any introduced gene products or modified traits, compared to the parent LMOs and to relevant non-modified recipient organisms (plants);

(ii) Compositional analysis (levels of expression in the LMO and persistence and accumulation in the environment (e.g. in the food chain) of potentially harmful substances produced by the LMO, including the levels of toxins, allergens or anti-nutritional factors known to be present in the parent LMOs or non-modified recipients;

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

Also, indirect effects due to changed agricultural management procedures, combined with the use of the transgenic stacked event LMO, should be taken into consideration.

Intentional and unintentional StaEv LMOs may have altered environmental impacts as a result of cumulative and combinatorial synergistic or antagonistic effects of the stacked traits prevalent in different LMOs of the same species in the receiving environment. Unintentional StaEv LMOs may occur via outcrossing with other LMOs of the same species or cross compatible relatives (see "Use of Terms") If a number of different StaEv LMO are cultivated in the same environment a number of varying unintentional StaEv LMOs may occur. Changed impacts on non-target organisms or a change in the range of non-target organisms in the likely receiving environment should be taken into account.

Development of specific methods for detecting individual transgenes combined in stacked events *(see Step 5, Point to consider (d) of the Roadmap for Risk Assessment)*

Rationale:

Some of the risk management strategies for LMOs with stacked genes may involve methods for the detection and identification of these LMOs in the context of environmental monitoring. Currently, many detection methods for LMOs rely on DNA-based techniques, such as polymerase chair reaction (PCR) or protein based ELISA tests targeted to single transformation events. The methods used to detect the transgene in the parental lines may not be sensitive or specific enough to differentiate between single parental transformation events and the same event being part of a stacked event. A special problem may arise particularly in the cases where the StaEv contains multiple transgenes with similar DNA sequences. Therefore, the detection of each and all individual transgenes in a StaEv may become a challenge and need special consideration.

USE OF TERMS

Stacked event (StaEv)

A stacked event (StaEv) contains the combination of the inserted recombinant DNA sequence of two or more single parental transformation events (TraEvs). These are typically physically unlinked (i.e. located separately in the genome) and segregate independently. These may be generated by re-transformation of an existing LMO or by the consecutive crossing of two or more LMO plants with different TraEvs. **Only those StaEvs produced by the conventional crossing of LMOs are being considered under this guidance document.**

Transformation event (TraEv)

A transformation event (TraEv) is the result of a transformation using *in vitro* nucleic acid techniques, for example, but not limited to, transformations using either single or a multi-gene transformation cassettes. In either case, the result will be one transformation event.

Unintentional stacked event

Unintentional stacked events are the result of outcrossing of stacked events into other LMOs or compatible relatives in the receiving environment. Depending on the segregation pattern of the stacked events this may result in new and/or different combinations of TraEvs.

BIBLIOGRAPHIC REFERENCES

See references relevant to the "*Guidance Document on Risk Assessment and Risk Management of LMOs with Stacked Genes or Traits*".
