TRAINING MANUAL ON BIOSAFETY

Implementation of the National Biosafety Framework of Bangladesh Project
Department of Environment
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Training Manual on Biosafety

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FOREWORD

The Training Manual on Biosafety of Genetically Modified Organism (GMOs) is prepared based on Cartagena Protocol on Biosafety, Biosafety Guidelines of Bangladesh, Biosafety Rules of Bangladesh, Guidelines for the Environmental Risk Assessment (ERA) of Genetically Engineered Plants, Guidelines for the Safety Assessment of Foods Derived from Genetically Engineered Plants and National Biosafety Framework. The Training Manual constitutes a conceptual guide for trainers that can be used to lead them through the issues biosafety and experiences.

In the recent years biotechnology based technology development has a significant importance in the technology systems. But developments of biotec products have potential risks which can be avoided through safety assessment. Development and use of GM products requires appropriate policy, legislation, controls and cross border movements to protect human health, bio-diversity and the environment. Bangladesh has approved number of biosafety regulatory documents for better handling GMO products. Lack of awareness and proper knowledge about GMOs at different levels has created confusion on the use GMO products. Thus, it is necessary to create awareness and disseminate appropriate knowledge at all levels.

A national biosafety system to regulate production and release of genetically modified organisms is established in the country in order to safe use of GMOs. All stakeholders, policy makers, border control, field worker must know the biosafety regulatory system in the country. A training manual on biosafety has been developed to provide basic and important biosafety regulatory system so that safe use of GMO in insured in the country.

This kind training manual will be a important document for trainers as well trainees. The biosafety issues is clearly explained in this manual. In addition, the Manual gives an overview of the step wise framework at all level that may affect human health and the environment in general.

This manual also provides a participatory process for more effective and sustainable risk assessment and risk management of GMOs in Bangladesh through personal skill and institutional capacity improvement.

Project Director
INBF Project

TRAINING MANUALS ON BIOSAFETY

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1.1 Importance of Modern Biotechnology

Modern biotechnology has tremendous potential for the well being of the world particularly in meeting the impending needs of improved and value added agricultural crops, food products, medicine, industry and environment. While there is an enormous prospect of modern biotechnology, there also exists apprehension as it may pose some certain or uncertain risks to biological world including the human being. Government of Bangladesh has put the emphasis on positive development of biotechnology in the policy regime. Harvesting the beneficial aspects of modern biotechnology is very crucial for the overall development of a country like Bangladesh. The essence of the precautionary approach to mitigate, avoid or prevent the potential adverse or harmful effects of Genetically Modified Organisms (GMOs) to the biodiversity, environment and human health must be taken into account while working with modern biotechnology.

1.2. The Cartagena Protocol on Biosafety

The Cartagena Protocol on Biosafety (CPB) was adopted by the international community in Montreal on 29 January 2000 in order to fulfil one of the important objectives of the Convention on Biological Diversity (CBD), 1992: the conservation and sustainable use of biological diversity.
The Convention takes a comprehensive approach to the conservation of biological diversity. It addresses the threats that might arise from the transfer, handling and use of living modified organisms (LMOs) resulting from modern biotechnology. Article 8(g) of the CBD deals with domestic measures generally. It requires each Contracting Party to take steps to regulate, manage or control the risks associated with the use and release of LMOs resulting from modern biotechnology which are likely to have adverse impacts on the conservation and sustainable use of biological diversity, taking into account the risks to human health.

Article 19(3) of the CBD provides the legal basis for the adoption of the CPB in order to establish an international regulatory regime on LMOs. It obliges the parties to the CBD to 'consider the need for and modalities of a protocol setting out appropriate procedure(s) in the field of the safe transfer, handling and use of any living modified organism resulting from biotechnology that may have adverse effect on the conservation and sustainable use of biological diversity'. In the Article 19(4) of the CBD, states dealing with the transfer of LMOs from one Party to another. It requires each Party to provide information on domestic regulations concerning use and safety to any other Party to which a LMO is provided, as well as any available information on the adverse effects, which the introduction may have for this party. Article 28 of the CBD mandates Parties to cooperate in the formulation and adoption of protocols.

Accordingly, the Conference of the Parties (COP) to the CBD at its first meeting held in 1994 in Nassau, Bahamas, authorised two meetings to consider the need for and modalities of a protocol on biosafety. A panel of experts met in Cairo in May 1995 and an open-ended Ad Hoc Group of Experts on Biosafety met in Madrid in July 1995. The large majority of delegations present at the

Madrid meeting favoured the development of a protocol on biosafety. At its second meeting held in 1995 in Jakarta, Indonesia, the COP decided to establish an open-ended Ad Hoc Working Group on Biosafety (BSWG) to elaborate a protocol on biosafety (Decision II/5). The BSWG was chaired by Veit Koester of Denmark. Six meetings of the BSWG were held between July 1996 and February 1999.

The Sixth and final meeting of the BSWG, held in Cartagena, Colombia, in February 1999, forwarded a draft consolidate text of the Protocol to the first Extraordinary Meeting of the Conference of the Parties (ExCOP) to the CBD for its consideration. However, the ExCOP failed to reach an agreement on certain issues of the Protocol such as the scope of the protocol, LMOs intended for direct use as food or feed, or for processing (LMO-FFPs), the precautionary principle, identification and documentation requirements and the relationship between the protocol and other international agreements, notably the World Trade Organization (WTO). The final negotiation of these core issues took place at the resumed session of the ExCOP which immediately followed the January 2000 informal meeting in Montreal. Ultimately the Protocol was adopted by the COP to the CBD on 29 January 2000 and entered into force on 11 September 2003.

1.3. Bangladesh's obligation to establish biosafety regulatory regime as a party to the Cartagena Protocol on Biosafety to CBD

Bangladesh signed the Protocol on 24 May 2000 and ratified it on 5 February 2004. According to Article 36 (4) of the Convention, the Protocol came into force for Bangladesh on 5 May 2004, on the ninetieth day after the date of deposit of the instrument of ratification. Bangladesh ratified the Convention on Biological Diversity on 20 March 1994.

Bangladesh ratified the CPB on 5 February 2004, which came into force in 5 May 2004. Being a party to the CPB**,** it is an obligation for each party to develop the National Biosafety Framework (NBF). The prime objective of this effort is to provide the basis of establishing regulatory regime to ensure safe transfer, handling, transit, transboundary movement, development, field trial and commercial release of Genetically Modified Organisms (GMOs). Developing National Biosafety Framework, from part of the government was the step taken in 2004 towards ensuring safe transfer, handling, transbounadry movement, import, transit and introduction of GMOs into the environment. NBF is a complimentary to our national commitments towards implementation of multilateral environmental agreement like the Cartagena Protocol on Biosafety (CPB) to the Convention on Biological Diversity.

Genetically modified Organism (GMO) is basically an organism (plant, animal or microorganism) created by application of biotechnology. The application of which a new genetically characterised organism is created by introducing a new character or genetic carrier or gene in any organism found from that organism or from any wild species thereof or from completely different type of organism.
The term GMO or LMO (Living Modified Organism) are used interchangeably to denote the same thing pertaining to modern biotechnology. In the context of Bangladesh, GM crops and foods are highly debated issue over the last few years. This issue at first drew the attention of

Bangladeshi people after the agreement between Monsanto and Grameen Bank in 1998. On 30th October 2013 Government by a notification approved to cultivate Bt. Brinjal and by that Bangladesh became the first country in South Asia to grow a GM food crop.

If we consider the context of Bangladesh, we find our population is increasing whereas cultivable land is decreasing. Agriculture is also threatened by adverse impacts of climate change related stressors (i.e. salinity, drought, flood, and storm), as well as, insects and diseases. So, adoption of GMOs will be the alternative solution to meet demands of food production and nutrition. While we are in the process to adopt GMOs, we must ensure the obligations came upon the country to implement the rules-regulations and procedures pertaining to risk assessment and management of GMOs.

In 2012 Government has enacted the Bangladesh Biosafety Rules, in exercising rules making power delegated under Section 20 of the Bangladesh Environment Conservation Act, 1995 towards regulating GMOs in the country.

Rule no. 3 imposes restrictions on import and export of GMOs as 'no person or institution can import, export, buy, sell or commercially use the Genetically Modified Organism and products thereof, without prior permission from the Ministry of Environment and Forests. This Rules also provides for mandatory provision for identification or labelling of GMOs in Rule 5 as 'any box or cover, which carries Genetically Modified Organism or Products thereof, shall have detail identification or labelling on it relating to the nature of Genetically Modified Organism or products thereof, which is additional provision, notwithstanding anything contained in any other law regarding this'.

This Rules included the adverse impacts of GMOs as with the provision that 'if any environmental pollution is created or ecosystem is damaged by the Genetically Modified Organism or Products thereof, the producer institution, exporter, importer, store keeper, supplier and retailer, all shall be liable for the offence of environmental pollution or ecosystem damage, unless he/they proves that he/they does not have direct involvement with such pollution or damage' (Rule 9).
In respect of biosafety related to GMOs, Bangladesh has developed a Biosafety Guidelines of Bangladesh in 2007, which is endorsed by the Biosafety Rules, 2012. The Guidelines of 2007 formed the basis of the regulatory framework of monitoring and enforcement processes in respect to biosafety in Bangladesh and it also structured the institutional frameworks. This Guideline provides different biosafety regulatory committees and their composition, powers, functions and responsibilities.

In accordance with the mandate of National Biosafety Framework, 2007, the Government with the support of UNEP-GEF is implementing a development project. A National Biosafety Policy, Monitoring and Enforcement Manual of Biosafety, and other related regulatory documents are underway to be published under this project. GMO detection facility has been established in DOE with modern equipments.

1. Biosafety Systems as per Biosafety Rules and Biosafety Guidelines

2.1 Introduction

Biosafety is used to provide safe and environmentally sustainable use of all modern biotechnology derived products towards ensuring safety towards human health and biodiversity. Application of modern biotechnology requires appropriate legislation and controls for their testing, release, use and cross border movements to protect human health, biodiversity and the environment. Before releasing a GM crop, a comprehensive analysis is required that includes an assessment of the relative benefits and risks of GM crops. Strong biosafety regulatory system should be in place to avoid any kind of unintended effects of new crop to the environment and human health.

Bangladesh as a party to the Cartagena Protocol on Biosafety to Convention on Biological Diversity and, Bangladesh is committed to ensure safe management of biotechnology activities including research, development, introduction and any other uses of GMOs. To promote and safe management of biotechnological activities including research, development, introduction and use of GMOs, Bangladesh has institutionalized various committees:

- National Committee on Biosafety
- Biosafety Core Committee
- Institutional Biosafety Committee

An effective regulatory and institutional framework is essential to coordinate, regulate and enforce biosafety issues in the country. Ministry of Environment and Forests is working as National Focal Point for and the competent authority to implement the Cartagena Protocol on Biosafety and performing the responsibility of establishing regulatory system in Bangladesh. Development and application GMOs are regulated by the National Committee on Biosafety, as described in the Biosafety Rules and Guidelines of Bangladesh.

The National Committee on Biosafety (NCB) is headed by the Secretary, Ministry of Environment and Forests as the Chairperson and it includes members/ representatives from the ministries and institutes associated with biotechnological development. The Biosafety Core Committee is headed by the Director General of the Department of Environment that includes experts from the disciplines of biosciences, environmental science, medical science. The responsibilities of NCB includes identification and evaluation of potential risk of GM products and recommend measures to minimize risk, and formulate, review or amend national policies and guidelines on biosafety, risk assessment of biotechnology and supervise the implementation of the guidelines.

Ministry of Environment and Forest has adopted Guidelines for the Safety Assessment of Foods Derived from GE Plants and Guidelines for the Environmental Risk Assessment GE Plants. Several other biosafety related documents viz. a) Crop wise Standard Operating Procedures (SOPs) for CFT, b) Inspector Manuals for confined field trials of Genetically Engineered plants, c) Crop wise Data recording formats for CFT, and d) Biology document of Eggplant are available for ready reference.

2.2 Scope of Biosafety Rules and Guidelines

The Biosafety Guidelines of Bangladesh has been gazetted in 2008 by the MOEF taking into account Cartagena Protocol and other international obligations. Biosafety guidelines are applicable to all research and development activities of modern biotechnology conducted in the laboratories of the government research institutes, state enterprises, universities, international organizations located in Bangladesh, private companies and non-governmental organizations. It applies to all laboratories, contained and confined trial, open field trial, trans-boundary movement, transit, handling and use of all GMOs that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. The Guidelines contains the following chapters:
Chapter-I : Scopes and Objectives of Biosafety Guidelines
Chapter-II : Institutional Arrangements
Chapter-III: General Provisions on Biosafety (risk assessment and risk management)
Chapter-IV: Physico-chemical and Biological Containments: Procedures and Facilities.

In order to conduct research or taking research project and implementation of the project on genetically modified organism or products should follow the instructions described in the biosafety guidelines.

The Biosafety Rules has been enacted by the Ministry of Environment and Forests on 29 August 2012 under the Environmental Protection Act 1995. As per the gazette notification the Rules is titled as the "Bangladesh Biosafety Rules, 2012". Biosafety Rules contains 13 articles describing different aspects of successful implementation of biosafety in the country. In order to conduct research or taking research project and implementation of the project on genetically modified organism or products would follow the instructions described in the biosafety guidelines. Biosafety Rules describes in Article-3 that no person or organization can import, export, buy, sell or commercially use any genetically modified organism or products without obtaining permission from the Ministry of Environment and Forest. In Article-10 of Rules, there is a provision of punishment for any violation of Biosafety Rules and Guidelines.

2.3 Implementation Mechanism of Biosafety

In order to ensure safe management of biotechnology activities including research, development, introduction and use of GMOs, Bangladesh has institutionalized various biosafety committees with responsibilities. Powers, functions and responsibilities of biosafety committees are described in the Biosafety Guidelines and Rules. The Biosafety Committees are as follows:

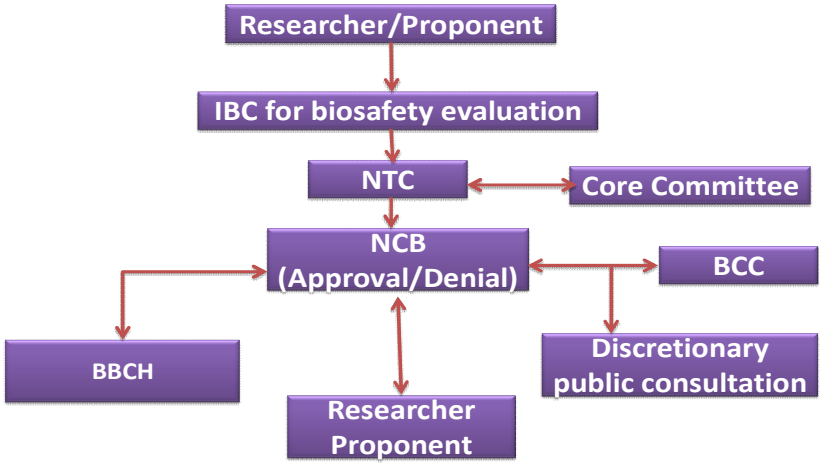
1. National Committee on Biosafety (NCB)
2. Biosafety Core Committee (BCC)
3. Institutional Biosafety Committee (IBC)
4. Biological Safety Officer (BSO) to be designated in the institute or organization
5. Field Level Biosafety Committee (FBC)

The Secretary, Ministry of Environment and Forests chair the National Committee on Biosafety (NCB). The main functions of NCB are to draft and adopt policies; approve applications for GMO research, introduction, commercial use, transboundary movement, and release into the environment, review, and monitor and recommend measures to minimize potential risks of

GMOs. Biosafety Core Committee is chaired by Director General of Department of Environment which provides technical supports to the NCB. An institution engaged in genetic engineering research has an Institutional Biosafety Committee (IBC) to evaluate and monitor the Biosafety aspects of their research and development activities. A Field level Biosafety Committee (FBC) is formed by the NCB to monitor each field trial/release, consisting of a minimum of three (3) members with relevant expertise necessary to monitor and ensure compliance during the field trial/release.

The procedure of application for GMO research and introduction is shown in Fig. 1. Bangladesh follows several steps to allow any applicant to conduct research, introduction, import or any other activities related to GMOs.

Fig.1. Notification system of Crop related GMOs in Bangladesh



3. Biosafety Concerns in the Laboratory and Contained Tests, Confined and Open Field Trials of Genetically Engineered Crops

3.1 Safety Levels in the Laboratories: The Difference in Laboratory Biosafety Levels 1, 2, 3 & 4

A **biosafety** level is a set of biocontainment precautions **required** to isolate dangerous biological agents in an enclosed **laboratory** facility. The levels of containment range from the lowest **biosafety** level 1 (BSL-1) to the highest at level 4 (BSL-4). Biological Safety Levels (BSL) are a series of protections relegated to the activities that take place in particular biological labs. They are individual safeguards designed to protect laboratory personnel, as well as the surrounding environment and community. These levels, which are ranked from one to four, are selected based on the agents or organisms that are being researched or worked on in any given laboratory setting. For example, a basic lab setting specializing in the research of nonlethal agents that pose a minimal potential threat to lab workers and the environment are generally considered BSL-1—the lowest biosafety lab level. A specialized research laboratory that deals with potentially deadly infectious agents like Ebola would be designated as BSL-4—the highest and most stringent level.

These lab levels are determined by the following:

- Risks related to containment
- Severity of infection
- Transmissibility
- Nature of the work conducted
- Origin of the microbe
- Agent in question
- Route of exposure

The reason biosafety levels are so important is because they dictate the type of work practices that are allowed to take place in a lab setting. They also heavily influence the overall design of the facility in question, as well as the type of specialized safety equipment used within it.
The following is an explanation of each biosafety level—what they mean and how they differ in safety measures and best practices.

BSL–1
As the lowest of the four, biosafety level 1 applies to laboratory settings in which personnel work with low-risk microbes that pose little to no threat of infection in healthy adults. An example of a microbe that is typically worked with at a BSL-1 is a non-pathogenic strain of *E. coli*.
This laboratory setting typically consists of research taking place on benches without the use of special contaminant equipment. A BSL-1 lab, which is not required to be isolated from surrounding facilities, houses activities that require only standard microbial practices, such as:

- Mechanical pipetting only (no mouth pipetting allowed)
- Safe sharps handling
- Avoidance of splashes or aerosols

- Daily decontamination of all work surfaces when work is complete
- Hand washing
- Prohibition of food, drink and smoking materials in lab setting
- Personal protective equipment, such as; eye protection, gloves and a lab coat or gown
- Biohazard signs

BSL-1 labs also require immediate decontamination after spills. Infection materials are also decontaminated prior to disposal, generally through the use of an autoclave.

BSL–2

This biosafety level covers laboratories that work with agents associated with human diseases (i.e. pathogenic or infections organisms) that pose a moderate health hazard. Examples of agents typically worked with in a BSL-2 include equine encephalitis viruses and HIV, as well as *Staphylococcus aureus (staph infections).*

BSL-2 laboratories maintain the same standard microbial practices as BSL-1 labs, but also include enhanced measures due to the potential risk of the aforementioned microbes. Personnel working in BSL-2 labs are expected to take even greater care to prevent injuries such as cuts and other breaches of the skin, as well as ingestion and mucous membrane exposures.

In addition to BSL 1 expectation, the following practices are required in a BSL 2 lab setting:

- Appropriate personal protective equipment (PPE) must be worn, including lab coats and gloves. Eye protection and face shields can also be worn, as needed.
- All procedures that can cause infection from aerosols or splashes are performed within a biological safety cabinet (BSC).
- An autoclave or an alternative method of decontamination is available for proper disposals.
- The laboratory has self-closing, lockable doors.
- A sink and eyewash station should be readily available.
- Biohazard warning signs

Access to a BSL-2 lab is far more restrictive than a BSL-1 lab. Outside personnel, or those with an increased risk of contamination, are often restricted from entering when work is being conducted.

BSL-3

Again building upon the two prior biosafety levels, a BSL-3 laboratory typically includes work on microbes that are either indigenous or exotic, and can cause serious or potentially lethal disease through inhalation. Examples of microbes worked with in a BSL-3 include; yellow fever, West Nile virus, and the bacteria that causes tuberculosis.

The microbes are so serious that the work is often strictly controlled and registered with the appropriate government agencies. Laboratory personnel are also under medical surveillance and could receive immunizations for microbes they work with.

Common requirements in a BSL-3 laboratory include:

- Standard personal protective equipment must be worn, and respirators might be required
- Solid-front wraparound gowns, scrub suits or coveralls are often required
- All work with microbes must be performed within an appropriate BSC
- Access hands-free sink and eyewash are available near the exit

- Sustained directional airflow to draw air into the laboratory from clean areas towards potentially contaminated areas (Exhaust air cannot be re-circulated)
- A self closing set of locking doors with access away from general building corridors

Access to a BSL-3 laboratory is restricted and controlled at all times.

BSL-4

BSL-4 labs are rare. However some do exist in a small number of places in the US and around the world. As the highest level of biological safety, a BSL-4 lab consists of work with highly dangerous and exotic microbes. Infections caused by these types of microbes are frequently fatal, and come without treatment or vaccines. Two examples of such microbes include Ebola and Marburg viruses.

In addition to BSL-3 considerations, BSL-4 laboratories have the following containment requirements:

- Personnel are required to change clothing before entering, shower upon exiting
- Decontamination of all materials before exiting
- Personnel must wear appropriate personal protective equipment from prior BSL levels, as well as a full body, air-supplied, positive pressure suit
- A Class III biological safety cabinet

A BSL-4 laboratory is extremely isolated—often located in a separate building or in an isolated and restricted zone of the building. The laboratory also features a dedicated supply and exhaust air, as well as vacuum lines and decontamination systems.

Knowing the difference in biosafety lab levels and their corresponding safety requirements is imperative for anyone working with microbes in a lab setting.

3.2 Tests of GMOs under Containment

Once the GMO has been innovated, it can be subjected to laboratory tests to gain information on its characteristics and behaviour under controlled conditions. All research, development and laboratory or greenhouse testing procedures are performed under *Containment*. Containment means that all contact of genetically modified material or organisms with the external environment is prevented, to the extent required by the risks posed by that material or organism. This is usually achieved by a combination of physical and biological barriers. Containment, or **contained use**, refers to measures and protocols applied to reduce contact of GMOs or pathogens with the external environment in order to limit their possible negative consequences on human health and the environment (FAO, 2001). Containment measures have to be adjusted to the highest level of risk associated with the experiment, especially when the risk category of the material being worked with is not certain. The risk associated with each GMO should be assessed on a case-by-case basis; accordingly, GMOs are classified into four different risk groups in relation to the risks they pose.

Containment can be achieved by a combination of physical containment structures and safe work procedures (also referred to as good laboratory practices). As an additional feature, biological containment can be included, i.e. “built-in” features of the organism being worked with that prevent its spread, survival or reproduction in the external environment

Transgenic Greenhouse facilities present opportunities to study plant growth and performance in a desired contained environment. The level of containment is determined by the degree of bio-safety concern associated with the studies being conducted. The transgenic greenhouses are specially designed greenhouses they have to be in compliance to the strict guidelines from national and international authorities.

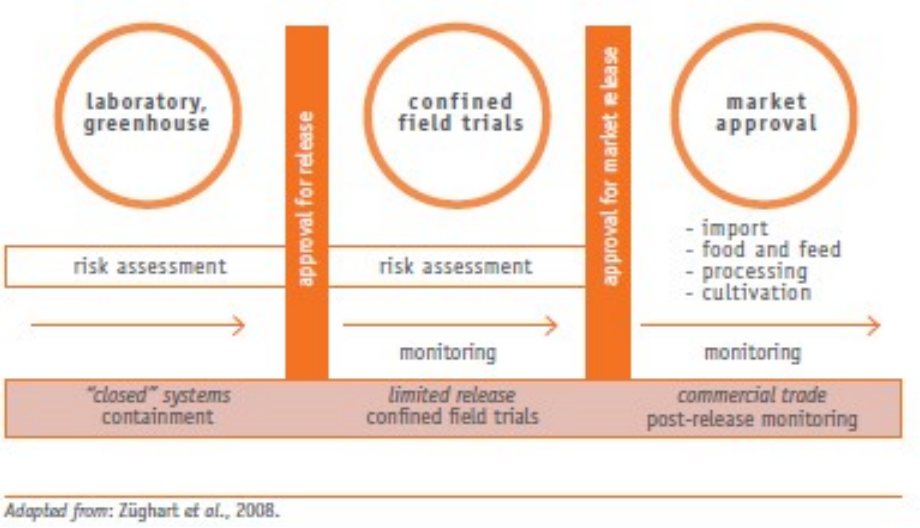
3.3 Trial of GMOs under Confined Field (CFT)

If we find the performance of the GMO under containment is promising and the potential risks it poses are found to be manageable, the testing can proceed to *confined field trials*. Here, the GMO is tested in the open environment, preferably under conditions that resemble its future area of use. However, stringent measures are put in place to confine the release, i.e. to prevent any escape of the GMO or the transgene into the environment and to prevent genetically modified (GM) material from entering human or animal food supplies. Confined field trials are repeated at different scales until all the needed information is acquired.

Once a GMO has passed all testing stages, the risk analysis has been performed with a positive outcome and the approval from the responsible national or international authority has been granted, it may be placed upon the market and released into the environment.

From this point on, no measures are put in place that limits the contact between the GMO and the receiving environment, even if specific risk management measures can be requested by the national biosafety authorities. However, it is important to implement *post-release monitoring* procedures to monitor the risks identified in the risk assessment of the GMO, recognize possible new, unanticipated risks and adverse effects, and to quantify the performance and benefits of the GMO. The overall goal of a monitoring programme should be the protection of the productivity and ecological integrity of farming systems, the general environment and human and animal health.

The relation between containment, confined field trials and post-release monitoring of GMOs



Adapted from: Züghart et al., 2008.

4. Risk Assessment, Risk Management and Risk Communications

4.1 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.

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".

Five steps of Risk Assessment

Paragraph 8 of Annex III of the Protocol describes the risk assessment process as a sequence of *five steps*, in which the results of one step are relevant to the others:

- Step 1: "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;
- Step 2: "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: "Evaluation of the consequences should these adverse effects be realized";
- Step 4: "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: "Recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks".

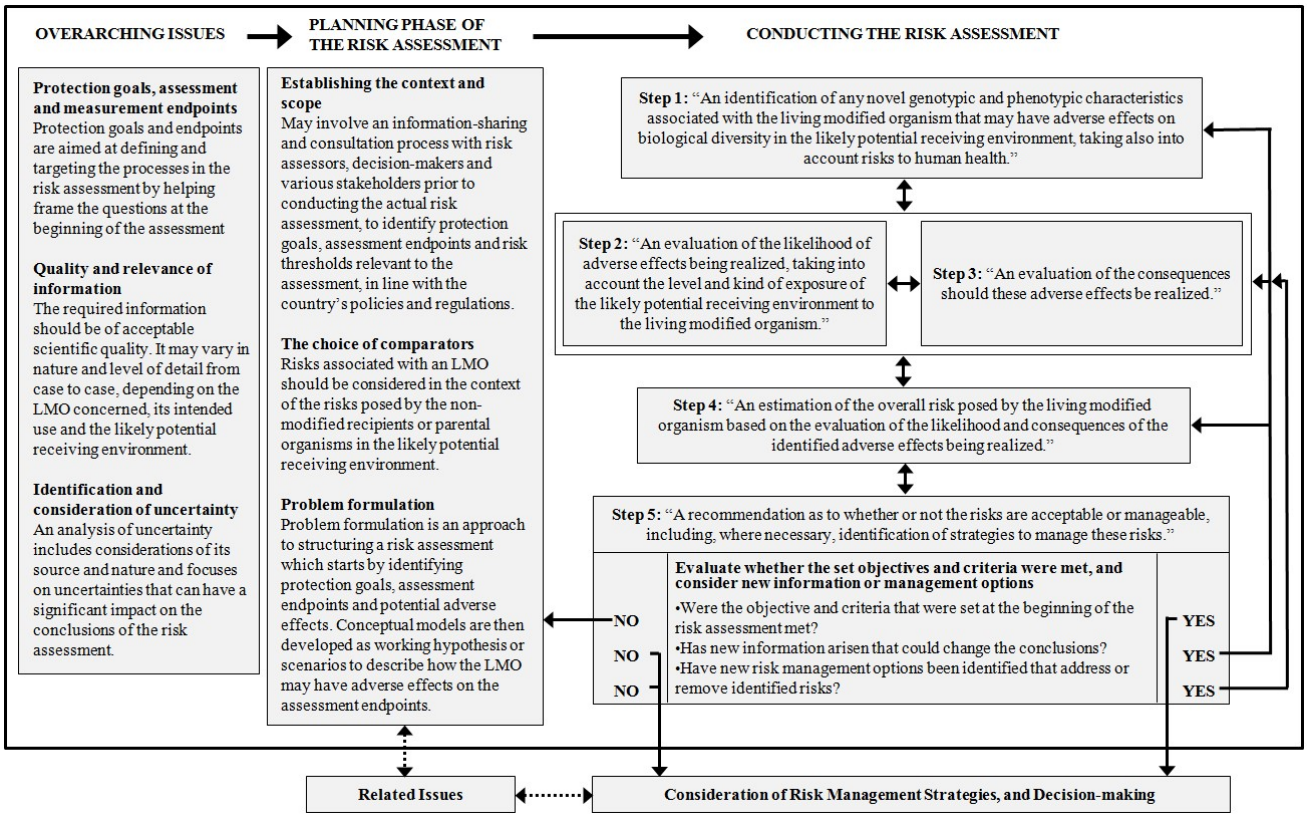


Figure-2: The Roadmap for Risk Assessment.

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

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

4.2 Protection goals, assessment endpoints and measurement endpoints

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*.

Adverse effects and protection goals are closely interlinked concepts. Protection goals are broadly defined and valued environmental outcomes (e.g. biodiversity conservation or ecological functions), sometimes called general protection goals or generic endpoints.
Examples of protection goals that focus on biodiversity conservation include species of conservation value or cultural value, species in the IUCN Red List (IUCN Red List of Threatened Species), and protected habitats and landscapes. Protection goals that focus on ecological functions include soil, water and production systems. Sustainable ecosystems as protection goals include both biodiversity conservation and ecological functions.

Protection goals and the conservation of centres of origin and genetic diversity

Among widely recognized protection goals is the conservation of centres of origin and of genetic diversity. In accordance with the International Treaty on Plant Genetic Resources for Food and Agriculture*, a "centre of origin" is defined as a geographical area where a plant species, either domesticated or wild, first developed its distinctive properties, and a "centre of crop diversity" is defined as a geographic area containing a high level of genetic diversity for crop species in in situ conditions.

Centres of origin and centres of genetic diversity contain unique genetic resources, such as *crop wild relatives* and are important areas for in situ conservation of biological diversity in the context of article 7(a) and annex I of the CBD.

In line with article 8 of the CBD, with special consideration of article 8(j), it should be recognized that within centres of origin and centres of genetic diversity are important pools of genetic resources that are valuable to humankind. Given their biological, cultural, social and economic significance, centres of origin and centres of genetic diversity transcend national protection goals and geographic borders, and are seen as a form of human heritage. They are continuously changing through ongoing domestication and diversification processes through a close and intricate relationship with indigenous and local communities embodying traditional lifestyles with traditional knowledge, innovations and practices relevant for the conservation and sustainable use of biological diversity.

A consideration to be addressed during the risk assessment is whether wild relatives or landraces of the LMO exist in the likely potential receiving environment and, if so, whether gene flow could occur and what would be the consequences. Another consideration is whether the LMO would have genetic characteristics or would be managed in such a way that could give it an

advantage over other organisms and which could lead to adverse effects such as displacement and higher mortality of other species (see step 1).

Risk assessments of the introduction of an LMO into a centre of origin or centre of genetic diversity should be conducted in such a way that a high degree of certainty is achieved in all steps of the process (steps 1-5) to ensure that no adverse effects on relevant species are expected, while taking into account the conservation and genetic variability of the original genotypes.
In order to adequately answer these considerations and to perform a sound risk assessment that can properly inform decision making, it is fundamental to have access to adequate *baseline* data, models to simulate gene flow, and methods to identify and quantify possible consequences related to the introduction of LMOs in centres of origin and centres of genetic diversity.
Due to the importance of centres of origin and centres of genetic diversity as repositories of wild relatives, landraces and genetic resources, if any potential adverse effects are identified during the risk assessment, they are typically considered to have major consequences.

Source: Plant treaty.

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.

Assessment endpoints and measurement endpoints are derived from the relevant protection goals. "Assessment endpoints" and "measurement endpoints" are important concepts and understanding the difference between these 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 (see below). Examples could include the abundance of an endangered bird species in a defined agricultural ecosystem or abundance of bees in the same area.

"Measurement endpoints" is a quantifiable indicator of change in the assessment endpoint, and constitutes measures of *hazard* and exposure. Examples include fitness, growth and density of species being used as assessment 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.

Using the ecosystem services approach to identify specific protection goals

At the beginning of a risk assessment, components of the environment – species, habitats, services, etc. – that are valued by civil society and/or protected by relevant laws or policies are

identified. This exercise establishes the so-called environmental policy protection goals: environmental components that should be protected and taken into account when conducting risk assessments to support regulatory decision-making. These protection goals can vary between jurisdictions, but their overall aim is to limit harm to the environment, including biodiversity and ecosystems, caused by human activities.

However, policy protection goals, such as protecting biodiversity, are often too generic and vague to be useful for a risk assessment, and need to be translated into assessment endpoints that are specific and operational. One way to translate policy protection goals into assessment endpoints for the risk assessment of LMOs is to use an ecosystem services approach. Ecosystems support human societies through functions and processes known as ecosystem services.

Investigating the environment through the framework of ecosystem services enables us to recognise the wide range of benefits to humans provided by ecosystems and biodiversity, to identify how changes in these environmental components influence human well-being, and to account for both economic and environmental considerations.

For example, the European Food Safety Authority (EFSA) is exploring ways to use the ecosystem services approach to define operational protection goals by: (1) identifying relevant ecosystem services potentially impacted by the use of LMOs; (2) identifying service providing units – structural and functional components of biodiversity – that provide or support these ecosystem services; and (3) specifying the level of protection for these service providing units. The level of protection is defined by the ecological entity of the service providing unit and its attribute, as well as the maximum magnitude and spatial/temporal scale of tolerable impact.

The ecosystem services approach provides an easy-to-understand tool and a common language, which facilitates communication among stakeholders (including government agencies, citizens, academia, risk assessment bodies, industry and non-governmental organisations). Improved communication will help to clarify the often divergent positions on what is of value and why, and reveal the underlying values and ideals held by the different actors. Communication among stakeholders will also be essential to reach agreement on operational protection goals, which should be set before risk assessments are conducted, as they define the framework in which scientists and risk assessors operate when performing the risk assessments

Source: BCH.CBD[a]

4.3 Quality and relevance of information[1]

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 where possible, supported by evidence-based information, including peer-reviewed data, as well as specialized knowledge, indigenous and traditional knowledge.

1 The term “information” is being used in a broad sense and includes, for example, experimental data, both raw and analysed.

In some regulatory frameworks, the criteria for evaluating the quality of scientific information are set out in policies developed by the competent authorities. Furthermore, risk assessors will bring professional expertise and will be capable of making determinations on the quality and relevance of information using their own experience. A number of points that are typically considered to ensure the quality and relevance of the information used as well as the outcome of the risk assessment include:

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

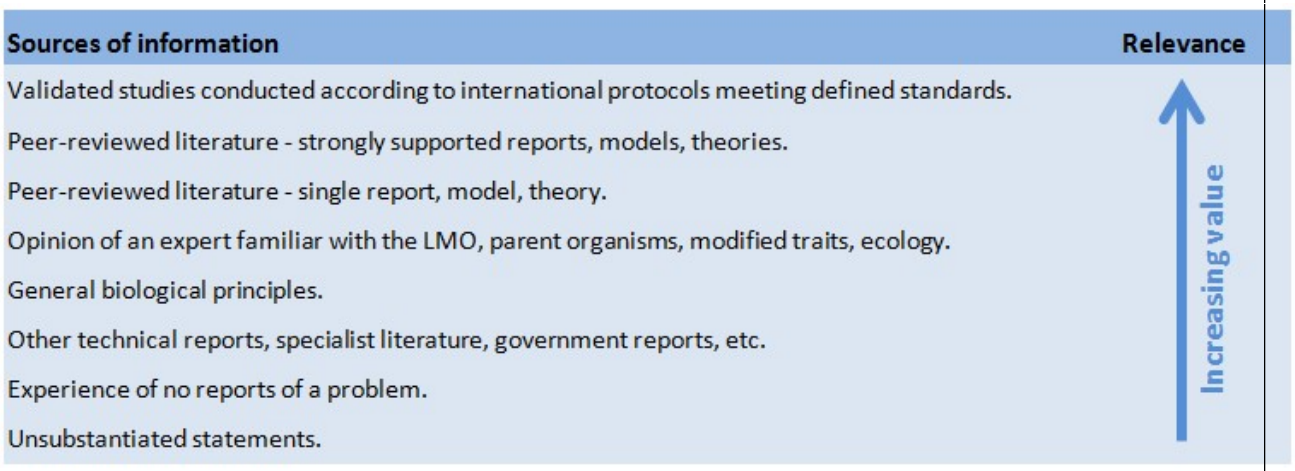
Sources and relevance of information for the risk assessment:

Information to be used throughout the risk assessment may be derived from a variety of sources such as new experiments, peer-reviewed scientific literature, expert opinions, data gathered during the development of the LMOs, as well as from previous risk assessments, in particular for the same or similar LMOs introduced in similar receiving environments (*Risk Assessment*[1)] Information from national and international standards and guidelines may also 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 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. As such, not all information on the LMO or its parental organisms available in the literature may be considered relevant to the risk assessment. Likewise, not all sources of information may be considered of equal relevance.

e.g. Sources of information and their relevance

The figure below illustrates how the risk assessor may view the value of some different types of information. The overall value of the data for the risk assessment is open to the risk assessor's judgment.



Source: BCH.CBD[b].

Moreover, information that is considered relevant to a risk assessment will vary from case to case depending on the nature of the modification of the LMO, on its intended use, intended receiving environment, 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.

i 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 one of the objectives 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 and, therefore, information on measures to minimize the exposure of the environment to the LMO is particularly relevant.

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.

4.4 Identification and consideration of uncertainty

Uncertainty is an inherent element of scientific analysis and risk assessment. Risk assessments cannot provide definitive answers regarding safety or risk as there is always some degree of uncertainty.

There are no internationally agreed guidelines to determine "scientific uncertainty", nor are there internationally agreed general rules or guidelines to determine its occurrence. As such, the consideration of uncertainty and its importance to effective decision making are subject to much discussion, and the importance assigned to uncertainty and the determination of its occurrence, are dealt with differently under different regulatory frameworks.

According to annex III to the Protocol, "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" and "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 and communication of uncertainty may improve the understanding of the outcomes of a risk assessment, 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 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 can be reduced or eliminated with more or better data obtained through 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). Furthermore, uncertainties associated with specific adverse effects may not allow the completion of a risk assessment or conclusions regarding the level of overall risk.

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.

4.5 Planning phase of the risk assessment

4.5.1 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.

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[2] and to the particular risk assessment. These include the relevant:

(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, including for example ecosystems functions and services, as well 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, habits 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);

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

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

(x) Information from previous risk assessments of the same or similar LMOs and modified trait(s) in other types of LMOs;
(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).

4.5.2 Problem formulation

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".

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 potential adverse effects of the LMO and its use. After identifying the potential adverse effects, conceptual models are developed as working hypothesis to describe how the LMO may have adverse effects on the assessment endpoints. This means describing and modelling scenarios and pathways on how the LMO may cause harm to a protection goal. For example, if the protection goal is conservation of biodiversity, a risk hypothesis could assess what novel characteristics of the LMO might affect specific assessment endpoints, such as a component of the food web or the population size of certain species in the likely potential receiving environment. The unambiguous specification of the assessment endpoints is crucial to focus the risk assessment. Finally, an analysis plan is developed for obtaining the needed data and how to test these hypothetical scenarios and pathways.

Using problem formulation to frame the risk assessment

Problem formulation helps framing the entire process. It also helps identifying available and missing information, and scientific uncertainties that may limit the assessment. Problem formulation has therefore proven adequate to maximise the usefulness of risk assessments for decision-making.
For example, problem formulation at EFSA involves several elements: (1) the definition of operational protection goals, which are explicit and unambiguous targets for protection extracted from legislation and public policy goals (see box on protection goals); (2) the identification of characteristics of the LMO capable of causing potential adverse effects (hazards) and pathways of exposure through which the deployment of the LMO may adversely affect human health, animal health or the environment; and (3) outlining specific hypotheses to guide the generation and evaluation of data in the subsequent risk assessment steps. Problem formulation also requires: (4) the identification of methods – through a conceptual model and analysis plan – that will help to direct the risk characterisation and produce information that will be relevant for decision-making. The provision of a conceptual model will underpin the usefulness of scientific information to the risk assessment. It would explain how the deployment of the LMO could lead to adverse effects on something of value through a chain of events taking account of both hazard and exposure.

Source :BCH.CBD[c]

4.5.3 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 local landraces and undomesticated species.

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 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, an assessment of the potential adverse effects of an LMO (for example, a Bt crop) to beneficial organisms (for example, honey bees) should reflect the standard management practices that are expected to be applied to the LMO (for example, different pesticide types/application regimes).

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 may be taken into consideration when deciding on appropriate comparators.

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 may, therefore, not be the most appropriate non-modified comparator. Furthermore, it may be necessary to modify the comparative approach when dealing with LMOs whose recipient organism is, for example a non-domesticated species.

An alternative to the comparative approach may become necessary when considering LMOs developed through future techniques where appropriate comparators will not exist[3] In such situations, the characterization of an LMO may be similar to that carried out for alien species, where the whole organism is considered a novel genotype in the receiving environment.

Challenges to the selection of comparators

LM plants are being developed with quality traits modified by major modifications in metabolic pathways, possibly leading to extensive compositional alterations. Examples include nutritionally enhanced foods with qualitative and quantitative changes in proteins, amino acids, carbohydrates, oils/lipids, vitamins and minerals. Other LM plants will have new traits which facilitate adaptation to environmental stress conditions such as drought or high salinity. These crops may be cultivated in areas where they have never been grown before.

The selection of appropriate comparators for the risk assessment of these LM plants with complex modifications may be difficult. When no appropriate comparator is available, the risk assessment should be based primarily on the evaluation of the characteristics of the LM plant and derived products themselves.

For example, the main focus of an environmental risk assessment at the European Food Safety Authority (EFSA) is on the environmental impacts and the management of the LM plant compared to what is currently grown and/or against environmental protection goals. Comparators should be chosen on a case-by-case basis. Dependent on the issue(s) under consideration, choices might include: a non-LM line derived from the breeding scheme used to develop the LM plant; a non-LM plant with agronomic properties as similar as possible to the LM plant under assessment; and/or a non-LM line having other characteristics as close as possible to those of the LM plant, except for the intended modification. Some of such comparators may be genetically more distant from the LM plant than the recipient organism, but can still serve as appropriate comparators. Additional comparators could be considered on a case-by-case basis, including plants of other species appropriate to the environmental conditions. Applicants should justify their choice in all cases and uncertainty arising from these non-standard comparators should be discussed.

Source: BCH.CBD[d]

4.6 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 any step during risk assessment can be reviewed to incrementally build on previous findings, for example, as a result of ongoing accumulation of information (data from applicant, expert advice, literature search) or when new information suggests that new issues need to be considered.
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.

Risk assessment is a science-based process where steps 1 to 4 of annex III are similar to "*hazard identification*", "*exposure assessment*", "*hazard characterization*", and "*risk characterization*", as described in some other risk assessment frameworks. In step 5 a recommendation is made as to whether or not the risks are acceptable or manageable, and, where necessary, strategies to manage these risks are identified.

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 element will depend on the case being 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.

4.6.1 Step 1: "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" [4]

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.
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).
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. This is done by

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

examining if any of the novel characteristics of the LMO and/or its intended use could give rise to adverse effects in the likely potential receiving environment. 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, as well as changes in its use and management in relation to the non-modified counterpart. The changes are considered in the context of the non-modified recipient or parental organisms in the likely potential receiving environment using the environmental conditions prior to the release of the LMO as baseline. 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 section 'The choice of comparators' above).

Furthermore, it is important to define clear links or pathways, both direct or indirect, between the LMO and possible adverse effects in order to focus on generating information that will be useful in the decision-making. 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. Potential adverse effects may be direct or indirect, immediate or delayed, combinatorial or cumulative, as well as predicted or unpredicted (see below).

Types of adverse effects

The types of adverse effects on the environment or human health may be:

Direct: primary effects which are a result of the LMO itself and which do not occur through a causal chain of events;

Indirect: effects occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management. Observations of indirect effects are likely to be delayed;

Immediate: effects which are observed during the period of the release of the genetically modified organism. Immediate effects may be direct or indirect;

Delayed: effects which may not be observed during the period of the release of the genetically modified organism, but become apparent as a direct or indirect effect either at a later stage or after termination of the release;

Cumulative: effects due to the presence of multiple LMOs or their products in the receiving environment;

Source: BCH.CBD[e]

Identifying potential adverse effects to human health arising through environmental exposure

Risks associated with toxicity and allergenicity of foods originated from LMOs are typically assessed separate from environmental risks (guidance on how to assess the risk of foods derived from LMOs and exposure through ingestion is available elsewhere[*]).

However, food safety assessments do not evaluate potential adverse effects of LMOs to human health due to environmental exposure through means other than by food consumption and

incidental ingestion of LMOs. Consequently, and in accordance with the Cartagena Protocol, environmental risk assessments also examine potential adverse effects to human health arising from environmental exposure.

The potential adverse effects on humans due to environmental exposure may be direct or indirect such as through dermal contact, inhalation of dust, flour or pollen, consumption of animals who fed on LMOs not intended for use as food or feed, or via drinking water.

The kind of experimental studies needed to assess potential adverse effects to human health is determined on a case-by-case basis depending on the nature of the product(s) synthesized by the transgene(s), the intended use of the LMO and the likely potential receiving environment.

Identifying potential direct adverse effects to human health during the problem formulation phase or in step 1 requires the development of a risk hypothesis and a causal chain of events, even if it is a rather simple chain such as contact (exposure) with the LMO by humans, followed by expression of adverse effects. The remaining of the risk assessment follows the other steps as described below.

Identifying potential indirect adverse effects to human health is more challenging as causal chains of events are more complex or the effects may only be expressed after a long period of time. For example, people may develop ailments by indirect and/or long-term exposure to an LMO. Monitoring strategies, particularly of long-term effects, may play a role in identifying indirect adverse effects of LMOs on human health.

BCH.CBD[f]

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;
   (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, terminators and selection markers, 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, including potential toxicity of the gene products to non-target organisms and clinical significance of any antibiotic resistance genes inserted into the LMO; 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, including changes in native/endogenous gene expression and regulation at the transcriptional, translational and post-translational levels (for example, toxic products of endogenous upregulated genes).

Characterization of LMOs developed through RNAi-based methods

RNA interference (RNAi) refers to a set of pathways that alter gene expression. RNAi pathways usually inhibit the translation of messenger RNA (mRNA) into proteins and involve different types of double-stranded RNA (dsRNA) such as small interference RNA (siRNA) and micro-RNA (miRNA).

Several LM plants have been developed using RNAi to silence the expression of target genes in planta (for example the Arctic Apple OKA-NBØØ1-8 and OKA-NBØØ2-9) and pests and pathogens (for example the common bean modified for resistance to Bean Golden Mosaic Virus EMB-PVØ51-1).

The intended outcomes resulting from the use of RNAi is the silencing of targeted gene(s) (also known as "on-target gene silencing"), however, the small RNA fragments may bind to the mRNA of genes other than those being targeted, based on their sequence complementarity. This may result in the unintended silencing of the other genes (also known as "off-target gene silencing"). The unintentional silencing of genes may occur within the LMO itself or in organisms exposed to the LMO, including targeted pests as well as other organisms that may be exposed to the LMO which are not considered pests (i.e. non-target organisms). Furthermore, non-target organisms may express genes that share enough sequence similarity with the genes being targeted for silencing, leading to their silencing as well.

Therefore, in addition to the "elements for consideration" under step 1 which are related to the molecular characterization of LMOs, further considerations that are relevant to the characterization of an LMO that was developed through RNAi methods include: i) potential silencing of "on-target" and/or "off-target" genes in the LMO as well as in the targeted pests and non-target organisms; ii) dsRNA and small RNA expression levels in different parts of the LMO; and iii) capacity of non-target organisms to take up the dsRNA and small RNA molecules.

Bioinformatic tools may be used to analyse the genomes of the LMO, targeted pests and potential non-target organisms in order to identify if these organisms contain mRNA sequences which are complementary to the dsRNA or small RNA therefore making predictions of potential "on

target" and "off-target" genes that could be unintentionally silenced through RNAi. However "omics" technologies, such as transcriptomics and proteomics, could also be used to monitor the expression levels of the dsRNA or small RNA in the LMO, targeted pests and non-target organisms, and to measure the inhibition of "on-target" and "off-target" genes.
Furthermore, additional considerations for the assessment of the interaction between LMOs developed through RNAi-based methods and the likely potential receiving environment may include: i) horizontal transfer of the genetic element, dsRNA and/ or small RNA into other non-target organisms; ii) persistence of the dsRNA and small RNA in the environment and the effects of such persistence.

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

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

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

(h) 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;

e.g. ***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) scale of the introduction (small, medium or large); (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.

(i) Potential of pests or pathogens developing resistance to the target trait (e.g. insect or disease resistance trait).

(j) Potential indirect adverse effects to biodiversity as a result of weeds developing resistance to the herbicide, if appropriate in the particular regulatory framework where the risk assessment is being conducted.

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 possible *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.

LM crops and the use of herbicides

In many countries, the safety of the active ingredients found in herbicides is assessed primarily through regulations for the use of chemical products. These regulations typically assess the use of herbicides, both in isolation and mixed with other plant protection products, in the presence or absence of LMOs. However, regulations related to chemical products may not necessarily require studies on changes in agricultural management practices and their effects on biodiversity. As such, changes in agricultural practices due to the cultivation of LM crops, including changes arising from the use of different herbicides, are evaluated as part of the biosafety environmental risk assessments. This means that, for LM crops that are resistant to herbicides, their risk assessments should also evaluate the overall environmental impact arising from expected changes in cultivation practices due to the use of the herbicides to which the LM crop is resistant, in addition to evaluating the potential environmental impacts directly associated with the LM crops themselves.

The risk assessment of LM crops may also include considerations of potential consequences arising from the use of multiple herbicides since their use in the same area, applied either simultaneously or in sequence, may result in additive or synergistic adverse effects.

While the considerations noted throughout the Roadmap are applicable to the assessment of LM crops with herbicide resistance, the following considerations are particularly relevant during the assessment of LMOs that may result in the use of two or more herbicides:

- Volunteers and outcrossed relatives may exhibit more persistence and invasiveness and require additional measures for control, which may be more difficult if they contain several resistance genes;
- Effects on non-target organisms may be different due to adverse effects of mixtures of herbicides and additional studies may be needed to both identify and assess those risks;
- The overall adverse effect on biodiversity may arise from different changes, for example from declines in the populations of a particular species and from changes in the survival of other weed species.

Detailed information on agricultural practices and the herbicide regime that will be applied along with the cultivation of the LM crop with herbicide resistance are needed in order to identify the differences in relation to conventional practices and to identify possible adverse effects of herbicide mixtures. For example, when, how often, and in what combinations will the herbicides be used? What is known of the effects of the herbicides being used and their active ingredients when used in isolation and/or in different combinations? What is known of the herbicides' fate and behaviour in the environment and could any potential adverse effect be amplified by mixing the herbicides?

In order to answer these questions, the comparative approach for the assessment of LM crops with herbicide resistance may need to be adapted, for instance, by including additional comparators in cases where a single comparator cannot be used under different management conditions.

Source: BCH.CBD[g] relevant to "Step 1".

4.6.2 Step 2: "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.

An assessment of exposure is done in this step to determine which organisms in the receiving environment could be adversely affected by being exposed, directly or indirectly, to the LMO. During the exposure assessment, factors that may affect the spread, persistence and establishment of the LMO, as well as its potential for outcrossing and expression levels of the transgenes in different tissues of the LMO are considered.

Questions addressed during the exposure assessment

The exposure assessment describes exposure pathways, and intensity, spatial and temporal extent of co-occurrence or contact. It also describes the impact of variability and uncertainty on exposure estimates and reaches a conclusion about the likelihood that exposure will occur. The following questions may help address these issues:

- How does exposure occur?
- What is exposed?

- How much exposure occurs? When and where does it occur?
- How does exposure vary?
- How uncertain are the exposure estimates?
- What is the likelihood that exposure will occur?

Source : BCH.CBD[h]

For each of the risk scenarios and risk hypotheses identified in step 1, pathways of exposure to the LMO and its transgenes are determined, taking into account the intended handling and use of the LMO, as well as the expression level, dose and environmental fate of transgene products. Conceptual models describing relationships between the LMO and pathways of exposure can be built to define a causal link between the LMO 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.

e.g. Exposure characterisation

Risk assessments of biological systems are often complex and dynamic, and the variable nature of such systems limits the degree of certainty that can be ascribed to our knowledge of them. There is often a degree of uncertainty about the mechanisms that may lead to an adverse outcome, making it impossible to estimate the probability or likelihood of each identified potential adverse effect in precise terms.

Likelihood of exposure can be expressed either qualitatively using an ordered categorical description (such as "high", "moderate", "low" or "negligible") or quantitatively as a relative measure of probability (from zero to one, where zero represents impossibility and one certainty). However, if qualitative terms are used to express such likelihoods, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of 0 to 1, to which the term is intended to refer. For example, "the likelihood of exposure of a non-target lepidopteran species to Bt toxin (Cry1Ab protein) in field margins was estimated to be moderate, where 'moderate' in this context means within the range 0.1 to 0.4".

Sources: BCH.CBD[i]

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 "high", "moderate", "low" or "negligible" or "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.

In some risk assessment frameworks or when a high level of uncertainty makes it difficult to assess the likelihood of the adverse effects the order of steps 2 and 3 may be reversed (see above and

Figure 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;
(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).

Source: BCH.CBD[j] *relevant to "Step 2*,

4.6.3 Step 3: "Evaluation of the consequences should these adverse effects be realized."

Rationale:

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

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).

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.

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 recovery in the event of an adverse 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 xx) 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/high', 'intermediate/moderate', 'minor/low' or 'marginal/negligible' may be used. Parties may consider describing these terms and their uses in risk assessment guidelines published or adopted by them.

Hazard characterisation

The following are suggested as illustrative and qualitative examples in a very broad sense. They are not intended to be definitive or exclusive, but to give an indication of the considerations that might be taken into account when weighing up the consequences:

— 'high level consequences' might be significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species in the short or long term. Such changes might include a reduction in or complete eradication of a species leading to a negative effect on the functioning of the ecosystem and/or other connected ecosystems. Such changes would probably not be readily reversible and any recovery of the ecosystem that did take place would probably be slow;

— 'moderate consequences' might be significant changes in population densities of other organisms, but not a change which could result in the total eradication of a species or any significant effect on endangered or beneficial species. Transient and substantial changes in populations might be included if likely to be reversible. There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem;

— 'low level consequences' might be non-significant changes in population densities of other organisms, which do not result in the total eradication of any population or species of other organisms and have no negative effects on functioning of the ecosystem. The only organisms that might be affected would be non-endangered, non-beneficial species in the short or long term;

— 'negligible consequences' would mean that no significant changes had been caused in any of the populations in the environment or in any ecosystems.

Source:BCH.CBD[k]

Elements for consideration:

(a) Potential consequences based on experience with the non-modified recipient or parental organisms, or with similar organisms in the likely potential receiving environment, and their interactions with other species, including:

(i) The effects of agricultural practices on gene flow within the same species as well as with other compatible species;

(ii) Pathways for dissemination and spread;

(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;

(d) Results from laboratory experiments examining, as appropriate, dose-response relationships or particular effect levels (e.g., EC_{50}, LD_{50}, *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).

Source: BCH.CBD[1] *relevant to "Step 3".*

4.6.4 Step 4: "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.

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*'. The characterization of overall risk is often the best estimate which is derived from the combination of the likelihood and consequences of the identified individual risks. Risk matrixes, risk indices or models are typically used for this purpose (see below).[5]

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.

5 See references in the list of background materials.

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

e.g. ***Risk determination matrix***

		Likelihood of adverse effect			
		Highly likely	**Likely**	**Unlikely**	**Highly unlikely**
Consequence of adverse effect	**Major**	High	High	Moderate	Moderate
	Intermediate	High	Moderate	Moderate	Low
	Minor	Moderate	Low	Low	Negligible
	Marginal	Low	Low	Negligible	Negligible

Source: BCH.CBD[m]

Elements for consideration:

(a) Individual risks and possible interactions among them, such as *synergism* or *antagonism*;

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

(c) Broader considerations based on the ecosystem services approach, including cumulative effects due to the presence of various LMOs in the receiving environment, taking into account potential environmental changes caused by human activities.

Source: BCH.CBD[n] relevant to "Step 4".

4.6.5 Step 5: "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 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. On the other hand, the "acceptability" of risks is decided at a policy level and the threshold of what is considered "acceptable" may vary from country to country, for instance, some countries may choose to accept different levels of risk associated with the development of a certain technology while others may not.

In making a recommendation regarding 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.

Balancing risk acceptability with potential benefits is not laid out in the provisions of the Protocol. However, in some jurisdictions 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. 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.

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.

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 strategies for risk management and monitoring to reduce uncertainty, where appropriate, for consideration in the decision-making process.

Elements for consideration related to the risk management strategies and/or monitoring:

(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;
(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, taking into account that the proposed risk management strategies may introduce different risks.

Elements for consideration related to the acceptability of risks:

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

Source: BCH.CBD[o] *relevant to "Step 5".*

5. RISK ASSESSMENT FOR SPECIFIC TYPES OF LMOS AND TRAITS

The training module 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[6] 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 with cross-reference to related sections or steps in the Roadmap. 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.

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

6.1 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.[7] 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 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

6 Paragraphs 8 and 9 of Annex III.

7 See different processes for producing stacked LMOs at http://www.isaaa.org/resources/publications/pocketk/42/.

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.[8] In some regulatory frameworks, the information requirements in cases of risk assessment of LMOs with stacked genes may be adjusted if individual transformation events have already gone through risk assessments, and if evidence shows that there are no interactions between the genes/proteins expressed.

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, i.e. considered as single events and assessed case-by-case.

6.2 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

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

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.

Elements for consideration:

(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.

6.3 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:

(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 and human health *(see "Step 1", "Element for consideration (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:

(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[9] 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.

9 See definitions in the "Use of Terms" section.

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, with the stacked LM plant(s) such as compositional analyses and toxicity studies on target and non-target organisms, including monitoring of potential adverse effects to human health through incidental exposure. Where appropriate, in-depth genotypic and phenotypic characterization of the stacked LM plant may be conducted.

Elements for consideration:

(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;
(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
(e) Effects on native and local biodiversity.

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)*

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.

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:

(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;
(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, including quantitative PCR (qPCR), 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:

(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.

Source: BCH.CBD[p] relevant to "*Risk Assessment of Living Modified Plants with Stacked Genes or Traits*".

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

7.1 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,[10] 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-

10 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.

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.

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.

7.2 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. These may include non-modified organisms that share, with the LMO, similar biochemical, physiological or phenotypical responses under the relevant stress conditions such as photosynthesis and accumulation of protective pigments, stress hormones, reactive oxygen species, and anti-oxidative species. 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", "metabolomics" and "ionomics", may help to detect phenotypic and compositional changes (e.g., the production of a novel allergen or anti-nutrient) that cannot be detected using a comparison with field grown plants under suboptimal conditions.

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

(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.

7.3 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:

(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:

(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)*

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:

(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)*

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:

(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.

Source: BCH.CBD[q] relevant to Risk Assessment of LM Plants with Tolerance to Abiotic Stress.

8 RISK ASSESSMENT OF LIVING MODIFIED TREES

8.1 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".[11] Moreover, forest biodiversity is one of the seven thematic programmes of work under the Convention on Biological Diversity (CBD).
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".[12] This guidance focuses on forest and plantation trees. Some considerations contained here may also be applicable to risk assessment of orchard trees. This section does not cover any additional species such as palms, bamboos and shrubs.

8.2 INTRODUCTION[13]

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.
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 propagules, such as branches or roots.

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,

11 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).

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

13 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.

the root systems of trees can be extensive and are often associated with microorganisms and fungi, such as mycorrhizae (symbiotic associations).

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.[14]

A number of LM trees have been developed through the use of modern biotechnology and introduced into the environment.[15] The majority of these LM trees are species of economic interest used in managed orchards, forests and plantations. The modified traits include herbicide tolerance, wood composition (e.g., lignin), growth rate and phenology (including flowering and fruiting), resistance to pests and diseases, and abiotic stress tolerance.

8.3 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.

14 Further information on the biology of different tree species can be found at http://www.oecd.org/env/ehs/biotrack/consensusdocumentsfortheworkonharmonisationofregulatoryoversightinbiotechnologytrees.htm

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

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)[16] is of great importance because they may show better adaptive capabilities and consequently better performance than unselected germplasm.[17] 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.

Elements for consideration:

(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.

8.4 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

16 A comparable concept for crop plants would be regionally adapted crop varieties.

17 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".

cases, the removal of undesirable genetic elements in LM trees through cross-breeding would not be feasible.

Elements for consideration:

(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. As a result, appropriate modelling may be particularly useful for the risk assessment of LM trees.

Elements for consideration:

(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)*

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:

(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)*

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:

(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)*

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:

(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.

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:

(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.

9 RISK ASSESSMENT OF LIVING MODIFIED MOSQUITOES SPECIES THAT ACT AS VECTORS OF HUMAN AND ANIMAL DISEASES

9.1 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.[18] 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

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

the target population and, thus, to persist in the ecosystem at least for the medium term. Hence, 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.

9.2 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.
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.
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.

9.3 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.
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.

9.4 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:

(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.

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[19] 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.

  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.,

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

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:

(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 organisms in other trophic levels (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) could 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. .

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:

(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:

(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.

Elements for consideration:

(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:

(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[20]

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.

Elements for consideration:

(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.

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

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

Rationale:

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

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

Elements for consideration:

(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.

Containment of the living modified mosquito

Rationale:
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.

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 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.

Source:BCH.CBD[r] relevant to Risk Assessment of LM Mosquitoes.

10 MONITORING OF LIVING MODIFIED ORGANISMS RELEASED INTO THE ENVIRONMENT

This document provides guidance on monitoring of living modified organisms released in the environment, and complements the Roadmap for Risk Assessment of Living Modified Organisms (see sections on "Identification and consideration of uncertainty" and "Step 5" in the Roadmap).

10.1 INTRODUCTION

Ecosystems are constantly changing as part of natural processes without necessarily causing adverse impacts on biodiversity. However, monitoring of living modified organisms (LMOs) released into the environment may allow for the identification, in a timely manner and as early as possible, of changes that have led or that could lead to adverse effects. 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.

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".[21]

10.2 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 accordance with the objective of the Protocol.[22] This guidance may be applicable to all types of LMOs, and scales of release into the environment (i.e., small- and large-scale releases).

10.3 MONITORING AND ITS PURPOSES

As established in Article 7 of the CBD, Parties shall, as far as possible and as appropriate, monitor the components of biological diversity important for its conservation and sustainable use, and identify processes and categories of activities which have or are likely to have significant adverse impacts, and monitor their effects through sampling and other techniques.

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

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

For the purposes of this document, monitoring is categorized as “case-specific monitoring”, or “general monitoring”.
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 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 environmental monitoring networks, including those that may not focus primarily on biosafety, for the surveillance of broader protection goals and assessment endpoints that are relevant to identifying adverse effects linked to LMOs. 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.

10.4 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 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.[23]

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.[24] 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.[25]

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.

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).

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,

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

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

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

monitoring the exposure of the environment to LMOs may be a highly relevant element of an overall monitoring approach.

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:

(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.

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/or 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.

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:

(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;[26]
(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.

Elements for consideration:

(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,[27] transgenic traits introduced), and

26 See also considerations on "Quality and relevance of information" in the Roadmap.

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

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:

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

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:

(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.

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:

(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.[28]

Source: BCH.CBD[s] relevant to "*Monitoring of LMOs Released into the Environment*":

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

11 RISK COMMUNICATION

Interactive exchange of information about (health or environmental) risks among risk assessors, managers, news media, interested groups, and the general public.

Risk communication is the process of informing people about potential hazards to their person, property, or community. Scholars define risk communication as a science-based approach for communicating effectively in situations of high stress, high concern or controversy.
From the risk manager's perspective, the purpose of risk communication is to help residents of affected communities understand the processes of risk assessment and management, to form scientifically valid perceptions of the likely hazards, and to participate in making decisions about how risk should be managed. Risk communication tools are written, verbal, or visual statements containing information about risk.

They should put a particular risk in context, possibly add comparisons with other risks, include advice about risk reduction behavior, and encourage a dialogue between the sender and receiver of the message. The best risk communication occurs in contexts where the participants are informed, the process is fair, and the participants are free and able to solve whatever communication difficulties arise. Ideally, risk communication is a two-way conversation in which an agency or organization informs, and is informed by, affected community members.

Risk communication is "the interactive exchange of information and opinions throughout the risk analysis process concerning hazards and risks, risk-related factors and risk perceptions among risk assessors, risk managers, consumers, industry, the academic community and other interested parties, including the explanation of risk assessment findings and the basis of risk management decisions" (Codex Alimentarius Commission, 2003).

Risk communication in this sense is also addressed in Article 23 of the Cartagena Protocol on Biosafety on public awareness and public participation which states that: *The Parties shall: 1(a) Promote and facilitate public awareness, education and participation concerning the safe transfer, handling and use of living modified organisms in relation to the conservation and sustainable use of biological diversity, taking also into account risks to human health. In doing so, the Parties shall cooperate, as appropriate, with other States and international bodies; (b) Endeavour to ensure that public awareness and education encompass access to information on living modified organisms identified in accordance with this Protocol that may be imported.*
2. *The Parties shall, in accordance with their respective laws and regulations, consult the public in the decision-making process regarding living modified organisms and shall make the results of such decisions available to the public, while respecting confidential information in accordance with Article 21. Each Party shall endeavor to inform its public about the means of public access to the Biosafety Clearing-House.*

There is wide agreement that effective risk communication is essential at all phases of risk assessment and risk management. It is also recognized that risk communication involves not only

risk assessors and risk managers, but also other interested parties like government, industry, academia, consumers, public interest groups and individuals concerned with risk.

Risk communication is essential in making decisions (ILGRA, 1999). It enables all interested parties, not only risk assessors and risk managers, to participate in deciding how risks should be managed.

Communication is also a vital part of implementing decisions - whether explaining mandatory regulations, informing and advising people about risks which they can control by themselves, or dissuading people from risky, antisocial behaviour. Therefore, the main goals of risk communication are: (1) to improve knowledge and understanding on all aspects of the risk analysis process by all interested parties concerned with risk; and (2) to promote interactive communication between risk assessors, risk managers and other interested parties concerned with risks in order to achieve the desired outcomes.

Risk does not have to turn into a crisis if it can be identified, planned for, and dealt with effectively. Good communication is the key. Good Risk Communication is the presentation of a *scientific assessment of risk* in such a way that the public can understand the information of the risk without becoming emotionally involved.
Good risk communication must:

- translate the scientific findings and probabilistic risk assessment into understandable terms;
- explain the uncertainty ranges, knowledge gaps, and ongoing research programmes;
- address issues of credibility and trust;
- understand the public's concern with regard to risk issues, and acknowledge their questions and concerns;
- analyze the conditions needed for the public to acquire relevant information, skills, and participatory opportunities.

Good communication with the public can also help responsible agencies to handle risk more effectively:

» **Lead to better decisions about how to handle risks**

Considering and integrating a wide number of public and stakeholders' opinions may contribute to formulating well-suited and adequate decisions about the management of a certain risk.

» **Preventing crises**

Early discussions with stakeholders and the public can help to inform responsible authorities of potential areas of public concern early on. This can enable them to take early action to address those concerns, before they turn into crises. It can be particularly valuable where there are public concerns about risks associated with new technologies, such as GMOs.

Engaging a wide range of stakeholders and the public in risk decisions can help ensure that decisions take account of a wide range of views and experience. It can also help responsible authorities to spot aspects of a risk that might otherwise have gone unnoticed. This can be particularly important where action taken to tackle a risk could have a knock-on effect on others.

» **Smoother implementation**

A key feature of risk management, and of policy-making, is the need to deal with different and often conflicting perspectives. Engaging stakeholders and the public at an early stage in decisions about risks can help ensure that decisions better reflect public values and can reduce the scope for misunderstanding, disagreement and bitterness later on. This can make it easier to implement measures to address risks, particularly where these require the public to take action.

» **Empowering and reassuring the public**

Providing clear and accurate information about the nature of risks can help people to make realistic assessments of the risks they face and, where appropriate, to make informed judgments on how to handle risks by themselves. This can in turn help to foster a climate of greater empowerment and reassurance, and reduce the risk of scares.

» **Building trust**

Over time, communication with stakeholders can help to reduce suspicion, and build trust in the information government provides. Open communication can help by bringing people "inside the tent", and by enabling them to see for themselves that decisions have been made on the best available evidence and with the public interest in mind.

Also, effective risk communication can help responsible agencies to:

- explain technical risks more effectively;
- understand the multi-dimensionality of risk;
- anticipate community responses to the intended activities;
- respond to public concerns and misinformation;
- increase the effectiveness of risk management decisions by involving concerned community members;
- improve dialogue and reduce tension between communities and companies;
- build relationships based on trust and respect;
- develop a good reputation with regulators and the public;
- build a foundation for dialogue and shared problem solving before operations begin.

When to communicate about risk

It is widely acknowledged that risk communication is an integral part of the risk analysis process.

It is embedded into the risk assessment and risk management processes; two key steps – hazard identification and selection of risk management measures – require effective risk communication to help build trust, reduce conflicts and achieve desired outcomes. In hazard identification, the views and opinions of interested parties about the potential hazards can help define the issues of concern and reduce potential points of conflict. During the selection of risk management options, the risk managers may need to consider factors in addition to the scientific input in the evaluation of a risk. This should involve active participation of stakeholders and other interested parties. Finding a common language that will be clearly understood by all parties is needed in explaining the results and the procedures of the risk assessment and risk management processes.

Applying risk communication principles in risk analysis

The joint FAO/WHO expert consultation on the application of risk communication to food standards and safety matters identified the elements, principles, barriers and strategies for effective risk communication (FAO, 1999). The principles, applied to risk assessment and risk management processes, are illustrated below:

Know the audience. In the risk analysis process, the different types of audience may include risk assessors, risk managers, government, interest groups and the general public. It is important to listen to and understand their motivations, opinions, concerns and feelings. These are important in the development and delivery of credible information on the risks identified, the decisions made, and the processes used. Understanding the audience's perception of risk can be done through surveys, interviews and focus groups.

ANNEX-1

EXAMPLE OF RELEVANT ELEMENTS FOR CONSIDERATION UNDER EACH STEP WHEN ASSESSING A RISK SCENARIO INVOLVING NON-TARGET ORGANISMS

Note: This example shows only the elements to consider under each step that are most relevant for assessing a risk scenario involving non-target organisms. Elements for consideration that are not specific to non-target organisms, but are relevant for other risk scenarios, are not included in this example.

The following information was used in this example:

Risk scenario: The LMO, which is a Bt maize producing Cry1Ac and Cry2Ab2, may have adverse effects on lacewing populations

Protection goal: Conservation and sustainable use of biodiversity.

Assessment endpoint: Numbers and health of green lacewing (Chrysoperla carnea) populations because they are an ecosystem service

Measurement endpoint: reduction in number or diversity of lacewings; change in lacewing vitality or behaviour resulting in lower overall predation rates

Proposed risk management strategy: Refuge areas to provide lacewings with prey that had not fed on the LMO.

Relevant elements for consideration:

Regarding the characterization of the LMO:

- Molecular characteristics of the LMO related to the modification, such as characteristics of the modified genetic elements, including potential toxicity of the gene products to non-target organisms…

Regarding the intended use and the likely potential receiving environment:

- Characteristics of the likely potential receiving environment including relevant ecosystem functions and services…

Regarding the potential adverse effects resulting from the interaction between the LMO and the likely potential receiving environment:

- Potential adverse effects on possible non-target organisms …

- Potential consequences based on experience with the non-modified recipient or parental organisms, or with similar organisms in the likely potential receiving environment, and their interactions with other species, including:
- Changes in the abundance of… beneficial organisms…
- The behavior of populations of other species, including interactions between predators and prey, their role in food webs and other ecological functions…
- Results from laboratory experiments examining, as appropriate, dose-response relationships or particular effect levels…

Related to the risk management strategies and/or monitoring:

- 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… crop rotation

Related to the acceptability of risk:

- Protection goals and assessment endpoints as identified when establishing the context and scope for a risk assessment and/ or point
- 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

12 USE OF TERMS

Definitions that are used in internationally accepted risk assessment guidance to the context of environmental risk assessment conducted under the Cartagena Protocol are described below:

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.

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/)

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.

Behavioural sterility – A type of reproductive sterility that is caused by changes in behaviour rather than to physiological changes.

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)

Combinatorial effects – Effects that arise from the interactions between two or more genes in one organism. The effects may occur at the level of gene expression, or through interactions between RNA, or among gene products. The effects may also be referred to as antagonistic, additive or synergistic effects (see also "Cumulative effects" for distinction).

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.

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).

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

Co-transformation – Techniques of modern biotechnology using two or more transformation vectors to produce an LMO.
Crop wild relative – Crop wild relatives include crop ancestors as well as other species more or less closely related to crops. They are a critical source of genes for resistance to diseases, pests and stresses such as drought and extreme temperatures, among others. *From:* http://www.bioversityinternational.org/uploads/tx_news/Crop_wild_relatives_1217.pdf

Cross-talk – Instances in which one or more components of a signal transduction pathway affect a different pathway.

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

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/)

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.

Exposure – The route and level of contact between the likely potential receiving environment and the LMO or its products.

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)

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)

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.

Gene product – The RNA or protein that results from the expression of a gene.

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

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)

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)

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 AssessmentTerminology, http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf)
Heterozygous (genomes) – Having different alleles at the corresponding chromosomal loci

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).

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.

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

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.

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.

Multi-trophic (effects) – Involving more than two trophic levels in a food web

Non-target organisms – All living organisms that are not meant to be affected by newly expressed compounds in LMOs, and that can be potentially exposed, directly or indirectly, to the LMO and/or its products in the ecosystem where LMOs will be released or in adjacent habitats (adapted from Arpaia S., 2010, Genetically modified plants and "non-target" organisms: analysing the functioning of the agro-ecosystem. Collect. Biosafety Rev. 5: 12-80, http://www.researchgate.net/publication/228421663_Genetically_Modified_Plants_and_Non-Target_Organisms_Analysing_the_Functioning_of_the_Agro-ecosystem).

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)

"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.

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")

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

Pleiotropic effects – Effects of a single gene on multiple phenotypic traits.

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)

Protection goal –Defined and valued environmental outcomes that guide the formulation of strategies for the management of activities that may affect the environment.

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

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)

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.

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)

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)

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.

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

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.

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".

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.

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.

***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.

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.

Unintended gene product – Gene products (e.g., RNA, proteins), which are different from those originally intended.

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.

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".

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