

Annex 2

**GUIDANCE ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS AND
MONITORING IN THE CONTEXT OF RISK ASSESSMENT**

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101 **PREFACE**

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

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

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

- 115 • “Risk assessment should be carried out in a scientifically sound and transparent manner, and
116 can take into account expert advice of, and guidelines developed by, relevant international
117 organizations”.
- 118 • “Lack of scientific knowledge or scientific consensus should not necessarily be interpreted
119 as indicating a particular level of risk, an absence of risk, or an acceptable risk”.
- 120 • “Risks associated with living modified organisms or products thereof should be considered in
121 the context of the risks posed by the non-modified recipients or parental organisms in the
122 likely potential receiving environment”.

¹ “In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation” (Principle 15 of the Rio Declaration on Environment and Development) at:

(<http://www.unep.org/Documents/Multilingual/Default.asp?DocumentID=78&ArticleID=1163>), and in line with Articles 10.6 (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-10>) and 11.8 (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-11>) of the Protocol.

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

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

- 123 • “Risk assessment should be carried out on a case-by-case basis. The required information
124 may vary in nature and level of detail from case to case, depending on the LMO concerned,
125 its intended use and the likely potential receiving environment”.

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

133 **OBJECTIVE AND SCOPE OF THIS GUIDANCE**

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

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

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

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

⁵ Decision BS-V/12.

147 **PART I:**

148 **ROADMAP FOR RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS**

149 **BACKGROUND**

150 This “Roadmap” provides guidance on identifying and evaluating the potential adverse effects of
151 living modified organisms (LMOs)⁶ on the conservation and sustainable use of biological diversity in
152 the likely potential receiving environment taking into account risks to human health, consistent with
153 the Cartagena Protocol on Biosafety (hereinafter “the Protocol”) and in particular with its Article 15
154 and Annex III (hereinafter “Annex III”).⁷ Accordingly, this Roadmap supplements Annex III and
155 may also supplement national biosafety policies and legislations. Specifically, the Roadmap is
156 intended to facilitate and enhance the effective use of Annex III by elaborating on the steps and
157 points to consider in identifying and evaluating the potential adverse effects and by pointing users to
158 relevant background materials. The Roadmap may be useful as a reference for designing and
159 planning risk assessment approaches. It may also be useful for risk assessors when conducting or
160 reviewing risk assessments and as a tool for training.⁸ Based on its use, the Roadmap may also be
161 useful for identifying knowledge gaps.

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

165 This Roadmap provides information that is relevant to the risk assessment of all types of LMOs and
166 their intended uses within the scope and objective of the Protocol. However, it has been developed
167 based largely on living modified (LM) crop plants because most of the available knowledge has been
168 gained from these organisms.⁸

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

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

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

⁸ Decisions on LMOs may be found, *inter alia*, in the BCH (<http://bch.cbd.int>) and links to national and intergovernmental websites relevant for this purpose. In accordance with BCH records, **XX** LM crop plants, **XX** LM trees, **XX** LM animals and **XX** LM microorganisms have been released into the environment to date.

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173 **INTRODUCTION**

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

- 182 • Overarching Issues in the Risk Assessment Process
183 • Planning Phase of the Risk Assessment
184 • Conducting the Risk Assessment

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

189 Adverse effects and protection goals are closely interlinked concepts. Assessment endpoints and
190 measurement endpoints are derived from the relevant protection goals. The choice of protection
191 goals may be informed by the Party's national policies and legislation as well as Annex I to the
192 Convention on Biological Diversity as relevant to the Party responsible for conducting the risk
193 assessment.

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Protection goals, assessment endpoints and measurement endpoints

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

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Examples of protection goals include...

Comment [A1]: Outstanding: include examples

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

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‘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).

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Assessment endpoints are sometimes called specific protection goals or operational protection goals.

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Assessment endpoints may serve as starting point for the “problem formulation” step of the risk assessment.

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‘Measurement endpoints’...

Comment [A2]: Outstanding: include explanation

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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.

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

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

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- 221 • Step 2: “An evaluation of the likelihood of adverse effects being realized, taking into account
222 the level and kind of exposure of the likely potential receiving environment to the living
223 modified organism”;
- 224 • Step 3: “An evaluation of the consequences should these adverse effects be realized”;
- 225 • Step 4: “An estimation of the overall risk posed by the living modified organism based on the
226 evaluation of the likelihood and consequences of the identified adverse effects being
227 realized”;
- 228 • Step 5: “A recommendation as to whether or not the risks are acceptable or manageable,
229 including, where necessary, identification of strategies to manage these risks”.

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

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

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

241 In addition to the approach described in the Roadmap, other approaches to risk assessment exist.

242 » *See references relevant to “Introduction”:*

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

244 **OVERARCHING ISSUES IN THE RISK ASSESSMENT PROCESS**

245 This section provides guidance on matters that are relevant to all the steps of the risk assessment. It
246 focuses on provisions related to the quality and relevance of information to be considered in the risk
247 assessment, as well as means to identify and describe the degree of uncertainty that may arise during
248 the risk assessment.

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

255 Independent experts with a background in relevant scientific disciplines can serve in an advisory
256 capacity during the risk assessment process or perform the risk assessment themselves, in line with
257 Article 21 of the Protocol.

258 **Quality and relevance of information⁹**

259 An important question in a risk assessment is whether the available information that will be used to
260 characterize the risk posed by the LMO is relevant, and where possible, supported by evidence-based
261 information, including peer-reviewed data, as well as specialized knowledge, indigenous and
262 traditional knowledge.

263 In some regulatory frameworks, the criteria for evaluating the quality of scientific information are set
264 out in policies developed by the competent authorities. A number of points that are typically
265 considered to ensure the quality and relevance of the information used as well as the outcome of the
266 risk assessment include:

- 267 • Criteria for the quality of scientific information:
- 268 ○ The information used in the risk assessment should be of acceptable scientific quality
269 and consistent with best practices of scientific evidence-gathering and reporting. An
270 independent review of the design and methods of studies used in the risk assessment,
271 and of the quality of reporting may be conducted to ensure appropriate data quality.
 - 272 ○ Appropriate statistical methods should be used where appropriate, to strengthen the
273 scientific conclusions of a risk assessment and be described in the risk assessment
274 report. Risk assessments frequently use data generated from multiple scientific fields;
 - 275 ○ The reporting of the information, including its source and methods used, should be
276 sufficiently detailed and transparent to allow independent verification and

⁹ The term “information” is being used in a broad sense and includes, for example, experimental data, both raw and analysed.

277 reproduction. This would include ensuring that relevant information and/or sample
278 and reference materials are available and accessible to risk assessors, as appropriate,
279 taking into account the provisions of Article 21 of the Protocol on the confidentiality
280 of information.

- 281 • The relevance of information for the risk assessment:
- 282 ○ Information is considered relevant if it is linked to protection goals or assessment
283 endpoints, or if it contributes to the identification and evaluation of potential adverse
284 effects of the LMO, outcome of the risk assessment or decision-making;
 - 285 ○ The information that is relevant to perform a risk assessment will vary from case to
286 case depending on the nature of the modification of the LMO, on its intended use, and
287 on the scale and duration of the environmental introduction, as well as on the risk
288 assessors' level of familiarity with the trait or organism being assessed;
 - 289 ○ Relevant information may be derived from a variety of sources such as new
290 experiments, peer-reviewed scientific literature, as well as from previous risk
291 assessments, in particular for the same or similar LMOs introduced in similar
292 receiving environments;¹⁰
 - 293 ○ Information from national and international standards and guidelines may be used in
294 the risk assessment, as well as knowledge and experience of, for example, farmers,
295 growers, scientists, regulatory officials, and indigenous peoples and local
296 communities;

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¹⁰ Risk assessments can be found, *inter alia*, in the BCH (<http://bch.cbd.int>) and ICGEB (<http://rasm.icgeb.org>).

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Information requirements in the case of field trials or experimental releases

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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.

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

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Identification and consideration of uncertainty

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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.

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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.

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According to paragraph 8(f) of annex III to the Protocol, “where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate *risk management* strategies or monitoring the living modified organism in the receiving environment”. Furthermore, paragraph 6 of article 10 of the Protocol states

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327 that, “Lack of scientific certainty due to insufficient relevant scientific information and knowledge
328 regarding the extent of the potential adverse effects of a living modified organism on the
329 conservation and sustainable use of biological diversity in the Party of import, taking also into
330 account risks to human health, shall not prevent that Party from taking a decision [...] in order to
331 avoid or minimize such potential adverse effects”. Furthermore, paragraph 4 of annex III states that
332 “lack of scientific knowledge or scientific consensus should not necessarily be interpreted as
333 indicating a particular level of risk, an absence of risk, or an acceptable risk”.

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

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

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

350 In cases where uncertainty cannot be addressed through the provision of more information, where
351 appropriate, it may be dealt with by the implementation of risk management and/or ~~monitoring~~ in
352 accordance with paragraphs 8(e) and 8(f) of Annex III to the Protocol (see step 5 and Part III).
353 Furthermore, uncertainties associated with specific adverse effects may not allow the completion of a
354 risk assessment or conclusions regarding the level of overall risk.

355 The various forms of uncertainty are considered and described for each identified risk and under the
356 estimation of the overall risk. In addition, when communicating the results of a risk assessment, it is

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357 important to describe, either quantitatively or qualitatively, those uncertainties that may have an
358 impact on the overall risk, as well as on the conclusions and recommendations of the risk assessment
359 in a way that is relevant for decision-making.

360 » *See references relevant to “Identification and consideration of uncertainty”:*

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

362 **PLANNING PHASE OF THE RISK ASSESSMENT**

363 **Establishing the context and scope**

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

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

376 In establishing the context and scope, several points may be taken into consideration, as appropriate,
377 that are specific to the Party involved¹¹ and to the particular risk assessment. These include the
378 relevant:

- 379 (i) Regulations and international obligations of the Party involved;
- 380 (ii) Environmental and health policies and strategies;
- 381 (iii) Guidelines and regulatory frameworks that the Party has adopted;

¹¹ See Protocol provisions with regard to whose responsibility it is to ensure that risk assessments are carried out.

- 382 (iv) Protection goals, including for example ecosystems functions and services, as well
383 assessment endpoints, risk thresholds and management strategies derived from (i) to (iii)
384 above;
- 385 (v) Intended handling and use of the LMO, including practices related to the use of the
386 LMO, taking into account user practices, habits and traditional knowledge;
- 387 (vi) Availability of baseline information for the likely potential receiving environment;
- 388 (vii) The nature and level of detail of the information that is needed (see above), which may,
389 among other things, depend on the biology/ecology of the recipient organism, the intended use
390 of the LMO and its likely potential receiving environment, and the scale and duration of the
391 environmental exposure (e.g., whether it is for import only, field testing or for commercial
392 use);
- 393 (viii) Identification of methodological and analytical requirements, including requirements for
394 review mechanisms, that must be met to achieve the objective of the risk assessment as
395 specified, for instance, in guidelines published or adopted by the Party that is responsible for
396 conducting the risk assessment (i.e., typically the Party of import according to the Protocol);
- 397 (ix) Experience and history of use of the non-modified recipient or parental organism, taking
398 into account its *ecological function*;
- 399 (x) Information from previous risk assessments of the same or similar LMOs, including the
400 use of related surrogate systems, modified traits in other organisms;
- 401 (xi) Criteria to characterize the likelihood (step 2) and magnitude of consequences (step 3) of
402 individual risks, and for combining them into the overall risk (step 4), and the acceptability or
403 manageability of risks (step 5);
- 404 (xii) Proposed limits and controls to restrict the spread and persistence of the LMO
405 (particularly relevant for field trials).

406 Some risk assessment frameworks combine the process of establishing the context and scope of the
407 risk assessment with the identification of potential adverse effects associated with the modifications
408 of the LMO into a single step called “Problem formulation” (see step 1).

409 ***Problem formulation***

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

417 » See references relevant to “Establishing the context and scope”:

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

419 **The choice of comparators**

420 In a comparative risk assessment, risks posed by an LMO are considered in the context of the risks
421 posed by the non-modified recipients or parental organisms, in the likely potential receiving
422 environment, including local landraces and undomesticated species.¹²

423 In practice, a comparative approach aims at identifying, in relation to the appropriate *comparator(s)*,
424 the phenotypic and genotypic changes of an LMO that may lead to adverse effects, and changes in
425 the nature and levels of risk of the LMO. The choice of comparators can have large effects on the
426 relevance, interpretation and conclusions drawn from the risk assessment process. Therefore, the one
427 or more comparators that are chosen should be selected on the basis of their capacity to generate
428 information that is consistent and relevant for the risk assessment.

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

435 Choosing the appropriate comparator(s) may, in some cases, be difficult or challenging. On the one
436 hand, some risk assessment approaches require the use a non-modified genotype with a genetic
437 background as close as possible to the LMO being assessed, e.g. a *(near-)isogenic line*, as the

Comment [A3]: Outstanding (editorial): improve text

¹² Annex III, paragraph 5.

/...

438 primary comparator, with additional comparators, such as defined non-modified reference lines,
439 being used depending on the biology of the organism and types of modified traits under assessment.
440 In these risk assessment approaches, the (near-)isogenic non-modified organism is used in step 1 and
441 throughout the risk assessment, whereas broader knowledge and experience with additional
442 comparators is used, along with the non-modified recipient organism, when assessing the likelihood
443 and potential consequences of adverse effects. Results from experimental field trials or other
444 environmental information and experience with the same or similar LMOs in the same or similar
445 receiving environments may also be taken into account.

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

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

463 Furthermore it may be necessary to modify the comparative approach when dealing with LMOs
464 whose recipient organism is, for example a non-domesticated species. In cases where appropriate
465 comparators do not exist, an alternative to the comparative approach may be needed.

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466 **CONDUCTING THE RISK ASSESSMENT**

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

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

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

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

487 » See references relevant to “*Conducting the Risk Assessment*”:
488 http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

489

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

493 *Rationale:*

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

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494 The purpose of this step is to identify changes in the LMO, resulting from the use of modern
495 biotechnology, that could cause adverse effects on the conservation and sustainable use of biological
496 diversity, taking also into account risks to human health.¹⁴

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

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

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

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

520 In this step, a comparison of the LMO should be considered in the context of the non-modified
521 recipient or parental organisms in the likely potential receiving environment and the baseline

¹⁴ See also article 2, paragraph 2(b) of the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress (<http://bch.cbd.int/protocol/nkl/article2/>).

522 environmental conditions prior to the release of the LMO. Choosing appropriate comparators is
523 particularly relevant for this step in order to enable the consideration of the new trait(s) of the LMO,
524 and any associated changes in management practices (see ‘The choice of comparators’ in the chapter
525 entitled ‘Planning Phase of the Risk Assessment’).

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

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

534 Elements for consideration may help the risk assessor in determining if, for example, (i) any toxic
535 sequences have been inserted into the host organism, (ii) any endogenous toxic gene could have been
536 upregulated resulting from the genetic modification, (iii) any antibiotic resistance gene sequence
537 have been inserted into the host genome that have clinical significance, (iv) potential genotypic
538 instability could result in a specific potential adverse effect, etc.

539 *Elements for consideration regarding characterization of the LMO:*

- 540 (a) Relevant characteristics of the non-modified recipient or parental organism, such as:
- 541 (i) Its biological characteristics and agronomic traits, in particular those that, if changed
542 or resulting in an interaction with the new *gene products* or traits of the LMO, could
543 lead to changes that may cause adverse effects;
 - 544 (ii) Its taxonomic relationships;
 - 545 (iii) Its provenance, centre(s) of origin and centre(s) of genetic diversity;
 - 546 (iv) Its ecological function; and
 - 547 (v) Whether it is a component of biological diversity that is important for the conservation
548 and sustainable use of biological diversity in the context of Article 7(a) and Annex I
549 of the Convention;

/...

- 550 (b) Relevant characteristics of the donor organism(s), such as:
- 551 (i) its taxonomic status and common name;
- 552 (ii) its provenance;
- 553 (iii) relevant biological characteristics;
- 554 (iv) Relevant characteristics of the genes and of other functional sequences, such as
555 promoters, terminators and selection markers, that have been inserted into the LMO,
556 including functions of the genes and their gene products in the donor organism with
557 particular attention to characteristics in the recipient organism that could cause
558 adverse effects;
- 559 (c) Characteristics related to the transformation method, including the characteristics of the
560 vector such as its identity, source or origin and host range, and information on whether the
561 transformation method results in the presence of (parts of) the vector in the LMO, including any
562 marker genes;
- 563 (d) Molecular characteristics of the LMO related to the modification, such as characteristics of
564 the modified genetic elements; insertion site(s) and copy number of the inserts; stability, integrity
565 and genomic organization in the recipient organism; specificity of the genetic elements (e.g.,
566 transcription factors); levels and specificity of gene expression and intended and *unintended gene*
567 *products*, such as novel proteins being encoded by sequences put together at the insertion sites or
568 elongation of the intended protein due to faulty or lacking terminator sequences;
- 569 (e) Genotypic (see point (d) above) and phenotypic changes in the LMO, either intended or
570 unintended, in comparison with the non-modified recipient, considering those changes that could
571 cause adverse effects. These may include changes in native/endogenous gene expression and
572 regulation at the transcriptional, translational and post-translational levels.
- 573 *Elements for consideration regarding the intended use and the likely potential receiving*
574 *environment:*
- 575 (f) Protection goals and assessment endpoints relevant to the likely potential receiving
576 environment (see “Planning phase of the risk assessment”, “Establishing the context and scope”);

/...

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

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

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

586 ***Attributes of the receiving environment***

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

597

/...

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

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

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

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

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

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

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

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

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

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

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

/...

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

630 (u) Cumulative effects with any other LMO present in the environment.

631 » See references relevant to “Step 1”:

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

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

636 *Rationale:*

637 In this step the risk assessors evaluate the likelihood that each of the potential adverse effects
638 identified in step 1 will occur. The evaluation of likelihood may be undertaken at the same time as
639 the evaluation of the consequences should the adverse effects be realized (step 3). While steps 2 and
640 3 are independent of each other, in some frameworks they are carried out in a reverse order.

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

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

651 Experimental studies and models may be used for an assessment of the potential level and type of
652 exposure, combined with the use of statistical tools relevant for each case. Past experience with
653 similar situations (e.g., same recipient organism, LMO, trait, receiving environment, etc), if available,

/...

Comment [A4]: Outstanding (here and elsewhere in the document): attempt to reconcile different comments with regard to “cumulative” effects

654 may also be used in assessing the level and type of exposure, taking into account user practices and
655 habits.

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

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

662 *Elements for consideration:*

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

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

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

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

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

/...

683 (f) When assessing the likelihood of outcrossing from the LMO to sexually compatible species
684 as a step in the pathway to an adverse effect, the following issues are relevant:

- 685 (i) The biology of the sexually compatible species;
- 686 (ii) The potential environment where the sexually compatible species may be located;
- 687 (iii) Persistence of the LMO in the environment;
- 688 (iv) Introgression of the transgene into the sexually compatible species;

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

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

694 » *See references relevant to “Step 2”:*

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

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

697 *Rationale:*

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

705 The evaluation of consequences of adverse effects should be considered in the context of the adverse
706 effects caused by the non-modified recipients or parental organisms in the likely potential receiving
707 environment (see Planning Phase of the Risk Assessment). The evaluation of consequences may also
708 consider the adverse effects associated with the existing practices or with practices that will be

/...

709 introduced along with the LMO (such as various agronomic practices, for example, for pest or weed
710 management).

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

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

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

725 *Elements for consideration:*

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

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

730 (ii) Dissemination of the recipient organism;

731 (iii) Abundance of volunteers in crop rotation;

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

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

/...

- 737 (vi) The behaviour of populations of other species, including interactions between
738 predators and prey, their role in food webs and other ecological functions, disease
739 transmission, allergies and interaction with humans or other species;
- 740 (b) Potential adverse effects resulting from **combinatorial and cumulative effects** in the likely
741 potential receiving environment;
- 742 (c) Relevant knowledge and experience with the LMO and non-modified organisms with
743 similar phenotypic characteristics in similar receiving environments;
- 744 (d) Results from laboratory experiments examining, as appropriate, dose-response relationships
745 or particular effect levels (e.g., *EC₅₀*, *LD₅₀*, *NOEL*) for acute, chronic or sub-chronic effects
746 including immunogenic effects;
- 747 (e) Results from field trials containing information about the potential for invasiveness and
748 impacts in the environment; and
- 749 (f) Potential adverse effects resulting from outcrossing/interbreeding to sexually compatible
750 species and introgression of the transgene(s).

751 » *See references relevant to “Step 3”:*

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

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

755 *Rationale:*

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

Comment [A5]: Outstanding: reconcile different comments on combinatorial and cumulative effects

/...

763 To date, there is no universally accepted approach for estimating the overall risk but rather a number
764 of approaches are available for this purpose. As indicated in paragraph 8(d) of Annex III of the
765 Protocol, the estimation of the overall risk is ‘*based on the evaluation of the likelihood and*
766 *consequences of the identified adverse effects being realized*’. For example, the characterization of
767 overall risk is often the best estimate which is derived from the combination of the identified
768 individual risks. By combining evidence from each identified risk, the overall risk may be supported
769 by multiple lines of evidence. These lines of evidence may be quantitatively or qualitatively
770 weighted and combined. Risk matrixes, risk indices or models may be used for this purpose.¹⁵

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

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

778 *Elements for consideration:*

- 779 (a) The identified potential adverse effects (step 1);
780 (b) The assessments of likelihood (step 2);
781 (c) The evaluation of the consequences should the adverse effects be realized (step 3);
782 (d) Individual risks and any interaction among them, such as *synergism* or *antagonism*;
783 (e) Any risk management strategies (see step 5) that may affect risk estimates if implemented;
784 (f) Broader ecosystem and landscape considerations, including cumulative effects due to the
785 presence of various LMOs in the receiving environment, taking into account potential
786 environmental changes caused by human activities.

787 » See references relevant to “Step 4”:

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

Comment [A6]: Outstanding: reconcile different comments on cumulative effects, and clarity what is meant with the last part of the sentence

¹⁵ See references in the list of background materials.

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

Comment [A7]: Outstanding: add text to clarify the difference between step 5, as per the Protocol, and decision-making.

791 *Rationale:*

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

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

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

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

/...

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

824 In accordance with Annex III paragraph 8(f) “where there is uncertainty regarding the level of risk, it
825 may be addressed by requesting further information on the specific issues of concern or by
826 implementing appropriate risk management strategies and/or monitoring the living modified
827 organism in the receiving environment”.

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

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

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

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

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

/...

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

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

855 (d) Methods for evaluating the proposed risk management and monitoring strategies for
856 feasibility, efficacy and effectiveness, taking into account that the proposed risk management
857 strategies may introduce different risks.

858 *Elements for consideration related to the acceptability of risks:*

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

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

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

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

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

870 » *See references relevant to “Step 5”:*

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

872 **RELATED ISSUES**

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

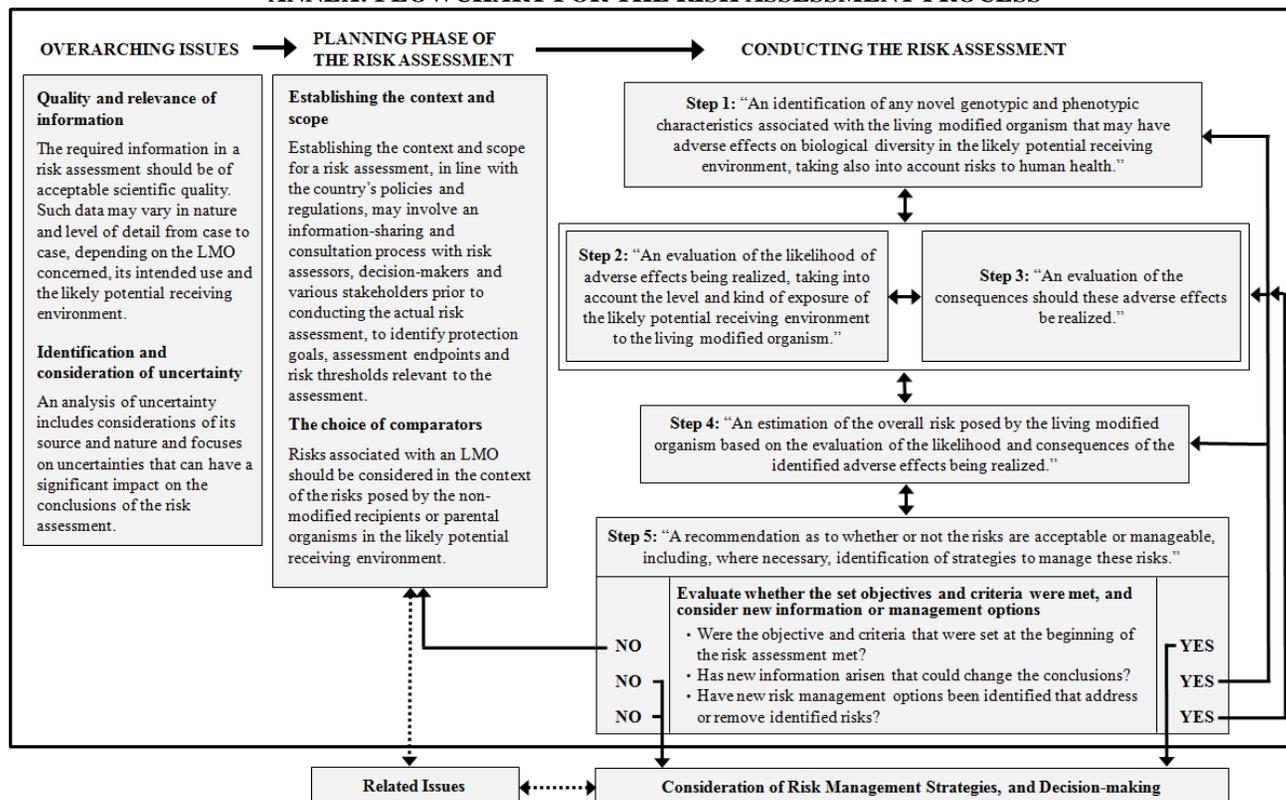
- 876 • Risk Management (Article 16);
- 877 • Capacity-building (Article 22);
- 878 • Public Awareness and Participation (Article 23);
- 879 • Socio-economic Considerations (Article 26);
- 880 • Liability and Redress (Article 27).

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

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ANNEX: FLOWCHART FOR THE RISK ASSESSMENT PROCESS



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

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PART II:
SPECIFIC TYPES OF LMOS AND TRAITS

895 The guidance contained in this section, Part II, should be considered in the context of the
896 Cartagena Protocol on Biosafety. The elements of Article 15 and Annex III of the Protocol apply
897 to these specific types of LMOs and traits. Accordingly, the methodology and points to consider
898 contained in Annex III¹⁶ are also applicable to these types of LMOs and traits. The guidance in
899 the sub-sections below complements the Roadmap for Risk Assessment of LMOs, giving
900 emphasis to issues that may be particularly relevant when assessing the risks of the respective
901 types of LMOs and traits.

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

906 **A. RISK ASSESSMENT OF LIVING MODIFIED PLANTS WITH**
907 **STACKED GENES OR TRAITS**

908 **INTRODUCTION**

909 Worldwide, a growing number of LMOs with stacked transgenic traits, particularly LM plants,
910 are being developed. As a result, the number of stacked genes in a single LM plant and the
911 number of LM plants with two or more transgenic traits is growing.

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

916 This guidance complements the Roadmap for Risk Assessment of LMOs, with emphasis on
917 issues that are of particular relevance to the risk assessment of LM plants with stacked traits
918 generated through cross-breeding. Some issues already covered in the Roadmap are further

¹⁶ Paragraphs 8 and 9 of Annex III.

919 elaborated on this section in an attempt to emphasize points that may need particular
920 consideration when assessing risks which may result from the combination of genetic elements
921 from two or more parental LM plants. As such, risk assessments of this type of LM plant follow
922 the general principles outlined in Annex III and the Roadmap, but also take into account the
923 specific issues outlined in this section of the present document.

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

929 It is assumed that the individual transformation events making up the stacked event have either
930 been assessed previously or are being assessed concomitantly to the stacked event in accordance
931 with Annex III of the Cartagena Protocol on Biosafety and as described in the Roadmap.¹⁷

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

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

939 **PLANNING PHASE OF THE RISK ASSESSMENT**

940 **The choice of comparators** (see “*Planning Phase of the Risk Assessment*”, “*The choice of*
941 *comparators*” in the Roadmap)

942 *Rationale:*

943 As seen in the Roadmap, choosing the appropriate comparator(s) is a crucial step for conducting
944 a comparative assessment. In the case of stacked LM plants, in addition to using non-modified

¹⁷ 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.

945 recipient organisms as comparators (see “The choice of comparators” in the Roadmap), the LM
946 plants that were involved in the cross-breeding process leading to the stacked LM plant under
947 consideration may also be used as comparators, as appropriate and according to national
948 regulations.

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

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

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

965 *Elements for consideration:*

- 966 (a) Level of heterozygosity among the non-modified recipient organisms used to produce
967 the parental LM plants;
- 968 (b) Phenotypic variability among non-modified hybrids produced through crosses between
969 the non-modified recipient organisms;
- 970 (c) Number of crossings and the use of intermediate stacked LM plants as additional
971 comparators.

972

973 **CONDUCTING THE RISK ASSESSMENT**

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

976 *Rationale:*

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

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

993 *Elements for consideration:*

- 994 (a) Whether or not methods to carry out molecular characterization are available, for
995 example PCR-based methods, and if they are specific and sensitive enough for the
996 characterization of the stacked LM plant;
- 997 (b) Phenotypic changes that may indicate underlying changes to any of the transgenes and
998 genetic elements present in the stacked LM plant (e.g., loss of a trait present in the
999 parental LM plants).

1000 **Potential interactions among the stacked genes, their resulting phenotypic changes and**
1001 **effects on the environment** (see “Step 1”, “Element for consideration (e)” in the Roadmap)

1002 *Rationale:*

1003 The expression level of transgenes or endogenous genes in a stacked LM plant may be changed
1004 as compared to the parental LM plant due to *trans-regulation*. Such changes are more likely to
1005 occur if the parental LM plants contain transgenes or regulatory elements that share similarities
1006 among them or with endogenous sequences (e.g., same binding sites for transcriptional factors).

1007 The products of transgenes and endogenous genes may also interact. This is most likely to occur
1008 if the gene products belong to the same metabolic pathway or physiological process. Some of the
1009 interactions may lead to changes that can be detected during the phenotypic characterization of
1010 the stacked LM plant, whereas other interactions may not be detectable through a typical
1011 phenotypic characterization. Previous risk assessments of the parental LM plants provide useful
1012 information on the mode of action and molecular characteristics of the individual genes as a
1013 starting point to assess the potential for interactions.

1014 In addition to information about the characteristics of the parental LM plant, specific information
1015 on potential for interactions among transgenes and other genetic elements (e.g., promoters and
1016 other regulatory elements), proteins, metabolites or modified traits and endogenous genes and
1017 their products in the stacked LM plant should be considered and assessed, paying particular
1018 attention to transgenes that belong to the same biochemical pathways or physiological processes.

1019 *Elements for consideration:*

- 1020 (a) Effects of the parental LM plants on the environment;
- 1021 (b) Information on transcriptional and post-transcriptional regulation of genes and their
1022 products that may be predictive of interactions between the novel and endogenous genes
1023 and/or DNA elements in the stacked LM plant;
- 1024 (c) Whether transgenes with similar functions or belonging to the same metabolic pathways
1025 were stacked;

1026 (d) Levels of expression of the transgenes and their products compared to the parental LM
1027 plants and to the non-modified recipient organisms.

1028 **Combinatorial and cumulative effects** (see “Step 1”, “Point to consider (d) and (q)”, “Step
1029 2”, “Point to consider (e)” and “Step 3”, “Point to consider (b)” in the Roadmap)

1030 *Rationale:*

1031 An assessment of the risks of a stacked LM plant to cause **combinatorial and cumulative effects**¹⁸
1032 should be considered in the context of the closely related non-modified recipient organism(s) and
1033 the parental LM plants in the likely potential receiving environment, taking into account the
1034 results of the genotypic and phenotypic assessments outlined above.

1035 Combinatorial effects may occur due to interactions among the proteins and metabolites
1036 produced by the transgenes or endogenous genes of a stacked LM plant. For example, the
1037 stacking of various insecticidal proteins in an LM plant could have a synergistic effect on non-
1038 target organisms that could be broader than the sum of the effects of the individual parental LM
1039 plants. Likewise, the evolution of resistance in target organisms (e.g., insect pests) to such
1040 stacked LM plants could happen faster than the development of resistance to the parental LM
1041 plants.

1042 The risks of multiple stacked LM plants being cultivated in the same environment to cause
1043 cumulative adverse effects (e.g., due to changes in agricultural practices) may also be
1044 considered.

1045 An assessment of potential **combinatorial and cumulative effects** may be performed, for instance,
1046 by conducting specific tests with the stacked LM plant(s) such as compositional analyses and
1047 toxicity tests on target and non-target organisms. Where appropriate, in-depth genotypic and
1048 phenotypic characterization of the stacked LM plant may be conducted.

1049 *Elements for consideration:*

1050 (a) Effects of the use of pesticides, other chemicals or agricultural practices commonly used
1051 in the cultivation of the parental LM plants;

Comment [A8]: Outstanding: reconcile different comments with regard to combinatorial and cumulative effects

Comment [A9]: Outstanding: reconcile different comments with regard to combinatorial and cumulative effects

Comment [A10]: Outstanding: reconcile different comments with regard to combinatorial and cumulative effects

¹⁸ See definitions in the “Use of Terms” section.

- 1052 (b) Phenotypic characteristics compared to the parent LM plants and to the non-modified
1053 recipient organisms;
- 1054 (c) Interactions between the stacked transgenes or their products, or interactions among the
1055 physiological pathways in which the transgenes are involved, taking into account the
1056 possibility that these interactions could result in potentially harmful substances (e.g.,
1057 anti-nutritional factors), some of which may persist or accumulate (e.g., via the food
1058 chain) in the environment;
- 1059 (d) **Combinatorial and cumulative effects** arising from the presence of two or more
1060 insecticidal proteins that could result in increased toxicity to non-target organisms or
1061 faster development of resistance in the target organisms.

Comment [A11]: Outstanding: reconcile different comments with regard to combinatorial and cumulative effects

1062 **Crossing and segregation of transgenes** (see “Step 1”, “Element for consideration (l)” and
1063 “(m)”, “Step 2”, “Element for consideration (f)”, “Step 3”, “Element for consideration (f)” in
1064 the Roadmap)

1065 *Rationale:*

1066 Due to genetic recombination, the offspring of a crossing will have combinations of genes that
1067 differ from those found in either parent. In the case of stacked events, the number of new
1068 combinations of transgenes that may result from a cross will depend on the number transgenes
1069 involved in a crossing, their location in the genome and their distance from each other.

1070 As a result, a set of new stacked LM plants may arise in the environment through crossings
1071 between a stacked LM plant and other LM plants. Successive crossings with non-modified
1072 sexually-compatible relatives in the receiving environment may also result in the stacking of
1073 genes and traits. These crossings can either be mediated by man or occur naturally through
1074 pollination and may result in a range of new stacked LM plants containing new and/or different
1075 combinations of transgenes and other genetic elements.

1076 The larger the number of different sexually-compatible LM plants, stacked or not, being
1077 cultivated in the same environment, the more variations and complexity of new stacked LM
1078 plants may occur. The presence of sexually-compatible LM plants being cultivated in the likely

1079 potential receiving environment of the stacked LM plant under consideration is to be taken into
1080 account when establishing risk scenarios or hypotheses during step 1 of the risk assessment.

1081 *Elements for consideration:*

- 1082 (a) Presence of other single-event and stacked LM plants of the same species;
- 1083 (b) Possible new combinations of transgenes and other genetic elements should the stacked
1084 event under consideration cross, intentionally or unintentionally, with other LM plants,
1085 stacked or not, or with non-modified relatives;
- 1086 (c) Potential adverse effects of the new stacked LM plants, including enhanced fitness as
1087 compared to the non-modified recipient or parental organisms, invasiveness, effects on
1088 non-target organisms, allergenicity and toxicity to humans;
- 1089 (d) Scientifically plausible risk scenarios or risk hypotheses involving the stacked events
1090 with different combinations of transgenes and DNA fragments.

1091 **Methods for distinguishing the combined transgenes in a stacked event from the parental**
1092 **LM plants** (*see “Step 5”, “Point to consider (b)” in the Roadmap*)

1093 *Rationale:*

1094 In the context of paragraphs 8(f) and 9(f) of Annex III of the Protocol, some of the risk
1095 management strategies for stacked events may require methods for the detection and
1096 identification of these LM plants in the context of environmental monitoring. Currently, many
1097 detection methods for LM plants rely on DNA-based techniques, such as polymerase chain
1098 reaction (PCR) or protein-based ELISA tests.

1099 Several of the current PCR-based detection methods are designed to be specific to a single
1100 transformation event. While these methods may be used to detect and identify single
1101 transformation events, when the analysis is carried out in bulk (i.e., mixing material collected
1102 from various test individuals), these methods are not sensitive or specific enough to differentiate
1103 between single transformation events and a stacked event arising from a cross between these
1104 single transformation events. For example, although some software may help predict the
1105 presence of stacked LM seeds in a bulk sample, it is not possible to unequivocally distinguish a

1106 sample containing material from different single transformation events from another sample
1107 containing one or more stacked LM events.

1108 PCR-based detection methods that are specific to a single transformation event often rely on the
1109 amplification of DNA sequences that flank the insertion sites and that are unique to a single
1110 transformation event. In the future, it may become a challenge to detect single transformation
1111 events produced through site-specific insertions because the flanking sequences could be the
1112 same among different LMOs. This could become challenging particularly in cases where the
1113 stacked event contains multiple transformation cassettes with similar DNA sequences.

1114 Based on the considerations above, the detection of each and all individual transgenes in a
1115 stacked event, if needed or required, may become a challenge and may need special
1116 consideration.

1117 *Elements for consideration:*

1118 (a) Level of similarity/difference between different transformation constructs in the stacked
1119 LM plant;

1120 (b) Availability, specificity and reliability of methods to detect stacked LM plants in the
1121 context of risk management strategies.

1122 **BIBLIOGRAPHIC REFERENCES**

1123 See references relevant to “*Risk Assessment of Living Modified Plants with Stacked Genes or*
1124 *Traits*”:

1125 http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

1126

1127 **B. RISK ASSESSMENT OF LIVING MODIFIED PLANTS WITH TOLERANCE TO**
1128 **ABIOTIC STRESS**

1129 **INTRODUCTION**

1130 While the same general principles used in the risk assessments of other types of LMOs also
1131 apply to LM plants with increased tolerance to abiotic stress,¹⁹ there are a number of specific
1132 issues that may be of particular importance when assessing the risks of LM plants tolerant to
1133 abiotic stresses.

1134 As outlined in the section on “Establishing the context and scope” and in step 1 of the Roadmap,
1135 identifying protection goals, assessment endpoints and establishing scientifically plausible risk
1136 scenarios are some of the first actions to be taken during a risk assessment.

1137 An important consideration in performing a risk assessment of an LM plant with tolerance to
1138 abiotic stress is the possibility of multiple interactions between the new trait and the receiving
1139 environment, and the associated need to design a properly controlled field experiment.

1140 In plants, any gene (or gene product) or gene combinations providing increased tolerance to
1141 abiotic stress may have *pleiotropic effects* on the stress physiology of the plant. For example,
1142 drought, temperature and salt stress are interconnected by common metabolic and signal
1143 transduction pathways. Such pleiotropic effects may be classified as "unintended predicted
1144 effects" (see the Roadmap, step 1) and may be evaluated during the risk assessment by
1145 considering the *cross-talk* mechanisms between different stress responses of the plant, and by
1146 evaluating whether or not the identified changes may cause adverse effects. Disciplines such as
1147 plant physiology, plant pathology and entomology may provide useful context based on non-
1148 modified crops to clarify cross-talk mechanisms among abiotic stress responses and how these
1149 responses may change susceptibility to biotic stresses (e.g., predators, pests and pathogens) in an
1150 LM plant that is tolerant to abiotic stresses.

¹⁹ 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 CO₂ 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.

1151 The stress tolerance of the LM plant should be assessed with respect to an appropriate range of
1152 potential environmental conditions that reflect the potential conditions to which the LM plant is
1153 likely be exposed, including for example variation in the duration and periodicity of the stressor
1154 (e.g., drought, flood, suboptimal temperatures, salinity or heavy metals). These variations pose
1155 difficulties for (i) controlling and measuring conditions in field experiments and (ii)
1156 characterizing the phenotype of the LM plant itself, which in many cases may be subject to the
1157 interaction between external and physiological parameters.

1158 Some of the issues that could arise from the introduction of LM plants tolerant to abiotic stress
1159 into the environment and which may lead to adverse effects include, for example: a) increased
1160 selective advantage(s), other than the intended tolerance trait, which may lead to potential
1161 adverse effects (e.g., resulting from the introduction of a transcription factor affecting more than
1162 one trait); b) increased persistence in agricultural areas and increased invasiveness in natural
1163 habitats; c) adverse effects on organisms exposed to the LM plant; and d) adverse consequences
1164 of potential gene flow to wild or non-modified relatives. While these potential adverse effects
1165 may exist regardless of whether the tolerant plant is a product of modern biotechnology or
1166 conventional breeding, some specific issues may be more relevant in the case of abiotic stress
1167 tolerant LM plants.

1168 In this context, questions that may be relevant to the risk assessment of LM plants with tolerance
1169 to abiotic stress in connection with the intended use and the receiving environment include:

- 1170 • Does the tolerance trait have the potential to affect other tolerance and/or resistance
1171 mechanisms of the LM plant, for example, via pleiotropism?
- 1172 • Does the tolerance trait have the potential to cause an increase of the invasiveness,
1173 persistence or weediness of the LM plant that could cause adverse effects to other
1174 organisms, food webs or habitats?
- 1175 • Does an LM plant arising from outcrossing with the abiotic stress tolerant LM plant
1176 have the potential to change or colonize a habitat or ecosystem beyond the intended
1177 receiving environment?
- 1178 • Does an LM plant expressing tolerance to a particular abiotic stress have other
1179 advantages in the targeted receiving environment that could cause adverse effects?

- 1180 • What are the adverse effects in regions that have not been exposed to commercial
1181 agriculture but may become exposed to stress tolerant LM plants?

1182 The following sections elaborate on specific issues that may be taken into account, on a case-by-
1183 case basis, when assessing the risks of LM plants tolerant to abiotic stress and the potential
1184 adverse effects to conservation and sustainable use of biodiversity, taking also into account risks
1185 to human health.

1186 **PLANNING PHASE OF THE RISK ASSESSMENT**

1187 **The choice of comparators** (see “*Planning Phase of the Risk Assessment*”, “*The choice of*
1188 *comparators*” in the Roadmap)

1189 *Rationale:*

1190 As outlined in the Roadmap, the first step in the risk assessment process involves the
1191 characterization of genotypic or phenotypic changes, either intended or unintended, associated
1192 with the abiotic stress-tolerant LM plant, that may have adverse effects on biodiversity in the
1193 likely potential receiving environment, taking into account risks to human health.

1194 The identification of genotypic and phenotypic changes in the abiotic stress tolerant LM plant,
1195 either intended or unintended, is typically carried out in comparison with the non-modified
1196 recipient organism and/or plants which are not LMOs but exhibit a similar abiotic stress
1197 tolerance. The non-modified comparator provides the baseline information for comparison
1198 during trials when it is grown at the same time and location as the LM plant. Comparisons should
1199 also be made, as appropriate, in a range of environments with different stressor intensities and
1200 durations.

1201 While the comparative approach should be used to assess whether or not the LM plants with
1202 tolerance to abiotic stress have increased fitness advantages under non-stress conditions,
1203 additional approaches (and comparators) for risk assessment need to be implemented for
1204 assessing potential adverse effects under abiotic stress.

1205 LM plants with tolerance to abiotic stress may present specific challenges in the experimental
1206 design to generate data for the risk assessment. In some cases, for instance, an approach uses
1207 different reference plant lines, which typically include a range of genotypes representative of the

1208 natural variation in the plant species. Another important consideration is whether the
1209 experimental design is properly controlled for the effect of the abiotic stress trait. In the extreme
1210 case, when the non-modified plant cannot be grown in the range of conditions of the receiving
1211 environment because the abiotic stress conditions prevent or severely affect the growth of the
1212 non-modified plant, a comparative approach between the LM plant and the non-modified plant
1213 will need to be adjusted. In such cases, non-modified varieties or distant relatives that are
1214 tolerant to abiotic stress may become useful comparators. It is noted however that, in situations
1215 where the non-modified recipient organism, or (near-)isogenic or closely related lines cannot be
1216 used for a comparative risk assessment, the use of non-isogenic lines or distant relatives as
1217 comparators can make it more difficult to identify statistically meaningful differences.

1218 In situations where a suitable comparator is not available, the characterization of the abiotic
1219 stress tolerant LM plant may be similar to that carried out for alien species, where the whole
1220 plant is considered a novel genotype in the receiving environment. On a case by case basis,
1221 available information from “*omics*” *technologies*, for example, “transcriptomics” and
1222 “metabolomics”, may help to detect phenotypic and compositional changes (e.g., the production
1223 of a novel allergen or anti-nutrient) that cannot be detected using a comparison with field grown
1224 plants under suboptimal conditions.

1225 Where non-modified organisms are unsuitable as comparators, insight may be gained by
1226 comparing LM individuals grown under stress to individuals grown under normal conditions.

1227 *Elements for consideration:*

- 1228 (a) Characteristics of the LM plant with and without the influence of the abiotic stress or
1229 other stresses, if applicable; and
- 1230 (b) Whether comparators that can generate meaningful data are available and can be used
1231 in appropriately designed experiments.

Comment [A12]: Outstanding: further discussion needed on how to best address this issue

1232 **CONDUCTING THE RISK ASSESSMENT**

1233 **Unintended characteristics including cross-talk between stress responses** (*see “Step 1” in*
1234 *the Roadmap*)

1235 *Rationale:*

1236 The abiotic-stress-tolerant LM plant may have characteristics such as tolerance to other types of
1237 biotic and abiotic stresses (i.e., cross-talk in biochemical signalling), which could lead to a
1238 selective advantage of these plants under stress conditions other than that related to the modified
1239 trait. For instance, plants modified to become tolerant to drought or salinity may be able to
1240 compete better than their counterparts at lower or higher growing temperatures. The
1241 characteristics of an LM plant with increased tolerance to an abiotic stress may affect its general
1242 biology (e.g., if the genes alter multiple characteristics of the plant) or its distribution range in
1243 the likely potential receiving environment, which may cause adverse effects. Other changes
1244 could influence seed dormancy, viability, and/or germination rates under other types of stresses.
1245 Particularly in cases where genes involved in abiotic stress are also involved in crucial aspects of
1246 physiology, modifications involving these genes may have pleiotropic effects. If the stress
1247 tolerance trait leads to an increased physiological fitness, introgression of the transgenes for
1248 stress tolerance may occur at higher frequencies than observed among non-modified plants.

1249 The response mechanisms to abiotic and biotic stresses in plants may have interactions and
1250 cross-talk mechanisms. For that reason, an LM plant modified to acquire drought or salinity
1251 tolerance may, for example, also acquire modified tolerance to biotic stresses, which could result
1252 in changes in interactions with its herbivores, parasitoids and pathogens. Such cross-talk between
1253 the different types of stress-response mechanisms could, therefore, have both direct and indirect
1254 effects on organisms that interact with them.

1255 *Elements for consideration:*

- 1256 (a) Any intended or unintended change that may lead to selective advantage or
1257 disadvantage acquired by the LM plant under other abiotic or biotic stress conditions
1258 that could cause adverse effects;
- 1259 (b) Any change in the resistance to biotic stresses and how these could affect the population

1260 of organisms interacting with the LM plant; and

1261 (c) A change in the substances (e.g., toxin, allergen, or nutrient profile) of the LM plant that
1262 could cause adverse effects.

1263 **Testing the living modified plant in representative environments** (*see “Step 1” in the*
1264 *Roadmap*)

1265 *Rationale:*

1266 LM plants with tolerance to abiotic stress are intended to be cultivated under abiotic stress
1267 conditions. Therefore, in accordance with the general principles of Annex III to the Protocol that
1268 risk assessments should be carried out on a case-by-case basis, it is of particular importance that
1269 the assessment of potential adverse effects of LM plants with tolerance to abiotic stress be
1270 conducted in relation to the ‘likely potential receiving environment’ of the LM plant under
1271 consideration.

1272 Regional variation and differences in receiving environments that may influence the
1273 characteristics and the behaviour of the LM plant as well as its interactions with the environment
1274 should be taken into account during the risk assessment. Regions and locations where data are
1275 collected or field trials are conducted should represent the range of agricultural, plant health and
1276 environmental conditions the LM plant is expected to encounter.

1277 Different environments may be distinguished, for example, by differences in flora and fauna, soil
1278 property/chemistry, agricultural practices, climatic and geographic conditions, etc. Relevant
1279 characteristics of a specific region such as agricultural practice, climatic and geographic
1280 conditions should be determined at the start of the risk assessment as these characteristics may
1281 lead to differences in potential adverse environmental effects which only become evident if
1282 assessed on a regional level.

1283 *Elements for consideration:*

1284 (a) The likely potential receiving environment where exposure to the LM plant may occur
1285 and its characteristics such as information on geographical, climatic and ecological

1286 characteristics, including relevant information on biological diversity, centres of origin
1287 and centres of genetic diversity;

1288 (b) Regional variation and differences in the likely potential receiving environments that
1289 may influence the characteristics and the behaviour of the LM plant with tolerance to
1290 abiotic stress including, for example, agricultural practices and agronomic structures
1291 (e.g., input of nitrogen fertilizers), cultivation systems (e.g., low-tillage farming), crop
1292 rotation practices, climatic conditions, occurrence of non-target organisms, as well as
1293 other abiotic and biotic conditions;

1294 (c) Locations where field trials have been conducted to generate data for the risk
1295 assessment, if applicable, and how the conditions of the field trials represent the range
1296 of conditions expected in the likely potential receiving environment(s) in different
1297 regions;

1298 (d) Relatives which can crossbreed with the LM plant in the likely receiving environment
1299 and the possible consequences of introgressing the abiotic stress tolerance traits into
1300 these species;

1301 (e) How the LM plant behaves when the tolerance trait is not expressed because of the
1302 absence of the stressor, e.g., drought tolerance under normal water regimes.

1303 **Persistence in agricultural areas and invasiveness of natural habitats** (see “Step 1”, “Step
1304 2”, “Elements for consideration (b), (f) and (g)”, and “Step 4”, “Element for consideration (e)”
1305 in the Roadmap)

1306 *Rationale:*

1307 Climate conditions, water availability and soil salinity are examples of factors that limit the
1308 growth, productivity, spread or persistence of a plant species. Expression of the genes for abiotic
1309 stress tolerance could result in an unwanted increased persistence of the LM plant in agricultural
1310 areas. Expression of these genes may also change the capacity of LM plants to establish in
1311 climatic and geographic zones beyond those initially considered as the likely potential receiving
1312 environments.

1313 In the event where the modified gene is a transcription factor conferring tolerance to abiotic
1314 stress, the transcription factor may also affect the response mechanisms to other forms of abiotic
1315 stress. For example, the seeds of a plant modified for drought or salinity tolerance may acquire in
1316 addition tolerance to cold resulting in an increased winter survivability of the seeds. Therefore,
1317 an abiotic stress-tolerant LM plant may acquire the potential to persist better than its non-
1318 modified counterpart and other species under different abiotic-stress conditions.

1319 Most tolerance traits can be expected to have a “metabolic cost” associated with them – usually
1320 an energy cost – which may impact the potential for the plant to persist under conditions of low
1321 selection pressure (i.e., low abiotic stress). The metabolic cost can have a significant impact on
1322 the potential of the LM plant to survive and persist in an environment over time and should be
1323 taken into account when assessing the potential of the LM plant to persist in agricultural areas
1324 and natural habitats.

1325 *Elements for consideration:*

- 1326 (a) Consequences of any increased potential for persistence of the modified plant in
1327 agricultural habitats, and invasiveness and persistence in natural habitats;
- 1328 (b) Need for and feasibility of control measures if the abiotic stress-tolerant LM plant
1329 shows a higher potential for persistence in agricultural or natural habitats, that could
1330 cause adverse effects;
- 1331 (c) Characteristics, such as prolonged seed dormancy, long persistence of seeds in the soil,
1332 germination under a broad range of environmental conditions, rapid vegetative growth,
1333 short lifecycle, very high seed output, high seed dispersal and long-distance seed
1334 dispersal;
- 1335 (d) Effects of climate change that could change the ecological range of the LM plant; and
- 1336 (e) Implications of modified agricultural practices associated with use of the LM plant
1337 expressing tolerance to abiotic stress.

1338 **Effects on the abiotic environment and ecosystem** (see “Step 3”, “*Elements for consideration*
1339 *(a) and (e)*” in the Roadmap)

1340 *Rationale:*

1341 Changes to the abiotic environment resulting from the use of LM plants will depend largely on
1342 the introduced trait, and may be relevant for LM plants with modified tolerance to certain
1343 environmental conditions.

1344 The development of LM plants with tolerance to abiotic stress(es) may allow for an expansion of
1345 arable lands and cultivation areas of these plants in natural environments. The increase in the
1346 area of land for agriculture and consequences to biodiversity should be assessed.

1347 The cultivation of LM plants with tolerance to abiotic stress may lead to changes at the
1348 ecosystem-level, for example by allowing certain pests associated with the LM plant species to
1349 breed in ecosystems where they were not previously present.

1350 *Elements for consideration:*

1351 (a) Changes in the geography, and extension of arable lands;

1352 (b) Agricultural practices related to the LM plant and how these may change the abiotic
1353 environment and ecosystem;

1354 (c) Modelling tools, if available, to predict how the changes in agricultural practices due to
1355 the LM plant may affect the abiotic environment.

1356 **BIBLIOGRAPHIC REFERENCES**

1357 See references relevant to “*Risk Assessment of LM plants with Tolerance to Abiotic Stress*”:
1358 http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

1359

1360 **C. RISK ASSESSMENT OF LIVING MODIFIED TREES**

1361 **BACKGROUND**

1362 During its eighth and ninth meetings, the Conference of the Parties to the CBD recognized “the
1363 uncertainties related to the potential environmental and socio-economic impacts, including long-
1364 term and transboundary impacts, of genetically modified trees on global forest biological
1365 diversity”, recommended “Parties to take a precautionary approach when addressing the issue of
1366 genetically modified trees”, and urged Parties to undertake a number of actions with regard to
1367 LM trees, such as “to develop risk-assessment criteria specifically for genetically modified
1368 trees”.²⁰ Moreover, forest biodiversity is one of the seven thematic programmes of work under
1369 the Convention on Biological Diversity (CBD).

1370 According to the Food and Agriculture Organisation of the United Nations (FAO), a tree is: “a
1371 woody perennial with a single main stem, or, in the case of coppice, with several stems, having a
1372 more or less definite crown”.²¹ This guidance focuses on forest and plantation trees. Some
1373 considerations contained here may also be applicable to risk assessment of orchard trees. This
1374 section does not cover any additional species such as palms, bamboos and shrubs.

1375 **INTRODUCTION**²²

1376 Tree species belong to many different taxonomic orders and families of angiosperms (flowering
1377 plants; e.g., mahogany, poplar, apple) and gymnosperms (“naked seed” plants; e.g., pine, spruce,
1378 cedar). Trees differ from other plants, such as annual crops, due to characteristics such as size,
1379 perennial growth habit with a long lifespan, and delayed onset of reproductive maturity.

1380 High fecundity together with seed dormancy, many pathways for dispersal of propagules, and
1381 high seed viability are important aspects of the reproductive capacity of many, although not all,
1382 tree species. Moreover, the potential for vegetative propagation in certain trees raises the
1383 possibility that new individuals can be established from propagules, such as branches or roots.

²⁰ 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>).

²¹ “Training manual on inventory of trees outside forests (TOF)” available at <ftp://ftp.fao.org/docrep/fao/006/AC840E/AC840E.pdf>.

²² 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.

1384 Because of their perennial growth and, in many cases, long lifespan and large size, trees develop
1385 complex, direct, indirect and multi-level ecological interactions with other organisms ranging
1386 from decomposers to birds and from insect pollinators to large wild animals. Those interactions
1387 may span over several generations of the other species if they have shorter lifespans. Moreover,
1388 the root systems of trees can be extensive and are often associated with microorganisms and
1389 fungi, such as mycorrhizae (symbiotic associations).

1390 Regarding reproductive maturity and breeding systems, many tree species undergo a distinct
1391 juvenile phase which may last from several years to more than a decade before the onset of
1392 reproductive maturity. As a result, some tree species have gone through only a limited number of
1393 breeding cycles by the time they are planted for commercial purposes. Additionally, some tree
1394 species are dioecious (i.e., plants that are either male or female) and cannot undergo selfing (i.e.,
1395 common practice for increasing homogeneity of many crops), leading to the increased use of
1396 methods for vegetative propagation to ensure uniformity of the propagated trees for plantation
1397 use. By using cuttings from some tree species, in particular some fruit trees, a desirable selected
1398 genotype may be grafted onto a rootstock of a different genotype. For many forest and fruit tree
1399 species, clonal multiplication of identical individuals can be achieved through regeneration of
1400 entire trees from vegetative propagules such as cuttings or somatic embryos.

1401 Tree species and genotypes are highly diverse and exhibit a wide range of distribution and
1402 complex associations with other organisms, as well as significant ecological, economic,
1403 environmental, climatic and socio-economic values. Fruit, ornamental, and forest tree species of
1404 economic interest grow in various regions of the world from temperate to tropical climates.
1405 Thirty one per cent of the total global land area or more than 4 billion ha, is covered by forests.
1406 Minimally managed forest habitats and non-managed forests like tropical rainforests or boreal
1407 forests are of high conservation value. Accordingly, many countries regard trees as important
1408 components of biodiversity and have protection goals to ensure their conservation. Such
1409 protection goals should be taken into account when assessing the possible adverse effects of LM
1410 trees and emphasis should be given to the precautionary approach.

1411 A number of LM trees have been developed through the use of modern biotechnology and
1412 introduced into the environment.²³ The majority of these LM trees are species of economic

²³ See the LMO registry in the BCH (<http://bch.cbd.int/database/organisms/>) and background documents for this section.

1413 interest used in managed orchards, forests and plantations. The modified traits include herbicide
1414 tolerance, wood composition (e.g., lignin), growth rate and phenology (including flowering and
1415 fruiting), resistance to pests and diseases, and abiotic stress tolerance.

1416 **PLANNING PHASE OF THE RISK ASSESSMENT**

1417 **The choice of comparators** (see “*Planning Phase of the Risk Assessment*”, “*The choice of*
1418 *comparators*” in the Roadmap)

1419 *Rationale:*

1420 As with the risk assessments of any other type of LMO, a comprehensive planning phase is
1421 needed to define, among other things, how a comparative approach can be carried out in the risk
1422 assessment of an LM tree.

1423 In instances where LM tree species have a long lifespan and high potential for dispersal,
1424 outcrossing and establishment beyond the intended receiving environment (e.g., into natural or
1425 less managed ecosystems) should be taken into account.

1426 In forestry, the use of well adapted provenances (i.e., trees that have evolved or been bred within
1427 the region where they will be grown commercially)²⁴ is of great importance because they may
1428 show better adaptive capabilities and consequently better performance than unselected
1429 germplasm.²⁵ These regional provenances, whether naturally occurring, domesticated or
1430 introduced but locally bred and adapted, may provide appropriate comparators for LM trees in
1431 accordance with national protection goals and good forest management practices.

1432 For those LM tree species for which there is little or no information with regard to their
1433 ecological functions and interactions in the likely potential receiving environment, the
1434 comparative approach may be challenging. In such cases, the assessment of the overall risk of
1435 the LM tree may involve a high degree of uncertainty which must be described in the
1436 conclusions of the risk assessment and communicated to decision makers.

²⁴ A comparable concept for crop plants would be regionally adapted crop varieties.

²⁵ 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”.

1437 *Elements for consideration:*

- 1438 (a) Availability of information and knowledge of the biology and ecological interactions of
1439 the species and/or genotype (including regional provenances or ecotypes as appropriate)
1440 that can be used as a comparator;
- 1441 (b) Whether one or more suitable comparators are available and the possibility of their use
1442 in the appropriate experimental design;
- 1443 (c) Design of field trials in relation to established methodologies for the non-modified trees,
1444 including for example the length of the period before flowering, the length/age of trials,
1445 testing in different environments and exposure to multiple biotic and abiotic stresses.

1446 **CONDUCTING THE RISK ASSESSMENT**

1447 The information provided in this section aims at covering different tree species and management
1448 practices and may be taken into account on a case-by-case basis.

1449 **Presence of genetic elements and propagation methods** (*see “Step 1”, “Point to consider (b)”*
1450 *in the Roadmap*)

1451 *Rationale:*

1452 The transformation method used may lead to the presence of modified genetic elements in an
1453 LM tree that could be linked to potential adverse effects (e.g., some antibiotic resistance genes).
1454 The cross-breeding process (including back-crossing) is an option to reduce the presence of such
1455 genetic elements.

1456 Many tree species have a long juvenile period and, for the purposes of forestry and plantations,
1457 their multiplication is typically achieved through clonal and vegetative propagation. In such
1458 cases, the removal of undesirable genetic elements in LM trees through cross-breeding would not
1459 be feasible.

1460

1461 *Elements for consideration:*

1462 (a) Transformation methods used which may possibly lead to the presence of genetic
1463 elements that may have an adverse effect;

1464 (b) Propagation method(s) used – cross-breeding (including the degree of back-crossing, if
1465 possible, in that species) and/or vegetative propagation.

1466 **Long lifespan, genetic and phenotypic characterisation and stability of the modified genetic**
1467 **elements** (see “Step 1”, “Point to consider (d) and (e)” in the Roadmap)

1468 *Rationale:*

1469 In unmanaged ecosystems, the lifespan of some trees can range from several decades to several
1470 centuries or longer. Such trees can tolerate and adapt to the different biotic and abiotic conditions
1471 they encounter during their lives. The phenotypic characterization of an LM tree should consider
1472 its developmental stage and a range of environmental conditions. To the extent possible, it may
1473 also be important to consider whether and how management practices, that could affect the
1474 characterization of the LM tree, would change over time.

1475 Taking into account the long lifespan of some trees, transgene instability, including those
1476 causing gene silencing and variable expression levels, should be considered in the context of its
1477 possible relevance for risk assessment. Similarly, genetic/environmental interactions, that may
1478 play a role in the expression level of the transgenes, should be duly considered. Consequently, an
1479 assessment of the stability of the transgenes and their levels of expression at different points
1480 during the lifespan of the LM tree may be important considerations, in particular where
1481 transgenic approaches are used for containment strategies (e.g., male sterility or ablation of floral
1482 organs).

1483 Due to the large size and long lifespan of many tree species, data obtained from glasshouse
1484 experiments may be limited with regard to, for example, the number of generations and
1485 experimental replications that can be observed. This may present a challenge when the risk
1486 assessment of an LM tree calls for data to reflect the changing characteristics of the LM tree and
1487 the likely potential receiving environment over time. The risk assessment of LM trees may
1488 benefit from a broader approach using mathematical modelling.

Comment [A13]: Outstanding: provide examples.

1489 *Elements for consideration:*

- 1490 (a) Changes in the interactions with other organisms, and changes in the ability to maintain
1491 role and function in ecosystems;
- 1492 (b) Phenotypic changes over time in response to different stressors and different
1493 developmental stages;
- 1494 (c) Potential for variability in transgene expression levels, including gene silencing over
1495 time;
- 1496 (d) Availability of data from glasshouse experimentation (including exposure to biotic and
1497 abiotic stresses).

1498 **Dispersal mechanisms** (see “Step 1”, and “Step 2”, “Elements for consideration (d), (e) and
1499 (h)” in the Roadmap)

1500 *Rationale:*

1501 Forest trees, like other plants, have developed a variety of ways to reproduce and disseminate via
1502 seeds, pollen and/or vegetative propagules. Trees often produce large amounts of pollen and seed
1503 per individual and propagules may be designed to spread over long distances (e.g., by wind,
1504 water, or animals including insects). The potential for vegetative propagation in certain trees
1505 raises the possibility of establishing new individuals from branches or root parts.

1506 Seeds inside fruits may travel as commodities around the globe and be released at the place of
1507 consumption such as road margins, railways or touristic areas, as well as in farmers’ fields and
1508 local gardens.

1509 Many trees are capable of vegetative propagation which increases the exposure of the
1510 environment, both in terms of time and space, particularly in the case of large trees with a long
1511 lifespan. Therefore, the potential for and means of vegetative propagation are relevant
1512 considerations during the risk assessment of LM trees.

1513

1514 *Elements for consideration:*

- 1515 (a) Available information on the dispersal mechanisms and viability of pollen and seed for
1516 the non-modified and LM tree species;
- 1517 (b) Potential for and mechanisms of vegetative propagation in the non-modified and LM
1518 tree species;
- 1519 (c) Climatic conditions, or management practices that affect reproductive biology;
- 1520 (d) Potential for dispersal mechanisms from anthropogenic activities (e.g., trade and
1521 consumption of fruits);
- 1522 (e) Expansion of the distribution area of an LM tree due to dispersal mechanisms
1523 throughout its lifespan.

1524 **The likely potential receiving environment(s)** (see “Step 1”, “Elements for consideration (f)
1525 and (g)”, “Step 2”, “Elements for consideration (b), (d), (f) and (h)”, “Step 3”, “Elements for
1526 consideration (a) and (e)” in the Roadmap)

1527 *Rationale:*

1528 The identification and characterisation of likely potential receiving environment(s) may be
1529 dependent on the LM tree in question, its habitats, the traits and modified characteristics and its
1530 mechanisms for dispersal. With some trees the intensity of management in the likely potential
1531 receiving environment may be less than for some annual plants. The domestication level of some
1532 forest trees may be low and trees can often survive without human intervention. Therefore, the
1533 potential for dispersal of propagative material into environments other than the intended
1534 receiving environment is an important consideration during the risk assessment.

1535 Many tree species (e.g., poplars and eucalyptus) can propagate through vegetative means. When
1536 characterizing the likely potential receiving environment during the risk assessment of such an
1537 LM tree, the movement of seeds as well as the movement of vegetative propagules should be
1538 taken into account. Issues related to unintentional transboundary movements may also be taken
1539 into account in cases where LM trees could cross national boundaries through, for example,

1540 pollen or seed dispersal by physical and biological vectors, including the international trade of
1541 fruits with seeds.

1542 *Elements for consideration:*

1543 (a) Environments and their degree of management which offer the potential for seeds
1544 and/or vegetative propagules to establish;

1545 (b) Presence and proximity of species in the receiving environment with which the LM tree
1546 may hybridize;

1547 (c) Proximity of protected areas, centres of origin and genetic diversity or ecologically
1548 sensitive regions;

1549 (d) Ecosystem functions and services of the potential receiving environment (e.g., relevant
1550 components of food webs);

1551 (e) Change in landscape patterns and sensitivity of the receiving environment to human
1552 activities.

1553 **Exposure of the ecosystem to living modified trees and potential consequences** (*see “Step 2”*
1554 *and “Step 3” in the Roadmap*)

1555 *Rationale:*

1556 Some trees remain relatively undisturbed for much of their life cycle and may engage in a variety
1557 of ecological interactions, such as providing habitat for other organisms and functioning as part
1558 of complex and elaborate food webs. In determining the likelihood of an adverse effect of an LM
1559 tree, an assessment of the exposure to the LM tree should take into account the expected duration
1560 of the trees’ presence in the receiving environment, the nature of the transgenic traits, the
1561 intended use of the LM tree (e.g., processing, trade routes), as well as dispersal mechanisms.
1562 Given the late onset of reproductive maturity of a number of tree species, pollen and seed
1563 production may not occur during field trials.

1564 The expansion of tree cultivation areas for bioenergy may also increase the diversity of
1565 environments exposed to LM trees including those modified to mitigate potential invasiveness.

1566 *Elements for consideration:*

- 1567 (a) Duration of the presence of the LM trees in the likely potential receiving environment;
- 1568 (b) Persistence and potential long-term adverse effects of the LM trees in the environment
1569 including potential for the non-modified recipient organism to be invasive;
- 1570 (c) Consequences of the modified trait on invasive characteristics;
- 1571 (d) Long-term interactions that could lead to adverse effects to other organisms including
1572 via food web interactions;
- 1573 (e) Consequences on ecosystem functions and biodiversity arising from the changes in land
1574 use for the cultivation of LM trees.

1575 **Risk management strategies** (see “Step 4”, “Point to consider (e)” and “Step 5” in the
1576 *Roadmap*)

1577 *Rationale:*

1578 The need for risk management strategies designed for LM trees will depend on the results of risk
1579 assessment, and may vary depending on the LM tree and the conditions under which it is grown.
1580 When the recommendations of the risk assessment include measures for limiting or preventing
1581 dispersal of forest or plantation LM trees, strategies that may be used include delaying or
1582 preventing flowering (e.g., fast-growing trees for pulp or biomass/bioenergy production being
1583 cut before reaching the reproductive phase) and biological confinement (e.g., induction of male
1584 sterility or flower ablation). While complete flower ablation is not desirable for many fruit or
1585 horticultural tree species, male sterility may be appropriate in some species (e.g., apples) where
1586 pollen from a different variety (which could be non-modified) is usually required. However,
1587 male sterility approaches will not prevent the production of seeds by LM trees fertilized by
1588 fertile trees. Where applications involve genetic modification of only the rootstock in grafted
1589 trees, dispersal may be managed by ensuring that the rootstocks do not produce shoots or
1590 flowers.

1591 *Elements for consideration:*

- 1592 (a) Type and intended use of the LM tree;

- 1593 (b) Degree and type of management (e.g., grafting of fruit trees, rotation period of forest
1594 trees);
- 1595 (c) Specific effects and risks of any containment strategy achieved through the use of
1596 modern biotechnology.

1597 **BIBLIOGRAPHIC REFERENCES**

1598 See references relevant to “*Risk Assessment of LM Trees*”:

1599 http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

1600

1601

1602 **D. RISK ASSESSMENT OF LIVING MODIFIED MOSQUITOES**

1603 **INTRODUCTION**

1604 Living modified (LM) mosquitoes are being developed through modern biotechnology to reduce
1605 transmission of vector-borne human pathogens, particularly those that cause malaria, dengue and
1606 chikungunya. Control and reduction of such diseases is a recognized public health goal. The
1607 impacts of such diseases on human health are staggering. For instance, in 2008, there were 247
1608 million cases of malaria and nearly one million deaths.²⁶ Therefore, specific and comprehensive
1609 considerations should be undertaken with regard to the potential benefits and adverse effects of
1610 LM mosquitoes.

1611 The biology and ecology of mosquitoes, on the one hand, and their impact on public health as
1612 vectors of human and animal diseases, on the other hand, pose specific considerations and
1613 challenges during the risk assessment process.

1614 Two strategies of modern biotechnology, namely self-limiting and self-propagating strategies,
1615 are being developed to produce LM mosquitoes to control vector-borne diseases.

1616 Self-limiting strategies are being developed to control mosquito vectors by suppressing their
1617 population or reducing their competence by developing LM mosquitoes that are unable to
1618 produce viable offspring. This can be achieved, for instance, by interrupting larval development
1619 of the offspring. As such, LM mosquitoes developed under self-limiting strategies are not
1620 expected to pass the modified trait to subsequent generations. Modern biotechnology techniques
1621 for the development of self-limiting LM mosquitoes populations (e.g., “Release of Insects
1622 carrying a Dominant Lethal” or RIDL) are different from those based on the use of irradiation to
1623 induce male sterility because they aim to produce populations that are *behaviourally sterile*.
1624 Other self-limiting strategies target metabolic processes of the mosquito vectors and aim at
1625 lowering their fitness and thereby reducing their populations.

1626 Self-propagating strategies, also known as self-sustaining strategies, rely on *gene-drive systems*
1627 that promote the spread and persistence of the transgene through populations of the same
1628 mosquito species. As opposed to the self-limiting strategy, the modifications in LM mosquitoes

²⁶ WHO (2010) Malaria fact sheet. Available at <http://www.who.int/mediacentre/factsheets/fs094/en/>.

1629 produced through self-propagating strategies are intended to be heritable and to spread through
1630 the target population and, thus, to persist in the ecosystem at least for the medium term. Hence,
1631 the objective of self-propagating strategies is the replacement of the non-modified mosquito
1632 population by the LM mosquitoes that have been modified to render them less capable of
1633 transmitting a disease. In a related approach, gene-drive systems may be used to promote the
1634 spread of a gene that confers a fitness load or a male bias in the offspring ratio. In this way, gene-
1635 drive systems may be used to suppress vector population sizes or induce a cascade of population
1636 crashes. An example of such a system is an X-shredding homing endonuclease gene (HEG)
1637 which can be driven into a population at the same time as biasing the offspring ratio towards
1638 males and hence potentially inducing an all-male population crash.

1639 Another strategy, the so-called paratransgenesis, is under development to control, reduce or
1640 eliminate the capacity of vectors to transmit pathogens mainly, but not exclusively, by blocking
1641 the development of the pathogen in the vector. Paratransgenesis focuses on utilizing symbionts
1642 of insects to express molecules, within a vector, that are deleterious to the pathogens transmitted
1643 by the vector. In the case of paratransgenesis for the control of diseases transmitted by
1644 mosquitoes, the mosquito itself will not be genetically modified, but the microorganism that
1645 inhabits the mosquito (e.g. in its mid-gut) will be the product of modern biotechnology. Such
1646 microorganisms may have a specific, symbiotic relationship with the mosquito, or may be
1647 commonly associated with the mosquito but not have an obligate relationship. Paratransgenesis
1648 can be used as a self-limiting strategy for population suppression or as a limited self-propagating
1649 strategy for population replacement (see above).

1650 The mosquitoes developed through the different strategies will differ, for example, in their
1651 ability to persist in the environment and to spread the inserted transgenes into the local mosquito
1652 population, or even into other organisms. Therefore, the risk assessment requirements and
1653 criteria will depend on the specific characteristics of the LM mosquito and the strategy used.

1654 Since this guidance is not focused on one particular type of technology or genetic mechanism,
1655 additional and more specific guidance may be necessary when conducting the risk assessment of
1656 a particular LM mosquito depending, among other things, on the strategy used. The risk
1657 assessment of LM mosquitoes performed on a case-by-case basis may also benefit from a
1658 broader approach using laboratory and confined field tests together with mathematical
1659 modelling.

1660 **OBJECTIVE AND SCOPE**

1661 The objective of this section is to give additional guidance on the risk assessment of LM
1662 mosquitoes in accordance with Annex III to the Cartagena Protocol on Biosafety. Accordingly, it
1663 complements the Roadmap for Risk Assessment of LMOs, giving emphasis to specific issues
1664 that may need special consideration for the environmental release of LM mosquitoes.

1665 This section focuses on the risk assessment of LM mosquitoes of the family *Culicidae*,
1666 developed through self-limiting and self-propagating strategies to be used in the control of
1667 human and zoonotic diseases such as malaria, dengue, chikungunya, yellow fever and West Nile.

1668 This section does not consider the potential adverse effects of LM microorganisms released into
1669 the environment. Thus, paratransgenesis is not in the scope of this guidance.

1670 **PLANNING PHASE OF THE RISK ASSESSMENT**

1671 In addition to the considerations raised in the Roadmap, the risk assessment of LM mosquitoes
1672 focuses on ecological and epidemiological processes that may be adversely affected by the
1673 introduction of the LM mosquito, taking into account the species of the mosquito, the LM trait,
1674 the intended and unintended receiving environment, and the objective and scale of the intended
1675 release. The biology and, to some extent, the ecology of the mosquito species that transmit
1676 malaria and dengue are rather well known in many regions of the world. However, in certain
1677 regions and in the environment where LM mosquitoes are likely to be introduced, more
1678 information may be needed depending on the nature and scale of the LM strategy to be deployed.
1679 In many of these environments few studies have been conducted to examine gene flow among
1680 disease-transmitting vectors, their mating behaviour, the interactions among vectors sharing one
1681 habitat, how pathogens respond to the introduction of new vectors, etc. Such information may be
1682 needed to establish a baseline in order to assess the risks of LM mosquitoes. Additionally,
1683 methods for the identification of specific ecological or environmental hazards are also needed.

1684 Identification of the likely potential receiving environment of an LM mosquito will depend on
1685 several factors, including whether specific release sites have been planned and whether natural or
1686 artificial barriers are present that could limit the dispersal of the LM mosquito. In some cases,
1687 risk assessors may need to consider the entire national territory or even neighbouring countries as

1688 the likely potential receiving environment (see also “Unintentional Transboundary Movement”
1689 below).

1690 **The choice of comparators** (see “*Planning Phase of the Risk Assessment*”, “*The choice of*
1691 *comparators*” in the Roadmap)

1692 *Rationale:*

1693 The line/strain used as a recipient organism for transformation may serve as a comparator for the
1694 risk assessment of LM mosquitoes. The approach of using a (near-)isogenic line may be a
1695 challenge. Where successive passages are used to develop a strain of the LM mosquito, the
1696 parental LM strain may be used as an additional comparator.

1697 **CONDUCTING THE RISK ASSESSMENT**

1698 **Characterization of the living modified mosquito** (See “*Step 1*” in the Roadmap)

1699 *Rationale:*

1700 Description of the mosquito species should include its sub-species and strains, including their
1701 bio-geographical distribution, ecological niche, and capacity to transmit the pathogen, and may
1702 include the use of reliable molecular markers.

1703 *Elements for consideration:*

1704 (a) Description of the genetic modification, and the molecular characterization associated
1705 with the relevant technologies with particular attention to sequences which might
1706 influence the mobility of the insert in the mosquito (such as transposable elements);

1707 (b) *Stability of the transgene* and the likelihood of mutations in the transgene(s) and
1708 changes in the insertion site(s) (in the case of mobile DNAs) in response to selection in
1709 the receiving environment.

1710

1711 **Unintended effects on biological diversity (species, habitats, ecosystems, and ecosystem**
1712 **function and services)** (See “Step 2” and “Step 3” in the Roadmap)

1713 *Rationale:*

1714 The role of mosquitoes in natural ecosystems should be assessed, as the release of LM
1715 mosquitoes may have unintended effects on the target vector and pathogen²⁷ and other non-target
1716 species which may lead to adverse effects. Potential unintended effects will vary from case to
1717 case and may include:

1718 • *New or more vigorous pests, especially those that have adverse effects on human health:*

1719 The released LM mosquitoes may not function as expected, for example due to gene
1720 silencing or undetected failures in the development of self-limiting LM mosquitoes, which
1721 could result in the release of sexually competent mosquitoes and thus increase the vector
1722 population or disease transmission.

1723 Mosquito species are currently able to transmit several pathogens, such as viruses and filaria,
1724 to human beings and animals. An LM mosquito, in which the capacity of transmission of one
1725 of these pathogens has been modified, may enhance the transmission of other pathogens.

1726 Suppression of the target mosquito population might cause the population of another vector
1727 species to increase, resulting in higher levels of the target disease or the development of a
1728 new disease in humans and/or animals. These other vector species may include other
1729 mosquito vectors of other diseases.

1730 The released LM mosquito may become a more vigorous pest by, for example, becoming a
1731 host to a broader range of pathogens.

1732 The released LM mosquitoes may cause other pests to become more serious, including
1733 agricultural pests and other pests that affect human activities. For example, the replacement
1734 of *Aedes aegypti* by *Aedes albopictus* could occur as the result of a release. Such risks should
1735 be monitored through time and at the appropriate geographical scale.

1736

²⁷ 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.

1737 • *Harm to or loss of other species:*

1738 The released LM mosquitoes might cause other species (for instance, birds, bats or fish that
1739 rely seasonally on mosquitoes for food) to become less abundant. These include species of
1740 ecological, economic, cultural and/or social importance such as wild food, endangered,
1741 keystone, iconic and other relevant wildlife species. Ecological effects might result from
1742 competitive release if the target mosquito population is reduced, or from trophic
1743 consequences of species that rely on mosquitoes for food at specific times of the year. Effects
1744 may also occur if (i) the target mosquitoes transmit a disease to animal species, (ii) the
1745 released LM mosquitoes transmit a disease to animal species more efficiently, (iii) another
1746 vector of an animal disease was released from control when the target mosquito population
1747 was reduced, or (iv) the target pathogen's abundance is reduced or eliminated, leading to
1748 effects on other organisms that interact with it, for example, by changing the population of
1749 another animal that hosts the pathogen.

1750 Mosquitoes, like other insects, typically have strong reproductive isolating mechanisms that
1751 will not allow interspecific gene flow. However, if interspecific mating between released LM
1752 mosquitoes and other mosquito species occurs, it could disrupt the population dynamics of
1753 these other species. Moreover, cessation of transmission of pathogens to other animals (e.g.,
1754 West Nile virus to birds, Rift Valley fever virus to African mammals) might change the
1755 population dynamics of those species, favouring increases in their numbers.

1756 • *Disruption of ecological communities and ecosystem processes:*

1757 The ecological communities in the ephemeral, small aquatic habitats occupied by the non-
1758 LM mosquitoes are unlikely to be disrupted beyond the possibilities already addressed above
1759 under "harm to or loss of other species." However, if the released LM mosquitoes were to
1760 inhabit natural habitats (e.g., tree-holes), disruption of the associated community is a
1761 possibility.

1762 The introduction of LM mosquitoes may have adverse effects on valued ecosystem
1763 processes, often referred to as "ecosystem services", such as pollination, or on processes that
1764 support normal ecosystem functioning. The adult male and female mosquitoes feed on nectar
1765 of flowers and participate in the pollination of plants in a similar way as butterflies,
1766 Hymenoptera and other Diptera. In cases where mosquito species are significant pollinators,

1767 mosquito control of any kind may reduce the rate of pollination of some plant species or
1768 cause a shift to different kinds of pollinators.

1769 Moreover, mosquitoes, both adults and larvae, are a food source for many predators (e.g.,
1770 insects, lizards and birds), and are responsible for the transfer of large amounts of biomass
1771 from aquatic to terrestrial ecosystems. As such, habitats in which mosquitoes are the
1772 dominant insect fauna (e.g., high Arctic tundra) could be affected if mosquitoes were
1773 eliminated. However, common target vector species are usually associated with human
1774 activity and therefore not as closely tied to ecosystem services.

1775 *Elements for consideration:*

- 1776 (a) The natural dispersal range and seasonality of the host mosquito in relation to the likely
1777 potential receiving environment where the LM mosquito may be released;
- 1778 (b) Effects on the target mosquitoes and pathogens resulting from the management and use
1779 of the strategy under consideration;
- 1780 (c) Whether the LM mosquitoes have the potential to cause adverse effects on other species
1781 which may result in the other species becoming agricultural, aquacultural, public health
1782 or environmental pests, or becoming a nuisance or a health hazard;
- 1783 (d) The effect of the transgene on the fitness of the LM mosquito in the receiving
1784 environment, including the areas to which the LM mosquito may spread, in particular if
1785 a self-sustaining technology is implemented;
- 1786 (e) Whether the target mosquito species is native or exotic to a given area;
- 1787 (f) The normal and potential habitat range of the target mosquito species and whether the
1788 habitat range is likely to be affected by climate change;
- 1789 (g) Whether the LM mosquitoes would be more susceptible to infection by other vector-
1790 borne disease pathogens;
- 1791 (h) Whether the mosquito is a member of a species complex in which inter-specific mating
1792 occurs;

- 1793 (i) Whether the introduction of LM mosquitoes is likely to affect other mosquito species
1794 that are pollinators or otherwise known to be beneficial to ecosystem processes;
- 1795 (j) The consequences of likely mutations resulting from the mosquito's interactions with
1796 other organisms in the environment, and any potential changes in its response to abiotic
1797 stresses;
- 1798 (k) Whether the LM mosquitoes are likely to affect other organisms with which they
1799 interact (e.g., predators of mosquitoes), and whether that could lead to an adverse effect
1800 (e.g., on the food chain);
- 1801 (l) Whether, in the absence of the target mosquito, niche displacement by other disease
1802 vector species may occur, and if so, whether that can result in an increased incidence of
1803 the target disease or other diseases in humans or animals;
- 1804 (m) Whether the LM mosquito has potential for natural long-distance transboundary
1805 dispersal or transport by anthropogenic mechanisms (e.g., used tires, aircraft, ships);
- 1806 (n) Whether changes in land management in the receiving environment (e.g., wetland
1807 drainage, irrigation practices) would occur as a result of the introduction of LM
1808 mosquitoes, and what consequences these changes could have on biodiversity.

1809 **Vertical gene transfer** (*See "Step 2" and "Step 3" in the Roadmap*)

1810 *Rationale:*

1811 For self-propagating LM mosquitoes, gene-drive systems for moving genes into wild populations
1812 may be the initial focus when assessing the likelihood of vertical gene transfer from LM
1813 mosquitoes to non-LM mosquitoes through cross-fertilization. The likelihood of vertical gene
1814 transfer in self-limiting LM mosquitoes is likely to be lower than for self-propagating LM
1815 mosquitoes, but should be assessed on a case-by-case basis (see below). Various factors may
1816 influence gene flow and any associated adverse effects, such as the strategy used in the
1817 development of the LM mosquito, characteristics of the transgenes, characteristics of the gene-
1818 drive system, the stability of the trait(s) carried by the mosquito over generations, and
1819 characteristics of the receiving environment.

1820 Some LM mosquitoes are being developed to spread the introduced trait rapidly through the
1821 target mosquito population. For instance, when introduced into *Anopheles gambiae*, the trait may
1822 be expected to spread throughout the *A. gambiae* species complex. Other LM mosquito
1823 technologies are designed to be self-limiting and, in such cases, spread of the transgenes or
1824 genetic elements in the target mosquito population is not intended or expected. For the self-
1825 limiting technologies, the potential for an unexpected spread of the introduced trait should be
1826 considered by focusing on the assumption that any management strategy to limit the spread could
1827 fail. The likelihood and consequences of this hazard can be evaluated by assessing the fitness of
1828 the LM mosquito with the transgene should the self-limiting mechanism fail to prevent spread of
1829 the transgene. .

1830 Gene flow between different species may be considered for all of the LM mosquito technologies
1831 in spite of the fact that mosquitoes, like other insects, typically have strong reproductive isolating
1832 mechanisms that will not allow interspecific gene flow. Identifying the key reproductive
1833 isolating mechanisms and possible conditions that could lead to the breakdown of such
1834 mechanisms is of particular importance in the risk assessment of LM mosquitoes with this trait.
1835 In addition, the fitness (dis)advantage conferred by the introduced trait to the LM mosquito and
1836 frequency of the introduction of the LM mosquito into the environment will affect its population
1837 size as well as the likelihood and rate of spread of the transgenes or genetic elements.

1838 For self-sustaining strategies, the initial numbers of LM mosquitoes released may be small,
1839 however their persistence in the environment will provide continuing opportunities for novel
1840 interactions and mutations that may not be detected in limited trials. Although sexual sterility
1841 (cytoplasmic incompatibility) may prevent the transfer of the microorganism to some species, the
1842 risks due to rare exceptions to the normal mating pattern should be considered.

1843 *Elements for consideration:*

- 1844 (a) Whether LM mosquitoes have the potential to transfer the modified traits to wild
1845 mosquito populations (when it is not an intended strategy), and if so, the occurrence of
1846 any potential undesirable consequences;

1847 (b) Whether LM mosquitoes have the potential to induce undesirable characteristics,
1848 functions or behaviour within the target mosquito species or a sexually compatible
1849 species complex.

1850 **Horizontal gene transfer**

1851 *Rationale:*

1852 LM mosquitoes may be associated with symbionts and/or parasites such as microorganisms. In
1853 particular, potential adverse effects as a result of the interaction between LM mosquitoes and
1854 *Wolbachia* could warrant attention because mosquitoes are currently infested by these bacteria.
1855 Empirical evidence suggests that horizontal gene transfer between mosquitoes and *Wolbachia*
1856 may occur. Since *Wolbachia* seems to reduce host fitness and to hamper virus transmission, such
1857 as for the Dengue viruses, potential adverse effects to the *Wolbachia* could change the capacity
1858 of the mosquitoes to transmit diseases.

1859 *Elements for consideration:*

- 1860 (a) Presence of symbionts and parasites in the LM mosquitoes and whether there may be
1861 exchange of genetic information between the host and the microorganism;
- 1862 (b) Whether LM mosquitoes have the potential to induce undesirable characteristics,
1863 functions, or behaviour in other organisms, particularly in bacteria living in symbiosis;
- 1864 (c) Nucleic acid sequences in the LM mosquito which might influence the mobility of the
1865 insert and transgenes (such as mobile elements) through recombination with genes in
1866 the microorganisms.

1867 **Persistence of the transgene in the ecosystem** (See “Step 2”, “Point to consider (f)” and “Step
1868 3”, “Point to consider (a)(iii)” and “Point to consider (b)” in the Roadmap)

1869 *Rationale:*

1870 Some of the transgenes in LM mosquitoes are designed not to persist in a population whereas
1871 others are expected to spread rapidly and/or persist in wild populations. In cases where LM
1872 mosquitoes have been found through the risk assessment process to have the potential to cause

1873 adverse effects to biological diversity, taking into account human health, methods to reduce the
1874 persistence of the transgene in the ecosystem need to be considered.

1875 *Point to consider:*

1876 (a) Any undesirable consequence should the transgene persist in the ecosystem;

1877 (b) Methods to reduce the persistence of the transgene.

1878 **Evolutionary responses (especially in target mosquito vectors or pathogens of humans and**
1879 **animals)** (See “Step 1” in the Roadmap)

1880 *Rationale:*

1881 Any strong ecological effect also exerts an evolutionary selection pressure on the human and
1882 animal pathogens and the mosquito vectors. The main evolutionary effects of concern are those
1883 that could result in a breakdown in the effectiveness of the technology and the resumption of
1884 previous disease levels. Some LM mosquito strategies aim at modifying the mosquito vector’s
1885 ability to transmit diseases by altering its physiological mechanisms. An evolutionary effect
1886 resulting in the development of resistance to modified physiological mechanisms in the targeted
1887 pathogen might occur when modifying mosquito vector competence. This might harm the
1888 effectiveness of the strategy used and result in a population of pathogens that may be transmitted
1889 more easily by additional vectors.

1890 Other evolutionary effects could be hypothesized, including effects resulting from climate
1891 change, but they would first imply the occurrence of some adverse effect on a species,
1892 community or ecosystem.

1893 *Elements for consideration:*

1894 (a) Whether the target mosquito vector has the potential to evolve and avoid population
1895 suppression, regain vector competence or acquire new or enhanced competence against
1896 another disease agent, and if so, the occurrence of any possible undesirable
1897 consequences;

1898 (b) Whether the trait has the potential to evolve and thus lose its effectiveness, or the
1899 pathogen to evolve and overcome the limitation posed by the genetic modification, and
1900 if so, the occurrence of any possible undesirable consequences.

1901 **Unintentional transboundary movements²⁸**

1902 *Rationale:*

1903 Mosquitoes, being LM or not, have very broad geographical distribution. Individual mosquitoes
1904 however within their lifetime have dispersal distances commonly of less than 5 km and for some
1905 urban species, as short as 200 meters. Confinement will therefore be highly dependent upon the
1906 species and the strategy used to develop the LM mosquito. Self-limiting sterile male types of
1907 technologies are expected to be highly confined temporally and spatially. On the other extreme,
1908 confinement of self-propagating LM mosquitoes to a particular receiving environment or to a
1909 country is unlikely and may result in transboundary movement between countries.

1910 The risk of dispersal due to anthropogenic activities, such as transport and trade of potential
1911 sources of breeding sites such as tyres or lucky bamboos should be considered. The
1912 consequences of water management practices, such as irrigation or sewage water treatment, on
1913 the introduced LM mosquito strains should also be taken into account.

1914 In cases where LM mosquitoes are modified with gene-drive systems, confinement may not be
1915 possible even when efforts are made to reduce long-distance dispersal due to anthropogenic
1916 activities.

1917

²⁸ See Article 17 of the Protocol (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-17>).

1918 *Elements for consideration:*

1919 (a) The type of strategy used in the development of the LM mosquito (i.e., self-limiting or
1920 self-propagating with gene-drive systems);

1921 (b) Presence of natural or artificial barriers that could limit the spread and unintentional
1922 transboundary movement of the LM mosquito.

1923 **Risk management strategies** (*See “Step 5” in the Roadmap*)

1924 *Rationale:*

1925 Where there is uncertainty regarding the overall level of risk of the LM mosquito, risk assessors
1926 may consider recommending strategies to monitor the LM mosquitoes to ensure that the
1927 technology is functioning as intended and to identify unintended adverse effects. Strategies for
1928 halting release or recalling the LM mosquitoes, as well as mitigation methods if an unanticipated
1929 effect occurs, should be considered. Careful implementation of the technology including the
1930 planning of mitigation measures (such as an alternative set of control measures should a problem
1931 occur) and the integration of other population control methods should also be taken into account.
1932 In some circumstances methods to reduce the persistence of the transgene in the environment or
1933 to mitigate adverse effects resulting from the expression of the transgene might be needed.
1934 Monitoring during and after the environmental release of the LM mosquitoes to enable prompt
1935 detection of unexpected adverse effects may also be considered.

1936 In the development of LM mosquitoes, male and female mosquitoes are commonly segregated at
1937 the pupal stage, according to the size of pupae. Some self-limiting strategies rely on releasing
1938 male LM mosquitoes only and require that no female LM mosquitoes are released.
1939 Understanding and measuring the reliability and failure rate of this segregation process and
1940 having quality control measures in place will be important in such cases.

1941 *Elements for consideration:*

1942 (a) Availability of monitoring methods to:

1943 (i) Measure the efficacy and effectiveness of LM mosquito technology, including
1944 gene-drive systems and segregation of male LM mosquitoes;

- 1945 (ii) Detect the transgene and other markers that distinguish the LM mosquito from
1946 non-LM mosquitoes in the receiving environment;
- 1947 (iii) Detect the spread of the transgenes into mosquito strains other than the target
1948 strain, for example by using reliable molecular markers to distinguish the strains;
- 1949 (iv) Assess the potential evolutionary long-term effects of the LM mosquito
1950 technology (monitoring for transgene stability and proper function over time);
- 1951 (v) Determine the level to which the identified adverse effects may be realized,
1952 including detection of unexpected and undesirable spread of the transgenic trait
1953 (e.g., monitor for undesirable functions or behaviours within target species and
1954 other wild related species);
- 1955 (b) Availability and feasibility of mechanisms to recall or confine the LM mosquitoes and
1956 transgenes in case they spread unexpectedly (e.g., mass release of wild-type mosquitoes
1957 above a certain threshold, alternative control methods including genetic control);
- 1958 (c) Effectiveness and availability of conventional methods of mosquito control (e.g.,
1959 insecticides, larval site destruction, trapping) to control LM mosquito strains as
1960 compared to the non-modified strain;
- 1961 (d) Availability of methods for managing the dispersal of the LM mosquitoes and ensuring
1962 that they do not establish themselves beyond the intended receiving environment (e.g.,
1963 vegetation-free zones, traps, high threshold gene-drive systems);
- 1964 (e) Availability of methods to manage potential development of resistance (e.g., in the
1965 target vector or pathogen);
- 1966 (f) Whether the release of an LM mosquito would affect pest control activities, such as the
1967 use of personal protection and insecticides that control other vectors.

1968 **Containment of the living modified mosquito**

1969 *Rationale:*

1970 Different strategies for the containment of LM mosquitoes can be applied, including physical,
1971 biological and chemical containment. In cases where there are uncertainties with regard to the
1972 potential adverse effects of a widespread release of LM mosquitoes into the environment, a

1973 release limited to in a particular geographic zone may be desirable. Any containment measures
1974 used as a means of limiting the release of the LM mosquito, either in location or in duration,
1975 must be taken into account in each of the steps of the risk assessment.

1976 *Elements for consideration:*

- 1977 (a) The containment strategy (physical, biological and chemical) and its effectiveness;
- 1978 (b) Success rate of separating sexes or induction of sterility in cases of biological
1979 containment, as appropriate;
- 1980 (c) Potential for spread of the genes responsible for the biological containment.

1981 **RELATED ISSUES**

1982 There are other issues that may be taken into consideration in the decision for environmental
1983 releases of LM mosquitoes which are not covered by Annex III of the Protocol. They encompass,
1984 *inter alia*, the potential social, economic, cultural and health benefits associated with the use of
1985 LM mosquitoes to control wild-type mosquitoes that are vectors of human and animal pathogens
1986 and parasites or, alternatively, the use of chemical pesticides or other means to achieve the same
1987 result. The use of LM mosquitoes will require broader considerations of how target-disease risk
1988 affects human behaviour, veterinary medicine, public health practices and national health
1989 priorities in order to address the risks to human and animal health caused by the exposure to
1990 wild-type mosquitoes that are vectors of pathogens and parasites.

1991 **BIBLIOGRAPHIC REFERENCES**

1992 See references relevant to “*Risk Assessment of LM Mosquitoes*”:
1993 http://bch.cbd.int/onlineconferences/ra_guidance_references.shtml

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

1999 In accordance with the terms of reference for the AHTEG, this document provides guidance on
2000 monitoring of living modified organisms released in the environment,²⁹ and complements the
2001 Roadmap for Risk Assessment of Living Modified Organisms (LMOs).

2002 **INTRODUCTION**

2003 Monitoring of LMOs released into the environment may allow for the identification of changes
2004 that are or that may lead to adverse effects, in a timely manner and as early as possible.
2005 Monitoring may also inform on the need for appropriate response measures such as changes to
2006 risk management strategies, emergency response measures, a new risk assessment, or re-
2007 evaluation of prior decisions.

2008 Paragraph 8(f) of Annex III to the Protocol states that “where there is uncertainty regarding the
2009 level of risk, it may be addressed by requesting further information on the specific issues of
2010 concern or by implementing appropriate risk management strategies and/or monitoring the living
2011 modified organism in the receiving environment”. Article 16 of the Protocol and, in particular,
2012 paragraphs 2 and 4 may also be relevant with respect to the implementation of monitoring. The
2013 Convention on Biological Diversity (CBD) covers monitoring in its article 7, “Identification and
2014 Monitoring”.³⁰

2015 **OBJECTIVE AND SCOPE**

2016 This document aims at offering science-based practical guidance for monitoring adverse effects
2017 of LMOs released into the environment that could affect the conservation and sustainable use of
2018 biological diversity, taking into account risks to human health. In this guidance, monitoring of
2019 LMOs refers to the systematic observation, collection, and analysis of data undertaken based on
2020 the risk assessment and following the release of an LMO into the environment, and in

²⁹ Decision BS-IV/11 of the Conference of the Parties serving as the meeting of the Parties to the Protocol (<http://bch.cbd.int/protocol/decisions/decision.shtml?decisionID=11690>).

³⁰ See CBD article 7(a) to (d) (<http://www.cbd.int/convention/articles/?a=cbd-07>).

2021 accordance with the objective of the Protocol.³¹ This guidance may be applicable to all types of
2022 LMOs, and scales of release into the environment (i.e., small- and large-scale releases).

2023 Although monitoring of potential adverse effects to human health is within the context of the
2024 Cartagena Protocol, it is not the focus of this section of the Guidance, and requires additional
2025 methods or approaches. Literature relevant to monitoring in the context of human health can be
2026 found among the background documents for this section (see below).

2027 This document does not address decisions as to whether or not monitoring should be
2028 implemented, or who bears the responsibility and costs for implementation.

2029 **MONITORING AND ITS PURPOSES**

2030 As established in Article 7 of the CBD, Parties shall, as far as possible and as appropriate,
2031 monitor the components of biological diversity important for its conservation and sustainable
2032 use, and identify processes and categories of activities which have or are likely to have
2033 significant adverse impacts, and monitor their effects through sampling and other techniques.

2034 For the purposes of this document, monitoring is categorized as “case-specific monitoring”, or
2035 “general monitoring”.³²

2036 Case-specific monitoring may be conducted to address uncertainty in the level of risk for effects
2037 anticipated in the risk assessment. The purpose of case-specific monitoring may vary, depending
2038 on the type, duration (e.g., short- or long-term) and scale (e.g., small- and large-scale) of the
2039 release, as well as on uncertainties regarding the level of risk or its management:

2040 • *Monitoring during experimental, short-term and/or small-scale environmental releases*

2041 Monitoring can generate data during experimental, short-term and small-scale releases in
2042 order to provide supporting information (e.g., to test specific risk scenarios) for future risks
2043 assessments that may involve a larger scale of release of the same LMO. When
2044 environmental releases of an LMO are conducted in a step-wise manner, monitoring at

Comment [A14]: Outstanding (editorial): consider if this sentence can be deleted vis-a-vis the outcomes of the revisions to the background materials

³¹ See Article 1 of the Protocol (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-1>).

³² Some experts in the Open-ended Online Forum and AHTEG are of the view that “general monitoring” should not be part of this Guidance.

2045 smaller scales may increase the scientific strength or certainty of risk assessments for
2046 subsequent larger scale releases.

2047 • *Monitoring during long-term and/or large-scale environmental releases*

2048 During long-term and large-scale releases of an LMO (e.g., for commercial purposes),
2049 monitoring may be conducted in order to gather further information to address uncertainties
2050 regarding the level of risk, or to confirm that conclusions of the risk assessment are accurate
2051 once the environmental release has taken place. In some cases, effects may be identifiable
2052 but difficult to estimate or address in the framework of a risk assessment (e.g., these may
2053 include long-term, multi-trophic, or cumulative effects, as well as changes to management
2054 practices and effects on human health). Using broader approaches to monitoring may be
2055 useful in such cases (see considerations on general monitoring below).

2056 • *Monitoring to evaluate the efficacy of specific risk management strategies*

2057 In cases where risk management strategies are implemented along with an environmental
2058 release, monitoring may be used to evaluate the effectiveness of these risk management
2059 strategies.

2060 General monitoring is used in some approaches to account for effects that were not anticipated in
2061 the risk assessment. General monitoring starts with general observations of changes in indicators
2062 and parameters, such as assessment endpoints, which are often defined within national protection
2063 goals or are related to the conservation and sustainable use of biological diversity, taking into
2064 account risks to human health.

2065 General monitoring may utilize existing environmental monitoring networks, including those
2066 that may not focus primarily on biosafety, for the surveillance of broader protection goals and
2067 assessment endpoints that are relevant to identifying adverse effects linked to LMOs. In case
2068 changes that could lead to an adverse effect are detected through general monitoring, possible
2069 causes for the observed changes are examined and, where appropriate, a more specific
2070 hypothesis is developed and tested to establish whether or not a causal relationship exists
2071 between LMO(s) and the adverse effect, and be followed up by case-specific monitoring or
2072 further research.

2073 **DEVELOPMENT OF A MONITORING PLAN**

2074 A monitoring plan is developed when the recommendation of a risk assessment and/or the
2075 national biosafety policy calls for monitoring activities to be carried out in conjunction with the
2076 environmental release of the LMO. In such cases, the competent authority(ies) or the entity
2077 responsible for the risk assessment may outline the requirements of a monitoring plan (including
2078 the reporting of monitoring data). The monitoring plan should be transparent, of scientific quality
2079 in the context of well constructed hypotheses, and in sufficient detail so that the relevance of the
2080 data can be appraised.³³

2081 If a monitoring plan is to be developed by the notifier, it may be evaluated by the competent
2082 national authority and may be subject to modification before a decision for release is granted.
2083 Importantly, the proposed activities for case-specific monitoring should be relevant to the
2084 identified uncertainties regarding the level of risk posed by the LMO under consideration.³⁴
2085 Information relevant for developing the monitoring plan may be available from the risk
2086 assessment and, if applicable, from previous monitoring activities, including those from other
2087 countries. For example, the choice of protection goals and assessment endpoints (which may
2088 include the selection of indicators and parameters) may often be derived from the context and
2089 scoping phase of the risk assessment (See Roadmap, “Establishing the context and scope”). The
2090 scientific and technical details of the specific LMO, including detection methods, would in many
2091 cases be available from the information required for conducting the risk assessment as outlined
2092 in Annex III of the Protocol.³⁵

2093 When developing (or evaluating) a monitoring plan, the following may be considered:

- 2094 1. Choice of indicators and parameters for monitoring (“what to monitor?”);
- 2095 2. Monitoring methods, baselines including reference points, and duration of
2096 monitoring (“how to monitor?”);
- 2097 3. Monitoring sites and regions (“where to monitor?”);
- 2098 4. Reporting of monitoring results (“how to communicate?”).

³³ See Roadmap “Overarching issues in the risk assessment process”, “Quality and relevance of information”.

³⁴ See Roadmap “Overarching issues in the risk assessment process”, “Identification and consideration of uncertainty”.

³⁵ See paragraph 9 of Annex III to the Protocol (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-43>).

2099 The sections below address these issues in terms of rationales and elements for consideration.

2100 **1. Choice of indicators and parameters for monitoring (“what to monitor?”)**

2101 *Rationale:*

2102 Monitoring for potential adverse effects of an LMO involves the observation of changes to
2103 *indicators* (e.g., species, populations, soil, environmental processes, etc.) and/or *parameters* (i.e.,
2104 a component to be measured in the observation of an indicator, such as species abundance or soil
2105 organic matter).

2106 Results obtained from monitoring may assist in evaluating the estimates of environmental
2107 exposure which were made during the risk assessment (see step 2 in the Roadmap). Therefore,
2108 monitoring the exposure of the environment to LMOs may be a highly relevant element of an
2109 overall monitoring approach].

2110 The selection of indicators and parameters to be monitored will vary from case to case,
2111 depending on the LMO, characteristics of the likely potential receiving environment, specific
2112 risk scenarios established during the risk assessment, (see the Roadmap), and on the protection
2113 goals and biosafety legislation or policies of each country

2114 *Elements for consideration:*

2115 (a) The potential of the indicators and parameters to signal changes related to adverse effects
2116 as early as possible and/or before the consequences are realized;

2117 (b) Characteristics of the indicators and their level of exposure to the LMO, as well as
2118 parameters for the distribution and abundance of those indicators that are organisms;

2119 (c) Quantitative and qualitative variability of the indicators and parameters to be observed
2120 and how this variability could affect the ability of these indicators and parameters to
2121 signal changes that may lead to potential adverse effects;

2122 (d) The usefulness of the candidate indicators and parameters to establish relevant baselines,
2123 including reference points;

2124 (e) The importance of the candidate indicators and parameters to relevant key ecological
2125 processes and functions or to the identified protection goals;

2126 (f) Whether sampling and analysis would be easy or difficult and how these would affect the
2127 choice of indicators and parameter.

2128 **2. Monitoring methods, baselines including reference points, and duration of monitoring**
2129 **(“how to monitor?”)**

2130 **i. Selecting monitoring methods**

2131 *Rationale:*

2132 Monitoring methods are largely dependent on the indicators and parameters chosen in the
2133 preceding step, as well as the ability of these indicators and parameters to address uncertainty
2134 regarding the level of risk and to signal changes that could lead to an adverse effect. The
2135 selection of monitoring methods should also take into account the level of sensitivity and
2136 specificity needed to detect changes in the indicators and parameters.

2137 The description of the monitoring methodology includes the means for sampling and observing
2138 indicators and parameters, and for the analysis of the resulting data. Appropriate methods for
2139 collecting monitoring data may include observations, descriptive studies and questionnaires
2140 addressed to those who are exposed to or are handling to the LMO. For ecological issues, or
2141 effects occurring outside of the receiving environment, additional knowledge and tools may be
2142 required to gather relevant data.

2143 The best available science should always be used for monitoring. In some cases, the
2144 harmonization of methods, data formats, and analytical approaches facilitates the comparison of
2145 results from monitoring in different environments. When the use of existing surveillance
2146 programs is to be considered, the monitoring plan should guide the choice and use of these
2147 programs.

2148 *Elements for consideration:*

- 2149 (a) Relevance of the monitoring methodology to generate the necessary information to
2150 address uncertainty related to the level of risk;
- 2151 (b) The nature of the effect to be monitored (e.g., whether short- or long-term, delayed or
2152 indirect, cumulative, etc.);
- 2153 (c) Relevance, suitability and adaptability of existing surveillance programs, as well as the
2154 accessibility to those data, in the context of broader environmental monitoring;
- 2155 (d) The specification of the range or magnitude of changes in a parameter or indicator to
2156 signal changes that could lead to an adverse effect;
- 2157 (e) The scientific quality of the sampling, analytical and statistical methods to be
2158 employed;³⁶
- 2159 (f) The availability of relevant standardized methods, and whether and how these could be
2160 taken into account;
- 2161 (g) Whether methods are adequate to meet the objectives of the proposed monitoring plan;
- 2162 (h) The availability and use of descriptive studies or questionnaires, taking into account
2163 their replicability and verifiability;
- 2164 (i) Findings from ongoing and/or other monitoring activities, if relevant;
- 2165 (j) Relevant local, regional and international monitoring practices.

2166 **ii. Establishing baselines, including reference points**

2167 *Rationale:*

2168 The establishment of relevant baselines, including reference points is necessary for observing
2169 and analysing changes during monitoring. A baseline is a measurement or description of the

³⁶ See also considerations on “Quality and relevance of information” in the Roadmap.

2170 existing conditions of the likely potential receiving environment, and/or comparable reference
2171 environment, including the relevant indicators and parameters. Therefore, the methodology by
2172 which the baseline is derived should be described in the monitoring plan in order to verify that it
2173 will provide useful information in relation to the environment where the LMO may be released.
2174 Natural and human induced variation that may occur in baseline data should be taken into
2175 account when analysing monitoring data.

2176 *Elements for consideration:*

- 2177 (a) The scientific quality of methods used for generating baseline data including reference
2178 points;
- 2179 (b) The appropriate spatial scale of the baseline including reference points to be established;
- 2180 (c) Effects of temporal and spatial variation (i.e., human induced or natural variation in the
2181 physical environment);
- 2182 (d) The scale of the likely potential spread of the LMO.

2183 **iii. Establishing the duration and frequency of monitoring**

2184 *Rationale:*

2185 The duration of the monitoring, including the frequency at which observations or measurements
2186 need to be made, is determined on a case-by-case basis and will depend on the type of changes
2187 that may lead to adverse effects that are to be monitored (e.g., immediate or delayed, short- or
2188 long-term), the type of LMO (e.g., short or long life cycles,³⁷ transgenic traits introduced), and
2189 the duration of the proposed environmental release. Where general monitoring is used, the type
2190 of changes to be monitored may be broader to account for unanticipated effects. The duration or
2191 frequency of monitoring may be adjusted, if appropriate, on the basis of the results of on-going
2192 monitoring activities.

2193

³⁷ See article 16.4 of the Protocol (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-16>).

2194 *Elements for consideration:*

- 2195 (a) How long it would take for changes in a parameter to likely become apparent;
- 2196 (b) Characteristics of the indicators to be measured or described (e.g., persistence, life-cycle
2197 and generation time of species when used as indicators);
- 2198 (c) Life-cycle and generation time of the LMO as it is being used in the environment;
- 2199 (d) Whether variability in the monitored parameters over time could affect the results and
2200 conclusions of monitoring;
- 2201 (e) Potential for environmental changes, both biotic and abiotic.

2202 **3. Choice of monitoring sites (“where to monitor?”)**

2203 *Rationale:*

2204 Monitoring sites are selected on a case-by-case basis depending on the geographical location of
2205 the release in the likely potential receiving environment, the parameters and indicators that will
2206 be used in the monitoring, as well as the intended use of the LMO, and taking into account the
2207 associated management practices.

2208 The choice of monitoring site may include areas beyond the intended receiving environment
2209 where the LMO may be introduced.

2210 Relevant information regarding the sites to be monitored includes, for example, specific
2211 locations, their size and relevant environmental characteristics. In this context location registries
2212 (e.g., national and regional databases) may be a useful information tool for LMO-monitoring and
2213 the selection of relevant monitoring sites or regions.

2214 *Elements for consideration:*

- 2215 (a) Dissemination and establishment of the LMO in the likely potential receiving
2216 environment;

- 2217 (b) The type of LMO as well as indicators and parameters to be monitored and, in case of
2218 indicators that are species, their biological or ecological characteristics and life cycles;
- 2219 (c) Appraisal of suitable, relevant reference sites where the LMO is not present for
2220 comparison over the duration of the monitoring, if applicable;
- 2221 (d) Pathways through which the environment is likely to be exposed to the LMO(s);
- 2222 (e) The distribution patterns, including seasonal distribution (e.g., migration), of the
2223 selected indicators that are species, in the likely potential receiving environment for
2224 consistent detection and observation;
- 2225 (f) Appraisal of protected areas and centres of origin and genetic diversity or ecologically
2226 sensitive regions, particularly in the context of monitoring the presence of LMOs;
- 2227 (g) The appropriate number of monitoring sites and the statistical power of the conclusions
2228 that can be drawn;
- 2229 (h) The continued availability of the monitoring sites throughout the duration of
2230 monitoring;
- 2231 (i) Current management practices and possible changes to those practices over the duration
2232 of monitoring.
- 2233 (j) Sites that were previously used for field trials or experimental releases.

2234 **4. Reporting of monitoring results (“how to communicate?”)**

2235 *Rationale:*

2236 Reporting of monitoring results serves four main objectives: i) to inform competent authorities of
2237 any changes that can be related to adverse effects; ii) to allow verification of the quality and
2238 relevancy of data derived from monitoring to ensure the activities have been carried out in a
2239 manner that meets the intended objectives set out in the monitoring plan; iii) to indicate, if
2240 appropriate, the need for changes to the monitoring plan and/or other risk management strategies

2241 (or for follow-up studies or risk assessments); and iv) to recommend, if appropriate, the re-
2242 evaluation of a decision and the necessity of any emergency measures.

2243 The report of monitoring activities may be communicated in different forms, for example,
2244 depending on the target audience. From the report, the regulatory authority should be able to
2245 interpret the results and decide whether or not a specific action is required.

2246 *Elements for consideration:*

2247 (a) Reporting requirements set out by the competent authority(ies) or in national biosafety
2248 regulations, if available;

2249 (b) The completeness of the report, including transparency in presentation of methods, data
2250 and analytical tools used to draw conclusions;

2251 (c) Accessibility to raw data accrued during the monitoring activities, taking into account
2252 information that may be confidential.³⁸

2253

³⁸ See article 21 of the Protocol (<http://bch.cbd.int/protocol/text/article.shtml?a=cpb-21>).

USE OF TERMS

Comment [A15]: Outstanding: check consistency of use of terms with the text and the need for additional terms to be added on the basis of comments from the testing

2254

2255 This section provides a working glossary of key terms used in this document. An attempt was
2256 made to adapt definitions that are used in internationally accepted risk assessment guidance to
2257 the context of environmental risk assessment conducted under the Cartagena Protocol.

2258 **Antagonism** – An interaction of elements that when combined produce a total effect that is less
2259 than the sum of the effect of the individual elements. [\[back to the text\]](#)

2260 **Assessment endpoint** – An explicit expression of the environmental value that is to be
2261 protected, operationally defined as an entity (such as salmon or honeybees, soil quality) and its
2262 attributes (such as their abundance, distribution or mortality). (Adapted from IPCS, 2001,
2263 Integrated Risk Assessment, http://www.who.int/ipcs/publications/new_issues/ira/en/) [\[back to the](#)
2264 [text\]](#)

2265 **Baseline** – A description or a measurement of existing conditions of an environment, or its
2266 attributes or components without the LMO under consideration and taking into account different
2267 practices in use (e.g., agricultural practices). The baseline description or measurement may
2268 provide quantitative (e.g., number of organisms, variability of abundance) and/or qualitative
2269 information about the receiving environment as a reference for estimating effects of the LMO or
2270 its use including, if applicable, information on the assessment endpoints. [\[back to the text\]](#)

2271 **Behavioural sterility** – A type of reproductive sterility that is caused by changes in behaviour
2272 rather than to physiological changes. [\[back to the text\]](#)

2273 **Case-by-case** – A commonly accepted approach where each LMO is considered relative to the
2274 environment in which the release is to occur and to the intended use of the LMO. (Adapted
2275 IUCN, 2003, An Explanatory Guide to the Cartagena Protocol on Biosafety,
2276 <http://bch.cbd.int/database/record-v4.shtml?documentid=41476>) [\[back to the text\]](#)

2277 **Combinatorial effects** – Effects that arise from the interactions between two (or more) genes in
2278 one organism, including epistatic interactions. The effects may occur at the level of gene
2279 expression, or through interactions between RNA, or among gene products. The effects may be
2280 analysed as qualitative or quantitative; quantitative effects are often referred to as resulting in
2281 antagonistic, additive or synergistic effects (see also “Cumulative effects” for distinction). [\[back to](#)
2282 [the text\]](#)

2283 **Comparator** – Non-modified recipients or parental organisms of the LMO. A comparator is
2284 used as an element to establish the basis for a comparative assessment in accordance with Annex
2285 III. [\[back to the text\]](#)

2286 **Consequence (of the adverse effect)** – The outcome, extent and severity of an adverse effect
2287 associated with exposure to an LMO, its handling and use, or its products (in the context of
2288 Annex III paragraph 5). [\[back to the text\]](#)

2289 **Conventional breeding** – Not involving the use of modern biotechnology as defined in Article 3
2290 of the Cartagena Protocol on Biosafety. [\[back to the text\]](#)

2291 **Co-transformation** – Techniques of modern biotechnology using two or more transformation
2292 vectors to produce an LMO. [\[back to the text\]](#)

2293 **Cross-talk** – Instances in which one or more components of a signal transduction pathway affect
2294 a different pathway. [\[back to the text\]](#)

2295 **Cumulative effects** – Effects due to the presence of multiple LMOs or their products in the
2296 receiving environment (see also “Combinatorial effects” for distinction). [\[back to the text\]](#)

2297 **EC50 (median effective concentration)** – A concentration that is statistically or graphically
2298 estimated to cause a specified effect in 50% of a group of test organisms under specified
2299 experimental conditions. (IPCS, 2001, Integrated Risk Assessment,
2300 www.who.int/ipcs/publications/new_issues/ira/en/) [\[back to the text\]](#)

2301 **Ecological function** – the role of an organism in ecological processes. The relevance of specific
2302 ecological functions in the risk assessment will depend on the protection goals. For example,
2303 organisms may be part of the decomposer network playing an important role in nutrient cycling
2304 in soils, or may be important as a pollen source for pollinators and pollen feeders. [\[back to the text\]](#)

2305 **Exposure** – The route and level of contact between the likely potential receiving environment
2306 and the LMO or its products. [\[back to the text\]](#)

2307 **Exposure assessment** – Evaluation of the exposure of the environment, including organisms, to
2308 an LMO or products thereof. (Adapted from WHO, 2004, IPCS Risk Assessment Terminology,
2309 <http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf>) [\[back to the](#)
2310 [text\]](#)

2311 **Gene-drive system** – Method of introducing and spreading a desired gene into populations, e.g.,
2312 mosquito. (Adapted from Hood E, 2008, Selfish DNA versus Vector-Borne Disease,
2313 Environmental Health Perspectives 116: A69;
2314 www.ncbi.nlm.nih.gov/pmc/articles/PMC2235231/pdf/ehp0116-a00066.pdf) [\[back to the text\]](#)

2315 **Gene flow** – The transfer of genetic material from one organism to another by vertical or
2316 horizontal gene transfer; or the movement of an organism from one environment to another. [\[back](#)
2317 [to the text\]](#)

2318 **Gene product** – The RNA or protein that results from the expression of a gene. [\[back to the text\]](#)

2319 **Genotypic (characteristics)** – Relating to “genotype” as all or part of the genetic constitution of
2320 an organism. [\[back to the text\]](#)

2321 **Hazard** – The potential of an organism to cause harm to human health and/or the environment.
2322 (UNEP, 1995, International Technical Guidelines for Safety in Biotechnology,
2323 www.unep.org/biosafety/Documents/Techguidelines.pdf) [\[back to the text\]](#)

2324 **Hazard characterization** – The qualitative and/or quantitative evaluation of the nature of the
2325 adverse effects associated with an LMO. (Adapted from CODEX, 2001, Definitions of Risk
2326 Analysis Terms Related to Food Safety,
2327 <http://www.fao.org/DOCREP/005/Y2200E/y2200e00.htm>) [\[back to the text\]](#)

2328 **Hazard identification** – The identification of the type and nature of adverse effects that an LMO
2329 could cause to an organism, system or (sub)population. (Adapted from WHO, 2004, IPCS Risk
2330 Assessment Terminology,
2331 <http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf>) [\[back to the](#)
2332 [text\]](#)

2333 **Heterozygous (genomes)** – Having different alleles at the corresponding chromosomal loci. [\[back](#)
2334 [to the text\]](#)

2335 **Horizontal gene transfer** – The transfer of genetic material from one organism to another
2336 through means other than inheritance from parent to offspring (i.e., vertical). [\[back to the text\]](#)

2337 **Introgression** – Movement of a gene or genetic element from one species into the gene pool of
2338 another species or population, which may result in a stable incorporation or some fertile
2339 offspring. [\[back to the text\]](#)

2340 **Isogenic line, (Near-)** – Isogenic lines: two or more lines differing from each other genetically at
2341 one locus only; near-isogenic lines are two or more lines differing from each other genetically at
2342 several loci [\[back to the text\]](#)

2343 **LD50 (median lethal dose)** – A statistically or graphically estimated dose that is expected to be
2344 lethal to 50% of a group of organisms under specified conditions. [\[back to the text\]](#)

2345 **Likelihood (of the adverse effect)** – Probability of the adverse effect occurring, taking into
2346 account the level and kind of exposure of the likely potential receiving environment to the LMO.
2347 [\[back to the text\]](#)

2348 **Multi-trophic (effects)** – Involving more than two trophic levels in a food web. [\[back to the text\]](#)

2349 **No-observed-effect level (NOEL)** – Greatest concentration or amount of a substance, found by
2350 experiment or observation, that causes no alterations of morphology, functional capacity, growth,
2351 development, or life span of target organisms distinguishable from those observed in normal
2352 (control) organisms of the same species and strain under the same defined conditions of
2353 exposure. (IUPAC, 2007, Glossary of Terms Used in Toxicology, 2nd edition, Pure Appl. Chem.
2354 79: 1153-1344, <http://sis.nlm.nih.gov/enviro/iupacglossary/frontmatter.html>) [\[back to the text\]](#)

2355 **“Omics” technologies** – A collection of - usually high-throughput - techniques to study an
2356 organism or group of organisms at the level of the genome, gene transcripts, proteins or
2357 metabolites, which depending on the level are specifically called “genomics”, “transcriptomics”,
2358 “proteomics” and “metabolomics”, respectively. [\[back to the text\]](#)

2359 **Outcrossing** – The transmission of genetic elements from one group of individuals (e.g.,
2360 population, crop variety) to another. In plants, outcrossing most commonly results from cross-
2361 pollination. (Adapted from GMO Compass, www.gmo-compass.org/. See also “Vertical gene
2362 transfer”) [\[back to the text\]](#)

2363 **Phenotypic (characteristics)** – Relating to “phenotype” as the observable physical or
2364 biochemical characteristics of an organism, as determined by both genetic and environmental
2365 factors. [\[back to the text\]](#)

- 2366 **Pleiotropic effects** – Effects of a single gene on multiple phenotypic traits. [\[back to the text\]](#)
- 2367 **Potential receiving environment** – The range of environments (ecosystem or habitat, including
2368 other organisms) which are likely to come in contact with a released organism due to the
2369 conditions of the release or the specific ecological behaviour of the organism. (Adapted from
2370 UNEP, 1995, International Technical Guidelines for Safety in Biotechnology,
2371 www.unep.org/biosafety/Documents/Techguidelines.pdf) [\[back to the text\]](#)
- 2372 **Protection goal** – Defined and valued environmental outcomes that guide the formulation of
2373 strategies for the management of activities that may affect the environment. [\[back to the text\]](#)
- 2374 **Re-transformation** – Use of modern biotechnology, as defined in the Protocol, to produce an
2375 LMO where the recipient organism is already an LMO. [\[back to the text\]](#)
- 2376 **Risk** – The combination of the magnitude of the consequences of a hazard and the likelihood that
2377 the consequences will occur. (Adapted from UNEP, 1995, International Technical Guidelines for
2378 Safety in Biotechnology, www.unep.org/biosafety/Documents/Techguidelines.pdf) [\[back to the text\]](#)
- 2379 **Risk assessment** – The process of estimating risks that may be associated with an LMO on the
2380 basis of what adverse effects may be caused, how likely the adverse effects are to occur, and the
2381 consequences should they occur. (Adapted from UNEP, 1995, International Technical
2382 Guidelines for Safety in Biotechnology,
2383 www.unep.org/biosafety/Documents/Techguidelines.pdf) Risk assessment is often considered as
2384 part of a broader process called ‘risk analysis’ which may also include considerations such as
2385 risk management and risk communication. [\[back to the text\]](#)
- 2386 **Risk characterization** – The qualitative and/or quantitative estimation, including attendant
2387 uncertainties, of the overall risk. (Adapted from CODEX, 2001, Definitions of Risk Analysis
2388 Terms Related to Food Safety, <http://www.fao.org/DOCREP/005/Y2200E/y2200e00.htm>) [\[back to](#)
2389 [the text\]](#)
- 2390 **Risk management** – The measures to ensure that risks identified in the risk assessment are
2391 reduced, controlled, or eliminated. (Adapted from UNEP, 1995, International Technical
2392 Guidelines for Safety in Biotechnology,
2393 www.unep.org/biosafety/Documents/Techguidelines.pdf) [\[back to the text\]](#)

2394 **Risk threshold** – The level of tolerance to a certain risk or the level of change in a particular
2395 variable beyond which a risk is considered unacceptable. [\[back to the text\]](#)

2396 **Stability (of the transgene)** – Permanence of the transgene in a defined genomic context and
2397 without changes to its structure or phenotypic expression. [\[back to the text\]](#)

2398 **Synergism** – An interaction of elements that when combined produce a total effect that is greater
2399 than the sum of the effect of the individual elements. [\[back to the text\]](#)

2400 **Transformation cassette** – A transformation cassette comprises a group of DNA sequences
2401 (e.g., parts of a vector and one or more of the following: a promoter, the coding sequence of a
2402 gene, a terminator, other regulatory sequences), which are physically linked and often originated
2403 from different donor organisms. The transformation cassette is integrated into the genome of a
2404 recipient organism through methods of modern biotechnology to produce an LMO. A
2405 transformation cassette may also be called “expression cassette” (mainly when a specific
2406 expression pattern is aimed at), “DNA cassette” or “gene construct”. [\[back to the text\]](#)

2407 **Transformation event** – An LMO with a specific modification that is the result of the use of
2408 modern biotechnology according to Article 3 (i) (a) of the Protocol. [\[back to the text\]](#)

2409 **Transgene** – A nucleic acid sequence in an LMO that results from the application of modern
2410 biotechnology as described in Article 3 (i) (a) of the Protocol. [\[back to the text\]](#)

2411 **Trans-regulation** – Transcriptional regulation of gene expression by regulatory elements that
2412 were themselves transcribed in a different region of the genome. For example, a transcriptional
2413 factor transcribed in one chromosome may regulate the expression of a gene located in another
2414 chromosome. [\[back to the text\]](#)

2415 **Unintended effects** – Effects that appear in addition to, or in some cases instead of, the intended
2416 effects. Some unintended effects may be foreseen while others are unanticipated. [\[back to the text\]](#)

2417 **Unintended gene product** – Gene products (e.g., RNA, proteins), which are different from those
2418 originally intended. [\[back to the text\]](#)

2419 **Unmanaged and managed ecosystems** – An “unmanaged ecosystem” is an ecosystem that is
2420 free from significant human intervention. As opposed to a “managed ecosystem” which is an
2421 ecosystem affected by varying degrees of human activities. [\[back to the text\]](#)

2422 **Vector** – In the context of genetic modification, a vector is an organism (e.g., virus) or a DNA
2423 molecule (e.g., plasmid, nucleic acid cassettes) used to assist the transfer of genetic material from
2424 a donor organism to a recipient organism. (Adapted from UNEP, 1995, International Technical
2425 Guidelines for Safety in Biotechnology,
2426 www.unep.org/biosafety/Documents/Techguidelines.pdf) In the context of epidemiology, a
2427 vector is an organism, often an arthropod (e.g., mosquito), that transmits a pathogen (e.g.,
2428 plasmodium) to a host (e.g., humans). [\[back to the text\]](#)

2429 **Vertical gene transfer** – Transfer of genetic material from one organism to its offspring via
2430 asexual, parasexual or sexual reproduction. Also referred to as “vertical gene flow”. [\[back to the text\]](#)

2431