

Convention on Biological Diversity

Distr.: General
6 October 2023

English only

Ad Hoc Technical Expert Group on Risk Assessment First meeting

Montreal, Canada, 1–3 November 2023

Items 3, 4 and 5 of the provisional agenda*

**Comments on the detailed outline of additional
voluntary guidance materials to support the
case-by-case risk assessment of living modified
organisms containing engineered gene drives**

**Working modalities for the development of
additional voluntary guidance materials**

Review of the detailed outline

Detailed outline of the voluntary additional voluntary guidance materials on risk assessment of living modified organisms containing engineered gene drives

1. In its decision CP-10/10, the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety agreed to develop additional voluntary guidance materials to support case-by-case risk assessments of living modified organisms containing engineered gene drives in accordance with annex III to the Protocol. It also decided that a specific focus of the materials should be engineered gene drive mosquitoes, taking into account the current experience with the organism, the type of gene drive and specific issues of risk assessment, as identified in annex I to decision CP-9/13, including existing reports, general considerations of living modified organisms containing engineered gene drives and existing national and regional risk assessment experiences.
2. In the same decision, the Conference of the Parties serving as a meeting of the Parties to the Protocol established an ad hoc technical expert group on risk assessment to develop the additional voluntary guidance materials and invited Parties, other Governments, indigenous peoples and local communities and relevant organizations to submit to the Executive Secretary information relevant to the work of the expert group. In addition, it requested the Executive Secretary to convene online discussions of the Open-ended Online Forum on Risk Assessment and Risk Management and to commission, subject to the availability of resources and applying decision 14/33 on the procedure for avoiding or managing conflicts of interest in expert groups, the preparation of a detailed outline of additional voluntary guidance materials on risk assessment of living modified organisms containing engineered gene drives.

* CBD/CP/RA/AHTEG/2023/1/1.

3. The Secretariat commissioned the International Centre for Biotechnology and Genetic Engineering to prepare the detailed outline of the additional voluntary guidance materials. In support of the drafting process, the Secretariat also convened the Open-Ended Online Forum on Risk Assessment and Risk Management, from 10 to 25 April 2023, to provide inputs to be taken into consideration by the International Centre during the drafting of the outline.
4. Following the online discussions, a first draft of the outline was produced and made available on 18 August 2023. The Secretariat then convened a second session of the Open-Ended Online Forum on Risk Assessment and Risk Management, from 21 to 30 August 2023, to facilitate a review of the draft.
5. In response to the interventions made during the second session of online discussions, the International Centre for Genetic Engineering and Biotechnology prepared a draft of the detailed outline of additional voluntary guidance materials for the risk assessment of living modified organisms containing engineered gene drives, with a view to facilitating the work of the Ad Hoc Technical Expert Group on Risk Assessment.
6. The detailed outline is presented in the annex below. The Expert Group is invited to review the draft detailed outline provided in the annex and to use it as a basis for its work in the development of the additional voluntary guidance materials.

ADDITIONAL VOLUNTARY GUIDANCE MATERIALS ON RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS CONTAINING ENGINEERED GENE DRIVES

DRAFT OUTLINE

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1. Objective and scope of the additional voluntary guidance materials

In its decision CP-10/10, the Conference of the Parties serving as a meeting of the Parties to the Cartagena Protocol on Biosafety agreed to develop additional voluntary guidance materials to support case-by-case risk assessment of living modified organisms (LMOs) containing engineered gene drives in accordance with annex III to the Protocol.¹ The Conference of the Parties decided that this material should have a special focus on engineered gene drive mosquitoes taking into account the current experience with the organism, the type of gene drive and specific issues of risk assessment, identified in annex I to decision CP-9/13, including existing reports, general considerations of LMOs containing engineered gene drives (EGD-LMOs) and existing national and regional risk assessment experiences. The decision also established an ad hoc technical expert group on risk assessment and requested the convening of the Online Forum on Risk Assessment and Risk Management to contribute to this process. The Subsidiary Body on Scientific, Technical and Technological Advice will consider the draft prepared by the Ad Hoc Technical Expert Group on Risk Assessment.

As a response, with the financial support of the Government of Finland and the European Union, the Secretariat of the Convention on Biological Diversity commissioned the International Centre for Genetic Engineering and Biotechnology to develop a comprehensive outline encompassing supplementary guidance materials concerning the risk assessment of EGD-LMOs. The objective is to facilitate a meticulous case-by-case risk assessment process for EGD-LMOs, thereby complementing annex III and existing guidelines while building upon the established road map.²

To achieve this objective, an extensive literature review was conducted to compile relevant scientific publications pertaining to the risk assessment of EGD-LMOs. The literature search methodology employed for this review is outlined in annex I. By synthesizing the latest scientific knowledge and evidence on the subject, this outline seeks to contribute substantially to the enhancement of risk assessment practices for EGD-LMOs and further the understanding of potential implications for biodiversity and ecosystems.

¹ The Cartagena Protocol on Biosafety 2003. <https://bch.cbd.int/protocol/>.

² See decisions BS-IV/11 BS-V/12, on risk assessment and risk management, of the Conference of the Parties serving as a meeting of the Parties to the Cartagena Protocol, which support the drafting of and describe the objectives of the guidance on risk assessment of living modified organisms and monitoring in the context of risk assessment.

PART A. RISK ASSESSMENT OF LIVING MODIFIED ORGANISMS CONTAINING ENGINEERED GENE DRIVES

2. Introduction

According to the Protocol, the risk assessment of LMOs shall be carried out in a scientifically sound manner, in accordance with annex III and taking into account recognized risk assessment techniques. Such risk assessments shall be based, at a minimum, on information provided in accordance with Article 8 and other available scientific evidence in order to identify and evaluate the possible adverse effects of LMOs on the conservation and sustainable use of biological diversity, taking also into account risks to human health. The primary objective is to identify, evaluate and quantify the potential risks of LMOs, while considering any associated uncertainties. The outcome of the risk assessment serves as a foundation for informed decision-making regarding the use and release of LMOs.

In 2016, the CBD published general guidance on the risk assessment of LMOs,³ which included LM mosquitoes among the examples of specific types and traits of LMOs. However, it lacked specific guidance regarding the assessment of risk from the use of the technology. Engineered gene drives spread genetic modifications at a rate greater than the 50 per cent expected from normal Mendelian inheritance. This distinctive characteristic enables them to proliferate rapidly within interbreeding populations. Consequently, engineered gene drives hold considerable promise in tackling enduring challenges concerning the current management of rodent and insect disease vectors, agricultural pests and invasive species, or to help save endangered species ({Devos and others, 2022}). Unlike other LMOs, those containing gene drive constructs are often specifically designed to disperse beyond their initial release locations and persist in target populations over extended periods and generations. This unique characteristic necessitates a comprehensive assessment of ecological risks with a broader scope (Connolly and others, 2022). Moreover, the release of even a small number of EGD-LMOs, depending on their design, can result in the genetic trait spreading throughout the entire population of the targeted species in the wild. As a result, the ecological and health consequences of EGD-LMOs have the potential to be far-reaching (Kuzma and others, 2019). Therefore, an effective risk assessment protocol must be capable of addressing this distinct and challenging situation, ensuring a thorough evaluation of the potential impacts on ecosystems.

This document presents an integrated risk assessment process for EGD-LMOs, comprising three sections:

- Section 3. Overarching Issues in the Risk Assessment Process,
- Section 4. Planning Phase of the Risk Assessment,
- Section 5. Conducting the Risk Assessment.

Within the “Conducting the Risk Assessment” section, the guidance draws from paragraph 8 of annex III to the Protocol to outline a sequential five-step risk assessment process. Each step builds upon the results of the preceding step, ensuring a comprehensive assessment of potential risks:

- Step 1. Identification of any novel genotypic and phenotypic characteristics associated with the living modified organism that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health;
- Step 2. Evaluation of the likelihood of adverse effects being realized, taking into account the level and kind of exposure of the likely potential receiving environment to the living modified organism;
- Step 3. Evaluation of the consequences should these adverse effects be realized;
- Step 4. Estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized;

³ Guidance on risk assessment of living modified organisms and monitoring in the context of risk assessment, UNEP/CBD/BS/COP-MOP/8/8/Add.1., 14 September 2016. www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf.

- Step 5. Recommendation as to whether or not the risks are acceptable or manageable, including, where necessary, identification of strategies to manage these risks.

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

To help place the risk assessment, with any associated issues and questions, in the context of a specific EGD-LMO, this document provides a case study, an EGD-LM *Anopheles* mosquito. This case study should not be considered general guidance for risk assessments of all EGD-LMOs.

Ultimately, the conclusions and recommendations derived from the risk assessment are essential inputs in the decision-making process concerning the use and release of LMOs. In line with country-specific policies and protection goals, other relevant aspects of the Protocol and related issues listed in the final section of this guidance are also considered during the decision-making process (“Related issues” section).

This guidance incorporates informative text boxes with additional information on specific topics or encompassing illustrative examples. While this guidance presents a specific approach to risk assessment, it is worth noting that alternative approaches also exist.

3. Overarching issues in the risk assessment process on living modified organisms containing engineered gene drives

This section provides updated guidance specifically addressing issues relevant to the risk assessment of EGD-LMOs. These considerations aim to complement the overarching issues related to protection goals, measurement end points, and the quality and relevance of information to be considered in the risk assessment.

Gene drives

Gene drives are genetic elements that function to increase the frequency of their own transmission to the next generation, beyond normal Mendelian inheritance. This increase in frequency results in preferential multiplication of a particular genotype within a population. Gene drives exist naturally in all organisms and operate through a variety of mechanisms, such as the modification of gametogenesis to cause the overrepresentation of specific genes in eggs or sperm. Other natural gene drives may be autosomal or linked to one of the sex chromosomes (Bier, 2012; Wendell, 2019).

Engineered gene drives (EGDs) have been developed in recent years using a variety of methods and technologies that produce results analogous to gene drives found in nature. In addition, EGDs have been developed to cause a deleterious trait to spread through the population of a harmful species, in order to suppress its growth, increase, spread or to permanently modify a population, through the insertion of a desirable trait, such as the inability to vector a harmful disease, such as malaria. Several different types of EGDs exist, with new versions in continual development. Below, a few of the more common EGDs are described briefly.

Underdominance systems: Underdominance is described as negative heterosis, where heterozygotes are less fit than homozygotes. When introduced in a population of wild types by regular releases, the population will evolve either into all wild-types or all gene drive-bearing individuals. They are high threshold-dependent gene drives that act locally and can be removed through dilution of the population with wild-type individuals.

Meiotic drive: In a meiotic drive, the transmission of certain alleles is biased during meiosis, leading to increased frequencies of those alleles in the gametes, and therefore in the resultant offspring. Sex-linked meiotic drives work through altering the sex ratios of offspring of affected individuals. They may be applied as a suppression drive.

Maternal-effect dominant embryonic arrest: Maternal-effect dominant embryonic arrest (MEDEA) is a system where embryo development is arrested (lethal effect) in all progeny except for those embryos that paternally or maternally inherited an “antidote” gene. The system may be used to replace a population. Synthetic MEDEA systems may also include a gene which the developer wants to add to the host genome.

Homing endonuclease genes: Homing endonucleases genes are able to selectively disrupt a specific DNA target sequence and copy itself, or “home,” into this target sequence. The phenomenon transforms a heterozygote organism into a homozygote for the gene drive. If this successfully happens anywhere in the lineage of the cells that will form the germline, the frequency of the changes allele in the progeny will be higher than expected according to Mendelian rules (CBD, 2020).

Some engineered gene drive terminology

EGDs are categorized in broad terms by their overall effect on the target population. *Population suppressive (or suppression)* gene drives act to reduce the size of a population, analogous to the treatment of a population of insects with an insecticide. *Population replacement (or modification)* gene drives operate by replacing wild-type individuals by the EGD-bearing individuals over time.

EGDs may also be categorized by the number of EGD-LMOs which must be introduced into the target population in order to have the desired effect. *Low-threshold EGDs* can be released in relatively small numbers to suppress or replace a population. *High-threshold EGDs* must be released in large numbers, relative to the size of the target population, to have the desired effect.

In addition, EGDs could also be categorized by their predicted geographical reach, either being non-localized (e.g. for low-threshold systems) or localized (e.g. for self-limiting systems and many high-threshold drives). (Alphey and others, 2020; Raban and others 2020).

Given the unique characteristics of EGD-LMOs, their risk assessment demands special attention to several critical aspects. The Ad Hoc Technical Expert Group on Risk Assessment⁴ has duly recognized these generic aspects encompassing the lack of data to inform the risk assessment procedure, the limited applicability of certain risk assessment methodologies to EGD-LMOs, such as challenges in the comparative approach and monitoring methods, as well as the lack of guidance on how to assess uncertainty, the lack of validated modelling tools and the lack of regulatory experience or capacity. Consequently, a careful assessment of uncertainties and knowledge gaps that arise during the risk assessment process becomes imperative (Kuzma and others, 2021), incorporating whenever strategies to identify, describe and appropriately account for the degree of uncertainty associated with the assessment.

Apart from the aforementioned generic challenges, a multitude of intricate specific challenges were also comprehensively detailed by the Ad Hoc Technical Expert Group on Risk Assessment. These challenges are multifaceted and may intersect across different categories, while not being universally applicable to all types of gene drives.

Primarily, the characteristics of the EGD system itself present formidable hurdles. Factors such as super-Mendelian inheritance, genetic and phenotypic stability and potential invasiveness demand meticulous scrutiny. Furthermore, the unpredictability of emergent genomic effects in subsequent generations, coupled with complex interactions with receiving environments, adds to the complexity of the assessment. Addressing the controllability of EGD systems post-release is also crucial, as is evaluating potential off-target changes and their cumulative impact on various genetic backgrounds.

A comprehensive risk assessment also necessitates a deep understanding of the target species. This includes gaining insight into the genetic diversity of the target species, its functional role within diverse ecosystems and possible interactions with interfertile species. Consideration of reproductive strategies, population dynamics and life cycle attributes are equally vital. Moreover, addressing pathogen resistance development in relation to vector control adds further dimensions to the assessment.

The receiving environment constitutes another critical aspect, entailing a careful evaluation of potential interactions with natural environments and long-term evolutionary processes. Assessing the possibility of cross-hybridization with non-target species and accounting for the diverse range of potential receiving environments are essential components.

⁴ Report of the Ad Hoc Technical Expert Group on Risk Assessment, Montreal, Canada, 30 March-03 April 2020, CBD/CP/RA/AHTEG/2020/1/5. www.cbd.int/doc/c/a763/e248/4fa326e03e3c126b9615e95d/cp-ra-ahteg-2020-01-05-en.pdf.

1 Lastly, risk management and monitoring demand careful attention. Post-release environmental monitoring
2 poses complexities, requiring the evaluation of impacts over prolonged periods. Developing supranational-
3 level monitoring plans to track the EGD spread could become imperative. In addition, formulating proven
4 strategies for controlling the spread in response to adverse health or environmental impacts is critical, as is
5 creating management plans to address the potential reversion of EGDs.

6 Throughout the risk assessment process, it may become evident that additional information is required on
7 specific subjects. In such cases, requesting supplementary data and documentation from the EGD-LMO
8 notifier or developer ensures a comprehensive evaluation.

9 Consultative meetings between regulators and the developers of the EGD-LMO may be helpful in the
10 planning phase of the risk assessment. These meetings provide an opportunity to discuss and align the
11 approaches and methodologies to be employed in the assessment. Discussions may also be held during the
12 assessment process to facilitate a common understanding among stakeholders and enhance the overall
13 completion of the assessment.

14 Independent experts with a background in relevant scientific disciplines can be considered to provide
15 advisory support during the risk assessment process. In accordance with Article 21 of the Protocol, these
16 experts can contribute to the assessment or act as assessors themselves, ensuring a robust and scientifically
17 sound evaluation.

18 **3.1. Protection goals, assessment end points and measurement end points**

19 The potential effects caused by an EGD-LMO may vary depending on its characteristics, how is used, and
20 the environment in which it is present. These effects may be intended or unintended, and their classification
21 as beneficial, neutral or adverse dependent upon their impact on a protection goal.

22 Adverse effects and protection goals are closely interlinked concepts. Protection goals encompass a broad
23 range of valued environmental outcomes, sometimes called general protection goals or generic end points.
24 These protection goals, as dictated by national policies and annex I to the Convention on Biological
25 Diversity,⁵ encompass various aspects, such as human and animal health, biodiversity, ecosystems,
26 ecosystem services, soil health and water quality. To assess and safeguard these goals, specific assessment
27 end points are identified, including the ecological entity and attribute to be protected, the maximum
28 tolerable impact and the spatial and temporal scale of protection. In addition, measurement end points are
29 established to quantify the assessment end points. In their work, Devos and others (2022a) highlight the
30 significance of operationalizing protection goals in risk assessment through the specification of assessment
31 end points and measurement end points.

32 Examples of protection goals that focus on biodiversity conservation include species of conservation value
33 or cultural value, species in the IUCN Red List and protected habitats and landscapes. Protection goals that
34 focus on ecological functions include soil, water and production systems. Sustainable ecosystems as
35 protection goals include both biodiversity conservation and ecological functions. Assessment end points
36 and measurement end points are derived from the relevant protection goals and represent important concepts
37 in risk assessment. Understanding the difference between these terms is crucial for the risk assessment
38 process.

39 Assessment end points define, in operational terms, the environmental values that are to be protected. An
40 assessment end point must include an entity (such as mosquito, invasive mice or soil quality) and a specific
41 attribute of that entity (such as their abundance, distribution, mortality, or gene flow). Assessment end points
42 are sometimes called specific protection goals or operational protection goals and may serve as starting
43 points for the “problem formulation” step of the risk assessment (see below). Examples could include the

⁵ The Convention on Biological Diversity 1992, annex I. Identification and monitoring www.cbd.int/convention/articles/?a=cbd-a1.

abundance of an endangered bird species in a defined agricultural ecosystem or abundance of bees in the same area.

A measurement end point is a quantifiable indicator of change in the assessment end point and constitutes measurements of hazard and exposure. Examples include fitness assessment, sex ratio monitoring, growth and density of the species used as the assessment end points.

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

3.2. Quality and relevance of information

The relevance and quality of information used to characterize the risks posed by an EGD-LMO are crucial considerations. Such information should be, where possible, supported by evidence-based information, including peer-reviewed data, specialized knowledge, indigenous and traditional knowledge, as well as subjective probabilities (Kuzma and others, 2021).

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

3.2.1. Criteria for the quality of scientific information

- The information used in the risk assessment should be of acceptable scientific quality and consistent with best practices of scientific evidence-gathering and reporting.
- Independent review of the design, methods, and reporting quality may be conducted to ensure appropriate data quality.
- Appropriate statistical methods should be used when applicable to strengthen the scientific conclusions of the risk assessment, and these methods should be clearly described in the assessment report.
- Acknowledge when the risk assessment draws on data from multiple scientific fields.
- Information should be reported in a detailed and transparent manner to allow independent verification and reproduction.
- Relevant information, samples and reference materials should be accessible to risk assessors, while respecting the confidentiality provisions of Article 21 of the Protocol.

3.2.2. Sources and relevance of information for the risk assessment

- Information used in the risk assessment may be derived from a variety of sources, such as new experiments, peer-reviewed literature, expert opinions, data from LMO development and previous risk assessments, in particular for similar EGD-LMOs introduced in similar receiving environments.
- Information from national and international standards and guidelines may also be used in the risk assessment, as well as knowledge and experience of, for example, farmers, growers, scientists, regulatory officials, and indigenous peoples and local communities.
- Information is considered relevant if it is linked to protection goals, assessment end points, the identification and evaluation of potential adverse effects of the EGD-LMO, or decision-making process.

- Not all information on the EGD-LMO or its parental organisms available in the literature may be considered relevant to the risk assessment. Likewise, not all sources of information may be considered of equal relevance.

Moreover, information that is considered relevant to a risk assessment will vary from case to case depending on the nature of the modification of the EGD-LMO, on its intended use, intended receiving environment, and on the scale and duration of the environmental introduction, as well as on the risk assessors' level of familiarity with the trait or organism being assessed.

3.3. Identification and consideration of uncertainty

Uncertainty is an inherent element of the scientific analysis and risk assessment of LMOs, and it is especially important in risk assessments involving new technologies, such as EGD-LMOs. The proposed release of these organisms is likely to raise questions of uncertainty and unpredictability, including some novel questions regarding their potential unintended effects on biodiversity. Consequently, caution and a thorough assessment of uncertainty are imperative for the effective governance of EGD-LMOs (Connolly and others, 2022; Devos and others, 2021; Rabitz and others, 2022).

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 treatment of uncertainty and its importance to effective decision-making varies across regulatory frameworks.

Annex III to the Protocol recognizes that the lack of scientific knowledge or consensus should not indicate a particular level of risk, the absence of risk, or an acceptable risk. In situations where there is uncertainty regarding the level of risk, further information may be requested or appropriate *risk management* strategies or monitoring of the EGD-LMO in the receiving environment can be implemented. Furthermore, paragraph 6 of Article 10 of the Protocol indicates that Parties may proceed with risk assessment and risk management strategies despite uncertainties surrounded the extent of potential adverse effects.

Considerations and communication of uncertainty may improve the understanding of the risk assessment outcomes, strengthen the scientific validity of the assessment and provide transparency in the decision-making process. Relevant considerations include the source and nature of uncertainties, focusing on those that can significantly impact the risk assessment conclusions.

Each identified EGD-LMO uncertainty can be categorized based on its *nature*, including: (a) lack of information; (b) incomplete knowledge; and/or (c) biological or experimental variability. Uncertainty resulting from lack of information includes, for example, the lack of knowledge about the long-term ecological impacts, or the potential for EGD-LMO to evolve and develop resistance to control measures (Kuzma and others, 2021; Frieß and others, 2019). Uncertainty arising from incomplete knowledge may manifest as an incomplete understanding of off-target effects or a limited knowledge of EGD persistence in natural populations (Frieß and others, 2023; Cisnetto and others, 2020). Lastly, uncertainties resulting from biological or experimental variability may involve variations in EGD efficiency and stability, as well as discrepancies in ecological or intergenerational responses (Then and others, 2020; Rabitz, 2022). In some cases, more information will not necessarily contribute to a better understanding of potential adverse effects, therefore risk assessors should look to ensure that any further information requested will contribute to better evaluations of the risks. For example, uncertainties originating from lack of information can be reduced or eliminated with more or better data obtained through further testing or by requesting additional information from the developers of the EGD-LMO. However, in cases of incomplete knowledge or inherent variability, the provision of additional information will not necessarily reduce the uncertainty.

In cases where uncertainty cannot be addressed through the provision of more information, appropriate risk management and monitoring measures, as outlined in subparagraphs 8 (e) and 8 (f) of annex III to the Protocol, can be employed. Furthermore, uncertainties associated with specific adverse effects may not allow the completion of a risk assessment or conclusions regarding the level of overall risk.

Modelling plays a pivotal role in addressing uncertainties associated with risk assessments of EGD-LMOs (Devos and others, 2022b; Golnar and others, 2021; Ickowicz and others, 2021; Rode and others, 2019; Zapletal and others, 2021;). By employing various robust modelling techniques, researchers and applicants can simulate different scenarios, test hypotheses, anticipate long-term and ecosystem effects and make informed predictions about the behaviour and potential risks of EGD-LMOs.

One key contribution of modelling is its ability to predict population dynamics (Combs and others, 2023; Golnar and others, 2021; Ickowicz and others, 2021; Sanchez and others, 2020). Through simulations that consider factors such as reproductive rates, population sizes, migration patterns and genetic interactions, models can provide insights into the spread and persistence of the EGD-LMO within target populations and environments. Moreover, modelling allows for the assessment of potential ecological and evolutionary impacts. By simulating interactions between the EGD-LMO and non-target species, as well as potential disruptions to ecosystems, models can quantify the risks and uncertainties associated with these impacts (Frieß and others, 2023; Golnar and others, 2021; Rode and others, 2019). Moreover, through simulations that incorporate various intervention approaches, such as different gene drive mechanisms or parameter variations, models help identify optimal strategies that minimize risks and maximize effectiveness (Connolly and others, 2021; Devos and others, 2022b; Zapletal and others, 2021). This information can support decision-making processes and can assist in the development of risk management plans.

The various forms of uncertainty are considered and described for each identified risk and under the estimation of the overall risk. In addition, when communicating the results of a risk assessment, it is important to describe, either quantitatively or qualitatively, those uncertainties that may have an impact on the overall risk, as well as on the conclusions and recommendations of the risk assessment in a way that is relevant for decision-making.

4. Planning phase of the risk assessment of living modified organisms containing engineered gene drives

4.1. Establishing the context and scope

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

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

In establishing the context and scope, several points may be taken into consideration, as appropriate, that are specific to the Party involved⁶ and to the particular risk assessment. These include the relevant:

- i. Regulations and international obligations of the Party involved;
- ii. Environmental and health policies and strategies;
- iii. Guidelines and regulatory frameworks that the Party has adopted;

⁶ See Protocol provisions with regard to whose responsibility it is to ensure that risk assessments are carried out.

- iv. Protection goals, including ecosystems functions and services, as well assessment end points, risk thresholds and management strategies derived from (i) to (iii) above;
- v. Robust mathematical models that predict long-term and ecosystem effects;
- vi. Availability of baseline information for the likely potential receiving environment;
- vii. The nature and level of detail of the information that is needed (see above), which may, among other things, depend on the biology/ecology of the recipient organism and the intended use of the EGD-LMO (suppression versus population modification), its temporal characteristics (self-limiting versus self-sustaining), and the spatial characteristics (localizing versus non-localizing);
- viii. Identification of methodological and analytical requirements, including requirements for review mechanisms, that must be met to achieve the objective of the risk assessment as specified, for instance, in guidelines published or adopted by the Party that is responsible for conducting the risk assessment;
- ix. Experience and history of use of other modified organisms similar to the EGD-LMO (e.g. in the case of vector control programmes, sterile insect technique, on incompatible insect technique), taking into account its ecological function;
- x. Information from previous risk assessments of the same or similar EGD-LMOs, modified trait(s) in other types of LMOs, or with similar vector control programmes in the case of insects;
- xi. Criteria to characterize the likelihood (step 2) and magnitude of consequences (step 3) of individual risks, and for combining them into the overall risk (step 4), and the acceptability or manageability of risks (step 5);
- xii. Monitoring strategies proposed for tracking the population dynamics, spread, and potential interactions with non-target species to ensure the desired outcomes and minimize potential risks to ecosystems.

Stakeholder engagement

Stakeholder engagement is a fundamental part of the risk assessment process, and guidance exists for conventional LMOs, such as food and fibre crops (CBD, 2016). New genetic modification technologies, such as gene drives, are likely to raise new questions and concerns for stakeholders, including indigenous peoples and local communities, who have an interest in technologies that may impact their traditional knowledge, innovation, practices livelihood and use of land and water. Therefore, risk assessors should anticipate and plan for an expanded engagement process to ensure the risk assessment has an appropriate scope that examines potential adverse effects which may be unique to EGD-LMOs (CDB, 2018).

As the risk assessment involves an evolving technology, an early stage in the engagement process should be the development and distribution of explanatory materials to ensure that all stakeholders have a sufficient understanding of the technology and how it will function in the environment. The technical complexity of these materials will need to be scaled to match the technical expertise of the various stakeholder audiences. Achieving meaningful engagement with indigenous peoples and local communities will depend upon providing them with sufficient information to enable them to understand what is being proposed so that they can raise questions and concerns relevant to their cultural experiences.

Regulators should use a wide variety of engagement methods and media to ensure that information is made available to indigenous peoples and local communities and other groups in ways that are accessible and culturally appropriate (Kokotovich and others 2022). The engagement process should account for a wide spectrum in risk tolerance, especially when compared with the expected benefits to result from the release of the EGD-LMO. For example, the suppression of an invasive rodent species may or may not be perceived as a significant benefit, depending on the stakeholder's aversion/tolerance to risk.

A particular stakeholder's perception of risk from the release of an EGD-LMO may also depend on the stakeholder's personal and cultural relationship with the environment, for example, whether the environment is a resource to be utilized or stewarded (Hartley and others 2023).

Lastly, when the broad dispersion of an EGD-LMO is likely, it may be challenging to obtain the free, prior and informed consent of indigenous peoples and local communities, as covered in United Nations Decision 14/19 on synthetic biology. Several recent papers have provided examples and proposed tools to define acceptance.

4.2. Problem formulation

Risk assessment frameworks often combine the process of establishing the context and scope with the identification of potential adverse effects associated with the EGD-LMO into a single step called "Problem formulation".

Problem formulation serves as a structured approach to risk assessment. It starts by identifying protection goals and defining assessment end points, which are specific measurable criteria used to evaluate potential adverse effects. This is followed by the identification of potential adverse effects of the EGD-LMO and its use. Conceptual models (sometimes referred to as "Pathways to harm") are then developed as working hypothesis to describe how the EGD-LMO may have adverse effects on the assessment end points. These models involve the description and modelling of scenarios and pathways on how the EGD-LMO may cause harm to a protection goal.

For instance, if the protection goal is the conservation of biodiversity, a risk hypothesis may assess how specific characteristics of the EGD-LMO could impact assessment end points. This includes assessing the consequences of the reduction of abundance of the EDG-LMO on predators, competitors or prey, as well as the potential replacement of ecological niches by other organisms within the receiving environment.

Precisely defining the assessment end points is crucial to focus the risk assessment and guide subsequent analyses.

Lastly, an analysis plan is developed for obtaining the needed data and how to test the hypothetical scenarios and pathways identified during problem formulation. This plan also outlines the approach for conducting experiments or studies to address the identified risk hypothesis and refine the understanding of potential adverse effects.

4.3. Choice of comparators

The choice of comparators plays a significant role in the risk assessment of EGD-LMOs. A comparative approach is aimed at identifying the phenotypic and genotypic changes that may lead to adverse effects, and changes in the nature and levels of risk associated to the EGD-LMO. The choice of comparators can have large effects on the relevance, interpretation and conclusions drawn from the risk assessment process. The one or more comparators that are chosen should therefore be selected on the basis of their capacity to generate information that is consistent and relevant for the risk assessment.⁷ However, for EGD-LMOs targeting non-domesticated or wild species, there may be limited information available on potential comparators.

The selection of appropriate comparators depends on the species of the EGD-LMO under assessment. In some cases, the line/strain used as a recipient organism for transformation may serve as a comparator. For example, when assessing an EGD-LMO is designed to control an invasive rodent population, the recipient strain of rodent could be an appropriate comparator. In the context of EGD-LM mosquitoes, although different from EGDs, the *Wolbachia* system can provide insights that inform the risk assessment process.⁸ In addition, when a strain of the EGD-LMO is developed through successive passages, the parental strain may be used as an additional comparator. Of course, consistent and relevant information could also be derived from use of the wild-type species as a comparator and should be considered in the risk assessment.

It is important to consider that an alternative to the comparative approach may become necessary when considering EGD-LMOs where appropriate comparators do not exist. In such situations, the characterization of an EGD-LMO may be similar to that carried out for alien species, where the whole organism is considered a novel genotype in the receiving environment.⁹

5. Conducting the risk assessment

To fulfil the objective under annex III to the Protocol, as well as provisions under other relevant articles, a risk assessment is conducted in a stepwise process and in an iterative manner, where any step during risk assessment can be reviewed to incrementally build on previous findings, for example, as a result of ongoing accumulation of information (data from applicant, expert advice, literature search) or when new information suggests that new issues need to be considered. In this section, the steps indicated in subparagraphs 8 (a) to (e) of annex III are described in further detail and elements for consideration are provided for each step.

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

⁷ Guidance on risk assessment of living modified organisms and monitoring in the context of risk assessment, UNEP/CBD/BS/COP-MOP/8/8/Add.1, section 1.4.3.

⁸ Study on Risk Assessment: Application of annex I to decision CP-9/13 to Living Modified Organisms Containing Engineered Gene Drives. Ad Hoc Technical Expert Group on Risk Assessment and Risk Management Online, 30 March-3 April 2020. CBD/CP/RA/AHTEG/2020/1/4. https://bch.cbd.int/protocol/risk_assessment/cp-ra-ahteg-2020-01-04-en-2.pdf.

⁹ Guidance on Risk Assessment of Living Modified Organisms and Monitoring in the Context of Risk Assessment, UNEP/CBD/BS/COP-MOP/8/8/Add.1, 14 September 2016. www.cbd.int/doc/meetings/bs/mop-08/official/bs-mop-08-08-add1-en.pdf.

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

5.1. Step 1. Identification of any novel genotypic and phenotypic characteristics associated with the EGD-LMO that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health

Rationale

The purpose of this step is to identify changes in the EGD-LMO that could cause adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health. The question that risk assessors ask in this step is “what could go wrong, why and how?” This step is very important in the risk assessment process as the answers to this question will determine what risk scenarios are considered in all subsequent steps. In many cases, this step is performed as part of a problem formulation process when establishing the context and scope of the risk assessment (see above).

In this step, risk assessors identify scientifically plausible risk scenarios and risk hypotheses to predict if the EGD-LMO could have an adverse effect on the assessment end points. This is done by examining if any of the novel characteristics of the EGD-LMO and/or its intended use could give rise to adverse effects in the likely potential receiving environment. The novel characteristics of the EGD-LMO to be considered can include any changes in the EGD-LMO, such as the specific gene drive mechanism employed, the nucleic acid alterations made (including any deletions), gene expression levels (if appropriate) and morphological and behavioural changes. The changes are considered in the context of the non-modified recipient or parental organisms in the likely potential receiving environment using the environmental conditions prior to the release of the EGD-LMO as baseline. Choosing appropriate comparators is particularly relevant for this step, in order to enable the consideration of the new trait(s) of the EGD-LMO (see section on the choice of comparators above).

Furthermore, it is important to define clear links or pathways, both direct or indirect, between the EGD-LMO and possible adverse effects in order to focus on generating information that will be useful in the decision-making. Potential adverse effects could arise, for example, from changes in the potential of the EGD-LMO to: (a) affect non-target organisms; (b) cause unintended effects on target organisms, such as the development of resistance to the engineered gene drive; (c) develop unintentional changes in fitness in ecosystems; (d) transfer genes to other organisms/populations, such as wild species with some sexually compatibility with the EGD-LMO; and (e) become genotypically or phenotypically unstable. Potential adverse effects may be direct or indirect, immediate or delayed, combinatorial or cumulative, as well as predicted or unpredicted (see below).

ILLUSTRATIVE EXAMPLE

Types of potential adverse effects on the environment or human health from the use of an engineered gene drive in the released EGD-LMO:

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

Indirect: effects occurring through a causal chain of events, through mechanisms such as interactions with other organisms or transfer of genetic material.¹⁰ Observations of indirect effects are likely to be delayed;

Immediate: effects which are observed during the period of the release of the EGD-LMO. Immediate effects may be direct or indirect;

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

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

ILLUSTRATIVE EXAMPLE

Identifying potential adverse effects to human health arising through environmental exposure.

In accordance with the Protocol, environmental risk assessments examine potential adverse effects to human health arising from environmental exposure. The potential adverse effects on humans due to environmental exposure may be direct or indirect.¹¹

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

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

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

Elements for consideration regarding characterization of the EGD-LMO:

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

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

(ii) Its taxonomic relationships;

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

¹⁰ The 2016 “Guidance on risk assessment” (CBD, 2016) refers to the “use and management” of LMOs, which is appropriate for LMO crops but is not appropriate in the context of EGD-LMOs.

¹¹ In the context of a harmful organism to which humans are normally exposed, the risk assessment may consider whether human exposure to an EGD-LMO version of the organism would cause different or more severe impacts.

- 1 (iv) Its ecological function;
- 2 (v) Whether it is a component of biological diversity that is important for the conservation and
- 3 sustainable use of biological diversity in the context of Article 7(a)¹² and annex I to the Convention;
- 4 (b) Relevant characteristics of the donor organism(s), such as:
 - 5 (i) Its taxonomic status and common name;
 - 6 (ii) Its provenance;
 - 7 (iii) Relevant biological characteristics;
 - 8 (iv) Relevant characteristics of the genes and of other functional sequences, such as promoters,
 - 9 terminators and selection markers, that have been inserted into the EGD-LMO, including functions
 - 10 of the genes and their gene products in the donor organism with particular attention to characteristics
 - 11 in the recipient organism that could cause adverse effects;
- 12 (c) Characteristics related to the transformation method, including the characteristics of the vector, such as
- 13 its identity, source or origin and host range, and information on whether the transformation method
- 14 results in the presence of (parts of) the vector in the EGD-LMO, including any marker genes;
- 15 (d) Molecular characteristics of the EGD-LMO related to the modification, such as characteristics of the
- 16 modified genetic elements, including potential toxicity of the gene products to non-target organisms and
- 17 clinical significance of any antibiotic resistance genes inserted into the EGD-LMO; insertion site(s) and
- 18 copy number of the inserts; stability, integrity and genomic organization in the recipient organism;
- 19 specificity of the genetic elements (e.g. transcription factors); levels and specificity of gene expression
- 20 and intended and unintended gene products, such as novel proteins being encoded by sequences put
- 21 together at the insertion sites or elongation of the intended protein owing to faulty or lacking terminator
- 22 sequences;
- 23 (e) Genotypic (see point (d) above) and phenotypic changes in the EGD-LMO, either intended or
- 24 unintended, including changes in native/endogenous gene expression and regulation at the
- 25 transcriptional, translational and post-translational levels (for example, toxic products of endogenous
- 26 upregulated genes).
- 27 Elements for consideration regarding the intended use and the likely potential receiving environment:
- 28 (f) Availability of data on the likely receiving environment which may serve as a basis for the risk
- 29 assessment;
- 30 (g) The intended spatial scale, duration and level of confinement (such as biological confinement) of the
- 31 environmental release, taking into account user practices and habits;
- 32 (h) Characteristics of the likely potential receiving environment, including relevant ecosystem functions
- 33 and services, in particular its attributes that are relevant to potential interactions of the EGD-LMO that
- 34 could lead to adverse effects (see also paragraph (k) below), taking into account the characteristics of
- 35 the components of biological diversity, in particular in centres of origin and centres of genetic diversity.

¹² The Convention on Biological Diversity 1992, Article 7. Identification and monitoring, www.cbd.int/convention/articles/?a=cbd-07.

ILLUSTRATIVE EXAMPLE

Attributes of the receiving environment.

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

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

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

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

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

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

(o) Potential for outcrossing and transfer of transgenes, by vertical gene transfer, from an EGD-LMO to other sexually compatible species that could lead to introgression of the transgene(s) into populations of sexually compatible species, and whether these would lead to adverse effects;¹³

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

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

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

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

Rationale

In this step the risk assessors evaluate the likelihood that each of the potential adverse effects identified in step 1 will occur.

An assessment of exposure is done in this step to determine which organisms in the receiving environment could be adversely affected by being exposed, directly or indirectly, to the EGD-LMO. During the exposure

¹³ The topic of vertical gene transfer is discussed below.

assessment, factors that may affect the spread, persistence and establishment of the EGD-LMO, as well as its potential for outcrossing are considered. For each of the risk scenarios and risk hypotheses identified in step 1, pathways of exposure to the EGD-LMO and its transgenes are determined, taking into account the intended handling and use of the EGD-LMO, as well as the expression level, dose and environmental fate of transgene products. Conceptual models describing relationships between the EGD-LMO and pathways of exposure can be built to define a causal link between the EGD-LMO and potential adverse effects in the environment, taking also into account risks to human health.

ILLUSTRATIVE EXAMPLE

Questions addressed during the exposure assessment.

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

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

ILLUSTRATIVE EXAMPLE

Exposure characterization

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

Likelihood of exposure can be expressed either qualitatively using an ordered categorical description (such as “high”, “moderate”, “low” or “negligible”) or quantitatively as a relative measure of probability (from zero to one, where zero represents impossibility and one certainty). However, if qualitative terms are used to express such likelihoods, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of 0 to 1, to which the term is intended to refer.

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

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

In some risk assessment frameworks or when a high level of uncertainty makes it difficult to assess the likelihood of the adverse effects the order of steps 2 and 3 may be reversed (see above and Figure 1).

Elements for consideration:

- (a) The relevant characteristics of the likely potential receiving environment that may be a factor in the occurrence of the potential adverse effects (see also step 1 (f), (g) and (i)), taking into account the variability of the environmental conditions and long-term adverse effects related to the exposure to the EGD-LMO;
- (b) Levels of expression in the EGD-LMO and persistence and accumulation in the environment (e.g. in the food chain) of substances with potentially adverse effects newly produced by the EGD-LMO, such as toxins, allergens and some insecticidal proteins. In the case of field trials, the level of persistence and accumulation in the receiving environment may be low depending on the scale and temporary nature of the release, and the implementation of management measures;
- (c) Information on the location of the release and the receiving environment (such as geographical and biogeographical information, including, as appropriate, geographical coordinates);
- (d) Factors that may affect spread of the EGD-LMO, such as its ecological range and ability to move; its reproductive ability, and its ability to spread using natural means (e.g. wind, water) or through human activities;
- (f) When assessing the likelihood of outcrossing from the EGD-LMO to sexually compatible species as a step in the pathway to an adverse effect, the following issues are relevant:
 - (i) The biology and reproductive biology of the sexually compatible species;
 - (ii) The potential environment where the sexually compatible species may be;
 - (iii) Persistence of the EGD-LMO in the environment;
 - (iv) Introgression of the transgene into the sexually compatible species;

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

Persistence in the context of living modified organisms containing engineered gene drives

In the experimental phase of the development of an EGD-LMO, the organism may be exposed to the environment through contained releases. Such releases provide valuable data to researchers regarding the efficacy of the gene drive and interactions of the EGD-LMO with the environment. When a contained release is properly designed and maintained, there should be no spread or environmental persistence of the EGD-LMO. Any indication that the EGD-LMO has escaped its containment could trigger a variety of mitigation measures and short- and long-term monitoring to minimize adverse impacts. The monitoring plan should include measures that could be quickly implemented should such an escape occur.

Once an EGD-LMO has been thoroughly tested in the laboratory and in contained field trials, unconfined releases may be proposed. At this point, the risk assessment for an EGD-LMO differs from that of a traditional LMO, such as a field crop. In the context of LMO crops, the intention is that the crop will be harvested and removed from the environment, limiting opportunities for the LMO crop to persist in the environment. However, in the context of an EGD-LMO, an unconfined release will likely be done with the intention that the organisms, or their progeny, will persist in the environment for an extended period of time (in the case of species suppression) or indefinitely (in the case of species replacement). Of course, transgene persistence in the environment will be of concern to the applicant since persistence will directly relate to the efficacy of the engineered gene drive in use. Transgene persistence in the environment may also be a matter of concern to regulators because any disparity in the predicted persistence and the actual persistence could indicate problems with the engineered gene drive system that could ultimately cause adverse impacts to biological diversity.

Unintentional prolonged persistence could indicate that an engineered gene drive designed to have only temporary presence in the environment may have an impact on the environment for a longer period of time than expected.

Unexpected disappearance of the transgene earlier than expected might indicate instability of the engineered gene drive system.

5.3. Step 3. Evaluation of the consequences should these adverse effects be realized

Rationale

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

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

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

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

The evaluation of the consequence of adverse effects may be expressed qualitatively or quantitatively. For instance, such qualitative terms as “major/high”, “intermediate/moderate”, “minor/low” or “marginal/negligible” may be used.

ILLUSTRATIVE EXAMPLE

Hazard characterization

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

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

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

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

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

Elements for consideration:

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

(i) Pathways for dissemination and spread;

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

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

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

(c) Relevant knowledge and experience with similar LMOs without the EGD (if any) and non-modified organisms with similar phenotypic characteristics in similar receiving environments;

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

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

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

5.4. Step 4. Estimation of the overall risk posed by the living modified organism based on the evaluation of the likelihood and consequences of the identified adverse effects being realized

Rationale

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

As indicated in subparagraph 8 (d) of annex III to the Protocol, the estimation of the overall risk is “based on the evaluation of the likelihood and consequences of the identified adverse effects being realized”. The characterization of overall risk is often the best estimate which is derived from the combination of the likelihood and consequences of the identified individual risks. Risk matrices, risk indices or models are typically used for this purpose (see below).

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

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

1 Elements for consideration:

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

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

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

7 **5.5. Step 5. Recommendation as to whether or not the risks are acceptable or**
8 **manageable, including, where necessary, identification of strategies to manage these risks**

9 Rationale

10 In step 5, risk assessors prepare a report summarizing the risk assessment process, identified individual
11 risks and the estimated overall risk, and provide recommendation(s) as to whether or not the risks are
12 acceptable or manageable and, if needed, recommendation(s) for risk management options that could be
13 implemented to manage the risks associated with the EGD-LMO. The recommendation is made in the
14 context of criteria for the acceptability of risk that were identified in the planning phase of the risk
15 assessment, taking into account established protection goals, assessment end points and risk thresholds, as
16 well as risks posed by the non-modified recipient organism and its use.

17 This step is an interface between the process of risk assessment and the process of decision-making.
18 Importantly, while the risk assessor provides a recommendation as to whether or not the risks are acceptable
19 or manageable, the ultimate decision about whether or not to approve the EGD-LMO notification is a
20 prerogative of the decision maker. On the other hand, the “acceptability” of risks is decided at a policy level
21 and the threshold of what is considered “acceptable” may vary from country to country, for instance, some
22 countries may choose to accept different levels of risk associated with the development of a certain
23 technology while others may not.

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

30 In accordance with annex III, subparagraph 8 (f), “where there is uncertainty regarding the level of risk, it
31 may be addressed by requesting further information on the specific issues of concern or by implementing
32 appropriate risk management strategies and/or monitoring the living modified organism in the receiving
33 environment”.

34 Environmental monitoring (see section 3) can be a means to reduce uncertainty, to address assumptions
35 made during the risk assessment, to validate conclusions of the assessment on a wider level of application,
36 and to establish a causal link or pathway between EGD-LMOs and adverse effects. Monitoring may also
37 be used to evaluate whether risk management strategies are being implemented effectively, including
38 whether those strategies are able to detect potential adverse effects before the consequences are realized.
39 Monitoring can also be applied as a tool to detect effects that were not anticipated in the risk assessment
40 and long-term adverse effects.

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

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

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

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

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

(c) Management options and their feasibility in the context of the intended and expected use (e.g. isolation distances to prevent outcrossing);

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

Elements for consideration related to the acceptability of risks:

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

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

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

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

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

6. Related issues

A key element concerning the conclusion of any risk assessment is whether the determined level of assessed risk is acceptable or not, and in many cases, this will be judged against the level of potential benefits that are expected to result from the deployment of the LMO in question. Balancing risk acceptability with

1 potential benefits is not laid out in the provisions of the Protocol. However, in some jurisdictions the
2 recommendation on the acceptability of risk(s) may take into account any available scientific analysis of
3 potential benefits for the environment, biodiversity, and human health (e.g. reduction of infections in the
4 case of EDG-LM mosquitoes) and may also take into account risks associated with other existing user
5 practices and habits.

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

11 In addition to the elements considered in a risk assessment process, other elements may be taken into
12 account in decision-taking. Resource materials can help guide decision makers. For example, the “Guidance
13 on the assessment of socioeconomic considerations in the context of Article 26 of the Cartagena Protocol
14 on Biosafety”¹⁴ provides a process and important principles for assessing socio-economic considerations
15 arising from the impact of LMOs on the conservation and sustainable use of biological diversity, especially
16 with regard to the value of biological diversity to indigenous peoples and local communities.

17

¹⁴ CBD/CP/MOP/9/10, annex.

PART B. RISK ASSESSMENT OF LIVING MODIFIED ANOPHELES CONTAINING AN ENGINEERED GENE DRIVE FOR MALARIA CONTROL

7. Introduction

Almost half of the world's population is at risk of malaria and, in 2021, there were 619,000 deaths from malaria¹⁵ and the control and reduction of malaria is a recognized public health goal. Research groups worldwide are currently engaged in the development of *Anopheles* mosquitoes bearing engineered gene drives designed to reduce the transmission of malaria. Several of these efforts have advanced to the point of confined releases into the environment, but so far there have been few unconfined releases.

Although numerous mechanisms have been developed to create a gene drive effect, to date, engineered gene drives in *Anopheles* have one of two broad purposes: the suppression of wild populations of *Anopheles* or the replacement of a wild *Anopheles* population with an EGD-LMO version that cannot vector malaria. While both of these purposes rely on self-sustaining gene drive strategies, population suppression involves a self-sustaining gene drive of temporary duration, while population replacement involves a self-sustaining gene drive of indefinite duration.

The impact of *Anopheles* mosquitoes on public health as vectors of malaria pose specific considerations and challenges during the risk assessment process. The risk assessment process will therefore depend both on the biology and ecology of non-modified *Anopheles* as well as on the specific characteristics of the EGD-LM *Anopheles* in question.

8. Objective and scope

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

9. Planning phase of the risk assessment of engineered gene drives in *Anopheles*

9.1. Choice of comparators

In a comparative risk assessment, risks posed by an EGD-LMO are considered in the context of the risks posed by the non-modified recipients or parental organisms, in the likely potential receiving environment, including local landraces and undomesticated species. In practice, a comparative approach is aimed at identifying, in relation to the appropriate comparator(s), the phenotypic and genotypic changes of an EGD-LMO that may lead to adverse effects, and changes in the nature and levels of risk of the EGD-LMO. The choice of comparators can have large effects on the relevance, interpretation and conclusions drawn from the risk assessment process. The one or more comparators that are chosen should therefore be selected on the basis of their capacity to generate information that is consistent and relevant for the risk assessment.

The mosquito line/strain used as a recipient organism for transformation may serve as a comparator for the risk assessment of EGD in *Anopheles* mosquitoes. Where successive passages are used to develop a strain of the EGD-LM mosquito, the parental LM strain may be used as an additional comparator (Connolly and others, 2021).

As technologies for genetic modification continue to advance and as the range of organisms subject to genetic modification grows, risk assessors should consider the need to expand their concept of what constitutes a useful comparator for the risk assessment. To date, the focus has been on comparator organisms, but there may also be a need for comparator activities. For example, EGD-LM mosquitoes

¹⁵ www.who.int/mediacentre/factsheets/fs094/en/.

designed for malaria control have modes of action that do not have exact comparators outside the realm of genetic modification, such as species suppression or species replacement. However, there are comparator activities, such as large-scale insecticide applications, the release of Wolbachia-infected, self-limiting mosquitoes, or the release of a predator species, which may generate information that is consistent and relevant to the risk assessment process of EGD-LMOs and could be considered by risk assessors.

10. Conducting the risk assessment of living modified *Anopheles* containing an engineered gene drive

10.1. Step 1. Characterization of the living modified mosquito containing an engineered gene drive

The first step in characterizing an EGD-LMO mosquito is to fully understand the unmodified species.¹⁶ This information forms an essential baseline against which the characteristics of the EGD-LM mosquito are compared. For example,

- What habitats are suitable to sustain *Anopheles* populations, and how do *Anopheles* colonize these habitats?
- What is the reproductive biology of *Anopheles*, and is there evidence of interspecific crossing, including with known species in the complex?
- What is the role of *Anopheles* in the environment (pollinator, food source, etc.) and what key interactions does it commonly have with other organisms?

Once the biology of unmodified *Anopheles* and its relationship with the environment is well understood, the risk assessor can begin to formulate questions regarding changes to the mosquito (both intended and unintended), resulting from genetic modification, that might cause harm to the environment (Gray, 2021). The applicant will be the primary source of information regarding the specific genetic modifications that were made to the mosquito, how these modifications function, and the genotypic and phenotypic outcomes of the modifications.

10.2. Step 2. Unintended effects on biological diversity (species, habitats, ecosystems, and ecosystem function and services)

10.2.1. Vertical gene transfer

Vertical gene transfer (VGT) is the transmission of one or more genetic elements from an organism to its progeny. VGT is essential to the function of gene drive systems, which are designed to accurately and efficiently transfer specific genetic elements to progeny (Bier, 2012). VGT is there an expected outcome in the deployment of organisms carrying a gene drive system. However, unintended outcomes from an engineered gene drive system that have an impact on VGT that may affect biological diversity, such as the following:

¹⁶ Because the *Anopheles* mosquito is a species with global importance, owing to its vectoring of malaria, there is a wealth of information regarding the biology of this organism. Examples of authoritative descriptors of unmodified host organisms are the biology documents published by the Organisation for Economic Co-operation and Development (OECD), such as the Consensus Document on the Biology of Mosquito *Aedes Aegypti*, Series on Harmonization of Regulatory Oversight in Biotechnology No. 65, [https://one.oecd.org/document/ENV/JM/MONO\(2018\)23/En/pdf](https://one.oecd.org/document/ENV/JM/MONO(2018)23/En/pdf). At the time of the submission of the current draft guidance for risk assessment of EDG-LMOs it is known that an advance draft of an OECD consensus biology document for *Anopheles gambiae* is in the last stages of review prior to publication.

- The EGD may completely fail to function, so that the desired VGT does not occur, and the intended effect of the EGD is not realized.
- The EGD may function correctly at first, having the desired effect, but after several generations, the EGD stops working.
- Conversely, an EGD system intentionally designed to stop working after a number of generations continues to operate past the intended limit.
- The EGD system acquires mutations which cause unwanted genetic material to be transferred to progeny.

10.2.2. Horizontal gene transfer

Horizontal gene transfer (HGT) is the transmission of genetic material from one species to another. HGT is common and well understood in prokaryotic organisms where mobile genetic elements, such as plasmids, facilitate the movement of genetic material between organisms of different species. In eukaryotes, the movement of genetic material is complicated by the presence of both the cell membrane and nuclear membrane and mechanisms to degrade foreign DNA, but specific instances of eukaryotes incorporating DNA fragments from prokaryotes are known to have occurred (Husnik, 2018). In the case of a species bearing an engineered gene drive, the genetic elements comprising the EGD are expected to spread by HGT within the species from the LMO individual to wild-type individuals, but unintended HGT of the EGD or its components could, theoretically, impact biological diversity.

- The organism carrying the EGD system could transfer it to a sexually compatible species.
- DNA fragments released into the environment from dead and decaying EGD-LMOs could be taken up by prokaryotes in soil or water and subsequently be incorporated into their genomes.

10.2.3. Persistence of the transgene in the ecosystem

In the experimental phase of the development of an EGD-LMO, the organism may be exposed to the environment during contained trials. Such releases provide valuable data to researchers regarding the efficacy of the gene drive and interactions of the EGD-LMO with the environment. When a contained release is properly designed and maintained, there should be no spread or persistence of the EGD-LMO. Any indication that the EGD-LMO has escaped its containment could trigger a variety of mitigation measures and short- and long-term monitoring to minimize adverse impacts. The monitoring plan should include measures that could be quickly implemented should such an escape occur.

Once an EGD-LMO has been thoroughly tested in the laboratory and in contained field trials, unconfined releases may be proposed. Generally, an unconfined release is done with the intention that the organisms, or their progeny, will persist in the environment for an extended period of time (in the case of species suppression) or indefinitely (in the case of species replacement.) Of course, transgene persistence in the environment will be of concern to the applicant since persistence will directly relate to the efficacy of the EGD in use. Transgene persistence in the environment may also be a matter of concern to risk assessors because any disparity in the predicted persistence and the actual persistence could indicate problems with the gene drive system that could ultimately cause adverse impacts to biological diversity.

- Unexpected persistence could indicate that an EGD designed to have only temporary presence in the environment may have an impact on the environment for a longer period of time than expected.
- Unexpected disappearance of the transgene earlier than expected might indicate instability of the EGD system.

10.2.4. Evolutionary response

If the release of an EGD-LMO causes a significant and prolonged ecological effect, there is the possibility for the development of evolutionary selection pressures. Such pressures could cause a variety of physiological and ecological changes to the EGD-LMO itself, as well as other organisms in the environment.

- When using a suppressive gene drive system in a disease vector, the long-term decline in vector population could create a selection pressure resulting in other species filling the ecological niche of the missing vector.
- When employing a suppressive gene drive approach, some individuals in the wild population may not be susceptible to the gene drive. Such individuals would be positively selected, resulting in the failure of the gene drive (Bier, 2022).

10.2.5. Unintentional transboundary movements

When EGD-LMO is released into a habitat under contained conditions, and those conditions are effectively and properly maintained and monitored, it would not be expected that the organism would move from the release site. However, if such an organism were released without containment, it would be expected that the organism would migrate away from the release site as long as the habitat conditions remained favourable for the survival of the organism. The rate of migration would depend on the biology of the organism in question. Theoretically, such movement could result in the EGD-LMO interacting with species, including alternate hosts for the gene drive that was not considered by the country where the release was authorized.

Mosquitoes, regardless of whether LM or not, have a very broad geographical distribution. Individual mosquitoes, however, within their lifetime have dispersal distances commonly of less than 5 km and for some urban species, as short as 200 metres. Confinement will therefore be highly dependent upon the species and the strategy used to develop the EGD-LM mosquito. For some EGD-LM mosquitoes, confinement may not be possible even when efforts are made to reduce long-distance dispersal due to anthropogenic activities. A dispersal spanning an international border would likely constitute an unpermitted release in the neighbouring country. As today EGD-LM mosquitoes are being designed to mostly target non-domesticated species, they will not be bound by national borders or territorial agreements. Many countries with less experience in LMO risk assessment are confronted, or anticipate being confronted, with possible applications of EGD-LMOs by transboundary movement, and they may be uncertain as to how to evaluate certain aspects (e.g. gene flow, unknown effects). However, there is currently no clear mechanism for transboundary considerations for EGD-LMOs (CBD 2020).

Elements for consideration:

- The type of strategy used in the development of the EGD-LM mosquito (i.e. “suppressive” or “species replacement”);¹⁷
- Presence of natural or artificial barriers that could limit the spread and unintentional transboundary movement of the EGD-LM mosquito.

10.3. Steps 4 and 5. Risk management strategies

Rationale

Where a risk has been identified that warrants a response through risk management or where there is uncertainty regarding the overall level of risk of the EGD-LM mosquito, risk assessors may consider

¹⁷ As gene drive technology is an evolving field, the terminology is similarly evolving. At the time of drafting, the two principal gene drive technologies are commonly called “suppression” and “species replacement”.

recommending such strategies as monitoring the EGD-LM mosquitoes to ensure that the technology is functioning as intended and to identify unintended adverse effects. The feasibility of any strategies for halting additional releases or destroying the EGD-LM mosquitoes that have been released, as well as mitigation methods if an unanticipated effect occurs, should be considered before any uncontained releases are permitted. Careful implementation of the technology, including the planning of mitigation measures (such as an alternative set of control measures should a problem occur) and the integration of other population control methods should also be taken into account. In some circumstances methods to reduce the persistence of the transgene in the environment or to mitigate adverse effects resulting from the expression of the transgene might be needed. Monitoring during and after the environmental release of the EGD-LM mosquitoes to enable prompt detection of unexpected adverse effects may also be considered.

Elements for consideration:

(a) Availability of monitoring methods to:

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

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

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

(iv) Determine transgene stability and proper function over time;

(v) Detect undesirable functions or behaviours within target species and other wild related species);

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

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

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

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

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

10.3.1. Containment of the EGD-LM mosquitoes

Rationale

Different strategies for the containment of EGD-LM mosquitoes can be applied, including physical, biological and chemical containment. In cases where there are uncertainties with regard to the potential adverse effects of a widespread release of EGD-LM mosquitoes into the environment, a release limited to in a particular geographical zone may be desirable. Any containment measures used as a means of limiting the release of the EGD-LM mosquito, either in location or in duration, must be taken into account in each of the steps of the risk assessment.

Elements for consideration:

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

11. Monitoring living modified *Anopheles* containing engineered gene drives released in the environment

The topic of monitoring LMOs released into the environment has been covered in general terms applicable to all species bearing any genetic modification (CBD, 2016). In this section, additional guidance will be provided specific to monitoring appropriate for the release of EGD-LM mosquitoes.

Monitoring associated with the planned release of EGD-LM mosquitoes begins before the release occurs and continues during and following the release. Some monitoring may be needed regardless of the species of mosquito and the genetic modification employed; however, the mechanism underlying the engineered gene drive and the specific genetic modifications used to implement that mechanism may necessitate the need for additional types of monitoring. The monitoring plan should take into account both these generic and specific information needs. The plan should also identify any non-target species that may affect, or be affected by, the release of the EGD-LM mosquito (Rasic and others, 2022).

Pre-release monitoring

Data collected prior to the release of the EGD-LMO mosquito can help determine the optimum release conditions, anticipate and avoid potential adverse impacts, and fine tune monitoring that occurs later during release and post release. Important pre-release data to be collected could include data on the population size, density, and dynamics (both spatial and temporal) of the target mosquito species and any non-target mosquito species expected in the release location, especially species competing for the same ecological niches as the target species (Rasic and others, 2022). Useful guidance for collecting these data may be found in the scientific literature from related fields, such as mosquito population control using *Wolbachia* (Hoffman, 2011). Other useful data would include whether there is gene flow between the mosquito target and non-target species known to exist in the release location and whether any of the non-target species are alternate vectors of the pathogen. Data regarding DNA polymorphisms in the sequence targeted by the EGD system would also be useful in the pre-release phase, as these could affect the long-term efficacy of the EGD system.

Monitoring during the release stage

During the time when the release of the EGD-LM mosquito is ongoing, monitoring can provide data on both the efficacy of the gene drive system and the effectiveness of any confinement measures in place during the release. Monitoring mosquito populations within the designated release area can indicate whether the EGD-LMs have established within the release area and whether the size of the native population of vector mosquitoes is decreasing. Monitoring for EGD-LM mosquitoes outside the designated release area is necessary to identify any potential failures of the prescribed confinement measures and provide guidance for mitigation measures.

Post-release monitoring

After the planned releases of the EGD-LM mosquito have been completed, the monitoring plan will include data collection to verify that any confinement measures imposed were effective. Results of initial post-release monitoring should be evaluated to determine how often and for how long additional monitoring should be repeated, and whether the monitoring plan should be updated. It is likely that data will also be collected during the post-release period to assess whether the gene drive was effective in reducing the population of pathogen-carrying mosquitoes, and that the gene drive spread spatially within the release zone as expected. The development of resistance to the gene drive within the vector population may also be assessed.

12. Related issues

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

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Annex I

Literature search

The methodology followed for the literature search is described by the following steps:

1. Formulation of the research questions

- a. How do risk assessments for Living Modified Organisms (LMOs) containing Engineered Gene Drive (EGD) differ from those for conventional LMOs, and what unique challenges do they pose?
- b. What is the current state of knowledge about the environmental risks associated with the release of LMOs containing EGD into the wild?
- c. What are the current guidelines and recommendations for environmental risk assessments of LMOs containing EGD? And for EGD *Anopheles*?

2. Development of keywords and search strategy (search strings)

The following keywords were chosen:

Engineered, LMO, GMO, Genetically modified, Gene drive, Genetic control, Biosafety, Biological control, Vector control, Ecological factor, Environmental harm, Environmental risk assessment, Field release, Governance, Hazard analysis, Modelling, Pathways to harm, Precautionary principle, Problem formulation, Regulation, Risk analysis, Uncertainty, Biocontrol, Alien species, Pest control, Management.

These were then combined in search strings using Boolean operators:

(Engineered OR LMO OR GMO OR “Genetically modification” OR Modified) AND (“Gene drive” OR “Genetic control”) AND (Biosafety OR “Biological control” OR “Vector control” OR “Ecological factor” OR “Environmental harm” OR “Environmental risk assessment” OR “Field release” OR Governance OR “Hazard analysis” OR Modelling OR “Pathways to harm” OR “Precautionary principle” OR “Problem formulation” OR Regulation OR “Risk analysis” OR Uncertainty OR Biocontrol OR “Alien species” OR “Pest control” OR Management)

3. Inclusion and exclusion criteria

Inclusion criteria: Gene drives; and Considerations on risks and risk assessment; and/or Extra challenges (or lack of) compared to other LMOs/GMOs; and/or Extra impact on the environment compared to other LMOs/GMOs; and/or Effects on biodiversity; and/or Release in the field/environment, and/or Aspects to limit spread/safeguards.

Exclusion criteria: Technical achievements, or Communication with stakeholders, or Social, economic, and ethical aspects, or Lab/cage containment.

4. Data collection

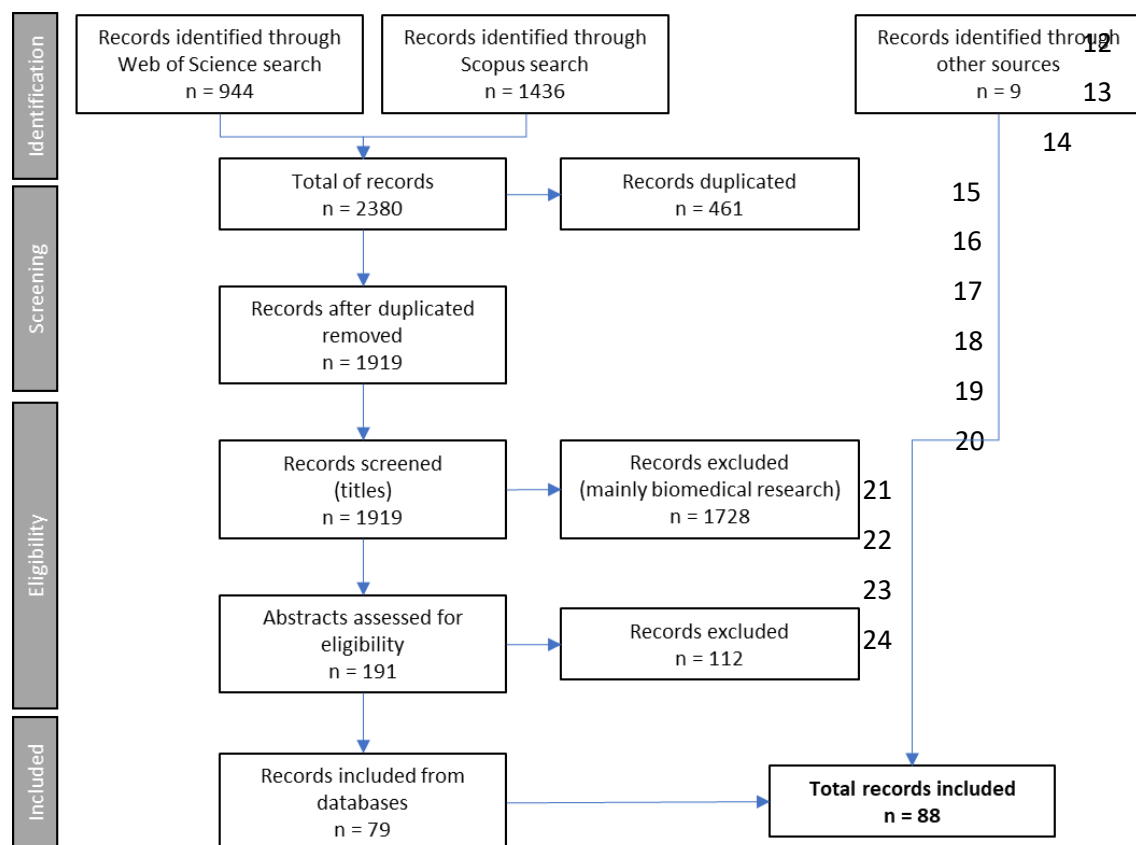
Web of Science and PubMed databases were chosen to search for relevant publications. The search was performed on 28 May 2023. After the elimination of the duplicate entries retrieved, 1919 publications were selected.

5. Selection based on title and abstract

The collection of publications was further narrowed down to 79 publications by employing the inclusion/exclusion criteria to title and abstract. There were nine publications that were identified separately manually.

6. PRISMA chart

The figure summarizes the key findings derived from the literature search.



Annex II

Conflict of interest disclosure

The following personnel were involved in the development of this detailed outline:

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In accordance with the rules and procedures specified in decision 14/33 of the Conference of the Parties to the Convention on Biological Diversity, the authors declare no conflict of interest.
