Secretariat
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BIOSAFETY TECHNICAL SERIES 07

Additional voluntary guidance materials to support case-by-case risk assessments of living modified organisms containing engineered gene drives

Convention on
Biological Diversity

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CBD BIOSAFETY TECHNICAL SERIES 07

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Secretariat of the Convention on Biological Diversity

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Foreword

I am delighted to contribute this foreword for a publication that we hope will advance the implementation of relevant provisions of the Cartagena Protocol on Biosafety and the Kunming-Montreal Global Biodiversity Framework, especially Target 17. "Biosafety Technical Series 07" compiles the *additional voluntary guidance materials to support case-by-case risk assessment of living modified organisms containing engineered gene drives*, which the Parties to the Cartagena Protocol on Biosafety welcomed at COP-MOP 11 on 30 October 2024 in Cali, Colombia.

This publication comes at a time when biotechnology is evolving at a rapid pace. This is epitomized by living modified organisms (LMOs) containing engineered gene drives, which are envisaged for crucial public health and conservation purposes: from tackling vector-borne diseases, such as malaria, or bolstering disease resistance in threatened species, to addressing invasive alien species — one of the major drivers of global biodiversity loss.

It is essential to ensure that tapping the potential of LMOs containing engineered gene drives does not come at the expense of people and nature. The challenge is to reconcile these biotechnological innovations with the precautionary approach and other provisions of the Cartagena Protocol on Biosafety. Rigorous environmental risk assessment as fostered by these additional voluntary guidance materials is key. The goal is to equip decision-makers with the information they need regarding the potential adverse effects that the release of LMOs containing engineered gene drives might provoke.

This publication rests on a solid foundation: Annex III to the Cartagena Protocol. The guidance materials it contains are aimed at strengthening the risk assessment provisions of the Protocol, while also building upon seminal work by the World Health Organisation, the Organisation for Economic Co-operation and Development and the European Food Safety Authority, as well as over 300 scientific publications.

Grounded in the universal recognition that the environmental risk assessment of LMOs containing engineered gene drives should be science-based and transparent, the guidance materials were also crafted to endure. Thanks to the approach it takes to risk assessment and the tools it provides, Biosafety Technical Series 07 will remain relevant even as further novel applications of engineered gene drives emerge.

Finally, Biosafety Technical Series 07 also crowns the process initiated under the Cartagena Protocol for the identification and prioritization of specific issues of risk assessment of LMOs that may warrant consideration (decision CP-9/13). This publication builds on the foundational work of the "Study on Risk Assessment: Application of Annex I of decision CP-9/13 to Living Modified Organisms containing Engineered Gene Drives" and the report of the 2020 meeting of the Ad Hoc Technical Expert Group on Risk Assessment. As such, it illustrates the ability of processes established under the Protocol to adequately meet the needs and priorities expressed by the Parties.

On behalf of the Secretariat of the Convention on Biological Diversity and its Protocols, I wish to express sincere gratitude to everyone who contributed to shaping this publication, including the members of the Ad Hoc Technical Expert Group on Risk Assessment, the Open-Ended Online Forum on Risk Assessment and the International Centre for Genetic Engineering and Biotechnology. We owe this publication to their labour and dedication, and to the support of the European Union and the Government of Finland.

Astrid Schomaker
Executive Secretary of the Convention on Biological Diversity

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Abbreviations

AHTEG	Ad Hoc Technical Expert Group
Cas	CRISPR-associated protein
CCA	Council of Canadian Academies
CRISPR	clustered regularly interspaced short palindromic repeats
CSS	Critical Scientists Switzerland
e-CHACR	erasing construct hitchhiking on the autocatalytic chain reaction
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EFSA GMO Panel	European Food Safety Authority Panel on Genetically Modified Organisms
EGD	engineered gene drive
EGD-LMOs	living modified organisms containing engineered gene drives
EGD-LMMs	living modified mosquitoes containing engineered gene drives
ENSSER	European Network of Scientists for Social and Environmental Responsibility
ERACR	element reversing the autocatalytic chain reaction
FAO	Food and Agriculture Organization of the United Nations
GDMM	gene drive-modified mosquito
GMM	genetically modified mosquito
ICGEB	International Centre for Genetic Engineering and Biotechnology
ISO	International Organization for Standardization
IUCN	Internation Union for Conservation of Nature
LMM	living modified mosquito
LMO	living modified organism
NASEM	National Academies of Sciences, Engineering, and Medicine
OECD	Organisation for Economic Co-operation and Development
SCBD	Secretariat of the Convention on Biological Diversity
US EPA	United States Environmental Protection Agency
VDW	Vereinigung Deutscher Wissenschaftler
VGT	vertical gene transfer
WHO	World Health Organization

1. Objective and scope

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 the case-by-case risk assessments of living modified organisms (LMOs) containing engineered gene drives (EGDs; EGD-LMOs) in accordance with annex III to the Protocol.[1] The Conference of the Parties serving as the meeting of the Parties to the Protocol decided that this material should have a special focus on living modified mosquitoes (LMMs) that contain an EGD (EGD-LMMs) taking into account the current experience with the organism, the type of EGD and specific issues of risk assessment identified in annex I to decision CP-9/13, including existing reports, general considerations of EGD-LMOs and existing national and regional risk assessment experiences. In decision CP-10/10, the Conference of the Parties serving as the meeting of the Parties to the Protocol also decided to establish an ad hoc technical expert group (AHTEG) on risk assessment that would develop the additional voluntary guidance materials, 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 would consider the outcomes of the work of the AHTEG at its twenty-sixth meeting.

As a response, and with the financial support of the European Union and the Government of Finland, the Secretariat of the Convention on Biological Diversity commissioned the International Centre for Genetic Engineering and Biotechnology (ICGEB) to develop a detailed outline to support the development of the additional voluntary guidance materials on the risk assessment of EGD-LMOs. The AHTEG revised the outline, then developed the detailed content of the guidance materials. The objective was to facilitate case-by-case risk assessment for EGD-LMOs, thereby complementing annex III to the Protocol and existing guidelines, while considering the established road map.[2]

Structure

The additional voluntary guidance materials were developed in accordance with annex III to the Protocol, in particular paragraph 8, which outlines the sequential steps of the risk assessment process.

These materials are structured as follows:

- Section 1 provides an overview of decision CP-10/10 and of the process by which the additional voluntary guidance materials were developed.
- Section 2 introduces EGD-LMOs, explains the precautionary approach, and establishes the context of the document.
- Section 3 provides details on EGD strategies, as well as opportunities and risk concerns.
- Section 4 outlines general risk assessment considerations for EGD-LMOs, steps of the

1 United Nations, Treaty Series, vol. 2226, No. 30619.

2 See decisions BS-IV/11 and BS-V/12 of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety, on risk assessment and risk management, 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.

problem formulation approach, and testing of risk hypotheses, including sources and quality of information, modelling, comparators, tier-based testing, limits of concern, weight of evidence, and uncertainties.
- Section 5 considers the making of a recommendation of acceptability of risk and identification of risk management strategies.
- Section 6 addresses monitoring of EGD-LMOs, taking into account general surveillance and case-specific monitoring.
- Section 7 describes related issues to risk assessment.
- Annexes I to VII provide further details on modelling, uncertainties, the WHO guidance framework for testing genetically modified mosquitoes, taxonomic classification of mosquitoes, mosquito vectors of diseases, the current landscape for the development of EGD-LMOs for disease vector control, and types of EGD systems.
- Annex VIII provides a list of terms with citations to assist the reader; it is not intended to constitute a glossary or list of definitions.
- References are listed at the end of the document.

2. Introduction

Advances in molecular and synthetic biology are enabling the engineering of living organisms with engineered gene drives. EGDs can be described as genetic elements that are sexually transferred to subsequent generations at a frequency greater than the 50 per cent expected by Mendelian inheritance (Burt, 2003; Burt and others, 2018; Champer and others, 2021; Hay and others, 2021; Raban and others, 2023; Wang and others, 2022), thereby biasing their own inheritance. This preferential inheritance may allow EGD systems (i.e. the EGD along with any genetically linked cargo/payload genes) to rapidly spread in sexually reproducing populations,[3] increasing their prevalence. EGD systems can be designed either to suppress or reduce interbreeding target populations or to modify them with an altered genotype. Depending on the design of the EGD system, a genetic modification of interest could potentially spread through target populations or species and persist indefinitely, or be restricted in its spread or persistence.

Due to the nature of EGDs, EGD-LMOs may differ significantly from non-EGD LMOs in their potential to spread, increase in frequency, persist in and/or suppress interbreeding target populations. EGD-LMOs may also differ from LMOs used in agriculture, as EGDs are generally designed to be applied in wild organisms (such as pests, disease vectors, invasive or endangered species), which commonly have higher genetic variability than domesticated organisms, and which may occur in receiving environments that are less well characterized and/or not managed by humans (Legros and others, 2021). It has also been noted that some EGD-LMOs may belong to species complexes that contain both vector and non-vector species, some combinations of which are capable of producing fertile interspecific hybrids. Such "semi-permeable" or "porous" species boundaries facilitate introgression and could plausibly lead to vertical EGD transfer among sibling species (Connolly and others, 2023; Courtier-Orgogozo and others, 2018). Depending on the EGD system, the envisaged effect of an intentional release may encompass several generations of the recipient organism. In comparison to non-EGD LMOs, an additional difference may pertain to the potential inability to halt the spread of the EGD (and EGD-LMO) or to reverse its action and effects.

While research on EGDs and their applications in living organisms is advancing, applications may take some years of technological development to move to practical applications for intentional release into the environment. Some living modified insects that contain an EGD have been tested experimentally in the laboratory, as well as in cage facilities (e.g. Hammond and others, 2021; Raban and others, 2020), but to date (February 2024) none have been released in small-scale confined or open release field trials.

Irrespective of their intended applications, concerns have been raised that the intentional release of EGD-LMOs into the environment may have adverse, unexpected and/or irreversible effects. These effects could include direct and immediate effects, as well as indirect, cumulative and/or long-term effects. Therefore, discussions have been held at various levels among

3 Analogous gene drive systems have also been developed in asexually reproducing bacteria with a view, for example, to control antimicrobial resistance (Valderrama and others, 2019).

indigenous peoples and local communities and various stakeholders, including policymakers, risk assessors, risk managers, developers and potential applicants, to determine whether there is a need to develop new or additional guidance for the risk assessment of EGD-LMOs for intentional release into the environment (Devos and others, 2021a, 2021b; Keiper and Atanassova, 2020; Simon and others, 2018).

Overall, it has been recognized that there are specific areas where further guidance is needed for the risk assessment of EGD-LMOs to ensure appropriate levels of safety. In 2016, the Secretariat of the Convention published general guidance on the risk assessment of LMOs,[4] which included mosquitoes among the examples of specific types and traits of LMOs. However, it did not contain specific guidance on EGD-LMOs. In addition, there are other guidance materials available that may also provide information relevant to EGD-LMOs (e.g. EFSA GMO Panel, 2020; NASEM, 2016; WHO, 2021).

2.1. Precautionary approach

In Principle 15 of the Rio Declaration on Environment and Development[5] it is stated that "in order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation."

Accordingly, in Article 1, the Cartagena Protocol provides as follows: "In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements."

In addition, in paragraph 6 of Article 10 of the Protocol, it is further articulated that "lack of scientific certainty due to insufficient relevant scientific information and knowledge regarding the extent of the potential adverse effects of a living modified organism on the conservation and sustainable use of biological diversity in the Party of import, taking also into account risks to human health, shall not prevent that Party from taking a decision, as appropriate, with regard to the import of the living modified organism in question as referred to in paragraph 3 above, in order to avoid or minimize such potential adverse effects".

2.2. Establishing the context

In most jurisdictions worldwide, the intentional release of LMOs into the environment is subject to risk assessment and regulatory approval. In this process, the role of risk assessors is to assess and provide scientific advice to risk managers on potential risks that the deployment of an LMO may pose to biodiversity, and to human and animal health. Risk assessment evaluates the various potential adverse effects and their associated likelihoods, taking into account the kinds and levels of exposure, to determine risks that might be associated with the use of an LMO for a particular purpose. The primary objective of a risk assessment is to identify and evaluate the potential risks of LMOs, while considering any relevant uncertainties and knowledge gaps. The outcome of the risk assessment serves as a foundation for informed decision-making regarding the use and the intended release of LMOs into the environment.

The risk assessment process starts by establishing the context and scope in a way that is consistent with the country's protection goals[6] (i.e. component of value that must be protected), the specific level of protection to achieve, and relevant policies. Establishing the context and scope for a risk assessment, in line with national policies and regulations as well as

4 UNEP/CBD/BS/COP-MOP/8/8/Add.1.

5 *Report of the United Nations Conference on Environment and Development, Rio de Janeiro, 3–14 June 1992*, vol. I, *Resolutions Adopted by the Conference* (United Nations publication, Sales No. E.93.I.8 and corrigendum), resolution 1, annex I.

6 Also termed general protection goals or generic endpoints.

international obligations, may involve an information-sharing and consultation process with risk assessors, risk managers, decision makers, indigenous peoples and local communities, and various stakeholders prior to conducting the actual risk assessment.

Several publications have elaborated on challenges related to the risk assessment of EGD-LMOs for intentional release into the environment (e.g. CSS, ENSSER and VDW, 2019; Dolezel and others, 2020; EFSA GMO Panel, 2020; NASEM, 2016; SCBD, 2020;[7] Then, 2020; Then and others, 2020; WHO, 2021).

Challenges in the risk assessment of EGD-LMOs may arise due to large spatial and temporal scales, as well as the heterogeneity in key factors such as target population genotypes and likely potential receiving environments, making it more difficult to characterize variability. In addition, a limited availability of knowledge and understanding regarding the behaviour in the laboratory versus the behaviour in the field over a large space and time may challenge the assessment. Genotype by environment interactions, as well as evolutionary effects, may contribute to the challenges in the risk assessment of EGD-LMO.

Agreed general principles of the risk assessment of LMOs are laid down in paragraphs 3 to 6 of annex III to the Cartagena Protocol. Risk assessment:

- Is ***science***-based. According to the Protocol, the risk assessment of LMOs shall be carried out in a scientifically sound and transparent manner, in accordance with annex III and taking into account recognized risk assessment techniques. Such risk assessment shall be based, at a minimum, on information provided in accordance with paragraph 9 of annex III to the Protocol and other available scientific evidence in order to identify and evaluate the potential adverse effects of LMOs on the conservation and sustainable use of biological diversity, taking also into account risks to human health.
- Is carried out on a ***case-by-case*** basis, meaning that it varies depending on the biology and ecology of the species under consideration, the introduced modifications and traits; the intended uses of the LMO (the scale and frequency of the intended release), the likely potential receiving environments (covering the likely potential receiving environments where the LMO will be released and spread), and the interactions among these variables. Thus, the potential adverse effects caused by an LMO on protection goals will vary depending on its characteristics, how it is used, the environment in which it is present, and across time.
- Uses a ***comparative*** approach, whereby the level of risk is estimated through comparison with the non-modified recipient or parental organism in the likely potential receiving environment.
- Is ***transparent***, *and* ***iterative*** when examining previous conclusions in the light of new information. Hence, a risk assessment may be revisited when new information arises or a change in circumstances has occurred that could change its conclusions.

There are some additional approaches that are also used in practice; they typically include:

- When appropriate, following the ***step-by-step*** principle, in which the deployment of an LMO proceeds iteratively through multiple phases, with each phase involving a larger spatial and temporal scale and a higher degree of human, animal or environmental exposure and realism. Relevant information gathered under controlled, contained conditions would provide confidence that the LMO can safely progress to the next testing and release phase (Hayes and others, 2018a; James and others, 2018; NASEM, 2016; WHO, 2021).
- Considering ***familiarity***, as it plays a key role in setting the context for the risk assessment (OECD, 2023). Familiarity arises from knowledge of and experience with the biology of the non-LMO, the introduced trait, and the receiving environment (OECD, 1993).
- Evaluating risk hypotheses in a ***tier-based*** test system because the likelihood of detecting potential hazards is higher in well-controlled lower-tier studies than in more complex field studies (Sanvido and others, 2012; see section

7 CBD/CP/RA/AHTEG/2020/1/5.

4.2.4). Using this approach, tests are initially conducted representing worst-case scenarios of exposure and/or consequence and are then progressively made more realistic, as appropriate. In so doing, hazards are evaluated within different tiers that progress from worst-case exposure and/or consequence scenario conditions (e.g. framed in highly controlled laboratory environments) to more plausible scenarios (e.g. under semi-field or field conditions). The underlying rationale is that when risks are acceptable under high-exposure conditions, they would be also acceptable at more realistic levels of exposure; for instance, if toxicity testing in a laboratory with high doses indicates no toxicity, there is no need for further testing at larger scales where doses will be much lower (EFSA GMO Panel, 2010).

- Using ***problem formulation*** as a way to frame the risk assessment process by clarifying policy goals and scientific criteria for assessing risks and devising risk hypotheses that meet those criteria. This approach enables risk assessors to identify a spectrum of potential adverse effects derived from the deployment of an LMO, to devise one or more plausible pathways to such harms, and to define the actual information needed to assess the likelihood of these potential adverse effects occurring and their seriousness.

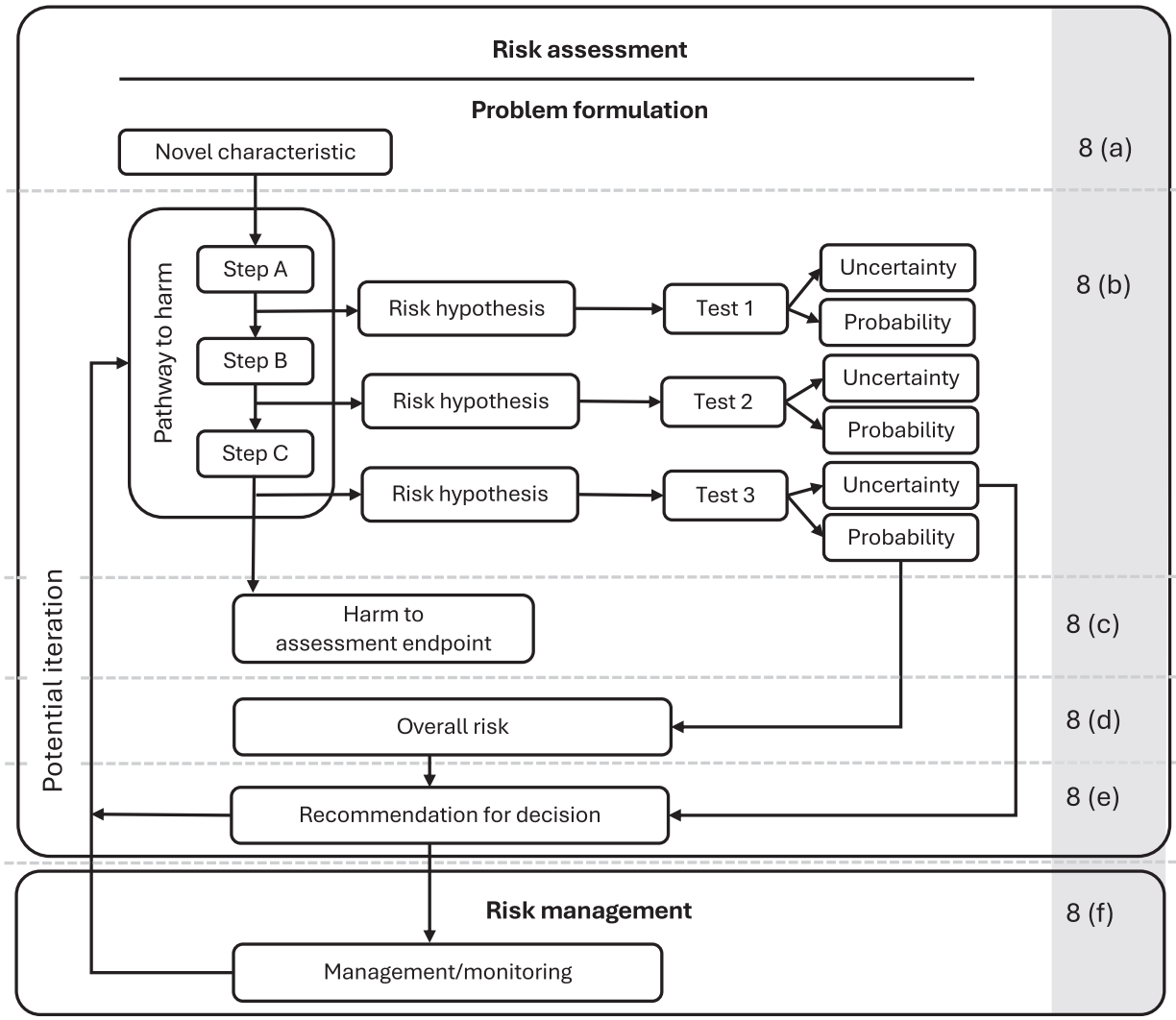

FIGURE 1
Risk assessment steps presented in this guidance and their linkages to paragraph 8 (a) to (f) of annex III to the Protocol (shown on grey background). Iteration in the light of new information may be performed to support decision-making. Steps A to C depict a single pathway to harm.

The present additional voluntary guidance materials introduce problem formulation as the first step of risk assessment; this approach is being widely applied by governments and relevant international organizations (e.g. CCA, 2023; EFSA GMO Panel, 2020; European Union, 2018; NASEM, 2016; OECD, 2023; WHO, 2021). The testing of the risk hypotheses of the plausible pathways to harm would be performed in the subsequent risk assessment steps consistent with paragraph 8 of annex III to the Protocol, as outlined in figure 1. At each step of the plausible pathway to harm, more detailed information on probabilities and uncertainties is provided. In addition, participation and engagement of stakeholders and indigenous peoples and local communities can be included at all points in the process, as appropriate.

3. Engineered gene drives

Recent advances in molecular and synthetic biology, including the discovery of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) systems (referred to hereafter as CRISPR-Cas, with CRISPR-Cas9 being a specific example), have delivered molecular tools, in combination with computational tools, that enable the design and development of a wide range of EGD systems in diverse organisms, with most initial focus on insects and rodents (Sanz Juste and others, 2023).

Scientists are working to utilize gene drives, either by modifying, redesigning and repurposing naturally occurring drive systems, or by designing and engineering novel systems, resulting in EGDs.[8] The use of EGD-LMOs is proposed to address challenges related to disease vectors and improvement of human and animal health (e.g. mosquitoes and ticks), agricultural production and pests (e.g. various fruit flies, screwworms and beetles), and invasive species (e.g. rodents) and conservation of species, as well as help to rescue endangered species (Devos and others, 2022; Raban and others, 2020; Wells and Steinbrecher 2023a,b). EGD systems can be categorized into two main mechanisms, namely over-replication mechanisms or interference mechanisms (see list of terms, annex VIII).

BOX 1: Mosquitoes: an introduction[9]

Mosquitoes belong to the family Culicidae in the order Diptera. The family is composed of at least 3,722 species (Harbach, 2023) under 41 recognized genera (Foster and Walker, 2019), grouped into two subfamilies, namely, Anophilinae (3 genera) and Culicinae (38 genera) (see annex IV). Mosquitoes have four life stages: the egg, larva, pupa and adult. Their life cycle is completed in aquatic (eggs, larvae and pupae) and terrestrial (adults) environments.

For a number of species, adult female mosquitoes require a blood meal (male mosquitoes do not bite) to provide the necessary nutrients for the successful development of viable eggs. Depending on the species, they blood feed on vertebrates such as amphibians, birds, mammals including humans, and reptiles (Clements, 1992). This behaviour presents major health risks to humans, livestock, and wild animals, as it can contribute to the transmission of pathogens from infected hosts (Foster and Walker, 2019). A non-exhaustive list of mosquitoes reported to transmit pathogens is presented in annex V.

Once the adults emerge, they shelter in vegetation, cavities and resting sites, or forages a few dozen meters away from their larval habitats (Foster and Walker, 2019). Several factors influence adult dispersal, such as larval predation risk (Alcalay and others, 2021), light (Bailey and others, 1965; Wellington, 1974), temperature (Marinho and others, 2016; Reinhold and others, 2018), and vegetation (Dufourd and Dumont, 2013). In addition, depending on the species, mosquitoes may travel hundreds of kilometres via wind dispersal (Yaro and others, 2022), human transport (Eritja and others, 2017), mass migration (Hume and others, 2003; Talapko and others, 2019) or international trade (Swan and others, 2022).

While the majority of research has focused on the role of mosquitoes as vectors of diseases, more recent studies have been investigating their roles in the ecosystem (Collins and others, 2019).

8 See also annex VII.

9 In line with the request in decision CP-10/10, mosquito-specific content has been included in boxes throughout the text.

BOX 2: Mosquitoes: mosquito-borne diseases

Malaria and dengue are among the most significant mosquito-borne diseases. The dynamics of these diseases are the result of a complex interplay between a number of biological, demographic, environmental, cultural and socioeconomic factors such as, insecticide resistance, land use, urbanization, globalization, climate change and limited access to health care (ECDC, 2023b; Institute for Health Metrics and Evaluation, 2024; Messina and others, 2019; WHO Regional Office for South-East Asia, 2022).

Malaria

Almost half of the world's population is at risk of malaria. According to a recent WHO report, of the 249 million new cases and 608,000 deaths in 2022 (WHO, 2023c), Africa shared the highest burden. Globally, 76 per cent of recorded malaria deaths (nearly 1,300 deaths daily) were of children under the age of five. In 2022, four countries in the African region, Nigeria (26.8 per cent), Democratic Republic of the Congo (12.3 per cent), Uganda (5.1 per cent) and Mozambique (4.2 per cent), accounted for nearly half of all malaria cases globally (WHO, 2023c).

Out of the 500 *Anopheles* species described in the world, more than 30 species are recorded as vectors of the five human malaria pathogen species (*Plasmodium falciparum, P. vivax, P. ovale*, *P. malariae* and *P. knowlesi*) (WHO, 2023c). These *Anopheles* vectors tend to prefer to feed on humans (Jeyaprakasam and others, 2022, Piedrahita and others, 2022; Massey and others, 2016).

Dengue

WHO (2023a) reported that 3.9 billion people are at risk of getting dengue fever. In 2023, more than 6 million dengue cases with more than 6,000 dengue-related deaths were reported in 92 countries/territories (ECDC, 2024). At least eleven *Aedes* species are recorded to vector the dengue virus (annex V).

Aedes aegypti is the primary vector of the dengue virus. Its current distribution includes the tropics and a number of sub-tropical regions (including the south-eastern United States, the Middle East, South-East Asia, the Pacific and Indian islands, and northern Australia) (ECDC, 2023a). *Aedes albopictus* is considered the secondary vector of dengue virus and has been included recently in the top 100 invasive species list of the Invasive Species Specialist Group (IUCN, 2024). Both are opportunistic feeders, but prefer human blood meals (Takken and Verhulst, 2013).

The control and reduction of mosquito-borne diseases is a recognized public health goal, and a range of novel strategies are currently being developed. Included in these are the development of *Anopheles* and *Aedes* mosquitoes bearing EGDs designed to reduce the transmission of diseases.

3.1. Engineered gene drive strategies

Strategies for EGD-LMOs can be differentiated on the basis of: (a) the intended outcome; and (b) the potential for the genetic modification to spread in target populations by mating and persistence in the environment after release (figure 2). Strategies aiming for population modification require the genetic modification of interest to persist in the population over an extended period (James and others, 2018).

Depending on the design of the EGD system (whose composition and mode of action are diverse), the genetic modification of interest could spread through interbreeding target populations (non-localized) and persist indefinitely (self-sustaining), or be restricted in its spread (localized) or persistence (self-limiting)

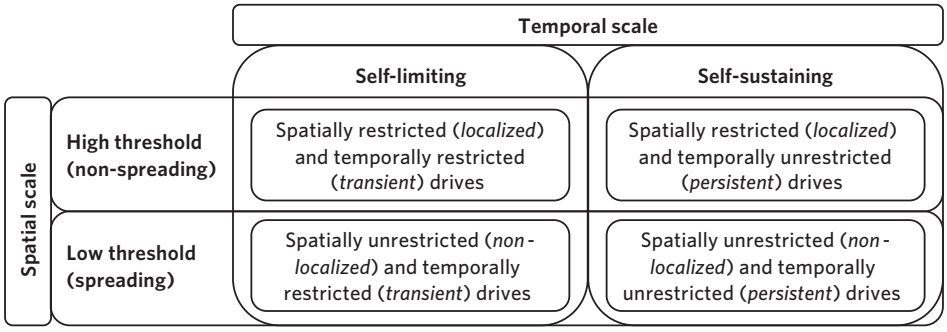

FIGURE 2
Possible elements to categorize engineered gene drive strategies

(CCA, 2023; EFSA GMO Panel, 2020; WHO, 2021). While the binary divides between localized/non-localized and self-sustaining/self-limiting systems are informative, it is important to consider that there is a spectrum of spreading and persistence within and between each category (Alphey, 2014), which can be affected by ecological factors (Backus and Delbourne, 2019; Dhole and others, 2018, 2020). Moreover, some types of EGDs are not clearly distinct, and they can be used alone or in combination with other types of EGDs. EGD-LMO approaches and applications will likely continue to expand as gene editing tools become more refined (Guichard and others, 2019; Holman, 2019; NASEM, 2016). Consequently, the initial "prototype" EGDs reported in the scientific literature may not necessarily be representative of the EGD systems that are currently under development or progressing to field testing, which aim to be more specific, stable and controllable systems (Friedman and others, 2020; NASEM, 2016; Raban and others, 2020).

Current research efforts also focus on the development of EGDs that would be confinable (i.e. limited in spread and/or persistence) and reversible (i.e. recallable from the environment) (e.g. Backus and Delborne, 2019; Buchman and others, 2021; Hay and others, 2021; Kandul and others, 2021; Li and others, 2020; Maselko and others, 2020; Oberhofer and others, 2021; Sánchez and others, 2020b; Terradas and others, 2021; Webster and others, 2020; Willis and Burt, 2021). Several approaches, some of which have already been tested experimentally under laboratory settings, have been proposed to restrict either spread of EGDs within a specified target population or geographic region, or their persistence (Raban and others, 2020). Examples include high-threshold EGD systems such as underdominance (heterozygote inferiority) EGDs, tethered homing-based EGDs, and split rescue EGDs (Hay and others, 2021).

Other localization approaches under development and/or investigation are EGD systems that target alleles that are only present in a genetically isolated (local) subpopulation of the target species or fixed in such isolated subpopulations (Sudweeks and others, 2019; Willis and Burt, 2021), and split homing-based EGDs, in which the Cas9 nuclease is separated from the guide RNA at different loci on chromosomes or lines of insects that would need to be crossed (Kandul and others, 2021; Li and others, 2020; Terradas and others, 2021). Nash and others (2019) evaluated the concept of integral EGDs that are based on multiple interacting components, each one of which could be tested separately or in combination. The modularity and interdependence of integral gene drive components may enable testing from self-limited to self-sustaining components in the field by modulating the propensity to spread in target populations (Nash and others, 2019).

BOX 3: Mosquitoes: engineered gene drive systems for living modified mosquitoes

Currently, two distinct intended uses are being explored to control mosquito-borne diseases using EGDs. EGDs for use in disease-transmitting mosquitoes are designed either to suppress target populations and potentially species, or to modify them with a new genotype (see figure 3).

- Population suppression strategies aim to reduce a target population by imposing a substantial fitness cost via the inactivation of important genes involved in the survival (non-developing offspring) or reproduction of the target population (e.g. reducing fertility of offspring, bias of the sex ratio toward males), or through the introduction of a new gene or genes that reduce(s) lifespan or bias(es) sex ratios (Buchman and others, 2018b; Galizi and others, 2014, 2016; James and others, 2018; Kyrou and others, 2018; Leitschuh and others, 2018; Simoni and others, 2020). These suppression strategies are expected to result in population decline/reduction or even collapse (local elimination) over a period of a few generations and may in some cases aim for (global) eradication of a disease vector species (Comité scientifique du Haut Conseil des Biotechnologies, 2017). In the case of disease-transmitting mosquitoes, model predictions suggest that it is unlikely that population suppression strategies would completely eliminate a species in the field (North and others, 2019). Strategies aiming for population suppression from a single release would require the genetic modification of interest to persist, despite the fact that EGD-LMMs are expected to decrease to low numbers as the overall target population is reduced. Alternatively, repeated releases over time would be required to reach and maintain suppression.
- Population modification strategies are used to modify a current genotype with one that is designed to be less able to transmit disease (impaired vector competence), or that is more resistant to pathogen infection (disease refractory) (Buchman and others, 2019, 2021; Carballar-Lejarazú and James, 2017; Carballar-Lejarazú and others 2020; Franz and others, 2006; Hedge and Hughes, 2017; Jupatanakul and others, 2017; Mathur and others, 2010; Pham and others,

2019). These strategies can be based on the inactivation of a gene or genes that are required for the target organism to transmit the pathogen (e.g. a tendency to feed on humans in the case of mosquitoes), or that are involved in pathogen survival in the mosquito. They can also involve the introduction of a new gene or genes, such as those that produce molecules that block pathogen development, or that kill the pathogen in the mosquito (Gantz and others, 2015; Hoermann and others, 2021; James and others, 2018; Carballar-Lejarazú and James, 2017). In order to be spread by an EGD, cargo/payload genes must be co-inherited with the EGD (i.e. be genetically linked to it). Strategies aiming for population modification require the genetic modification of interest to persist (James and others, 2018).

Depending on the design of the EGD system, the genetic modification of interest could spread through interbreeding target populations (non-localized) and persist indefinitely (self-sustaining) or could be restricted in its spread (localized) or persistence (self-limiting):

- Self-sustaining EGD systems can be described as those in which the genetic modification is intended to become stably established in target populations. They can be designed to spread a genetic modification of interest in target populations rapidly, w idely and for an indeterminate time or until the target population is eliminated (Alphey, 2014). Since self-sustaining EGDs can be engineered to be spatially and temporally unrestricted (non-localized and persistent, respectively), they could move to any interbreeding target population that has vertical gene flow with the target population where the EGD-LMMs are released, within a relevant timeframe (Noble and others, 2018). Once established, such self-sustaining approaches are intended to be relatively stable and require only smaller and infrequent secondary releases.
- Self-limiting EGD systems can be described as those in which the genetic modification of interest is expected to be temporally limited (transient) and disappears from the target population in the absence of additional periodic releases. The number of generations over which the genetic modification of interest will remain apparent will vary according to the genetic control system employed. Conceptually, EGDs could be engineered to increase the frequency of the genetic modification of interest in a population for a limited number of generations, after which the frequency of the genetic modification of interest in the population decreases and is then lost from the target population. Genetic modifications of interest could either change harmful population characteristics or suppress population density (Gould and others, 2008; Noble and others, 2019).

Inherent in many EGD systems is the requirement for individuals to be released above a certain threshold frequency before they will drive the genetic modification of interest through the target population (Alphey, 2014; Backus and Delborne, 2019; Dhole and others, 2020; Leftwich and others, 2018). This threshold refers to the proportion of EGD-LMM individuals with respect to the total target population that will reliably initiate spread of the genetic modification of interest. This threshold is determined as a combination of the action of the EGD system and its fitness load (Alphey, 2014; Leftwich and others, 2018).

- Low-threshold (non-localized) EGDs may spread from very low initial population frequencies, requiring only a small number of EGD-LMM individuals to be released to spread (Noble and others, 2018). Such types of EGDs have a higher potential to spread into neighbouring populations for an indeterminate time (Alphey, 2014; Champer and others, 2016). The lower the threshold, the more likely that dispersal of low numbers of EGD-LMM individuals could be sufficient to initiate spread of the genetic modification of interest in neighbouring target populations.

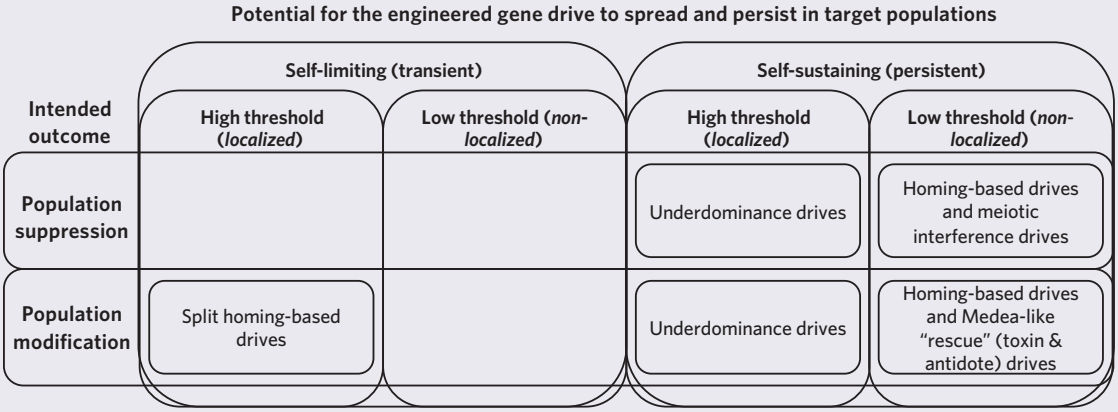

FIGURE 3
Selected examples of engineered gene drive approaches for mosquitoes

- High-threshold (localized) EGDs spread only if the number of EGD-LMM individuals reaches a high proportion in the target population, requiring a larger introduction (or proportion) of EGD-LMM individuals to be successful, compared to threshold-independent EGDs. These types of EGDs may enable local confinement. Simple population models predict spread to a high frequency in areas connected to the target area (in which the EGD-LMM individuals would be released broadly) but low levels of dispersal would be inhibited, as the genetic modification of interest fails to reach the threshold frequency needed (Marshall and Hay, 2012). However, as dispersal to neighbouring populations increases, spatial restriction to the targeted population may not be assured (e.g. Champer and others, 2020c; Dhole and others, 2018, 2020; Marshall and Hay, 2012).

The degree of persistence and, in particular, the spread of a specific EGD in target mosquito populations represent key considerations in the case-by-case risk assessment of EGD-LMMs, given their inherent implications for exposure and hazard characterizations. For current examples of EGD-LMMs, including their intended effect in terms of spread and persistence, see annex VI.

3.2. Opportunities and risk concerns

The ability to engineer gene drives has sparked both enthusiasm and concerns (Brossard and others 2019; Deplazes-Zemp and others, 2020; Esvelt and others, 2014). Some examples of opportunities and risk concerns are given below.

3.2.1. Opportunities

The use of EGDs could achieve goals that are otherwise challenging to attain, such as reaching parts of target populations that are missed by conventional methods, ensuring high target specificity compared to most conventional methods, and providing ongoing effects with relatively little or no further input.

There is potential for the use of EGDs to achieve biodiversity protection and conservation goals, agricultural management, and/or to positively impact human and animal health (Kelsey and others, 2020; Leitschuh and others, 2018; Neve, 2018; Preston and others, 2019). EGDs may be one of the most promising tools to control invasive species, which are a significant driver of species extinctions (Bellard and others, 2016; Clavero and Garcia-Berthou, 2005). For example, EGDs could be used to limit the reproductive capabilities of invasive species that have adverse impacts on an ecosystem, where they may provide a more sustainable and/or targeted solution compared to traditional methods like chemical or physical control.

EGDs may also be leveraged for disease vector control, including for non-native diseases with significant adverse impacts, including extinction, on native species (e.g. avian malaria in Hawai'i). Specifically with regard to insect pests, some other control strategies, such as sterile insect technique, often require multiple releases of large number of organisms to overwhelm the target pest and achieve efficacy, which may not be feasible. In contrast, self-sustaining gene drives aim to allow for a small number of individuals to be released into the population. Thus, the use of EGDs aim to reduce disease-transmitting insect populations, which could benefit ecological and human health outcomes.

Gene drives may also be beneficial for the management of agricultural pests. Pests destroy more than 40 per cent of the worldwide food supply (Oerke and others, 1994; Pimentel, 1997). The common methods to control these pests are via chemical pesticides, which can be toxic to wildlife and humans. EGDs may offer a unique opportunity to alter pests to reduce their fitness or their pest potential, while requiring a limited release of individuals (dependent on the type of EGD) and with low levels of off-target toxicity compared to chemical approaches (Legros and others 2021). Given that rodent pests have proliferated further with recent shifts to conservation agriculture, rodents are organisms where gene drives could be useful and are currently in research and development (Ruscoe and others, 2021, 2023). Gene drive development also shows promise for invasive weed and insect control and may contribute to reducing food supply breakdown. For example, the use of EGDs could enable effective control of aphids, which are pests, as well as vectors for plant viruses, of agricultural plants in many countries (Guo and others, 2022; Legros and others, 2021).

Finally, a key opportunity for the use of EGDs is in the fight against malaria and other vector-borne diseases. This opportunity may help to improve human health in many developing countries and economies, particularly among children under the age of 5 years. Based on challenges experienced with vector control interventions to reduce mortality linked to the spread of diseases such as malaria and dengue, the need for additional methods to combat mosquito-borne diseases is widely recognized. Currently available methods to control mosquito vectors are based on the use of insecticides, bed nets, mass release of sterile males, housing improvements, addressing social determinants of health, and elimination of mosquito larval breeding sites. Therefore, for both operational and economic reasons, there is a recognized need to add new, sustainable and cost-effective vector control tools. Recent research offers the possibility that LMMs, including EGD-LMMs, could be used as a complementary tool to prevent pathogen transmission (Fouet and others, 2024; WHO, 2021).

3.2.2. Risk concerns

Unlike other LMOs, EGD-LMOs are specifically designed to disperse beyond their initial release locations and persist in target populations over extended periods and generations in order to control disease vectors, agricultural pests and invasive species, or to rescue endangered species.

Concerns have been raised that EGDs may adversely impact biodiversity and human and animal health, lead to undesired side effects and uncontrolled spread, and alter organisms, populations or species and ecosystems in unwanted, unanticipated and irreversible ways with no current ability for recall (e.g. Cotter and others, 2020; CSS, ENSSER and VDW, 2019; Dolezel and others, 2020; Esvelt, 2014; Simon and others, 2018; Then, 2020; Then and others, 2020). Those unique characteristics necessitate a comprehensive assessment of ecological risks with a broader spatio-temporal scale (Connolly and others, 2022; SCBD, 2020).[10]

A concern is that the release of a small number of EGD-LMOs, depending on their design, could result in the genetic modification of interest spreading throughout the entire population of the targeted species in the wild. As a result, the potential ecological and health consequences of certain EGD-LMOs could be far-reaching (Kuzma, 2019). Moreover, some EGDs may raise novel risk assessment and risk management challenges (Connolly and others, 2021; CSS, ENSSER and VDW, 2019; Devos and others, 2021a, 2021b; Dolezel and others, 2020; EFSA GMO Panel, 2020; Hayes and others, 2018b; NASEM, 2016; SCBD, 2020;[11] Simon and others, 2018; Then, 2020; Then and others, 2020). There is also evidence suggesting that some EGDs are functioning under molecular mechanisms or behaviours different from the intended design. For example, population reduction EGDs may potentially result in mixed populations with unpredictable chasing dynamics[12] (Champer and others, 2021). Homing EGD systems designed to operate via the expected CRISPR-based homing process may instead function via an unintended meiotic mechanism at least in part, and in some studies, exclusively, via unintentionally decreasing the inheritance of the non-drive recipient chromosome (Li and others, 2020; Terradas and others, 2021; Verkuijl and others, 2022; Xu and others, 2020). Certain designs of EGD aim to reduce risks in terms of controllability by intended self-limiting or threshold-dependent behaviour. Depending on ecological conditions and receiving population these design goals may not be realized in the wild, resulting in unlimited or low-threshold EGDs. Therefore, effective risk assessment and risk management protocols must be capable of addressing these concerns, ensuring a thorough evaluation of the potential impacts of EGD-LMOs.

The above-mentioned risk concerns and associated uncertainty have led some scientists, scientific and non-governmental organizations to call for the strict application of the precautionary approach to gene drive research, including for field tests (Cotter and others, 2020; CSS, ENSSER and VDW, 2019; NASEM, 2016). Calls have also been made for a better understanding of the potential ecological and evolutionary

10 CBD/CP/RA/AHTEG/2020/1/5.

11 CBD/CP/RA/AHTEG/2020/1/5.

12 An outcome of a release of a suppression drive predicted by modelling whereby wild type individuals recolonize an area where the drive has locally eliminated the population (Champer and others, 2021).

impacts associated with the intentional release of EGD-LMOs to inform risk assessment (e.g. CSS, ENSSER and VDW, 2019; Dolezel and others, 2020; Giese and others, 2019; NASEM, 2016; Rode and others, 2019). In parallel to this dialogue, established guidance for LMMs had provided a basis for developing further recommendations for the phased testing of EGD-LMOs (e.g. Hayes and others, 2018b; James and others, 2018, 2020; NASEM, 2016; WHO, 2014, 2021), as well as recommendations for the responsible and sustainable deployment of the technology (James and others, 2018, 2020; Warmbrod and others, 2020), and engagement of all concerned Parties, stakeholders and indigenous peoples and local communities (NASEM, 2016; WHO, 2021).

The preferential inheritance of a transgenic construct, along with the intended spatial and temporal scale of spread of the genetic modification(s) of interest, may lead to potential adverse effects across large spatial and/or temporal scales in specific cases. Moreover, EGDs may enable the modification of target populations in the field, and expand the means to achieve population modification (including the spectrum and nature of novel cargo/payload genes, along with the diversity of target organisms). Further consideration in any future risk assessment is required to scrutinize whether the aspects mentioned above (or others) are potential novel adverse effects, and whether they may introduce additional factors into the risk assessment of some EGD-LMOs. The hazardous potential of any novel aspect identified will need to be assessed on a case-by-case basis using the problem formulation approach.

4. General risk assessment considerations for living modified organisms containing engineered gene drives

4.1. Problem formulation

An explicit problem formulation is a key starting point for a robust risk assessment. It serves as a rigorous-science-based analysis that defines the overall parameters for a risk assessment and facilitates the systematic identification of potential adverse effects, as well as routes of exposure or pathways to harm, while being transparent about the assumptions that have been made during the process (OECD, 2023). Problem formulation addresses novel characteristics, as well as both intended and unintended behaviour, of the EGD-LMO.

Problem formulation can be made operational through a multi-step process involving:

(a) The identification of protection goals, and making them operational for use in risk assessment through the definition of assessment endpoints;
(b) The identification of potential adverse effects on assessment endpoints (hazard identification);
(c) The derivation of plausible pathways to harm[13] that describe how the intentional release of an EGD-LMO could be harmful;
(d) The formulation of risk hypotheses about the likelihood and consequences of such events;
(e) The participation and engagement of stakeholders and indigenous peoples and local communities can be included at all points in the process, as appropriate.

For further information, see, for instance, Devos and others (2019), EFSA GMO Panel (2010), OECD (2023), Raybould (2006, 2010), Raybould and Macdonald (2018), US EPA (1998), and Wolt and others (2010).

While problem formulation is conceptually straightforward, its implementation can be challenging when protection goals and scientific criteria for assessing risks are not clearly defined. Hence, reaching a common understanding of the relevant protection goals and scientific criteria is a prerequisite for conducting risk assessment. Data collection and interpretation can then be directed towards evaluating the impact of any observed effect on what is to be protected.

Transparency in how problem formulation is conducted is important. Thus, sufficient detail about the methods, data, assumptions and uncertainties should be reported to ensure transparency, facilitate an appropriate assessment of the quality of the problem formulation, ensure relevance, and enable reproducibility. Moreover, the problem formulation is an iterative process, enabling the revision of each step of the process as evidence becomes available. This process should also involve deeper engagement with stakeholders such as impacted communities at the relevant steps, to complement protection goals and draw upon knowledge (CCA, 2023).

13 Also termed adverse outcome pathways. A pathway to harm is a causal or conditional chain of events that need to occur for a harm to be realized.

4.1.1. Identification and operationalization of the protection goals

A crucial step in problem formulation is to identify protection goals and more specifically those that could possibly be harmed as the result of the deployment of an EGD-LMO. Protection goals can vary among jurisdictions, but their overall aim is to reduce or avoid potential harm caused by human activity to the environment, to human, animal, plant and soil health, and to water quality (OECD, 2023). As dictated by national policies and further clarified in annex I (Identification and monitoring) to the Convention on Biological Diversity,[14] protection goals encompass various aspects, such as biological diversity, genetic diversity, human and animal health, ecosystems, ecosystem functions and services, soil health, water quality and habitats. Examples of protection goals that focus on biodiversity conservation include goals related to species of conservation value or cultural values, including those of indigenous peoples and local communities, species on the IUCN Red List, and protected habitats and landscapes. Protection goals that focus on ecological functions include fertile soil, clean water and sufficient biological diversity to withstand environmental change. Sustainable ecosystems as protection goals include both biodiversity conservation and ecological functions.

National policies and legislative frameworks generally define protection goals broadly. Consequently, refinement is required to make them operational for use in risk assessment; they must be translated into specific, operational goals (referred to hereafter as assessment endpoints) (Devos and others, 2015, 2019; Garcia-Alonso and Raybould, 2014; Nienstedt and others, 2012; OECD, 2023; Suter II, 2006). This process requires the delineation of what must be protected, where and over what time period, and definition of the maximum tolerable impact, also termed limits of concern. Three sequential steps can be followed to define assessment endpoints: (a) identification of relevant species (ecosystem units), habitats/ecosystems and ecosystem services that could be at risk from the intentional release of an EGD-LMO; (b) identification of service-providing units (populations or communities) – structural and functional components of biodiversity – that provide or support these ecosystem services; and (c) specification of the level of protection for habitats/ecosystems and these service-providing units. The level of protection is then defined by the ecological entity of the service-providing unit and its attributes, as well as the maximum tolerable impact (Devos and others, 2015, 2019; EFSA GMO Panel, 2010; Nienstedt and others, 2012). The assumption is that the general protection goal, represented by specific assessment endpoints, will be achieved through the protection of the habitats/ecosystems and service-providing units of ecosystem services.

Risk hypotheses for testing are subsequently established for identified assessment endpoints, which lead to measurement endpoints that define the relevant experimental data or evidence required for the assessment (Devos and others, 2015; Sanvido and others, 2012). Measurement endpoints determine the information to be collected to test the formulated risk hypotheses. Thus, measurement endpoints are used as indicators of potential harm, but they are not part of a definition of harm. Measurement endpoints are rather a measurable (quantifiable) biological characteristic that can be related to a particular assessment endpoint (Sanvido and others, 2012; see table 1).

Protection goals and assessment endpoints are aimed at defining and targeting the initial processes in the risk assessment by helping frame relevant questions, especially during the problem formulation phase. Precisely defining the assessment endpoints is crucial to focus the risk assessment and guide subsequent analyses. The choice of the protection goals and assessment endpoints may change after an objective analysis of the characteristics of the EGD-LMO or as the risk assessment progresses and new information emerges.

Since some EGD-LMOs may spread across jurisdictional boundaries, regional approaches that would facilitate multi-country/international regulatory oversight and governance have been suggested (James and others, 2018; Kelsey and others, 2020; Rabitz, 2019). A point that would likely require further consideration is whether the risk assessment should therefore be framed only by the specific protection goals

14 United Nations, *Treaty Series*, vol. 1760, No. 30619.

1. Protection goals		2. Assessment endpoints						3. Measurement endpoints		
		Criteria for the operational definition of the protection goal						Criteria for the type of effect to be measured		
Area of protection		Ecological entity	Attribute	Unit of protection	Spatial scale of protection	Temporal scale of protection	Definition of harmful effect	Indicator	Parameters Early tiers	Parameters Higher tiers
Biodiversity conservation	Red List species Species of high conservation / cultural value	Mammals Birds Amphibians	Abundance				Relevant decrease in abundance	Selected species		
		(Valued) insects (e.g. butterflies)		Population	Non-agricultural habitats	10 years			Mortality	Abundance
		(Valued) plants								
	Protected habitats	Habitats listed in legislation						Selected habitats		
Ecosystem services	Pollination	Pollinating insects	Ecological function	Guild	Arable land and non-agricultural habitats	Following cropping season	Relevant disturbance in ecological function	Direct or indirect indicator able to demonstrate failures in ecosystem function	Mortality	Abundance
	Pest regulation	Predators and parasitoids								
	Decomposition of organic matter	Soil invertebrates, soil microorganisms		Guild	Crop fields	Following cropping season			Decomposition rate	Abundance
	Soil nutrient cycling (N, P)	Soil microorganisms								
	Soil structure	Soil invertebrates								
	Water regulation and purification	Fish Aquatic invertebrates Algae								

TABLE 1

Matrix for an operational definition of environmental harm with some selected examples of its application (adapted from Sanvido and others, 2012)

established by the jurisdictions that would host the intentional release, or should address those of the entire area of potential spread to cover the potential for transboundary movements.

4.1.2. Identification of potential adverse effects on the assessment endpoints

This step involves the identification of any features of the EGD-LMO that may have potential adverse effects on the conservation and sustainable use of biological diversity in the likely potential receiving environment, taking also into account risks to human health. In addition, this can include the identification of potential adverse effects on plant and animal health. The potential adverse effects caused by the intentional release of an EGD-LMO will vary depending on its characteristics, how it is used and the environment in which it is present. 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 these questions will determine what risk scenarios are considered in all subsequent steps. In this step, risk assessors postulate and identify scientifically plausible risk scenarios to predict whether the EGD-LMO may have an adverse effect on the assessment endpoints. This is done by examining whether any of the novel or altered characteristics of the EGD-LMO and/or its intended use could give rise to potential adverse effects in the likely potential receiving environment. The novel characteristics of the EGD-LMO to be considered should include any changes in the EGD-LMO, such as at the DNA level, gene expression level and morphological and behavioural changes. The changes are then considered in the context of the comparators (e.g. the non-modified recipient or parental organisms; see section 4.2.3) in the likely potential receiving environment, using the environmental conditions prior to the intentional release of the EGD-LMO as baseline.

Potential adverse effects may be direct or indirect, immediate or delayed, cumulative, local or long distance, as well as predicted or unpredicted. Direct or indirect effects on individual organisms that the EGD-LMO itself generates may be caused via predation, competition, habitat alteration, hybridization (see also box 8 on gene flow at the end of section 4.1.4) and introduction of new parasites and diseases.

Potential adverse effects could be caused by the ability of the EGD-LMO to: (a) affect non-target organisms; (b) cause unintended effects on target organisms; (c) develop unintentional changes in fitness; (d) transfer genes to other organisms/populations, such as sexually compatible wild species; (e) become genotypically or phenotypically unstable; (f) lead to unintended phenotypes; and (g) affect the food web.

4.1.3. Devising plausible pathways to harm

In the risk assessment process, it is important to define clear links or pathways between the EGD-LMO and potential adverse effects in order to focus on generating information that will be useful in the decision-making. Plausible pathways to harm constructed using the problem formulation approach are based on the available information on the biology and ecology of the species under consideration, the EGD design and strategy, the introduced traits, the intended uses of the EGD-LMO (the scale and frequency of the intentional release), the likely potential receiving environments (covering the likely potential receiving environments where the EGD-LMO will be released and spread) and the interactions among these variables. Pathways to harm are used as a conceptual model to describe how the intentional release of an EGD-LMO could lead to possible harm to assessment endpoints.

A pathway to harm describes the plausible and necessary steps that would need to occur for the environmental release of an EGD-LMO to result in an adverse effect on the assessment endpoint (OECD, 2023). In effect, a causal chain of events is required for a hazard to be realized. Such a pathway can be the function of a simple linear chain of events, or a complex one that is branched. A risk assessment typically includes many pathways (Connolly and others, 2021), because the proposed activity may affect different protection goals and assessment endpoints, and could lead to different harms, or because a particular hazard could arise in different ways, or both. Moreover, there may be multiple interconnected pathways to be considered that may share some of the same steps.

When planning the risk assessment, one or more pathways to harm may be postulated for each potential adverse effect identified for an assessment endpoint (OECD, 2023). Different techniques may be used to

BOX 4: Mosquitoes: characterization of a living modified mosquito containing an engineered gene drive and its likely potential receiving environments

The characterization of an EGD-LMM aims to identify any novel genotypic and phenotypic characteristics that may have adverse effects on biological diversity in the likely potential receiving environment, taking also into account risks to human health. Depending on the case, risk assessment takes into account the relevant technical and scientific details regarding the characteristics of the subjects outlined in paragraph 9 (a) to (h) of annex III to the Protocol.

In the case of an EGD-LMM, this includes the unmodified target mosquito and associated pathogen(s), the EGD-LMM (including the genetic modification), and the likely potential receiving environment (including interactions between the EGD-LMM and its likely potential receiving environments) in which the EGD-LMM will be released and spread.

Challenges in the characterization of an EGD-LMM may arise due to knowledge gaps regarding the biology of the parental species, such as life cycle, reproductive strategies, population dynamics and the potential cross-compatible species. Access to information on the functional role of the target organism in the various ecosystems and the potential genetic and behavioural diversity of the target species may be limited.

Challenges in the characterization of the likely potential receiving environments may arise due to their diversity, limited environmental and ecological data, and knowledge gaps regarding ecological interactions of the EGD-LMM.

Examples of characteristics that may require further consideration on a case-by-case basis are given below:

(a) Characteristics of the unmodified target mosquito and associated pathogen(s):
    (i) Biology, genetic diversity, species status (existence of a complex of species, species barriers, anatomy, physiology) and behaviour of the target mosquito population;
    (ii) Ecological niches occupied by a species at different stages of development;
    (iii) Species' contribution to biodiversity, ecosystem functions and services, and food webs;
    (iv) Seasonal dynamics of the target mosquito population;
    (v) Aquatic and terrestrial habitats;
    (vi) Reproductive biology of target mosquito populations;
    (vii) Interactions with other organisms;
    (viii) Contribution of the target population to disease transmission;
    (ix) Biological (including genotypic and phenotypic) characteristics of the pathogen;
    (x) Host-pathogen interactions;

(b) Characteristics of the EGD-LMM and associated pathogen(s):
    (i) Vector species and disease targeted;
    (ii) Intended entomological objective (e.g. suppression or modification of the target mosquito populations);
    (iii) Degree of spread of the EGD in target mosquito populations, from localized to non-localized;
    (iv) Degree of persistence of the EGD in target mosquito populations, from self-limiting to self-sustaining;
    (v) Threshold ratio of EGD-LMMs to be released relative to wild mosquito target populations, from low to high;
    (vi) Molecular and biological mechanisms underpinning the EGD in the LMM, such as:
        a. Nature of the genomic target sequence (e.g. within a conserved domain);
        b. The EGD and its design, covering both the underlying mechanisms involved and their components;
        c. Stability and specificity of expression of the EGD system;
        d. Characteristics of any cargo/payload gene(s) linked to the EGD, and its/their function;
        e. Homing and/or transmission rate of the EGD (e.g. efficiency of EGD ratio of non-homologous end joining to homologous repair and cleavage efficiency of the target sequence);
    (vii) Effects of the genetic modification on the biology (e.g. genotype, phenotype) of the EGD-LMM;
    (viii) Effects of the genetic modification on the pathogen, in terms of genotype and phenotype, in the EGD-LMM;
    (ix) Effects of the genetic background on the EGD, including in sibling species;

(c) Characteristics of the likely potential receiving environments (including interactions between the EGD-LMM and its likely potential receiving environment):
    (i) Geographic, demographic, entomological, seasonal and climatic characteristics of the likely potential receiving environment;
    (ii) Effects of the likely potential receiving environment (e.g. abiotic factors) on the EGD-LMM;
    (iii) Effects of the genetic modification on interactions with the target and non-target pathogens.

BOX 5: Mosquitoes: postulated adverse effects of living modified mosquitoes containing engineered gene drives

Several publications have previously postulated adverse effects on broad protection goals (such as the environment, and human and animal health) associated with the intentional release of the EGD-LMMs (e.g. Collins and others, 2019; CSS, ENSSER and VDW, 2019; Dolezel and others, 2020; EFSA GMO Panel, 2013, 2020; James and others, 2018, 2020; NASEM, 2016; Roberts and others, 2017; Rode and others, 2019; Smets and Rüdelsheim, 2020; Teem and others, 2019; Then, 2020; Then and others, 2020; WHO, 2021). Some of these previously postulated adverse effects on human and animal health and the environment associated with the intentional release of EGD-LMMs are summarized below.

The identification of adverse effects is inevitably hypothetical to some extent, as no EGD-LMM application has been submitted for regulatory approval in any jurisdiction globally as at February 2024.

Postulated adverse effects related to human and animal health include:

(a) Increased disease transmission:
    (i) Increased abundance of disease-transmitting mosquitoes;
    (ii) Increased competence for transmission of the pathogen or other vector-borne pathogens and thus the prevalence of other mosquito-transmitted diseases;
    (iii) Altered mating, host seeking, or feeding behaviours, or geographic range (broader temperature tolerance) of disease-transmitting mosquitoes;
    (iv) Reduced capability to control the target species by conventional methods;

(b) Increased potential for resistance to evolve in the target organism:
    (i) Reduced efficacy of the EGD-LMM in the target population(s);

(c) Increased toxicity and/or allergenicity:
    (i) Transmission of toxic or allergenic substances (related to the components of an EGD) either directly by biting or indirectly by exposure from such substances released into the environment (e.g. incidental exposure through inhalation or ingestion);
    (ii) Increased pathogen virulence in case of population modification.

Postulated adverse effects related to the environment (biodiversity, food webs, ecosystems and ecosystem services) include:

(a) Increased persistence and invasiveness potential:
    (i) A competitive advantage of EGD-LMMs as compared to the wild type, causing increased persistence and invasiveness and leading to the displacement of other mosquito species;

(b) Increased potential for resistance to evolve in the target organism:
    (i) Management responses to reduced efficacy of the EGD-LMM;

(c) Increased potential for vertical and horizontal gene transfer:
    (i) Spread of the genetic modification of interest to non-target organisms through vertical and horizontal gene transfer that results in harm to the wider ecosystem;

(d) Increased toxicity:
    (i) Transmission of substances (related to the components of an EGD) that are toxic to non-target organisms that consume the EGD-LMM;

(e) Adverse effects associated with the suppression of the target organism:
    (i) Suppression of the target organism that serves as food source (e.g. prey) for non-target organisms (e.g. predator);
    (ii) Suppression of the target organism may harm non-target organisms that rely on the species for the delivery of ecosystem services (such as pollination, biological control, decomposition);
    (iii) Invasion of the ecological niche vacated by suppression of the target organism by other mosquito species (niche replacement);

(f) Decreased water quality:
    (i) Suppression of the target organism that results in reduced larval consumption of algae causing increased levels of algae and associated toxins produced from algal blooms. This is in turn could lead to adverse effects on non-target organisms in the aquatic habitat, and negative effects on water quality;

(g) Decreased genetic diversity in target populations.

The above-mentioned postulated adverse effects represent areas of concern for further consideration in the risk assessment. Any adverse effect will need to be identified on a case-by-case basis using the problem formulation approach and assessed as part of the risk characterization process (i.e. testing of risk hypotheses). Wider environmental mediators are also known to impact vectorial capacity and could be considered, in the context of conservation and sustainable use of biological diversity, taking into account the EGD-LMM capacity to spread and persist over time and space.

postulate pathways to harm (e.g. Hayes and others, 2018a; Roberts and others, 2017; Teem and others, 2019; Wolt and others, 2010). The nature and formality of this exercise, which may include stakeholder engagement, may reflect priorities based on policies and approaches of the responsible authorities. When devising pathways to harm, potential pathways to harm should be systematically explored in a broad fashion. In principle, only those pathways to harm that are plausible according to existing knowledge, expert judgment and at least potentially consequential should be carried forward into the analysis. However, if the validity or consequences of a pathway to harm cannot be sufficiently defined, one can expand efforts to consider existing knowledge and/or carry that pathway forward into the analysis. Due consideration should be given to having both broad and detailed knowledge and expertise from different disciplines for the identification of potential pathways to harm.

Since it can be challenging to adequately devise multiple, complex pathways to harm over long time periods, a wide area, and/or a heterogeneous environment, it is important that all potential pathways be reported transparently. Moreover, a rationale justifying why potential pathways to harm are not considered sufficiently plausible and/or consequential should be reported transparently.

The main aim of the pathway to harm approach is to focus the risk assessment process and to improve transparency in the risk assessment by making these pathways explicit and thereby amenable to comparison and independent review. This is typically achieved by using block diagrams to portray pathways to harm. Several authors (e.g. Alcalay and others, 2021; Connolly and others, 2021; Kormos and others, 2023; Roberts and others, 2017; Romeis and others, 2020; Teem and others, 2019) have reported some relevant pathways to harm associated with the intentional release of EGD-LMOs (mostly insects) that can be considered further when devising such pathways. Other types of conceptual models that may also be useful include fault trees and event trees (Hayes and others, 2018a,b; Hosack and others, 2023). Pictorial conceptual models, such as block diagrams showing pathways to harm, have many useful properties beyond improving transparency. They are relatively easy to construct, allowing multiple models to be developed, and are a recommended approach for tackling deep uncertainty (section 4.2.7, "Uncertainties"), without excessive resource commitments. Moreover, they do not require specialized skills to develop or understand, and hence can be used to engage stakeholders, who may have different backgrounds and training, in the risk assessment by capturing the views and beliefs on relevant assessment endpoints and pathways.

4.1.4. Formulation of risk hypotheses

Each step in a pathway to harm enables the formulation of risk hypotheses that can then be tested to characterize risk. For instance, if the protection goal is biodiversity, a risk hypothesis may assess how specific characteristics of the EGD-LMO could impact different assessment endpoints related to biodiversity. This could include assessing the consequences of the reduction of EGD-LMO abundance on predators, competitors or prey, as well as the potential replacement of ecological niches by other organisms within the likely potential receiving environment.

In practice, a careful first scrutiny of the pathway to harm can usually help to identify which of the risk hypotheses may be the most decisive or easiest to test, while minimizing uncertainty. A particularly useful feature of this analysis is that it decisively determines with sufficient confidence whether a critical step is highly unlikely or not. If one step in the pathway is highly unlikely this would cause the entire pathway to harm to be equally unlikely.

There may be cases for which the available evidence may not be sufficient to show that the pathway is blocked at any step. The testing of each step in the pathway to harm will help to assess the probability of each step occurring, the severity of outcomes and the associated level of uncertainty, and thus a hazard to be realized through the postulated pathway to harm. In some cases, evidence from a series of risk hypotheses may together produce weight of evidence to indicate rejection or acceptance of that pathway, or uncertainty may be so high that no reliable conclusions can be drawn.

Some pathways to harm may need to be re-examined on a case-by-case basis, as new or altered pathways to

BOX 6: Mosquitoes: an illustrative pathway to harm and how to test the underlying risk hypotheses

The figure below presents a pathway to harm by which the consumption of EGD-LMM adults by insectivorous bats could have toxic effects on the bats. In this example, insectivorous bats feed on EGD-LMM adults, potentially causing acute or chronic toxicological effects on the bats, which in turn reduces their abundance significantly, leading to a reduction in the ecosystem services they provide, such as biological (pest) control, pollination (Connolly and others, 2021; Ramírez-Fráncel and others, 2022) and cultural services (e.g. the value of biological diversity and the relationship with land, waters and territories for indigenous peoples and local communities).

The protection goal chosen for illustration was ecosystem services (in this case biological (pest) control, pollination and cultural services) and more specifically within that the assessment endpoint of bat abundance. The plausible pathway describes steps by which the intentional release of EGD-LMMs could adversely impact this assessment endpoint via acute or chronic toxicity through consumption of EGD-LMM adults.

A risk hypothesis was built around this step in the pathway and methods were explored by which data and information could be obtained to test it. The methods proposed are illustrative examples. Alternative methods to test the risk hypothesis could be considered on a case-by-case basis.

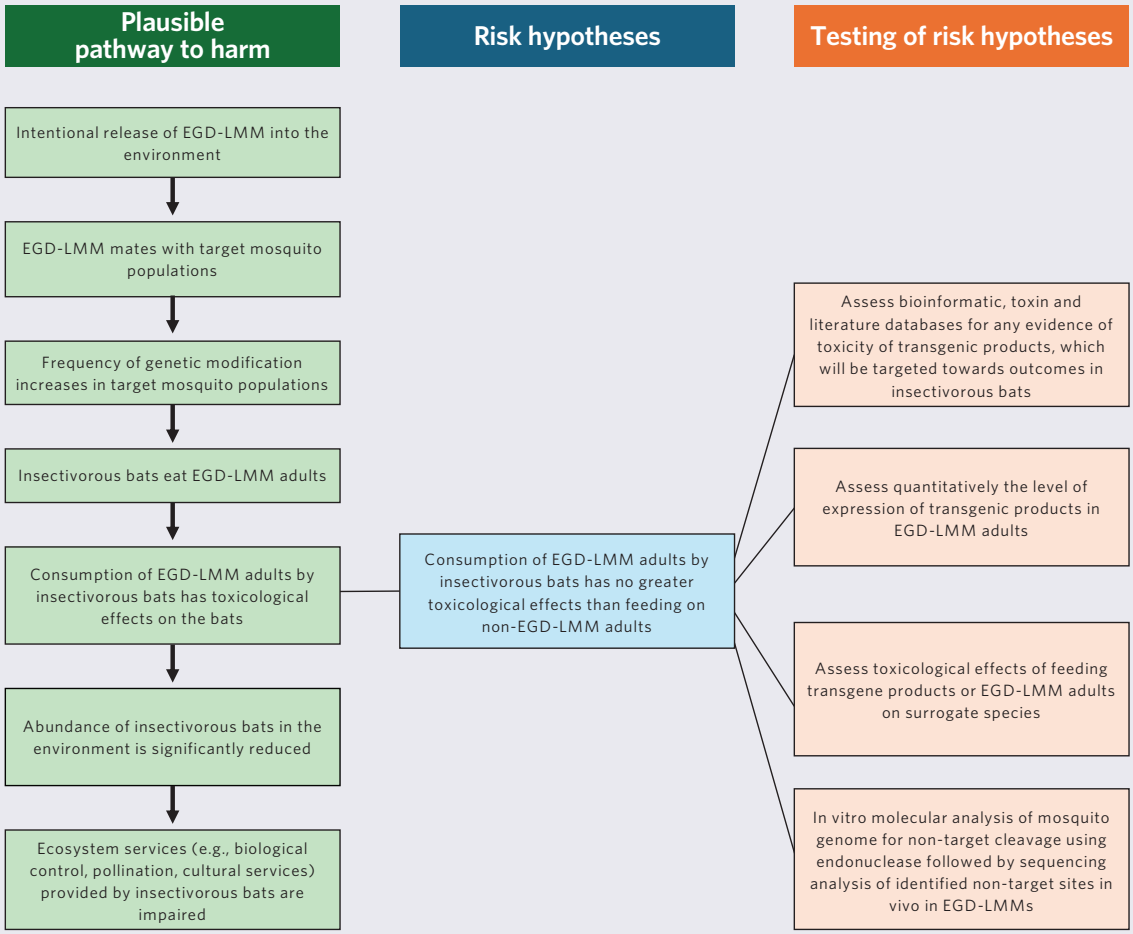

FIGURE 4

An illustrative pathway to harm and how to test the underlying risk hypotheses

BOX 7: Mosquitoes: illustrative examples of some potential adverse effects of living modified mosquitoes containing engineered gene drives

Illustrative examples of some potential adverse effects are provided in headings A to C below. These examples are not exhaustive and reflect elements that could be considered in the construction of a pathway to harm.

A. Potential adverse effects on biodiversity and ecosystem services (niche replacement, competition, disease transmission)

Competitive interactions

In the case of population suppression (which can eventually be partial and lead to long-term mixed populations of wild type and EGD-LMMs), where the target mosquito population is in competition with a non-target species, its niche, in particular its aquatic habitat which is a rate-limiting resource for mosquito populations, could be filled by another non-target species, in a process known as niche expansion or niche replacement (Connolly and others, 2021). If that non-target species is in competition, predates or is a species that provides ecosystem services, then this could lead to a reduction in those ecosystem services. If that non-target species is another disease vector, this could lead to increased or novel disease transmission. Niche replacement of one species of *Anopheles* with another has been observed in a number of instances when insecticide-based vector control measures have been applied (Qureshi and Connolly, 2021).

In the case of population replacement, reductions in the abundance of the species of pathogen in target mosquito populations could lead to niche expansion or replacement by non-target species of pathogens. This could potentially lead to increased or novel disease transmission.

Interactions involving predators

Where target mosquito populations make up a substantial component of the diet of a predator, with population suppression where less prey would be available, or with both population suppression and modification where a predator could avoid consumption of target mosquito populations containing the EGD, the predator would have reduced levels of nutrition from its typical predominant source. This could lead to compensatory consumption by the predator, and consequently, reduced abundance of non-target species that contribute valuable ecosystem services, leading to reduced ecosystem services (Connolly and others, 2021).

With population suppression, reduced abundance of target mosquito populations could also have indirect effects on the abundance or density of non-target species in the ecosystem with whom they share a predator, as a result of "apparent competition" (Holt and Bonsall, 2017). Here, the predator consumes both the target mosquito population and a non-target species that has negative effects on biodiversity. Reduction in abundance of the target mosquito population leads to reduction in the abundance of the predator because of its reduced food resources. This reduction in the predator is also accompanied by increases in the density of the non-target species, with concomitantly increased negative impacts on biodiversity.

Exposure of predators to suppression drives may however arise, when there is a failure in the drive to consistently suppress populations, e.g. if chasing dynamics occur, whereby local elimination would result in gaps in populations and wild-type rebounds to fill the localized empty niches (Champer and others, 2021).

B. Potential adverse toxic effects on water quality or human health

The expressed components of the EGD or newly expressed endogenous products in EGD-LMMs could cause acute or chronic toxicological effects on non-target populations. For example, a predator could eat EGD-LMMs which cause acute or chronic toxicological effects on that species, which in turn reduces its abundance, leading to a reduction in ecosystem services provided by that predator. Alternatively, the accumulation of expressed products from the EGD could lead to toxicity to detritivores that consume detritus in aquatic mosquito habitats, leading to negative effects on water quality for aquatic flora and fauna. Increased larval or pupal mortality of EGD-LMMs in aquatic habitats could lead to the accumulation of detritus and decreased water quality for other species, including humans and other animals (Connolly and others, 2021).

Apart from this direct potential toxicity, unintended alterations of the genome could lead to aberrant protein production (Tuladhar and others, 2019). Moreover, as unintended effects of genome editing machineries vary depending on the genetic background, they could change over time and space (Cancellieri and others, 2023) and this highlights the need to consider next-generation impacts.

C. Potential increased human and animal disease transmission, either from increased vectorial capacity or from competitive releases of other mosquito vector species

The EGD could directly affect the vectorial capacity of the EGD-LMM by: (a) affecting its vector competence for a particular pathogen; (b) causing an increase in the biting rate of the EGD-LMM on mammalian hosts; (c) extending the longevity of EGD-LMM females; or (d) decreasing the extrinsic incubation period of the EGD-LMM.

The intended impact of the EGD on target mosquito populations could also cause potential adverse effects by increased or novel disease transmission. For example, in the case of population suppression, the EGD-LMMs could lead to competitive releases of a non-target species. If that non-target species were to be another disease vector, this could lead to increased or novel disease transmission. As noted earlier, niche replacement of one species of *Anopheles* with another has been observed in a number of instances when insecticide-based vector control measures have been applied (Qureshi and Connolly, 2021).

In the case of population replacement, reductions in the abundance of the species of pathogens in target mosquito populations could lead to niche expansion or replacement by non-target species of pathogens. This could potentially lead to increased or novel disease transmission.

BOX 8: Gene flow

There are two main mechanisms of gene flow: vertical gene transfer and horizontal gene transfer.

Vertical gene transfer

Vertical gene transfer (VGT) refers to the sexual transmission of genetic material between genetically distinct populations, including the movement of genes from a population into other populations of the same species or other sexually compatible species. Some mosquitoes (e.g. most malaria vectors) belong to species complexes that contain both vector and non-vector species, some combinations of which are capable of producing fertile interspecific hybrids, making VGT to sibling species biologically plausible (Connolly and others, 2023).

Vertical gene transfer is a natural process mediated by sexual reproduction through which (trans)genes can be transferred from parents to offspring. While VGT is not an adverse effect per se, it could serve as an "exposure pathway" that lead to potential adverse effects. Therefore, a consideration for the risk assessment of an EGD-LMM would include the evaluation of the potential for transfer of transgenes via VGT to sexually compatible mosquitoes to result in potential adverse effects on humans, animals and the environment, relative to the comparator.

A plausible consequence of the use of some EGD-LMMs in species complexes is VGT of the transgenes to both vector and non-vector sibling species. Depending on how the target organism and protection goals are defined, the potential adverse effects due to VGT may differ across the spectrum of such a complex. This would require further consideration in the risk assessment (Connolly and others, 2023).

Horizontal gene transfer

Genetic material can also be naturally transferred from one species to another (Houck and others, 1991) via a phenomenon called horizontal gene transfer, making its consideration relevant in the case of EGD-LMOs (Courtier-Orgogozo and others, 2018).

harm may be identified as the scientific evidence base expands. Therefore, some pathways to harm are likely to be revised and updated periodically, with feedback from stakeholders and the wider scientific community.

4.1.5. Participation of and engagement with stakeholders

New technologies, such as EGDs, are likely to raise new questions, expectations and concerns among stakeholders as well as among indigenous peoples and local communities, whose traditional knowledge, innovation, practices, livelihood and use of land and waters may be impacted by the technology. Therefore, risk assessors should anticipate and plan for an expanded engagement process to ensure that the risk assessment has an appropriate scope and wide input from stakeholders.[15]

A particular stakeholder's perception of risk from the intentional 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).

Active stakeholder participation, including consultations and engagement, on problem formulation (including the identification of both the protection goals that are relevant for the specific case and the assessment endpoints) can improve the value of risk assessment, as it may help to ensure that the process is meaningful and informative to the environmental decisions that affect them (NASEM, 2016).

Experience gained from consultations between developers and/or potential applicants and risk assessment bodies has shown that this could be potentially helpful to frame the problem formulation by clarifying policy goals (including protection goals), decision-making criteria and information requirements, advise on study designs, and navigate the regulatory process. As the risk assessment involves an evolving technology, an early stage in the engagement process should include the development and distribution of explanatory materials to ensure that stakeholders and indigenous peoples and local communities have a sufficient understanding of the technology, its potential risks and how it will function in the environment.

15 UNEP/CBD/BS/COP-MOP/8/8/Add.1.

Regulators and/or other government officials should use a wide variety of appropriate engagement methods and media to ensure that information is made available to interested stakeholders, indigenous peoples and local communities and other groups, in ways that are sufficient, accurate, easy to understand, accessible and culturally appropriate (Kokotovich and others, 2022).

4.2. Testing risk hypotheses to characterize (overall) risk(s)

With risk hypothesis testing, the risk assessment moves from problem formulation to risk characterization in order to estimate the overall risk posed by the EGD-LMO based on the evaluation of the likelihood and consequences of the identified adverse effects being realized. This is achieved through the testing of the risk hypotheses of the plausible pathways to harm, which enables the characterization and analyses of potential adverse effects being realized, their likelihood and consequences and combines them into an estimation of the overall risk, taking into consideration any relevant uncertainty that was identified in each of the steps of the plausible pathway to harm and how it could affect the estimation of the overall risk of the EGD-LMO. Risk matrices, risk indices or models are typically used for this purpose (see table 2 below).

Likelihood should be expressed quantitatively, for example as a percentage, or, if this is not possible, qualitatively. For example, qualitative terms could include "highly likely", "likely", "unlikely" and "highly unlikely". The evaluation of the consequence of the potential adverse effects may be expressed qualitatively or quantitatively. For instance, qualitative terms, such as "major", "intermediate", "minor" or "marginal", may be used.

A characterization of the risk may also be expressed quantitatively, or, if this is not possible, qualitatively. Qualitative terms such as "high", "moderate", "low", "negligible" may be used if they are defined in detail, together with the uncertainties that are associated with the particular risk assessment (Mastrandrea and others, 2011; Spiegelhalter and Hauke, 2011). A description of the risk characterization always needs to include the assumptions of certain scenarios or provide a range of estimates rather than a single number or ordinal value that has been used to characterize the overall risk of an EGD-LMO.

Some risk hypotheses, despite being relevant for the assigned protection goals and assessment endpoints, may be difficult to test, or testing using available information may not produce desired reliability regarding the likelihood of a particular step in a pathway to harm. As part of the risk assessment, such uncertainty may be addressed and reduced through an iterative and tier-based testing approach, by consideration of multiple lines of evidence (including modelling predictions) in a weight of evidence approach, and/or by new studies being undertaken (EFSA GMO Panel, 2020; Hayes and others, 2018a; James and others, 2018; NASEM, 2016; Romeis and others, 2020; WHO, 2021). In general, some degree of uncertainty may still need to be addressed by risk managers and decision makers.

TABLE 2

Example of a risk matrix used to estimate the level of risk

Likelihood assessment	Consequence assessment: Marginal	Minor	Intermediate	Major
Highly likely	Low	Moderate	High	High
Likely	Low	Low	Moderate	High
Unlikely	Negligible	Low	Moderate	Moderate
Highly unlikely	Negligible	Negligible	Low	Moderate

Level of risk

4.2.1. Sources and quality of information

The testing of risk hypotheses uses information from various sources, including, but not limited to, using existing information, previous risk assessments, information submitted in applications by developers, peer-reviewed literature, modelling, new empirical investigations, expert opinions, indigenous peoples and local communities, indigenous and traditional knowledge, innovation and practices, or any combination thereof. Information required for testing the risk hypotheses is likely to be specific for different species, traits and/or environments, and it will vary dependent on the risk hypothesis and measurement endpoints.

Reliability of data is based on the methods by which the information was obtained, especially the suitability of the experimental methods to provide findings that are clear and plausible. Reliable information can be obtained by using internationally recognized standards and test guidelines. Peer-reviewed data may also be a source of reliable information. It is therefore important to determine the risk of bias, which refers to the likelihood that features of the study design or conduct of the study will give misleading results. The introduction of bias into studies can be due to methodological insufficiencies to prevent biases related to vested interests such as financial interests, academic interests, industry and interest group influence, or other biases related to the generation of the data.

Relevance relates to the ability of the information to test the risk hypotheses, and thus the extent to which information and/or tests are appropriate for a particular hazard identification or risk characterization. Information is considered relevant if it is linked to protection goals, assessment endpoints, and the identification and evaluation of potential adverse effects of the EGD-LMO. Information that is considered relevant to a risk assessment will vary from case to case depending on the organism being modified, the trait, nature of the modification of the EGD-LMO, its intended use, intended receiving environment, and/or the scale and duration of the environmental introduction.

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 and/or that of recognized scientific experts, according to national policies.

If sufficient relevant and reliable data are available to test the risk hypotheses, the risk assessor may conclude that there are adequate data to complete the risk assessment. Data can be judged as adequate if they are technically suitable for inclusion in the analysis and allow testing of the hypotheses with the desired certainty. If further data are required, because existing data either inadequately corroborate the hypotheses of the identified risk or reject it, then the same criteria used to evaluate existing data may be used to design new studies (Raybould, 2020).

Information derived from experimental studies that are not directly applicable, fully conclusive, or of lower reliability may at times still be useful as supporting evidence as part of a weight of evidence approach that can contribute to understanding risk.

A prerequisite for the appraisal of evidence is that the information should be reported in a sufficiently detailed and transparent manner.

4.2.2. Modelling

Models will likely play an important role in the assessment of EGD-LMOs because they can be used to predict the effects of specific EGD-LMOs inside and outside laboratory conditions and at spatio-temporal scales that are too large to study empirically prior to their intentional release (Golnar and others, 2021). Information gathered at one step within a phased release strategy, can be used by modellers to predict outcomes in the next step and thereby help direct experimental studies and monitoring strategies within an iterative process of model-driven data collection and data-driven model prediction (Restif and others, 2012). Using outcomes observed at one step (e.g. physically confined laboratory) to predict outcomes in the next step (e.g. small-scale field trial), however, inevitably introduces uncertainty which should be acknowledged and wherever possible accounted for (Ickowicz and others, 2021).

In the risk assessment of EGD-LMOs, challenges that may arise from assessing long-term evolutionary changes and their potential consequences in the target organisms, including those with different genetic backgrounds, and the prediction of off-target effects in wild populations, may be addressed by modelling.

Models can help to address uncertainty by highlighting how different model structures, or variation in model parameters, influence risk predictions, and thereby delineate the drivers of (un)acceptable outcomes for specific assessment endpoints. Models may enable analysts to: (a) identify parameters that have the most influence on the persistence, spread and effects of the EGD; (b) test and refine risk hypotheses; and (c) simulate outcomes under different future scenarios, to help anticipate long-term evolutionary and ecosystem effects. In this manner, models can be used to potentially predict the behaviour and risks of EGD-LMOs, guide post-release environmental monitoring efforts and contribute to the weight of evidence in a risk assessment (EFSA GMO Panel, 2020; Golnar and others, 2021; WHO, 2021).

A key contribution of modelling is its ability to predict the population dynamics of EGD-LMOs in the field (Beeton and others, 2022; Eckhoff and others, 2017; North and others, 2019, 2020; Sánchez and others, 2020a) and to address some of the challenges arising from potential evolutionary effects in the target organisms (Morozov, 2013). By considering parameters such as reproductive rates, dispersion patterns and genetic interactions, models may provide insights into the spread and persistence of the EGD-LMO within target populations and environments. Moreover, modelling could allow 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 potential impacts (Golnar and others, 2021). Furthermore, through simulations that incorporate various intervention approaches, such as different EGD mechanisms or parameter variations, models help identify optimal strategies that may minimize risks while also considering effectiveness of the EGD system (Connolly and others, 2021; Devos and others, 2022b; Zapletal and others, 2020). This information could then support decision-making processes and assist in the development of risk management plans.

When modelling the spread of an EGD-LMO, care should be taken to include, on a case-by-case basis, all relevant ecological processes. Realistic model predictions may require inclusion of a range of ecological considerations such as confinement by interaction with other species, long-range migration, habitat heterogeneity over space, mating complexity, aestivation, and local population structure (Combs and others, 2023; Frieß and others, 2023; Kim and others, 2023; Olejarz and Nowak, 2024; Verma and others, 2023). Furthermore, to date most models have focused on the spread of different EGDs to assess and predict EGD effectiveness, rather than how the EGD-LMO effects the environment. Additional modelling may therefore be needed to predict population dynamics of biodiversity potentially affected by the EGD-LMO (Frieß and others, 2023). See additional information in annex I.

Models use assumptions to simplify real-world systems to help understand and predict outcomes in what would otherwise be overwhelmingly complex situations. These assumptions, together with the use of inappropriate parameter values, may limit the model's ability to accurately predict outcomes or recreate the full patterns of behaviour of a system's individual components. The accuracy of model predictions can be tested by comparing them to independent data, that is, observed outcomes that were not used to train or parameterize the model. It is important that the assumptions used to guide the structure of the model and its parameters values be clearly documented so that users can gauge its limitations and the circumstances under which the model may or may not be fit for purpose. An interdisciplinary approach, including mathematical or statistical training, however, may be required to fully appreciate the limits or utility of a model. Users should also be aware that certain types of models can require significant computational resources to run, which may limit their application under certain circumstances, such as real-time decision support.

BOX 9: Mosquitoes: choice of comparators for living modified mosquitoes containing engineered gene drives

The mosquito line/strain used as a recipient organism for transformation may serve as a comparator for the risk assessment of an EGD-LMM. Where successive passages are used to develop a strain of the EGD-LMM, the parental living modified 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-LMM 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, that may generate information that is consistent and relevant to the risk assessment process for EGD-LMMs and could be considered by risk assessors. Such comparators may provide information on the impacts of intended aims of population suppression or modification. However, there are limitations in the use of such comparators with regard to addressing unintended impacts. For example, pesticide application may provide information on impacts of population reduction, but not on risk of exposure of non-target organisms to suppression drives. Similarly, *Wolbachia* applications may provide certain relevant insights but are limited in relevance when taking into account that *Wolbachia* is a high-threshold approach. Moreover, it does not allow for assessing issues such as the potential risk of pathogen evolution in response to a population modification drive. Such comparators are also not relevant in assessment of next-generation effects of gene drive technologies and the potential for evolutionary responses post-release.

Depending on the intended outcome of the EGD-LMM application and focus of the comparison, relevant comparators may include: (a) the LMM (without an EGD) of the same species with a genetic background that is as close as possible to that of the EGD-LMM; (b) the target (non-modified) organism; and (c) other disease vector/pest control systems (e.g. species-specific genetic control methods involving the release of insects, insecticides, insecticide treated bed-nets), to enable comparisons at both the organismal and (management) systems level.

The selection of comparators may need to consider issues relevant to offspring of the EGD-LMM and include comparisons with heterozygotes and homozygotes of the EGD-LMM, where relevant.

4.2.3. Comparators

When testing risk hypothesis, a comparative approach is often used, whereby the level of risk is estimated through comparison, most often with a non-LMO counterpart or parental organism that has a history of (safe) use for humans and/or animals and/or familiarity for the environment. A comparative approach is aimed at identifying the phenotypic and genotypic changes that may lead to potential adverse effects, and changes in the nature and levels of risk associated with the LMO. The differences identified between a particular LMO, and a comparator provide a starting point for determining whether the intentional release of the LMO might result in potential adverse effects on the environment. When a relevant difference is identified between the LMO and a comparator, it is evaluated to determine whether it is significant and has biological relevance related to protection goals.

The choice of comparators can have large effects on the relevance, interpretation and conclusions drawn from the risk assessment process. Therefore, comparators should be selected based on their capacity to generate information that is consistent and relevant for the risk assessment. Typically, the LMO is compared to a non-LMO with a genotype that is as closely related as possible to the LMO. However, there is no single concept of an appropriate comparator that is agreed upon internationally (OECD, 2023). In some instances, where the regulatory framework permits, an appropriate comparator may be another LMO. Furthermore, more than one comparator may be used in a risk assessment. For a given intentional release of an EGD-LMO, there may be a range of relevant comparators (such as the non-EGD LMO of the same species with a genetic background as close as possible and relevant to that of the EGD-LMO, the target organism, or other disease vector/pest control systems) to inform a risk assessment and contextualize risks.

Different comparators may be relevant for different component properties of an EGD-LMO. Thus, more emphasis may need to be given to the purpose of risk assessment studies and comparisons when selecting relevant comparators. Given that some EGD-LMOs

BOX 10: Mosquitoes: stepwise testing

The stepwise testing approach may leave some uncertainty before open field testing or field implementation of some LMOs, including some EGD-LMMs, as it may be challenging to collect data from experimental systems that would be fully applicable to field conditions. Mathematical modelling may help to fill this gap in data. Moreover, greater use of models may help to address the long temporal scale and wide spatial scale of specific EGD-LMM applications. Monitoring may also be needed.

The WHO framework (WHO, 2021, section 1.5; also see annex III to the present document) advocates a phased testing approach for LMMs:

(a) Phase 1: small-scale laboratory studies for efficacy and safety testing, followed by testing in larger population cages in an indoor setting;
(b) Phase 2: physically, ecologically or genetically confined field trials, or small-scale isolated releases;
(c) Phase 3: staged open-field releases;
(d) Phase 4: post-implementation surveillance.

WHO recognizes that the characteristics of persistence and spread for self-sustaining, non-localizing, low-threshold EGD-LMMs could make it difficult to distinguish the specific transition between phases 2 through 4 (WHO 2021, section 1.5.1). Moreover, for self-sustaining, non-localizing, low-threshold EGD-LMMs, WHO does not consider phase 2 semi-field testing to be a required step in the development pathway (WHO, 2021, section 3.8.2). This means that the data obtained in phase 1 or 2 becomes a major driver for the decision to proceed to field testing or release (WHO, 2021, section 3). WHO recommends that initial small-scale releases of EGD-LMMs should focus on the assessment of the biological function and activities of the EGD-LMMs, including their potential effects on native mosquitoes and the local ecosystem. While noting that absolute ecological containment cannot be guaranteed for EGD-LMMs, WHO advises that initial small-scale releases should aim for some level of isolation (WHO, 2021, section 1.5.1).

Gathering relevant data for self-sustaining and low-threshold (independent) EGDs in open release trials may be challenging due to their spatially and temporally unrestricted nature and the inability to be recalled. Since self-sustaining EGDs are designed for widespread and long-standing control, spatially and/or temporally restricting their spread would not necessarily be in keeping with the intended outcome of their intentional release. Therefore, the utility of prior field testing of a related self-limiting strain may be considered as an intermediate step to reduce uncertainties in risk assessment (e.g. Benedict and Robinson, 2003; James and others, 2018). Self-limiting EGD systems may enable localized and temporally restricted spread of the genetic modification of interest, resembling other self-limiting approaches for disease vector/pest control.

will operate at an ecosystem level, the definition of the comparator may need to be broadened from endpoints that solely consider genetic and phenotypic changes to those that can be indicative of potentially harmful ecosystem impacts. At the population and system level, multiple comparators may be needed to allow robust comparisons across a range of factors that are not sufficiently covered by any single comparator (EFSA GMO Panel and others, 2022).

The choice of comparators will depend on the risk hypothesis to be tested and other factors, such as the availability of appropriate comparators and specific regulatory requirements (OECD, 2023). For EGD-LMOs targeting non-domesticated or wild species, there may be limited information available on potential comparators. Further, decades of experience and research on invasive species and biological control agents have provided insight into the complexities, dynamics and effects that new organisms in ecosystem may have and the often low predictability of these effects.

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.

4.2.4. Tiered testing

Tiered testing starts by testing conservative risk hypotheses (in which the likelihood of detecting potential hazards is high) and only moves to more realistic tests if trigger values are exceeded (Romeis and others, 2008; Sanvido and others, 2012). According to the tiered approach, information collected in lower tiers directs the extent and nature of any experimentation conducted in higher tiers: hazards are evaluated within different tiers that progress from worst-case exposure scenario conditions, framed in highly controlled laboratory environments, to more realistic scenarios under semi-field or field conditions. Progression

to larger-scale experiments in higher tiers aims to provide increasingly refined estimates of exposure. Within each tier, all relevant information is gathered to determine whether there is enough evidence to conclude the risk assessment at that tier. The conclusion can only be made if any residual uncertainty has been defined; otherwise, additional investigations to generate further information at one or more higher tiers are conducted. Should potential hazards be detected in early tier tests or if unacceptable uncertainties concerning possible hazards remain, additional information is required to confirm whether the observed effect might still be detected at more realistic rates and routes of exposure (Devos and others, 2019).

4.2.5. Limits of concern

A comprehensive and consistent progression from one tier to another requires the definition of limits of concern that trigger either additional studies (if the initial assessment indicates a potential for harm) or a decision to stop further testing (Raybould, 2010). Limits of concern may be set conservatively and categorically (more, few, no more than, no less than, etc.) early in the risk assessment. They are only set precisely (quantitatively) if a conservative assessment indicates the potential for harm. Limits of concern are directly related to whether the studies are performed in the laboratory or in the field. For laboratory studies, limits of concern are conservative trigger values (i.e. low values) which if exceeded indicate potential harm and the need for exposure assessments and determination of field-scale effects (Raybould, 2010). For field studies, the lower limit will usually be defined by a threshold effect, i.e. the lowest effect to cause environmental harm (Perry and others, 2009). Knowing in advance the size of the effect to be determined is crucial because this information will enable an assessment of the ability of the study to detect harm. Limits of concern are estimated from literature data, modelling and existing knowledge (Dolezel and others, 2017, 2018; Perry and others, 2009).

4.2.6. Weight of evidence

The weight of evidence approach can be defined as a process in which information is integrated to determine the relative support for possible answers to a question (EFSA Scientific Committee and others, 2017). Concretely, it means using a combination of information derived from several independent sources to give sufficient evidence to fulfil an information requirement. This approach is helpful when: (a) the information from a single piece of evidence alone is not sufficient to fulfil an information requirement; and (b) individual studies using similar methodologies provide different or conflicting conclusions. The weight given to the available evidence depends on factors such as the quality of the data, consistency of results, epistemic uncertainty and variability, nature and severity of effects, and relevance of the information. The weight of evidence approach requires the use of scientific judgment and, therefore, it is essential to provide adequate and reliable documentation.

4.2.7. Uncertainties

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

Uncertainty in risk assessment arises in the language, input data, models and parameters of the assessment. It may also arise in the context of the problem and in the values, intentions and behaviour of human beings. Risk assessors can encounter three types of uncertainty:

(a) Linguistic uncertainty: the uncertainty created by language that is either deliberately or inadvertently imprecise;
(b) Epistemic uncertainty: the uncertainty created by imperfect knowledge about something that is in principle knowable, and therefore in principle reducible with additional research and observation;
(c) Variability: the uncertainty caused by randomness that is often associated with the inherent diversity or heterogeneity in a population over space and time.

Each identified uncertainty should be categorized based on its nature, including: (a) lack of information or incomplete knowledge; and/or (b) biological or experimental variability. Uncertainty resulting from lack of information or incomplete knowledge includes, for example, an incomplete understanding of off-target effects, long-term ecological impacts, potential for EGD to evolve and develop resistance to control measures, or a limited knowledge of EGD persistence in natural populations (Cisnetto and Barlow, 2020; Frieβ and others, 2019, 2023; Kuzma, 2019). Uncertainties resulting from biological or experimental variability may involve variations in EGD efficiency and stability, as well as discrepancies in ecological or intergenerational responses (Rabitz, 2022; Then and others, 2020).

The various forms of uncertainty should be 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.

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. More information will not necessarily contribute to a better understanding of potential adverse effects.

In cases where uncertainty cannot be addressed through the provision of more information, appropriate risk management measures and post-release environmental monitoring of the EGD-LMO in the likely potential receiving environment, as outlined in paragraph 8 (e) and (f) of annex III to the Protocol, can be employed. Furthermore, uncertainties associated with specific adverse effects may not allow for the estimation of the overall risk, thus complicating the final recommendation regarding the acceptability of risk.

Consideration 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.

See additional information in annex II.

5. Recommendation of acceptability of risk and identification of risk management strategies

Following the risk characterization, risk assessors prepare a report summarizing the risk assessment process, identified individual risks and related uncertainties and the estimated overall risk. Further, they provide one or more recommendations as to whether or not the risks are acceptable or manageable and, where necessary, identification of risk management options that could be implemented to manage the risks associated with the EGD-LMO. Such recommendations are made based on the overall risk identified in the context of the scientific criteria for risks that were identified in the problem formulation of the risk assessment, considering established protection goals, assessment endpoints and risk thresholds and what uncertainty remains after potential management of risks.

In making a recommendation regarding the overall risk of the EGD-LMO, it is important to include, where necessary, identification of strategies to manage these risks as well as information on uncertainty regarding the level of risk. These measures shall be imposed to the extent necessary. The need, feasibility and efficacy of the management options, including the capacity to implement them, should be considered on a case-by-case basis. If such measures are identified, the preceding steps of the risk assessment may need to be revisited to evaluate how the application of the proposed risk management measures would change the outcome of the steps including the capacity to undertake them.

Further, while the risk assessor provides a recommendation as to whether or not the risks are acceptable or manageable, the ultimate decision about whether or not to approve the EGD-LMO release is the prerogative of the decision makers (also see section 7).

BOX 11: Mosquitoes: risk management strategies

Where a risk has been identified that warrants a response through mitigation of the EGD-LMM, risk assessors may consider recommending such strategies as monitoring the EGD-LMM 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-LMMs that have been released, as well as mitigation methods if an unanticipated adverse effect occurs, should be considered before any uncontained releases are carried out.

Planning of mitigation measures (such as an alternative set of control measures that could be employed) and the integration of other population control methods may also be considered. Monitoring during and after the environmental release of the EGD-LMM may also be considered to enable estimation of mitigation effects on identified risks (see section 6).

Apart from monitoring, the risk management may need to consider the recall or suppression of the drive. The question of countermeasures has been discussed by Rode and others (2020).

6. Monitoring

Uncertainty, in its various forms, is an important consideration in risk assessment of modern biotechnologies, such as EGD-LMO applications. In accordance with paragraph 8 (f) of annex III to the Cartagena Protocol on Biosafety, "where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment". Furthermore, Article 16 of the Protocol, and in particular, paragraph 2 (which deals with risk management) and 4 (which deals with observation requirements), is relevant with respect to the implementation of risk management. Further, Article 7 (Identification and monitoring) of the Convention on Biological Diversity establishes that Parties shall, as far as possible and as appropriate, monitor the components of biological diversity important for its conservation and sustainable use, and identify processes and categories of activities which have or are likely to have significant adverse impacts, and monitor their effects through sampling and other techniques.

Remaining uncertainties in the risk assessment due to long-term evolutionary changes and their potential consequences in the target organisms, including those with different genetic backgrounds, and the prediction of off-target effects in wild populations, could be addressed by monitoring.

Monitoring of LMOs refers to the systematic observation, data collection, and data analysis during and after the intentional release of an LMO into the environment in accordance with the objectives of the Protocol. It should be noted that monitoring efforts should be imposed to the extent necessary to prevent adverse effects. Furthermore, where there is uncertainty regarding the level of risk, it may be addressed by implementing appropriate monitoring of the EGD-LMO in the receiving environment.

Monitoring can be categorized as case-specific monitoring and general surveillance monitoring. Case-specific monitoring is hypothesis-driven and should be targeted at the assessment endpoints and protection goals identified in the risk assessment conclusions as being at risk, or where levels of unresolved uncertainty were identified in relation to potential risks associated with the EGD-LMO. While case-specific monitoring may be conducted to address uncertainty in the level of risk for effects anticipated in the risk assessment, general surveillance monitoring is used to account for effects, especially residual or unresolved or unanticipated risks, and typically forms the basis for the monitoring plan. General surveillance monitoring is carried out without any preconceived hypothesis to detect effects that were not anticipated in the risk assessment. Should any such effects be observed, they are studied in more detail to determine whether the effect is adverse and whether it is associated with the deployment of an EGD-LMO.

In certain situations, statistical or process-based models may be used to simulate outcomes under a proposed sampling design and thereby calculate its statistical power (Arnold and others, 2011). In this regard, clear triggers for management responses, based on modelling, for particular monitoring results/events may be considered.

Monitoring measures may be implemented to trace and identify any direct or indirect, immediate, delayed or unforeseen adverse effects on the environment, taking into account human health, of LMOs as or in products after they have been intentionally released into the environment. This can also include monitoring potential adverse effects on plant and animal health. Monitoring data may feed back into the risk assessment process.

Environmental monitoring may be a means to:

(a) Address/reduce uncertainties;
(b) Confirm assumptions made during the risk assessment, including efficacy and safety for human, animal and plant health and the environment;
(c) Validate conclusions of the risk assessment on a wider spatio-temporal level of application;
(d) Determine the causal link between an environmental change observed and the specific use of an EGD-LMO;
(e) Evaluate whether risk management strategies are efficacious and being implemented effectively;
(f) Detect effects that were not anticipated in the risk assessment, including cumulative and long-term adverse effects;
(g) Establish a causal link between EGD-LMOs and any observed adverse effects.

In addition, monitoring can be considered to also serve as an early warning system that could lead to the activation of additional risk management actions. Hence, monitoring results inform decision-making about continued testing and implementation of the EGD-LMO and its ongoing use and management.

BOX 12: Mosquitoes: considerations for monitoring

There is substantial experience with releasing insects for genetic and biological control of disease vectors and pests, including with their monitoring. It may be advisable/appropriate to draw on the experience from current disease vector/pest control strategies that involve the release of insects, seek precedents from more or less similar situations, and use this experience to inform the monitoring of EGD-LMMs. However, caution is required as the systems compared differ in various aspects.

6.1. Considerations for monitoring

A monitoring plan is either developed by competent national authorities based on relevant national biosafety laws, regulations and policies and the recommendations derived from the risk assessment, or developed by the developer/applicant and evaluated and agreed upon by national authorities. This plan should be relevant to uncertainties identified in the risk assessment and the level of risk posed by the specific EGD-LMO. The plan should relate to the context and scope of the risk assessment and may utilize related monitoring data and activities, including from other countries/areas, as appropriate.

6.1.1. What to monitor

Indicators (e.g. species, soil, water, unintended persistence) and parameters (components within a given indicator such as species density) should be capable of reliably signalling a change as proximal as possible to the adverse effect occurring. Parameter prioritization may relate to ease of sampling and collection of required material as well as assaying for the parameter. Consideration should be given to the interrelationships of the indicator with a pathway to harm; that is, the indicator should signal an adverse effect relevant to a step or steps within a causal pathway considered in the risk assessment and thereby tie back to the assessment endpoints and protection goals. Pre-exposure baseline data and reference points may be available or collected for the chosen indicators and parameters.

Other considerations may include time to develop signal, temporal and spatial variability of the indicators (e.g. seasonality of occurrence), signal sensitivity (i.e. signal-to-noise ratio appropriate for the early and effective verification and determination of adverse effect), throughput, cost, and impact of natural and human induced changes to the environment. Depending on the EGD strategy, the genetic and phenotypic stability may need to be assessed over multiple generations under confined conditions as

part of the risk assessment, as well as in the field as part of monitoring.

Methods could be considered whereby existing surveillance data collected for other purposes, such as integrated vector management or ecosystem or wildlife management, could be analysed for sources of signal determination.

Particularly for human health-related pathways to harm, resistance development to the drive mechanism and pathogen resistance could be considered in the monitoring plan, as appropriate.

6.1.2. How to monitor

Methods are dependent on and directly applicable to case-specific indicators and parameters chosen (see previous section on "what to monitor"), their inherent variability, specificity, sensitivity, and ability to signal change resulting in an adverse effect. Monitoring methodology should provide sufficient details on sampling, collecting, and analysing the samples, as well as on the relevant data analysis. Monitoring data could be collected from various sources, including but not limited to surveys, questionnaires, field observations, ongoing/existing monitoring for other considerations such as public health, invasive species, biocontrol, disease surveillance, integrated vector management, resistance to pesticides, etc. Methodology for both collection and analysis could differ for areas outside the expected spread and dispersal range versus within the expected release environment. In addition, monitoring methodology should also consider effective identification and detection of EGD-LMOs in the likely potential receiving environment.

Considerations could include:

(a) The nature of the effect being measured (e.g. acute/short-term, chronic/long-term, immediate or delayed, direct or indirect);
(b) The range or amplitude of change required to signal an adverse event;
(c) Analytical methodology (i.e. molecular methods, trapping/sampling/collection methods, adaptive methods);
(d) Statistical methodology (e.g. sample size, power, etc.);
(e) Weight of evidence of the data type;
(f) Replicability and standardization of studies, questionnaires, methods;
(g) Ease of use in various environments and/or countries (including resource considerations such as capacity, personnel training, equipment, logistics, sample and reagent availability and shipping);
(h) Potential for scaling and use of high-throughput methods;
(i) Cost and duration of the monitoring activities, and identification of who will cover the costs;
(j) Potential for method improvement, ability to include new techniques or methods over time;
(k) Ability for real-time feedback into models, future risk assessments, and/or decision making to stop the monitoring or alter the monitoring plan;
(l) Pre-exposure baselines for informing the monitoring.

6.1.3. Where to monitor

Monitoring sites should be chosen based on the specific case and indicators and parameters being sampled and measured, as well as specifics of the intended receiving environment and ongoing land use and management practices. Initial sites should be chosen such that they include indicators with the potential to be exposed to or impacted by the presence of the EGD-LMO and are relevant to the pathway to harm. Monitoring site locations, size, and density/distribution, and timing of the monitoring, should be determined prior to release based on the biology and life cycle of the EGD-LMO, its potential spread, dispersal, and establishment; the likely potential receiving environment, including geography, land use, and local wild population size, density and distribution; seasonality (migration, impact of rain or temperature), etc.

In cases where species are used as indicators, their biology, life cycle, abundance, seasonality, and interactions with other ecosystem features, including the EGD-LMO and other organisms, should be considered. In addition, specific monitoring of environmental effects may need to take place in representative

areas where the EGD-LMO is intentionally released. The spatial and temporal scale of specific monitoring will need to be adapted according to the spatial and temporal distribution of the EGD-LMO in the environment.

Consideration should be given to protected areas, biodiversity hotspots, wildlife reserves, genetic centres of origin, and access and availability throughout the duration of monitoring, that is, through the different times of the year and for all the years that are required (for long-term monitoring).

Other considerations could include the potential for change in management practices or land use and their impact on the indicator/parameter over the duration of monitoring, statistical power based on the number and density of measurement sites, baseline data sources or control/reference sites versus treatment sites, and impact of modelling approaches on site choice, density, and duration.

6.1.4. How long to monitor

Duration of monitoring would be related to factors such as frequency, number, and periodicity of observations or measurements required to reliably encounter the change in a parameter (time to signal observation), the type of changes that are being sought to be measured (e.g. short-term or long-term, immediate

BOX 13: Mosquitoes: specific guidance for the monitoring of releases of living modified mosquitoes containing engineered gene drives

Monitoring of EGD-LMMs begins before the release occurs and continues during and following the release. Monitoring should be considered at multiple levels: for the presence of the released EGD-LMM and transgenic construct in the local population of the target mosquito species; and for environmental effects, taking into consideration human health, as they pertain to assessment endpoints and protection goals and pathways to harm identified in the risk assessment. Some monitoring may be needed regardless of the species of mosquito and the genetic modification employed; however, the mechanism underlying the EGD 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 (Rašić and others, 2022).

Clear description of specific monitoring is even more important for EGD-LMMs than for non-EGD LMMs, as the potential adverse effects of intentional releases may not be spatially or temporally constrained and any changes to the transgenic construct may require rapid management intervention. Spatial and temporal scales will be greater with most EGD-LMM applications than with non-EGD LMM applications, and reversibility may depend on the nature of the EGD. Large-scale and long-term impact is particularly relevant to self-sustaining EGDs because temporal/spatial scales are increased. Consequently, EGDs will require monitoring to be dynamic and spatially explicit, tracking spread and persistence over space and time, including areas beyond the expected range of the release, and possibly across jurisdictional boundaries.

Release and post-release monitoring

During the release of the EGD-LMM, monitoring or inspection should ensure compliance with the release conditions laid down in the authorization. Monitoring will also provide data on the efficacy of the EGD system, as well as on the identified pathways to harm in the risk assessment and any other requirements determined by the regulatory authorities for release. Post-release data can also be used to inform the generation of baseline data for the post-release monitoring.

Monitoring mosquito populations and intended phenotypic change within the designated release and dispersal area will support the primary indication that the product (e.g. the EGD-LMM) has been established within the release area and the size of the native population of target vector mosquitoes is decreasing (for population suppression applications) or that the construct is spreading through the target population (for population modification applications). Monitoring for the EGD-LMM outside the designated release area could identify dispersal range (temporal and spatial) of the EGD-LMM. These data could provide guidance for potential mitigation measures as well as information useful for validating and updating models used to inform risk assessment.

After the planned release(s) of the EGD-LMM have been completed, the monitoring plan is expected to include data to support spread and dispersal information described in the risk assessment, as well as related to safety and efficacy of the product based on its intended use (including product failure such as loss of drive or uncoupling of the drive element or failure of the effector). Moreover, it will provide data on any outstanding unresolved risk-related concerns outlined by competent authorities in the initial monitoring plan. Results of initial post-release monitoring should be evaluated to determine frequency and duration of any additional monitoring and reporting period if extended, and whether the monitoring and risk mitigation plan should be updated.

or late onset; i.e. time to signal generation), the life cycle, generation time, and biology of the EGD-LMO as well as of the indicator (of a species), duration of the release, and effect of the release on the environment over time. Monitoring duration should be sufficient to provide data that supports decision-making (i.e. providing data to further assess the identified uncertainty and level of risk). The anticipated time scale of the effect of the EGD-LMO is an additional parameter for consideration. Conditions for stopping, extending, or altering the monitoring plan, including duration, should be described a priori.

6.1.5. How to report data/findings

Monitoring data and results should be reported on the agreed upon frequency, to the agreed parties and in the appropriate format, described in a monitoring plan. Goals for reporting generally include reporting potential adverse effects; verifying prior observations and conclusions; reaffirming product safety and efficacy; addressing any remaining uncertainty in pathways to harm; providing data for re-evaluation of models or risk assessments; addressing any need to change, extend, or stop existing risk mitigation procedures; and supporting decision-making in any of these areas, including the need for emergency measures.

Reporting requirements are described by national competent authorities based on applicable laws and should provide frequency and format of the information to be reported as well as mitigation measures used.

Results and data may be shared with other stakeholders in formats appropriate to those audiences for transparency. Confidentiality of the data and information should respect national and international laws and agreements.

7. Related issues

7.1. Risk assessment and assessing the benefits as components of the decision-making process

A critical element in the conclusion of risk assessment is a recommendation as to whether or not the risks, including strategies to manage the risks, are acceptable or manageable, as outlined in paragraph 8 (e) of annex III to the Cartagena Protocol on Biosafety. Paragraphs 3 to 6 of annex III to the Protocol provide general principles of risk assessment, but no specific guidance is included on how to decide on risk acceptability and assess potential benefits.

Appropriate risk assessment and benefit analysis should also take into account potential benefits and potential risks associated with other existing alternatives to control mosquito vectors that are based on the use of insecticides and elimination of mosquito larval breeding sites. In considering the potential of new technologies, it is necessary to evaluate their potential risks and potential benefits in the context of the current situation. Therefore, when testing new strategies, they should be weighed against the risks to human health and the environment posed by maintaining the status quo, which includes both ongoing disease and insecticide exposure. This includes present user practices and habits, such as use of pesticides and integrated pest management, as well as others that may not directly affect the targeted organism population size. Such measures include vaccination campaigns, distribution of insecticide-treated mosquito nets, information campaigns regarding stagnant waters as breeding grounds for mosquitoes, and use of repellents, among others.

7.2. Consideration of the benefits to human health

According to the guidance framework for testing genetically modified mosquitoes (GMMs) published by WHO (2021),[16] a new product should be assessed in the regulatory review process on the basis of both the benefits and risks (see also annex III below). The primary potential benefit of a GMM/LMM would be the improvement of human health. In this regard, efficacy data will be an integral part of the decision-making regarding benefits in order to ensure measurable reductions in the incidence or prevalence of infection or disease relative to conventional control.

Decision makers may consider that other contextual factors should also be taken into account, such as severity of the health problem being addressed by the new technology and the availability and effectiveness of alternative disease control methods/measures. Some of these factors are discussed in detail in the 2021 WHO guidance framework.

According to WHO, the risk of novel technologies such as GMMs may be considered in the context of relevant alternatives, such as the risk of no action or the risk of conventional control methods. "Causes more harm" than current practice has been proposed as a reasonable benchmark for decision-making on GMM-based vector control systems. Other considerations

16 WHO (2021) refers to LMMs as genetically modified mosquitoes (GMMs) and to EGD-LMMs as gene drive modified mosquitoes (GDMMs).

may include conducting a "cost-effectiveness analysis", which expresses benefit as a measurement of a particular health gain.

There may be potential benefits of using GMMs in the fight against malaria and dengue, given the public health burden of these diseases. The number of deaths due to malaria, especially in sub-Saharan Africa, highlights that the current approaches (pesticides, impregnated mosquito nets, etc.) have not completely eliminated the burden. The 2023 WHO world malaria report stated that the number of confirmed malaria cases in West Africa in 2022 was 67.1 million, with 28,200 deaths in total, of which 20,600 deaths were children under five years of age. Further, more than 6 million cases of dengue and more than 6,000 dengue-related deaths were reported in 2023 (ECDC, 2024).

7.3. Socioeconomic, cultural and ethical considerations

EGD-LMOs may involve socioeconomic, cultural, traditional, religious, or ethical concerns that may be considered in the decision-making process. Article 26 of the Cartagena Protocol addresses socioeconomic considerations; in paragraph 1 it is stated that "the Parties, in reaching a decision on import under this Protocol or under its domestic measures implementing the Protocol, may take into account, consistent with their international obligations, socioeconomic considerations arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity to indigenous and local communities". In this regard, Parties may take into account their own domestic measures when identifying potential benefits and potential adverse effects of EGD-LMOs on the conservation and sustainable use of biodiversity, also focusing on the value of biodiversity to indigenous peoples and local communities. The guidance on the assessment of socioeconomic considerations in the context of Article 26 of the Cartagena Protocol on Biosafety annexed to CBD/CP/MOP/9/10 provides voluntary guidance to support decision-making. These may include economic (e.g. effects on income), social (e.g. effects on food security), ecological (e.g. effects on ecosystem functions), cultural/traditional/religious/ethical (e.g. effects on seed saving and exchange practices), and human health-related (e.g. effects on nutritional status) consideration.

Voluntary guidelines for the conduct of cultural, environmental and social impact assessment regarding developments proposed to take place on, or which are likely to impact on, sacred sites and land and waters traditionally occupied or used by indigenous peoples and local communities (the Akwé: Kon Voluntary Guidelines), were adopted by the Parties to the Convention in decision VII/16 and provide useful guidance. In particular, the potential adverse effects of EGD-LMOs on the land, waters and territories, sacred sites, wild species of fauna and flora, and on the relationship of indigenous peoples and local communities with Mother Nature and the reciprocity between them, may be considered. Assessment of such issues could draw on biocultural community protocols and customary laws of indigenous peoples and local communities, which take into account community identities, histories, territorialities, traditional or indigenous knowledge, practices, innovations and traditional technologies, depending on national circumstances of indigenous peoples and local communities. The knowledge and value systems of indigenous peoples and local communities are helpful when considering the behaviour of relevant species and their interactions with other species.

EGD-LMOs may increase dependency on technology; may alter biological components; and may adversely impact biodiversity, cultural and ethical values of indigenous peoples and local communities, socioeconomic situations, and the reciprocal relationship with Mother Earth in the long term. The possibility of impacts on non-target species such as wild species that are valuable for indigenous peoples and local communities should be assessed before releasing EGD-LMOs into the environment.

7.4. Free, prior and informed consent of indigenous peoples and local communities

In decision 14/19, the Parties to the Convention noted the conclusions of the AHTEG on Synthetic Biology that, "given the current uncertainties regarding engineered gene drives, the free, prior and informed consent of indigenous peoples and local communities might be warranted when considering the possible release of living modified organisms containing engineered gene drives that may impact their traditional knowledge, innovation, practices, livelihood and use of land and waters". As such, it is highly recommended that prior and informed consent, or national equivalents, of potentially affected indigenous peoples and local communities be obtained before considering introducing EGD-LMOs into the environment, including for experimental releases and research and development purposes. Relevant guidelines for the development of mechanisms, legislation or other appropriate initiatives to ensure the "prior and informed consent", "free, prior and informed consent" or "approval and involvement" of indigenous peoples and local communities when accessing their knowledge, innovations and practices, for fair and equitable sharing of benefits arising from the use of their knowledge, innovations and practices, and for reporting and preventing unlawful appropriation of traditional knowledge were adopted as the Mo'otz Kuxtal Voluntary Guidelines by the Parties to the Convention in decision XIII/18.

It is thus important to ensure the full and effective participation of potentially affected indigenous peoples and local communities and to ensure that free, prior and informed consent is sought when considering the possible release of EGD-LMOs according to national legislation and international obligations, as appropriate.

7.5. Consideration of public awareness, education and participation (including full and effective participation of indigenous peoples and local communities), and access to information and risk communication

Public awareness, education and participation, and access to information about the risk assessment of EGD-LMOs and their potential adverse effects, or related activities, including biosafety-related communication, are essential to ensure effective participation of indigenous peoples and local communities.

Indigenous knowledge, innovations and practices integrated with accessible and understandable science for effective communication, including use of local and indigenous languages for risk communication, may be useful for scientists and decision makers in regulation of EGD-LMOs. In addition, it should be added that communication should be done in a transparent manner that avoids creating a communication gap between, for instance, scientists and the public (the deficit model concept).

Public awareness and a participatory process, including full and effective participation of indigenous peoples and local communities in the risk assessment process while ensuring the inclusion of their knowledge and value systems, are important elements. It is also important to consider appropriate means to make data available in order to facilitate independent analysis of the risk assessment. In paragraph 1 (a) of Article 14 of the Convention, it is stated that "each contracting Party, as far as possible and as appropriate, shall introduce appropriate procedures requiring environmental impact assessment of its proposed projects that are likely to have significant adverse effects on biological diversity with a view to avoiding or minimizing such effects and, where appropriate, allow for public participation in such procedures." Public awareness, education and participation is addressed in Article 23 of the Protocol.

7.6. Comparisons of novel and alternative strategies

The control of vector-transmitted human diseases, invasive species and (agricultural) pests demands the development of a wide range of complementary strategies, currently in use or under development. These strategies can inform risk assessment, benefit analysis, risk-benefit analysis and decision-making for EGD-LMOs. Such comparisons shall reflect all existing alternative practices and habits (see section 7.1).

In addition to alternatives listed above, ethical governance of gene drives may also consider the range of alternative ways of formulating and framing the problems that the gene drive technology is addressing. This alternative framing of the problems (e.g. disease control, invasive species control) will encourage discussion on a range of alternative approaches. These alternatives approaches may cause fewer potential risks, may be more actionable in the short term, and may be more sensitive to local needs and resources.

Additional long-term human health impacts such as unintended evolution of pathogens, reduced capability to control target organisms with conventional methods, increased human and animal disease transmission, and compatibility with other vector control methods can also be considered in the comparisons.

The comparison of novel strategies with alternative interventions and current measures available should take into account the sources and nature of uncertainties regarding potential risks and potential benefits. The sources and nature of uncertainty that could not be addressed during the early steps of the risk assessment can be described in relation to how they could affect the conclusions of the risk assessment.

For risk assessment where uncertainties have been identified, they shall be made transparent to the decision makers. In such cases, it may also be useful to provide an analysis of alternative options to assist the decision makers. The outcome of the risk assessment should be evaluated in regard to a broad range of comparators for the decision-making process.

7.7. Transboundary movements

If an EGD-LMM were released in the field without any isolation, it would be expected that the EGD-LMM would spread to target mosquito populations distal to the release site. The rate of spread of the EGD-LMM would depend on: (a) dispersal of the target mosquito population; (b) threshold frequency with which the EGD is required to establish in distal target mosquito populations; (c) fitness costs of the EGD incurred by the EGD-LMM; (d) reproductive capacity; and (e) release sites.

For some EGD-LMMs, sufficient isolation may not be possible because of dispersal brought about by long-distance windborne migration (Huestis and others, 2019), or human-assisted transport links by road or water. Gene drives may eventually spread beyond release sites and establish across national borders, raising issues of transboundary movements and international governance. Regional approaches that would facilitate multi-country/international regulatory oversight and governance have been suggested (James and others, 2018; Kelsey and others, 2020; Rabitz, 2019).

7.8. Consideration of liability and redress elements

In the event of adverse effects being realized, the costs entailed may include those of potential response measures that may be undertaken in accordance with provisions of the Nagoya-Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety,[17] as appropriate for some Parties. The Supplementary Protocol applies to damage resulting from LMOs that find their origin in a transboundary movement as well as to damage within the limits of national jurisdictions. Damage is defined as an adverse effect on the conservation and sustainable use of biological diversity, also taking into account risks to human health, that is measurable or otherwise observable, taking into account, wherever available, scientifically established baselines recognized by a competent authority that takes into account any other human-induced variation and natural variation, and is significant.

17 See United Nations Environment Programme, document UNEP/CBD/BS/COP-MOP/5/17, annex, decision BS-V/11.

Annex I

Further information on modelling

Almost all risk assessments will utilize at least one of the following four types of models:

- Conceptual models: qualitative representations of the system components, and the interactions between these components, that are thought to be most relevant to the risk assessment problem (see section 4.1.3, "Devising plausible pathways to harm").
- Qualitative mathematical models: a special type of conceptual model that predicts how the relevant system's components will change (i.e. increase, decrease or remain unchanged) without specifying by how much, when one or more of the components is subject to a sustained change.
- Process-based models: models that use mathematical descriptions of the system to predict how, and by how much, the magnitude of the relevant system variables will change in time and/or space.
- Statistical models: models that use special types of mathematical descriptions to describe the properties and behaviour of system components that are inherently variable, with a particular emphasis on describing the observed patterns in data.

A. Conceptual models

All risk assessments begin with implicit mental models of the problem at hand. The principal aim of a conceptual modelling exercise is to improve transparency in the risk assessment by making these implicit models explicit and thereby amenable to comparison and independent review. In the problem formulation, this is typically achieved by using block diagrams to portray plausible pathways to harm. EGD-LMO relevant examples of this type of conceptual model can be found in Alcalay and others (2021), Connolly and others (2021) and Kormos and others (2023). Other types of conceptual models that may also be useful include fault trees and event trees (Hayes and others, 2018a,b; Hosack and others, 2023).

Many conceptual modelling techniques, including fault trees and block diagrams, use linear representations of a system, and are not therefore well suited to situations where feedback has an important influence on how a system responds to change. In these situations, qualitative mathematical models are a useful complement.

B. Qualitative mathematical models

Qualitative mathematical models possess the same useful properties as pictorial conceptual models; they are transparent, relatively easy to construct and hence a cost-efficient way to explore the effects of different model structures (an important type of epistemic uncertainty) and are a good way to engage with diverse stakeholder groups and indigenous peoples and local communities. In addition, they provide information that may be helpful in systems where negative (or positive) feedback – a process in which an initial change in a system variable will cause it to return to (or move away from) its original value – is an important feature (Levins, 1998).

Qualitative mathematical modelling describes systems using signed digraphs that portray the system as a series of nodes (system variables) linked by edges that depict interactions between the system variables that have either a positive or negative effect on the nodes they join. Once constructed, the signed digraph enables the analyst to study the stability properties of the model, predict the direction of change following a sustained change to one or more of the system's variables, and estimate the sign determinacy, an indication of the confidence in the qualitative model predictions (see for example Dambacher and others, 2003).

Training in quantitative mathematical methods is required in order to fully understand the theory, assumptions and utility of qualitative mathematical

modelling. Levins (1998) provide a good introduction, while Puccia and Levins (1986) provide a comprehensive description of the method and the underlying mathematics. Examples of its use that are relevant to EGD-LMO risk assessment include Hayes and others (2014) and Hosack and others (2023).

C. Process-based models

Process-based models represent systems using one of three types of mathematical equations: (a) a recursion equation which describes the value of variables in the next time unit as a function of their value in the current time unit; (b) a difference equation that specifies how much variables change between time points; and (c) a differential equation which describes the rate at which variables change in time (Otto and Day, 2007).

Process-based models enable analysts to identify the equilibrium properties of the system, and to predict how its variables will change, in both direction and magnitude, if the system is perturbed. A large number of process-based models have been used to describe systems that are relevant to EGD-LMO risk assessment, ranging from relatively simple models of populations in containment (e.g. Facchinelli and others, 2019) to more complex models that predict how multiple populations in the wild might vary in time and space (e.g. Beeton and others, 2022). None of the current EGD-LMO process models, however, approach the complexity of the large, whole-of-ecosystem models that are employed in other domains (Fulton, 2010).

When building process-based models, analysts must make important choices about: (a) which real-world processes and components to include in the model, and which to exclude; (b) how to mathematically describe the processes that are included; (c) the values or probability distribution models of the parameters; (d) the resolution of the model in time and space (e.g. are predictions made on daily, monthly or yearly time steps); (e) the parameter's initial values; and (f) the rules that govern what happens at the model boundaries. Among these choices, the first will usually have the greatest influence on the risk predictions and must therefore be taken carefully.

Guidance on this matter generally recommends simpler models, with the least number of uncertain parameters, rather than larger models, particularly if predictive accuracy is the ultimate goal. In addition, simpler models are easier to understand and interpret. Hilborn and Mangel (1997), however, caution that simpler models may underrepresent true uncertainty, and biological theory may dictate a more complex model with more realistic features as a better choice because this allows for a wider range of biologically plausible outcomes. In an EGD-LMO risk assessment, data on observed outcomes may be unavailable prior to (or even soon after) the assessment is completed. The predictive accuracy of the process-based models used within the assessment may therefore be unknown at the time when decisions regarding field release are to be made. In these circumstances the complexity of the process models must be guided by the range of plausible outcomes identified by the plausible pathways to harm and the ecological processes that enable these outcomes. The genetic, demographic and ecological phenomena that become increasingly relevant as EGD-LMOs progress through staged-release protocols, and examples of how these phenomena are (or currently are not) addressed within EGD-LMO models, are discussed in a number of recent reviews (Combs and others, 2023; Frieß and others, 2023).

D. Statistical models

A primary aim of a statistical model is to accurately reproduce the variation that exists in real-world phenomena. Statistical models enable the analyst to infer the variation that exists in a larger population from the variation observed in a (usually much) smaller sample, and thereby accurately predict the probability of all possible outcomes, including those outcomes that were not observed in the sample but which actually exist in the wider population. An important distinction in this context is the variation in a sample that is created by the imperfections in the way we observe and measure things (measurement error), and the variation created by a combination of environmental forces acting on, and the innate variability within, the things we observe (process error). Accurate inference about variability in population-level parameters, such as the parameters of a process-based model, requires

that these two sources of variability be separated in what are often termed "hierarchical models" (Bolker, 2008; Clark, 2007). EGD-LMO relevant examples of this approach can be found in Hosack and others (2023) and Ickowicz and others (2021).

The use of modern modelling techniques for EGD-LMO risk assessment requires a high degree of training in the process-based models used to represent ecological and biological systems, and the probabilistic theory used to assign probability distribution models to the parameters of these models, as well as in the computational methods that enable inferences to be made about population-level variability in the presence of measurement error. Furthermore, biosafety regulators without this training may find it difficult to judge the scientific quality and validity of any specific modelling approach, although guidance on these issues is currently available (Augusiak and others, 2014; Calder and others, 2018).

Annex II

Further information on uncertainty

Guidance on how to identify and address the different types of uncertainty is available from many sources. Hayes and others (2007) provide a non-technical introduction highlighting examples relevant to living modified fish. The EFSA GMO Panel (2013) provides a similar introduction in the context of living modified animals. The EFSA Scientific Committee and others (2018) recommend a suite of procedures for assessing uncertainty in scientific assessment. Good textbooks on how to address uncertainty within quantitative (probabilistic) risk assessment include Bedford and Cooke (2001), Cullen and Frey (1999), and Morgan and Henrion (1992).

A. Linguistic uncertainty

Linguistic uncertainty occurs for many reasons but principally because words can be vague and ambiguous, and our interpretation of a qualitative proposition depends on the context in which it is made (Regan and others, 2002). For these reasons the same word or phrase can mean different things to different people (EFSA Scientific Committee and others, 2018). Linguistic uncertainty is prominent in qualitative risk assessment because terms such as "small effect", "low likelihood" or "negligible risk" are open to interpretation, hence current guidance almost always recommends that these terms be carefully defined (see for example EFSA GMO Panel, 2013) and that language-based misunderstandings be minimized through careful facilitation of expert input wherever possible (Carey and Burgman, 2008).

Qualitative expressions of uncertainty are problematic for two reasons. First, the effect of the uncertainty on the risk assessment is confounded by linguistic uncertainty. This makes it difficult for decision makers to gauge how precise the risk prediction is, or how far it may be from a true value. Secondly, there is no principled way to combine qualitative expressions of uncertainty around individual components of a risk calculation into an overall expression of uncertainty. For these reasons, current guidance recommends that, wherever possible, expressions of epistemic uncertainty or variability should be quantified to the extent that is scientifically achievable (EFSA Scientific Committee and others, 2018).

For EGD-LMOs quantification of uncertainty could be more challenging than in other LMO risk assessment because their potentially larger spatio-temporal footprint could lead to exposure in more variable, heterogenous environments, and because of the relevant paucity of empirical data on their behaviour in the wild. It is a misconception, however, to assume that quantifying uncertainty requires extensive data. Uncertainty can be quantified by expert judgment (via formal elicitation) for any well-defined question or quantity provided there is at least some relevant evidence (EFSA Scientific Committee and others, 2018).

Guidance on how to quantify uncertainty through expert elicitation is available from several sources. For example, Burgman and others (2005) provides a helpful introduction, Morgan (2014) provides an excellent overview of key issues, while O'Hagan and others (2006) provide a comprehensive treatment. EFSA (2014) provides guidance on three approaches within the context of a food safety risk assessment, but the methods discussed are applicable to other domains. Hayes and others (2018a) and Hosack and others (2023) provide examples of how to use elicitation to conduct probabilistic risk assessment for LMMs.

B. Epistemic uncertainty

Risk assessment of EGD-LMOs will initially encounter epistemic uncertainty in the problem formulation phase, when identifying potential adverse effects (section 4.1.2.) and when devising plausible pathways to harm (section 4.1.3.). Both steps rely on conceptual

models to identify how things may go wrong if EGD-LMOs are released in the environment, and these models (like all models) will be subject to structural uncertainty (see annex I).

In this context, model structure uncertainty is manifested in two ways: (a) is the conceptual modelling exercise complete (i.e. has the risk assessment identified all the plausible pathways to harm); and (b) are the conceptual models adequate (i.e. do the identified plausible pathways to harm accurately capture all of the critical processes and intermediate events between release of the EGD-LMO and harmful outcomes). These sources of uncertainty are common to all risk assessments. Again, however, the paucity of experience, and potentially large spatial and temporal footprint, may accentuate them in an EGD-LMO risk assessment.

Structural uncertainty in the conceptual models that underlie a problem formulation approach can be addressed procedurally and methodologically. Ensuring that relevant stakeholders, indigenous peoples and local communities and experts are consulted when plausible pathways to harm are identified and described is a recommended procedure. Carefully comparing the adverse effects identified in an EGD-LMO problem formulation with those described in: (a) the biosafety regulations of relevant authorities; (b) relevant guidance developed by respected international authorities such as the EFSA GMO Panel (2013, 2020) and the National Academies of Sciences, Engineering, and Medicine (NASEM, 2016); and (c) documents produced by the scientific community (e.g. Benedict and others, 2018; Connolly and others, 2021; David and others, 2013; Hayes and others, 2018a; James and others, 2020; Rode and others, 2019; Teem and others, 2019) will also help ensure that potentially relevant pathways have not been inadvertently overlooked.

In addition to these recommended procedures, Hayes and others (2007, 2014) describe a variety of hazard identification methods that risk analysts can employ to help ensure that all plausible pathways have been comprehensively evaluated and described. These techniques encourage analysts to "think outside the box", and provide a framework that supports them to apply their expertise and imagination in a systematic manner to identify potential pathways to harm.

It is difficult to assess whether the structural uncertainty in the conceptual models that underlie a problem formulation has been comprehensively addressed. In particular, the number of plausible pathways to harm identified in the problem formulation is not of itself an infallible guide to how complete this part of the risk assessment is. Nonetheless, a problem formulation for a complex, new technology such as EGD-LMOs that only identifies very few, or very simple, pathways will likely be viewed with some scepticism. Ultimately, reviewers and decision makers must use their expertise, experience and judgment to decide whether this source of uncertainty in the problem formulation stage of an EGD-LMO risk assessment process has been adequately addressed.

C. Variability

Variability, often also referred to as aleatory uncertainty, occurs in LMO risk assessment because many of the relevant environmental and demographic processes or variables within the plausible pathways to harm will be inherently variable in time and space. It is possible that some of the sources of variability could in theory be explained with a very detailed mechanistic model or more precise measurements but in practice this may be unnecessary. Simply characterizing the variation, and propagating its effect through a risk assessment, is often a sufficient and much more cost-effective strategy.

The effect of variability on risk assessment predictions can be captured in several ways. A common approach is to repeat the risk calculations many times while allowing the parameters of the risk assessment's process-based models (see annex I) to vary with each repetition in a realistic manner. The realism is achieved by carefully assigning an appropriate probability distribution to each uncertain parameter. The choice of probability distribution is guided by theory, the observed variation in the parameter or by expert belief. Xu and others (2010), for example, use a very flexible probability distribution (the beta distribution) to capture expert beliefs in the variability of key mosquito life history parameters, including survival rates and fecundity. Similarly, Hosack and others (2021) use the common (but in this case transformed) normal distribution to capture experts' beliefs about how the

parameters that govern the vector competence of LMMs vary as the mosquitoes become increasingly habituated to laboratory conditions.

Probabilistic representations of variability in risk assessment models, and the associated methods necessary to propagate their effect on risk estimates, requires training and a good understanding of probability theory. Analysts wishing to employ these methods in EGD-LMO risk assessment should either complete training in the underlying theory and techniques or seek assistance.

D. Deep uncertainty and the "unknown unknowns"

Deep uncertainty arises in situations where epistemic uncertainty or variability is so large that analysts do not know, or the parties to a decision cannot agree on: (a) the appropriate models to describe the interactions among a system's variables; (b) the probability distributions to represent uncertainty about key variables and parameters in these models; and/or (c) how to value the desirability of alternative outcomes (Institute of Medicine, 2013). Risk assessment for EGD-LMOs that are designed to spread over large (continental) scales or persist for long (decades) periods of time, may encounter this type of uncertainty. Then and others (2020), for example, argue that the "next-generation effects" that may occur when EGD-LMOs replicate with genetically diverse target populations, within complex ecosystems, and effects triggered by genome × environment interactions, may introduce a high level of uncertainty into EGD-LMO risk assessment.

Current guidance for addressing deep uncertainty within risk assessment recommends that analysts compare or combine predictions from multiple models that are consistent with available knowledge (Cox, 2012). Alternatively, analysts may use scenario analysis to identify possible future states of the world by describing hypothetical, but conceptually feasible pathways to harm during the problem formulation, and through the use of multiple single-value (e.g. best-case/worst-case) or deliberately imprecise (e.g. order of magnitude) model parameter estimates (Spiegelhalter and Hauke, 2011). The emphasis in these situations may switch away from a reliance on the risk predictions and more towards the identification of risk management strategies that are effective across many (ideally all) scenarios, and towards the identification of post-release monitoring strategies that enable rapid feedback and learning about actual outcomes (Institute of Medicine, 2013).

Risk assessment models (see annex I) typically have many parameters, which may be understood to a greater or lesser extent; the variation in fecundity and mortality rates in an EGD-LMO population may be well understood, whereas interspecific competition coefficients and long-range dispersal rates may be poorly understood. In these circumstances a mixed strategy that combines probabilistic assessments of variability for well characterized parameters within scenarios that reflect possible best-case/worst-case situations for poorly characterized parameters may be advisable.

However, there is no operational definition for when a lack of consensus about an appropriate model or the range of values a parameter may take becomes a case of deep uncertainty (Institute of Medicine, 2013). Ultimately, risk analysts, reviewers and biosafety regulators must judge whether the models and parameter estimates used within a risk assessment are plausible, consistent with theory and defensible in the light of the available evidence base.

Finally, it is important to recognize that a rigorous and systematic analysis of uncertainty within a risk assessment requires specialist skills and computing resources, and the number of plausible pathways to harm that might be imagined during the problem formulation stage will always be subject to practical constraints. Furthermore, multiple models and scenario analyses cannot protect against the deepest form of uncertainty – the "unknown unknowns" – that is, the ignorance that lies beyond the things we know that we don't know. Current guidance on this topic, however, is very clear: decision makers should understand that by definition the "unknown unknowns" can be neither quantified nor described in any type of risk assessment and must therefore act accordingly (EFSA Scientific Committee and others, 2018).

Annex III

World Health Organization guidance framework for testing genetically modified mosquitoes

In 2021, WHO published the second edition of its guidance framework for testing genetically modified mosquitoes (it refers to LMMs as genetically modified mosquitoes (GMMs) and to EGD-LMMs as gene drive modified mosquitoes (GDMMs)). WHO recommends that a safety criterion for moving EGD-LMMs from laboratory to field testing is "a well-reasoned justification that they will do no more harm to human health than wild mosquitoes of the same genetic background and no more harm to the ecosystem than other conventional vector control interventions" (WHO, 2021).

As a matter of comparison (although the use of EGD-LMOs is not a biological control approach), WHO points out that a biologically relevant precedent already exists in trials of biological control agents, which also are expected to spread and persist in the environment, are capable of transboundary movement, and, moreover, cannot be recalled once released (WHO, 2021, section 5.3.5). Before the field release of biological control agents, the International Plant Protection Convention, overseen by the Food and Agricultural Organization of the United Nations, advocates rigorous science-based environmental risk assessment based on International Standards for Phytosanitary Measures. Numerous jurisdictions have established national regulatory systems based on this approach.

Here, a relevant example of field release of a biological control agent that also involved transboundary movement is the release of the neotropical parasitoid *Apoanagyrus* (*Epidinocarsis*) *lopezi* (Hymenoptera: Encyrtidae), from South America, in 22 countries in Africa to successfully control the damaging accidental introduction of the cassava mealybug *Phenacoccus manihoti*. In a similar vein, in Australia, Viet Nam and Indonesia, rigorous environmental risk assessment was conducted ahead of field studies of *Wolbachia*-infected *Aedes aegypti* which, although not regulated as GMMs, were incapable of being recalled once released into the environment (WHO, 2021). Note that for an introduced *Wolbachia*, in principle it could be "recalled" returning to the initially uninfected state by a "swamping" strategy bringing the infection frequency below a given threshold, but this seems implausible except for small and isolated populations (Turelli and Barton, 2017).

WHO sees environmental risk assessment ahead of any proposed field releases as essential, recognizing that this would occur at institutional and national levels and is typically underpinned by national biosafety legislation that, in the case of at least 172 countries, is itself derived from the Cartagena Protocol on Biosafety (WHO, 2021, section 5). In the context of self-sustaining, non-localizing, low-threshold GDMMs, WHO advises that considerations to move from physically confined indoor testing to field testing involve: (a) thorough environmental risk assessment informed by laboratory and insectary trials of the gene drive mosquitoes; (b) entomological, epidemiological, and ecological data from the proposed field locations; and (c) mathematical modelling simulating the behaviour of the gene drive system at the field location (WHO, 2021, section 1.5.1).

Annex IV

Taxonomic classification of the Culicidae (mosquitoes)[18]

Subfamily	Tribe	Genera
Anophilinae		*Anopheles* (*An.*), *Bironella* (*Bi.*), *Chagasia* (*Ch.*)
Culicinae	Aedeomylini	*Aedeomyia* (*Ad.*)
	Aedini	*Aedes (Ae.), Armigeres (Ar.), Eretmapodites (Er.), Haemagogus (Hg.), Heizmannia (Hz.), Opifex (Op.), Psorophora (Ps.), Udaya (Ud.), Zeugnomyia (Ze.)*
	Culicini	*Culex (Cx.), Deinocerites (De.), Galindomyia (Ga.)*
	Culisetini	*Culiseta (Cs.)*
	Ficalbiini	*Ficalbia (Fi.), Mimomyia (Mi.)*
	Hodgesiini	*Hodgesia (Ho.)*
	Mansoniini	*Coquillettidia (Cq.), Mansonia (Ma.)*
	Orthropodomylini	*Orthopodomyia (Or.)*
	Sabethini	*Sabethes (Sa.), Wyeomyia (Wy.), Phoniomyia (Ph.), Limatus (Li.), Trichoprosopon (Tr.), Shannoniana (Sh.), Runchomyia (Ru.), Johnbelkinia (Jb.), Isostomyia (Is.), Tripteroides (Tp.), Malaya (Ml.), Topomyia (To.), Maorigoeldia (Mg.)*
	Toxorhynchitini	*Toxorhynchites (Tx.)*
	Uranotaeniini	*Uranotaenia (Ur.)*

18 Adapted from Foster and Walker (2019).

Annex V

Non-exhaustive list of mosquito vectors of diseases

Host	Mosquito species	Disease	Pathogen	Reference(s)
Human	*Aedes aegypti*	Chikungunya	Virus	WHO, 2022a
		Dengue fever	Virus	WHO, 2023a
		Mayaro fever	Virus	Celone and others, 2021
		Lymphatic filariasis	Nematode	WHO, 2023b
		Rift Valley fever	Virus	Gregor and others, 2021
		Urban yellow fever	Virus	Shinde and others, 2022; WHO, 2023d
		Zika fever	Virus	Kauffman and Kramer, 2017
	Ae. africanus	Zika fever	Virus	Haddow and others, 1964
	Ae. albopictus	Chikungunya	Virus	WHO, 2022a
		Dengue fever	Virus	WHO, 2019
		Jamestown Canyon virus	Virus	Paupy and others, 2009
		Lymphatic filariasis	Nematode	WHO, 2023b
		Mayaro fever	Virus	Celone and others, 2021
		Potosi virus	Virus	Paupy and others, 2009
		Zika fever	Virus	Kauffman and Kramer, 2017; WHO, 2019, 2022b
	Ae. atropalpus	La Crosse encephalitis	Virus	Giunti and others, 2023
		West Nile fever	Virus	Giunti and others, 2023
	Ae. bromeliae	Dengue fever	Virus	Foster and Walker, 2019
		Yellow fever	Virus	Foster and Walker, 2019
	Ae. cantans	Tahyna virus	Virus	Cai and others, 2023
	Ae. caspius	Tahyna virus	Virus	Calzolari and others, 2022
	Ae. cinereus	Rabbit fever (Tularemia)	Bacterium	Petersen and others, 2009
	Ae. communis	Sindbis fever	Virus	Wilkman and others, 2023
	Ae. dorsalis	California encephalitis	Virus	Foster and Walker, 2019
	Ae. excrucians	Sindbis fever	Virus	Wilkman and others, 2023
	Ae. furcifer	Dengue fever	Virus	Foster and Walker, 2019
	Ae. hensilli	Zika fever	Virus	Duffy and others, 2009
	Ae. japonicus japonicus	Cache Valley fever	Virus	Waddell and others, 2019
	Ae. luteocephalus	Dengue fever	Virus	Foster and Walker, 2019
		Yellow fever	Virus	Foster and Walker, 2019
		Zika fever	Virus	Epelboin and others, 2017
	Ae. melanimon	California encephalitis virus	Virus	Foster and Walker, 2019
	Ae. niveus	Lymphatic filariasis	Nematode	Foster and Walker, 2019
	Ae. opok	Dengue fever	Virus	Foster and Walker, 2019
	Ae. polynesiensis	Chikungungya	Virus	Richard and others, 2016
		Dengue fever	Virus	Foster and Walker, 2019
		Lymphatic filariasis	Nematode	Foster and Walker, 2019

Host	Mosquito species	Disease	Pathogen	Reference(s)
Human	*Ae. pseudoscutellaris*	Dengue fever	Virus	Foster and Walker, 2019
		Lymphatic filariasis	Nematode	Foster and Walker, 2019
	Ae. rotumae	Dengue fever	Virus	Foster and Walker, 2019
	Ae. scapularis	Cache Valley fever	Virus	Waddell and others, 2019
	Ae. scutellaris	Dengue fever	Virus	Foster and Walker, 2019
	Ae. sollicitans	Cache Valley fever	Virus	Waddell and others, 2019
	Ae. taeniorhynchus	Cache Valley fever	Virus	Waddell and others, 2019
	Ae. taylori	Dengue fever	Virus	Foster and Walker, 2019
	Ae. triseriatus	La Crosse encephalitis	Virus	Giunti and others, 2023
	Ae. vexans	Cache Valley fever	Virus	Waddell and others, 2019
		Tahyna virus	Virus	Cai and others, 2023; Mravcova and others, 2023
	Ae. vittatus	Yellow fever	Virus	Sudeep and Shil, 2017
	Anopheles gambiae	Malaria	Plasmodium	Djihinto and others, 2022
		Lymphatic filariasis	Nematode	Foster and Walker, 2019
	An. arabiensis	Malaria	Plasmodium	Djihinto and others, 2022
		Lymphatic filariasis	Nematode	Foster and Walker, 2019
	An. barbirostris	Lymphatic filariasis	Nematode	Foster and Walker, 2019
	An. coluzzii	Malaria	Plasmodium	Djihinto and others, 2022
	An. funestus	Malaria	Plasmodium	Djihinto and others, 2022
	An. stephensi	Malaria	Plasmodium	Djihinto and others, 2022
	An. punctipennis	Cache Valley fever	Virus	Waddell and others, 2019
	An. quadrimaculatus	Cache Valley fever	Virus	Waddell and others, 2019
	Coquillettidia richiardii	Sindbis fever	Virus	Wilkman and others, 2023
	Culex annulirostris	Murray Valley encephalitis	Virus	Braddick and others, 2023
	Cx. antennatus	Rift Valley fever	Virus	Tantely and others, 2015
	Cx. nigripalpus	St. Louis encephalitis	Virus	Curren and others, 2018
	Cx. pipiens	Rift Valley fever	Virus	Foster and Walker, 2019
		St. Louis encephalitis	Virus	Curren and others, 2018
		Usutu virus	Virus	Braack and others, 2018
		West Nile fever	Virus	Colpitts and others, 2012
	Cx. quinquefasciatus	Lymphatic filariasis	Nematode	Foster and Walker, 2019
		St. Louis encephalitis	Virus	Curren and others, 2018
		West Nile fever	Virus	Colpitts and others, 2012
	Cx. rubinotus	Banzi virus	Virus	Braack and others, 2018; MacIntyre and others, 2023
	Cx. stigmatosoma	West Nile fever	Virus	Colpitts and others, 2012
	Cx. tarsalis	St. Louis encephalitis	Virus	Curren and others, 2018
		West Nile fever	Virus	Colpitts and others, 2012
	Cx. thriambus	West Nile fever	Virus	Colpitts and others, 2012
	Cx. tritaeniorhynchus	Japanese encephalitis	Virus	Lessard and others, 2021
	Cx. univittatus	West Nile virus	Virus	Cornel and others, 1993
	Cx. vishnui	Japanese encephalitis	Virus	Maquart and others, 2022
	Haemagogus janthinomys	Mayaro fever	Virus	Celone and others, 2022; Hoch and others, 1981; Pereira and others, 2021
		Yellow fever	Virus	Celone and others, 2022

Host	Mosquito species	Disease	Pathogen	Reference(s)
Human	*Hg. leucocelaenus*	Yellow fever	Virus	Silva da Silva and others, 2020
	Hg. lucifer	Yellow fever	Virus	Foster and Walker, 2019
	Mansonia annulifera	Lymphatic filariasis	Nematode	Foster and Walker, 2019
	Ma. uniformis	Lymphatic filariasis	Nematode	Foster and Walker, 2019
Other animals	*Ae. albopictus*	Eastern equine encephalitis virus	Virus	Little and others, 2021
		Canine heartworm	Nematode	Morchon and others, 2012
	Ae. circumluteolus	Wesselsbron virus	Virus	Foster and Walker, 2019
	Ae. mcintoshi	Wesselsbron virus	Virus	Foster and Walker, 2019
	Cx. tarsalis	Western equine encephalitis virus	Virus	Eldridge and others, 2004
	Cx. tritaeniorhynchus	Tembusu virus	Virus	Hamel and others, 2023
	Cx. taeniopus	Venezuelan equine encephalitis virus	Virus	Torres and others, 2017
	Culiseta melanura	Eastern equine encephalitis virus	Virus	Armstrong and Andreadis, 2010
	Psorophora confinnis	Venezuelan equine encephalitis virus	Virus	Torres and others, 2017

Annex VI

Current landscape for development of living modified mosquitoes containing engineered gene drives for disease vector control

Target vector-borne disease	Target mosquito vector species	EGD threshold for field releases	EGD persistence in target populations	EGD spread in target populations	Mechanism underpinning EGD	Intended impact on target populations	Stage of EGD development	References
Malaria	*An. gambiae* s.l.	Low	Self-sustaining	Non-localized	Homing	Suppression	Modelling; strains generated and tested in insectary in target species	Hammond and others, 2016, 2021; Kyrou and others, 2018; North and others, 2019
					Homing	Modification	Modelling; strains generated and tested in insectary in target species	Carballar-Lejarazú and others, 2023
					Homing with sex ratio distorter	Suppression	Modelling; strains generated and tested in insectary in target species	Simoni and others, 2020
					Homing based on integral and modular mechanism	Modification, potentially in conjunction with population suppression	Modelling; strains generated and tested in insectary in target species	Ellis and others, 2022; Hoermann and others, 2021, 2022; Nash and others, 2019
					Y drive	Suppression	Modelling only	Deredec and others, 2011

Target vector-borne disease	Target mosquito vector species	EGD threshold for field releases	EGD persistence in target populations	EGD spread in target populations	Mechanism underpinning EGD	Intended impact on target populations	Stage of EGD development	References
				Localized	Double drive; homing	Suppression or modification	Modelling only	Geci and others, 2022; Sudweeks and others, 2019; Willis and Burt, 2021
	An. funestus	Low	Self-sustaining	Non-localized	Homing	Suppression	CRISPR-Cas9-mediated genomic insertion of transgenes via homology directed repair in target species	Li and others, 2018; Quinn and others, 2021
	An. stephensi	Low	Self-sustaining	Non-localized	Homing	Modification	Strains generated and tested in insectary in target species	Gantz and others, 2015; Pham and others, 2019
					Toxin-antidote rescue system; homing	Modification	Strains generated and tested in insectary in target species	Adolfi and others, 2020
Dengue, Yellow fever, Chikungunya, Zika	*Ae. aegypti*	Low	Self-sustaining	Non-localized	Medea (maternal effect dominant embryonic arrest	Modification	Modelling	Legros and others, 2013
		High	Self-sustaining	Localized	Two-locus underdominance	Modification	Modelling	Edgington and Alphey, 2017, 2018; Sánchez and others, 2020b
			Self-limiting	Localized	Homing; split drive	Modification	Modelling; strains generated and tested in *Drosophila* model system; mosquito strains generated and tested	Anderson and others, 2023; Li and others, 2020; López Del Amo and others, 2020; Terradas and others, 2021
					Toxin-antidote rescue system	Modification	Modelling	Legros and others, 2013

Target vector-borne disease	Target mosquito vector species	EGD threshold for field releases	EGD persistence in target populations	EGD spread in target populations	Mechanism underpinning EGD	Intended impact on target populations	Stage of EGD development	References
Wuchereria bancrofti lymphatic filariasis, West Nile virus, St. Louis encephalitis	*Cx. quinquefasciatus*	High	Self-limiting	Localized	Homing; split drive	Modification	Strains generated and tested in insectary in target species	Harvey-Samuel and others, 2023
Potentially multiple other diseases (e.g. malaria or arboviral infections from South America or Asia-Pacific regions)	Potentially multiple other vectors (e.g. *Anopheles*, *Aedes,* or *Culex* species from South America or Asia-Pacific regions)	Low	Self-sustaining	Non-localized	Medea (maternal effect dominant embryonic arrest)	Modification	Modelling; strains generated and tested in *Drosophila* model system only	Buchman and others, 2018a; Chen and others, 2007
					Toxin-antidote rescue system	Modification	Modelling; strains generated and tested in *Drosophila* model system only	Oberhofer and others, 2019, 2020b
		High	Self-limiting	Localized	Toxin-antidote rescue system, Split drive	Modification or suppression	Modelling; strains generated and tested in *Drosophila* model system only	Akbari and others, 2013; Champer and others, 2020a,b; Gould and others, 2008; Oberhofer and others, 2020a, 2021
					One-locus underdominance	Modification or suppression	Modelling; strains generated and tested in *Drosophila* model systems only	Buchman and others, 2018b, 2021; Dhole and others, 2018, 2019; Reeves and others, 2014

Annex VII

Engineered gene drive systems

A. Homing

Here, an EGD results in germline expression of both the Cas9 endonuclease and guide RNAs, which together recognize and cleave specific sequences in the genome (Burt and others, 2018; Connolly and others, 2023). This EGD is inserted precisely into its genomic target location on one of a pair of homologous chromosomes of an LMM. In germline cells, the guide RNA and Cas9 act in concert to cause a double-stranded break in the target DNA site of the homologous chromosome that does not contain the EGD. Homology-directed repair mechanisms are activated by germline cells to repair the double-stranded break. These use the homologous chromosome containing the EGD as a repair template. The flanking sequences on either side of the EGD, along with the EGD itself, are repaired into the double-stranded break at the target site of the homologous, formerly wild-type, chromosome. This process of homing creates pairs of parental homologous chromosomes that are typically homozygous for the EGD, leading to super-Mendelian inheritance of the EGD in progeny. Thus, once introduced into mating populations of mosquitoes, the EGD is expected to increase in frequency, or drive, and spread in target mosquito populations.

B. Y-drive

This form of gene drive is also known as meiotic drive. As is the case in humans, male mosquitoes possess both X and Y chromosomes in their cells, while female cells possess two parental copies of the X chromosome only. The EGD is located on the Y chromosome, so it is only inherited by male mosquitoes. The EGD also expresses a DNA endonuclease in male germline cells that cleaves a genomic target site on the X-chromosome. This means that sperm with X chromosomes produced by the male mosquito are cut and become inviable; only Y-bearing sperm survive. When an EGD-LMM male mates with a wild-type female, only progeny possessing an X from their mother and Y from their father can be produced. So far, such a system has only been tested in the laboratory (Simoni, 2020) or via modelling (Metchanun and others, 2022).

C. Toxin-antidote rescue system

A variety of toxin-antidote EGD systems consist of a genetically linked pair of transgenes, one encoding a toxin and the other an antidote (Hay and others, 2021). Expression of the EGD in LMMs results in the death of gametes or progeny that do not contain the EGD, leading to an increase in the frequency of EGD-LMMs relative to wild type mosquitoes. For example, the *cleave and rescue* (ClvR) or *toxin-antidote recessive embryo* (TARE) systems use germline expression of the Cas9 nuclease and a guide RNA to introduce cuts into an endogenous mosquito gene required for viability. Cellular end-joining repair mechanisms produce loss-of-function mutations in this endogenous gene. When expressed in the germline, it creates loss-of function mutations in essential endogenous genes in the EGD-LMM. The antidote portion of the EGD supplies a recoded version of the endogenous gene that cannot be cleaved by the Cas9/guide RNA combination. Offspring that do not inherit the EGD will not survive because they do not possess the rescuing recoded version of the endogenous gene. Therefore, individuals possessing the EGD increase in frequency relative to wild type mosquitoes and spread in the population.

D. Maternal effect dominant embryonic arrest

The *maternal effect dominant embryonic arrest* (Medea) gene drive system consists of two genetically linked components: a maternally expressed toxin and an

antidote expressed in the zygote. The toxin consists of maternally expressed microRNAs that inhibit expression of an endogenous mosquito gene required for early embryogenesis. The antidote consists of a transgenic version of the same endogenous mosquito gene required for early embryogenesis, but which has been recoded so that it cannot be inhibited by the microRNA. When this antidote transgene is expressed in the early embryo, it rescues the loss of expression of the endogenous mosquito gene so that the embryos survive. Offspring of Medea EGD-LMM mothers that do not inherit the EGD die because they cannot express the rescuing transgene antidote, while those that do inherit the EGD express the rescuing transgene antidote and survive, leading to an increase in the frequency of EGD-LMMs relative to wild type mosquitoes and spread of the EGD through target populations (Hay and others, 2021).

E. Underdominance

Underdominance is a form of gene drive that has been proposed for population modification of mosquito vectors, which allows for localized spread in target mosquito populations (Wang and others, 2022). Because of its requirements for high release thresholds, it can be thought of as a form of localized gene drive. In one-locus underdominance, heterozygotes for the EGD are less fit than either wild types or homozygotes of the EGD, typically leading to self-limiting characteristics. In two-locus underdominance, mosquitoes carrying none or both of two different EGDs are fitter than those carrying only one of the two EGDs, typically producing self-sustaining gene drive.

F. Split drives

Split drives are a type of EGD consisting of two or more unlinked components inserted at different sites in the genome, which are only capable of increasing in frequency and spreading in target mosquito populations when coupled with each other (Champer and others, 2019; Li and others, 2020; Noble and others, 2019; Oberhofer and others, 2020a). They have principally been considered for mosquito population modification. Some modelling indicates that such EGD-LMMs would increase in frequency in target mosquito populations but persist for only a limited time before declining in frequency due to dissociation of both EGD elements. However, evidence also suggests that split-drives may persist beyond the intended design aim and behave like full gene drives (Terradas and others, 2023).

G. Double drives with private alleles

Double drives are comprised of two separate elements that together produce a functional EGD (Willis and Burt, 2021). The first element of the EGD encodes Cas9 that, when expressed alongside a guide RNA that recognizes a specific genomic target locus, or "private allele", that is present in target mosquito populations but not in other mosquito populations, causes homing of that EGD element at that target genomic locus. A separate genetically unlinked element of the EGD encodes a guide RNA that recognizes a second genomic target site. Alongside Cas9 expressed from the first element, this allows homing of the second EGD element that can be used in either population suppression or population modification applications. Together both elements act in EGD-LMMs as a "double drive" EGD for homing both at the genomic target locus required for population suppression or modification and at the genomic target locus restricted to the target mosquito population. This means the double drive EGD would be localized, acting as a self-sustaining, low-threshold EGD in target mosquito populations but a self-limiting, high-threshold split drive in non-target mosquito populations. By contrast, they act as a split drive in non-target populations. Modelling shows that such designs can restrict the spread and impact of the construct even if there is a relatively modest level of genetic differentiation between target and non-target populations (Willis and Burt, 2021).

H. Secondary drive

Examples of secondary drives include reversal drives, immunizing drives (Esvelt and others, 2014; Girardin and others, 2019), overwriting drives, e-CHACR and ERACR (Xu and others, 2020). Such mitigation strategies remain unproven. If considering the use of secondary drives, consideration of potential novel

genetic rearrangements is necessary, with evidence that interaction of the two systems may occur with unintended genetic effects, adding yet more unpredictability and complexity to potential outcomes (Xu and others, 2020).

Annex VIII

List of terms[19]

Term	Description/definition(s)	Source
Applicant	*An individual or organization that applies for approval or authorization of a regulated activity to a responsible government agency or regulatory body. The applicant may be the developer.* *Related term: developer*	Not applicable, N/A (original)
Assessment endpoint	*An expression of the environmental value that is to be protected, operationally defined as an entity (e.g. a species, population or habitat) and an attribute of that entity (e.g. abundance, distribution, mortality) that can be measured or modelled.* *Related term: measurement endpoint*	Adapted from EFSA GMO Panel, 2010; NASEM, 2016; OECD, 2023; WHO, 2001
Cargo/payload gene	*A functional gene or cassette that is linked to the engineered gene drive insert that is not necessary for the engineered gene drive to function but aims to spread the linked gene/cassette throughout a target population.* *Related terms: engineered gene drive, target population*	Alphey and others, 2020 (publication by the gene drive research community proposing a list of standardized definitions); the words "engineered" and "target" have been added to the published definition to link to other definitions in this list of terms.
Confinement measures	*A set of measures intended to prevent or minimize the unintentional release of organisms, such as a living modified mosquito (see living modified organism) containing an engineered gene drive, from a designated area into the surrounding environment. This may include studies conducted in physical confinement (also termed "containment"), with measures including physical barriers such as indoor laboratories, insectaries, or population cages. In outdoor settings, large cages may be used, and additional ecological confinement measures may include geographical/spatial and/or climatic isolation.* *Related terms: engineered gene drive, living modified organism*	Adapted from explanatory text in WHO, 2021
Developer	*An entity/entities undertaking research and development activities aimed at producing new or improved products (goods or services) or processes.*	Derived from descriptions in Beeckman and Rüdelsheim, 2020; OECD, 2015
Ecosystem	*A dynamic complex of plant, animal and microorganism communities and their non-living environment interacting as a functional unit.*	Article 2 (Use of terms) of the Convention on Biological Diversity

19 This list of terms is meant to assist the reader; it is not intended to constitute a glossary or list of definitive definitions.

Term	Description/definition(s)	Source
Ecosystem services	*Benefits people obtain from ecosystems; four categories of ecosystem services are distinguished (provisioning, regulating, cultural and supporting services), where the supporting services are regarded as the basis for the services of the other three categories.* *Related term: ecosystem*	Adapted from Devos and others, 2015; Reid, 2005
Engineered gene drive	*A gene drive system that is created through the application of recombinant DNA techniques.*	Adapted from Alphey and others, 2020; Australian Academy of Science, 2017
Habitat	*The place or type of site where an organism or population naturally occurs.*	Article 2 (Use of terms) of the Convention on Biological Diversity
Harm	*Actual injury or damage to the receiving environment or human or animal health. A harm may also be referred to as an "adverse effect".*	Adapted from Article 15 of the Cartagena Protocol on Biosafety; ISO, 2019; WHO, 2021
Hazard	*A source of potential harm.* *Related term: harm*	ISO, 2019; Office of the Gene Technology Regulator, 2005
Hazard identification	*A step in the risk assessment process involving the identification of potential sources of harm to protection goals, and the causal pathway giving rise to that harm.* *Related terms: harm, protection goals, risk assessment*	Adapted from Office of the Gene Technology Regulator, 2005; WHO, 2021
High-threshold drive	*Modelling indicates that gene drive systems may have a threshold level, which refers to the ratio of gene-drive-bearing organisms to wild-type organisms that must be exceeded for the gene drive to spread throughout a target population. For high-threshold drives, this ratio is relatively high (compare low-threshold drive), and in theory, they are likely to demonstrate restricted spread.* *Related terms: low-threshold drive, target population*	Adapted from Alphey and others, 2020; Australian Academy of Science, 2017; WHO, 2021
Integrated pest management	*The careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations. It combines biological, chemical, physical and crop specific (cultural) management strategies and practices to grow healthy crops and minimize the use of pesticides, reducing or minimizing risks posed by pesticides to human health and the environment for sustainable pest management.*	FAO, 2024
Interference mechanism	*A gene drive mechanism in which the transgenic construct biases its transmission by interfering with the inheritance or function of wild-type genes. A reported example is a meiotic drive.*	Adapted from NASEM, 2016; WHO, 2021
Limits of concern	*The level of environmental protection set for a measurement endpoint, expressed as the minimum ecological effects deemed biologically relevant and of sufficient magnitude to cause harm.* *Related terms: measurement endpoint, harm*	EFSA GMO Panel, 2010
Living modified mosquito	*Any living mosquito that possesses a novel combination of genetic material obtained through the use of modern biotechnology.*	Adapted from Article 3 (g) of the Cartagena Protocol on Biosafety

Term	Description/definition(s)	Source
Living modified organism	*Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.*	Article 3 (g) of the Cartagena Protocol on Biosafety
Low-threshold drive	*Modelling indicates that gene drive systems may have a threshold level, which refers to the ratio of gene-drive-bearing organisms to wild-type organisms that must be exceeded for the gene drive to spread throughout a target population. For low-threshold drives, this ratio is relatively low (compare high-threshold drive), and in theory, a low initial release of gene-drive-bearing individuals would be sufficient for the drive to spread throughout a large target population.* *Related terms: high-threshold drive, target population*	Adapted from Alphey and others, 2020; Australian Academy of Science, 2017
Measurement endpoint	*A measurable indicator of change in an assessment endpoint, e.g. the density and abundance of a species.* *Related term: assessment endpoint*	Suter II, 2006
Open release trial	*A field trial or series of sequential field trials of increasing size, duration and complexity, conducted at a single site or multiple sites; may involve confinement measures. The trials will aim to collect data including entomological and epidemiological efficacy, dispersal, trait behaviour and ecological interactions.* *Related term: confinement measures*	Adapted from WHO, 2021
Over-replication mechanism	*A gene drive mechanism in which the transgenic construct biases its transmission by replicating more often than other genes. Homing endonuclease genes are reported to achieve drive using this mechanism.*	Adapted from MacFarlane and others, 2023; WHO, 2021
Pathway to harm	*A scientifically plausible description of the necessary sequence of steps for a harm to be realized. These pathways are constructed during the problem formulation process.* *Related term: harm*	Adapted from EFSA GMO Panel, 2020; OECD, 2023
Protection goals	*Components of the environment (e.g. biological diversity, genetic diversity, human and animal health, habitats, ecosystems, ecosystem functions and services, soil health, water quality) that are valued and need to be protected from harm. They are usually identified in the relevant laws or policies of a jurisdiction and establish the context for the environmental risk assessment.* *Related terms: habitat, harm, risk assessment*	Adapted from EFSA GMO Panel, 2010; OECD, 2023
Regulator	*A regulatory entity or government body with responsibility for regulating certain activities; e.g. for activities with EGD-LMOs, a regulator may have responsibility for issuing regulatory approvals and authorizations, monitoring compliance, and enforcement of regulatory conditions.*	N/A
Risk	*The likelihood of a hazard causing harm.* *Related terms: harm, hazard*	EFSA, 2016

Term	Description/definition(s)	Source
Risk assessment	*A process that evaluates the potential risks associated with certain hazards. It involves four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization.* *Related terms: hazard, hazard identification, risk characterization*	EFSA, 2024; WHO, 2021
Risk assessor	*The entity that conducts the risk assessment; e.g. for an EGD-LMO regulatory application, a risk assessor would review the scientific data and information submitted by the applicant to evaluate the risks associated with the proposed regulated activity and may make recommendations for risk management.* *Related terms: applicant, risk, risk assessment, risk management*	N/A
Risk characterization	*The final step of the risk assessment process, with estimation of the overall risk posed to protection goals on the basis of the likelihood and consequences of adverse effects being realized.* *Related terms: protection goals, risk, risk assessment*	Adapted from WHO, 2021
Risk hypothesis	*For each postulated pathway to harm, corresponding risk hypotheses are formulated that will enable the risk assessor to determine whether the pathway is likely to occur.* *Related terms: pathway to harm, risk assessor*	Adapted from OECD, 2023
Risk management	*The management of risks identified by the risk assessment through the implementation of appropriate measures for reducing risk to an acceptable level.* *Related terms: risk, risk assessment*	Adapted from EFSA, 2024; WHO, 2021
Risk manager	*The entity that defines and/or implements risk management measures. In certain jurisdictions, e.g. in the European Union, the risk manager makes regulatory decisions (see also regulator).* *Related terms: regulator, risk management*	N/A
Signal	*A measurable change in an indicator or parameter of interest that can be linked to an adverse change in the environment.*	Adapted from Tofelde and others, 2021
Target population	*An individual population or interbreeding populations of the target organism on which the specifically designed characteristics of the EGD-LMO are intended to act.*	Adapted from Connolly and others, 2023; EFSA, 2024; WHO, 2021
Vector	*Agent which carries and transmits an infectious pathogen into another living organism.*	Adapted from WHO, 2020

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