UN environment programme

CBD/CP/RA/AHTEG/2024/1/2/Add.1

Distr.: General
21 February 2024

English only

Convention on Biological Diversity

Ad Hoc Technical Expert Group on Risk Assessment
Second meeting
Montreal, Canada, 27 February–1 March 2024
Item 4 of the provisional agenda*
Consideration of the draft additional voluntary guidance materials to support the case-by-case risk assessment of living modified organisms containing engineered gene drives

Draft additional voluntary guidance materials to support the case-by-case risk assessment of living modified organisms containing engineered gene drives

Note by the Secretariat

The present document contains the draft additional voluntary guidance materials for conducting case-by-case risk assessments of living modified organisms containing engineered gene drives in accordance with annex III to the Protocol. The Expert Group will need to further advance those materials with a view to completing a draft for consideration by the Subsidiary Body at its twenty-sixth meeting. The draft is being issued without formal editing.

* CBD/CP/RA/AHTEG/2024/1/1.

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

Contents

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 assessment of living modified organisms (LMOs) containing engineered gene drives **(**EGDs; EGD-LMOs**)** in accordance with annex III to the Protocol.[1, 2] The Conference of the Parties decided that this material should have a special focus on living modified mosquitoes (LMMs) that contain an EGD (EDG-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. Decision CP-10/10 also established an *Ad Hoc* Technical Expert Group (AHTEG) on risk assessment that is responsible to 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 The Subsidiary Body on Scientific, Technical and Technological Advice will consider the draft guidance materials prepared by the AHTEG at its twenty-sixth meeting (13–18 May 2024).

As a response, and with the financial support of the Government of Finland and the European Union, the Secretariat of the Convention on Biological Diversity commissioned the International Centre for Genetic Engineering and Biotechnology (ICGEB) to develop a detailed outline to support the development of the supplementary guidance materials on the risk assessment of EGD-LMOs. The AHTEG revised the outline, then developed the detailed content of the additional voluntary guidance materials. The objective was to facilitate a case-by-case risk assessment process for EGD-LMOs, thereby complementing annex III and existing guidelines, while considering the established roadmap.[3]

1.1. Structure

The guidance is developed in accordance with annex III to the Cartagena Protocol on Biosafety, in particular with its paragraph 8, which outlines the sequential steps of the risk assessment process. The evaluation of consequences of potential adverse effects may be undertaken at the same time as the evaluation of their likelihood. Each step in the process builds upon the results of the preceding step, ensuring a comprehensive assessment of potential risks.

Risk assessment frameworks can combine the process of establishing the context and scope with the identification of potential adverse effects associated with a LMO into an approach called “problem formulation”. This approach is followed in the additional voluntary guidance materials. Initiating the risk assessment with the problem formulation approach has gained wide acceptance globally and is increasingly applied and recognised as contemporary best practice by the risk assessors’ community (e.g., NASEM, 2016; European Union, 2018; EFSA, 2020; WHO, 2021; CCA, 2023; OECD, 2023).

Problem formulation helps to frame the risk assessment process and does so by clarifying policy goals and scientific criteria for assessing risks and devising risk hypotheses that meet those criteria. It enables risk assessors to identify a spectrum of potential adverse effects derived from the deployment of a LMO and to

[1] Decision CP-10/10: https://www.cbd.int/doc/decisions/cp-mop-10/cp-mop-10-dec-10-en.pdf

[2] The Cartagena Protocol on Biosafety 2003. https://bch.cbd.int/protocol/.

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

devise (a) potential pathway(s) to such harm and define the actual information needed to assess the likelihood of these hazards to occur and their seriousness. The additional voluntary guidance materials introduce problem formulation as the first critical step of risk assessment. The testing of the risk hypotheses of the pathways to harm would be performed in the subsequent risk assessment steps presented in paragraph 8 of annex III to the Protocol, as outlined in Figure 1. At each step of the pathway to harm more detailed information on probabilities and uncertainties are provided. In addition, stakeholder engagement can be included, as appropriate, at several points in the process.

Ultimately, the conclusions and recommendations derived from the risk assessment are essential inputs in the decision-making process concerning the use and intentional release of LMOs. In line with country-specific policies and protection goals, other relevant aspects of the Protocol and related issues discussed in section 7 of this guidance may also be considered during the decision-making process.

2. Introduction

Advances in molecular and synthetic biology are enabling the engineering of gene drives in LMOs. Such EGDs can be described as genetic elements that are sexually transferred to subsequent generations at a frequency greater than the 50% expected by Mendelian inheritance (WHO, 2021), thereby biasing their own inheritance. This preferential inheritance may allow EGD systems (i.e., the engineered gene drive along with any genetically linked cargo/payload genes) to rapidly spread in sexually reproducing populations[4], increasing their prevalence. EGD systems can be designed either to suppress or eliminate interbreeding target populations or modify them with an altered genotype. In theory, 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 LMOs with other (non-EGD) transgenes in their potential to spread, increase in frequency, persist in and suppress interbreeding target populations. EGD-LMOs may also differ from LMOs used in agriculture, as EGDs are often 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. It has also been noted that some disease vectors belong to species complexes that contain both vector and non-vectors species, where 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 amongst sibling species, including non-disease vectors (Connelly and others, 2023). Depending on the EGD system, the envisaged effect of a 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 LMOs 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 (EGD-LMO) have been tested experimentally in the laboratory (e.g., Raban and others, 2020), but to date none have been assessed 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 potential adverse, unexpected and/or irreversible effects. Further these effects, whether intended or not, could include not only direct and immediate effects, but also indirect, cumulative and long-term effects. Therefore, discussions have been held at different levels amongst various stakeholders, including policy makers, risk assessors, risk managers and potential applicants, to determine whether there is a need to develop new or additional guidance for the risk assessment of EGD-LMOs for intended release into the environment (Simon and others, 2018; Keiper and Atanassova, 2020; Devos and others, 2020, 2021).

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

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 Convention on Biological Diversity published general guidance on the risk assessment of LMOs,[5] 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 provide relevant information to EGD-LMOs as well (NASEM, 2016; EFSA, 2020; ESA, 2020; WHO, 2021).

2.1. Precautionary approach

Article 1 of 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".

Principle 15 of the Rio Declaration on Environment and Development (United Nations, 1992) states that: "In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities".

Additionally, Article 10, paragraph 6 of the Cartagena Protocol further articulates 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.

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." Should an organism containing an engineered gene drive provide a demonstrated benefit to biodiversity, particularly when compared to existing measures (e.g., pesticide use), scientific uncertainty should not be used to delay the development, approval, and adoption of that EGD organism.

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 plausible and/or relevant risks that the deployment of a LMO may pose to biodiversity, and human and animal health. Risk assessment evaluates the various potential adverse effects and their associated likelihood, taking into account the kinds and levels of exposure, to determine risks that might be associated with the use of a 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, evidence gaps and knowledge gaps. The outcome of the risk assessment serves as a

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

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

Agreed principles of the risk assessment of LMOs are laid down in annex III of the protocol.

Typically, risk assessments:

- Are ***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 assessments shall be based, at a minimum, on information provided in accordance with annex III, paragraph 9 of 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.;
- Are carried out on a ***case-by-case*** basis, meaning that they vary 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 receiving environments (covering the receiving environments where the LMO will be released and spread), and the interactions amongst these variables. Thus, the potential adverse effects caused by a LMO on protection goals will vary depending on its characteristics, how it is used, and the environment in which it is present, and across time.
- Use a ***comparative*** approach, whereby the level of risk is estimated through comparison, most often with a non-LMO counterpart or parental organism in the likely receiving environment. Previous experience with the assessment of LMOs has typically involved comparators that have a history of safe use for humans and/or animals and familiarity for the environment;
- When appropriate, follow the ***step-by-step*** principle, in which the deployment of a 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 (NASEM, 2016; Hayes and others, 2018; James and others, 2018; WHO, 2021). However, in some cases (e.g., for organisms that cannot be maintained in colonies), this approach using smaller trials may not feasible;
- Consider ***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);
- Follow a ***tiered-based*** testing approach, where tests are initially conducted representing worst-case scenarios of exposure and/or consequence and are then progressively made more realistic, as

[6] Also termed: general protection goals or generic endpoints.

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 (e.g., 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);

- Are ***iterative*** and ***transparent*** 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.

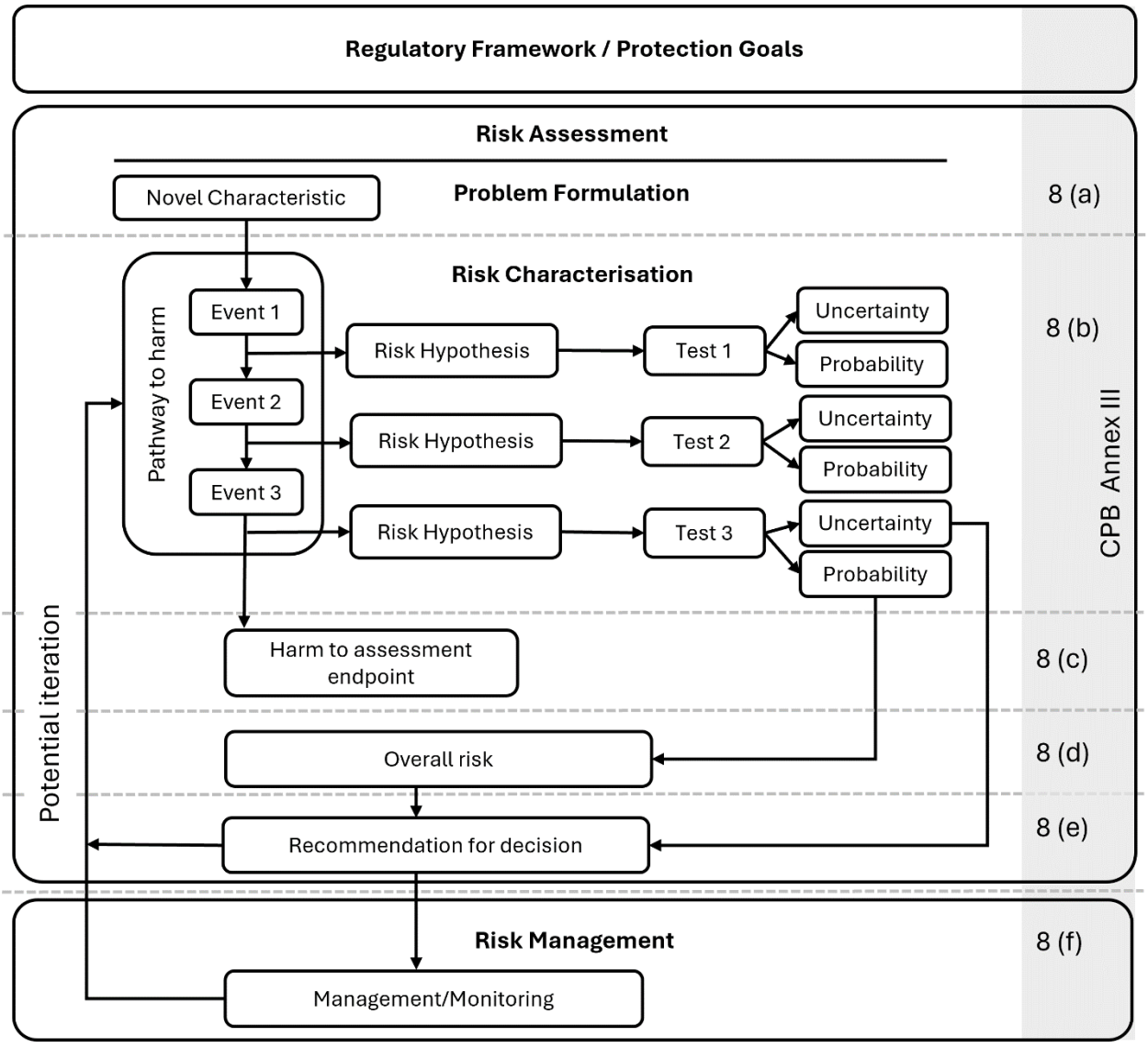

Figure 1: Integration of the problem formulation and risk hypothesis testing as complementary steps in the risk assessment process outlined in annex III of the Protocol

3. Explaining 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 and 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. Other current and proposed targets for gene drive development include snails, fungi, fish and mammals such as rabbits, feral cats and grey squirrel (Wells and Steinbrecher, 2023). While research on EGDs and their applications in insects are advancing at a

relatively faster pace, it is generally accepted that it will likely take several more years for technological developments to move to practical applications for intentional release into the environment.

Scientists are working to utilize gene drives, either by modifying, redesigning and re-purposing naturally occurring drive systems, or by designing and engineering novel systems, resulting in EGDs. The use of EGD-LMOs is proposed to address challenges related to disease vectors (e.g., mosquitoes and ticks), agricultural pests (e.g., various fruit flies, and beetles) and invasive species (e.g., rodents), as well as help to rescue endangered species (Raban and others, 2020; Devos and others, 2022; Wells and Steinbrecher 2023a,b). A variety of EGDs are in the research and development. EGD systems can be categorised into two main mechanisms, namely: over-replication mechanisms or interference mechanisms**.**

3.1. Engineered gene drive strategies

Strategies for EGD-LMOs can be differentiated based on: (1) the intended outcome; and (2) the potential for the genetic modification to spread in target populations by mating and persistence in the environment after release (table 1). EGD systems can be designed either to suppress[7] or reduce target populations and potentially species, or to modify[8] or replace them with a new genotype. This can be achieved either through the inactivation of an endogenous gene, or by the introduction of a new (engineered) genetic trait in a target population. Strategies aiming for population modification require the genetic modification of interest to persist in the population over an extended period (James and others, 2018). Moreover, depending on the design of the EGD system (whose composition and mode of action are diverse), theoretically, the genetic modification of interest could spread through interbreeding target populations (non-localised[9]) and persist

[7] 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 (James and others, 2018). These suppression strategies are expected to result in population decline/reduction or even collapse (local elimination) over the period of a few generations and may in some cases aim for (global) eradication of a disease vector species.

[8] Population modification strategies, mostly for disease vector control, are used to modify a current genotype with one that is less able to transmit disease (impaired vector competence), or that is more resistant to pathogen infection (disease refractory). 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 insect (James and others, 2018). 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 insect.

[9] Also termed: Low threshold or threshold independent gene drives. Threshold independent gene drives may spread from very low initial population frequencies, requiring only a small number of EGD-LMO individuals to be released to spread (Noble and others, 2018). Such types of EGDs have a higher potential to spread into neighboring populations for an indeterminate time (Alphey, 2014; Champer and others, 2016).

indefinitely (self-sustaining[10]), or be restricted in its spread (localised[11]) or persistence (self-limiting[12]) (EFSA, 2022; WHO, 2022; CCA, 2023) (table 1). While the binary divides between localised/non-localised 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 (Dhole and others, 2018, 2020; Backus and Delbourne, 2019). 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 (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 progress to field testing, which aim to be more specific, stable and controllable systems (NASEM, 2016; Friedman and others, 2020; Raban and others, 2020).

Table 1

Possible dimensions to categorize EGD strategies based on their potential to spread and persist

		Time scale	
		Self-limiting	**Self-sustaining**
Spatial scale	**High threshold (non-spreading)**	Spatially restricted (*localized*) and temporally restricted (*transient*) drives	Spatially restricted (*localized*) and temporally unrestricted (*persistent*) drives
	Low threshold (spreading)	Spatially unrestricted (*non-localized*) and temporally restricted (*transient*) drives	Spatially unrestricted (*non-localized*) and temporally unrestricted (*persistent*) drives

3.2. Concerns and opportunities

The ability to engineer gene drives has sparked both enthusiasm and concerns (Esvelt and others, 2014; Brossard and others 2019; Deplazes-Zemp and others, 2020). Unlike other LMOs, EGD-LMOs are specifically designed to disperse beyond their initial release locations and persist in target populations over

[10] Also termed: Self-propagating or global drives. Self-sustaining genetic control systems can be described as those in which the genetic modification is intended to become stably established in target populations. In the case of engineered gene drives, they can be designed to spread a genetic modification of interest in target populations rapidly, widely and for an indeterminate time, perhaps many generations or until the target population is eliminated (Alphey, 2014). Since self-sustaining gene drives can be engineered to be spatially and temporally unrestricted (non-localised and persistent, respectively), they could move to any interbreeding target population that has vertical gene flow with the target population where the gene drive modified individuals are released, within a relevant time frame (Noble and others, 2018). Once established, such self- sustaining approaches are intended to be relatively stable and require only smaller and infrequent secondary releases.

[11] Also termed: High threshold or threshold dependent gene drives. Threshold dependent gene drives only spread if the number of EGD-LMO individuals reaches a high proportion in the target population, requiring a larger introduction (or proportion) of transgenic individuals to be successful, compared to threshold independent gene drives. These types of EGDs may enable local confinement.

[12] Also termed: Self-exhausting gene drives. Self-limiting genetic control 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 will vary according to the genetic control system employed. Conceptually, gene drives 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 subsequently lost from the target population.

extended periods and generations in order to control disease vectors, agricultural pests and invasive species, or rescue endangered species. The use of EGDs could achieve goals that are otherwise challenging to address, such as reaching parts of target populations that are missed by conventional methods, ensure high target specificity compared to most conventional methods and provide ongoing effects with relatively little or no further input.

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., Esvelt, 2014; Simon and others, 2018; CSS–ENSSER–VDW, 2019; Cotter and others, 2020; Dolezel and others, 2020a,b; Then and others, 2020a,b). Those unique characteristics necessitate a comprehensive assessment of ecological risks with a broader spatio-temporal scope (AHTEG, 2020; Connolly and others, 2022).

A chief concern is that the release of a small number of EGD-LMOs, dependent on their design, can theoretically result in the genetic modification of interest to 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 and others, 2019). Moreover, some EGDs may raise novel risk assessment and risk management challenges (NASEM, 2016; Hayes and others, 2018; Simon and others, 2018; CSS–ENSSER–VDW, 2019; AHTEG, 2020; Devos and others, 2020, 2021; Dolezel and others, 2020; Then and others, 2020; Connolly and others, 2021; EFSA, 2022). 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 on ecosystems.

The above-mentioned risk concerns and associated uncertainty have led some scientists, scientific and non-governmental organisations and politicians to call for the strict application of the precautionary approach on gene drive research, including field tests (NASEM, 2016; CSS–ENSSER–VDW, 2019; Cotter and others, 2020). Calls are also made for a better understanding of the potential ecological and evolutionary impacts associated with the intentional release of EGD-LMOs to inform risk assessment (e.g., NASEM, 2016; CSS–ENSSER–VDW, 2019; Giese and others, 2019; Rode and others, 2019; Dolezel and others, 2020a,b). In parallel to this dialogue, established guidance for living modified mosquitoes provided a basis for developing further recommendations for the phased testing of EGD-LMOs (e.g., WHO, 2014, 2021; NASEM, 2016; Hayes and others, 2018; James and others, 2018, 2020), 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 (NASEM, 2016; WHO, 2020). Since some EGD systems may spread across jurisdictional boundaries, regional approaches that would facilitate multi-country/international regulatory oversight and governance have been suggested (James and others, 2018; Rabitz, 2019; Kelsey and others, 2020).

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, theoretically, EGDs may enable modifying 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 scrutinise 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 organism 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, whilst being transparent about the assumptions that have been made during the process (OECD, 2023). Problem formulation addressed intended and unintended behaviour of the EDG-LMO.

Problem formulation can be made operational through a five-step process (e.g., US EPA, 1998; Raybould, 2006, 2007, 2010; EFSA 2010; Wolt and others, 2010; Raybould and Macdonald, 2018; Devos and others, 2019; OECD, 2023), 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 harm13 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; and
(e) The engagement with stakeholders.

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 assessments. 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 a problem formulation approach 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).

4.1.1. Identifying protection goals and making them operational

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 and human, animal, plant, and soil health and water quality (OECD, 2023). As dictated by

[13] Also termed: adverse outcome pathways.

national policies and further clarified in annex I to the Convention on Biological Diversity[14], protection goals encompass various aspects, such as biological 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 species of conservation value or cultural value, species in 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 (termed hereafter as assessment endpoints) (Suter and others, 2006; Nienstedt and others, 2012; Garcia-Alonso and Raybould, 2014; Devos and others, 2015, 2019b; OECD, 2023). This process requires the delineation of what must be protected, where and over what time period, and defining the maximum tolerable impac.t.[15] Three sequential steps can be followed to define assessment endpoints: (1) identify relevant species (ecosystem units), habitats/ecosystems and ecosystem services that could be at risk from the intentional release of an EGD-LMO; (2) identify service-providing units (populations or communities) – structural and functional components of biodiversity – that provide or support these ecosystem services; and (3) specify 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 (EFSA, 2010a,b, 2016; Nienstedt and others, 2012; Devos and others, 2015, 2019b). 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 (Sanvido and other, 2012; Devos and others, 2015). 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 other, 2012).

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

[15] Also termed: limits of concern. Limits of concern are defined in EFSA (2013) as the minimum ecological effects that are deemed biologically relevant and that are deemed of sufficient magnitude to cause harm. These limits of concern are set for each assessment endpoint in the problem formulation (see also Dolezel and others, 2017, 2018).

Table 2

Matrix for an operational definition of environmental harm with some selected examples of how the matrix could be applied. Between the definition of assessment endpoints (step 2) and the definition of measurement endpoints (step 3), risk hypotheses need to be formulated according to conceptual models and exposure scenarios (Sanvido and others, 2012).

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 habits	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 & 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								

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; Rabitz, 2019; Kelsey and others, 2020). A point that would likely require further consideration is whether the risk assessment should therefore be framed only by the specific protection goals established by the jurisdictions that would host the intentional release, or address those of the entire area of potential spread to cover the potential for transboundary movements.

4.1.2. Identifying potential adverse effects on the assessment endpoints

This step involves the identification of any features of the EGD-LMO that may have 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. 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 if the EGD-LMO may have an adverse effect on the assessment endpoints. This is done by examining if any of the novel or altered characteristics of the EGD-LMO and/or its intended use could give rise to adverse effects in the potential receiving environment. The novel characteristics of the EGD-LMO to be considered should include any changes in the EGD-LMO, such as DNA-level changes, gene expression levels and morphological and behavioural changes which may relate to competition and fitness. 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 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, hybridisation and introduction of new parasites and diseases.

Potential adverse effects could arise, for example, from changes in the ability of the EGD-LMO to: (1) affect non-target organisms; (2) cause unintended effects on target organisms, such as the development of resistance to the EGD; (3) develop unintentional changes in fitness; (4) transfer genes to other organisms/populations, such as wild species with some sexually compatibility with the EGD-LMO**,** or via an intermediate; (5) become genotypically or phenotypically unstable; and (6) lead to unintended phenotypes.

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 possible adverse effects in order to focus on generating information that will be useful in the decision-making. 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 receiving environments (covering the receiving environments where the EGD-LMO will be released and spread) and the interactions amongst these variables, plausible pathways to harm[16] are constructed in the problem formulation process. 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 realised. 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 postulate pathways to harm (e.g., Wolt and others, 2010; Roberts and others, 2017; Hayes and others, 2018; Teem and others, 2019). The nature and formality of this exercise, which may include stakeholder and rightsholder 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, and then prioritised based on their likelihood and consequences. In principle, only those pathways to harm that are valid according to existing knowledge, expert judgement 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.

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

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., Roberts and others, 2017; Teem and others, 2019; Romeis and others, 2020; Alcalay and others, 2021; Connolly and others, 2021; Kormos and others, 2023) 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, a recommended approach for tackling deep uncertainty (Section 4.2.7. "Uncertainties"), without excessive resource commitments. Moreover, they do not require specialised skills to develop or understand, and hence can be used to engage stakeholders, who may have different

[16] Also termed: adverse outcome pathway (AOP). A pathway to harm is a causal or conditional chain of events that need to occur for a harm to be realized.

backgrounds and training, into the risk assessment by capturing the views and beliefs on relevant assessment endpoints and pathways.

4.1.4. Formulating risk hypotheses

Each step in a pathway to harm enables the formulation of risk hypotheses that can then be tested to characterise 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 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 minimising uncertainty. A particularly useful feature of this analysis is that it decisively determines with sufficient confidence if 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 to occur, the severity of outcomes and the associated level of uncertainty, and thus a hazard to be realised 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 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. Engaging with stakeholders for risk assessment

New technologies, such as EGDs, are likely to raise new questions and concerns for stakeholders, including indigenous peoples and local communities, who have an interest in technologies that may impact their traditional knowledge, innovation, practices, livelihood and use of land and water. Therefore, risk assessors should anticipate and plan for an expanded engagement process to ensure that the risk assessment has an appropriate scope and wide input from stakeholders (CBD, 2018).

Active stakeholder 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 risk assessments are 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 have a sufficient understanding of the technology, its potential risks and how it will function in the environment.

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 stakeholder, including 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).

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).

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

With risk hypothesis testing, the risk assessment moves from problem formulation to risk characterisation 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 pathways to harm, as they enable the characterization and analyses of potential adverse effects being realized, their likelihood and consequences and combine them into an estimation of the overall risk, taking into consideration any relevant uncertainty that was identified in each of the steps of the 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.

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 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 which uncertainties are implicit as it relates to the particular risk assessment. 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.

Table 3: Risk matrix used to estimate the level of risk

		CONSEQUENCE ASSESSMENT			
		Marginal	**Minor**	**Intermediate**	**Major**
LIKELIHOOD ASSESSMENT	**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			

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 tiered-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 (NASEM, 2016; Hayes and others, 2018; James and others, 2018; EFSA, 2020; Romeis and others, 2020; WHO, 2021, 2022). In general, some degree of uncertainty may still need to be addressed by risk managers and decision makers.

4.2.1. Information sources and quality

The testing of risk hypotheses uses information from various sources, including, but not limited to, using existing information, previous risk assessment, information submitted in applications by developers, peer-reviewed literature, modelling, new empirical investigations, expert opinions, indigenous and traditional knowledge or any combination thereof. Information required for testing the risk hypotheses is likely to be specific for different species, traits 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 recognised 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 hypothesis, 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, on its intended use, intended receiving environment, and on 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 recognised scientific experts, according to national policies.

If sufficient relevant and reliable data are available to test the risk hypothesis, 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 to be included into the analysis and allow testing the hypothesis with the desired certainty. If further data are required, because existing data either inadequately corroborate the hypothesis 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 spatial-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).

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 analyst to: (1) identify parameters that have the most influence on the persistence, spread and effects of the EGD; (2) test and refine risk hypotheses; (3) 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, 2020; Golnar and others, 2021; WHO, 2021).

A key contribution of modelling is its ability to predict the population dynamics of EDG-LMOs in the field (Eckhoff and others, 2017; North and others, 2019; North and others, 2020; Sanchez and others, 2020; Beeton and others, 2022). 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 (Frieβ and others, 2023; Golnar and others, 2021; Rode and others, 2019). 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; Zapletal and others, 2021; Devos and others, 2022b). 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 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 to be included (Frieß and others, 2023; Combs and others, 2023; Kim and others, 2023; Olejarz and Nowack, 2023;

Verma and others, 2023)**.** Furthermore, to date most models have focussed 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 of the present document.

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 adverse effects, and changes in the nature and levels of risk associated to the LMO. The differences identified between a particular LMO, and a comparator provide a starting point for determining if 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 if 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 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, 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-based testing

Tiered testing starts by testing conservative risk hypothesis (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; Raybould, 2011). Risk hypotheses can be evaluated in a tiered 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). 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 (a) higher tier(s) 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). So, the sequence of testing continues only if potential effects are detected, or if unacceptable uncertainties about possible effects remain.

4.2.5. Limits of concern

A comprehensive and consistent progression from one tier to another requires the definition of limits of concern that either trigger additional studies (if the initial assessment indicates a potential for harm) or a decision to stop further testing (Raybould, 2011). 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, 2011). 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 (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, 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: (1) the information from a single piece of evidence alone is not sufficient to fulfil an information requirement; and (2) 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 assessments 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 (Devos and others, 2021; Connolly and others, 2022; Rabitz and others, 2022).

Uncertainty in risk assessments 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: (1) lack of information or incomplete knowledge; and/or (2) 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 (Frieβ and others, 2019; Cisnetto and others, 2020; Kuzma and others, 2021; Frieβ and others, 2023). Lastly, uncertainties resulting from biological or experimental variability may involve variations in EGD efficiency and stability, as well as discrepancies in ecological or intergenerational responses (Then and others, 2020; Rabitz, 2022).

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-market environmental monitoring of the EGD-LMO in the receiving environment, as outlined in subparagraphs 8 (e) and 8 (f) of annex III to the Protocol, can be employed. Furthermore, uncertainties associated with specific adverse effects may not allow the completion of a risk assessment or conclusions regarding the level of overall risk.

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 of the present document.

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

It is important for risk managers to have a clear understanding of protection goals, assessment endpoints and to define decision-making criteria (e.g., what constitutes harm, limits or thresholds of concern, trigger values for action or acceptability of risk, judging the sufficiency of scientific knowledge and the extent to which uncertainty should be reduced for decision-making) that are needed to guide the interpretation of scientific information and results of the risk assessment (Devos and others, 2019a,c). Consequently, enhanced dialogue between risk assessors and risk managers is advocated to clarify how risk assessment can address specific protection goals and decision-making criteria.

Following the risk characterisation, risk assessors prepare a report summarizing the risk assessment process, identified individual risks and the estimated overall risk, and provide (a) recommendation(s) 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. This recommendation is 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 consider whether risk management options can be identified that could address identified individual risks and the estimated overall risk as well as uncertainties. The need, feasibility and efficacy of the management options, including the capacity to enact them, should be considered on a case-by-case basis. If such measures are identified, the preceding steps of the risk assessment may need to be revisited 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 a prerogative of the decision makers The “acceptability” of risks is decided at a policy level and the threshold of what is considered “acceptable” may vary from Party to Party (also see section 7).

B. Living modified mosquitoes containing engineered gene drives

Kindly note that the sections below will be integrated into the appropriate sections above using boxes

B.1. Introduction

In line with the decision CP-10/10 of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety, the AHTEG focused its activities on disease-transmitting insects, mainly mosquitoes, as they represent the most likely cases of EGD-LMOs moving to practical applications for intentional release into the environment in the near future. Although the use of EGD systems is under consideration in mammals (Leitschuh and others, 2018; Conklin, 2019; Godwin and

others, 2019; Grunwald and others, 2019; Manser and others, 2019; Faber and others, 2020) and plants (Neve, 2018; Barrett and others, 2019; Gardiner and others, 2020), basic technical challenges need to be overcome before an EGD will be possible in these taxa (NASEM, 2016; Godwin and others, 2019; Pixley and others, 2019; Scudellari, 2019).

In insects, the most likely EGD-LMO cases for intentional release into the environment are expected to be those that are directed at human, livestock and wildlife disease vectors, followed by agricultural and horticultural pests in highly managed ecosystems and non-native invasive insect species. To reduce their threat to human or animal health, agricultural production and biodiversity, humans have aimed at controlling insect disease vectors (such as mosquitoes), agricultural pests and invasive species through a variety of methods, including the use of biological or chemical insecticides, resistant crop varieties, biological control, and genetic control methods such as the sterile insect technique (SIT) and the incompatible insect technique (IIT) (e.g., Ritchie and Staunton, 2019; Caragata and others, 2020; Romeis and others, 2020).

Controlling disease transmission by mosquitoes is a long-standing public health goal (Feachem and others, 2019; Masterson, 2019). While effective on a local/regional scale and despite diligent application, current control methods (e.g., removal of standing water for mosquito breeding and resting sites, use of insecticides delivered via bed-nets and indoor residual spraying, outdoor insecticide fogging, applications of chemical larvicides, mass release of sterile males, IIT) have not prevented the proliferation of mosquito-vectored diseases, in part due to evolution of resistance to commonly used insecticides, difficulty in reaching all mosquito breeding and resting sites, and global climate change that facilitates mosquito spread (e.g., Ritchie and Staunton, 2019; WHO, 2019; Fouet and others, 2020). This has prompted the development of new genetic approaches to combat the spread of mosquito vector-borne diseases. One of these approaches utilises EGD-LMMs (e.g., Windbichler and others, 2007, 2008, 2011; Gantz and others, 2015; Hammond and others, 2016; Kyrou and others, 2018).

B.1.1. Possible cases of living modified mosquitoes containing engineered gene drives

The additional voluntary guidance materials focus on EGD-LMMs, as they represent the most likely cases of EGD-LMOs moving to practical applications for intentional release into the environment. Various EGD systems (whose intended uses, design, composition and mode of action are diverse) are currently under development and/or have been proposed for use in mosquitoes in the scientific literature.

(a) Self-sustaining threshold-independent (non-localised) EGDs to suppress or modify Malaria-transmitting *Anopheles* mosquitoes (e.g., *An. gambiae*, *An. coluzzii*) or Aedes mosquitoes (e.g., *Ae. aegypti*)
    (i) Homing-based EGDs for either population suppression or modification
    (ii) Meiotic interference EGDs for population suppression
    (iii) Medea and other rescue (Medea-like) EGDs for population modification
(b) Self-sustaining threshold-independent (localised) EGDs to suppress to or modify Malaria-transmitting *Anopheles* mosquitoes (e.g., *An. Gambiae*, *An. Coluzzii*) or Aedes mosquitoes (e.g., *Ae. aegypti*)
    (i) Underdominance EGDs for either population suppression or modification
    (ii) Tethered homing-based EGDs for either population suppression or modification
(c) Self-limiting threshold-independent (non-localised) EGDs
    (i) Daisy-chain EGDs for either population suppression or modification

(d) Self-limiting threshold-independent (localised) EGDs
    (i) Split homing-based EGDs for either population suppression or modification
    (ii) Split rescue EGD systems for either population suppression or modification
(e) Reversal EGDs

B.1.1.1 Mosquitoes

Mosquitoes belong to the family of Culicidae in the Order Diptera. Culicidae is composed of at least 3,722 species (Harbach, 2023) under the 41 recognized genera (Foster and Walker, 2019). Currently, it is comprised of two subfamilies (annex IV namely, Anophilinae (3 genera) and Culicinae (38 genera). Mosquitoes exhibit a holometabolous type of development, with covering four different life stages, namely, the egg, larva, pupa and adult. Their life cycle is completed in aquatic (egg, larvae, and pupae) and terrestrial (adult) environments. Depending on the species, females lay their eggs on or in standing water or on the inner walls of containers with water. The oviposition behaviour of mosquitoes is very diverse, and the choice of oviposition sites is dependent on the species (Day, 2016) as well as the presence of microorganism (Girard and others, 2021) and other abiotic and biotic factors (Wachira and others, 2010).

While the production of eggs does not require a bloodmeal for a number of genera of mosquitoes (*Toxorynchites* (Donald and others, 2020), *Topomyia*, *Malaya*), a phenomenon called autogeny, a majority of species are not able to reproduce autogenously, as adult female mosquitoes require a blood meal (male mosquitoes do not bite) to provide the necessary nutrients for egg development in a gonotrophic cycle (de Swart and others, 2023). Depending on the species of mosquitoes, they feed on the vertebrates such as amphibians, birds, humans, mammals, and reptiles (Molaei and others., 2008; Molaei and others, 2007). This behaviour presents major health risks to humans, livestock and wild animals, as it contributes to the transmission of viruses, protozoa and nematodes from infected hosts (Melgarejo-Colmenares and others, 2022). Mosquitoes could acquire pathogens during blood feeding. These pathogens can reproduce within the mosquito digestive tracts or other tissues to subsequently migrate to the salivary glands (Ohm, 2018) or mouthparts (Anderson, 2000). Not all pathogens can be transmitted via mosquito, since the pathogen needs to be able to reproduce within the mosquito, and not all pathogens are able to successfully do so. 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, 2018), light (Wellington, 1974; Bailey and others, 1965), temperature (Reinhold and others, 2018; Marinho and others, 2016), and vegetation (Dufourd and Dumont, 2013).

Recent reports revealed windborne migration of mosquitoes (Yaro and others, 2022), enabling them to travel substantial distances (Huestis and others, 2019; Sanogo and others, 2020; Wadman, 2019). Besides wind dispersal, other factors can influence mosquito dispersal. These include human transport (Eritja and others, 2017), human mass migration (Hume and others, 2003; Talapko and others, 2019) and international trade (Swan and others, 2023). It is generally true that mosquitoes have limited ability to disperse over long distances without human assistance, e.g., rapid global colonisation of *Aedes albopictus* being linked to human transport (Eritja and others, 2017). However, this has recently been challenged by evidence of longer range wind dispersal being linked to the seasonal colonization surges of the Sahel region, with Anopheles mosquitoes travelling hundreds of kilometres in a single night (Huestis and others, 2019; Sanogo and others, 2020; Wadman, 2019) as well as human mass migration

(Hume and others, 2003; Talapko and others, 2019) or international trade (Swan and others 2023). These external factors can greatly affect the dispersal of mosquitoes with recent studies highlighting the possible displacement up to 300 km for *Anopheles spp*. in West Africa (Huestis and others 2019) or continental wide movement as shown for *Aedes* species (European Centre for Disease Prevention and Control, 2023).

B.1.1.2 Mosquito-borne diseases

The emergence and resurgence of viral mosquito borne diseases, such as dengue (Dieng and others, 2022; Sankar and others, 2021), West Nile virus (WNV; Ruiz-Lopez and others, 2023; Hadfield and others, 2019), Yellow Fever (Nomhwange, and others, 2021; Lindsey and others, 2022; Rosser and others, 2022) and Zika (Islam and others, 2023; Sharma and others, 2019) have been reported in the recent years. This emergence/resurgence may be attributed to a number of parameters with mismanagement of insecticides resulting to resistance development as the major contributing factor (Dahmana and Mediannikov, 2020). These diseases are mainly vectored by mosquitoes belonging to *Aedes or Culex* (for WNV), major genera that transmit numerous pathogens of humans and animals (annex V).

Dengue

WHO (2022a) reported that 3.5 billion people are at risk of getting dengue fever. From January to November 2023, more than 4.5 million dengue cases with more than 4,000 dengue-related deaths had been reported in 80 countries/territories in the European Centre for Disease Prevention and Control (2023). Dengue is the major disease transmitted by Aedes mosquitoes. At least 11 *Aedes* species are recorded to vector the dengue virus (annex V).

Aedes aegypti is the primary vector of the dengue virus and mosquitoes that transmit the disease prefer human as blood meal source (Saifur and others, 2012). While historically present in southern continental Europe, its current distribution includes the tropics and a number of sub-tropical regions, south-eastern United States, the Middle East, southeast Asia, the pacific and Indian islands and northern Australia (European Centre for Disease Prevention and Control, 2023). Further, *Ae. aegypti*, there is great variability in susceptibility to arboviral infections across geographic populations and even for the same population with different viral species and strains, based on these complex and evolving interactions between the pathogen, host and symbionts (Souza-Neto, Powell and Bonizzoni, 2019; Dada and others, 2021).

Aedes albopictus is considered the secondary vector of dengue viruses. *Aedes albopictus* has been included recently in the top 100 invasive species list of the Invasive Species Specialist Group (International Union for Conservation of Nature, 2024). While *Ae. albopictus* is reported as an opportunistic feeder (Turell and others, 2005), it also prefers humans blood meals (Paupy and others, 2009). Engineered gene drive systems are currently being developed to manage dengue vectors (annex VI).

Malaria

Almost half of the world's population is at risk of malaria. In 2022, 608,000 deaths were attributed to malaria (WHO, 2023) with a case incidence of 58.4 per 1,000 population at risk and a mortality rate of 14.3 per 1,000,000 in 2022. Apart from a slight increase in 2021 due to disruptions in access to malaria prevention and case management tools during the COVID-19 pandemic or humanitarian emergencies,

the different indicators of malaria epidemiology show a decrease since 2000, with a number of territories reporting zero malaria deaths or indigenous cases in 2022 (Cabo Verde, Sao Tome and Principe, the Comoros, Bhutan, Timor-Leste and Thailand). Other territories have been certified malaria free for a couple of years (Argentina, Belize, El Salvador and Paraguay). The WHO Malaria report of 2022 states that of the global 247 million new malaria cases and 619,000 deaths recorded in the year 2021, Africa shares the highest burden (95% new cases and 96% malaria deaths). Out of the recorded deaths 77% are children where daily average deaths reported are about 1,000 children under the age of five. Currently, ten African countries (Burkina Faso, Cameroon, the Democratic Republic of the Congo, Ghana, Mali, Mozambique, the Niger, Nigeria, Uganda and the United Republic of Tanzania) have been classified as high burden, high impact countries and they account for 68% of all malaria cases and 70% of malaria deaths reported. In 2021, the population at risk of malaria in Africa was estimated at 1,031,000,000 persons in 42 countries and the situation seems to be getting worse with the years. As reported by the WHO, between 2020 and 2021, malaria cases in high burden, high impact countries increased from 163 million to 168 million people, an increase associated with disruption to services during the COVID-19 pandemic.

Five malaria pathogen species (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi*) are transmitted to humans by *Anopheles* mosquitoes. Out of the 500 *Anopheles* species described in the world, more than 30 species are recorded as vectors of the human malarial parasite (Escobar and others, 2020). These *Anopheles* vectors also prefer human as blood meal source (Jeyaprakasam and others, 2022, Piedrahita and others, 2022). Knowledge gaps remain however, regarding completing our understanding of all relevant malaria vector species, with for example new species of *Anopheles gambiae* complex being recently discovered (Barrón and others, 2019). Gene drive research is actively working on numerous malaria vector species, however challenges remain regarding the lack of amenability of some important vectors, such as *Anopheles funestus*, to laboratory techniques and modification (Odero and others, 2023**)**.

B.1.1.3 Engineered gene drive systems for living modified mosquitoes

The common feature of EGDs is that they are capable of biasing their own inheritance (Burt, 2003; Burt and others, 2018; Champer and others, 2021; Hay and others, 2021; Raban and others, 2023; Wang and others, 2022b). Currently, two distinct intended uses are explored to control mosquito vector-borne diseases. 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.

- **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 (Galizi and others, 2014, 2016; Buchman and others, 2018b; Hammond and others, 2018; James and others, 2018; Kyrou and others, 2018; Leitschuh and others, 2018). These suppression strategies are expected to result in population decline/reduction or even collapse (local elimination) over the period of a few generations and may in some cases aim for (global) eradication of a disease vector species (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 less able to transmit disease (impaired vector competence), or that is more resistant to pathogen infection (disease refractory) (Franz and others, 2006; Mathur and others, 2010; Hedge and Hughes, 2017; Jupatanakul and others, 2017; Carballar-Lejarazú and James, 2017, 2020; Buchman and others, 2019, 2020a; 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; Lejarazú and James, 2017; James and others, 2018; Buchman and others, 2019, 2020a; Hoermann and others, 2020). 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 (whose composition and mode of action are diverse), theoretically, the genetic modification of interest could spread through interbreeding target populations (non-localised) and persist indefinitely (self-sustaining) or be restricted in its spread (localised) or persistence (self-limiting).

- **Self-sustaining engineered gene drive 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, widely and for an indeterminate time, perhaps many generations or until the target population is eliminated (Alphey, 2014). Since self-sustaining EGDs can be engineered to be spatially and temporally unrestricted (non-localised 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 engineered gene drive 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 be those that 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; Leftwich and others, 2018; Backus and Delborne, 2019; Dhole and others, 2020). 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).

- **Threshold independent (non-localised**) 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.
- **Threshold dependent (localized) engineered gene drives** only spread 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 for drive (Alrockand others, 2010; Marshall and Hay, 2012). However, as dispersal to neighbouring populations increases, spatial restriction to the targeted population may not be assured (e.g., Marshall and Hay, 2012b; Dhole and others, 2018, 2020; Champer and others, 2020c).

Current research efforts 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; Li and others, 2020; Maselko and others, 2020; Sánchez and others, 2020b; Webster and others, 2020; Buchman and others, 2021; Hay and others, 2021; Kandul and others, 2021; Oberhofer and others, 2021; Terradas and others, 2021; Willis and Burt, 2021). Several theoretical approaches – some of which have already been tested experimentally under laboratory settings – have been proposed to restrict 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 localisation 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 and would need to be crossed (Li and others, 2020; Kandul and others, 2021; Terradas and others, 2021). Nash and others (2019) evaluated theoretically 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).

Examples of EGDs currently under development for mosquito vector control are briefly presented in annex VI to illustrate the different approaches followed for EGD-LMMs and their characteristics. For some EGD systems, it must be recognised that there may be a spectrum of spread, persistence and dispersal characteristics dependent on the specific design, fitness costs and context in which the EGD will be used. Moreover, some types of EGDs are not clearly distinct, and they could be used alone or in combination with other types of EGDs. EGD approaches and applications will likely continue to expand as gene editing tools become more refined (NASEM, 2016; Guichard and others, 2019; Holman, 2019).

The different EGD-LMM approaches have been designed to possess a broad spectrum of characteristics based on:

(a) The disease and vector species targeted;
(b) Whether the intended entomological objective is the suppression or modification of the target mosquito populations;
(c) The threshold ratio of EGD-LMMs to be released relative to wild mosquito target populations, from low to high;
(d) The degree of spread in target mosquito populations, from localized to non-localized;
(e) The degree of persistence in target mosquito populations, from self-limiting to self-sustaining; and
(f) The molecular and biological mechanisms underpinning the EGD in LMMs. While some of the more familiar examples of EGDs in LMM might be those which are designed to be 'low threshold'[17, 18], 'self-sustaining'[19], and 'non-localized'[20], a diverse array of EGD-LMMs have been or are currently under development. These different EGD-LMM initiatives are presented in a table in annex 6.

The different EGD-LMM approaches have been designed to possess a broad spectrum of characteristics based on:

(a) The mosquito species and associated disease/pathogen targeted;
(b) The threshold ratio of EDG-LMMs to be released relative to wild mosquito target populations, from low to high;
(c) The degree of spread in target mosquito populations, from localized to non-localized;
(d) The threshold ratio of EGD-LMM individuals to be released relative to wild mosquito target population(s), from low to high;
(e) The molecular and biological mechanisms underpinning the EGD in LMMs; and
(f) Whether the intended entomological objective is modification or suppression of target mosquito populations.

There is evidence suggesting that some drives are functioning under different molecular mechanisms or behaviours to the intended design. For example, population reduction drives may potentially result in mixed populations with unpredictable chaser dynamics (Champer and others, 2021a). Homing drive 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 (Verkujil and others, 2022; Terradas and others, 2021; Xu and others, 2020; Li and others, 2019).

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

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

[17] see definitions in Part A and Glossary
[18] only small release numbers are required for their propagation in target mosquito populations
[19] they can propagate in target mosquito populations without the need for additional releases
[20] they have the capacity to spread to neighboring target mosquito populations given their low threshold requirements

design goals may not be realised in the wild, resulting in unlimited or low-threshold drives. A risk assessment therefore needs to reflect on the consequences of potential unintended behaviour.

B.2. Risk assessment considerations for living modified mosquitoes containing engineered gene drives for intentional release into the environment

In the following sections, previously postulated adverse effects associated with the intentional release into the environment of EGD-LMMs, and postulated challenges related to the risk assessment and monitoring of EGD-LMMs are briefly addressed. The identification of adverse effects, and potential challenges is inevitably hypothetical to some extent, as no EGD-LMM application has been submitted for regulatory approval in any jurisdiction globally to our knowledge.

B.2.1. Potential adverse effects associated with living modified mosquitoes containing engineered gene drives

The identification of potential adverse effects may be informed by characteristics of the mosquito, trait and receiving environment. The conduct of a scientific risk assessment relies on the a priori identification of protection goals (e.g., biodiversity or ecosystem-services) and the identification of assessment endpoints that can be objectively measured to determine if harm is taking place (e.g., species or habitats). This adds transparency to the risk assessment process, as well as validation that risk determination and the resulting decisions are done correctly.

B.2.1.1. Characterising the unmodified target mosquito and associated disease(s)/pathogen(s)

The characterization of an EGD-LMM facilitates the understanding of the unmodified species into which the EDG has been introduced.[21] This information forms an essential baseline against which the characteristics of the EGD-LMM are compared. For example,

(a) What do we understand about the basic biology, genetic diversity, species status (existence of a complex of species, species barriers, anatomy, physiology) and behaviour of the target mosquito population? Is the target mosquito population part of a species complex? (Besansky and others, 2003; Connolly, 2023; Connolly and others, 2023).
(b) What do we know about ecological niches occupied by a species at different stages of ontogenesis, a set of conditions under which a given species can exist and reproduce, and food webs?
(c) How well understood is the contribution of the target population to disease transmission?
(d) What are the seasonal dynamics of the target mosquito population?
(e) What aquatic and terrestrial habitats are suitable to sustain target mosquito populations, and how do target mosquito populations colonize these habitats? (Diabaté and others, 2005; Diabaté and others, 2008; Epopa and 2019).
(f) What is the reproductive biology of target mosquito populations? (Baeshen, 2022; Oliva and others, 2014).

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

(g) Is there evidence that target mosquito populations can currently produce fertile interspecific hybrids in the wild? (Elnour and others, 2022; Futami and others, 2020; Harbach and Wilkerson, 2023; Small and others, 2020; Soghigian and 2020).
(h) What is the role of the target mosquito population in ecosystem services (pollinator food source, etc.)? (Collins and others 2019; Lahondère and 2020).
(i) What key interactions does the target mosquito population have with other organisms? (Bonds and 2022; Collins and others, 2019; Marini and others, 2017).
(j) What are the genotypic and phenotypic characteristics of the human pathogen associated with the unmodified target mosquitoes?

When characterizing an EGD-LLM it is necessary to establish what are the known mechanistic impacts of the EGD, on the biology, anatomy, physiology and/or behaviour of the recipient mosquito species.

B.2.1.2. Characterising the living modified mosquito containing an engineered gene drive and associated disease(s)/pathogen(s)

The complete description of the EGD-LMM should also be provided as well as its impact on the mosquito-borne human pathogens. The following information can be considered:

(a) What are the modifications in the EGD-LMM?
(b) How do the modifications affect the genotype and the phenotype of the EGD-LMM?
(c) How do the modifications affect the biology and fecundity of the EGD-LMM?
(d) How do the modifications affect the vectorial capacity of the EGD-LMM?
(e) How do the modifications affect the behaviour of the EGD-LMM?
(f) How does the EGD affect the pathogen, in terms of genotype and phenotype, in the EGD-LMM?
(g) How do modifications affect interactions with the target and non-target pathogens?

Interaction of introduced EGD elements with the genome may change across genetic backgrounds and in subsequent generations.

Unintended effects are observed in the laboratory when generating LMOs but might be difficult to monitor once these organisms are released into the wild. With potential target species such as *Anopheles* belonging to species complexes, characterization of the EGD-LMM should be conducted across all species that are connected by gene flow. Genetic stability and expression of EGDs, for example, can only be calculated in regard to the strains used in the laboratory and under defined conditions.

B.2.1.3. Characterising the receiving environment

The receiving environment into which the EDG-LMM will be introduced is likely to be case-specific, depending on the field release protocol and locations. Geographic, demographic, entomological, seasonal and climatic characteristics of the receiving environment may be considered. In particular, the degree to which the EGD-LMM is expected to spread into target mosquito populations (in time and space) may determine the range of the receiving environments in which the EGD will be expected to be present.

B.2.2. Postulated adverse effects of living modified mosquitoes containing engineered gene drives

Several publications have previously postulated adverse effects on broad protection goals (such as human and animal health, and the environment) associated with the intentional release of the EGD-

LMMs (e.g., NASEM, 2016; Roberts and others, 2017; James and others, 2018, 2020; Collins and others, 2019; CSS–ENSSER–VDW, 2019; Rode and others, 2019; Teem and others, 2019; Dolezel and others, 2020a,b; Smets and Rüdelsheim, 2020; Then and others, 2020a,b; EFSA, 2021; WHO, 2021). Some of these previously postulated adverse effects to 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 to our knowledge.

Postulated adverse effects to human and animal health include:

(a) Increased disease transmission;
(b) Increased abundance of disease-transmitting mosquitoes;
(c) Increased competence for transmission of the pathogen or other vector-borne pathogens and thus the prevalence of other mosquito-transmitted diseases;
(d) Altered mating, host seeking, or feeding behaviours, or geographic range (broader temperature tolerance) of disease-transmitting mosquitoes;
(e) Reduced capability to control the target species by conventional methods;
(f) Increased potential for resistance to evolve in the target organism;
(g) Reduced efficacy of the EGD-LMM in the target population(s);
(h) 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); and
(j) Increased pathogen virulence in case of population modification.

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

(a) Increased persistence and invasiveness potential;
(b) 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;
(c) Increased potential for resistance to evolve in the target organism;
(d) Management responses to reduced efficacy of the EGD-LMM;
(e) Increased potential for vertical and horizontal gene transfer;
(f) 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;
(g) Increased toxicity;
(h) Combinatorial effects of gene drive systems;
(i) Transmission of substances (related to the components of an EGD) that are toxic to non-target organisms that consume the EGD-LMM;
(j) Suppression of the target organism that serves as food source (e.g., prey) for non-target organisms (e.g., predator);
(k) 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);
(l) Invasion of the ecological niche vacated by suppression of the target organism of other mosquito species (niche replacement);
(m) Suppression of the target organism which results in reduced larval consumption of algae causing levels of algae to increase and their associated toxins produced from algal bloom. This

is in turn could lead to adverse effects on non-target organisms in the aquatic habitat, and negative effects on water quality;

(n) The effects of gene drive use on genetic diversity in target populations. Genetic diversity may be more susceptible to natural or anthropogenic pressures (Oye and others, 2014); and

(o) Unintended spread and/or persistence due to complexities such as 'chaser' dynamics, or shadow drive behaviour.

The abovementioned 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 (i.e., testing of risk hypotheses) process. 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, considering the EGD-LMM capacity for spread and persist over time and space.

Examples of plausible pathways to harm:

Adverse effects on biodiversity and ecosystem services (niche replacement, competition, disease transmission)

Competitive interactions

In the case of population suppression (that 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, or 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.

Predator interactions

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). An example pathway to harm is provided in figure 2 below.

For 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 another 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 chaser dynamics occur, whereby local elimination would result in gaps in populations and wild-type rebounds to fill the localised empty niches (Champer and 2021).

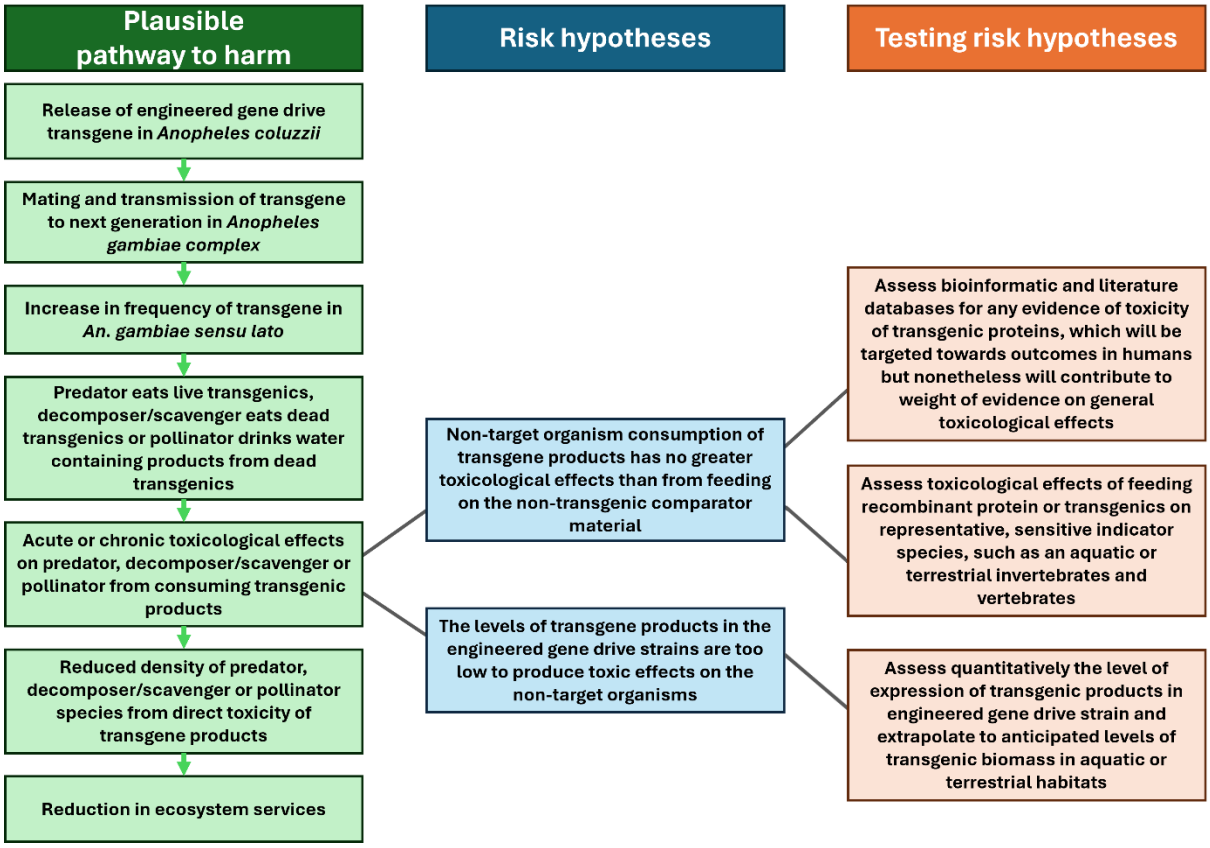

Figure 2. Plausible pathway to reduced biodiversity and ecosystem services.

Adverse toxic effects on water quality or human health

The expressed components of the EGD or newly expressed endogenous products in EDG-LMMs could cause acute or chronic toxicological effects to non-target populations. For example, a predator could eat EDG-LMMs which cause acute or chronic toxicological effects to that species, which in turn reduced its abundance, leading to a reduction in ecosystem services[22] provided by that predator. Alternatively, the accumulation of expressed products from the EGD could lead to toxicity in detritivores, which 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,

[22] Ecosystem services are delivered by species within an ecosystem, including 'provisioning services' such as water availability, 'regulating services' such as pollination, and 'supporting services' such as nutrient recycling. Devos, Y., Romeis, J., Luttik, R., Maggiore, A., Perry, J.N., Schoonjans, R., Streissl, F., Tarazona, J.V., Brock, T.C. (2015) Optimising environmental risk assessments: Accounting for ecosystem services helps to translate broad policy protection goals into specific operational ones for environmental risk assessments. EMBO Rep 16, 1060-1063.

including humans and other animals (Connolly and others, 2021). An example pathway to harm is provided in figure 3 below.

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.

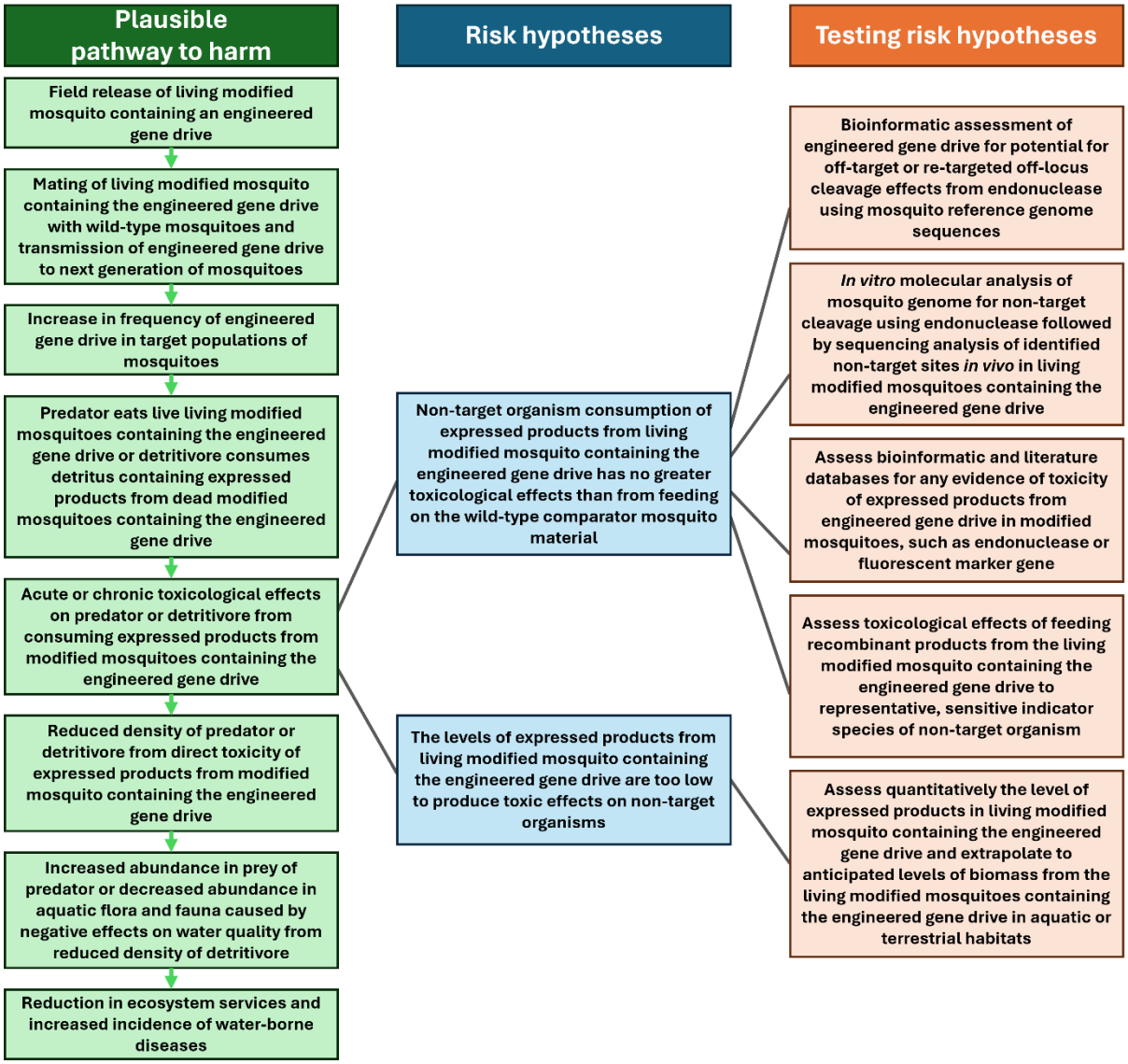

Figure 3. Plausible pathway to reduce water quality and increase incidence of water-borne diseases in humans.

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-LMMs.

The intended impact of the EGD on target mosquito populations could also cause potential harm 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. 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). An example pathway to harm is provided in figure 4 below.

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.

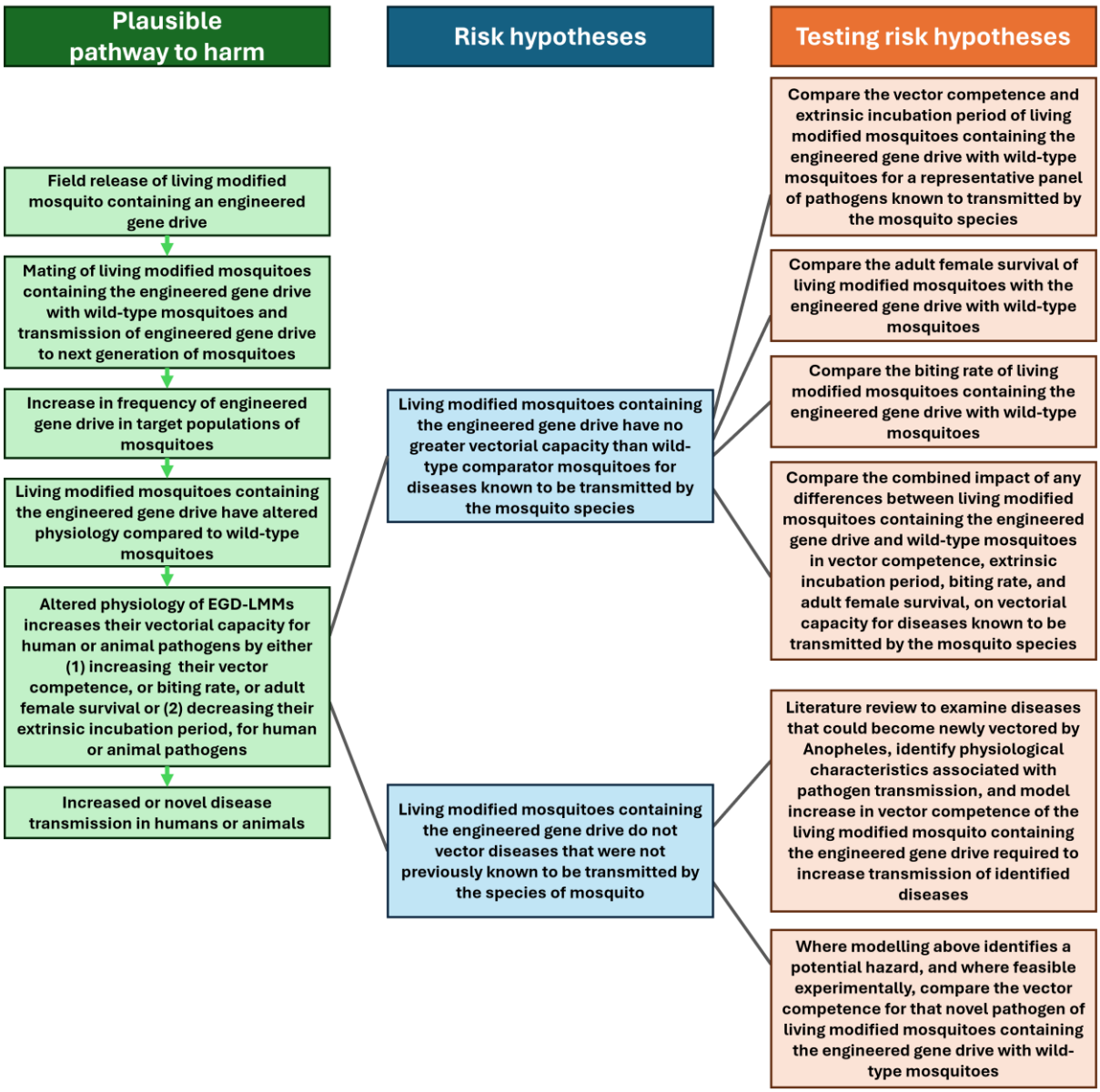

Figure 4. Plausible pathway leading to increased or novel disease transmission.

Gene flow as a mechanism through which adverse effects may occur

Vertical gene transfer

Vertical gene transfer (VGT) is the transmission of genetic material from an organism to its progeny. VGT therefore occurs via sexual reproduction. A number of mosquito disease vector species that are expected targets of engineered gene drive vector control belong to species complexes and thus are capable of producing fertile hybrids with other species from their species complex.

For example, the *Anopheles gambiae* species complex, or *Anopheles gambiae senso lato* (*s.l.*), is currently considered to be made up of nine morphologically indistinguishable species (Besansky and others, 2003; Connolly, 2023; Connolly and others, 2021; Connolly and others, 2023). Of these, *Anopheles coluzzii*, *Anopheles gambiae sensu stricto*. and *Anopheles arabiensis* have been identified as dominant malaria vectors, while others are considered to be minor vectors of malaria or non-malaria vector (*Anopheles quadriannulatus*), often because of their preference for blood meals from animal hosts. Species of the *An. gambiae* complex show only partial reproductive isolation and, for at least some of the species that live in sympatry, hybrids have been observed in nature at low frequencies, and gene flow has been inferred from genomic analyses.

The other dominant species of malaria mosquito vector in Africa is *Anopheles funestus*, which is a species complex consisting of at least seven morphologically similar species with evidence of extensive introgression of genetic material by hybridisation and VGT (Small and others, 2020). Similarly, the dengue vector Ae. aegypti is now considered to represent a group of three species, *Ae. aegypti*, *Aedes formosus* and *Aedes mascarensis*, all of which are capable of hybridisation and VGT (Delatte and others, 2011; Harbach and Wilkerson, 2023), while *Anopheles stephensi* is now considered to be a complex of three species (Khan and others, 2022).

Therefore, the transfer of an EGD from the EDG-LMM to sibling species in its species complex by VGT may lead to functional population suppression or modification. This would be considered a desirable and intended outcome for malaria vector control interventions based on EDG, but might be considered as an undesirable and unintended outcome potentially impacting sibling species that play little if any role in disease transmission (Connolly and others, 2023). Such a situation would relax the assumption of a so-called taxonomic controllability. This would require an increased number of studies for secondary vectors or non-vectors such as *An. quadriannulatus* B. Alternatively, the terms “target species complex” and “target species complex organism” have been created to recognize and accommodate the fact that mosquito disease vectors have semi-permeable species boundaries (Besansky and others, 2003; Fontaine and others, 2015), so that VGT of the EGD could be considered an biological consequence of the use of some kinds of EDGs for mosquito vector control (others, 2023).

Due to VGT of the EGD in target species complexes the risk assessment will need to be considered in the context of other sibling species, particularly when they are dominant disease vectors. These considerations could apply not only to the EDG-LMM species that would be released in the field, but also to other target species complex organisms where VGT of the EGD is plausible (Connolly and others, 2023).

Horizontal gene transfer

Horizontal gene transfer (HGT) is the transmission of genetic material from one species to another without the involvement of sexual reproduction. HGT is common and well understood in prokaryotic organisms where mobile genetic elements, such as plasmids, facilitate the movement of genetic material between organisms of different species. DNA fragments released into the environment from dead and

decaying EGD-LMOs could be taken up by prokaryotes in soil or water and subsequently be incorporated into their genomes. Specific instances have been reported of eukaryotes incorporating DNA fragments from prokaryotes (Husnik, 2018), and from eukaryotes (Li and others, 2022; Xia and others, 2021).

In the case of a EGD designed for population suppression, HGT and germline expression of the intact and functional EGD could lead to reduced abundances of non-target species if they were to (1) possess the same genomic target sequence as the EDG-LMM, (2) have as high levels of homology-directed repair in their germline as the EGD-LMM, (3) employ the same biological mechanisms underpinning the target of the EGD, such as female fertility. In the case of a population modification EGD, it would be important to consider whether it is plausible for the abundance of potentially beneficial endosymbiont non-target species to be reduced in non-target recipients of the EGD HGT.

B.2.3. Postulated challenges for risk assessment

Several publications have previously postulated challenges related to the risk assessment of EGD-LMOs for intentional release into the environment (e.g., NASEM, 2016; CSS–ENSSER–VDW, 2019; ATHEG, 2020; Dolezel and others, 2020a,b; Then and others, 2020a,b; EFSA, 2021; WHO, 2021). These EGD-LMOs cover more organisms than mosquitoes only. Some of the previously postulated challenges for risk assessment are summarized below.

Postulated risk assessment challenges related to the EGD system include:

(a) Prediction of all relevant genomic effects that could emerge in the next and subsequent generations, and from interactions with the receiving environments;
(b) Evaluation of off-target changes and their consequences over time in different genetic backgrounds and their potential accumulation in populations; and
(c) The potential for the EGD to evolve after intentional release, including through unexpected genetic drift.

Postulated risk assessment challenges related to the target organism include:

(a) Need for information on the potential genetic diversity of the target species;
(b) Need for information on the functional role of the target organism and potential cross-compatible species in the various ecosystems that may be encountered;
(c) Consideration of the reproductive strategies, population dynamics and life cycle of the target organism; and
(d) Consideration of possible evolution of resistance in pathogens regarding disease vector control.

Postulated risk assessment challenges related to the receiving environment include:

(a) Need for information on the potential for hybridisation with non-target organisms;
(b) Diversity of potential receiving environments, and limited information on the potential interactions with natural receiving environments; and
(c) Limited information on long-term evolutionary processes occurring in ecosystems.

Postulated risk assessment challenges related to risk assessment methodologies include:

(a) Difficulties of applying the stepwise approach for risk assessment;
(b) Challenges to the comparative risk assessment framework;
(c) Assessing and taking into consideration uncertainty;
(d) Need to address the broader temporal and spatial scale;

(e) Higher dependency on model-based predictions (for example, to address the long temporal and wide spatial scale of some EGD applications and to anticipate the range of scenarios for the possible evolution of the EGD in the environment);
(f) Difficulty to predict the non-linear, exponential effects of EGDs;
(g) Difficulties in assessing next generation effects of organisms containing EGDs;
(h) The need to develop knowledge and procedures for assessing the EGD's long-term effects on ecosystems; and
(i) Difficulty to comprehensively assess risks prior to intentional release.

Postulated risk assessment challenges related to data collection and analysis include:

(a) Additional information needed on the molecular characterisation of both the EGD mechanism and the EGD-containing organism;
(b) Information to predict off-target effects and potential consequences in the target organism;
(c) Advances in conceptual approaches are required to understanding the novel evolutionary and ecological couplings and feedback that EGD-organisms generate;
(d) Lack of environmental and ecological data;
(e) Difficulties with obtaining data for relevant modelling; and
(f) Difficulties with validation and calibration of modelling data before the occurrence of an environmental release.

It is important not to generalise the postulated potential risk assessment and monitoring challenges, as they may not apply to all types of EGD-LMMs.

B.2.4. Choice of comparators

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

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

There will often not be a single comparator (i.e., the non-LMM (without an EGD) with a genetic background as close as possible and relevant to the EGD-LMM) for a given proposed intentional release into the environment of an EGD-LMM, but a range of relevant comparators to inform risk assessment and contextualise risks. The choice of comparators should put more emphasis on the focus of the risk assessment studies conducted and thus the purpose of comparisons.

Depending on the intended outcome of the EGD-LMM application and focus of the comparison, relevant comparators may include: (1) 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; (2) the target (non-modified) organism; and (3) 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.

Given that some EGD systems will operate at an ecosystem level, the definition of comparator needs to be broadened from endpoints that solely consider genetic and phenotypic changes to those that can be indicative of potentially harmful ecosystem impacts. Multiple comparators may be needed to allow robust comparisons across a range of factors that are not sufficiently matched by a single comparator.

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.

B.2.5. Stepwise/staged/tiered-based testing

The stepwise/staged/tiered-based testing approach may leave some uncertainty before open field testing or field implementation of 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 to address the long temporal scale and wide spatial scale of specific EGD-LMM applications, and monitoring may be needed.

The WHO framework (WHO 2021, section 1.5; also see annex III of 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: Leading to physically-, ecologically- or genetically-confined field trials, or small-scale isolated releases.
(c) Phase 3: Staged open-field releases; and
(d) Phase 4: Post-implementation surveillance.

The WHO recognises that the characteristics of persistence and spread for self-sustaining, non-localizing, low-threshold EGD-LMMs may make it theoretically 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 the 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). The 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, it 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 for 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). Theoretically, self-limiting EGD systems may enable localised and temporally restricted spread of the genetic modification of interest, resembling other self-limiting approaches for disease vector/pest control.

B.2.6. 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 estimating that mitigation reduces 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

6.1. General

Uncertainty, in its various forms, is an important consideration in risk assessments of modern biotechnologies, such as EGD-LMO applications. In accordance with annex III of the Cartagena Protocol on Biosafety, subparagraph 8(f), “where there is uncertainty regarding the level of risk, it may be addressed by requesting further information on the specific issues of concern or by implementing appropriate risk management strategies and/or monitoring the living modified organism in the receiving environment”. Furthermore, Article 16 of the Protocol and in particular, paragraph 2 (which deals with risk management) and 4 (which deals with observation requirements) are 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.

Monitoring of LMOs refers to the systematic observation, data collection, and data analysis during and after the intentional release of a LMO into the environment and in accordance with the objectives of the Protocol.

Monitoring can be categorised 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. In general surveillance monitoring, the general status of the environment that is associated with the deployment of the EGD-LMO is monitored 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.

Mathematical modelling may potentially be deployed as a design tool for sampling protocols to define expectations of intended outcomes, deviations, and responses. In this regard, clear triggers for management responses, based on modelling, for particular monitoring results/events may be considered. It should, however, be noted that monitoring efforts should be proportionate to the level of risk/uncertainty identified.

Monitoring measures may be implemented to trace and identify any direct or indirect, immediate, delayed, or unforeseen effects on the environment, taking into account human health, of LMOs as or in products after they have been intentionally released into the environment. In this regard, 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 health and the environment;
(c) Validate conclusions of the 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; and
(g) Establish a causal link between EGD-LMOs and any observed adverse effects.

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

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.

6.2. Considerations for monitoring

A monitoring plan is developed either by competent national authorities based on relevant national biosafety laws, regulations and policies and recommendations derived from the risk assessment, or 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 utilise related monitoring data and activities, including from other countries/areas, as appropriate.

There is substantial experience with releasing insects for genetic and biological disease vector/pest control, including their monitoring . It may be advisable/appropriate to draw on the experience from current insect disease vector/pest control strategies that involve the release of insects, seek precedence for 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.

The monitoring plan could consider the following:

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 to the adverse effect occurring as possible. Parameter prioritisation may relate to ease of sampling and collection of required material as well as assaying for the parameter. Consideration should be given to the interrelation of the indicator with a pathway to harm i.e., 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 (IVM), 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.

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 describe sufficient information on sampling, collecting, and analysing the samples as well as the data resulting from undertaking the method. 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 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 standardisation 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 for carrying out the monitoring activities, including 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; and
(l) Pre-exposure baselines for informing the monitoring.

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 such that indicators have the potential to be exposed to or impacted by the presence of the EGD-LMO and relevant to the pathway to harm. Monitoring site locations, size, density/ distribution, and timing should be determined prior to release based on the biology and life cycle of the EGD-LMO, its potential spread, dispersal, and establishment; the 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, 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 i.e., through the different times of the year and for all the years that are required (long term).

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.

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 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. 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*.

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 that is 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 reported information 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.

6.3. 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 (Rasic 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 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.

Monitoring during the 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. P-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 of the product (e.g., the EGD-LMM) have 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 EGD-LMM outside the designated release area could identify dispersal range (temporal and spatial) of the EGD-LMMs. 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 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.

7. Related issues

7.1. Risk assessment and assessing the benefits as component 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 annex III 8(e) of the Cartagena Protocol on Biosafety (CPB). In many cases, this decision/recommendation is made by assessing the potential benefits and comparing them to the estimation of overall risk posed by the LMO. The CPB does not give specific guidance 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 do 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 of human health

According to guidance framework for testing genetically modified mosquitoes published by the WHO (2021), a new product should be assessed in the regulatory review process on the basis of both the benefits and risks (also see annex III of the present document). The primary potential benefit of a Genetically Modified Mosquito (GMM)/Living Modified Mosquito (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, factors 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 for testing genetically modified mosquitoes.

According to the 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. Moreover, other considerations may include conducting a "cost-effectiveness analysis", which expresses benefit as a measurement of a particular health gain.

There are potential benefits of using GMM in the fight against malaria, which could extend to other vector-borne diseases. The number of deaths due to malaria in West African countries is proof that current approaches (pesticides, impregnated mosquito nets, etc.) have not produced satisfactory results. In 2022, WHO estimated that there would be 8 million cases of malaria and over 16,669 deaths attributable to malaria.

7.3. Socioeconomic considerations, and cultural and ethical considerations including participation of indigenous people and local communities

Living modified organisms containing engineered gene drives may have socioeconomic, cultural, traditional, religious, or ethical concerns that may be considered in the decision-making process. Article 26, para 1 of the Cartagena Protocol addresses socioeconomic considerations and states 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, socio-economic 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 Socio-Economic Considerations in the Context of Article 26 of the Cartagena Protocol on Biosafety" that was adopted

in annex 1 to CBD/CP/MOP/9/10 provides additional guidance. These issues 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).

Voluntary guidelines (i.e., Akwé: Kon 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 lands and waters traditionally occupied or used by indigenous people and local communities, was adopted by Parties to the Convention in decision VII/16 provide useful guidance. In particular, the potential adverse effects of EGD-LMOs on the lands, waters and territories, sacred sites, wild species of fauna and flora, and on the relationship of indigenous people 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 people 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 people and local communities. The knowledge and value systems of indigenous people and local communities and their knowledge and value systems are helpful when considering the behavior of relevant species and their interaction with other species.

Living modified organisms containing engineered gene drives may increase dependency on technology, alter biological components and may adversely impact biodiversity, cultural, and ethical values of indigenous people and local communities, socio-economic situations, and the reciprocal relationship with Mother Nature in the long term. The possibility of conflicts with non-target species such as wild species that are valuable for indigenous people 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, Parties to the Convention noted the conclusions of the AHTEG on Synthetic Biology that, given the current uncertainties regarding EGDs, the free, prior and informed consent of indigenous people and local communities might be warranted when considering the possible release of EGD-LMOs that may impact their traditional knowledge, innovation, practices, livelihood and use of land and water. As such, it is highly recommended to obtain prior and informed consent, or national equivalents, of potentially affected indigenous people and local communities before considering introducing EGD-LMOs into the environment, including for experimental releases and research and development purposes. Relevant guidance 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 people 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, has been adopted as the Mo'otz Kuxtal Voluntary Guidelines by Parties to the Convention in decision XIII/18.

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

7.5. Consideration of public awareness, education and participation (e.g., 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 activities, including biosafety related communication, is essential to ensure effective participation of indigenous people and local communities.

Indigenous knowledge, innovations and practices integrated with accessible and understandable science for effective communication including use of local 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 scientists and the public (the deficit model concept).

Inclusion of public awareness, participatory process, including full and effective participation of IPLCs 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. Article 14 1(a) of the Convention states 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 strategies with alternative interventions, current measures and cost of inaction

Vector-transmitted human disease control as well as invasive species control and (agricultural) pest control demands the development of a wide range of complementary strategies, currently in use or under development. These strategies can be used as comparators for EGD-LMO risk assessment or risk benefit analysis alone and in combination. These comparators 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 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 assessments 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 the EDG-LMM were released in the field without any isolation, it would be expected that EGD-LMM would spread to target mosquito populations distal to the release site. The rate of spread of the EGD-LMM would depend on the (1) dispersal of the target mosquito population, (2) threshold frequency with which the EDG is required to establish in distal target mosquito populations, (3) the fitness costs of the EGD incurred on the EDG-LMMs, (4) reproductive capacity, and (5) 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.

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 as appropriate for some Parties. The Supplementary Protocol applies to damage resulting from living modified organisms 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.

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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 1.4.3 “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: 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: 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.

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 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 in steps 1 and 2 of a risk assessment include fault trees and event trees (Hayes and others, 2018a; 2018b; 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.

Qualitative mathematical models

Qualitative mathematical models possess the same useful properties of 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. In addition, they provide information that may be helpful in systems where negative (positive) feedback – a process in which an initial change in a system variable will cause it to return to (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, whilst Puccia and Levins (1985) 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).

Process-based models

Process-based models represent systems using one of three types of mathematical equations: (i) 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; (ii) a difference equation that specifies how much variables change between time points; and (iii) 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 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, analyst must make important choices about: (i) which real-world processes and components to include in the model, and which to exclude; (ii) how to mathematically describe the processes that are included; (iii) the values or probability distribution models of the parameters; (iv) the resolution of the model in time and space (e.g., are predictions made on daily, monthly or yearly time steps); (v) the parameter's initial values; and, (vi) 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 better to understand and interpret. Hilborn and Mangel (1997), however, caution that simpler models may under-represent 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-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 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 a stage-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 (Comb and others, 2023; Frieß and others, 2023).

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

The use of modern modelling techniques to EGD-LMO risk assessment requires a high degree of training in the process-based models used to represent ecological and biological systems, the probabilistic theory used to assign probability distribution models to the parameters of these models, as well the computational methods that enable inference 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 (2007a) provide a non-technical introduction highlighting examples relevant to LM fish. The EFSA GMO Panel (2013) provides a similar introduction within the context of LM animals. EFSA (2018a,b) recommends a suite of procedures for assessing uncertainty in scientific assessments. Good textbooks on how to address uncertainty within quantitative (probabilistic) risk assessments include Morgan and Henrion (1992), Cullen and Frey (1999), and Bedford and Cooke (2001).

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, 2018). Linguistic uncertainty is prominent in qualitative risk assessments because terms such as “small effect”, “low likelihood” or “negligible risk” are open to interpretation, hence current guidance almost always recommends that these terms are carefully defined (see for example EFSA, 2013) and where-ever possible language-based misunderstandings minimized through careful facilitation of expert input (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 2018a,b).

For EGD-LMOs quantification of uncertainty could be more challenging than in other LMO risk assessments because of 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 judgement (via formal elicitation) for any well-defined question or quantity provided there is at least some relevant evidence (EFSA, 2018a,b).

Guidance on how to quantify uncertainty through expert elicitation is available from several sources. For example, Burgman (2005) provides a helpful introduction, Morgan (2014) provides an excellent overview of key issues, whilst O’Hagan and others (2006) provides 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 (2018b) and Hosack and others (2023) provide examples of how to use elicitation to conduct probabilistic risk assessments for living modified mosquitoes.

Epistemic uncertainty

Risk assessments 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 the 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: (i) is the conceptual modelling exercise complete – i.e., has the risk assessment identified all the plausible pathways to harm; (ii) are the conceptual models adequate – i.e., do the identified 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 can be addressed procedurally and methodologically. Ensuring that relevant stakeholders and experts are consulted when 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 EFSA (2013, 2020) and the National Academy of Sciences Engineering and Medicine (NASEM, 2016); and (c) documents produced by the scientific community - such as Benedict and others (2008), David and others, (2013), Hayes and others (2018b), James and others (2020), Rode and others (2019), Teem and others (2019), and Connolly and others (2021) – will also help ensure that potentially relevant pathways have not been inadvertently overlooked.

In addition to these recommended procedures, Hayes and others (2007b, 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 if the structural uncertainty in the conceptual models that underlie a problem formulation has been comprehensively addressed. In particular, the number of 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 judgement to decide if this source of uncertainty in the problem formulation stage of an EGD-LMO risk assessment process has been adequately addressed.

Variability

Variability, often also referred to as aleatory uncertainty, occurs in LMO risk assessments because many of the relevant environmental and demographic processes or variables within the 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 whilst 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) used the common (but in this case transformed) normal distribution to capture expert's beliefs about how the parameters that govern the vector competence of living modified mosquitoes varies 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 assessments should either complete training in the underlying theory and techniques or seek assistance.

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: (i) the appropriate models to describe the interactions among a system's variables; (ii) the probability distributions to represent uncertainty about key variables and parameters in these models; and/or (iii) how to value the desirability of alternative outcomes (Institute of Medicine, 2013). Risk assessments 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, argued 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 $\times$ environment interactions, may introduce a high level of uncertainty into EGD-LMO risk assessments.

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 typically have many parameters (See annex I) 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 inter-specific 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 characterised parameters, within scenarios that reflect possible best-case/worst-case situations for poorly characterised 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 if the models and parameter estimates used within a risk assessment are plausible, consistent with theory and defensible in light of the available evidence base.

Finally, it is important to recognise that a rigorous and systematic analysis of uncertainty within a risk assessment requires specialist skills and computing resources, and the number of 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 or described in any type of risk assessment and must therefore act accordingly (EFSA, 2018a,b).

Annex III

World Health Organisation Guidance framework for testing genetically modified mosquitoes

The WHO published a couple years ago, the second edition of its 'Guidance framework for testing genetically modified mosquitoes' (WHO, 2021), in which it refers to LMMs as "genetically modified mosquitoes (GMMs)" and to EDG-LMMs as "gene drive modified mosquitoes (GDMMs)". The WHO recommends that a safety criterion for moving an EDG-LMM from laboratory to field testing is "a well-reasoned justification that the GDMMs 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." (section 3.7 of WHO guidance framework).

As a matter of comparison (the use of EGD-LMO is not a biological control approach), the 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 (section 5.3.5 of WHO guidance framework). 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, and numerous jurisdictions have established national regulatory systems based on this approach.

Here, a relevant example of field release of 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, Vietnam, and Indonesia, rigorous environmental risk assessment was conducted ahead of studies in the field 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 & Barton, 2017).

The 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 172 countries, is itself derived from the Cartagena Protocol on Biosafety to the Convention on Biological Diversity (CBD; WHO 2021 section 5). In the context of self-sustaining, non-localizing, low-threshold GDMMs, the WHO advises that considerations to move from physically confined indoor testing to field testing involve (i) thorough environmental risk assessment informed by laboratory and insectary trials of the gene drive mosquitoes, (ii) entomological, epidemiological, and ecological data from the proposed field locations and (iii) mathematical modelling simulating the behaviour of the gene drive system at the field location (section 1.5.1 of WHO guidance framework).

Annex IV

Taxonomic classification of Culicidae[23]

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.*)

[23] 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, 2022
		Dengue fever	Virus	WHO, 2023
		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, 2023
		Zika fever	Virus	Kauffman & Kramer, 2017
	Ae. africanus	Zika fever	Virus	Haddow and others, 1964
	Ae. albopictus	Chikungunya	Virus	WHO, 2022b
		Dengue fever		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 & Kramer, 2017; WHO 2019
	Ae. atropalpus	La Crosse encephalitis	Virus	Giunti and others, 2023
		West Nile fever	Virus	Giunti and others, 2023
	Ae. bromeliae	Dengue fever	Virus	Foster & Walker, 2019
		Yellow fever	Virus	
	Ae. cantans	Tahyna virus**	Virus	Cai and others, 2023
	Ae. caspius	Tahyna virus	Virus	Calzolari and others, 2022
	Ae. cinereus	Rabbit fever (Tularemia)	Bacteria	Petersen and others, 2008
	Ae. communis	Sindbis fever	Virus	Wilkman and others, 2023
	Ae. dorsalis	California encephalitis	Virus	Foster & Walker, 2019
	Ae. excrucians	Sindbis fever	Virus	Wilkman and others, 2023
	Ae. furcifer	Dengue fever	Virus	Foster & 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 & Walker, 2019
		Yellow fever	Virus	
		Zika fever	Virus	Epelbion and others, 2017
	Ae. melanimon	California encephalitis virus	Virus	Foster & Walker, 2019
	Ae. niveus	Lymphatic filariases	Nematode	Foster & Walker, 2019
	Ae. opok	Dengue fever	Virus	Foster & Walker, 2019
	Ae. polynesiensis	Chikungungya	Virus	Richard and others, 2016

		Dengue fever	Virus	Foster & Walker, 2019
		Lymphatic filariasis	Nematode	
	Ae. pseudoscutellaris	Dengue fever	Virus	Foster & Walker, 2019
		Lymphatis filariasis	Nematode	Foster & Walker, 2019
	Ae. rotumae	Dengue fever	Virus	Foster & Walker, 2019
	Ae. scapularis	Cache Valley fever**	Virus	Waddell and others, 2019
	Ae. scutellaris	Dengue fever	Virus	Foster & 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 & Walker, 2019
	Ae. triseriatus	La Crosse encephalitis	Virus	
	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 & Shil, 2017
	Anopheles gambiae	Malaria	Plasmodium	Djihinto and others, 2022
		Lymphatic filariasis	Nematode	Foster & Walker, 2019
	An.arabiensis	Malaria	Plasmodium	Djihinto and others, 2022
		Lymphatic filariasis	Nematode	Foster & Walker, 2019
	An. barbirostris	Lymphatic filariasis	Nematode	Foster & 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
	Anopheles 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, 2015b
	Cx. nigripalpus	St. Louis encephalitis	Virus	Curren and others, 2018
	Cx. pipiens	Rift Valley fever	Virus	Foster & 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 & 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	Hoch and others, 1981; Periera and others, 2021; Celone and others, 2022
		Yellow fever	Virus	Celone and others, 2022
	Hg. leucocelaenus	Yellow fever	Virus	Da Silva and others, 2020
	Hg. lucifer	Yellow fever	Virus	Foster & Walker, 2019
	Mansonia annulifera	Lymphatic filariasis	Nematode	Foster & Walker, 2019
	Ma. uniformis	Lymphatic filariasis	Nematode	Foster & 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 & Walker, 2019
	Ae. mcintoshi	Wesselsbron virus	Virus	Foster & 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 equie encephalitis virus	Virus	Torres and others, 2017
	Culiseta melanura	Eastern equine encephalitis virus	Virus	Armstrong & Andreadis, 2010
	Psorophora confinnis	Venezuelan equine encephalitis virus	Virus	Torres and others, 2017

Note: *Known/competent vector; **Wild infection

Annex VI

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

This list of EGD-LMMs that have been developed does not necessarily reflect the successful ones not their current successful development. For example, the product developed in Kyrou and others, 2018 has been reported to have instability (WHO, 2022)

Target vector-borne disease	Target mosquito vector species	EDG threshold for field releases	EDG 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-localised	Homing	Suppression	Modelling, Strains generated and tested in insectary in target species	(Hammond and others 2016; Hammond and 2021; Kyrou and others 2018* ; North and others.
					Homing	Modification	Modelling, Strains generated and tested in insectary in target species	(Carballar-Lejarazu 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, 2022; Hoermann and others, 2021; Nash and others, 2019)

					Y drive	Suppression	Modelling only	(Deredec and others, 2011)
				Localised	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-localised	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-localised	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,
Dengue, Yellow fever, Chikungunya, Zika viruses	*Ae. aegypti*	Low	Self-sustaining	Non-localised	Medea (Maternal effect dominant embryonic arrest	Modification	Modelling	(Legros and others, 2013)
		High	Self-sustaining	Localised	Two-locus underdominance	Modification	Modelling	(Edgington and Alphey, 2017, 2018; Sánchez

								and others, 2020)
			Self-limiting	Localised	Homing Split drive	Modification	Modelling, Strains generated and tested in *Drosophila* model system, Mosquito strains generated and tested	(Anderson and others, 2023; Anderson and others, 2022; Li and others, 2020; López Del Amo and others, 2020; Terradas and 2021)
					Toxin antidote rescue system	Modification	Modelling	(Legros and others, 2013)
Wuchereria bancrofti lymphatic filariasis, West Nile virus, St. Louis encephalitis	*Cx. quinquefasciatus*	High	Self-limiting	Localised	Homing, Split drive	Modification	Strains generated and tested in insectary in target species	(Harvey-Samuel and others, 2023)
Potentially multiple other vectors (e.g., *Anopheles*, *Aedes*, or *Culex* species from South America or Asia Pacific regions)	Potentially multiple other diseases (e.g., malaria or arboviral infections from South America or Asia Pacific regions)	Low	Self-sustaining	Non-localised	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	Localised	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; Champer and

					others, 2020b; Gould and others, 2008; Kandul and others, 2021; Oberhofer and others, 2020a; Oberhofer and others, 2021)
			One-locus underdominance	Modification or suppression	Modelling, Strains generated and tested in *Drosophila* model systems only	(Buchman and others, 2021; Buchman and others, 2018b; Dhole and others, 2019; Dhole and others, 2018; Reeves and others, 2014)

Annex VII

Engineered gene drive systems

A. Homing

Here, an EGD results in germline expression of both the CRISPR-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 EDG, 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 EDG is located on the Y chromosome, so it is only inherited by male mosquitoes. The EDG 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 system has only been tested in 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 who 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. Medea

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 EDG 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 EDG 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 localised spread in target mosquito populations (Wang and others, 2022a). Because of its requirements for high release thresholds, it can be thought of as a form of localised 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 EDGs are fitter than those carrying only one of the two EGDs, typically producing self-sustaining gene drive.

F. Split drives

Split drives consist of two or more unlinked EGDs, 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 EDG-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 (Teradas and others, 2023).

G. Double drives with private alleles

Double drives are comprised of two separate elements to produce a functional EGD (Willis and Burt, 2021). The first element of the EGD encodes Cas9 that, when expressed alongside a guide RNA that recognises 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 recognises 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 homig both at the genomic target locus required for population suppression or modifcation and at the genomic target locus restricted to the target mosquito population. This means the double drive EGD would be localised, acting as a self-sustaining, low-threshold EGD in target mosquito populations but a self-limiting, high-threshold split drive in non target mosqutio populations. By contrast, they act as a split drive in non-target populations. Modeling 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 including reversal drives, immunizing drives (Girardin, Calvez & Debarre, 2019; Esvlet and others, 2014), overwriting drives and e-CHACR, 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).

Glossary of terms

#	Term	Draft definition(s)	Source
	Applicant	*An individual or organisation that applies for approval or authorisation of a regulated activity to a responsible government agency or regulatory body. The applicant may be the developer.*	N/A (original)
		Related definition: developer	
	Assessment endpoints	*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, or gene flow) that can be measured or modelled.*	Adapted from: Group A proposal, EFSA GMO Panel 2010, NASEM 2016, OECD 2023, WHO 2001
		Related definition: measurement endpoint	
	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.*	Alphey and others, 2020 – publication by the gene drive research community proposing a list of standardised definitions. The words "engineered" and "target" have been added to the published definition to link other definitions in this Glossary of Terms.
		Related definitions: engineered gene drive, target population	
	Causal pathway	*The sequence of events or mechanisms by which a hazard may lead to a specific harm. This is a consideration in the hazard identification process.*	Derived from explanatory text in: EFSA GMO Panel 2010, OGTR 2005
		Related definitions: harm, hazard, hazard identification	
	Chaser dynamics	Definition needed	Reference needed

#	Term	Draft definition(s)	Source
	Contained use	Any operation, undertaken within a facility, installation or physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment.	Article 3 of the Cartagena Protocol on Biosafety
	Containment	*Utilisation of a set of measures to prevent the unintentional release of organisms from a designated area (e.g., laboratory or facility) into the surrounding environment. Measures may include biological containment, operational practices, safety equipment, and facility safeguards.*	Adapted from: Beeckman and Rudelsheim 2020, WHO 2021a ("confinement"), WHO 2020 Original texts: *WHO 2021a* *(confinement) utilisation of measures that seek to prevent unplanned or uncontrolled release of organisms into the environment.* *WHO 2004 and Beeckman and Rudelsheim 2020:* *A set of measures including biological containment, practices, safety equipment, and facility safeguards that protect workers, the community and the environment from exposure to and/or unintentional escape of biological material*
	Confinement measures	*A set of measures intended to prevent or minimise 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*	Derived from explanatory text in: WHO 2021a

#	Term	Draft definition(s)	Source
		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 definitions: containment, engineered gene drive, living modified organism*	
	Daisy-chain drive	*A daisy drive system involves introducing several genetic changes at different places in an organism's genome. It is a gene drive made up of multiple independent drive elements, where each element, except one, biases the inheritance of another, forming a chain.*	Adapted from Nash and others (2019)
	Developer	*An entity/entities undertaking research and development activities aimed at producing new or improved products (goods or services) or processes.*	Derived from descriptions of Beeckman and Rudelsheim 2020, OECD 2015
	Ecosystem	*A dynamic complex of plant, animal and micro-organism communities and their non-living environment interacting as a functional unit.*	Article 2 (Use of terms) of the Convention on Biological Diversity
	Ecosystem services	*Benefits people obtain from ecosystems and distinguishes four categories of ecosystem services, where the supporting services are regarded as the basis for the services of the other three categories. These four categories of ecosystem services are: provisioning, regulating, cultural and supporting services.*	The Millennium Ecosystem Assessment report 2005, The Millennium Ecosystem Assessment (MA), and Ehrlich and Ehrlich 1981.
	Engineered gene drive (EGD)	*A gene drive system that is created through the application of recombinant DNA techniques.* *Related definition: gene drive*	Adapted from: Alphey and others (2020), Australian Academy of Sciences (2017) Original texts: *Alphey and others, 2020* *A gene drive system that is created through*

#	Term	Draft definition(s)	Source
			recombinant DNA techniques. *Australian Academy of Science 2017* *An application of gene technology that increases the prevalence of a genetic variant within a population.*
	EGD-LMO	*Abbreviation representing "living modified organism" (LMO) containing an "engineered gene drive" (EGD).* *Related definitions: engineered gene drive, living modified organism*	
	Event	*An event consists of the DNA sequence that has been incorporated into the genome of a living modified organism through the application of recombinant DNA techniques and the specific site of insertion. May also be referred to as a "transgenic event" or "transformation event".* *Related definition: living modified organism*	Adapted from: Mumm 2013
	Gene drive	*Genetic elements capable of biasing their own inheritance within a population through sexual reproduction. Thus, the result of a gene drive is the preferential increase of a specific genotype that determines a specific phenotype from one generation to the next, and potentially throughout a population.*	Adapted from: Alphey and others (2020), EFSA GMO Panel (2020) Original texts: EFSA: *Genetic elements capable of biasing their own inheritance.* Alphey and others, 2020: *a gene drive is any genetic element able to bias its inheritance within a population.* Others reviewed: *NASEM 2016: A system of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring*

#	Term	Draft definition(s)	Source
			through sexual reproduction is enhanced. *WHO 2021a: A mechanism that increases the transmission of a transgene in a population above that which would be expected based on Mendelian inheritance.* Drafting Group A: *Any genetic elements capable of biasing their own inheritance to gain a transmission advantage over the rest of the genome can be referred to as gene drives*
	Geographic controllability	*Theoretical capacity to limit the spatial spread of functional drive inserts to only target populations*	[Reference missing]
	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: Cartagena Protocol (Art 15), ISO 14791:2019, WHO 2021a Original texts: *ISO 14791:2019: injury or damage to the health of people, or damage to property or the environment.* *WHO 2021a: … hazards being actualised to harms in the receiving environment … realisation of hazards … hazards that could lead to harms to the environment or human or animal health.* *Cartagena Protocol (Art 15): … adverse effects of LMOs on the conservation and sustainable use of biological diversity, taking*

#	Term	Draft definition(s)	Source
			also into account risks to human health
	Hazard	*A source of potential harm.*	ISO 14791:2019, OGTR 2005
		Related definition: harm	
	Hazard identification	*The first 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.*	Derived from definitions and explanatory text in: OGTR 2005, WHO 2021a Original OGTR 2005 text*: Hazard identification: the process of analysing hazards and the events that give rise to harm.* *… involving analysis of what, how, where and when something could go wrong and the causal pathway leading to that adverse outcome.*
		Related definitions: harm, causal pathway, protection goals, risk assessment	
	High-threshold/ High threshold systems (both terms requested) *Suggested edit: 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 (see also localised drives and self-limiting drives).*	Adapted from: Alphey and others, 2020, AAS 2017, WHO 2021a
		Related definitions: gene drive, low threshold drive, localised drives, self-limiting, target population	
	Incident/incidental exposure *Suggested edit: incidental exposure*	*Unintended or accidental exposure in the receiving environment that may impact protection goals, e.g., incidental ingestion or inhalation of EGD-LMOs is relevant to human and animal health.*	Derived from: Roberts and others, 2017
		Related definitions: EGD-LMOs, protection goals	

#	Term	Draft definition(s)	Source
	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.*	Food and Agriculture Organization of the United Nations (2024)
	Interference mechanisms	*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.* *Related definition: gene drive*	Adapted from: NASEM 2016, WHO 2021a
	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 definitions: measurement endpoint, harm*	EFSA GMO Panel 2010
	Living modified organism (LMO), Living modified mosquito (LMM)	*Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.*	Cartagena Protocol Article 3(g)
	Localized EGD in target population *Suggested edit: localized drive*	*Gene drives that are expected to be geographically confined and not spread substantially beyond the target population or area. Also known as spatially restricted gene drives. Potential means to restrict spread include use of high-threshold drives or self-limiting drives.* *Related definitions: gene drive, high-threshold drives, self-limiting, target population*	Adapted from: Alphey and others, 2020

#	Term	Draft definition(s)	Source
	Low-threshold *Suggested edit: 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. Also known as threshold-independent drives, or non-localized drives.*	Combined definitions/text from: Alphey and others, 2020, AAS 2017
		Related definitions: gene drive, high threshold drive, non-localized drive	
	Measurement endpoints	*A quantifiable indicator of change in the assessment endpoint and constitutes measurements of hazard and exposure. Examples may include fitness assessment, sex ratio monitoring, and growth and density of species used as assessment endpoints.*	Proposed by drafting Group A – based on EFSA GMO Panel 2010
		Related definitions: assessment endpoint, hazard	
	Modelling	*Mathematical modelling is a tool that may be utilised to predict EGD- LMO behaviour in the receiving environment to support risk assessment, e.g., simulation of the spread of a gene drive system in a wild population beyond the initial release site and estimation of the likelihood and impact of hazards.*	Adapted from explanatory text in: WHO 2021a
		Related definitions: EGD- LMO, gene drive, risk assessment, hazards	
	Monitoring	*Post-implementation monitoring may be utilised used as a risk management measure to address remaining uncertainties identified in the risk assessment, and/or confirm at the operational level that the conclusions of previous risk assessments were accurate.*	Adapted from: WHO 2021a

#	Term	Draft definition(s)	Source
		Related definitions: risk assessment, risk management	
	Mosquitoes	*A common holometabolous insect found in a diverse range of environments on all continents except for Antarctica. Mosquitoes have four distinct life stages: egg, larva, pupae, and adult. The juvenile phase is strictly aquatic, with eggs laid on or near a water source. Once pupae are fully developed, adult mosquitoes emerge at the water surface, and soon after are mature and capable of flight and mating.* *Adult male and female mosquitoes will feed on plant-based sugars, and females of certain species will seek a blood meal as it contains the requisite nutrients for egg development.* *Certain species of mosquito are vectors of diseases, e.g., malaria is a parasitic infection transmitted by Anopheline mosquitoes, and Aedes mosquitoes are vectors of dengue, chikungunya, Zika and yellow fever.*	Information combined from multiple sources: Hawkes and Hopkins 2022 OECD 2018 WHO website: "Vector-borne diseases" https://www.who.int/news-room/fact-sheets/detail/vector-borne-diseases
	Non – localized EGD in target population *Suggested edit: non-localized drive*	*Gene drives that are expected to spread widely through interbreeding populations of the target species (compare localized drive). Types of gene drives that are likely to be non-localising include low-threshold drives and self-sustaining drives.* *Related definitions: gene drives, low-threshold drive, localized drive, self-sustaining*	Adapted from: WHO 2021a, Alphey and others, 2020

#	Term	Draft definition(s)	Source
	Open release trial	*The development pathway defined for a living modified mosquito (see living modified organism) containing an engineered gene drive includes studies in open release field trials. These are likely to involve a series of sequential trials of increasing size, duration and complexity, conducted at a single site or multiple sites. The trials will aim to collect data including entomological and epidemiological efficacy, dispersal, trait behaviour and ecological interactions. Potential ecological confinement measures include geographical/spatial and/or climatic isolation.* *Initial small scale releases are expected to focus on assessing the biological and functional activity of the EGD-LMO, including its effect on nontarget organisms and the ecosystem.* *For an EGD-LMO intended for a public health intervention, large scale releases may focus on evaluating infection and/or disease in human populations.*	
	Over-replication mechanisms	*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.* *Related definition: gene drive*	Adapted from: MacFarlane and others, 2023, WHO 2021a
	Pathways to harm	*Describe the scientifically plausible and necessary sequence of steps for a harm to be realised. These pathways are constructed during the problem formulation process.*	Adapted from: EFSA 2020, OECD 2023

#	Term	Draft definition(s)	Source
		Related definitions: harm, problem formulation	
	Population modification/ Modification of target population (both terms requested)	Option – specific to vectors *Strategies that target vector competence with the intent to reduce the inherent ability of individual vectors to transmit a given pathogen. May also be referred to as "population replacement", "conversion", or "alteration".* Option – broader alternative *The spread of a genetic element that causes the genotype of a target population to change.*	Adapted from: WHO 2021a and Alphey and others, 2020 Adapted from: NASEM 2016
		Related definition: target population	

#	Term	Draft definition(s)	Source
	Population suppression/ Suppression of target population (both terms requested)	Option – specific to vectors *Strategies that target vector density with the intent to reduce the size of the natural vector population to the extent that it would not be able to sustain pathogen transmission. May also be referred to as "population reduction".* Option – broader alternative *The spread of a genetic element that causes the number of individuals in a population to decrease.*	Adapted from: WHO 2021a and Alphey and others, 2020 NASEM 2016
	Problem formulation	*A structured process for establishing the context and scope of the risk assessment. It involves identification of the protection goals that may plausibly be adversely impacted, determination of assessment endpoints, identification of potential adverse effects, identification of plausible pathways to harm and formulating corresponding risk hypotheses and determining information elements (measurement endpoints) relevant to hypothesis testing.*	Adapted from: Drafting Group A, OECD 2023 Original texts: *Drafting Group A: Combine the process of establishing the context and scope with the identification of potential adverse effects associated with a LMO into a single step* *OECD 2023: Consists of:* - *Identification of protection goals that may be plausibly adversely impacted* - *Determination of assessment endpoints* - *Identification of potential adverse effects (harm)* - *Identification of plausible pathways to harm and formulating corresponding risk hypotheses* - *Determining information elements relevant to evaluating risk hypotheses*

#	Term	Draft definition(s)	Source
		Related definitions: assessment endpoints, pathways to harm, protection goals, risk hypotheses, measurement endpoints	
	Protection goals	*Components of the environment (e.g., species, habitats, services) 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.*	Adapted from: EFSA GMO Panel 2010, OECD 2023,
		Related definitions: harm, risk assessment	
	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 authorisations, monitoring compliance, and enforcement of regulatory conditions.*	N/A
		Related definition: EGD-LMOs	
	Risk	*The likelihood of a hazard causing harm.*	EFSA website: Hazard vs. Risk | EFSA (europa.eu)
		Related definitions: harm, hazard	
	Risk assessment	*A process that evaluates the potential risks associated with certain hazards. It involves four steps: hazard identification, hazard characterisation, exposure assessment, and risk characterisation.*	Adapted from: EFSA glossary (risk assessment | EFSA (europa.eu)), WHO 2021a
		Related definitions: hazard, hazard identification, risk characterisation	
	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.*	N/A

#	Term	Draft definition(s)	Source
		Related definitions: applicant, EGD-LMO, risk, risk assessment, risk management	
	Risk characterization	*The final step of the risk assessment process, with estimation of the overall risk posed to protection goals based on the likelihood and consequences of adverse effects being realised.*	Adapted from: WHO 2021a
		Related definitions: protection goals, risk, risk assessment	
	Risk hypotheses	*For each postulated pathway to harm, a corresponding risk hypothesis is formulated that will enable the risk assessor to determine whether the pathway is likely to occur.*	Adapted from: OECD 2023
		Related definitions: pathway to harm, risk assessor	
	Risk management	*The management of risks identified by the risk assessment through the implementation of appropriate measures for reducing risk to an acceptable level.*	Adapted from: WHO 2021a, EFSA Glossary (Glossary | EFSA (europa.eu))
		Related definitions: risk, risk assessment	
	Risk manager	*The entity that defines and/or implements risk management measures. In certain jurisdictions, e.g., the European Union, the risk manager makes regulatory decisions (see also regulator).*	
		Related definitions: regulator, risk management	
	Self-limiting	*A transient drive mechanism that is expected to be temporally limited and effectively disappear from the target population in the absence of ongoing periodic releases (compare self-sustaining; also termed "self-exhausting").*	Adapted from: Alphey and others, 2020, EFSA 2020, Target Malaria 2022, WHO 2021a
		Related definitions: gene drive, self-sustaining, target population	
	Self-sustaining	*A gene drive system that is designed to cause specific sequences to increase in frequency in a target*	

#	Term	Draft definition(s)	Source
		population and potentially sustain a high frequency indefinitely (compare self-limiting).	Adapted from: Alphey and others, 2020, Target Malaria 2022
		Related definitions: gene drive, self-limiting, target population	
	Shadow drive	*The situation in which perduring Cas9–gRNA complexes are transmitted maternally for one generation in the absence of the Cas9 or gRNA transgenes. This may act to extend the observed engineered gene drive-related changes for one additional generation should they become separated from a Cas9 source.*	Adapted from Guichard and others, 2019
	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
	Split drive	*The necessary components for gene drive are split between two or more genetic loci. May also be referred to as a "Daisy drive".*	Adapted from: Alphey and others, 2020
		Related definition: gene drive	
	Target populations	*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: WHO 2021a, EFSA (reviewed in Connolly and others, 2023)
		Related definition: EGD-LMO	
	Target species complex *and* *Target species complex organism*	*The target organism for a gene drive intervention may be an individual target population, a single species, a species complex, or a partially reproductively connected species.* *In mosquitoes, a species complex includes strains and sibling species where reasonable levels of hybridisation or introgression can occur in the field, or a set of partially reproductively connected species.* *Species complexes can include both vector species that are likely to be the target organisms for the gene drive intervention, and non-vector (non-*	Adapted from: Connolly and others 2023, EFSA 2020

#	Term	Draft definition(s)	Source
		target) species. Due to the potential for vertical transfer from the original EGD-LMO released into the environment to other vector and non-vector species of the complex, all species in the complex can be considered members of the target species complex, or target species complex organisms.	
		Related definitions: gene drive, EGD-LMO, target population	
	Toxin anti-dote *Suggested edit: Toxin anti-dote drive system*	*A diverse set of systems, including a range of naturally-occurring gene drive systems, in which a cell or organism deposits a toxin into most or all of its offspring such that those that do not inherit an antidote gene have a significant fitness cost. This can lead to preferential inheritance of the antidote gene.*	Adapted from: Alphey and others, 2020
		Related definition: gene drive	
	Taxonomic controllability	*Theoretical capacity to limit functional gene drive inserts to target species or sub-species*	[Reference missing]
	Temporal controllability	*Theoretical capacity to limit how long functional gene drive inserts persist in target populations*	[Reference missing]
	Unconfined environmental release	*At the completion of EGD-LMO development, the final phase is implementation of the intervention through release into the open environment with limited or no restrictions (i.e., "unconfined"). This will require a regulatory authorisation in accordance with the applicable regulatory framework, which may be indeterminate or time-limited, and may or may not impose requirements such as post-implementation monitoring.*	Adapted from: CropLife International 2010
		Related definition: EGD-LMO	
	Vector	*Agent which carries and transmits an infectious pathogen into another living organism.*	Adapted from World Health Organisation (2020)

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