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Advancing insights for possible future guidance on the environmental risk assessment and post-market environmental monitoring of gene drive modified insects

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Abstract

Research and development on engineered gene drives (EGDs) has expanded rapidly over the past decade, driven in part by the advent of CRISPR-Cas9 genome editing technologies. Much of this work has focused on insects, including plant pests, agricultural disease vectors, and species that transmit pathogens affecting humans, animals, and plants. As technological advances accelerate, so does the recognition of the need for a careful and forward-looking evaluation of their potential impacts. Gene drive modified organisms, including insects, present characteristics that differ substantially from previously assessed genetically modified organisms (GMOs), prompting growing calls for thoughtful and robust environmental risk assessment (ERA) frameworks tailored to these novel technologies. In earlier work, the GMO Panel of the European Food Safety Authority (EFSA) identified several information gaps that would need to be addressed to provide an adequate foundation for possible future risk assessment guidance. This report brings together and synthesises key elements to help close those gaps, including: an overview of the molecular mechanisms currently being explored for gene drive modified insects (GDMIs); potential use cases relevant to the European Union and its Outermost Territories; considerations for selecting a broader set of comparators for ERA; the role of computational modelling and ecological dynamics in shaping ERA for GDMIs; and aspects to consider when designing post-market environmental monitoring.

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Keywords: Agricultural insect pests, disease-transmitting insects, disease vector control, environmental harm, gene drive, hazard, invasive insects, modelling, monitoring, problem formulation, risk assessment

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1 Summary

The Panel on Genetically Modified Organisms (GMOs) of the European Food Safety Authority (EFSA) published guidance in 2012 and 2013 on the risk assessment of genetically modified (GM) animals covering food and feed safety (EFSA, 2012) and environmental risk assessment (ERA) (EFSA, 2013). In its 2020 scientific opinion, EFSA concluded that these guidelines provide an appropriate basis for risk assessing GM insects containing engineered gene drives (i.e., gene drive modified insects, GDMIs), but also highlighted the need for more specific considerations to address the unique challenges posed by their potential environmental releases (EFSA, 2020). While the existing guidance was considered broadly adequate, it lacked sufficient detail to fully support risk assessments of certain GDMI applications and identified several knowledge gaps requiring attention ahead of any future GDMI risk assessments. In response, EFSA launched a call for tenders in 2023 to address these information gaps and inform the potential development of future guidance for the ERA of deliberate GDMI releases. This report presents the outcomes of that work.

This research report presents the results of an extensive literature review and subsequent analyses across five related but distinct domains, each selected to generate information relevant for addressing the identified gaps. The areas examined include: (1) molecular designs of engineered gene drives; (2) identification of GDMI cases relevant to the European Union and its Outermost Territories, including plausible pathways to harm associated with their potential environmental release; (3) the selection and application of comparators for GDMI risk assessment; (4) molecular, biological and ecological facts influencing GDMI spread and persistence; and (5) considerations for post-market environmental monitoring.

Molecular designs

A comprehensive literature review covering the period 2003–2024 was carried out to identify publications containing detailed information on engineered gene drive designs. From the 142 publications identified, data were extracted on multiple aspects of gene drive design and performance, including: the type and intended purpose of the drive; the molecular mechanisms and components employed; predicted behaviour based on modelling studies; observed performance under laboratory conditions; system stability; and the developmental stage of the research (conceptual design, proof of concept, or advanced experimental work).

Engineered gene drives have now been introduced into 17 species, spanning plants, animals, yeasts, bacteria, and viruses. Although much of the molecular design and experimental development has relied on the model organism *Drosophila melanogaster*—which accounts for approximately 40% of all publications reporting a species—engineered gene drive systems have also been developed in insects of major public health and agricultural importance, including *Anopheles gambiae*, *Aedes aegypti*, and *Drosophila suzukii*.

The analysis revealed that engineered gene-drive developers are exploring a broad spectrum of molecular designs. Most publications (73%) described homing-type engineered gene drives (which are systems that gain their transmission advantage by converting transgene heterozygotes into homozygotes through a gene-conversion process). The advent of CRISPR=Cas9 genome-editing technologies has significantly accelerated the development of such homing-based gene drive systems.

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However, 27% of reported engineered gene drive systems relied on mechanisms fundamentally different from homing drives. These designs do not depend on gene conversion or the transformation of heterozygotes into homozygotes. Instead, many achieve their transmission advantage by eliminating cells or individuals that fail to inherit the drive=mechanisms commonly referred to as toxin-antidote or killer-target systems. Other designs are based on the genetic principle of underdominance.

The diversity of molecular designs and mechanisms of engineered gene drives is substantial. This suggests that any future risk assessment guidance should avoid focusing exclusively on a single design, regardless of its current prevalence. Guidance on molecular characterisation will therefore need to accommodate a wide range of engineered gene drive types and operational principles and should not be tailored to any specific design or molecular toolkit.

An assessment of the adequacy and sufficiency of the molecular characterisation guidance provided in EFSA (2012, 2013) was carried out in light of the findings from the present literature review and analyses. This assessment concluded that the existing guidance remains both adequate and sufficient for the molecular characterisation of GDMIs. This differs somewhat from EFSA (2020), which judged certain aspects of the guidance to be adequate but not fully sufficient. These differences largely reflect minor variations in the interpretation of specific guidance sections and the less prescriptive approach adopted in the present analysis. Moreover, the current assessment found that a broader interpretation of "stability" within the existing guidance framework would address several of the information gaps previously identified by EFSA (2020).

Identification of relevant GDMIs for the European Union and its Outermost Territories and plausible pathways to harm

The literature review was also used to identify insect species for which GDMIs may plausibly be developed in the near future with the intention of environmental release within the European Union (EU) or its Outermost Territories (OMTs)[1]. Species identified in the review were evaluated for their relevance using a set of criteria that considered:

- The stage of technological development;
- The species' current or potential ability to establish in the EU or its OMTs;
- Their capacity to cause environmental or human health impacts in those regions.

Based on these criteria, four categories of insects emerged as potentially relevant: *Aedes aegypti*, *Anopheles* species (including *Anopheles stephensi*, *Anopheles gambiae* and *Anopheles coluzzii*), *Drosophila suzukii*, and *Plutella xylostella*.

Further evaluation led to the exclusion of *Anopheles* spp. from consideration. Although *Anopheles* mosquitoes are the subject of some of the most advanced gene drive research globally, their relevance to the EU context is limited. Their public health significance is primarily tied to malaria transmission, and the EU and its OMTs already have well-established, effective conventional approaches for malaria prevention and control. As a result, deployment of GDMIs targeting *Anopheles* spp. is considered unlikely in these regions. Moreover, current

[1] In 2024, the EU consisted of 27 countries and nine outermost regions or outermost territories (OMTs), which are geographically very distant from the European continent. The OMTs consist of: (1) French Guiana, Guadeloupe, Martinique, Mayotte, Reunion Island and Saint-Martin (France); (2) Azores, Madeira (Portugal); and (3) the Canary Islands (Spain).

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evidence indicates that *Anopheles* mosquitoes have a low likelihood of becoming established in the EU or its OMTs, even under changing climatic conditions.

For the remaining three insect species, information was compiled on their ecology and the characteristics of potential receiving environments in the EU and its OMTs in the event of a deliberate GDMI release. This information formed the basis for a problem-formulation exercise aimed at identifying plausible pathways to harm and corresponding risk hypotheses that would need to be addressed in any ERA of these GDMI species.

Use of comparators for GDMI risk assessments

EFSA (2020) concluded that the use of comparators in GMO risk assessment may need to be broadened to reflect the types of applications and potential pathways to harm associated with GDMIs. To support the development of potential future guidance for GDMI risk assessment, a review was conducted of relevant international and national regulatory frameworks governing control measures for the insect species identified as most relevant for the EU and its OMTs.

Because these species are primarily considered in the context of pest management—for human health protection or agricultural purposes—the review focused on regulatory provisions and guidance for chemical and biological control tools. This analysis shows that comparator use in these domains is already considerably broader—both implicitly and explicitly—than in traditional GMO risk assessments and is often employed to provide context for the interpretation of identified risks.

In line with the conclusions of EFSA (2020), it is recommended that any future risk assessment guidance applies a broader and more flexible range of comparators, selected according to the specific risk hypotheses under consideration. Comparisons between the GDMI and its parent (non-modified) organism remain essential for identifying unintended effects and for assessing potential environmental adverse effects linked to GDMI phenotypes—for example, altered susceptibility to chemical control measures or changes in disease-vectoring capacity.

However, additional comparators should also be incorporated. In particular, comparisons with the target population—the insect population intended to be controlled—are important, as these populations may differ genetically from the laboratory colonies from which the GDMI originates. Understanding these differences is critical for contextualising ecological performance, potential spread, and population-level effects.

Finally, because the most relevant GDMIs identified are proposed for use in agricultural pest management or disease-vector control, comparisons with alternative control approaches are both appropriate and informative. Such comparisons, including evaluation of environmental protection goals and human health impacts, can help decision-makers weigh the relative risks and benefits of GDMI deployment against currently available control strategies.

Review of molecular, biological, and ecological factors affecting GDMI spread and persistence

A literature review covering the period 2003-2024 was conducted to identify publications relevant to understanding the molecular, biological, and ecological factors that influence the GDMI spread and persistence, with particular emphasis on modelling approaches. A database

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of 177 publications was compiled and examined to identify existing models (and modelling practices) as well as approaches to validation.

The analysis revealed a strong imbalance in the current modelling landscape: over 95% of publications focus on model development, while fewer than 1% address model validation, highlighting a major gap in current practice.

Most identified models examine the genetic dynamics of engineered gene drives, focusing on the inheritance efficiency of engineered constructs and the behaviour of the gene drive elements within populations using population-genetics frameworks. A smaller, yet meaningful subset of studies applies modelling to ecological factors influencing the spread and spatial distribution of GDMOs.

Models exploring molecular and genetic efficiency have been used to demonstrate the potential performance of different engineered gene-drive architectures and to identify key parameters influencing gene drive success, such as negative selection against the gene drive constructs, fitness effects in heterozygotes, or rates of wild type-to-drive conversion in low threshold systems. These models are typically deterministic, discrete-time recursion models: they are straightforward to implement but offer only simplified representations of inheritance and do not capture ecological realism. For example, recursion models commonly assume discrete, non-overlapping generations, whereas many insect species exhibit overlapping generations and asynchronous development, even within a single season.

No comprehensive model currently exists that integrates both the genetic and ecological dynamics needed to predict GDMI spread. Furthermore, no modelling studies were identified that address ecological endpoints beyond spread and persistence, such as trophic interactions or ecosystem-level effects associated with population-suppression drives.

From this analysis, several key principles emerge for informing potential future guidance on modelling in risk assessment:

- ERA and PMEM require bespoke modelling tools, tailored to the specific properties and use cases of each GDMI;
- Models predicting GDMI spread and persistence should be stochastic, not purely deterministic, to allow quantification of uncertainty;
- Models should be embedded within appropriate statistical frameworks, enabling evaluation against empirical data and supporting transparent uncertainty analysis.

Post-market environmental monitoring of GDMIs

There is currently no practical experience with the release of GDMIs, and therefore no direct experience with post-market environmental monitoring (PMEM) for such organisms. However, a review of monitoring practices used in other insect-release applications provides a useful basis for considering how PMEM could be approached in future guidance for GDMI risk assessment.

Practices associated with conventional biological control, augmentative biological control, the sterile insect technique, the release of GM insects for inundative sterile-male programs, and *Wolbachia*-based methods (including the incompatible insect technique and pathogen-interference approaches) were reviewed. These techniques offer insight into both the mechanics and objectives of PMEM for insect releases. Monitoring typically focuses on:

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- Detecting the presence or persistence of the released insects and any associated genetic markers or elements;
- Assessing the response of the target population (e.g. suppression, replacement, or behavioural change);
- Evaluating the overall performance of the control programme against defined objectives.

Consistent with EFSA (2020), case-specific monitoring is expected to be particularly informative for GDMIs. Case-specific monitoring can be used to confirm predictions from the risk assessment or to collect data that address key uncertainties. In contrast, general surveillance is anticipated to pose significant challenges for GDMIs, primarily because establishing a causal link between observed environmental changes and the presence of the GDMI is likely to be extremely difficult.

Finally, examples are provided of how case-specific monitoring could be applied to GDMI species relevant to the EU and its OMTs, based on key steps in plausible pathways to harm identified through problem formulation.

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2 Introduction

The European Food Safety Authority (EFSA) is responsible for providing environmental risk assessments (ERA) for genetically modified organisms (GMOs) under Directive 2001/18/EC and its subsequent amendments. To fulfil this mandate—and to offer clarity to applicants, stakeholders, and the public regarding how EFSA and its GMO Panel conduct these assessments—EFSA, at the request of the European Commission, commissioned a series of reports addressing the ERA of genetically modified (GM) fish, insects, mammals, and birds. These reports were synthesised by the EFSA GMO Panel into the guidance on the ERA of GM animals (EFSA, 2013). Together with the guidance on the risk assessment of food and feed from GM animals and on animal health and welfare aspects (EFSA, 2012), which outlines requirements for molecular characterisation, the 2013 guidance provides applicants and risk assessors with a comprehensive framework describing how ERA should be conducted and what information should accompany any application for the deliberate release of a GM animal.

Following publication of EFSA (2013), the potential development and release of GM animals modified with genetic elements that bias their own inheritance—so-called gene drives—generated significant scientific, regulatory, and public interest due to both their potential utility and associated risks. In response to a request from the European Commission, EFSA evaluated the adequacy and sufficiency of its existing guidance for the risk assessment of gene drive modified insects (GDMIs), resulting in the scientific opinion "Adequacy and sufficiency of existing EFSA guidelines for the risk assessment of gene drive modified insects" (EFSA, 2020). This scientific opinion concluded that, while current guidance remains broadly adequate, it does not provide sufficient detail to fully support risk assessments of GDMIs and identified several knowledge gaps that would be important to address in future assessments.

To address these gaps and support the potential development of future GDMI-specific guidance, EFSA launched a call for tenders. This report presents the results of that work.

The research and analysis undertaken for this report are organised into five related but distinct areas, each selected to help close the gaps identified in EFSA (2020):

- Molecular designs of engineered gene drives;
- Identification of GDMI cases relevant to the European Union and its Outermost Territories, including plausible pathways to harm associated with their potential environmental release;
- The selection and application of comparators for GDMI risk assessment;
- Molecular, biological and ecological facts influencing GDMI spread and persistence;
- Considerations for post-market environmental monitoring (PMEM).

2.1 Background and terms of reference

This contract/grant was awarded by EFSA to the Agriculture & Food Systems Institute (AFSI).

Contractor/Beneficiary: The consortium was led by AFSI (Andrew Roberts) and supported by a group of internationally recognised experts. The consortium included: Michael Bonsall; Debora Glandorf; David O'Brochta from the Foundation for the National Institutes of Health; Geoff Ridley from Manaaki Whenua – Landcare Research, Jörg Romeis from Agroscope; and Patrick Rüdelsheim and Greet Smets from Perseus BV.

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Contract/Grant title: Preparatory work for the development of risk assessment guidance for gene drive modified insects.

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The background and terms of reference (ToRs) are those provided by EFSA in the tender specifications. They are reproduced and adapted (where needed) below, to frame the planned activities and agreed timelines.

2.1.1 Background

Recent advances in molecular and synthetic biology have enabled the engineering of gene drives in an increasingly broad range of organisms, with initial efforts focusing predominantly on insects. Gene drives are genetic elements that bias their own inheritance, allowing a genetic modification of interest to spread through interbreeding wild populations. Research on engineered gene drives and their potential applications in insects is progressing rapidly.

Engineered gene drive systems in insects can be designed either to suppress target populations or to modify them by introducing a new genotype. Depending on the design and mode of action—both of which vary widely—engineered gene drives may, at least in theory, spread through target populations without needing repeated interventions (non-localised, self-sustaining systems), or instead be restricted in their spatial spread (localised systems) or temporal persistence (self-limiting systems).

These technological possibilities have generated both enthusiasm and concern. Engineered gene drives in insects could potentially help address long-standing challenges in controlling disease vectors, agricultural pests, and invasive species, or be applied to the conservation of endangered species—thus offering potential public health, agricultural, and environmental benefits. At the same time, deliberate release of gene drive modified insects (GDMIs) may introduce new or different risks to human or animal health and the environment and may raise novel challenges for risk assessment. Against this backdrop, risk assessors, risk managers, developers, potential applicants, and other stakeholders are actively discussing whether new or additional risk assessment guidance may be needed for the deliberate release of gene drive modified organisms (GDMOs), including insects.

To support the European Union's work on GDMOs under the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, EFSA undertook a problem formulation exercise to evaluate whether:

- The environmental release of GDMIs could pose novel risks to human and animal health or to the environment;
- The scientific considerations included in previously published EFSA guidance for the risk assessment of GM animals (EFSA, 2012, 2013), including GM insects, are adequate and sufficient for GDMIs;
- There is a need for updated or additional guidance.

EFSA's analysis focused primarily on disease-transmitting insects, especially mosquitoes, as these represent the most likely cases of GDMOs intended for environmental release in the near term. However, agricultural pests and non-native invasive insects were also considered.

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In its 2020 scientific opinion (EFSA, 2020), EFSA concluded that existing guidelines for GM insects without engineered gene drives provide an appropriate foundation for the risk assessment of GDMIs but should be made more specific to address the distinctive challenges associated with their potential release into the environment. While risk assessments for GDMIs can build on the current framework for GM insects, EFSA identified several gaps—in molecular characterisation, ERA, and PMEM—where additional guidance would be necessary.

To ensure preparedness for future risk assessment challenges, EFSA seeks to collect and evaluate the information needed to fill these gaps and to provide recommendations on considerations for any potential development of additional guidance for the ERA of GDMIs intended for deliberate release. To this end, EFSA launched the call for tenders "Preparatory work for the development of risk assessment guidance for gene drive modified insects", which was awarded to AFSI (contract OC/EFSA/NIF/2023/01).

2.1.2 Terms of reference

The aim of the project is to gather and assess the information needed to address the gaps previously identified by EFSA in existing risk assessment guidelines for GM animals, and to provide recommendations on aspects to consider for the potential development of additional guidance for the ERA of GDMIs intended for deliberate release. The assignment focuses specifically on engineered gene drives in disease-transmitting insects—primarily mosquitoes—as well as agricultural pest species and non-native invasive insects that could be candidates for intentional environmental release.

The project is divided into five broad objectives (referred to as ToRs hereafter).

- ToR1 – Review relevant case studies and their risk to human, animal and environmental health
  - Based on the evidence available in the scientific literature, this task consists of reviewing all GDMI cases, including their mode of action, intended outcomes (suppression, eradication, replacement), temporal and spatial characteristics (self-sustaining or self-limiting, localised or non-localised), reversibility, specific features of the receiving environments in which they may be deliberately released, and their current stage of development;
  - The relevance of each identified GDMI case is to be assessed in the context of the European Union (EU) and its outermost territories (OMTs);
  - For the most relevant GDMI cases, risks to human, animal and environmental health should be identified and examined using a pathway-to-harm approach.
- ToR2 – *Review molecular designs*
  - This task involves reviewing the molecular designs of GDMIs reported in the scientific literature, including the characteristics of the molecular constructs, target genes, traits, intended uses and outcomes, efficacy, and potential for resistance to evolve in target organisms. The review also covers designs intended to restrict the spread and/or persistence of the engineered gene drive, to enable reversibility or recall, and/or to delay or prevent resistance evolution;
  - The analysis should also assess which aspects of molecular characterisation, as outlined in EFSA's risk assessment guidelines for GM animals, may require adjustment to account for the specific properties of GDMIs;
  - For any molecular characterisation elements identified as needing refinement, recommendations should be provided on how these aspects could be adapted.

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- ToR3 – Review comparators
  - This task involves reviewing comparators that could support the risk assessment of GDMIs, including the most appropriate endpoints and approaches for comparison, and determining for which risk assessment purposes single or multiple comparators may be utilised;
  - It also includes assessing the strengths and weaknesses of the comparators identified;
  - Based on this analysis, recommendations should be provided on the selection of single or multiple comparators suitable for the risk assessment of GDMIs.
- ToR4 – Review factors affecting engineered gene drive dynamics
  - This task focuses on reviewing how molecular, biological, and ecological factors influence the spread and persistence of engineered gene drives, as well as their potential invasiveness in both target and non-target populations or species;
  - It also includes mapping of existing models used to investigate engineered gene drive dynamics and assessing their strengths and weaknesses in terms of underlying assumptions, computational approaches, parameters, biological and ecological realism, and ability to predict ecological effects;
  - The usefulness of these models for ERA and PMEM of GDMIs should be evaluated;
  - Based on this appraisal, recommendations should be provided on which models could support the risk assessment of GDMIs, and how such models should be further developed to support both ERA and PMEM.
- ToR5 – Review monitoring approaches
  - This task includes identifying the objectives relevant to the PMEM of GDMIs, and identifying monitoring and surveillance approaches aligned with these objectives. The strengths and weaknesses of the identified approaches should be investigated;
  - Based on this analysis, recommendations should be provided on monitoring and surveillance approaches that could be used to support PMEM for GDMIs.

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3 Data and methodologies

3.1 Protocol

The protocol was developed in accordance with EFSA's Scientific Committee guidance on protocol development for EFSA's generic scientific assessments (EFSA, 2023). It sets out how the mandate's ToRs were interpreted and translated into operational assessment questions (AQs) and sub-questions (SQs), and describes the approach and methods applied for conducting the assessment.

The protocol is organised around the five ToRs outlined above. For each ToR, a problem formulation and a methods section are provided. In line with EFSA's guidance, the problem formulation clarifies ***what*** the assessment aims to achieve. ToRs were therefore translated into scientifically answerable AQs, which were then further broken down into SQs to ensure they are operational. Relationships between AQs and SQs are illustrated through a conceptual model (Figure 1).

The methods section of the protocol describes ***how*** the assessment was carried out. It specifies the evidence required and the methodological approaches applied to address the problem formulation and, ultimately, to deliver the assessment.

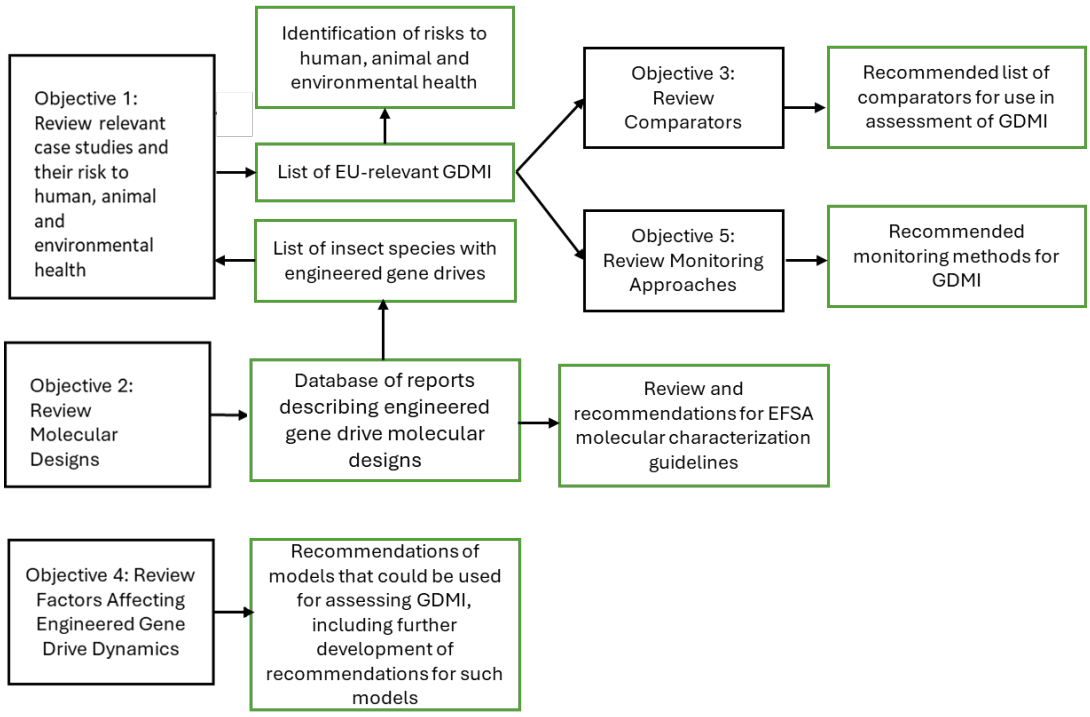


Figure 1: Conceptual model of the overall project, illustrating the overall objectives (black boxes representing the terms of references (ToRs)) and the outcomes or outputs of each ToR (green boxes). Arrows indicate how the outcomes/outputs of ToRs relate to one another.

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3.1.1 ToR1 – Review relevant case studies and their risks to human, animal and environmental health

Problem formulation

ToR1 focuses on identifying GDMI cases that could potentially be deliberately released into the environment and assessing their significance for the EU and its OMTs. It also aims to determine, for the most relevant cases, potential risks to human, animal, and environmental health using a pathway to harm approach. These aims were operationalised through three AQs (AQ1.1–AQ1.3) and their corresponding SQs (SQ1.1.1–SQ1.3.4).

AQs and SQs

To address ToR1, the following AQs and SQs were formulated.

AQ1.1 asks: Which GDMI cases, based on the available evidence, could be deliberately released into the environment? This descriptive assessment is addressed through the following SQs:

- SQ1.1.1 – Which GDMIs (species and trait) are being developed to suppress, eradicate, or modify:
  - Disease-transmitting insects (primarily mosquitoes);
  - Agricultural insect pests;
  - Non-native invasive insects.
  - And what are their mode(s) of action and development stage(s)?
- SQ1.1.2 – Which of the GDMIs identified in SQ1.1.1 are in the most advanced stages of development, and what are their key characteristics?
- SQ1.1.3 – What are the intended temporal and spatial scales (e.g., self-sustaining vs self-limiting; localised vs non-localised)?
- SQ1.1.4 – What reversal or recall methods are being developed to mitigate or undo gene drive effects following deliberate release?

To address the first question of ToR1, GDMI cases reported in the scientific literature (covering both insect species and engineered traits) were identified as those intended for deliberate environmental release to suppress or modify populations of harmful insect species. From this set, cases in the most advanced stages of development were prioritised, as these are considered the most likely to be ready for environmental release in the EU and its OMTs within the foreseeable future (e.g., over the next decade).

Development stages used in this assessment, include:

- Conceptual stage – proposal of a GDMI case;
- Genetic proof of concept – successful transformation of the insect;
- Phenotypic proof of concept – experimental evidence that the gene drive delivers the intended effect;
- Advanced research – population-level studies.

Preference was given to cases in advanced research and phenotypic proof of concept stages.

AQ1.2 asks: Which of the GDMI cases identified under AQ1.1 (specifically those in SQ1.1.2) are relevant for the EU and its OMTs? This is examined through:

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- SQ1.2.1 – Which GDMI cases involve insect species that:
  - Are already established in the EU and its OMTs?
  - Could establish in the EU and its OMTs in the near future?
- SQ1.2.2 – Which of these insect species could cause adverse effects on human health, animal health, or the environment in the EU and its OMTs?
- SQ1.2.3 – Based on development stage, (potential) establishment, and potential adverse effects, which GDMI cases are most relevant to the EU and its OMTs?
- SQ1.2.4 – For the most relevant cases, what are the likely receiving environments where GDMI insects might be deliberately released?

To address the second question of ToR1, the relevance of GDMI cases in the most advanced stages of development was assessed for their significance to the EU and its OMTs. A GDMI case was considered relevant when the insect species carrying the gene drive (i.e. the species into which the construct is introduced) was already established in the EU or its OMTs and had been shown to cause adverse effects on human or animal health or the environment. Species expected to establish soon were also considered relevant—for example, invasive insects that are increasingly detected but have not yet formed stable populations.

Relevance was informed by whether the target insect is established (or likely to establish) and whether it is associated with adverse impacts (e.g., vector-borne disease transmission, agricultural damage, ecological harm). Where specific pathogens or diseases were uncertain or variable across geographies, references were framed generically (e.g., "vector-borne diseases") to avoid over-specificity.

AQ1.3 asks: For the most relevant GDMI cases (for the EU and its OMTs), what risks to human, animal, and environmental health can be identified using a pathway-to-harm approach, considering their deliberate release into the environment? This is addressed via:

- SQ1.3.1 – What hazards (potential harms) can be identified for the most relevant GDMI cases?
- SQ1.3.2 – For each hazard, what exposure pathways could lead to potential harm following deliberate release?
- SQ1.3.3 – Based on exposure pathway analysis, what are the most plausible pathways to harm?
- SQ1.3.4 – What are the resulting risks for human, animal, and environmental health, and what is the likelihood and magnitude associated with these risks?

To address the third question of ToR1, the most relevant GDMI cases (for the EU and its OMTs) were examined to identify potential hazards, the most plausible pathways through which these harms could occur (e.g., via exposure routes), and the resulting risks to human, animal, and environmental health from their deliberate release.

Risk characterisation was based on both the likelihood that identified harms could occur and the magnitude of their potential consequences.

Conceptual model

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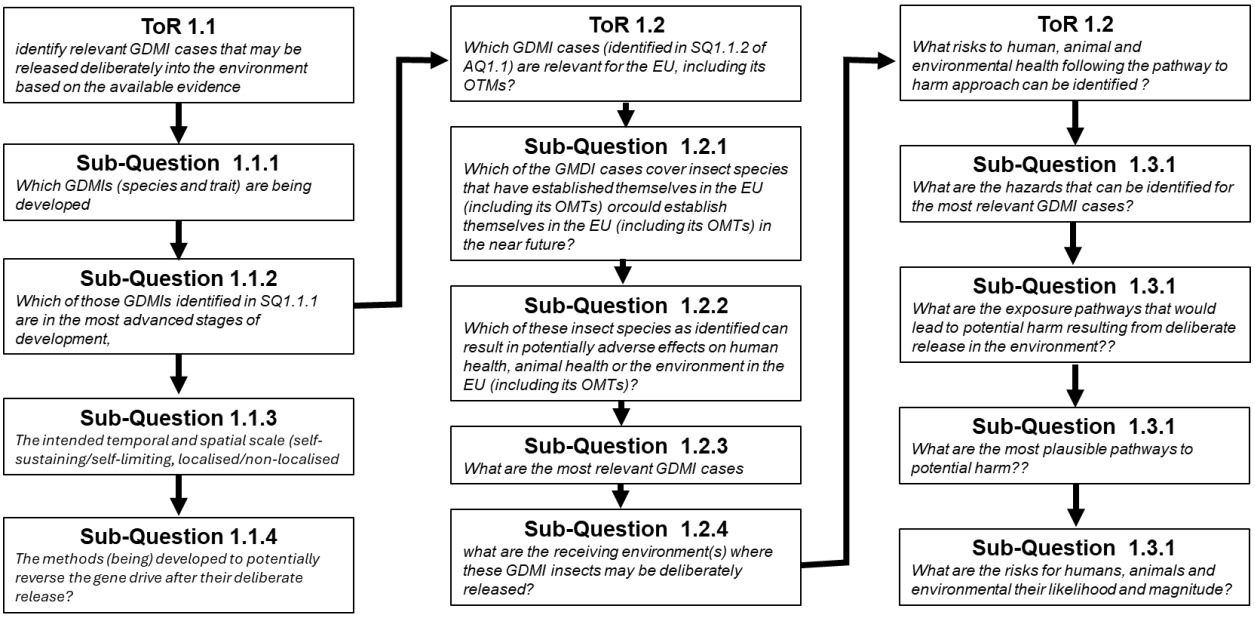


Figure 2: Conceptual model for the term of reference 1 (ToR1: Review relevant case studies and their risks to human, animal, and environmental health)

Methods applied for conducting the assessment

The assessment was based on evidence retrieved from extensive scientific literature searches and expert consultations (Table 1). The approach is organised by AQs and SQs, with methods tailored to each AQ.

Table 1: Overview of methods used for answering assessment questions (AQs) and sub-questions (SQs) of the term of reference 1 (ToR1: Review relevant case studies and their risks to human, animal, and environmental health)

AQs and SQs	Data collection	Study selection	Evidence appraisal	Evidence synthesis
AQ1.1; SQ1.1.1–SQ1.1.4	Extensive literature search (see ToR2 methods)	Structured process with predefined eligibility criteria	Narrative	Qualitative
AQ1.2; SQ1.2.1–SQ1.2.4	Literature search and expert deliberations	Structured process with predefined eligibility criteria	Narrative	Qualitative
AQ1.3; SQ1.3.1–SQ1.3.4	Expert deliberations (plus targeted literature as applicable)	—	Narrative	Qualitative

Note (AQ1.1 – Methods): See Methods for Objective 2 (ToR2) for the detailed literature-search protocol and screening/selection workflow used to compile the list of GDMI cases

Methods for AQ1.1 (identification of GDMI cases) and linkage with the term of reference 2 (molecular designs)

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The method used to address AQ1.1 (identification of GDMI cases (i.e., insect species into which engineered gene drives have been introduced)) was performed in conjunction with ToR2. A search of the scientific literature in the period 2003–2024 was performed for ToR2 to identify relevant scientific reports on designs and characteristics of gene drives developed in insect species, in the form of a searchable database (GDMD database ToR2; Annex 1). This search and the resulting database were also used for ToR1 to identify GDMI cases (insect species, traits) with the potential (or intention) of introduction into the environment to suppress or modify populations of harmful insect species (SQ1.1.1).

The ToR2 literature search and aggregation of publications on molecular designs identified:

- All insect species into which engineered gene drives had been introduced;
- The intended purpose(s) (e.g., suppression/replacement);
- The species for which development was most advanced.

To address SQ1.1.2, these GDMI cases were classified according to the stage of development they have reached, defined as follows:

- Conceptual stage with a theoretical design of a GDMI case;
- Proof of concept stage at the genetic level (e.g., transformation of an insect);
- Proof of concept stage at the phenotypic level (e.g., studies to show effectiveness of the gene drive in an insect);
- Advanced research (population level studies).

Among the different stages of development, the GDMI cases at: (1) the proof-of-concept stage at the phenotypic level; and (2) the advanced research stage were identified. Consequently, these cases were selected for further consideration in this project due to their high likelihood of being ready for environmental release within the next decade. Of these GDMI cases in the most advanced stages of development the intended temporal and spatial scale of the gene drive was indicated (SQ1.1.3). In addition, methods that are being developed to potentially reverse the gene drive after their deliberate release are given (SQ1.1.4).

The resulting species list directly informed AQ1.2 and AQ1.3.

The following predefined eligibility criteria were applied (see Tables 2–3).

Table 2: Eligibility criteria (study characteristics) for assessment question 1.1 (AQ1.1)

Key element	Inclusion	Exclusion
Gene drive type	Insects genetically modified to contain engineered gene drives (including reversal drives)	Non-GDMIs (e.g., *Wolbachia*-mediated incompatible insect technique (IIT) and pathogen interference (PI), sterile insect technique (SIT), release of insects carrying a dominant lethal gene (RIDL) and its female-specific variant (fsRIDL)); natural gene drives
Target organisms	Harmful insects (e.g., disease-transmitting insects, agricultural insect pests, non-native invasive insects)	Hypothetical species; model insects with limited field relevance; insects used solely for conservation; endangered insect species; non-

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Key element	Inclusion	Exclusion
		insect organisms; organism not specified
Intended uses	Gene drives developed for deliberate environmental release in insects	Gene drives developed only for contained use; purely hypothetical/theoretical designs with no identifiable pathway to release

Table 3: Eligibility criteria (report characteristics) for assessment question 1.1 (AQ1.1)

Key element	Inclusion	Exclusion
Temporal scope	Publications dated January 1, 2004-2024 (search start to end)	Publications before January 1, 2004
Language	English	Other languages
Type of publication	Peer reviewed literature; primary research (new data); conference abstracts/posters containing primary data; reviews (used to snowball additional sources)	Letters to the editor; editorials; opinion papers; PhD theses/dissertations

Methods for AQ1.2 (relevance to the EU and its OMTs)

To assess the relevance of the GDMI cases to the EU and its OMTs, the consortium team searched peer-reviewed and grey literature and consulted relevant databases for information on the (potential) establishment of the implicated insect species and their potential to cause adverse effects on human, animal, or environmental health. We also consulted national reports (where available) on disease-transmitting mosquitoes, agricultural insect pests, and non-native invasive insects in EU Member States and OMTs and conducted targeted expert consultations to validate relevance determinations.

- Below are illustrative

Methods for AQ1.3 (risk assessment using a pathway-to-harm approach)

For the most relevant GDMI cases and their plausible receiving environments, potential risks were assessed using a problem formulation framework following a pathway-to-harm approach. The assessment relied primarily on expert deliberation, involving specialists in molecular genetics, gene-drive technology, harmful insects (including invasive species, disease vectors, and agricultural pests), insect population dynamics, and ERA. To ensure robustness, risk assessors, risk managers, and other relevant stakeholders were also consulted, with explicit consideration given to disciplinary and worldview diversity.

Where available, publications describing pathways to harm for GDMI cases were incorporated and aligned with the selected species, characteristics of the engineered gene drive (e.g., low- or high-threshold systems), intended release purposes, and relevant receiving environments. Across all analyses, the focus remained on identifying the most plausible pathways to harm and characterising the associated risks to human, animal, and environmental health.

3.1.2 ToR2 – Review molecular designs

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Problem formulation

The first ToR2 objective is to gather and critically evaluate existing information on the molecular designs of engineered gene drives reported in the scientific literature. This includes molecular constructs, key functional characteristics, target genes, traits, intended uses and outcomes, efficacy, and the potential for resistance evolution. The review also encompasses designs intended to limit spread or persistence, enable reversibility/recall, or delay/avoid resistance evolution. Because most engineered gene drive architectures are species-agnostic and technically portable, and because innovation in this domain is rapidly evolving, the review cannot be restricted to molecular designs associated only with ToR1 cases.

The second ToR2 objective is to assess which aspects of molecular characterisation, as outlined in EFSA's risk assessment guidelines for GM animals may require modification for application to GDMIs. For each aspect identified as insufficient or in need of clarification, recommendations for adjustment are to be proposed.

AQs and SQs

To address ToR2, the following AQs and SQs were formulated.

AQ2.1 asks: What is the current state of the art regarding the molecular design and function of engineered gene drives? This descriptive assessment is addressed through the following SQs:

- SQ2.1.1 – Which scientific reports describe molecular designs of engineered gene drives?
- SQ2.1.2 – What are the reported molecular characteristics (form and function) of engineered gene drives in relation to intended use, efficacy, target and construct gene(s), molecular mechanism, controllability, susceptibility to resistance evolution, and intended or unintended fitness effects?

AQ2.2 asks: Are current EFSA guidelines for the risk assessment of GM animals sufficient and adequate for assessing GDMIs with respect to molecular design considerations?

AQ2.3 asks: How can current EFSA guidelines for the risk assessment of GM animals be improved or adapted to accommodate molecular design considerations specific to GDMIs?

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Conceptual model

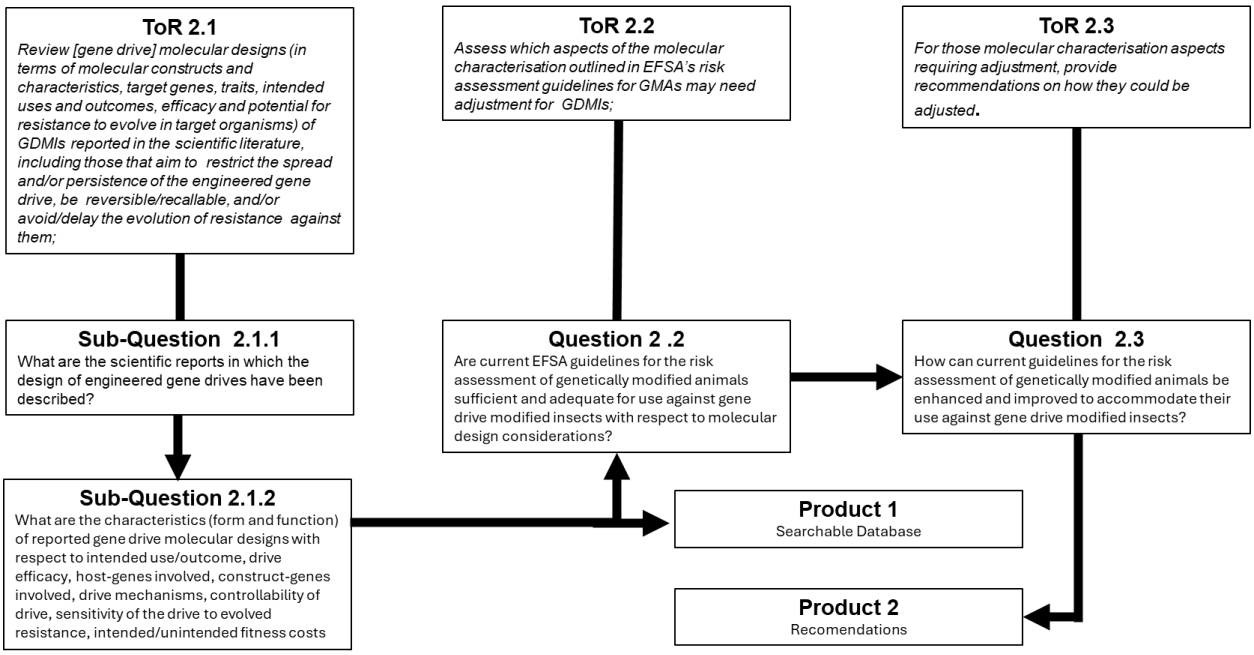


Figure 3: Conceptual model for the term of reference 2 (ToR2: Review molecular designs)

Methods applied for conducting the assessment

The methodological approach for addressing ToR2 was designed to ensure that the AQs could be answered in a transparent, systematic, and scientifically robust manner. Because engineered gene drive systems represent a rapidly evolving field with diverse molecular architectures and functional strategies, the methods needed to accommodate a wide range of empirical and theoretical designs while maintaining consistency with established EFSA risk assessment principles. The procedures applied therefore combine structured evidence retrieval, predefined eligibility criteria, systematic data extraction, and qualitative synthesis (Table 4). Together, these steps ensure that all relevant information on engineered gene drive molecular designs is captured, organised, and analysed in a manner that supports clear, defensible conclusions about the adequacy of current EFSA molecular characterisation guidance and the adjustments required for application to GDMIs.

Table 4: Overview of methods used for answering assessment questions (AQs) and sub-questions (SQs) of the term of reference 2 (ToR2: Review molecular designs)

AQs and SQs	Data collection	Study selection	Evidence appraisal	Evidence synthesis
AQ2.1; SQ2.1.1	Comprehensive scientific literature search	Structured process based on pre-defined eligibility criteria	Narrative	Integrative
AQ2.1; SQ2.1.2	Review individual reports and assess gene drive molecular design-content based	Structured process based on pre-defined eligibility criteria	Narrative	Qualitative

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AQs and SQs	Data collection	Study selection	Evidence appraisal	Evidence synthesis
	on pre-defined categorical criteria			
AQ2.2	Expert deliberations	Structured process based on pre-defined eligibility criteria	Narrative	Qualitative
AQ2.3	Expert deliberations	Structured process based on pre-defined eligibility criteria	Narrative	Qualitative

Note: Database creation for SQ2.1.2: A flat database (Excel) with rows of records (reports) and columns of data consisting of encoded characteristics of the engineered gene drive(s); filtering and search functions included

Evidence needs

A comprehensive scientific literature review was conducted to capture the breadth and depth of both theoretical and empirical engineered gene drives. Given the high rate of innovation and the largely species-independent nature of engineered gene drive molecular designs, no taxonomic restrictions were applied. All engineered molecular designs of potential relevance to ToR2 were therefore included.

Eligibility criteria to establish the relevance of evidence

Eligibility criteria were applied to determine the relevance of evidence for inclusion in the review (Tables 5–6). These criteria were used for screening literature retrieved through the search strategy. The criteria were tested on a subset of publications and refined as necessary if any ambiguities or risks of misinterpretation were identified.

Table 5: Eligibility criteria for study characteristics

Inclusion	Exclusion
Full English language text retrievable	Full text unavailable or not retrievable or not in English
Empirical or theoretical information describing engineered gene drive architecture, activity, or application, including underdominance systems and engineered systems using components of natural gene drives	Natural gene drive systems; symbiont/microbe-based drive systems; non-driving genetic biocontrol systems; ethical, governance, risk-assessment, risk-analysis, engagement, economics studies; studies not about gene drives

Table 6: Eligibility criteria for record characteristics

	Inclusion	Exclusion
Temporal scope	Publications dated 1 January 2003 – present (start date reflects first use of "gene drive" referring to selfish genetic elements)	Publications prior to 1 January 2003
Type of publication	Peer-reviewed literature	Book chapters; editorials; meeting abstracts; letters; news items;

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	Inclusion	Exclusion
		proceedings papers; retracted publications

Evidence sources

The main evidence sources used to identify the necessary evidence for the review were electronic bibliographic databases. Databases consulted:

- Web of Science;
- Scopus;
- PubMed.

The databases selected were identified in line with the defined scope of the review.

Search strategy

The search strategy for identifying evidence from the scientific literature has been designed to retrieve as many relevant studies as possible to address the AQs and SQs reported in Table 7.

The search strings as ran in the information sources are reported in Table 7. They combine search terms using Boolean and proximity operators. They were developed to capture the full scope of publications relevant to engineered gene drive molecular designs while excluding records unrelated to molecular aspects of gene drives.

Table 7: Search strings

Web of Science	((ALL=("gene drive" OR "gene drives")) NOT ALL=(ethic* OR govern* OR (risk AND assess*) OR (risk AND anal*) OR guidance OR rules OR engag* OR economic* OR commun*) NOT(DT==("EDITORIAL MATERIAL" OR "MEETING ABSTRACT" OR "LETTER" OR "NEWS ITEM" OR "PROCEEDINGS PAPER" OR "RETRACTED PUBLICATION")) NOT KP=("stem-cells"); Dates from 2003-01-01 to 2024-12-31
Scopus	TITLE-ABS-KEY({gene drive} OR {gene drives}) AND PUBYEAR > 2002 AND (EXCLUDE (SRCTYPE,"b") OR EXCLUDE (SRCTYPE,"k") OR EXCLUDE (SRCTYPE,"p") OR EXCLUDE (SRCTYPE,"d")) AND (LIMIT-TO (PUBSTAGE,"final")) AND (LIMIT-TO (DOCTYPE,"ar") OR LIMIT-TO (DOCTYPE,"re")) AND (EXCLUDE (SUBJAREA,"MATH") OR EXCLUDE (SUBJAREA,"PHYS") OR EXCLUDE (SUBJAREA,"SOCI") OR EXCLUDE (SUBJAREA,"PHAR") OR EXCLUDE (SUBJAREA,"NEUR") OR EXCLUDE (SUBJAREA,"COMP") OR EXCLUDE (SUBJAREA,"BUSI") OR EXCLUDE (SUBJAREA,"PSYC") OR EXCLUDE (SUBJAREA,"HEAL") OR EXCLUDE (SUBJAREA,"EART") OR EXCLUDE (SUBJAREA,"ECON") OR EXCLUDE (SUBJAREA,"ARTS") OR EXCLUDE (SUBJAREA,"ENER") OR EXCLUDE (SUBJAREA,"MATE") OR EXCLUDE (SUBJAREA,"NURS") OR EXCLUDE (SUBJAREA,"DECI")) AND (LIMIT-TO (LANGUAGE,"English"))
PubMed	("gene drive"[Title/Abstract] OR "gene drives"[Title/Abstract]) AND (2003/1/1:2024/12/31[pdat])

Search strategy appraisal

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Recent review publications published in the previous two years were used to evaluate the completeness of the search strategy (Bier, 2022; Gantz and Bier, 2022; Garrood et al., 2022; Verkuijl et al., 2022; Wang et al., 2022; Raban et al., 2023). All relevant references contained in these reviews were checked for presence or absence in the search results, allowing refinement of the strategy where needed. The appraisal confirmed that the search strings were effective in capturing the intended evidence base.

Publication selection process

The evidence retrieved through the literature searches was screened for its relevance against the eligibility criteria illustrated above (Tables 5–6). Relevant evidence needed to comply with all the eligibility criteria of the review.

The publication selection process was carried out in three sequential steps designed to ensure that all relevant molecular-design publications were captured while systematically excluding irrelevant material.

- **Step 1 – Broad electronic search**: A conservatively inclusive electronic search was conducted (e.g. Web of Science) using the predefined search string. The purpose of this step was to minimise the risk of omitting relevant reports on engineered gene drive molecular designs, while excluding categories of non-scientific or non-molecular material such as editorials, meeting abstracts, letters, news items, proceedings papers, and retracted publications. The lower temporal boundary was set to 2004, the first year in which "gene drive" was used in the scientific literature to refer to selfish genetic elements. As expected, the initial dataset contained many irrelevant records in which "drive" was used as a verb (e.g., "gene X drives cancer development"), requiring further refinement.
- **Step 2 – Electronic refinement of biomedical false positives**: To remove publications where "gene drive(s)" was captured erroneously due to the verb form "drives," the initial results were filtered using Web of Science citation meso-topic categories. These filters excluded biomedical and clinical research domains where the term "drive" is commonly used in unrelated contexts (e.g., cancer biology, immunology, neurology, stem cell research). This step substantially reduced false positives and enriched the dataset for records potentially relevant to engineered gene drive molecular designs.
- **Step 3 – Manual curation based on title and abstract**: The remaining publications were manually screened to retain only those explicitly describing or analysing the molecular design of engineered gene drives, whether theoretical or empirical. This was implemented by applying two initial questions: (1) Is a General Mechanistic Type (see below) of gene drive identifiable (homing-type, non-homing type); and (2) Is a General Molecular Mechanism of gene drive identifiable (homing, toxin antidote, underdominance, other; see below). Both questions needed to be answered in the affirmative to be included in this analysis. These questions were particularly useful in distinguishing general gene drive modelling studies from theoretical studies exploring specific GDMDs which are included in the final analysis. Regarding General Molecular Mechanism: Technologies currently considered as "gene drives" frequently are based on recapitulating or mimicking 'selfish-genetic' systems found in nature, but not always. Underdominance systems are not based on 'selfish-genetic' behaviour but are considered 'gene drive' because the population-level outcomes resemble those when 'selfish-genetic' elements are used. Other gene propagation/spreading systems are

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being designed/developed that are not based on 'selfish-genetic' behaviour although in some cases have been labelled 'gene drive'. All reports concerning these 'other' drive-like systems were included in the analysis. Published corrections were examined but not included in the final database because they have the same DOI as the original report. All published corrections encountered in this report concerned errors in basic citation data. Engineered systems incorporating components of natural gene drives (e.g., cytoplasmic incompatibility mechanisms recreated through transgenic constructs) were also included. Conversely, naturally occurring symbiont- or microbe-based systems capable of biased inheritance (e.g., natural Wolbachia-driven population replacement) were excluded unless they formed part of an engineered transgene-based system. Genetic biocontrol methods lacking drive properties—i.e., systems that may persist for more than one generation but do not increase in frequency—were also excluded.

This three-step process yielded the final curated set of publications, which was assembled into the searchable database used for all subsequent analyses, assessments, and recommendations associated with AQ2 and AQ3.

Data extraction

The final curated set of publications (fewer than 150 reports) was compiled into a flat, portable, searchable Excel database. Each publication was independently reviewed by consortium team members, and the following categories of data were extracted:

1. Design Status. Reports were classified as either Empirical, if the gene drive was introduced into an organism, or Theoretical, if the gene drive was only described on paper. Some reports described Both.

2. Research Category. The following research categories were defined by ToR1 and applied to reports in this database:

I. Conceptual stage
- a. A proposal for a target organism and a potential application
- b. Modelling studies

II. Proof of concept (genetic level)
- a. Producing a genetic construct
- b. Demonstrating that all or part of the genetic construct works in a model organism
- c. Transforming the target species (not with the gene drive genetic construct)
- d. Transforming the target species with components of the genetic construct to demonstrate they work in species (i.e., Cas-9)

III. Proof of concept (phenotypic level)

IV. Advanced Research (studies intended to develop a GDMI for release into the environment) population studies using captive populations (i.e., cage studies and reflecting realistic environmental conditions) to e.g.:
- a. To improve effectiveness of the gene drive or its component parts in the target organism
- b. To improve effectiveness of a reversal drive
- c. Test phenotypic behaviour
- d. Test unintended effects of gene drive
- e. Test unintended effects of "cargo" associated with a gene drive

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f. Test intended effects of a reversal drive

V. Related studies that are not being considered

a. Theoretical drive designs (molecular mechanisms without a target organism and specific population modification in mind)

3. Organism. While empirical studies were conducted in specific organisms, theoretical designs in some cases were species agnostic and could in principle be applied to any organism.

4. General Mechanistic Type (Drive Type – General). Gene drives fall into two broadly distinguishing categories. Homing type in which the drive element is copied into the same genomic location on a homologous chromosome. Non-Homing types that use any one of several alternative mechanisms to achieve a transmission advantage.

5. General Molecular Mechanism (Drive Type – Specific). Homing results from homology directed repair following a double strand DNA break in a chromosome. Toxin-Antidote refers to gene drive systems that rely on the drive element expressing genes that kill cells that do not contain the element while protecting those that do. Killer-Target systems are like Toxin-Antidote system but have elements whose gene product interacts with polymorphic sites on chromosomes that lack the drive element. Underdominance refers to the genetic situation in which a heterozygote is less fit than both corresponding homozygotes resulting in an unstable equilibrium leading to a population of homozygotes. Other unique approaches to 'driving' alleles into populations are being considered.

6. Homing Target Gene. Homing occurs at specific locations in the genome that are determined by the investigator. For homing-drive designs the target gene(s) was recorded.

7. Drive Design. The molecular components comprising engineered gene drives can be arranged colinearly in a group, in cis, or the components can be split into multiple elements, all of which are required for a functioning gene drive. Drive design in reports including the use of cis and split designs were classified as both.

8. gRNAs. Homing type drives depend on inducing double strand breaks at specific chromosomal locations (target genes) to induce homology directed repair. This is currently done using RNA-guided DNA endonucleases such as Cas9. The number of RNA guide molecules (gRNAs) used in the reported design was recorded. This category was not applicable to non-homing-type gene drives.

9. Mode of Transmission. Most gene drives are being designed for use in sexually reproducing organisms and transmission can be either through only Males, only Females or through Males and Females. These designations are not applicable to organisms that do not reproduce sexually (e.g., viruses, bacteria, yeast, fungi).

10. Homing Cas Driver. Homing type drives depend on inducing double strand breaks at specific chromosomal locations (target genes) to induce homology directed repair. This is often done using RNA-guided DNA endonucleases such as Cas9 whose expression must be regulated. The regulatory sequences used or proposed by the reported design were recorded and if not reported were indicated as not specified. This category was not applicable to non-homing-type gene drives.

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11. Site of Activity. Engineered gene drives might be active in only the Soma, only the Germline or in Soma and Germline. Where appropriate the site of activity was recorded. These designations are not applicable for some organisms (e.g., viruses, bacteria, yeast, fungi).

12. Drive Strength. The percentage of progeny containing the gene drive from a mating in which one parent was heterozygous for the gene drive was recorded. The strength of a drive varies depending on whether it is in a male or female and ranges of activity are shown in GDMDdb, where appropriate. Some studies tested or considered drives with varying activities, and these were recorded in the database as variable. Gene drive activity in viruses, bacteria and yeast could not be captured using metrics associated with sexually reproducing organisms.

13. Sustainability Characteristics. Gene drives can have design features that are intended to ensure their unrestricted sustained activity over time and space and are referred to as self-sustaining. Drives can also be designed that would result in restrictions on the drive's activity in some way and are referred to as self-limiting.

14. Containment Potential. Some drive designs (e.g., high activity, self-sustaining drives) are expected to be difficult to control and contain. Other drives (e.g., self-limiting drives) are inherently more controllable and containable. Containment potential can be unknown and in most cases containment potential is a subjective estimate.

15. Evolutionary Stability. Depending on the drive type and its design and purpose, natural selection may favour inactive drives resulting from cis or trans mutations. For some designs the evolutionary stability was empirically or theoretically investigated and was classified as high, medium or low. In some cases, evolutionary stability was not considered. There is no standard or metric in the research community for classifying or characterising evolutionary stability. Classifying evolutionary stability of a drive can, in many cases, be subjective at this point.

16. Purpose. Gene drives in some cases are being designed with specific end-uses in mind – either to suppress a standing population of organisms, modify a standing population of organisms, or to mitigate or reverse the actions of an initial gene drive. In other cases, the intention is to simply see if gene drive can be designed or created without having a specific end-use in mind. These drives were of a general nature and might be adapted for different uses.

17. Mode of Suppression, Modification, Mitigation. When a use-specific gene drive was described its mode of action was recorded.

18. Scale of Studies. While no engineered gene drive has been field-tested at present, the scale at which they have been tested in the laboratory can vary from limited crosses among relatively few individuals to assess transmission patterns to small and large laboratory cage populations involving hundreds or thousands of individuals, respectively. In instances where a drive design was described in an initial report and larger-scale studies were reported in a subsequent report, both reports were included in the final database.

19. Modelling Included. In some cases, empirical studies also included some mathematical modelling. Whether a case included modelling was recorded.

Evidence synthesis and integration (AQ2.2, AQ2.3)

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To determine which aspects of EFSA's molecular characterisation guidelines require adjustment for application to GDMIs, the consortium considered the requirements in:

- Sections 2.1.1 and 2.1.2 of EFSA (2012) and 4.2.1-4.2.5 of EFSA (2013);
- Section II of Annex IIIA of Directive 2001/18/EC;
- Additional considerations identified by the EFSA GMO Panel in EFSA (2020).

Specific attention was given to:

- Mechanisms and intended outcomes of gene drive releases;
- Interactions among multiple gene drive components;
- Efficiency, stability, and inheritance of the engineered system;
- Monitoring of intended and unintended molecular effects.

Because GDMOs are novel and risk assessors have limited experience evaluating them, the adequacy and sufficiency of existing guidelines were considered in light of the technology's novelty. More explicit guidance for GDMIs is justified to ensure that applicants provide appropriate molecular-level data and that risk assessors can identify data needs effectively.

Initial evidence synthesis for AQ2 was conducted by three persons of the consortium team led by the molecular design specialist. Draft analyses were reviewed by the full consortium team.

For AQ3, the internal working group drafted recommendations on how existing guidelines could be adapted. Recommendations were evaluated using criteria such as completeness, practicality, clarity, flexibility, and generalisability.

3.1.3 ToR3 – Review comparators

Problem formulation

ToR3 objectives focused on identifying and reviewing suitable comparators to support the risk assessment of GDMIs. They involved the examination of which comparators, endpoints and comparative approaches are most appropriate for different risk assessment purposes, an evaluation of the strengths and limitations of the identified comparators, and the provision of recommendations on when the use of single or multiple comparators is justified.

AQs and SQs

The following AQs and SQs were answered to address ToR3:

AQ3.1 asks: Which comparators could be used to support the risk assessment of GDMIs, including the most appropriate endpoints and comparative approaches, and for which risk assessment purposes single or multiple comparators may be applied? This question is addressed through the following SQs:

- SQ3.1.1 – Based on the GDMI applications identified as relevant for the EU and its outermost territories under ToR1, which existing activities are comparable to the proposed use of GDMIs, such as alternative approaches to pest population control?
- SQ3.1.2 – How are comparators used in risk assessment for comparable activities, including human health and ERAs?

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- SQ3.1.3 – For each identified GDMI, which organisms could serve as suitable biological comparators for risk assessment?
- SQ3.1.4 – For each identified GDMI, which activities, processes or methods used for similar purposes could serve as non-biological comparators for risk assessment?

AQ3.2 asks: What are the strengths and weaknesses of the comparators identified under AQ3.1? This question is addressed through the following sub-questions:

- SQ3.2.1 – For each identified comparator or comparator type, what are the strengths of using that comparator for risk assessment?
- SQ3.2.2 – For each identified comparator or comparator type, what are the weaknesses or limitations associated with its use in risk assessment?

AQ3.3 asks: Which single or multiple comparators are recommended as most appropriate for the risk assessment of GDMIs?

Conceptual model

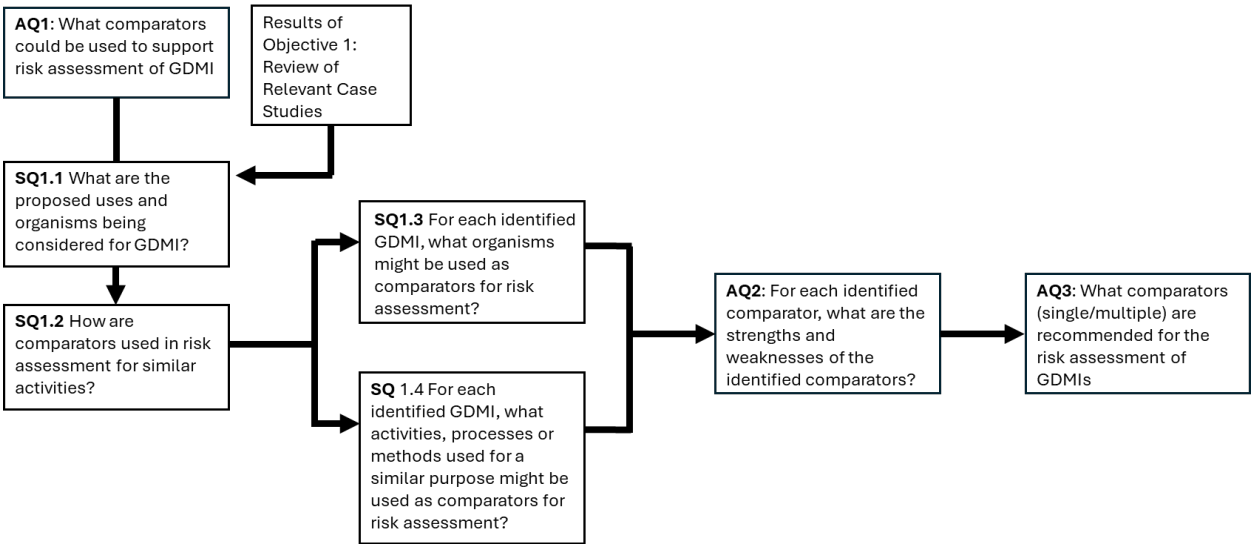


Figure 4: Conceptual model for the term of reference 3 (ToR3: Review comparators)

Methods applied for conducting the assessment

To address ToR3, a structured, qualitative analytical approach was applied that combined review of relevant GDMI applications, comparative analysis of existing risk-assessment practices, and expert judgement (Table 8). The approach involved identifying potential biological and non-biological comparators, examining how comparators are used in comparable human health and ERAs, evaluating their strengths and limitations, and integrating these insights to support recommendations on the appropriate use of single or multiple comparators for GDMI risk assessment.

Table 8: Overview of methods used for answering the questions (AQs) and sub-questions (SQs) of the term of reference 3 (ToR3: Review comparators)

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Assessment question(s)	Data collection	Study selection	Evidence appraisal	Evidence synthesis
AQ3.1; SQ3.1.1-3	Results of literature search and analysis from Objective Analysis of GDMI proposed uses and expert consultation	Compilation of National Reports, Risk Assessment Guidance Documents	Narrative	Qualitative
AQ3.2	Expert consultation	—	Narrative	Qualitative
AQ3.3	Expert consultation	—	Narrative	

Methods for addressing AQ3.1 (comparators for GDMI risk assessment)

Following the identification of GDMIs potentially relevant to Europe, including its OMTs territories, under ToR1, each GDMI was subsequently assessed and categorised according to the organism involved and its intended purpose. The consortium team working on this ToR collectively identified and evaluated key characteristics of each organism, including its status as a pest affecting humans, animals or the environment, whether it is invasive in Europe or elsewhere, its role as a disease vector of relevance to human, animal or environmental health, and other pathways through which the species may cause harm.

The proposed use of each GDMI was then categorised in relation to existing human activities in the environment (Table 9). Likely categories included, but were not limited to, biological control, disease control, pest control and population control. Because the final list of relevant GDMIs could only be established upon completion of ToR1, categorisation was performed after that stage. Each GDMI was assigned to one or more intended use categories (Table 10), reflecting the fact that a single application may serve multiple purposes. For example, a GDMI mosquito designed to reduce mosquito population size would be categorised both as a biological control measure and as a vector or pest control intervention. This inclusive categorisation approach was adopted to ensure that the widest possible range of relevant comparator uses could be explored in subsequent analyses.

Table 9: Categorisation of the gene drive modified insect (GDMI) species (SQ3.1.1)

Organism	Population status in Europe including its outermost territories	Disease vector status	Other potential for causing harm to humans, animals or the environment
Insect Species	Not present; native; introduced; naturalised; invasive	Human disease vector; animal disease vector; plant disease vector	Agricultural pest; aggressively invasive; harmful predator; damaging to plants outside of agriculture; toxic or allergenic to humans or animals

Table 10: Categorisation of gene drive modified insects (GDMIs) by proposed use (SQ3.1.1)

GDMI	Function of the engineered gene drive	Intended use
Insect species	Population suppression; population modification	Pest control; biological control; disease/vector control; environmental remediation; other uses as identified in specific cases

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Methods to identify how comparators are used for similar intended uses to that of the GDMI (SQ3.1.2)

To examine how risk assessment is conducted for processes and methods serving purposes comparable to those proposed for GDMIs, the consortium team identified relevant national regulatory frameworks and guidance documents from the EU and other jurisdictions. These documents address risk assessment for activities aligned with the intended uses of GDMIs. To ensure the analysis was both robust and manageable, consideration was limited to national level documents from OECD countries. The review focused on documents available in English and employed software-assisted text analysis to identify relevant references to the use of comparators, comparative approaches, alternatives, or other forms of comparative assessment.

Methods to identify organisms that might serve as comparators for GDMI risk assessment (SQ3.1.3)

The consortium team reviewed the GDMI species identified under ToR1 as potentially relevant for Europe, including its OMTs, and used the associated categorisations developed for SQ3.1.1 to identify potential organisms that could serve as comparators. These are expected to include the corresponding unmodified insect species but may also encompass organisms with similar ecological roles and distribution in Europe, as well as other organisms that may be introduced for the same intended purpose as the proposed GDMI. Once candidate comparator organisms were identified, the consortium team evaluated and proposed how each could be used to inform GDMI risk assessment.

Methods to identify processes or methods that might serve as comparators for GDMI risk assessment (SQ3.1.4)

Building on the categorisation of intended GDMI uses developed under SQ3.1.1 and informed by an analysis of how national regulatory frameworks employ comparators when assessing activities with the same or similar purposes, the consortium team identified additional activities, processes and methods that could serve as comparators for GDMI risk assessment. For example, where pest control was identified as an intended use for a GDMI relevant to Europe or its OMTs, the consortium team examined how comparators, including alternative processes and methods, are used in risk assessments of pest control strategies, as described under the methods for SQ3.1.2. Based on this analysis, the consortium team formulated recommendations on how such comparative approaches could be applied to GDMIs and identified which other activities, processes or methods would be appropriate comparators for their risk assessment.

Methods for addressing AQ3.2 (strengths and weaknesses of comparators)

The consortium team considered the comparators identified under SQ3.1.1–SQ3.1.4 in the context of EFSA guidance on the risk assessment of GM animals, together with the findings from the review of national regulatory frameworks addressing activities, processes, and methods with the same intended uses as the GDMIs identified under ToR1. Through expert consultation (see acknowledgments), including structured discussions with the full consortium team, it was evaluated how each comparator could be applied in risk assessment, while the associated strengths and limitations were identified. This evaluation considered the extent to

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which each comparator is applicable across different GDMI scenarios, as well as the level of practical experience available within the EU and internationally with the use of each comparator or comparative approach.

Methods for addressing AQ3.3 (comparator use recommendations)

Based on the work described above for addressing AQ3.2, the consortium team developed recommendations on the use of the identified comparators for GDMI risk assessment. These recommendations were informed by the practical utility of each comparator, its relevance for contextualising GDMI-related risks, its applicability across different GDMI scenarios, and its capacity to support and inform the risk-assessment process. The recommendations specify when and how individual comparators, or combinations of comparators, may be most appropriately used.

3.1.4 ToR4 – Review factors affecting engineered gene drive dynamics

Problem formulation

ToR4 addresses the factors that influence the behaviour of engineered gene drives after environmental release, with particular emphasis on how molecular, biological and ecological processes affect their spread, persistence and potential invasiveness. It also considers the role of existing modelling approaches in analysing gene drive dynamics and evaluates their suitability to support ERA and PMEM of GDMIs. These aims were operationalised through four AQs (AQ4.1–AQ4.4) and their corresponding SQs (SQ4.1.1–SQ4.4.4).

AQs and SQs

To address ToR4, the following AQs and SQs were formulated.

AQ4.1 asks: How do molecular, biological and ecological factors influence the spread, persistence and potential invasiveness of engineered gene drives in target and non-target populations or species? To address this question, a review of the relevant gene drive modelling literature was conducted, supported by predefined inclusion and exclusion criteria and targeted search terms. This question is addressed through the following SQs:

- SQ4.1.1 – Which molecular designs, as identified under ToR2, are currently under development and represented in gene drive modelling studies?
- SQ4.1.2 – Which biological factors influence the spread, persistence, and invasiveness of engineered gene drives?
- SQ4.1.3 – How should the boundaries between target organisms and non-target organisms, populations or species be defined for the purposes of modelling gene drive dynamics?
- SQ4.1.4 – Which approaches are appropriate for reviewing and analysing gene drive models in a risk assessment context?

AQ4.2 asks: *Which models are currently used to investigate engineered gene drive dynamics and what their strengths and weaknesses?* To address this question, a database of dynamic models identified through the literature review conducted under AQ4.1 was compiled. Given that few, if any, gene drive models have been developed explicitly for ERA, each model was reviewed with respect to its assumptions, computational structure, parameterisation, and capacity to inform risk assessment. This question is addressed through the following SQs:

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- SQ4.2.1 – What are the expected performance, limitations and variability associated with each model?
- SQ4.2.2 – What do the models predict regarding gene drive frequency, persistence, spread and impacts on target populations?
- SQ4.2.3 – What do the models predict regarding release strategies and field conditions required to achieve intended outcomes?
- SQ4.2.4 – What do the models predict regarding interactions with non-target populations or species?
- SQ4.2.5 – What do the models predict regarding resistance evolution, reversal or recall potential?

AQ4.3 asks: *What role could existing models play in supporting ERA and PMEM of GDMIs*? Each model identified under AQ4.2 was evaluated in terms of its usefulness for ERA and PMEM. This question is addressed through the following SQs:

- SQ4.3.1 – What challenges exist in obtaining data to parameterise the models, and how should uncertainty be addressed?
- SQ4.3.2 – What challenges exist in validating gene drive models, particularly prior to environmental release?
- SQ4.3.3 – Which tools and approaches are appropriate for analysing, interpreting, and presenting model outputs in a risk assessment context?

AQ4.4 asks: Which models are recommended for use in GDMI risk assessment and how such models should be further developed to support ERA and PMEM? This question is addressed through the following SQs:

- SQ4.4.1 – How useful are existing modelling strategies for supporting ERA of GDMIs?
- SQ4.4.2 – How useful are different modelling approaches for supporting PMEM of GDMIs?
- SQ4.4.3 – What gaps currently exist in the use of modelling for ERA and PMEM of GDMIs?
- SQ4.4.4 – What options and solutions could address these gaps and improve the use of modelling in ERA and PMEM for GDMIs?

Conceptual model

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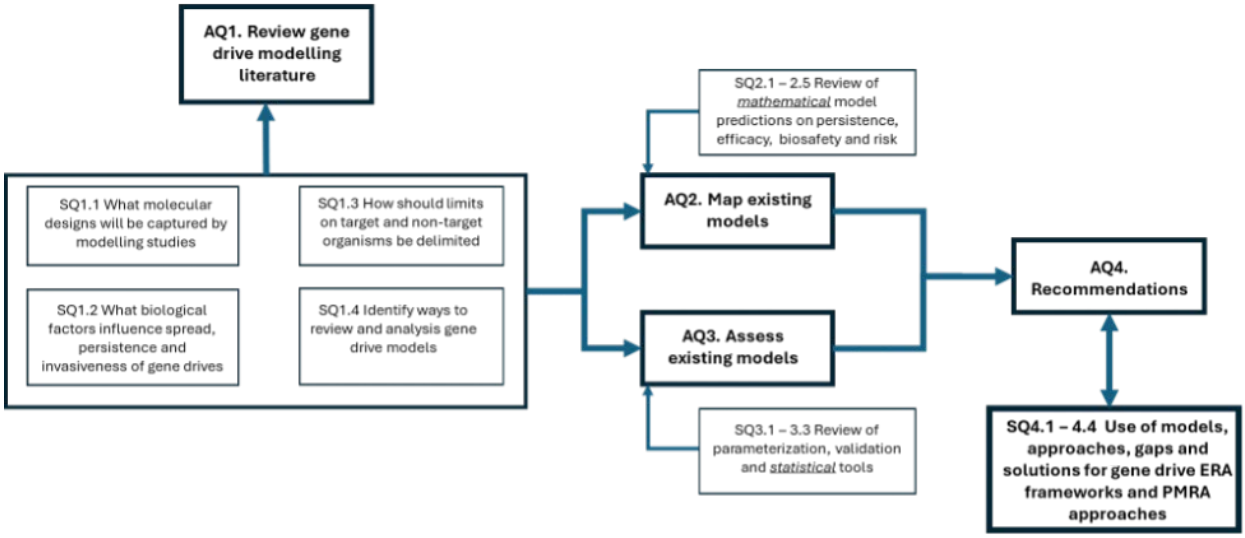


Figure 5: Conceptual model for the term of reference 4 (ToR4: Review factors affecting engineered gene drive dynamics)

Methods applied for conducting the assessment

The assessment was based on evidence retrieved from extensive scientific literature searches, relevant databases, national reports, expert consultations, and expert judgement (Table 11).

Table 11: Overview of methods used for answering assessment questions (AQs) and sub-questions (SQs) of the term of reference 4 (ToR4: Review factors affecting engineered gene drive dynamics)

Assessment question(s)	Data collection	Study selection	Evidence appraisal	Evidence synthesis
AQ4.1; SQ4.1-4	Extensive literature search	Structured study selection process based on pre-defined eligibility criteria	Structured, using critical appraisal tool(s)	Qualitative
AQ4.2; SQ4.2.1-5	Extensive literature search	Structured study selection process based on pre-defined eligibility criteria	Structured, using critical appraisal tool(s)	Qualitative
AQ4.3; SQ4.3.1-3	Extensive literature search	Structured study selection process based on pre-defined eligibility criteria	Structured, using critical appraisal tool(s)	Qualitative
AQ4.4; SQ4.4.1-4	Extensive literature search	Structured study selection process based on pre-defined eligibility criteria	Structured, using critical appraisal tool(s)	Qualitative

Evidence needs

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A comprehensive scientific literature review was conducted to capture the range of mathematical models that have been applied to engineered and natural gene drive systems, and to assess their relevance to the ERA and PMEM of GDMIs, including their use in assessing efficacy and in evaluating hypothetical gene drive designs.

Eligibility criteria to establish the relevance of evidence

Eligibility criteria were applied to determine the relevance of evidence for inclusion in the review (Tables 12–14). These criteria were used for screening literature retrieved through the search strategy. The criteria were tested on a subset of publications, and refined as necessary if any ambiguities or risks of misinterpretation were identified.

Table 12: Eligibility criteria for study characteristics

	Inclusion	Exclusion
Gene drives	Genetically modified; natural gene drives; selfish genetic elements	Specifics application to gene drives not explicit
Model	Model framework (e.g., deterministic, stochastic, statistically, agent-based, network) identified to allow determination of approach/application to GDMIs; use of models in natural gene drives	Model framework not explicitly defined
Use	Environmental risk assessment; post-market environmental monitoring; efficacy; hypothetical gene drive designs	Taxonomic specifics; application to the use of gene drive technologies in integrated pest management

Table 13: Eligibility criteria for study characteristics

Inclusion	Exclusion
Full text retrievable	Full text unavailable or not retrievable
Detailed information on gene drive, model and application available	No information on the mathematical model and gene drive system

Table 14: Eligibility criteria for record characteristics

	Inclusion	Exclusion
Temporal scope	Publications dated 1 January 2000 – present	Publications prior 1 January 2000
Language	English	Other languages
Type of publication	Peer-reviewed literature	Book chapters; editorials; meeting abstracts; letters; news items; proceedings papers; retracted publications

Evidence sources

The main evidence sources used to identify the necessary evidence for the review were electronic bibliographic databases. Databases consulted:

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- Web of Science;
- Scopus;
- Google Scholar.

The databases selected were identified in line with the defined scope of the review.

Search strategy

The search strategy for identifying evidence from the scientific literature has been designed to retrieve as many relevant studies as possible to address the AQs and SQs reported in Table 11.

The following search string was used (Table 15).

Table 15: Search string

Web of Science; Scopus; Google Scholar	(engineered OR synthetic OR modified OR revers* OR daisy* OR "meiotic drive" OR "homing drive" OR natural) AND "gene drive" AND (modeling OR modelling OR math*)

Search strategy appraisal

Relevant review publications were used to evaluate the completeness of the search strategy. All relevant references contained in these reviews were checked for presence or absence in the search results, allowing refinement of the strategy where needed. On this basis, the search string was considered effective in capturing the intended evidence base.

Publication selection process

The search results were compiled in an Excel file for screening. Initial screening (Selection A) was performed at the level of title, abstract and keywords, with relevance assessed against the eligibility criteria described in Tables 12–14. Screening was carried out by the consortium team, and full-text publications were retrieved in PDF format and evaluated according to Selection A and Selection B criteria.

Any differences in screening outcomes, whether concerning inclusion, exclusion, or interpretation of the criteria, were discussed. If disagreements could not be resolved, additional members of the consortium team were consulted.

Following compliance with copyright requirements, the final set of selected publications was uploaded into an accessible database for use. A complete list of included publications, with DOIs where available, was also compiled and shared.

Data extraction

Each publication was independently reviewed by consortium team members, and the following categories of data were extracted:

- Model type: deterministic, stochastic, statistical, agent-based, network;

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- Gene drive type: population suppression, population eradication, population replacement, other;
- Predicted target organism application: disease-transmitting insect, invasive insect, agricultural pest insect, other;
- Predicted non-target organism range: competitive interactions, trophic interactions, other;
- Type of model focus: ecological, molecular, biological, other;
- Predictions reported: contained laboratory studies, confined screenhouse studies, confined field studies, unconfined settings;
- Intended temporal scale: self-sustaining, self-limiting/temporal suppression;
- Intended spatial scale: localised, non-localised;
- Threshold dynamics: low-threshold, high-threshold, local suppression;
- Intended receiving environments: (as identified in the publication);
- ERA relevance: problem formulation, pathways to harm, ERA, receiving environments, protection goals, PMEM.

Evidence synthesis

Evidence synthesis and integration followed a semi-quantitative approach. The extracted data from all included publications formed the basis for answering AQ4.1–AQ4.3 and their associated SQs. Evidence was synthesised systematically against each SQ, and the findings are presented and explained narratively in the report. Uncertainties arising from data extraction, study selection, and interpretation of outcomes are also described narratively.

Recommendations under AQ4.4 were derived from a synoptic evaluation that integrates the mapping of existing models (AQ4.2) with the assessment of their usefulness for ERA and PMEM (AQ4.3), thereby identifying how mathematical models can best support gene drive ERA and PMEM.

3.1.5 ToR5 – Review monitoring approaches

Problem formulation

ToR5 addresses the monitoring of insect species with the potential to be GDMIs and identified under ToR1 as relevant for the EU, including its OMTs. This task focuses on determining the objectives of such monitoring and evaluating whether existing or proposed monitoring approaches are adequate to achieve those objectives in the context of GDMIs.

AQs and SQs

To address ToR5, the following AQs and SQs were formulated.

AQ5.1 asks: *What monitoring objectives are relevant* for *GDMI PMEM?* This question is addressed through the following SQs:

- SQ5.1.1 – What are the different objectives for which insect monitoring is conducted?
- SQ5.1.2 – Considering the GDMI examples most relevant for the EU (including its OMTs), which environmental protection objectives would PMEM approaches support?

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AQ5.2 asks: Which monitoring approaches would achieve the objectives identified under AQ5.1 for the GDMIs identified under ToR1? This question is addressed through the following SQ:

- SQ5.2.1 – What are the strengths and weaknesses of these approaches that determine their ability to meet the stated objectives?

AQ5.3 asks: Which monitoring and assessment approaches are recommended for use in the GDMI PMEM?

Conceptual model

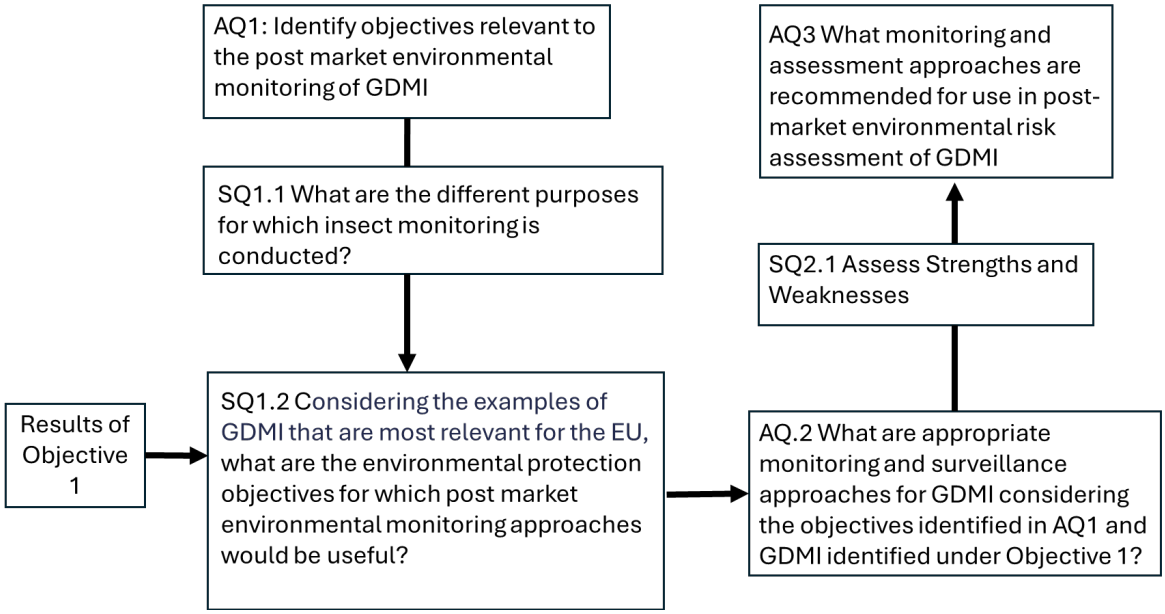


Figure 6: Conceptual model for the term of reference 5 (ToR5: Review monitoring approaches)

3.1.5.2 Methods applied for conducting the assessment

To answer ToR5, a structured, qualitative approach was applied that combined a review of relevant GDMI applications, a literature review, a comparative analysis of existing insect monitoring practices and expert judgement (Table 16). The approach involved identifying the objectives of insect monitoring, examining how these objectives are met, evaluating their strengths and weaknesses, and integrating these insights to support recommendations on the appropriate use of monitoring approaches for GDMIs relevant for the EU including its OMTs (Table 16).

Table 16: Overview of methods foreseen for answering assessment questions (AQs) and sub-questions (SQs) of the term of reference 5 (ToR5: Review monitoring approaches)

Assessment questions and subquestions	Methods used for answering the questions
AQ5.1 and SQ5.1.1-2	• Scientific literature review • Analysis of existing insect monitoring approaches • Expert deliberations

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Assessment questions and subquestions	Methods used for answering the questions
AQ5.2 and SQ5.2.1	• Expert deliberations
AQ5.3	• Expert deliberations

Methods for addressing AQ5.1 (monitoring objectives relevant for PMEM of GDMIs)

Building on the EU framework of PMEM of GMOs, and further detailed by EFSA, monitoring objectives for GDMIs were identified and compared to those for existing insect control methods and to those mentioned in guidance documents from other jurisdictions and advisory organisations (Table 17). Based on this, monitoring objectives for GDMIs relevant for the EU and its OMTs were identified.

Table 17: Categorisation of monitoring objectives for gene drive modified insects (GDMIs)

Insect monitoring objectives	Monitoring in EU	Experience with other insect control technologies	International guidance for monitoring of insects
Verification of intended effects; detection of unanticipated outcomes; support for adaptive management; reduction of residual uncertainty	Genetically modified organisms (GMO), genetically modified insects (GMI), gene drive modified insects (GDMI)	Classical biological control (CBC), augmentative biological control (ABC), sterile insect technique (SIT), insects carrying a dominant lethal gene ((fs)RIDL), *Wolbachia*-mediated incompatible insect technique (IIT) and pathogen interference (PI)	World Health Organization (WHO), North American Plant Protection Organization (NAPPO), Secretariat of the Convention on Biological Diversity (CBD), New Zealand Environmental Protection Authority (NZ EPA)

Methods for addressing AQ5.2 (monitoring approaches that would achieve the objectives under AQ5.1)

To determine which approaches would achieve the objectives identified under AQ5.1, the described monitoring strategies were examined for GDMIs relevant for the EU and its OMTs, taking into account the relevant protection goals for each of the insect species. Strengths and weaknesses of the main approaches were identified (Table 18).

Table 18: Categorisation of monitoring approaches that would achieve objectives under AQ5.1 and their strengths and weaknesses

Monitoring approaches	Strengths and weaknesses (criteria)
Case-specific monitoring (CSM); general surveillance (GS)	Hypothesis-driven; targeted to critical steps in pathways to harm; fit-for-purpose; flexible and adaptable over time; potential to link adverse effect to GDMI; early warning system; cost-effectiveness

Methods for addressing AQ5.3 (monitoring and assessment approaches recommended for use in PMEM of GDMIs)

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Based on the criteria mentioned under AQ5.2, the most relevant monitoring approach was selected. For the GDMIs relevant for the EU and its OMTs, it is described what should be monitored, how, where and for how long. Where relevant, more specific monitoring and assessment approaches were exemplified based on, e.g., ecology, receiving environment and purpose of the gene drive modification (population suppression or modification).

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4 Assessment and results

In the sections that follow, the results are presented separately for each ToR. For every ToR, the corresponding AQs and SQs are addressed in a structured manner. This organisation ensures that the evidence, analyses, and conclusions are transparently aligned with the specific questions defined under each ToR, thereby facilitating clarity, traceability, and consistency throughout the assessment.

4.1 ToR2 – Review of molecular designs

4.1.1 AQ2.1 – What is the current state of the art regarding the molecular design and function of engineered gene drives?

4.1.1.1 SQ2.1.1 – Which scientific publications have described the designs of engineered gene drives?

Across the period analysed (2003–2024), a total of 142 primary research publications described and/or experimentally tested specific gene drive molecular designs (GDMDs). Nearly ninety percent (88.7%) of these publications were published during the last decade (2014–2024), indicating a marked acceleration in research activity (Figure 7). This increase coincides with the emergence of RNA guided DNA endonuclease systems for genome engineering in 2012, which substantially expanded the technical feasibility of engineered gene drive systems and catalysed both theoretical and empirical advances in the field.

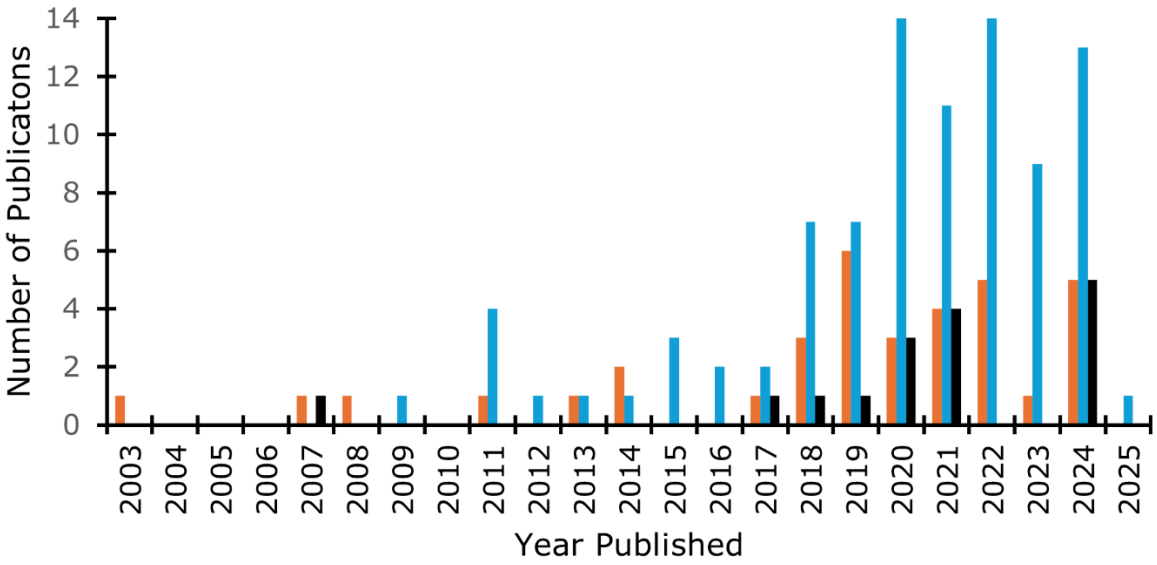


Figure 7: Time series of gene drive molecular design (GDMD) publications.

Theoretical (red): Publications proposing specific molecular designs or drive mechanisms without assembling or experimentally testing them. *Empirical (blue)*: Publications involving the assembly and laboratory testing of a molecular design. *Both (black):* Publications that combine both theoretical design elements with empirical testing

4.1.1.2 SQ2.1.2 –What molecular characteristics (form and function) of engineered gene drives have been reported in relation to intended use, efficacy, target and construct

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gene(s), molecular mechanism, controllability, susceptibility to resistance evolution, and intended or unintended fitness effects?

Species represented

Over the past 25 years, GDMDs were described and/or implemented in 17 species. Although insects account for nearly 60% of all species represented, gene drive designs have also been developed and evaluated in plants, fungi, mammals, yeasts, bacteria, and viruses (Figure 8). The fruit fly, *Drosophila melanogaster,* has been the main experimental system, featuring in approximately 40% of all molecular design publications and serving as a tractable platform for mechanistic studies and proof-of-concept testing.

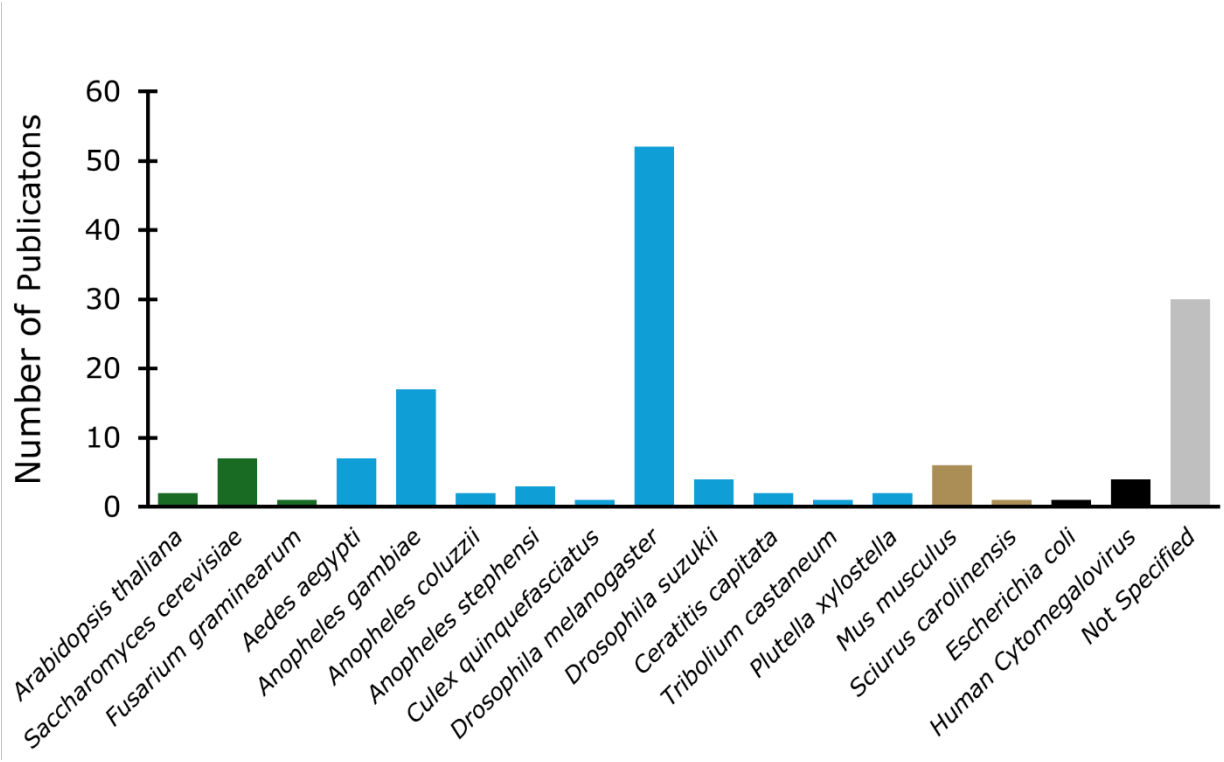


Figure 8: Species used in gene drive molecular design publications between 2003 and 2024.

Most theoretical studies addressing specific design concepts did not focus on individual species and are classified as Not Specified. Colours: Green = plants/fungi; Blue = insects; Brown = mammals; Black = bacteria/viruses.

Types of engineered gene drives being developed

Natural gene drive systems operate through a diversity of mechanisms, many of which have inspired engineered gene drives (Hurst and Werren, 2001). Among these, homing-type engineered gene drives—modelled after homing endonucleases (meganucleases) found in certain archaea, bacteria, fungi, algae and protozoa (Belfort and Roberts, 1997; Chevalier and Stoddard, 2001)—have been by far the most commonly studied. During the study period, 74% of all GDMD publications concerned homing-type systems (Figure 9).

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The remaining publications described a more diverse set of non-homing designs, inspired by natural systems that distort inheritance through mechanisms other than DNA-break-induced homing. In this report, these include systems fitting toxin–antidote, killer–target (Burga et al., 2020), and underdominance paradigms (Curtis, 1968) (Figure 9). In parallel, applied geneticists are also developing gene drive-like systems that rapidly shift allele frequencies in populations without fully recapitulating the behaviour of selfish genetic elements or classical gene drives (Rasgon, 2009; Auradkar et al., 2024; Johnson et al., 2024).

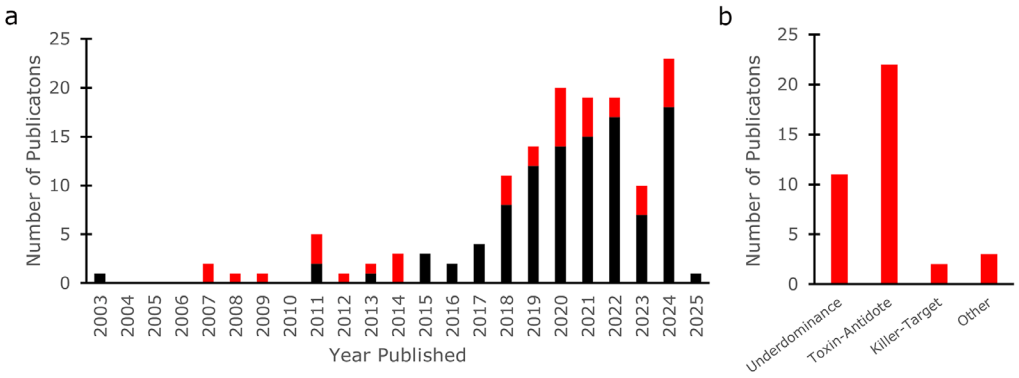


Figure 9: Gene drive molecular design publications (GDMD) between 2003 and 2024.

a) Frequency of gene drive molecular design publications by type and year. Homing-type (black), Non-homing-type (red). b) Non-homing-type gene drive publications between 2003 and 2024

Intended applications

The design, assembly and testing of engineered gene drives have largely been motivated by the goal of modifying or suppressing organisms that pose risks to agriculture, public health, or the environment. While a subset of GDMDs was designed specifically for population suppression (20%) or population modification (14%), the majority (58%) consisted of general-purpose designs aimed at preferentially increasing allele frequencies and potentially applicable to either outcome. By contrast, only a small minority of GDMD efforts (8%) focused specifically on mitigating or reversing the effects of a previously released GDMO (Figure 10)

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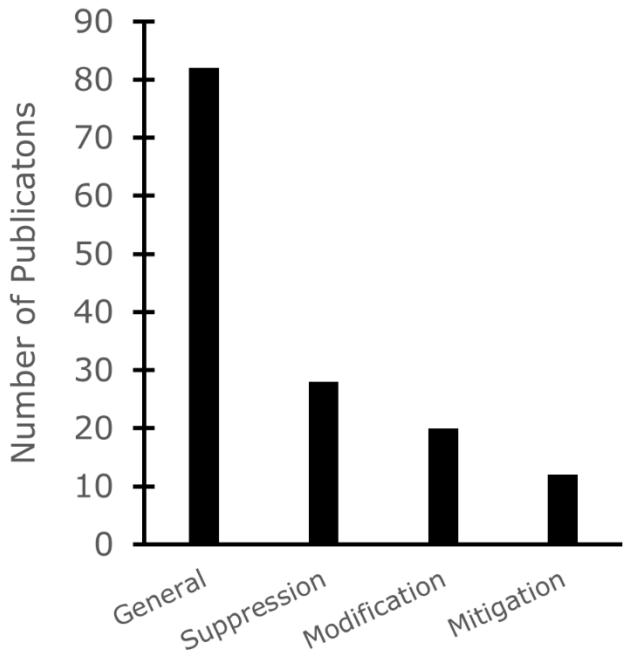


Figure 10: Intended applications of gene drive modified designs in publications between 2003 and 2024

Scale of empirical studies

To date (2025), there have been no reported field releases of a GDMO. All empirical testing and evaluation of GDMDs have so far taken place exclusively under laboratory conditions. However, the scale at which GDMIs have been evaluated has varied substantially (Figure 11). Most studies have involved controlled crosses with relatively small numbers of individuals, typically in the tens, and can be described as breeding experiments. Small-cage studies, usually involving populations in the hundreds, have also been reported and generally do not require specialised infrastructure. By contrast, large-cage studies, involving populations on the order of 10^3–10^4 individuals, require specialised room-sized enclosures capable of providing appropriate environmental conditions for the species concerned. To date, only a few GDMDs have been evaluated at this larger scale (Hammond et al., 2021; D'Amato et al., 2024).

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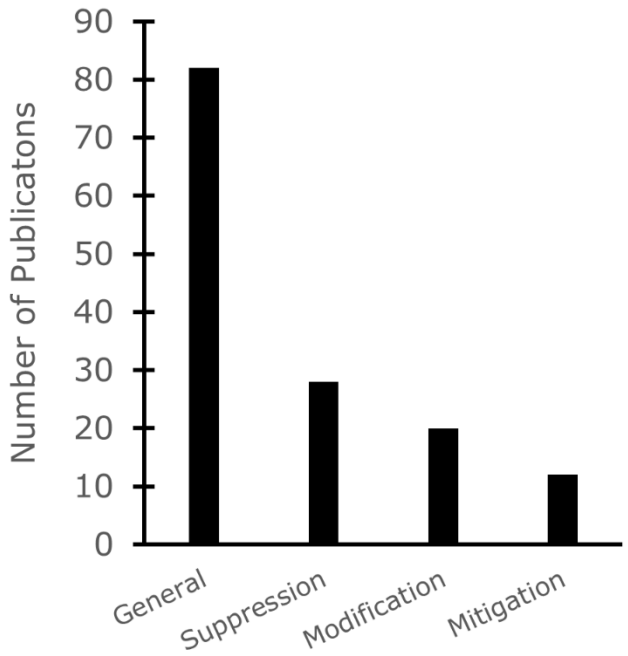


Figure 11: Number of studies reported in gene drive modified design publications between 2003 and 2024

Performance of engineered gene drives – Biological factors

DNA repair: Homing-type engineered gene drives rely fundamentally on the induction of site-specific double-strand breaks (DSBs) in chromosomal DNA, followed by repair through homology-directed repair (HDR). Cellular responses to DSBs vary substantially across species, as well as among different cell types and developmental stages (Shrivastav et al., 2008; Xue and Greene, 2021). Some of the variation in performance currently observed among insect homing drives may therefore reflect differences in how DNA repair pathways are regulated across taxa (Figure 12). At present, however, little research has directly addressed how DNA repair activities differ among insect species, developmental stages, or cell types.

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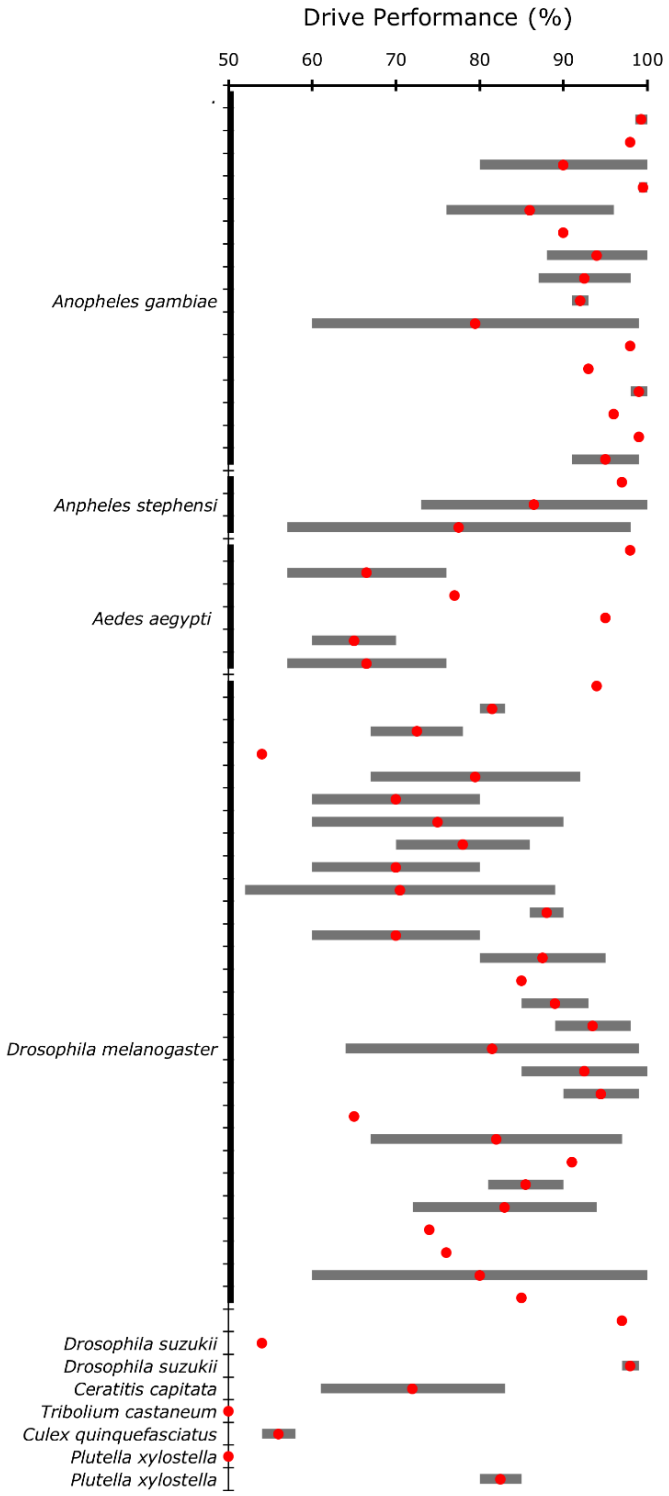


Figure 12: Empirical performance of homing-type engineered gene drives in insects.

Results from individual studies are shown. Ranges are shown as grey bars. Red points represent either the single reported value or the midpoint of the reported range.

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Resistance evolution: The DNA endonuclease expressed by a homing-type engineered gene drive introduces site-specific DSB that is repaired primarily through either HDR or non-homologous end joining (NHEJ). NHEJ can reduce drive performance because it often generates sequence variants at the target locus. In RNA-guided gene drives, some of these variants are no longer recognised or cleaved by the endonuclease (Hammond et al., 2016, 2017). These so-called resistance alleles may either disrupt the gene function or preserve its function.

Resistance alleles that disrupt target gene function can reduce the efficiency of the drive but still contribute to suppression. By contrast, resistance alleles that retain gene function can be especially problematic for suppression drives, because strong selection may favour their rapid spread, potentially leading to a gene drive-resistant population (Hammond et al., 2017; Champer et al., 2018).

Gene drive evolution over generations: Although selection for target-site resistance in Cas9-based suppression drives has been well documented, much less attention has been given to whether drive efficiency itself changes over successive generations. Hammond et al. (2016, 2017) examined this question in a 25-generation small-cage study of a Cas9-based homing drive in *Anopheles gambiae*. Homing efficiency was assessed at generations 2, 3, 4, and 5, and varied substantially (69–98%) between generations, but without evidence of a consistent decline across the monitored period (Hammond et al., 2016).

Performance of engineered gene drives – Technical factors

Promoters: Differences in the apparent strength of homing-type gene drives between *Anopheles* mosquitoes and other insects may partly reflect technical design choices, particularly the timing and level of expression of the gene drive-encoded DNA endonuclease.

Current homing-type designs aim to restrict endonuclease expression to the germ line, ideally during prophase of meiosis I, when homologous chromosomes are paired and HDR is the dominant repair pathway.

- Expression too early (before chromosome pairing) favours NHEJ, which introduces indels at the target site and reduces drive efficiency.
- Expression too late (after meiosis I) cannot support HDR because homologous chromosomes are no longer aligned, making NHEJ the default repair route.
- Somatic expression is also undesirable, as somatic DNA repair is strongly biased towards NHEJ, generating disruptive mutations and potentially imposing fitness costs.
- Maternal carry-over of endonuclease activity into early embryos can be mutagenic and may create resistance alleles in the developing germ line before the drive is active.

For these reasons, promoter choice is one of the most consequential molecular design decisions for homing-type engineered gene drives. In *Anopheles* mosquitoes, the *nos*, *vasa*, and *zpg* promoters have supported strong drive activity (>90% transmission from heterozygotes), although *nos* and *vasa* are not perfectly germline restricted. In *Aedes aegypti*, however, homing drives using *nos* or *zpg* have achieved <75% transmission. Of thirteen promoters tested in *Aedes*, only six improved transmission, and none matched the efficiencies observed in *Anopheles* (Anderson et al., 2023; Ang et al., 2025). Identifying and engineering optimal cis-regulatory sequences therefore remains a major challenge for robust gene drive development across insect species.

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When RNA-guided endonucleases are used, the timing and tissue specificity of gRNA expression also matter. Germline expression is essential, although precise timing relative to meiosis appears less critical than for endonuclease expression. To date, five Pol III promoters have shown utility for driving gRNA expression in insect gene drives, albeit with differing expression patterns (Ang et al., 2025; Gonzalez et al., 2025).

Transgene positioning effects: In addition to promoter choice, the genomic location of the drive construct can strongly influence performance. Transgene expression is subject to position effects, including local enhancer activity, chromatin context, and other genomic features (Boldyreva et al., 2022). Consequently, the performance of homing-type drives can vary unpredictably depending on where the endonuclease cassette is inserted (Anderson et al., 2023).

The degree of freedom available for selecting the insertion site depends strongly on the drive architecture. For example, if the objective is to minimise the evolution of functional resistance alleles, homing drives are often targeted to highly conserved regions within genes required for female fertility or sex determination (Hammond et al., 2016, 2017; Kyrou et al., 2018). In such cases, the insertion site is constrained by the biology of the target gene, leaving little room to optimise expression by relocating the construct.

By contrast, split-drive systems, in which the endonuclease and gRNA are encoded at separate loci, offer greater flexibility. Because only the gRNA must be placed within the target gene, the endonuclease transgene can be located in a region more conducive to strong and appropriately timed expression.

Unlike in mice, insects do not yet have well-characterised genomic safe harbour loci that reliably support stable transgene expression. As a result, identifying suitable insertion sites remains largely empirical (Zambrowicz et al., 1997).

Codon optimisation: Species differ in their preferred codon usage, and these differences can influence heterologous gene expression (Parvathy et al., 2022). Despite this, codon optimisation has not been widely prioritised in insect gene drive development. Where it has been implemented, its effect on drive performance has not always been demonstrated.

Codon-optimised Cas9 transgenes have occasionally been used in RNA-guided homing drives, including variants optimised for humans, insects generally, *Drosophila melanogaster*, and *Anopheles stephensi* (e.g. Gantz and Bier, 2015; Hammond et al., 2016, 2021a,b; Champer et al., 2017; Kyrou et al., 2018; Adolfi et al., 2020). Codon optimisation has also been applied to other construct components, such as effector genes (Taxiarchi et al., 2021; Carballar-Lejarazú et al., 2022; Green et al., 2023).

Its importance may be species dependent. For example, *Anopheles gambiae* homing drives can achieve very high efficiencies (>95%) even with human codon-biased Cas9 variants (Hammond et al., 2016, 2021a,b; Kyrou et al., 2018; Waters et al., 2018; Ellis et al., 2022). Nevertheless, codon optimisation remains a relatively simple and adjustable variable that may become increasingly important as evidence accumulates that Cas9 expression levels can strongly affect gene drive behaviour (Reid et al., 2022; Du et al., 2024; Xu et al., 2025).

Modulating DNA repair: There is broad interest in maximising HDR after Cas-mediated cleavage (Chu et al., 2015). One possible strategy is to reduce or eliminate the expression of

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genes required for NHEJ, such as *Ku70* and *Ligase IV*. Although genetics-based strategies of this kind have been proposed for insect gene drives, they have not yet been implemented in practice (Finney et al., 2022).

Off-target effects: Cas9 may cleave unintended genomic sites, generating DSBs that can lead to undesirable "off-target" effects through NHEJ or larger rearrangements (Zhang et al., 2015). These effects are relevant both to risk assessment (e.g. creation of harmful mutations) and to drive performance (e.g. fitness costs that alter allele frequency dynamics).

One major source of off-target cleavage is the tolerance of Cas9 for up to three mismatches between the sgRNA and the genomic target. These sgRNA-dependent off-targets can be reduced through careful in silico target selection, although prediction tools cannot fully account for chromatin state and therefore require experimental validation (Guo et al., 2023). Cas9 can also produce sgRNA-independent off-target effects, which cannot be predicted computationally and must be assessed using cell-free, cell-based, or in vivo assays (Guo et al., 2023).

Several strategies exist to reduce off-target activity, including the use of high-fidelity SpCas9 variants such as SpCas9-HF, eSpCas9(1.1), and HypaCas9 (Kleinstiver et al., 2016; Slaymaker et al., 2016; Chen et al., 2017). sgRNA design can also be modified to improve specificity, for example by truncating the 5′ end (Fu et al., 2014). However, chemically modified sgRNAs are generally impractical for insect gene drive applications because these systems typically rely on transgene-based, rather than ribonucleoprotein-based, delivery.

Because most insect gene drive studies have focused on proof-of-concept, off-target activity has rarely been characterised in depth. One notable exception is Garrood et al. (2021), who investigated off-target effects in four *Anopheles gambiae* strains carrying Cas9-based suppression drives. Off-target mutations were detected at frequencies of ≤1.42% in three strains and were highest in a strain with leaky somatic Cas9 expression, suggesting that somatic cleavage contributes measurably to off-target activity. By contrast, no off-target activity was detected in a fourth strain carrying a *doublesex*-targeting drive with strictly germline-restricted expression.

From a performance perspective, Langmüller et al. (2022) showed that a *Drosophila melanogaster* construct expressing both Cas9 and an sgRNA declined steadily in frequency over 20 generations, whereas control constructs expressing only Cas9 or a fluorescent marker remained stable. Although no physical evidence of cleavage at predicted off-target sites was detected, modelling suggested that Cas9/sgRNA-related off-target effects were the most plausible explanation for the observed fitness costs (Liu et al., 2019; Langmüller et al., 2022). Importantly, pairing the same sgRNA with SpCas9-HF eliminated these detectable fitness costs while preserving drive performance, suggesting that high-fidelity nucleases should be considered standard design options in future gene drive development.

Resistance management: Many homing-type designs, especially those intended for suppression, incorporate molecular features intended to reduce or delay resistance. One widely used strategy is to target highly conserved regions within essential genes. Because such sequences tolerate little natural variation, mutations are more likely to be deleterious and therefore less likely to spread (Kyrou et al., 2018; Morianou et al., 2024).

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A second major strategy is multiplexing, in which multiple gRNAs target adjacent sequences. This redundancy reduces the probability that the loss of one target site will fully prevent cleavage (Champer et al., 2018, 2020; Edgington et al., 2020a; Lewis et al., 2021; Green et al., 2023). However, multiplexing introduces design challenges, particularly when repeated Pol III promoters increase the risk of recombination. Using different promoters for distinct gRNAs may reduce this problem (Green et al., 2023).

The choice of endonuclease may also affect resistance evolution. Most current homing drives rely on Streptococcus pyogenes Cas9, but Cas12a has theoretical advantages because it cuts outside the gRNA recognition sequence, making it less likely that NHEJ-induced changes will generate resistance alleles (Lewis et al., 2021; Juste et al., 2023). Despite this promise, Cas12a and other variants remain little explored in insect gene drives.

Fitness costs: Whether a gene drive increases in frequency depends partly on whether its transmission advantage outweighs any associated fitness costs. In suppression drives, some fitness costs are intentional—for example, female infertility in homozygotes when the drive targets a haplosufficient fertility gene. However, additional unintended fitness costs, particularly in heterozygotes, can undermine performance and should be minimised.

These costs may arise from incomplete dominance of the target mutation, expression of linked transgenes, somatic effects, or off-target activity. In *Drosophila melanogaster*, Cas9 expression alone appears to impose limited direct costs, whereas combined Cas9 + sgRNA expression has been associated with moderate fitness reductions, likely due to off-target cleavage (Langmüller et al., 2022). This underlines the importance of designing systems that minimise unintended burdens on carriers.

Non-homing gene drives

Unlike homing-type drives, which bias inheritance by converting wild-type alleles into drive-bearing alleles ("drive by addition"), non-homing gene drives typically gain a transmission advantage by selectively eliminating cells, gametes, embryos, or individuals that do not inherit the drive ("drive by subtraction"). These systems are often described as toxin–antidote drives because the driving locus encodes both a toxin and its corresponding antidote, typically expressed at different stages relative to meiosis (Burga et al., 2020).

In many natural systems, the toxin is expressed before meiosis I such that all meiotic products are exposed to it, while the antidote is expressed only after meiosis I. As a consequence, only cells carrying the driving chromosome survive. Cells or individuals that do not inherit the drive are eliminated, generating transmission distortion in favour of the driver.

These systems are generally well suited to population modification, although some designs may also support population suppression under certain conditions.

Engineered toxin–antidote systems have been explored far less than homing-type drives. In some cases, natural toxin–antidote systems have been transplanted into new species while retaining gene drive activity (Gardiner et al., 2020; Li et al., 2023). In parallel, a range of synthetic systems has been developed, including Cleave-and-Rescue (ClvR) and Toxin-Antidote Recessive Embryo (TARE) designs, in which the drive carries both a nuclease that disrupts an essential gene and a recoded, nuclease-resistant version of that same gene that restores function (Oberhofer et al., 2019; Champer et al., 2020).

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Compared with homing drives, toxin–antidote systems are less constrained in their insertion site choice. Nevertheless, adequate expression of the rescue gene is crucial: incomplete rescue can impose fitness costs that reduce drive performance. Once fixed, these systems may be highly stable because the population becomes dependent on the drive-encoded rescue function. Their threshold properties can also be tuned to support different degrees of confinement.

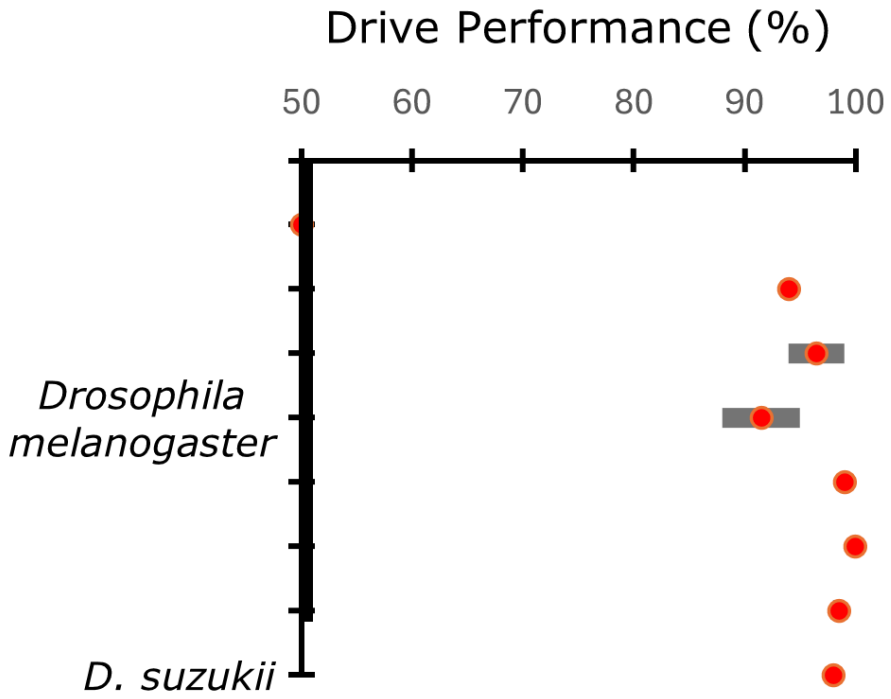


Figure 13: Empirical performance of non-homing-type engineered gene drives in insects.

Results from individual studies are shown. Ranges are shown as grey bars. Red points represent either the single reported value or the midpoint of the reported range.

To date, engineered toxin–antidote systems have been demonstrated mainly in *Drosophila*, where they have performed strongly, but their underlying logic should in principle be transferable to a wider range of species.

Fitness costs across engineered gene drives

Fitness costs associated with engineered gene drives may be either intentional or unintentional, depending on the drive mechanism and the intended application.

Homing- vs non-homing-type gene drives

In principle, homing-type drives can achieve a transmission advantage without imposing substantial fitness costs if the endonuclease and homing process do not adversely affect the host. Natural homing endonucleases often achieve this by targeting non-coding genomic

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regions. By contrast, engineered homing drives usually rely on the expression of heterologous transgenes, which may impose costs (Burt et al., 2004). For example, Cas9 expression and especially off-target cleavage may reduce host fitness (Langmüller et al., 2022).

Among non-homing systems, some mechanisms inherently depend on fitness costs to achieve distortion. Many natural meiotic-drive systems work by destroying gametes that do not inherit the drive, thereby reducing fertility (Lindholm et al., 2016; Zanders and Unckless, 2019). Most engineered non-homing systems developed so far similarly rely on toxin–antidote logic that eliminates gametes, embryos, or individuals lacking the drive construct (Oberhofer et al., 2019; Champer et al., 2020). Additional costs may arise if the rescue is incomplete or if the target gene is not fully haplosufficient.

Gene drive purpose: Suppression- vs modification-type engineered gene drives

Population modification drives typically combine the drive with cargo genes intended to confer a desired trait. Developers generally seek to minimise the fitness costs associated with these cargo elements, although suitable effector genes may be limited (Hoermann et al., 2022; Carballar-Lejarazú et al., 2023).

By contrast, suppression drives are intentionally designed to impose significant fitness costs on the target population by generating recessive lethality, infertility, or sex-ratio distortion. As these deleterious alleles rise in frequency, population size declines, and sufficiently potent systems may lead to local extinction. Such designs are especially sensitive to both intrinsic fitness costs and unintended maternal deposition of Cas activity, which can reduce efficacy (Pan and Champer, 2023).

Containment potential

The ability of a gene drive to spread following release depends strongly on its design and performance characteristics (Marshall and Akbari, 2018). Strong homing-type drives in which all components are located at a single locus are expected to require only a small introduction size—potentially even a single carrier—to achieve introgression. These low-threshold systems may in principle spread throughout the entire range of the host species and are therefore often described as potential global drives. Because of their low threshold, they are not expected to remain localised.

By contrast, split-drive versions of otherwise similar homing systems, where components are placed at two unlinked loci, generally have substantially higher release thresholds. A larger propagule is needed to initiate population-wide spread. Notably, approximately half of empirical studies involving homing drives have used split-drive designs, both to test alternative component combinations and to reduce the likelihood that accidental release would lead to uncontrolled spread.

Engineered toxin–antidote systems can likewise be designed with different threshold requirements and thus different containment properties. If the system imposes little or no fitness cost on carriers, thresholds may be low and containment potential limited. As with homing drives, splitting the nuclease and gRNA across different loci can raise thresholds and enhance confinement (Oberhofer et al., 2021a).

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Among current concepts, strong underdominance systems have the highest thresholds for successful introgression and are therefore expected to remain more localised, even under moderate migration (Champer et al., 2020).

Although no single metric fully captures containment potential, threshold dependence is currently used as a practical proxy. Actual containment will also depend on the ecology and biology of the target species, including dispersal, geographic range, natural history, and the environmental context of release (Edgington and Alphey, 2022; Xu et al., 2025).

Methods being developed to stop or reverse engineered gene drives

Efforts to stop, reverse, or neutralise gene drives after deployment have attracted growing interest, largely in response to biosafety concerns. The feasibility of reversal depends strongly on the type of drive and its design characteristics.

Underdominance-based systems can in principle be reversed by releasing sufficient numbers of wild-type individuals to push the population back across the relevant threshold (Akbari et al., 2013, 2014; Buchman et al., 2018, 2021; Dhole et al., 2019; Maselko et al., 2020; Sychla et al., 2022). This follows from the disruptive selection dynamics that characterise underdominance (Altrock et al., 2011).

Suppression drives exert strong selection for resistance. In homing-type systems, alleles that are no longer recognised by the endonuclease may gain a selective advantage and counteract the drive (Gomulkiewicz et al., 2021). Laboratory studies have also shown that transgenic individuals expressing proteins capable of inactivating the drive-encoded endonuclease can halt the spread of a suppression-oriented homing drive in *Anopheles gambiae* (Taxiarchi et al., 2021; D'Amato et al., 2024). Similar approaches have shown promise in *Drosophila melanogaster* (Wu et al., 2016). Toxin–antidote suppression drives could likewise be countered by alleles that neutralise the toxin or its effects (Gomulkiewicz et al., 2021).

By contrast, modification drives designed for persistence are expected to be less susceptible to resistance-based reversal because the fitness differences between drive-carrying and wild-type individuals are often smaller. Most proposed countermeasures for such systems therefore involve releasing a second engineered drive designed to inactivate or replace the first (Xu et al., 2020; Bier, 2022), an approach already demonstrated in *Drosophila melanogaster* (Xu et al., 2020).

Finally, some designs featuring inducible excision mechanisms, intended to remove the drive from the host genome while leaving only minimal residual sequences, have been described, although these remain theoretical and untested (Chennuri et al., 2024).

4.1.2 AQ2.2 – Are current EFSA guidelines for the risk assessment of GM animals sufficient and adequate for use against GDMIs with respect to molecular design considerations?

EFSA has published two guidance documents and one scientific opinion that are relevant to the risk assessment of GDMIs with respect to molecular characterisation (MC):

- EFSA (2012): Guidance on the risk assessment of food and feed from GMAs and on animal health and welfare
- EFSA (2013): Guidance document on the ERA of GM animals

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- EFSA (2020): Adequacy and sufficiency evaluation of existing EFSA guidelines for the MC, ERA and PMEM of GDMIs

Although EFSA (2012) was developed primarily for food and feed assessment, it provides a detailed framework for the MC of the genetic modification as part of hazard identification and characterisation, including information on both the genetic modification itself and the resulting GMA. EFSA (2013) describes a stepwise methodology for ERA of GMAs, with specific considerations for GMIs presented in Section 4.2. EFSA (2020) subsequently reviewed these guidelines and concluded that, although they provide a sound foundation for considering the MC of GDMIs as part of ERA, some sections are adequate but insufficient to address the specific realities of gene drive design and intended use.

More broadly, the EFSA (2020) analysis points to several recurring themes that are particularly relevant to GDMIs:

1. Persistence over time and across generations

The existing EFSA guidance documents were developed on the implicit assumption that the molecular characteristics of the released GMO would be transmitted largely unchanged to its offspring. This assumption does not hold well for many GDMIs. Population modification drives, for example, are intended to introduce and maintain new traits in populations over time, while other designs—such as daisy-chain systems—may function only when different components come together in subsequent generations. Future guidance should therefore make explicit the need to characterise not only the molecular properties of the released parent organisms, but also the expected and plausible molecular characteristics of their descendants over time.

2. Drive systems that intentionally alter DNA sequences in subsequent generations

EFSA (2012, 2013) were developed in the context of a regulatory paradigm in which transgenes directly conferred a trait and were inherited according to ordinary Mendelian principles. By contrast, homing drives introduce genome-editing machinery that is itself intended to modify DNA in the germ line of offspring, both to bias inheritance and, in some cases, to confer the intended phenotype. For such systems, MC should encompass not only the released individuals, but also the likely molecular outcomes in subsequent generations, including both intended homing events and unintended outcomes such as NHEJ-derived alleles. At the same time, future guidance should remain sufficiently broad and technology-neutral so as not to presume that all gene drives function through homing or genome editing.

3. Consequences of mutation and selection over time

For GDMIs, especially those intended to persist in the environment, the MC should also consider how the system may change under mutation and selection. This could include the emergence of resistance alleles, the evolution of pathogens in response to modification traits, or changes in the activity of the drive itself. While such dynamics are not unique to GDMIs, they are particularly relevant because persistence and inheritance bias are integral to the intended mode of action of many gene drive systems.

Table 19: Gaps in EFSA guidance for gene drive applications

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Guidance Document	Section (adequate but insufficient)	Additional elements requiring consideration
EFSA 2012	Source and characterisation of inserted nucleic acid (2.1.2.1.2)	• Full description of engineered gene drive design, mechanisms (e.g. CRISPR-Cas9) and components (Cas9, sgRNA, target sequence) • Functionality in non-target cells (relevant for HGT) • Stability and specificity of expression • Characteristics and function of cargo/payload genes • Molecular tools to detect, track and monitor spread and persistence
	Description of introduced/modified traits (2.1.2.2.1)	• Target sequence features, including polymorphism and functional context • DNA repair dynamics (NHEJ vs HR) and functional consequences • Pre-existing and emerging resistance alleles (payload and drive) • Design parameters (homology arms, number of targets, cleavage efficiency) • Functional characterisation of expressed proteins and downstream biological effects
	Inserted/modified sequences (2.1.2.2.2)	• Assessment of intended and unintended edits at target and off-target sites, including across generations
	Expression of inserted/modified sequences (2.1.2.2.3)	• Additional data for systems relying on complex molecular interactions (e.g., multi-locus targeting)
	Inheritance and genetic stability (2.1.2.2.4)	• Preferential (biased) inheritance • Outcomes of genome editing, including alternative repair pathways • Detection and tracking across generations • Characterisation of multi-component systems (e.g., daisy-chain drives)
	Conclusions of molecular characterisation (2.1.2.3)	• Integrated assessment of drive design, mechanism and intended outcome • Interactions among multiple genetic elements • Evidence of efficiency, stability and inheritance pre- and post-release • Monitoring approaches for intended and unintended effects
EFSA 2013	Unintended effects (2.2)	• Consideration of unintended effects in progeny across generations, not only released organisms
	Long-term effects (3.6)	• Influence of mutations, evolution and selection pressures on gene drive components over time
	Persistence and invasiveness (4.2.1)	• Explicit consideration of biased inheritance independent of fitness costs • Long-term spread and persistence in populations • Performance after vertical transfer to related species
	Horizontal gene transfer (4.2.2)	• Potential for HGT, particularly for homing-based drives, analogous to transposable elements
	Interactions with target organisms	• Interaction of persistent drives with heterogeneous populations • Mechanisms and dynamics of resistance development

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Guidance Document	Sections found to be adequate but insufficient	Elements highlighted by EFSA 2020 analysis as potentially requiring additional consideration
EFSA 2012	Source and characterization of the nucleic acid intended to be inserted (2.1.2.1.2)	• The engineered gene drive and its design covering both the underlying mechanisms involved (e.g. CRISPR-Cas9) and their (multiple) components (e.g. Cas9 protein and sgRNA and targeted sequence). In particular, information on the potential functionality of the engineered gene drive system in non-target cells may be needed for the HGT assessment (see Section 5.1.4.2); • The stability and specificity of expression of the engineered gene drive system; • The characteristics of any cargo/payload gene(s) linked to the engineered gene drive, and its/their function; • The molecular approaches used to detect and follow the intended and unintended spread of the genetic modification(s) of interest and its persistence in target populations.
	General Description of the trait(s) and characteristics introduced or modified (2.1.2.2.1)	• The target sequence (including any available information on the polymorphism in the population targeted); • The nature of the target sequence (e.g. within a conserved domain of a particular protein); • The ratio of NHEJ vs. HR repair resulting from the cleavage of the targeted sequence(s); • The characterisation of the NHEJ repair step following the cleavage of the targeted sequence including information about the consequences on the targeted sequence(s) (e.g. whether the targeted gene remains functional); • The pre-existence of resistance alleles to the cargo/payload genes in the target population; • The possible occurrence of resistance alleles to the engineered gene drive itself; • The size of the homologous sequences used for homing; • Single/multiple target sites (within the same gene or in multiple genes); • Cleavage efficiency of the target sequence including information on any additional steps to increase efficiency (e.g. activation/repression of other genes); • The characterisation of the protein(s) newly expressed in the GDMI or modified endogenous proteins including information on its/their biological role (e.g. protein structure/function); • Possible interruption of molecular pathways, possible metabolites accumulation, altered substrate specificity in case of enzymes, etc.
	Information on the sequences actually inserted/deleted or altered (2.1.2.2.2)	• Intended and unintended effects of genome editing machinery at both the target site and off-target sites in future generations
	Information on the expression of the	• "For engineered gene drives that are designed to achieve the expected phenotype through

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Guidance Document	Sections found to be adequate but insufficient	Elements highlighted by EFSA 2020 analysis as potentially requiring additional consideration
	inserted/modified sequence (2.1.2.2.3)	• molecular interactions (e.g. through multiple gene targeting, see section below) additional information • may be needed for the assessment of those GDMIs to assess those interactions."
	Inheritance and genetic stability of the inserted/modified sequence and phenotypic stability of the genetically modified insect (2.1.2.2.4)	• Preferential inheritance of gene drives • The function of the drive components and any genome editing activity – including the expected results of successful editing, potential results of NHEJ repair mechanisms in the context of the intended function of the drive • Detection of inherited traits or the results of gene drive inheritance in subsequent generations • Effectively characterizing multi-part gene drive systems (e.g. "daisy chain" drives)
	Conclusions of the molecular characterisation (2.1.2.3)	• The MC of the engineered gene drive system, including the underlying mechanisms involved and intended outcome of the release; • An assessment of possible interactions between the multiple engineered gene drive components, if the transgenic construct is composed of multiple elements that can segregate out intentionally as part of the gene drive strategy; • Proof of the efficiency, stability and inheritance of the engineered gene drive system before and during deliberate release; • MC approaches for the continued monitoring of untended and unintended effects.
EFSA 2013	Information to identify potential unintended effects (2.2)	• The current guidelines focus on the unintended effects on the gene drive modified individuals to be released and do not necessarily address unintended effects in their progeny after release.
	Long Term Effects (3.6)	• The effects of potential mutations and selection pressures over time on the molecular identity of the gene drive components
	Persistence and invasiveness of genetically modified insects, including vertical gene flow (4.2.1)	• Ensuring adequate consideration of biased inheritance of gene drives regardless of fitness cost • The potential for the spread and persistence of gene drive machinery over time in a population • Performance of the gene drive if transferred vertically to closely related species
	Horizontal Gene Transfer (4.2.2)	• Some gene drives, primarily homing-based engineered gene drives, may facilitate HGT the same way naturally occurring transposable elements do
	Interactions of genetically modified insects with target organisms	• Current guidelines may not adequately address how gene drives that are intended to persist (e.g. population modification drives) will interact across target populations that are heterogeneous • Current guidelines may not adequately address the potential mechanisms of resistance development in target populations

4.1.3 Themes emerging from EFSA 2020 Analysis

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The EFSA 2020 analysis of the individual elements in the EFSA guidance documents (2012, 2013) that may require additional consideration in future guidance for the MC of GDMI (Table 19) leads to a few generalizable themes. These include the consequences of the persistence of gene drives over time and across generations in populations in the environment, the potential of some gene drives (i.e. homing drives) to modify genomes including those of target populations, nontarget populations and other organisms through vertical or horizontal gene transfer, and the consequences of mutations and selection pressure over time on the molecular characteristics of the GDMI.

4.1.3.1 Persistence of GDMI over time and across generations

When the existing EFSA guidance documents were prepared in 2012 and 2013, gene drive organisms were considered outside the scope of the documents. As a result, the elements of the guidance documents related to molecular characterizations focus on identifying the molecular characteristics of the parent organism with the expectation that those molecular characteristics will be passed on to offspring without change. However, the intended purpose of gene drives may be to introduce new characteristics into a population (population modification) or in the case of some drive designs (e.g. Daisy Chains) to function in combination with other drive components that are introduced to the population separately and only function when a functional drive is assembled in subsequent generations. These do not fit the assumptions inherent in EFSA 2012 and 2013 that the MC of the organism to be released will essentially be the same as the MC of its offspring.

Although it is conceivable that applicants might recognize and address these considerations when using existing guidance documents, future guidance for GDMI can make explicit the need to fully capture not just the MC of the parent organisms but the expected and potential MC of offspring over generations and across time in populations.

4.1.3.2 Homing drives and other drive mechanisms that intentionally alter DNA sequences

EFSA 2012 and 2013 were written in the context of a paradigm where transgenes added to an organism were intended to directly confer a specific trait, and heredity between generations would function per Mendelian genetics. Homing gene drives instead introduce genetic engineering machinery which is intended to cause DNA modification in offspring as part of the mechanism to ensure biased inheritance, and potentially also to confer intended traits to offspring. In this context it is important for the MC to consider not only the MC of the individual to be introduced to the environment, but also the potential characteristics of offspring across generations depending on the intended use of the GDMI. This includes both the intended molecular characteristics but also any identifiable unintended characteristics. In the example of CRISPR-Cas mediated homing gene drives, this would mean providing an estimate of the likelihood and results of both the intended homologous DNA repair mechanism but also the likelihood of non-homologous end joining (NHEJ) in progeny and the potential consequences with respect to the molecular characteristics of the offspring.

All of these features would be expected to inform any discussion of the efficacy of a gene drive. However, explicit elicitation of these details in future guidance on the molecular characterization of GDMI could improve the utility of such guidance for developers of GDMI. A balance will need to be maintained, as guidance for MC of GDMI cannot presume that one particular technology (homing drives) will be employed, and many proposed drive mechanisms do not rely on DNA modification in subsequent generations. Therefore any

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specific requests for information on MC of subsequent generations should be specifically applied to GDMI where modification of DNA is a component of the drive machinery.

4.1.3.3 The consequences of selection pressure and mutation on GDMIs that persist in the environment over time

Particularly for drives that are intended for population modification, which may be intended to exist in the environment over an extended period of time, it may be important to address how the MC of the GDMI is likely to be impacted by selection pressure and mutation in the environment. It is important to note that GDMI would not be the first organisms that have been released into the environment with the intention that they would persist over time. Likewise, even GDMI employing a drive mechanism such as homing drives which are intended to function through the altering of DNA sequences in subsequent generations would not represent a unique phenomenon in the environment. Large proportions of genomes exist as fragments or non functioning elements of transposons or other selfish genetic elements that alter inheritance through the modification of DNA and are not inherited in Mendelian fashion. However, it is reasonable to expect the MC of a GDMI to address not only the molecular identity of released individuals but also what the expected and predictable characteristics of their offspring will be over the duration of the GDMI's persistence in the environment. Depending on the nature of the GDMI and its intended use, this could include the development of resistant genotypes, interactions with pathogens that evolve to overcome any resistance traits conferred by the drive, or potential mechanisms which might inactivate a drive.

4.1.4 AQ 2.3 – How can current guidelines for the risk assessment of GMAs be enhanced to better accommodate GDMIs?

Based on AQ2.2, the diversity of engineered gene drive concepts identified in the literature, the rapid pace of technological development, and the current lack of experience with concrete GDMI cases in the EU, the following recommendations are proposed.

1 **Retain EFSA (2012, 2013) as the baseline, technology-neutral framework, while recognising gaps identified in EFSA (2020):** EFSA should continue to rely on the existing MC framework set out in EFSA (2012), applied within the broader ERA framework of EFSA (2013), as the baseline for assessing GDMIs. Although engineered gene drives were originally considered out of scope in EFSA (2013), and EFSA (2020) subsequently identified several gaps—concluding that the MC-related guidance is adequate but not sufficient—the general principles and structure of the existing framework remain applicable when interpreted in light of GDMI-specific characteristics. This technology-neutral baseline provides flexibility across different GDMIs and offers a coherent foundation on which the gaps identified in EFSA (2020) can be addressed through interpretation, integration, and targeted clarification rather than wholesale revision.
2 **Apply existing guidance through ERA-driven problem formulation:** Rather than introducing gene drive-specific or mechanism-specific MC requirements, emphasis should be placed on ERA-driven problem formulation. This would help clarify how MC information supports key ERA considerations, including persistence, spread, invasiveness, inheritance patterns, unintended effects, and resistance evolution. Clearer articulation of these linkages would strengthen consistency and transparency without expanding or duplicating requirements. In practice, the nature of the gene drive (e.g., low-threshold versus high-threshold, suppression versus modification), the biology of

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the target insect species, and the characteristics of the receiving environments where the GDMIs will be released (e.g., agricultural or human-inhabited areas) should determine, through a problem formulation approach, which MC aspects require particular attention (Table 19).

3 **Explicitly consider expected molecular trajectories across generations:** For GDMIs—especially self-propagating or self-limiting systems whose properties may change over time—future guidance should explicitly request information not only on the molecular identity of the released organisms, but also on the expected molecular trajectories of the system across subsequent generations. This includes anticipated inheritance patterns, the consequences of intended genome editing events, the likelihood and implications of alternative repair outcomes such as NHEJ, and the plausible emergence of resistance or other molecular variants. Making this expectation explicit would improve the relevance of MC to ERA without necessitating a wholly separate framework.

4 **Use EFSA (2020) as contextual guidance for interpretation, not as a parallel framework:** The issues identified in EFSA (2020) should serve as a regulatory reference point that informs how existing MC and ERA requirements are interpreted and applied to GDMIs EFSA (2020) and should not be treated as a basis for establishing a stand-alone or parallel MC requirements, as this could undermine coherence and consistency. Instead, the elements identified as requiring additional consideration should, where appropriate, be integrated into existing guidance for GMAs. Elements that warrant additional consideration for GDMIs should therefore, where relevant, be integrated in existing guidelines for GMAs to come to a more refined and flexible risk assessment framework.

5 **Avoid premature mechanism-specific codification, while allowing for a stepwise approach:** Given the diversity of engineered gene drive concepts and the rapid evolution of the underlying technologies, caution is warranted in developing MC guidance that is narrowly tailored to specific gene drive types (e.g. homing-based drives) or molecular mechanisms (e.g., RNA-guided DNA endonucleases). Overly specialised guidance risks becoming overly prescriptive, quickly outdated, and misaligned with future technological developments. At the same time, early regulatory experience is likely to arise from a limited number of first-in-class cases that are closer to practical application. A stepwise approach may therefore be justified, whereby initial clarification focuses on the features of the most advanced candidate systems, while retaining sufficient flexibility to accommodate additional guidance as empirical evidence and regulatory experience accumulate.

6 Clarify the interface between MC and PMEM: A clearer delineation between MC and PMEM would help avoid conflation of objectives. MC should remain focused on characterisation, inheritance, expression, stability, and identification of potential hazards, while requirements related to detecting, following, and monitoring the spread, persistence, or resistance dynamics of GDMIs post-release should be explicitly anchored within PMEM. The interface between MC and PMEM may become more apparent by following a problem formulation approach to determine (additional)MC aspects that are most relevant for ERA of the specific intended GDMI release, while MC aspects related to PMEM are focused on actual detection of GDMI over time.

7 **Revisit and refine guidance in light of emerging regulatory needs and identified gaps:** Targeted refinements to MC or ERA guidance may become warranted as realistic deployment scenarios emerge, empirical evidence accumulates (for example from laboratory or semi-field studies), or formal applications are submitted within the EU.

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Any such updates should remain grounded in regulatory experience and evidence-based learning, rather than speculative future developments. Until such triggers arise, the existing EFSA (2012, 2013) framework—applied in an integrative, adaptive, and ERA-driven manner—can continue to serve as a proportionate baseline.

4.2 ToR1 – Review relevant case studies and their risks to human, animal, and environmental health

4.2.1 AQ1.1 Identify relevant GDMI cases that may be released deliberately into the environment based on available evidence (SQ1.1.1-SQ1.1.4)

4.2.1.1 SQ1.1.1 – Which GDMIs (species and traits) are being developed?

Insect species

Based on the selected literature (see the GDMD database, ToR2), the following insect species are currently being developed with engineered gene drives:

- Mosquitoes: *Aedes aegypti,* *Anopheles* species (*Anopheles gambiae*, *Anopheles stephensi* and *Anopheles coluzzii)* and *Culex quinquefasciatus* (all Diptera: *Culicidae*);
- Pest insects: *Ceratitis capitata* (Diptera: *Tephritidae*), *Drosophila suzukii* (Diptera: *Drosophilidae*), *Plutella xylostella* (Lepidoptera: *Plutellidae*) and *Tribolium castaneum* (Coleoptera: *Tenebrionidae*);
- Model insect: *Drosophila* melanogaster (Diptera: Drosophilidae).

Table 20 summarises the identified GDMIs, their intended traits, expected outcomes, and key references. Additional details on these traits and expected outcomes is provided below.

Table 20: Insect species engineered with gene drive constructs, their intended traits, and expected outcomes (as identified by the ToR2 literature search)

Insect species	Trait(s)	Expected outcome	Reference number[a]
Aedes aegypti	Trait not specified[b]	General[c]	6, 22, 34, 37 40, 80, 140
Anopheles gambiae	Female fertility, sex-ratio distortion (X-shredding), sex ratio and fertility	Suppression	7, 67, 71, 72, 75, 112, 120, 123
	Anti-*Plasmodium* effectors, *Plasmodium* infectivity	Modification	33, 48, 51, 65
	Reproductive load, trait not specified	General	91, 133
	Cas9 inhibition, anti-CRISPR	Mitigation	19, 59
Anopheles stephensi	Fertility	Suppression	124
	Anti-*Plasmodium*	Modification	1
	Trait not specified	General	92
Anopheles coluzzii	*Plasmodium* transmission, anti-*Plasmodium* effectors	Modification	31, 33
Ceratitis capitata	Female fertility, sex conversion	Suppression	9, 113
Culex quinquefasciatus	Trait not specified	General	30

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Insect species	Trait(s)	Expected outcome	Reference number[a]
Drosophila suzukii	Female fertility, sex ratio distortion	Suppression	13, 27
	Trait not specified	Modification	116
	Haplolethality	General	39
Drosophila melanogaster	Female lethality, female fertility, haploinsufficiency and female fertility, sex conversion	Suppression	2, 11, 35, 111, 113
	Insecticide susceptibility, allelic conversion, modifying unlinked genes, trait not specified	Modification	16, 23, 44, 70, 78, 101
	Recessive lethal haploinsufficiency, reduced female fertility, recessive lethality, overexpression lethality, trait not specified	General	15, 17, 25, 29, 32, 41, 42, 43, 49, 52, 58, 61, 62, 63, 64, 66, 74, 81, 85, 86, 88, 89, 97, 105, 108, 114, 115, 119, 121, 125, 128, 130, 131, 137, 141
	Conditional drive excision, Cas9 inactivation, drive deletion, overwriting drive, excision drive element	Mitigation	12, 73, 77, 90, 122
Plutella xylostella	Trait not specified	General	36, 53
Tribolium castaneum	Trait not specified	General	10

[a] Reference numbers correspond to those in the ToR2 GDMD searchable database
[b] Trait is listed as "not specified" or "not applicable" in the ToR2 GDMD database
[c] The term 'general' indicates that authors did not define a specific outcome (e.g., suppression, modification, mitigation); see ToR2; section Purpose.

Traits and expected outcome

Table 20 shows that for *Anopheles* species (primarily *Anopheles gambiae*), engineered gene drives are intended for both population modification and population suppression.

Population modification aims to reduce transmission of the *Plasmodium* malaria parasite to humans by introducing anti-*Plasmodium* effector genes or other mechanisms that decrease parasite infectivity.

Population suppression is foreseen through a reduction in female offspring, thereby decreasing the number of human-biting adults. This can be achieved either by lowering female fertility–thus reducing overall population size–or by inducing sex-ratio distortion or disruption, resulting in fewer females.

Similarly, population suppression of *Drosophila suzukii* is expected through reduced female fertility or via sex-ratio distortion or conversion. The introduction of traits causing haplolethality (i.e., genetic regions where a single functional copy is insufficient for viability) is expected to result in either population suppression or modification of *Drosophila suzukii* populations.

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In the model insect *Drosophila melanogaster*, engineered gene drives are also used for population suppression, for example by introducing traits that reduce female offspring (female fertility reduction or sex conversion). Population modification is achieved by altering insecticide susceptibility, enabling allelic conversion, or modifying unlinked genes. Additional traits introduced to suppress or modify *Drosophila melanogaster* populations include recessive haploinsufficiency, reduced female fertility, recessive lethality, and lethality due to overexpression. However, much of the research in this species focuses on developing efficient engineered gene-drive systems while preventing resistance to the gene drive.

The same general trend applies to work in *Aedes aegypti*, *Ceratitis capitata*, *Culex quinquefasciatus*, *Plutella xylostella*, and *Tribolium castaneum*, where the emphasis is similarly on establishing robust gene-drive systems and evaluating their performance.

With respect to reversal drives, research has been reported for *Anopheles gambiae* and *Drosophila melanogaster* (see SQ1.1.4).

4.2.1.2 SQ1.1.2 – Which of the GDMIs identified in SQ1.1.1. are at the most advanced stages of development?

Stages of development

To identify which GDMI cases (per species) are at the most advanced stage, the stages of development (see AQ1.1), the scale of experimentation, and the number of publications identified in the literature search were considered (Table 21).

Among the GDMI species identified in SQ1.1.1, the most advanced in development are *Aedes aegypti*, *Anopheles* species, *Drosophila suzukii* and *Drosophila melanogaster.*

Although *Drosophila melanogaster* ranks among the most extensively studied species for engineered gene drive research, it is a model organism used exclusively in laboratory settings, with no intention for deliberate environmental release. Therefore, *Drosophila melanogaster* is not considered relevant for the EU and its OMTs (see ToR1.2). Nonetheless, research in *Drosophila melanogaster* is directly informative for GDMI development in *Drosophila suzukii*, thereby supporting its inclusion in this report.

Other GDMI species identified in SQ1.1.1 remain at the conceptual or proof-of-concept stages and supported by only one or two publications (Table 21). For these species, deliberate environmental release is not expected in the coming decade(s).

Engineered gene drive development in *Plutella xylostella* is also in its early stages. However, as the only Lepidopteran species identified, *Plutella xylostella* provides a valuable case study and therefore its relevance for the EU and its OMTs is explored further (see AQ1.2).

Table 21: Insect species engineered with gene drive constructs identified in the database of gene drive molecular designs (Terms of Reference 2, ToR2), including number of publications, development stage, and experimental scale (as defined in ToR2).

Insect species	Number of publications	Stages of development[a]	Scale of studies
Aedes aegypti	7	Conceptual stage; proof of concept	Limited crosses; small cage trials

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Insect species	Number of publications	Stages of development[a]	Scale of studies
Anopheles gambiae	16	Conceptual stage; proof of concept; advanced research	Limited crosses; small cage trials; large cage trials
Anopheles stephensi	3	Proof of concept	Limited crosses; small cage trials
Anopheles coluzzii	2	Proof of concept; advanced research	Limited crosses; large cage trials
Ceratitis capitata	2	Proof of concept	Limited crosses
Culex quinquefasciatus	1	Proof of concept	Limited crosses
Drosophila suzukii	4	Proof of concept	Limited crosses; small cage trials
Drosophila melanogaster	51	Proof of concept	Limited crosses; small cage trials
Plutella xylostella	2	Proof of concept	Limited crosses
Tribolium castaneum	1	Proof of concept	Limited crosses

[a] 'Proof of concept' refers to studies conducted at the phenotypic level (e.g., demonstrating the functional effectiveness of the engineered gene drive in insects)

Based on the above considerations, the GDMI cases that will be further addressed in this report are those developed in the following insect species:

1) *Aedes aegypti*,
2) *Anopheles* species,
3) *Drosophila suzukii*
4) *Plutella xylostella.*

4.2.1.3 SQ1.1.3 – What is the intended temporal and spatial scale (self-sustaining/self-limiting, restricted/non-restricted)?

Both self-sustaining and self-limiting engineered gene drive systems are under development for *Aedes aegypti*, *Anopheles* species, *Drosophila suzukii* and *Plutella xylostella* (Table 22).

Self-sustaining engineered gene drives are expected to operate at larger temporal and spatial scale, meaning that such systems can theoretically spread indefinitely and without spatial restriction in the receiving environment, with little to no potential for containment.

By contrast, self-limiting systems, such as split gene drive designs, are generally intended to act over limited temporal and spatial scales. In these cases, the GDMI is expected to persist in the environment only temporarily, and its spread is constrained by the intrinsic properties of the system.

For some cases, the intended spatial scale is described as 'variable', reflecting reported variability in engineered gene drive strength or instability in the evolutionary persistence of the gene drive.

Table 22: Insect species engineered with a gene drive construct in the most advanced stages of development (including *Plutella xylostella*), their intended traits, expected outcomes, and intended temporal and spatial scales

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Insect species	Traits	Expected outcome	Intended temporal scale	Intended spatial scale[a]	Reference number[b]
Aedes aegypti	Trait not specified[c]	General[d]	Self-limiting	Restricted	34, 37, 80
			Self-sustaining	Non-restricted	6, 40
				Restricted	22
				Variable[e]	140
Anopheles gambiae	Female fertility, sex ratio distortion (X-shredding), sex ratio and fertility	Suppression	Self-sustaining	Non-restricted	7, 67, 71, 72, 75, 112, 120, 123
	Anti-Plasmodium effectors, Plasmodium infectivity	Modification	Self-sustaining	Non-restricted	33, 48, 51
				Restricted	65
	Trait not specified	General	Self-sustaining	Non-restricted	91
				Restricted	133
Anopheles stephensi	Fertility	Suppression	Self-sustaining	Non-restricted	1
	Anti-Plasmodium	Modification	Self-sustaining	Non-restricted	124
	Trait not specified	General	Self-sustaining	Non-restricted	92
Anopheles coluzzii	Anti-Plasmodium effectors, Plasmodium infectivity	Modification	Self-sustaining	Non-restricted	33
			Self-limiting	Variable	31
Drosophila suzukii	Female fertility	Suppression	Self-sustaining	Non-restricted	13
	Sex ratio distortion	Suppression	Self-limiting	Restricted	27
	Trait not specified	Modification	Self-limiting	Medium-restricted	116
	Haplolethality	General	Self-limiting or self-sustaining	Restricted	39
Plutella xylostella	Trait not specified	General	Self-limiting	Restricted	36
			Self-sustaining	Non-restricted	53

[a] Intended spatial scale is derived from the containment level defined in ToR2, section Containment Potential. "Restricted" corresponds to high containment; "non-restricted" corresponds to low containment.

[b] Publication numbers correspond to the reference numbers in the ToR2 GDMD database.

[c] Trait listed as "not specified" or "not applicable" in the ToR2 GDMD database.

[d] "General" indicates that no specific outcome (suppression, modification or mitigation) was defined (see ToR2, section Purpose).

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[e] "Variable" is used when gene drive strength or evolutionary stability is reported to be variable (see ToR2, section Containment Potential).

4.2.1.4 SQ1.1.4 - What methods are (being) developed to potentially reverse the gene drive after deliberate release?

Only a few of the species listed above have documented efforts to develop methods capable of reversing an engineered gene drive after release (Table 23). These species correspond to those with the highest number of GDMI-related publications, either because they represent advanced stages of research or are widely used as model organisms:

- *Anopheles gambiae*: Several approaches to reverse or neutralise a gene drive have been explored, including Cas9 inhibitors and anti-CRISPR elements, which are intended to mitigate or halt gene drive activity.
- *Drosophila melanogaster*: Gene drive reversal methods have also been investigated in the model organism *Drosophila melanogaster*. Although this species is not intended for deliberate environmental release, these methods are relevant because they may inform future developments in *Drosophila suzukii*. Reported reversal mechanisms comprise:
  - Inactivation or overwriting of the gene drive element;
  - (Conditional) Excision of the drive;
  - Inactivation of Cas9 activity.

Further discussion on these approaches is provided in ToR2, under "Methods that are (being) developed to potentially reverse the gene drive after their deliberate release".

Table 23: Insect species and methods under development to potentially reverse gene drive activity after environmental release.

Insect species	Methods/traits	Expected outcome	Publication number[a]
Anopheles gambiae	Cas9 inhibition, anti CRISPR	Mitigation	19, 59
Drosophila melanogaster	Conditional drive excision, Cas9 inactivation, drive deletion, overwriting drive, excision drive element	Mitigation	12, 73, 77, 90, 122

[a] Publication numbers refer to those in the ToR2 GDMD database.

4.2.2 AQ 1.2 Which GDMI cases (identified in SQ1.1.2 of ToR1.1) are relevant to the EU, including its OMTs?

To address AQ1.2, the GDMI cases identified in SQ1.1.2 as being in the most advanced stages of development (*Aedes aegypti*, *Anopheles* species, *Drosophila suzukii* and *Plutella xylostella*) were evaluated for their relevance to the EU and its OMTs.

A GDMI case is considered "relevant" for the EU and its OMTs when the insect species used to introduce the engineered gene drive construct:

1 Is established in the EU or its OMTs (SQ1.2.1); and
2 Has documented adverse effects on human, animal, or environment health (SQ1.2.2).

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4.2.2.1 SQ1.2.1 – Which GMDI cases involve insect species established in the EU (including its OMTs)?

Aedes aegypti

Aedes aegypti is an invasive mosquito species and a known vector of viral diseases such as West Nile virus, Zika virus and Chikungunya virus. It is closely monitored in the EU and in its OMTs by the European Centre for Disease prevention and Control (ECDC).

As of February 2026, *Aedes aegypti* is reported to be established in Cyprus and several EU OMTs, such as Madeira, the French Caribbean Islands and the Canary Islands (ECDC, 2026).

While *Aedes albopictus* (Diptera: Culicidae) is considered of higher concern for establishment in mainland Europe than *Aedes aegypti*, it is not included further in this report because no GDMI cases for *Aedes albopictus* were identified in the ToR2 GDMD database.

Aedes aegypti feeds primarily on mammalian hosts, preferably humans, even when alternative hosts are available. It thrives in urban habitats and exploits a wide range of artificial water-holding containers (e.g., vases, water tanks and tires), as well as aquatic underground systems such as septic tanks.

Climate projections indicate that *Aedes aegypti* may expand further into European regions with humid subtropical climates, such as parts of the Mediterranean basin, countries surrounding the Black Sea, and riverine lowlands (e.g. Ebro, Garonne, Rhone, and Po). Its establishment in more temperate regions is currently restricted by low winter temperatures and the high frost-sensitivity of its eggs (ECDC, 2025a).

Conclusion: *Aedes aegypti* is established in parts of the EU and in several OMTs.

Anopheles species

Anopheles gambiae, *Anopheles coluzzii* and *Anopheles stephensi* are important vectors of the *Plasmodium* spp., responsible for malaria transmission in tropical Africa. According to ECDC (2025b), these species have not been detected in the EU and its OMTs.

Their establishment is considered unlikely under present climatic conditions. However, for *Anopheles stephensi* future establishment cannot be entirely ruled out due to factors such as globalisation, climate change and increased trade (Acosta et al., 2025; Aksu, 2025).

Conclusion: The *Anopheles* species for which GDMI cases exist are not established, nor expected to establish in the EU or OMTs in the near future.

Drosophila suzukii

Drosophila suzukii (spotted-wing *Drosophila*) is endemic to Asia and was first detected as invasive in Hawaii in 1980 followed by simultaneous introductions into California and Europe in 2008. It has since rapidly spread across most temperate regions in Asia, Europe, and the Americas.

By 2026 *Drosophila suzukii* was reported to be widespread across the EU and present in several OMTs, including the Canary Islands, with limited presence in Madeira and Réunion (EPPO, 2026).

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Drosophila suzukii infests fruit and berry crops, with broad host range including thin-skinned berries (e.g., cranberries, blueberries, strawberries, raspberries), and stone fruits (e.g., cherries, peaches, plums), and even grapes. When healthy fruits are unavailable, *Drosophila suzukii* can exploit damaged or rotting fruits. Adults can recolonise orchards after surviving and reproducing in nearby semi-natural habitats (EPPO, 2026).

Conclusion: *Drosophila suzukii* is established in the EU and its OMTs.

Plutella xylostella

Plutella xylostella (diamondback moth) is a highly invasive, globally distributed Lepidopteran species. It is present wherever cruciferous host plants occur and is considered the most cosmopolitan Lepidoptera species.

Plutella xylostella is reported to be present in the EU and in OMTs (e.g., Azores, Guadeloupe, Martinique, Réunion)(CABI, 2025).

Plutella xylostella is predominantly associated with *Brassicaceae* crops. These homogeneous cropping systems provide abundant food resource supporting high survival, development, and population densities. *Plutella xylostella* has broad ecological tolerance and can reproduce under varied climatic conditions. Population dynamics are influenced by factors such as rainfall, food availability, and natural enemies (CABI, 2025).

Conclusion: *Plutella xylostella* is established in the EU and its OMTs.

4.2.2.2 SQ1.2.2 – Which of the insect species listed under SQ1.2.1 can result in potentially adverse effects on human, animal, or environmental health in the EU (including its OMTs)?

Aedes aegypti

Aedes aegypti is an invasive mosquito species and a competent vector of several arboviruses, including chikungunya, dengue, yellow fever and zika (ECDC, 2026).

Chikungunya

Chikungunya is not endemic in mainland EU or in non-EU countries (Iceland, Liechtenstein and Norway (together they form the European Economic area or EEA). Most reported cases involve travellers infected outside the EU/EEA and several chikungunya outbreaks have taken place in mainland EU/EEA since 2007. In 2025, two countries in Europe have reported cases of chikungunya virus disease: France (788) and Italy (384). In the OMTs, outbreaks have only taken place in the French parts (ECDC, 2026).

Dengue

Dengue is not endemic in mainland EU/EEA and the vast majority of the cases are travellers infected outside of mainland EU/EEA. In 2025 (as of 19 November 2025), three countries in mainland EU/EEA have reported cases of dengue: France (29), Italy (four), and Portugal (two) (ECDC, 2026).

In the OMTs:

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- Dengue is endemic in French Guiana, Guadeloupe, Réunion, Martinique, Mayotte and Saint-Martin
- Madeira experienced a major dengue outbreak in 2012, no dengue cases are reported in 2025.
- For the Azores and the Canary Islands no dengue cases were reported in 2025.

Yellow fever

Yellow fever is not endemic in mainland EU/EEA and the vast majority of the cases are travellers infected outside of mainland EU/EEA. Between 2005 and 2023, 16 imported yellow fever cases have been reported in the EU/EEA. In 2024, none of the EU/EEA countries reported an unusual increase in yellow fever cases (ECDC, 2026).

Climate change is expected to increase yellow fever transmission risk (Erazo et al., 2024).

Zika

Zika is not endemic in mainland EU/EEA and the vast majority of the cases are travellers infected outside of mainland EU/EEA. In 2024 about a hundred zika cases were reported, all linked to travel outside the EU/EEA (ECDC, 2026)

Although the above mentioned diseases vectored by *Aedes aegypti* are not endemic yet in the EU/EEA, the risk of local transmission is high (ECDC, 2026) due to:

- Importation through infected travellers;
- Widespread presence of competent vectors, especially in Mediterranean countries; and
- Population susceptibility.

Although *Aedes albopictus* is established more widely than *Aedes aegypti* in the EU/OMTs, and can transmit all four diseases (chikungunya, dengue, yellow fever and zika), *Aedes albopictus* is generally considered more efficient vector for dengue (Kristan et al., 2025).

Nevertheless, the further establishment of *Aedes aegypti* in the EU and it OMTs is expected to increase public health risk in affected EU/OMT regions.

Conclusion: The establishment of *Aedes aegypti* in the EU and OMTs is considered likely to cause adverse effects on human health.

Anopheles spp.

Malaria is transmitted by *Anopheles* species. Malaria is now largely eliminated in the EU and OMTs, except for parts of French Guyana where it remains endemic (WHO, 2024a). In 2022, 5,375 malaria cases were reported in the EU/EEA, from which 99.8% were travel-related.

Climate change may slightly increase vector suitability in Europe. However:

- Current public health systems are highly capable of rapid detection and treatment;
- Resurgence risk remains low;
- Multiple alternative control measures exist that would be preferred over GDMIs (Climate Adapt, 2026; ECDC, 2025b).

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Additionally, the Anopheles species for which GDMI cases exist (*Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles stephensi*) are not established and unlikely to establish in the EU or OMTs in the near future.

Conclusion: The Anopheles species relevant to GDMIs are not likely to cause adverse effects on human health in the EU/OMTs.

Drosophila suzukii

The spotted wing *Drosophila*, *Drosophila suzukii*, is a major invasive insect pest of berries and stone fruits in both the Americas and in the EU. Unlike most *Drosophila* species, *Drosophila suzukii* can oviposit into undamaged, ripening fruit with sufficiently soft skin. Larval development within the fruit renders produce unmarketable.

Control of *Drosophila suzukii* is challenging as developing larvae are protected from insecticide applications inside the fruit, and the species produces numerous overlapping generations. As a result, newly emerging adults must be targeted repeatedly (EPPO, 2026). Ineffective control therefore leads to serious revenue losses for berry, stone fruit and vine fruit producers.

Physical control measures such as intensive orchard sanitation, exclusion netting and the application of kaolin, lime or rock dusts to alter the fruit surface have shown to reduce fruit damage. However, these measures are often extremely labour-intensive, costly or insufficiently effective to meet markets' zero-tolerance thresholds for *Drosophila suzukii* infestation (Romeis et al., 2020). The economic impact of *Drosophila suzukii* is substantial: or example, in the United States of America, annual damage to fruit crops is estimated at approximately 500 million USD. While comparable data for the EU are lacking, economic losses are expected to be of a similar magnitude.

In conclusion, *Drosophila suzukii* is considered to cause significant adverse effects on agricultural crops (fruit and berries) in the EU and its OMTs.

Plutella xylostella

The diamondback moth, *Plutella xylostella,* is considered the main insect pest of cruciferous vegetables (e.g., cabbages, broccoli, and cauliflowers) and oilseed crops (e.g., canola and mustard) (CABI, 2025). It is a polyphagous pest that is of significant concern in Europe. The larvae are known for their voracious feeding habits, often causing extensive damage to crops.

Plutella xylostella, is one of the most extensively studied insect pests worldwide, yet it remains among the most difficult to control. It was the first crop insect reported to develop resistance to microbial *Bacillus thuringiensis* insecticides and has subsequently evolved resistance to nearly all major classes of chemical insecticides, including newer groups such as diamides.

In the EU and its OMTs, infestations can cause substantial agricultural damage, particularly under hot and dry weather conditions, resulting in significant economic losses. Globally, damage caused by *Plutella xylostella* is estimated to cost 4-5 billion USD annually, making it the most economically damaging insect pest worldwide (CABI, 2025).

In conclusion, *Plutella xylostella* is considered to cause adverse effects on agricultural crops (*Brassica* crops) in the EU and its OMTs.

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4.2.2.3 SQ1.2.3 – What are the most relevant GDMI cases based on their development stage, (potential) establishment of the insect species covered, and their potential adverse effects on human, animal, and environmental health in the EU (including its OMTs)?

In SQ1.2.2, the focus shifted to the potential adverse effects of GDMI cases in the EU and its OMTs, including on human, animal and environmental health. This analysis identified potential adverse effects on human health associated with *Aedes aegypti* and on agricultural health associated with *Drosophila suzukii* and *Plutella xylostella*. No adverse effects on human health are expected from GDMI cases involving *Plasmodium*-carrying *Anopheles* species, as malaria is generally not considered likely to reemerge in the EU and its OMTs due to the presence of functioning health systems.

Taking into account both the likelihood of establishment and the potential for adverse effects, the most relevant GDMI cases for the EU and its OMTs are those involving *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella*.

4.2.2.4 SQ1.2.4 – For the most relevant GDMI cases, what are the receiving environment(s) where these GDMIs may be deliberately released?

The potential receiving environments that may be considered for the deliberate environmental release of the GDMI cases identified in SQ1.2.3 include large parts of the EU mainland and its OMTs. These receiving environments are highly heterogeneous, differing substantially in climate, geography, land use, agricultural practices and biodiversity. General information on environmental conditions in the EU and its OMTs is therefore provided, together with references to publicly available sources more detailed information can be obtained. This information can support case-by-case ERA of GDMI cases (see Box 1).

In addition, more specific information is presented for the potential receiving environments of *Aedes aegypti, Drosophila suzukii and Plutella xylostella* in the EU and its OMTs, reflecting the ecological requirements, habitat preferences and host associations of these species.

Box 1. Information sources on receiving environments in the European Union (EU) and its outermost territories (OMTs).

Climate

The EU encompasses a wide range of climatic zones, including subtropical, temperate, polar and highland climates. The French OMTs in the Caribbean and Indian Ocean are characterised by tropical climates with high temperatures and significant rainfall, whereas the Azores and Madeira exhibit mild maritime climates with moderate temperatures and high humidity (https://www.britannica.com/place/Europe/Climate).

Geography

A general map of the EU displaying countries, capitals and major physical features is available at *https://gisgeography.com/europe-map/*. More detailed geographical information (including topography, waterbodies, rivers) can be obtained for individual countries or regions.

Ecosystems

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Marine ecosystems represent the most extensive ecosystem types in the EU. On land, forests and cropland each cover approximately 36% of the total surface area, followed by grasslands (11%), urban areas, (5%) heathlands and shrublands (4%), rivers and lakes (2.5%), inland wetlands (2%) and sparsely vegetated land (1.5%) (*https://publications.jrc.ec.europa.eu/repository/handle/JRC120383*). Detailed ecosystem information at national level is available via the Biodiversity Information System for Europe (BISE; *https://biodiversity.europa.eu*).

Biodiversity

Information on biodiversity in the EU and its OMTs, including protected areas, can be accessed through BISE. BISE serves as a central platform for data, information, and knowledge on biodiversity across Europe and its OMTs, with country-specific details available.

Receiving environments for the potential environmental release of Aedes aegypti, Drosophila suzukii and Plutella xylostella

The EFSA GMO Panel defines receiving environments as the specific ecological and geographical areas where GM organisms are intended to be released, including climate, local ecosystems, biodiversity, potential interactions with native species and habitats, and existing management systems (EFSA, 2013). This definition is applied here to GDMIs.

Aedes aegypti

Aedes aegypti is currently established in Cyprus, Madeira, the French Caribbean islands, and the Canary Islands. It is expected to expand its distribution to additional areas of the EU and its OMTs with humid subtropical climates, such as parts of the Mediterranean region, countries surrounding the Black Sea, and areas along major lowland river systems such as the Ebro, Garonne, Rhône and Po (Table 24).

Aedes aegypti feeds preferentially on humans and is adapted to urban and domestic environments. Therefore, any deliberate environmental release of GDMI *Aedes aegypti* in the EU or its OMTs would most likely occur in urban domestic settings with suitable climatic zones. Adult dispersal capacity is limited, with typical flight ranges of approximately 200m. Integrated control strategies combining insecticide use, source reductions and public health awareness have been reported primarily from Madeira.

Table 24: Receiving environments of *Aedes aegypti* in the European Union and its outermost territories.

Characteristics	Description	References, sources of information
Climate	Humid sub-tropical	ECDC (2025a, 2026)
Geographical areas	• Cyprus, Madeira, French Caribbean Islands and Canary Islands • Expected expansion to Mediterranean regions, Black Sea countries and major lowland river areas (Ebro, Garonne, Rhône, and Po)	ECDC (2025a, 2026); Erazo et al. (2024)
Local ecosystems	Urban and peri-urban ecosystems associated with human habitation	Box 1

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Characteristics	Description	References, sources of information
Biodiversity	Biodiversity of urban domestic habitats and adjacent semi-natural areas	Box 1
Potential interactions with native species and habitats	Limited interactions expected due to human association and limited dispersal	ECDC (2025a, 2026)
Management systems	Combined control strategy (Madeira) including insecticides, breeding site reduction and public awareness	ECDC (2017)

Drosophila suzukii

Drosophila suzukii is present in fruit and berry-producing regions across the EU and its OMTs and is adapted to a broad range of temperatures. It occurs primarily in orchards and other fruit and berry-producing agroecosystems. In 2022, the main fruit-producing EU countries were Spain, Poland, Greece, Italy and France, which are predominantly characterised by temperate climates (Fruitlogistica, 2024).

Accordingly, any deliberate environmental release of GDMI *Drosophila suzukii* would most likely initially occur in major fruit- and berry-producing regions within these countries (Table 25). The species is capable of active dispersal and can also be passively transported via infested fruit. Current management relies mainly on chemical insecticides and physical control measures.

Table 25: Receiving environments of *Drosophila suzukii* in the European Union and its outermost territories.

Characteristics	Description	References, sources
Climate	Temperate	
Geographical areas	Fruit- and berry-producing regions in major producing countries (such as Spain, Poland, Greece, Italy, France)	EPPO (2026)
Local ecosystems	Orchards, agro-ecosystems and adjacent semi-natural areas	EPPO (2026); Romeis et al. (2020)
Biodiversity	Biodiversity of orchards, agro-ecosystems and semi-natural areas and associated ecosystem services	Box 1
Potential interactions with native species and habitats	Hybridisation with wild relatives; effects on ecosystem services	Box 1
Management systems in place	Chemical control; physical control such (as sanitation, exclusion netting, or kaolin, lime and rock dusts)	EPPO (2026); Romeis et al. (2020)

Plutella xylostella

Plutella xylostella occurs mainly in Brassicaceae crop systems in the EU and its OMTs. The insect is capable of surviving and causing damage across a wide range of climatic conditions.

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The most widely cultivated Brassica crops in Europe include oilseed rape (*Brassica napus* and *Brassica rapa*) and mustard (*Brassica juncea* and *Sinapis alba*). Main *Brassica napus*-producing countries in the EU include France and Germany. *Brassica rapa* is mainly grown in Germany and Poland, while mustard crops are mostly cultivated in Hungary (EC, 2016).

If GDMI *Plutella xylostella* were to be deliberately released in the EU or its OMTs, initial releases would most likely occur in major Brassica-growing regions characterised by temperate climates (Table 26). The species has strong dispersal capacity and is considered invasive. Management currently relies on chemical and biological insecticides, including *Bacillus thuringiensis*, as well as integrated pest management (IPM), although resistance to most insecticide classes has been reported (CABI, 2025).

Table 26: Receiving environments of Plutella xylostella in the European Union and its outermost territories.

Characteristics	Description	References, sources
Climate	Temperate	
Geographical areas	Brassica-growing regions (e.g., Germany, Poland and Hungary)	EC (2016)
Local ecosystems	Agro-ecosystems	CABI (2025)
Biodiversity	Biodiversity of Brassica agro-ecosystems and associated ecosystem services	Box 1
Potential interactions with native species and habitats	Hybridisation with wild relatives; effects on ecosystem services	Box 1
Management systems in place	Chemical control; biological control (microbial *Bacillus thuringiensis*); integrated pest management	CABI (2025)

Conclusion

Based on AQ1.1, GDMI cases that may be deliberately released into the environment involve *Aedes aegypti*, *Anopheles* species and *Drosophila suzukii*. In addition, GDMI cases involving *Plutella xylostella* were included. Although GDMI development in *Plutella xylostella* is at an earlier stage, it represents the only Lepidopteran species relevant to the EU and its OMTs and therefore serves as an informative case study (AQ1.2).

Following AQ1.2, GDMI cases involving *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella* were identified as the most relevant for the EU and its OMTs. The receiving environments for potential releases of these GDMI insects are expected to include urban domestic environments with humid subtropical climates (*Aedes aegypti*), major fruit- and berry-producing regions (*Drosophila suzukii*), and major Brassica-growing areas (*Plutella xylostella*) across the EU and its OMTs.

4.2.3 AQ 1.3 What risks to human, animal and environmental health can be identified using the Pathway To Harm approach?

To address SQ1.3.1, examples of hazards to human health, animal health, and the environment were identified for GDMI cases involving *Aedes aegypti, Drosophila suzukii and Plutella xylostella* (Table 27). These hazards were derived from the most relevant findings in the scientific literature and were structured according to the areas of risk defined for GM

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insects, for example by EFSA (2013). These areas of risk reflect established protection goals for human and animal health and for the environment, including plant health.

To answer SQ1.3.2, examples of exposure pathways that could lead to potential harm (pathways to harm, PTHs) following the deliberate environmental release of the GDMI cases of *Aedes aegypti, Drosophila suzukii and Plutella xylostella* were identified. These exposure pathways were specified for each hazard listed in Table 27.

For SQ1.3.3, the most plausible PTHs were identified for the three GDMI cases, taking into account current knowledge of the ecology of the target insect species and the characteristics of their potential receiving environments in the EU and its OMTs (see AQ1.2; Table 27).

To address SQ1.3.4, an assessment was done to identify risks to human health, animal health, and the environment arising from these pathways, including a qualitative consideration of the likelihood and magnitude of such risks.

Examples of GDMI cases of Aedes aegypti, Drosophila suzukii and Plutella xylostella

Based on the literature search (ToR2) and the stage of development of relevant GDMI cases (SQ1.1.2), it can be concluded that no specific, fully characterised gene drive modified strains of *Aedes aegypti, Drosophila suzukii and Plutella xylostella* are currently available that are close to deliberate environmental release and could be directly assessed using a PTH approach. Therefore, in order to address SQ1.3, a set of assumptions was applied regarding the expected outcomes of the GDMIs (population suppression or modification), their spatial and temporal behaviour (e.g., self-sustaining, self-limiting), and their level of molecular characterisation.

Expected outcome

Aedes aegypti is a vector of viral diseases that pose significant risks to human health. Consequently, GDMI applications targeting *Aedes aegypti* may plausibly aim at either:

- Population modification, to reduce or prevent pathogen transmission, or
- Population suppression to reduce or eliminate vector populations.

By contrast, *Drosophila suzukii* and *Plutella xylostella* are both agricultural pests that cause direct damage to plants and do not act as vectors of plant diseases. The primary anticipated application of engineered gene drives in these species is therefore population suppression. However, it is acknowledged that population modification strategies may also be developed for these species in the future.

For the purpose of this report, potential PTHs focus on:

- Both population modification and population suppression for gene drive modified *Aedes aegypti*; and
- Population suppression only for gene drive modified *Drosophila suzukii and Plutella xylostella*.

Spatial and temporal scale

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Although not explicitly evident from the literature search (ToR2), it is assumed that both self-limiting (high threshold) and self-sustaining (low threshold) gene drive systems could potentially be developed and deployed for the GDMI cases considered.

Molecular characterisation

For the purpose of this assessment, it is assumed that any GDMIs deliberately released into the environment would be fully characterised at the molecular level prior to release. This includes confirmation that:

- Only the intended genetic modifications are present;
- The gene drive construct functions as designed;
- No unintended sequences (e.g., marker genes) are retained;
- No unintended effects on molecular level are expected.

These assumptions provide a transparent and consistent basis for applying the PTH framework in the absence of concrete GDMI case studies.

4.2.3.2 SQ1.3.1 – What hazards can be identified for the most relevant GDMI cases?

To address SQ1.3.1, examples of hazards for human health, animal health, and the environment were identified for GDMIs in general, including representative cases of *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella* (Table 27, second column). The listed hazards reflect those most consistently identified in the scientific literature on the ERA of GDMIs, drawing in particular on Roberts et al. (2017), James et al. (2018), Rüdelsheim and Smets (2018), Teem et al. (2019), EFSA (2020), Connolly et al. (2021), WHO (2021), Kormos et al. (2023), Hayes and Hosack (2025) and SCBD (2025).

In line with Kormos et al. (2023) and Hayes and Hosack (2025), hazards were structured according to the areas of risk for GM insects as defined by EFSA (2013). This guidance was selected because it provides a comprehensive and structured framework for hazard identification, includes risk areas directly relevant to insects, and has been considered applicable to GDMIs when supplemented with addition considerations (EFSA, 2020). Although much of the existing hazard identification literature focuses on GDMIs intended for the control of human disease vectors, many of the identified hazards are generic and equally applicable to agricultural pest species such as *Drosophila suzukii* and *Plutella xylostella* (Kormos et al., 2023).

In addition to the EFSA (2013) risk areas, two additional categories were included in Table 27:

- Evolutionary and stability considerations and
- Control, emergency measures.

The first category captures hazards associated with unintended genetic or phenotypic changes arising from the prolonged persistence of gene drive constructs in the environment, particularly relevant for self-sustaining gene drive systems (e.g., population modification strategies). The second category, while not strictly part of ERA sensu stricto, was included to acknowledge that hazards may also arise indirectly from follow-up or contingency control measures, such as changes in insecticide use or the deployment of reversal gene drives. Hazard associated with reversal gene drive were considered conceptually similar to those associated with engineered gene drives themselves.

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4.2.3.3 SQ1.3.2 – What exposure pathways could lead to potential harm following deliberate environmental release?

To address SQ1.3.2, examples of PTHs were identified for each hazard listed in SQ1.3.1. These pathways describe plausible sequences of events by which the deliberate environmental release of a GDMI could result in adverse effects on human health, animal health, or the environment. The identified PTHs are presented in Table 27 (third column) and are formulated at a generic level, applicable in principle to GDMIs irrespective of the target species.

Whether a given PTH is plausible in practice for a specific GDMI depends on multiple factors, including the characteristics of the gene drive construct, the biology and ecology of the target insect species, and the features of the receiving environments in the EU and its OMTs. These aspects are therefore considered explicitly in SQ1.3.3, where the plausibility of the identified PTHs is evaluated for *Aedes aegypti*, *Drosophila suzukii*, and *Plutella xylostella*.

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Table 27: Areas of risk, example hazards and plausible pathways to harm (PTHs) for gene drive modified insects (GDMIs), with species-specific plausibility and indicative assessment endpoints for the European Union (EU) and its outermost territories (OMTs)[a].

Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
1. Persistence and invasiveness, including vertical gene flow	Potential persistence or invasiveness in receiving environments in the EU and its OMTs Increased fitness of the GDMI relative to non-GM insect counterparts, enabling persistence or spread	Increased persistence or fitness of the GDMI resulting from the engineered gene drive construct leads to unintended spread and persistence of the construct beyond the intended receiving environments, resulting in harm in newly colonised regions (see risk areas 2-9)	Plausible • Fitness-related traits of GDMI compared with non-GDMI • Spread and persistence of the engineered gene drive construct	Plausible • Fitness-related traits of GDMI compared with non-GDMI • Spread and persistence of the engineered gene drive construct	Plausible • Fitness-related traits of GDMI compared with non-GDMI • Spread and persistence of the engineered gene drive construct
	Reproduction and hybridisation with non-GM insects of other species	Hybridisation of the GDMI with (closely) related species results in unintended spread and persistence of the engineered gene drive construct and suppression of recipient species	Not plausible, as hybridisation of *Aedes aegypti* and its only (closely) related species (*Aedes albopictus*) in the EU is considered unlikely (OECD, 2018)	Not plausible, as no hybridisation with (closely) related species is known in the EU (Wolf et al., 2023)	Not plausible, as no (closely) related species capable of hybridisation are known in the EU (CABI, 2025)
	Altered habitat use and/or geographic range of the GDMI or hybrid populations	Broader tolerance of the GDMI to biotic and/or abiotic conditions enables occupation of additional habitat(s) or an expanded geographic range, leading to unintended spread and	Plausible • Tolerance of GDMI to a broader range of biotic and abiotic conditions compared with non-GDMI	Plausible • Tolerance of GDMI to a broader range of biotic and abiotic conditions compared with non-GDMI	Plausible • Tolerance of GDMI to a broader range of biotic and abiotic conditions compared with non-GDMI

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
		persistence of the engineered gene drive construct beyond intended receiving environments (see risk areas 2-9)	• Spread and persistence of the engineered gene drive construct	• Spread and persistence of the engineered gene drive construct	• Spread and persistence of the engineered gene drive construct
2. Horizontal gene transfer	Effects following horizontal transfer of the engineered gene drive construct to other insects	Horizontal gene transfer of the engineered gene drive to other (not related) insect species leads to unintended spread, expression, or functionality of the engineered gene drive construct, resulting in suppression of non-target species and disruption of the ecosystem services they provide	Plausible[d] • Presence of target sequence of a homing-based gene drive in non-target insects • Potential functionality of the engineered gene drive construct following horizontal gene transfer (EFSA, 2020)	Plausible[d] • Presence of target sequence of a homing-based gene drive in non-target insects • Potential functionality of the engineered gene drive construct following horizontal gene transfer (EFSA, 2020)	Plausible[d] • Presence of target sequence of a homing-based gene drive in non-target insects • Potential functionality of the engineered gene drive construct following horizontal gene transfer (EFSA, 2020)
	Effects following horizontal transfer of engineered gene drive construct to microorganisms Effects arising from genetic exchange between GDMIs and associated symbionts	Horizontal gene transfer of the engineered gene drive to microorganisms and/or symbionts leads to unintended spread, expression, or functionality of the engineered gene drive construct in these microbes, resulting in suppression of these microbes and the	Not plausible as multiple conditions must be met for the transferred construct to remain intact, be expressed, and function as a gene drive after horizontal gene transfer	Not plausible as multiple conditions must be met for the transferred construct to remain intact, be expressed, and function as a gene drive after horizontal gene transfer	Not plausible as multiple conditions must be met for the transferred construct to remain intact, be expressed, and function as a gene drive after horizontal gene transfer

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	*Drosophila suzukii*	*Plutella xylostella*
		ecosystem services they provide			
3. Pathogens, infections and diseases for humans, animals and plants	Altered GDMI phenotype resulting in increased transmission of pathogen	An altered phenotype of the GDMI (e.g. changes in behaviour (e.g. biting rate), physiology, transcriptome or microbiome) increases transmission of human, animal or plant pathogens	Plausible • Comparison of GDMI and non-GDMI with respect to phenotype, vector competence and other parameters involved in disease transmission to humans and animals	Not plausible, as *Drosophila suzukii* does not transmit diseases to humans, animals or plants and is not a plant pathogen	Not plausible, as *Plutella xylostella* does not transmit diseases to humans, animals or plants and is not a plant pathogen
	Changes in pathogen population due to metabolites produced by the GDMI	A difference in metabolite production of the GDMI results in a change of vector competence or transmission of other pathogens for humans, animals and plants	Plausible • Comparison of GDMI and non-GDMI with respect to metabolite production	Not plausible, as *Drosophila suzukii* does not transmit diseases to humans, animals or plants and is not a plant pathogen	Not plausible, as *Plutella xylostella* does not transmit diseases to humans, animals or plants and is not a plant pathogen

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Emergence or selection of new pathogens or pathogenic strains with increased virulence	Rearing of GDMI results in the emergence or selection of new pathogens or more virulent strains, which may subsequently be released into the environment	Plausible • Presence and virulence of pathogens in GDMI compared to non-GDMI • Adequate quality control GDMI breeding facility	Not plausible, as *Drosophila suzukii* does not transmit diseases to humans, animals or plants and is not a plant pathogen	Not plausible, as *Plutella xylostella* does not transmit diseases to humans, animals or plants and is not a plant pathogen
	Emergence or selection of new pathogens or pathogenic strains with increased virulence	Reduced efficacy of the GDMI or malfunction of the GDMI release technology leads to increased prevalence of pathogens not targeted by the engineered gene drive, or to the emergence of more of more harmful pathogen strains	Plausible, especially in case of population modification • GDMI efficacy over prolonged time • Pathogen populations over prolonged time	Not plausible, as *Drosophila suzukii* does not transmit diseases to humans, animals or plants and is not a plant pathogen	Not plausible, as *Plutella xylostella* does not transmit diseases to humans, animals or plants and is not a plant pathogen
	Malfunction or reduced efficacy of GDMI release technology	Reduced efficacy of the GDMI or malfunction of the GDMI release technology leads to the persistence or resurgence of harmful target insect populations	Plausible, especially in case of population modification • GDMI efficacy and pathogen populations over prolonged periods	Plausible • GDMI efficacy and pathogen populations over prolonged periods	Plausible •GDMI efficacy and pathogen populations over prolonged periods

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
4. Interactions with target organisms	Unintended changes in target organism population (e.g., population size, age structure, sex ratio, fertility, mortality) following GDMI release	Changes in target organism population parameters (size, structure, sex ratio, fertility and mortality) reduce the efficacy of the GDMI, resulting in adverse effects, particularly for self-sustaining engineered gene drive systems	Plausible for self-sustaining engineered gene drive systems • GDMI efficacy over prolonged time	Plausible for self-sustaining engineered gene drive systems • GDMI efficacy over prolonged time	Plausible for self-sustaining engineered gene drive systems • GDMI efficacy over prolonged time
	Reduction in efficacy or resistance development in the target organism against the GDMI over time	Reduced efficacy of the GDMI leads to the implementation of alternative management measures (e.g. increased use of pesticides), which results in more adverse effects than the GDMI itself	Plausible • Development of GDMI efficacy	Plausible • Development of GDMI efficacy	Plausible • Development of GDMI efficacy
	Changes in interactions with the target organism arising from an altered genetic diversity of the released insect population	Release of GDMI with altered genetic diversity (e.g., mass-reared laboratory insects) changes interactions with target populations, potentially leading to adverse effects on human health or crop plants	Plausible • Interactions between GDMI and target populations	Plausible • Interactions between GDMI and target populations	Plausible • Interactions between GDMI and target populations

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Effects on target organisms resulting from the release of non-transformed insects	Release of non-transformed biting insects increases exposure of humans or animals, resulting in adverse effects	Plausible • Adequate quality control of GDMI breeding facilities • Development of field populations of target organisms	Plausible for animals, workers and bystanders in fruit and berry agro-ecosystems • Adequate quality control of GDMI breeding facilities • Development of field populations of target organisms	Plausible for animals, workers and bystanders in Brassica agro-ecosystems • Adequate quality control of GDMI breeding facilities • Development of field populations of target organisms
	Effects on target organisms resulting from the release of non-transformed insects	Release of non-transformed pest insects increases exposure of crops resulting in adverse effects on plant health	Not plausible, as *Aedes aegypti* does not interact with crop plants	Plausible • Adequate quality control of GDMI breeding facilities • Field populations of target organisms	Plausible • Adequate quality control of GDMI breeding facilities • Field populations of target organisms
5. Interactions with non-target organisms	Changes in abundance of species of conservation concern Changes in abundance or species composition of insectivorous vertebrates Changes in abundance or species composition of natural enemies, affecting pest regulation services	Toxic effects of the GDMI on non-target organisms (e.g., species of conservation concern, insectivorous vertebrates, natural enemies), lead to reductions in population size and loss of ecosystem services	Not plausible as *Aedes aegypti* is currently not a major component of diets of non-target organisms, and inhabits primarily urban domestic environments where few non-target organisms are present	Plausible for non-target organisms found in orchards and fruit- and berry-growing agro-ecosystems, including adjacent semi-natural areas (Table 25) • Potential toxicity of GDMI compared to non-GM insect counterpart through bioinformatic analysis	Plausible for non-target organisms found in *Brassica* agro-ecosystems (Table 26) • Potential toxicity of GDMI compared to non-GM insect counterpart through bioinformatic analysis

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Changes in abundance or species composition of pollinators and pollination services	Suppression of the target species reduces pollination of crops	Not plausible as *Aedes aegypti* does not pollinate crops	Not plausible as *Drosophila suzukii* does not pollinate crops	Not plausible as *Plutella xylostella* does not pollinate crops
	Changes in abundance or species composition of natural enemies, affecting pest regulation services	Suppression of the target species reduces food availability for non-target organisms (e.g., parasitoids, predators), resulting in population declines and loss of associated ecosystem services	Not plausible as *Aedes aegypti* is currently not a major component of diets of non-target organisms, and inhabits primarily urban domestic environments where few non-target organisms are present	Plausible • Strict dependency on *Drosophila suzukii* as food source (e.g. parasitic wasps)	Plausible • Strict dependency on *Plutella xylostella* as food source
	Change to ecosystem services such as water regulation and purification	Changes in availability of dead insect larvae alter aquatic food webs (e.g. algal growth), negatively affect water quality and aquatic non-target organisms	Not plausible as *Aedes aegypti* lays eggs in ephemeral water bodies (e.g. containers, tyres), limiting aquatic food-web interactions	Not plausible as larvae of *Drosophila suzukii* do not develop or live in aquatic environments	Not plausible as larvae of *Plutella xylostella* do not develop or live in aquatic environments
	Change to ecosystem services such as decomposition, nutrient cycling and disease suppression	Toxic effects of the GDMI on soil microorganisms involved in decomposition, nutrient cycling or disease suppression result in loss of these ecoservices	*Not plausible as Aedes aegypti* inhabits primarily urban domestic environments	Plausible • Potential toxicity of GDMI compared to non-GM insect counterpart through bioinformatic analysis	Plausible • Potential toxicity of GDMI compared to non-GM insect counterpart through bioinformatic analysis

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Reduced nutritional quality of GDMI	Reduced nutritional quality of the GDMI as prey diminishes predator performance and ecosystem functions	Not plausible as *Aedes aegypti* is currently not a major component of diets of non-target organisms, and inhabits primarily urban domestic environments where few non-target organisms are present	Plausible • Strict dependency on *Drosophila suzukii* as food source for predators in fruit-and berry-growing area	Plausible • Strict dependency on *Plutella xylostella* as food source for predators in *Brassica* agro-ecosystems
	Niche replacement	Population suppression of the target species leads to niche replacement by other species that reduce valued species or ecosystem services	Not plausible as no PTH can be envisaged how replacement of *Aedes aegypti* by *Aedes albopictus* (or other human disease vectors) will result in reduction of valued species or loss of ecosystem services in urban domestic habitats	Plausible • Potential niche replacement over prolonged time	Plausible • Potential niche replacement over prolonged time

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Changes in abundance or species composition of host organisms other than plants and the ecosystem services they provide	Population suppression results in changes in abundance or species composition of host organisms, leading to loss of ecosystem services	Not plausible as *Aedes aegypti* does not have a host organism other than humans	Not plausible as *Drosophila suzukii* has only plant hosts	Not plausible as *Plutella xylostella* has only plant hosts
	Changes in abundance or species composition of host plants and the ecosystem services they provide	Population suppression results in changes in abundance or species composition of host plants, leading to loss of ecosystem services	Not plausible as *Aedes aegypti* is not a plant pest, and inhabits urban domestic habitats where no crops are grown	Plausible • Range of host plants of *Drosophila suzukii* and ecosystem services that they provide	Plausible • Range of host plants of *Plutella xylostella* ecosystem services that they provide
6. Environmental impacts of specific techniques used for the management of GDMIs	Exploitation of different or additional environmental resources and management/control/recovery systems	GDMI release management requires the use of different environmental resources and/or different management, control, or recovery systems (e.g., changes in pesticide use) compared with current systems, resulting in adverse environmental impacts	Plausible • Release management of GDMIs compared with current management measures	Plausible • Release management of GDMIs compared with current management measures	Plausible • Release management of GDMIs compared with current management measures
	Resource use and waste production of GDMI production facilities	Production of GDMI requires different or greater resource inputs and generates different types or quantities of compared with current control methods, leading	Plausible • Production of GDMI compared with current control measures	Plausible • Production of GDMI compared with current control measures	Plausible • Production of GDMI compared with current control measures

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	*Drosophila suzukii*	*Plutella xylostella*
		to adverse environmental effects			
	Alteration of natural regulating ecosystem services (e.g. predation, parasitism)	Introduction of GDMI and associated changes in management practices alter natural regulating services (e.g. predation, parasitism) affecting ecosystem functioning, which requires other control methods, leading to adverse environmental effects	Not plausible as *Aedes aegypti* has no natural predators or parasites	Not plausible as *Drosophila suzukii* is not naturally suppressed by predators or parasites	Not plausible as *Plutella xylostella* is not naturally suppressed by predators or parasites
	Altered management and control of other (secondary) vector or pest species that arise through control of the primary target vector	Control of the primary target species using GDMI may lead to changes in management and control measures for secondary (non-target) vector or pest species, resulting in adverse environmental impacts	Not plausible, as management measures for *Aedes albopictus* are already established, and the measures applied for *Aedes aegypti* are expected to be the same; therefore, no additional or different management practices are anticipated	Plausible • Development of secondary vector/pest species populations	Plausible • Development of secondary vector/pest species populations

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Changes in agricultural or land management practices	GDMI release leads to changes in agricultural or land management practices compared with current practices, potentially resulting in adverse environmental effects	Plausible • GDMI-based management compared with current practices	Plausible • GDMI-based management compared with current practices	Plausible • GDMI-based management compared with current practices
	Changes in management responses to reduced efficacy of GDMIs	In cases of reduced GDMI efficacy, alternative or intensified management responses could be implemented, potentially leading to additional environmental impacts	Not plausible as management responses are expected to be the same as current measures	Not plausible as management responses are expected to be the same as current measures	Not plausible as management responses are expected to be the same as current measures
	Changes in program activities at release sites related to mosquito/pest species surveillance and trapping	GDMI release may lead to changes in program activities at release sites related to mosquito or pest surveillance and trapping, potentially resulting in adverse environmental effects	Not plausible as program activities are expected to be the same as current activities	Not plausible as program activities are expected to be the same as current activities animals	Not plausible as program activities are expected to be the same as current activities
7. Impacts on human, animal and plant health	Toxic, allergenic or other adverse effects of new compounds, their derived metabolic products and/or GDMI on humans and animals via biting	Changes in GDMI saliva composition (e.g., proteins/toxins/allergens /virulence factors expressed in saliva) lead to toxic, allergenic or other adverse effects in humans or animals following biting	Plausible • Screening of proteins expressed in saliva (bioinformatics for similarity to known toxins/allergens/virulence factors)	Not plausible as Drosophila suzukii does not bite humans or animals	Not plausible as *Plutella xylostella* does not bite humans or animals

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Toxic, allergenic or other adverse effects of new compounds, their derived metabolic products and/or GDMIs to humans and animals following accidental exposure (handling, ingestion or inhalation)	Accidental ingestion, inhalation, or contact with GDMIs or their products (e.g., scales, fragments, secretions) leads to toxic or allergenic effects in humans/animals	Plausible • Bioinformatics screening for toxins/allergens	Plausible for workers and bystanders in fruit and berry agro-ecosystems • Bioinformatics screening for toxins/allergens	Plausible for workers and bystanders in Brassica agro-ecosystems • Bioinformatics screening for toxins/allergens
	Water quality-mediated impacts	Aquatic life stages or larval presence in water bodies lead to release/exposure to harmful compounds, causing decreased water quality and adverse effects in humans/animals (e.g., drinking water exposure) or indirect impacts	Not plausible as *Aedes aegypti* lays eggs in ephemeral water bodies like pots and tyres	Not plausible as *Drosophila suzukii* does not breed or live in water	Not plausible as *Plutella xylostella* does not breed or live in water
	Loss of immunity in the human population following suppression by GDMI	Long-term suppression of a disease vector reduces population immunity ("herd immunity"), leading to higher disease incidence if the target vector resurges	Plausible • Disease incidence in relevant settings	Not plausible as *Drosophila suzukii* is not a vector for human or animal diseases	Not plausible as *Plutella xylostella* is not a vector for human or animal diseases

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
	Niche replacement	Suppression of the target species leads to niche replacement by other more harmful species (e.g., alternative vectors/pests), resulting in increased adverse impacts on human/animal health (disease)	Plausible • Identification of species with overlapping niches • Development of target and potential replacement species (e.g., *Aedes albopictus*)	Plausible for workers and bystanders in fruit and berry agro-ecosystems • Identification of species with overlapping niches • Development of target and potential replacement species	Plausible for workers and bystanders in Brassica agro-ecosystems • Identification of species with overlapping niches • Development of target and potential replacement species
	Niche replacement	Suppression of the target species leads to niche replacement by other more harmful species (e.g., alternative vectors/pests), resulting in increased adverse impacts on plant health (pest damage)	Not plausible as *Aedes aegypti* does not interact with plants	Plausible • Identification of species with overlapping niches • Crop damage/health	Plausible • Identification of species with overlapping niches • Crop damage/health
	Change in GDMI phenotype resulting in more crop damage	An altered phenotype of the GDMI (feeding/oviposition/larval development) increases crop damage	Not plausible as *Aedes aegypti* does not interact with plants	Plausible • Comparison of GDMI and non-GDMI with respect to phenotype • Development of crop damage, yield	Plausible • Comparison of GDMI and non-GDMI with respect to phenotype • Development of crop damage, yield

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Risk areas[b]	Hazard examples for GDMIs[c] Potential harm resulting from:	Examples of plausible PTHs for GDMIs	Plausibility for GDMI species relevant for the EU (and potential assessment endpoints) *Aedes aegypti*	 *Drosophila suzukii*	 *Plutella xylostella*
8. Evolutionary and stability considerations	Changes in the GDMI genotype and phenotype, including marker genes and other expressed genes; genetic rearrangements or mutations occurring at measurable rates; and synergistic interactions or unexpected phenotypic effects arising from stacked transgenic modifications	Unintended genetic changes in GDMIs arising over prolonged time periods may lead to: -phenotypic changes relevant for ERA, and/or -loss or reduced reliability of marker genes used for monitoring and surveillance, and/or -reduced efficacy of the GDMI (see also Interactions with target organisms and Pathogens, infections and diseases)	Plausible • Occurrence of genetic or phenotypic changes in GDMI populations over prolonged time • Use of alternative or redundant markers to ensure reliable monitoring and surveillance • Modelling of evolutionary dynamics and stability of the engineered gene drive construct over time	Plausible • Occurrence of genetic or phenotypic changes in GDMI populations over prolonged time • Use of alternative or redundant markers to ensure reliable monitoring and surveillance • Modelling of evolutionary dynamics and stability of the engineered gene drive construct over time	Plausible • Occurrence of genetic or phenotypic changes in GDMI populations over prolonged time • Use of alternative or redundant markers to ensure reliable monitoring and surveillance • Modelling of evolutionary dynamics and stability of the engineered gene drive construct over time
9. Control, emergency measures	Reduced control capability due to changes in insecticide susceptibility of the GDMI	Loss or reduction of susceptibility of the GDMI to insecticides as a consequence of the genetic modification may limit the effectiveness of emergency or back up control measures, thereby reducing the capacity to manage or contain the GDMI if needed	Plausible (as a potential consequence of the genetic modification) • Testing of susceptibility/resistance of the GDMI to the most relevant insecticides compared with the non-GDMI prior to release	Plausible (as a potential consequence of the genetic modification) • Testing of susceptibility/resistance of the GDMI to the most relevant insecticides compared with the non-GDMI prior to release	Plausible (as a potential consequence of the genetic modification) • Testing of susceptibility/resistance of the GDMI to the most relevant insecticides compared with the non-GDMI prior to release

[a] PTH considered plausible and potentially risk-relevant are indicated (grey shading), while PTHs considered not plausible and therefore very unlikely to result in risk are not highlighted.

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[b] Based on EFSA (2013), Legros et al. (2021), Kormos et al. (2023) and Hayes & Hosack (2025)
[c] Based on EFSA (2013) NASEM (2016), Roberts et al. (2017), James et al. (2018), Rüdelsheim & Smets (2018), Teem et al. (2019), Devos et al. (2020), EFSA (2020), Connolly et al. (2021), WHO (2021), Kormos et al. (2023), Hayes & Hosack (2025) and SCBD (2025)
[d] Horizontal gene transfer is considered biologically possible but highly unlikely, as multiple conditions must be met for the transferred construct to remain intact, be expressed, and function as a gene drive. The likelihood may be increased if homologous target sequences of homing-based gene drives are present in non-target insects (EFSA, 2020).

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4.2.3.4 SQ1.3.3 – Based on the outcome of SQ1.3.2, what are the most plausible PTHs?

To address SQ1.3.3, PTHs that were considered plausible were identified for GDMI cases of *Aedes aegypti, Drosophila suzukii* and *Plutella xylostella.* This identification was based on existing knowledge of the biology and ecology of these insect species, as well as the characteristics of their receiving environments in the EU and its OMTs (Tables 24-26).

As mentioned above, it is assumed that GDMI cases of *Aedes aegypti* (a human disease vector) may be intended for either population modification or population suppression, while GDMI cases of *Drosophila suzukii and Plutella xylostella* (agricultural pest insects) are intended only for population suppression.

The plausible PTHs identified for each species, together with the most relevant assessment endpoints that can be used to test these PTHs, are summarised in Table 27 (columns 4–6). The ecological characteristics of the three insect species that are relevant for determining the plausibility of the PTHs listed in Table 27 are described below.

4.2.3.5 Relevant ecological characteristics of the three insect species.

Aedes aegypti

Aedes aegypti is a highly anthropophilic mosquito species. Adults predominantly reside indoors in human dwellings and typically disperse over relatively short distances, generally not exceeding 100 m. The species breeds mainly in artificial containers provided by humans, including containers located indoors, and almost exclusively (99%) feeds on human blood. Only females bite, and blood meals are required for egg development (OECD, 2018).

Hybridisation potential

Viable hybrids between *Aedes aegypti* females and *Aedes albopictus* males have been produced under laboratory conditions, but such events are rare. In the field, interspecific mating has been observed at low frequencies (1.12–3.73%) in areas where both species occur in sympatry (Bargielowski et al., 2015). Overall, hybridisation between *Aedes aegypti* and *Aedes albopictus* is considered unlikely (OECD, 2018).

Niche overlap

The ecological niches of *Aedes aegypti* and *Aedes albopictus* can overlap, particularly in anthropogenic environments. In regions where both species occur, competitive displacement of *Aedes aegypti* by *Aedes albopictus* has been documented (Bargielowski et al., 2015).

Biotic interactions

Aedes aegypti has a relatively narrow ecological niche. Larvae and pupae develop in small, artificial water containers that are often only intermittently flooded. These habitats rarely sustain stable populations of aquatic predators, such as fish or amphibians, and trophic interactions are therefore limited. As a result, endemic species in natural subtropical ecosystems are only weakly or not at all trophically connected to *Aedes aegypti* populations (OECD, 2018).

Drosophila suzukii

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Drosophila suzukii is an invasive species originating from South East Asia and is now a major global pest of soft and stone fruits. Females oviposit in ripening fruit, and larvae feed within the fruit pulp. The species has a broad host range in both crop and non-crop plants, facilitating its spread across fruit-growing regions in Europe, Asia and the Americas (Kirschbaum et al., 2020).

Hybridisation potential

Hybridisation between *Drosophila suzukii* and closely related European *Drosophila* species (*Drosophila ananassae*, *Drosophila melanogaster*, *Drosophila simulans*; all Diptera: Drosophilidae) is considered highly unlikely under natural conditions, as experimental crosses did not result in viable hybrid offspring (Wolf et al., 2023).

Niche overlap

Although *Drosophila suzukii* and *Drosophila melanogaster* occupy partially distinct niches, *Drosophila melanogaster* may oviposit in fruit damaged by *Drosophila suzukii*. In direct interspecific competition, *Drosophila melanogaster* larvae often show higher survival, partly due to greater tolerance to ethanol produced during fruit fermentation (Gao et al., 2018).

Biotic interactions

Drosophila suzukii is subject to predation and parasitism by a range of natural enemies, including parasitoids, predators and entomopathogenic organisms. Parasitoids are the most extensively studied group, with several larval and pupal parasitoid species occurring in Europe. Generalist predators such as ants, spiders, earwigs and rove beetles have also been observed attacking *Drosophila suzukii* larvae and pupae in infested fruit (Kirschbaum et al., 2020).

Plutella xylostella

Plutella xylostella is a highly invasive pest of cruciferous crops and is considered one of the most widely distributed Lepidoptera species globally. Neonate larvae mine into leaf tissue immediately after hatching and later feed externally, causing substantial crop damage. Adults are highly migratory and can disperse over long distances, including transboundary and intercontinental movements facilitated by wind currents (CABI, 2025).

Hybridisation

There are no known closely related insect species present in the EU or its OMTs with which *Plutella xylostella* can hybridise (CABI, 2025).

Niche overlap

There are no known insect species inhabiting the same niches as *Plutella xylostella.*

Biotic interactions

All life stages of *Plutella xylostella* are subject to predation and parasitism. In Europe, a diverse complex of parasitoid species has been documented, with larval parasitoids being particularly abundant. Generalist predators, including ground beetles, lady beetles, lacewings,

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ants and spiders, also contribute to mortality of *Plutella xylostella* populations (Haye et al., 2021; CABI, 2025).

4.2.3.6 Plausible PTHs for Aedes aegypti, Drosophila suzukii and Plutella *xylostella*

Although some overlap exists in the types of PTHs identified for the three insect species, Table 27 clearly shows that the plausibility of PTHs differs markedly between them.

- PTHs related to human health are mainly plausible for GDMI cases of *Aedes aegypti*, reflecting its role as a disease vector and its close association with human habitats.
- PTHs related to plant and crop health are primarily plausible for GDMI cases of the agricultural pest species *Drosophila suzukii* and *Plutella xylostella*.
- Plausible PTHs for non-target organisms are considered limited for *Aedes aegypti*, given its urban, domestic habitat and weak trophic integration. In contrast, exposure of non-target organisms is more likely for *Drosophila suzukii* and *Plutella xylostella*, which occur in agro-ecosystems.

Illustrative examples of PTHs and their plausibility for each species are presented in Figures 1–3 for the areas of risk:

- Persistence and invasiveness,
- Interactions with non-target organisms, and
- Impacts on human, animal and plant health.

Persistence and invasiveness

For the risk area "Persistence and invasiveness" (Table 27, item 1), the assessment considers whether genetic modification with an engineered gene drive construct could result in increased persistence or invasiveness of GDMIs, for example through spread beyond the intended receiving environments, potentially leading to adverse effects on human, animal, plant, or environmental health (Figure 14).

In this context, it is important to recognise that engineered gene drive constructs and their associated genetic cargo are designed to spread through populations, even in the presence of fitness costs. Consequently, the focus of plausible PTHs is on unintended persistence or spatial spread of the engineered gene drive construct beyond the intended receiving environments. Such spread could arise, for example, from unintended increases in fitness, enhanced tolerance to environmental conditions, or hybridisation with closely related species present in the receiving environment.

Unintended persistence or spread may result in adverse effects occurring outside the intended receiving environments of the GDMI. Where such PTHs are considered plausible, the ERA is therefore extended beyond the intended receiving environments and addresses all subsequent risk areas (items 2–9 in Table 27) for these additional or extended environments.

The nature and potential severity of adverse effects associated with unintended spread and persistence depend on:

- The intended function of the gene drive (population suppression or population modification), and
- The temporal and spatial scale of its activity (self-limiting or self-sustaining).

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PTHs related to increased fitness or increased tolerance to (a)biotic conditions are considered plausible for all GDMIs, as these may arise from the genetic modification process itself. Such PTHs can be addressed under contained conditions by comparative testing of GDMIs and non-GDMIs for fitness-related traits (e.g., development, phenotype, reproduction) and tolerance to relevant (a)biotic conditions in the receiving environment. These assessments are required for all GDMIs prior to any environmental release.

By contrast, PTHs related to hybridisation with closely related species are not considered plausible for the three insect species assessed here, based on the available body of evidence for *Aedes aegypti* (OECD, 2018) and *Plutella xylostella* (CABI, 2025), and on experimental hybridisation studies for *Drosophila suzukii* (Wolf et al., 2023) (Table 27).

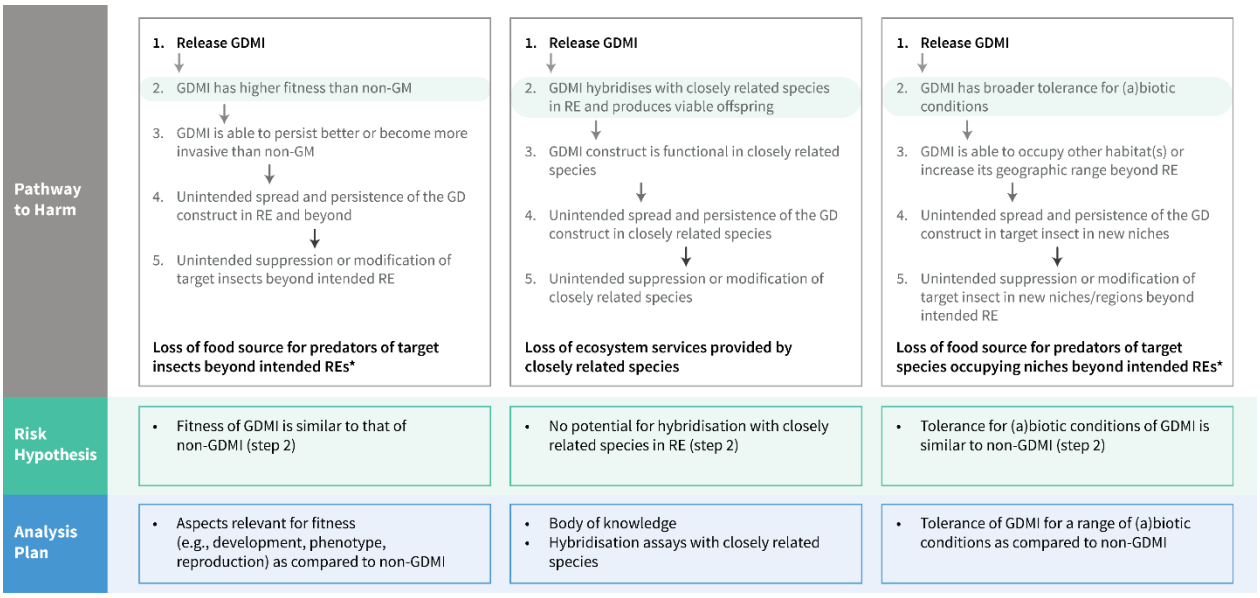


Figure 14: Examples of plausible pathways to harm (PTHs) for gene drive (GD) modified insects (GDMIs) related to persistence and invasiveness (including risk hypotheses and analysis plans). RE: receiving environment. Where unintended spread is plausible, the environmental risk assessment (ERA) is extended to include REs beyond the intended RE for all risk areas (2–9) listed in Table 27.

Interactions with non-target organisms

For the risk area "Interactions with non-target organisms" (Table 27, item 5), the assessment considers whether genetic modification of the insect with an engineered gene drive construct could result in adverse effects on non-target organisms, compared with the corresponding non-GM insect (Figure 15). Plausible hazards and PTHs for GDMIs are considered broadly similar to those described for GM insects (EFSA, 2013, 2020). These include, for example, loss of food resources for predators, niche replacement following population suppression, and reduction of valued species or ecosystem services due to potential toxicity of the GDMI (Table 27).

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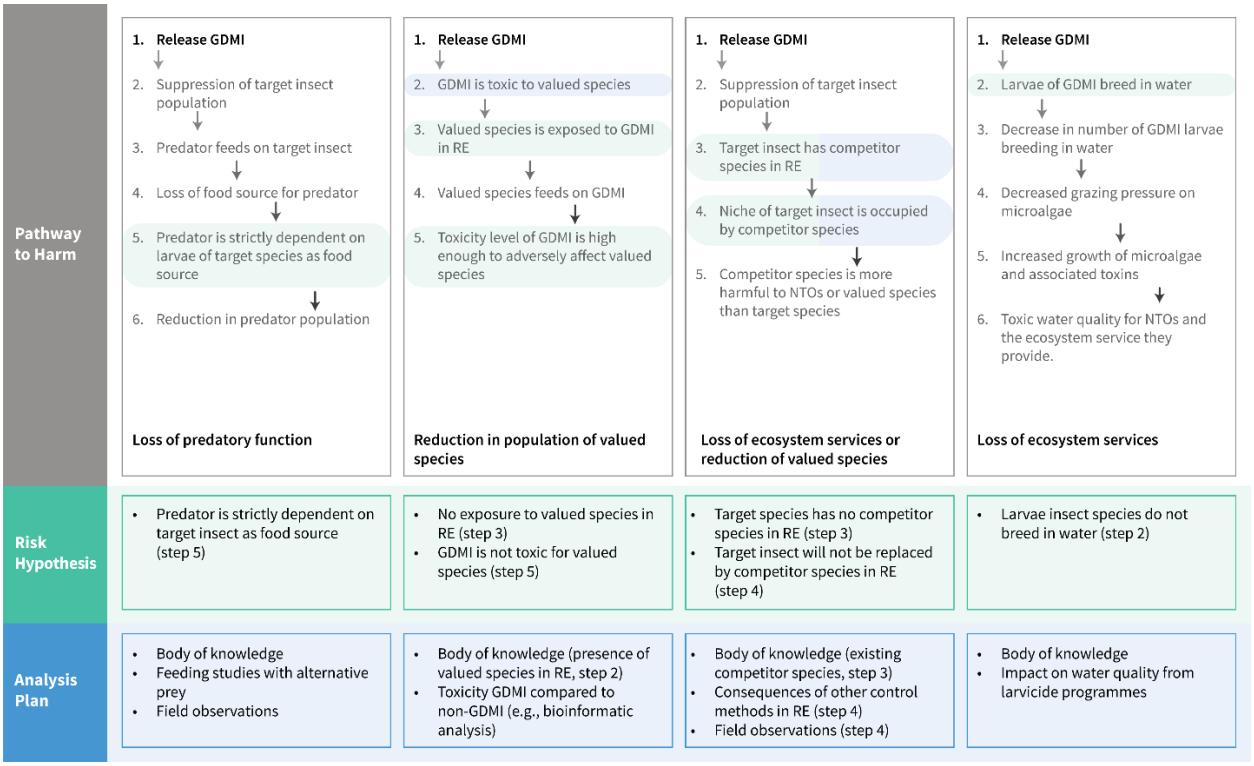


Figure 15: Examples of plausible pathways to harm (PTH) of gene drive modified insects (GDMIs) to non-target organisms (including risk hypotheses and analysis plans). RE: receiving environment.

For *Aedes aegypti*, PTHs leading to adverse effects on non-target organisms (including valued species or non-target organisms providing ecosystem services) are considered not plausible, due to the predominantly urban and domestic habitat of this species and the absence of credible exposure pathways for non-target organisms in such environments. The plausibility of adverse effects on aquatic non-target organisms and associated ecosystem services is also considered low, as *Aedes aegypti* larvae breed mainly in small, artificial containers where trophic interactions are limited or absent (OECD, 2018; Table 27).

In principle, water quality in urban water bodies could be affected indirectly through reduced grazing pressure on micro-algae if suppression of *Aedes aegypti* larvae were to occur, potentially leading to algal blooms and associated toxins. However, the plausibility of this PTH is considered very low, since *Aedes aegypti* larvae do not typically breed in natural or semi-natural urban water bodies but almost exclusively in artificial containers (Table 27).

Potential adverse effects on aquatic species or water quality are not considered plausible for *Drosophila suzukii* and *Plutella xylostella*, as larvae of these species do not breed or live in water (Table 27). Nevertheless, PTHs affecting other non-target organisms are considered plausible for these species, but are expected to be limited to non-target organisms inhabiting agro-ecosystems associated with fruit and berry production (*Drosophila suzukii*) or *Brassicaceae*-based cropping systems (*Plutella xylostella*), as well as adjacent semi-natural habitats.

Impacts on human, animal health and plant health

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For the risk area "Impacts on human, animal and plant health", the assessment considers whether genetic modification with an engineered gene drive construct may result in adverse effects on humans, animals, or plants, compared with non-GDMIs (Figure 16).

Adverse effects on human health may arise from changes in the capacity of the GDMI to transmit human diseases (Table 27, item 3), as well as from changes in the biological properties of the insect itself (Table 27, item 7). Examples of the latter include potential toxic or allergenic effects associated with proteins expressed in GDMI saliva that could affect human or animal health through biting, or adverse effects related to reduced water quality resulting from toxicity of larvae breeding in water bodies. These PTHs can be addressed by comparative testing of the potential toxicity of saliva or larvae from GDMIs and non-GDMIs, for example through bioinformatic analyses.

Such PTHs are considered plausible only for *Aedes aegypti* (with respect to human health), and not plausible for *Drosophila suzukii* or *Plutella xylostella*, as the latter species do not bite humans or animals and their larvae do not breed or live in water. Additional PTHs may arise from potential toxicity following ingestion or inhalation of GDMIs. These are considered plausible for humans in the case of *Aedes aegypti*, and for workers and bystanders in agro-ecosystems where *Drosophila suzukii* or *Plutella xylostella* are present.

Other adverse effects on human health may result indirectly from loss of population immunity to diseases vectored by the GDMI following long-term suppression of the target vector (loss of "herd immunity"). In the event of vector resurgence, disease incidence in humans could therefore increase. Such PTHs are considered plausible only for *Aedes aegypti* and are informed by existing surveillance activities.

Additional PTHs may result from niche replacement, whereby suppression of the target species allows other, potentially more harmful species to occupy the vacated ecological niche. This is most plausible for species known to compete for similar niches as *Aedes aegypti*, *Drosophila suzukii*, or *Plutella xylostella*. In the case of *Aedes aegypti*, the most likely candidate is *Aedes albopictus*, which vectors the same diseases but has a broader host range. Other vector species, such as *Anopheles* spp., may also occupy these niches in the EU's outermost territories. These PTHs can be evaluated using evidence from other *Aedes aegypti* control measures and through field observations conducted within existing surveillance programmes.

For *Drosophila suzukii* and *Plutella xylostella*, it is less clear which species might replace these pests following suppression, as current control methods have not proven fully effective. In such cases, monitoring of crop damage (e.g., in fruit, berry, or cruciferous crops) within existing agricultural surveillance frameworks represents the most appropriate approach addressing these PTHs.

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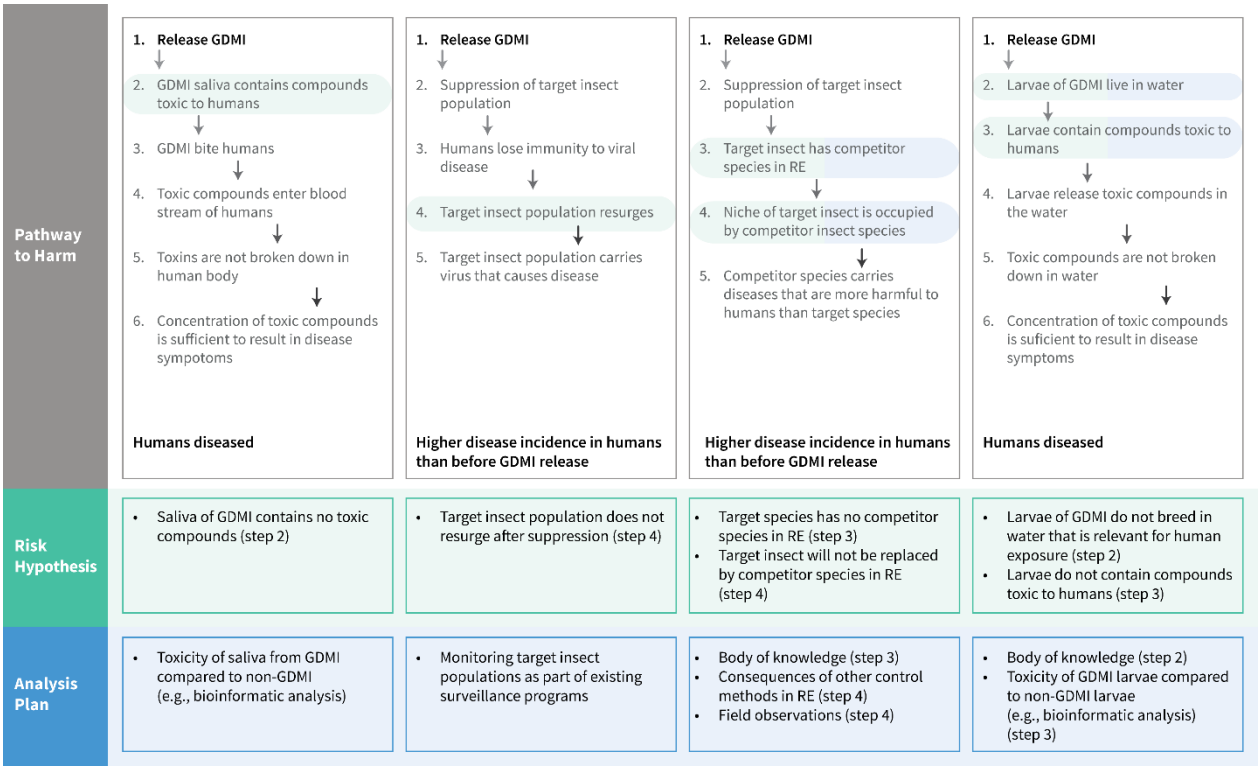


Figure 16: Examples of plausible pathways to harm (PTHs) to human and animal health (including risk hypotheses and analysis plans). RE: receiving environment.

4.2.3.7 SQ1.3.4 – What are the risks to human, animal and environmental health resulting from the PTHs identified in SQ1.3.3 and what is the likelihood and magnitude of these risks?

This SQ focuses on the risks to human, animal and environmental health that may arise from the plausible PTHs identified for *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella* in SQ1.3.3, and on the likelihood and magnitude of these risks. As no specific GDMI cases have yet been developed for these insect species and there is no experience with deliberate environmental release of GDMIs to date, this assessment can only be addressed at a generic level, based on existing knowledge of the species, their ecology and their receiving environments.

Risks and their likelihood

Although no concrete GDMI cases are currently available for deliberate environmental release for *Aedes aegypti*, *Drosophila suzukii* or *Plutella xylostella*, application of the problem formulation approach allows certain risks to human, animal and environmental health to be ruled out or considered very unlikely, based on well-established knowledge of these insect species and their receiving environments.

For example, PTHs related to viral infection of humans or animals are not plausible for agricultural pest insects such as *Drosophila suzukii* and *Plutella xylostella*, whereas such PTHs are plausible and may result in risks to human health for the disease vector *Aedes aegypti*.

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Conversely, based on ecological considerations, risks to plant health can be ruled out for *Aedes aegypti*, but not for *Drosophila suzukii* and *Plutella xylostella*.

It should be noted that some PTHs related to phenotypic differences arising from the genetic modification are considered plausible for all three insect species and could, in principle, result in risk. Examples include changes in fitness-related traits, increased tolerance to (a)biotic conditions, or altered susceptibility to insecticides, which could lead to increased persistence or reduced effectiveness of emergency control measures. However, the likelihood of such risks is expected to be very low, as phenotypic characterisation of GDMIs is a standard component of ERA. GDMIs that exhibit differences in characteristics relevant to environmental risk assessment or risk management, compared with their non-GDMI counterparts, would not be selected for deliberate environmental release.

For other potential risks associated with the deliberate environmental release of GDMIs, plausible PTHs can be addressed by focusing on assessment endpoints corresponding to the most critical step of the pathway, or to steps for which information can be obtained in the most reliable and cost-effective manner. These assessment endpoints are identified in Table 27.

Some potential risks are not directly related to the GDMI itself but rather to its intended effect, particularly in the case of population suppression. Examples include loss of food resources for predators that feed on *Aedes aegypti*, *Drosophila suzukii* or *Plutella xylostella*, or loss of ecosystem services provided by these insects. Such effects would not occur in the case of population modification and are not novel, as they are comparable to impacts associated with other control methods, such as insecticide use or biological control (Tables 24-26). Risks arising from suppression of these insect populations by GDMIs should therefore be evaluated in the broader context of risks associated with existing control strategies (Romeis et al., 2020; see also ToR3).

Risk and magnitude

The magnitude of potential risks to human, animal and environmental health depends on both the type of engineered gene drive construct (self-sustaining or self-limiting) and its intended function (population suppression or population modification).

In general, if risks are identified in a case-specific ERA, higher exposure—and therefore potentially higher risk—may be associated with self-sustaining engineered gene drives, as these constructs are expected to persist longer in the environment and to spread over larger geographical areas. In contrast, self-limiting engineered gene drives are expected to remain active for shorter periods and to exhibit more limited dispersal. The magnitude of risk associated with self-limiting engineered gene drives is therefore considered comparable to that of GM insects without an engineered gene drive, such as self-limiting technologies already authorised or tested under contained or semi-field conditions (e.g., Romeis et al., 2020).

The intended function of the engineered gene drive also influences exposure and risk magnitude. For population suppression, populations of the target insect—and consequently GDMI populations—are expected to decline over time, particularly for self-sustaining engineered gene drives, resulting in decreasing environmental exposure and potentially lower risk. By contrast, population modification using self-sustaining engineered gene drives may result in prolonged or continuous environmental exposure, which could lead to higher risks if

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such risks are identified in ERA (Table 27). Whether increased exposure translates into increased risk must be evaluated on a case-by-case basis.

As noted under Evolutionary and stability considerations (Table 27), prolonged exposure may increase the likelihood of adverse effects arising from unintended molecular or phenotypic changes accumulating over time, particularly for GDMIs intended for population modification.

Conclusion

Following AQ 1.3, hazards associated with GDMIs identified in the literature were structured according to established GM insect risk areas (EFSA, 2013) (SQ1.3.1), and plausible PTHs resulting from deliberate environmental release were identified (SQ1.3.2). These PTHs were evaluated for plausibility for *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella*, based on species-specific biology, ecology and receiving environments (SQ1.3.3). Using this approach, PTHs affecting human health were found to be most plausible for GDMI *Aedes aegypti*, whereas PTHs affecting agro-ecosystems and plant health were most plausible for GDMI *Drosophila suzukii* and *Plutella xylostella*. However, in the absence of concrete GDMI cases for these species, no definitive conclusions can be drawn regarding the risks or their magnitude following deliberate environmental release (SQ1.3.4).

4.3 ToR3 – Review of comparators

4.3.1 AQ3.1 – What comparators could be used to support the risk assessment of GDMIs, including the most appropriate endpoints and approaches of comparison, and for which risk assessment purposes such single or multiple comparators could be utilised.

4.3.1.2 Use of comparators in risk assessment and decision-making

Identifying appropriate comparators for the risk assessment of GDMIs requires an understanding of how comparators are used in existing risk assessment frameworks. This includes not only GMO risk assessment, but also comparator use across other regulatory and ERA contexts. Examining these approaches helps determine which comparator types, endpoints and comparison strategies are relevant and transferable to GDMIs.

4.3.1.3 Comparators in GMO risk assessment

Purpose and form of comparators

The comparative approach is a foundational element of modern GMO risk assessment (OECD, 1986) and is embedded in key international and regional frameworks, including Annex III of the Cartagena Protocol on Biosafety, Codex Alimentarius Guidelines, and EU risk assessment guidance. The primary purpose of this approach is to ensure that risk assessments focus on risks that are new, novel or different compared with those associated with the unmodified organism.

By situating the GMO within the context of its conventional counterpart, and potentially within the context of other organisms or alternative technologies that could deliver similar outcomes, the comparative assessment provides structure and proportionality to the evaluation. Comparisons are used to identify relevant differences rather than to demonstrate similarity

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per se, thereby supporting a focused assessment of those differences that could plausibly lead to harm. This approach extends across molecular characterisation (MC), ERA and, where relevant, PMEM.

Use of comparators in MC

Within MC, the comparative assessment aims to identify molecular-level differences between the GMO and its most closely related conventional comparator. This typically includes genotypic differences introduced by the genetic modification, as well as molecular-level phenotypic traits such as the presence, structure and expression of novel gene products.

The role of comparators at this stage is not to establish risk directly, but to support hazard identification by highlighting differences that may be biologically relevant. Only those differences for which a plausible PTH can be articulated are subsequently considered in the ERA. In this way, MC acts as an entry point for the comparative risk assessment, helping to prioritise further evaluation.

The most appropriate comparator for MC is usually a near-isogenic line or the closest available genetic relative. This minimises background genetic variation and ensures that observed differences can be attributed with confidence to the genetic modification rather than to natural variation within the species.

Use of comparators in ERA

The ERA places greater emphasis on phenotypic and ecological comparisons. These include comparisons of life-history traits, behaviour, fitness, and ecological interactions between the GMO and its comparator, as well as differences in intended use, management practices or receiving environments.

At this stage, the scope of comparators may broaden beyond a single near-isoline to include a wider range of genetic backgrounds representative of the species as it occurs in the environment. This supports the establishment of an appropriate baseline against which potential environmental effects can be evaluated. Comparisons during ERA focus on differences that may affect defined environmental protection goals, such as biodiversity, ecosystem functioning or the persistence and spread of the organism.

Depending on the nature and purpose of the GMO, ERA may also involve comparisons with alternative approaches or technologies that achieve similar objectives. Such comparisons can help contextualise potential environmental effects but do not replace the comparison with biologically relevant organisms.

Use of comparators in PMEM

Comparators can also play an important role in risk management and PMEM. In this context, the emphasis shifts from hypothesis-driven risk assessment to the practical detectability, monitoring and interpretation of potential effects under field conditions.

PMEM comparisons often focus on wild-type organisms of the same species rather than on specific near-isogenic lines. This reflects the need to understand how the GMO behaves, persists and interacts within the broader environmental context. Differences between the GMO and wild populations may be exploited to facilitate monitoring, for example through the

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use of molecular, phenotypic or behavioural markers that enable detection, tracking or discrimination.

Comparisons may also consider differences in collection, counting or surveillance methods, as these can influence the feasibility and sensitivity of PMEM. Overall, comparator use in PMEM supports both the identification of unexpected adverse effects and the refinement of monitoring strategies over time.

Summary of comparator use across risk assessment phases

Across MC, ERA and PMEM, the choice and role of comparators evolve in line with the objectives of each risk assessment phase. Close genetic comparators are most relevant for identifying modification-related differences during MC, whereas broader genetic and ecological comparators are needed to support ERA. PMEM relies on comparisons with wild populations to enable practical surveillance and environmental interpretation. Together, these complementary uses of single or multiple comparators underpin a coherent, proportionate and problem-formulated risk assessment framework that can be adapted to GDMIs.

4.3.1.4 SQ3.1.1 – What are the proposed uses and organisms being considered for GDMI, and how do these uses inform comparator selection?

Comparator selection in the context of proposed GDMI uses

The selection of appropriate comparators for GDMIs must be informed by the intended use of the GDMI and the biological and ecological characteristics of the target organism. Before comparators can be identified, it is therefore necessary to clarify the proposed uses and mechanisms of action of GDMIs under consideration.

Based on the outcomes of ToR1 and ToR2, GDMIs are being developed primarily for the control of insect populations that pose risks to human health or agriculture. These include vectors of human diseases as well as agricultural pest species. Across these potential GDMI applications, GDMIs are designed for the control of agricultural pests or vectors of human diseases, either by reducing pest populations or by reducing their ability to transmit disease. Accordingly, two broad categories of intended use can be distinguished: population suppression and population modification.

4.3.1.5 Considerations for selecting comparators for population suppression GDMIs

Population suppression GDMIs and implications for comparator selection

Population suppression refers to a range of approaches through which the release of a GDMI results in a reduction of the target insect population. This may be achieved through mechanisms such as reduced survival, impaired reproduction, sex-ratio distortion or other effects that lead to population collapse.

From a functional perspective, the intended use of population suppression GDMIs is analogous to established pest and vector control strategies. These include the use of biological control agents such as predators, parasitoids or pathogens, as well as chemical control methods including insecticides and larvicides. Comparable objectives are also pursued through sterile insect techniques (SIT), including classical SIT and genetically-based sterile insect release approaches.

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A defining feature of population suppression GDMIs is the expectation that the GDMI will be released into the environment, spread rapidly through the target population, exert its suppressive effect, and ultimately decline and disappear within a defined timeframe. The duration of persistence will depend on the molecular design of the engineered gene drive system and the demographic and ecological dynamics of the target population. Nevertheless, in contrast to population modification strategies, the intended endpoint is the elimination of the GDMI from the environment once the population suppression objective has been achieved.

In this context, comparator selection should focus on organisms or interventions that represent an appropriate baseline for evaluating environmental effects arising from population reduction. Comparators may include wild-type populations of the target species, populations subject to conventional SIT or biological control measures, or baseline environmental conditions in the absence of intervention. The relevance of these comparators lies in their ability to contextualise population level effects and associated ecological consequences, rather than in strict genetic similarity to the GDMI.

Population modification GDMIs and implications for comparator selection

Population modification GDMIs are designed to alter the genetic composition of an existing population through the introduction of one or more genetic elements that are intended to persist for a defined period. Rather than reducing population size, these systems aim to modify specific traits, such as vector competence or susceptibility to pathogens, thereby reducing the harmful impact of the population.

Although population modification may not always be intended to result in permanent genetic change, GDMIs developed for this purpose are generally expected to persist in the environment for longer periods than population suppression systems. This extended presence increases both the duration and scope of potential environmental exposure.

As a result, comparator selection for population modification GDMIs typically requires more extensive and sustained comparison with relevant wild-type populations. Such comparisons are necessary to capture long-term phenotypic, ecological and evolutionary effects and to assess interactions with the receiving environment over time. Comparators therefore play a critical role in establishing appropriate baselines for prolonged exposure scenarios and in supporting the identification of gradual or cumulative effects that may not be apparent over shorter timescales.

4.3.1.6 SQ3.1.1 Based on the results of GDMI identified under Objective 1 as relevant for the EU and its outermost territories, identify activities that are comparable to the use of GDMI insects for the proposed purposes (e.g. if GDMI are being proposed for pest population control, identify other pest population control activities).

Comparable activities and technologies relevant to GDMI use

Based on the GDMIs identified under ToR1 as potentially relevant for the EU and its OMTs, the predominant proposed use is the control of pest insect populations, including both medically relevant disease vectors and agricultural pests. To support the identification and selection of appropriate comparators for ERA, it is therefore informative to examine existing activities, technologies and regulatory frameworks that pursue similar objectives.

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A review of applicable laws and regulations in the EU and its OMTs, alongside comparable frameworks in other countries, indicates that several pest control approaches are relevant comparators in this context. These include biological control strategies such as classical biological control (CBC), augmentative biological control (ABC), sterile insect release, and genetic SIT, as well as chemical control methods including the use of pesticides, larvicides and chemical trapping systems.

Across these regulatory contexts, comparisons are often used to frame risk assessment by relating new interventions to established practices, baseline environmental conditions, or alternative control measures. Examining how such comparisons are applied in existing assessments provides useful insights into how comparator-based approaches can support the ERA of GDMIs, particularly where objectives, scales of action and environmental contexts overlap.

4.3.1.7 SQ3.1.2 – How are comparators used in risk assessment for similar activities, including risk assessments for human health, and ERAs?

Table 28: Summary of biocontrol regulations and guidance documents reviewed

Regulation / Guidance document	Type of risk or impact assessment	Use of comparators
FAO (2005) – ISPM 3: Guidelines for the export, shipment, import and release of biological control agents and other beneficial organisms	Pest Risk Analysis conducted in accordance with IPPC standards (ISPM 2 and ISPM 11, stage 2), focusing on identification and characterisation of potential risks associated with release	**No.** The framework assesses risks associated with the organism itself, without explicit comparison to alternative pest management approaches
OECD (2004) – Guidance for Information Requirements for Regulation of Invertebrates as Biological Control Agents (IBCAs)	Risk assessment for human health and safety and environmental risks	**Yes.** Comparative elements are explicitly included, requiring information on the beneficial effects of release compared to current or alternative pest management strategies; benefits should be described relative to alternative control methods, resistance management and level of control
EPPO (2024) – PM 6/2 (4) Import and release of non-indigenous biological control agents	Impact assessment covering risks and benefits associated with release	**No (implicit only).** While benefits are considered, a systematic comparison with alternative pest management options is not formally required
NAPPO (2015a) – RSPM 7: Guidelines for petition for first release of non-indigenous	Assessment of environmental and economic impacts, including effects on pests and non-targets, known impacts on humans, and impacts on the physical environment	**Yes.** Includes cost-benefit assessment comparing biological control agents with pesticide use

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Regulation / Guidance document	Type of risk or impact assessment	Use of comparators
phytophagous biological control agents		
NAPPO (2015b) – RSPM 12: Guidelines for petition for first release of non-indigenous entomophagous biological control agents	Assessment of environmental and economic impacts, including effects on pests and non-targets, known impacts on humans, and impacts on the physical environment	**Yes.** Includes cost-benefit assessment comparing biological control agents with pesticide use
IOBC/WPRS Recommendations (Bigler et al., 2005)	Assessment of risks to human and animal health, environmental risks, non-target effects, and potential direct effects on host plants of the targeted pest	**No.** The recommendations are hazard-focused and do not include explicit comparison with alternative pest management strategies

Biocontrol regulations

It is worthwhile to consider how BCAs have historically been regulated, as this provides useful context for the selection of comparators in risk assessment. The use of biocontrol organisms can broadly be divided into CBC and genetic control methods, the latter including SIT, non-driving GMIs, and Wolbachia-infected insects (Romeis et al., 2020).

Classical biocontrol

CBC involves the deliberate environmental release of non-native predators, parasitoids or other natural enemies of invasive pests, typically sourced from the pest's region of origin. The objective is that these introduced organisms establish self-sustaining populations in the receiving environment, providing long-term suppression of the target pest.

Although CBC has a long history, for much of that time deliberate environmental releases were carried out with little or no statutory or structured risk assessment. It is now widely accepted, however, that the intentional release of exotic organisms for CBC should be preceded by a formal ERA. Guidance documents have been developed by several international and regional organisations, including the Food and Agriculture Organization of the United Nations (FAO, 2005), the Organisation for Economic Co-operation and Development (OECD, 2004), the European and Mediterranean Plant Protection Organization (EPPO, 2024), the North American Plant Protection Organization (NAPPO, 2015a, 2015b), and the Western Palaearctic Regional Section of the International Organization for Biological Control of Noxious Animals and Plants (IOBC/WPRS) (Bigler et al., 2006). However, incorporation of these guidelines into binding regulatory frameworks remains inconsistent, and many countries still lack specific, dedicated regulation for CBC (Mason et al., 2017; Romeis et al., 2020).

Risk assessment for CBC focuses on the careful characterisation of the organism to be introduced, including its taxonomic identity, strain and biotype. Available evidence on host or prey specificity is reviewed using information from the organism's native range and from other regions where it has already been released. In addition, experimental studies are conducted

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to assess potential impacts on non-target organisms in the area of introduction. These studies typically follow a tiered testing approach, starting with conservative, worst-case no-choice laboratory experiments and, if necessary, progressing to more realistic and ecologically relevant assays to determine the likelihood and magnitude of potential environmental effects following release.

Genetic biocontrol

Genetic biocontrol encompasses a range of approaches that use genetic mechanisms as part of pest control programmes. The most established example is SIT, which involves the mass release of sterilised male insects to compete with wild-type males for mating opportunities, thereby reducing the reproductive output of the target population through unproductive matings. Sterilisation has traditionally been achieved using ionising radiation. Related approaches include the use of non-driving GMIs designed to produce sterile or self-limiting males, as well as the use of Wolbachia-infected insects to generate incompatible matings with uninfected populations, a strategy referred to as the incompatible insect technique (IIT).

Risk assessments for SIT have focused primarily on the safe rearing, handling and release of the target species, in particular to prevent the unintended release of fertile individuals. Assessments have also addressed potential human health and environmental risks associated with the sterilisation process itself. Sterile insects are generally regarded as posing low environmental risk, although some consideration has been given to the periodic release of very large numbers of insects into the environment and to the ecological consequences of sustained reductions in the target pest population.

For non-driving GMIs with self-limiting traits, risk assessments have emphasised potential environmental impacts of the modified insects, including effects mediated through food webs when the insects are consumed by predators or parasitoids. Additional considerations have included the characteristics and stability of the genetic construct, the likelihood of its persistence or spread within the target population, and the possibility of horizontal gene transfer (HGT). Ensuring that the genetic construct reliably confers the intended sterile or self-limiting phenotype has also been a key component of these assessments.

The use of Wolbachia-infected insects differs mechanistically from other genetic control approaches, although it can still be considered a form of genetic biocontrol. Wolbachia are maternally inherited intracellular endosymbionts that manipulate host reproduction to promote their own transmission. One such mechanism, cytoplasmic incompatibility (CI), results in reduced or absent offspring production when Wolbachia-infected males mate with uninfected females (Le Page et al., 2017; Lindsey, Rice, et al., 2018). As a consequence, Wolbachia can spread rapidly through insect populations, in a manner analogous to a gene drive, a phenomenon that has been observed under natural conditions (Turelli and Hoffmann, 1991; Weeks et al., 2007; Kriesner et al., 2013).

When Wolbachia-infected males are released for population suppression under IIT, the outcome is functionally similar to SIT or non-driving GMI-based self-limiting approaches, resulting in unproductive matings. Risk assessments for IIT have therefore focused on the effectiveness of sex-separation methods to avoid the release of Wolbachia-infected females, the likelihood of horizontal transfer of Wolbachia to non-target species, and the broader ecological consequences of reducing target pest populations, including food-web effects.

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An alternative application involves introducing Wolbachia into insect populations to reduce their capacity to transmit pathogens, known as the pathogen blocking technique (PBT) (Terradas et al., 2017; Lindsey, Bhattacharya, et al., 2018; Gong et al., 2023). This approach exploits the ability of Wolbachia to increase host resistance to viral and other infections. When applied independently of IIT, PBT leads to population replacement rather than suppression and therefore results in outcomes similar to those expected from GDMIs intended for population modification. Experience with risk assessments for PBT remains limited, but available assessments generally use the corresponding naturally occurring insect population, lacking Wolbachia infection, as the primary comparator (Buchori et al., 2022).

Experience with Genetically Modified Insects (non-driving GMI)

Previous regulatory experience with non-driving GMIs may be particularly informative when selecting appropriate comparators for GDMIs. According to EFSA (2020), comparators for GDMIs need not be restricted to non-modified insects of the same species. Instead, they may also include other pest control systems, such as alternative genetic control approaches that involve insect release, including non-driving GMIs, as well as non-genetic control measures such as chemical insecticides. This broader interpretation of comparators reflects the understanding that risk assessment should consider not only the modified organism in isolation, but also its role within the wider pest management context.

GM Aedes aegypti mosquitoes

Two genetically modified *Aedes aegypti* mosquito lines, OX513A and OX5034 (Bargielowski et al., 2011; Spinner et al., 2022), have been approved for release in Brazil. One or both lines have also been authorised for confined or open field research trials in several other jurisdictions, including the Cayman Islands, the Marshall Islands, and the USA states of Florida and California. Although based on different technological constructs, both lines are intended to suppress local *Aedes aegypti* populations through mechanisms that are functionally analogous to the classical SIT.

The OX513A line relies on the production, sex-sorting and release of males carrying a repressible dominant lethal gene, such that offspring derived from matings with wild females do not survive in the absence of the repressor. In contrast, OX5034 males carry a repressible, female-specific lethality trait, resulting in progeny composed exclusively of males. While these traits are not classified as engineered gene drive mechanisms, population level suppression approaches have been proposed for GDMIs, and the functional outcomes of these GM mosquito releases are comparable to self-limiting genetic control strategies.

Several of these releases have been accompanied by regulatory risk assessment reports, although the level of detail and transparency regarding assessment methodologies varies between jurisdictions. In Brazil, the National Technical Commission on Biosafety (CTNBio), which is responsible for GMO risk assessment, issued decision documents for both mosquito lines. In both cases, CTNBio concluded that the GM *Aedes aegypti* mosquitoes are equivalent to their wild-type counterparts, aside from the presence of the intended genetic modification and its associated phenotype.

Beyond public health applications, regulatory experience with non-driving GMIs has also expanded into agricultural pest management. Regulatory authorities in Brazil and Paraguay have approved the GM fall armyworm OX5382G (*Spodoptera frugiperda*; Lepidoptera:

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Noctuidae) for agricultural use (Reavey et al., 2022). This line employs a repressible, female-specific lethality construct analogous to that used in the OX5034 mosquito (EFSA, 2020), further illustrating the transferability of assessment concepts across sectors and reinforcing the relevance of previous non-driving GMI experience for informing comparator selection in GDMI risk assessments.

Plant protection products and chemical pesticide regulations

Table 29: Summary of plant protection product and chemical pesticide regulations and guidance reviewed, with respect to the use of comparative approaches

Guidance, regulation or legislation	Comparative approach applied
Regulation (EC) No 1107/2009	Recommends comparing risks with other "substances" as defined in the regulation and encourages prioritisation of substances presenting lower risk relative to available alternatives
Australia – Agricultural and Veterinary Chemicals Code Act (1994)	No explicit comparative risk assessment methodology specified
Canada – Canadian Environmental Protection Act (CEPA)	Emphasises prioritisation of risk management measures aimed at reducing exposure to more harmful chemicals
Canada – Pest Control Products Act (PCPA)	Supports access to pest control products that pose lower risk and includes comparison of value and risks relative to other pest control products
Japan – Agricultural Chemicals Regulation Act	Provides for priority registration of chemicals that are demonstrably safer than other agricultural chemicals with comparable pest and use profiles
New Zealand – Hazardous Substances and New Organisms Act (and amendments)	Allows rapid assessment for importation or manufacture of hazardous substances where at least one hazardous property is less severe than that of previously approved substances
United States – Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)	Mandates expedited review for products that reduce risks to human health, non-target organisms, or environmental resources such as water
United States – National Environmental Policy Act (NEPA)	Requires assessment of reasonable alternatives as part of environmental impact evaluations

Within the EU

Within the EU, Regulation (EC) No 1107/2009 governs the placing of plant protection products on the market. Under this Regulation, plant protection products are defined as products containing active substances intended, among other functions, to protect plants or plant products against harmful organisms or to prevent the action of such organisms. The scope of the Regulation explicitly includes "substances, including microorganisms, having general or specific action against harmful organisms".

Although GDMIs would not fall under the regulatory definition of a "substance", the intended use of certain GDMIs, namely the control of insect pests affecting agricultural production, clearly aligns with the functional purpose of plant protection products as described in Regulation (EC) No 1107/2009. This makes the Regulation relevant as a point of reference

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for understanding how comparative considerations are embedded in EU approaches to pest control risk management.

Several recitals and articles within Regulation (EC) No 1107/2009 explicitly or implicitly introduce the concept of comparative assessment. Paragraph (17) of the preamble states that the evaluation of an active substance may reveal that it poses lower risks compared with other substances, and that incentives should be provided to facilitate the placing on the market of low-risk plant protection products. This articulation implies that risks are not assessed in isolation, but rather in relation to the risk profile of alternative substances already in use.

This comparative logic is further developed in paragraph (19), which provides for the identification of substances that should be considered candidates for substitution. These are to be replaced by plant protection products containing active substances that require less risk mitigation, or by non-chemical control or prevention methods. Importantly, non-chemical control or prevention methods are defined under Article 3(8) to include biological pest control methods, a category that would encompass approaches relying on the release of insects, including GDMIs. This recital therefore explicitly recognises non-chemical biological control methods as valid comparators for chemical plant protection products.

Paragraph (20) extends this concept by noting that, in certain Member States, non-chemical control or prevention methods that are significantly safer for human and animal health and for the environment are already established and widely applied for specific uses. In such cases, Member States are allowed, in exceptional circumstances, to apply comparative assessment when granting authorisation for plant protection products. Together, these preambular paragraphs clearly frame comparative assessment as a mechanism for situating risks within the broader context of available pest control strategies.

The operative provisions of Regulation (EC) No 1107/2009 reinforce this approach. Article 50, together with Annex IV, establishes a formal comparative assessment procedure for plant protection products containing active substances identified as candidates for substitution. Under this procedure, authorisation decisions take into account several criteria, including the availability of authorised plant protection products or non-chemical control or prevention methods that are significantly safer for human or animal health or for the environment.

While the regulatory context of comparative assessment under Regulation (EC) No 1107/2009 differs from that of GMO risk assessment, these provisions nevertheless demonstrate a clear EU policy principle: risks should be evaluated relative to alternative methods capable of achieving similar pest control objectives. This principle provides both explicit and implicit justification for considering alternative control approaches, including biological and non-chemical methods, when contextualising risks associated with new technologies such as GDMIs.

International examples

Comparable concepts are reflected in plant protection product legislation and related regulatory frameworks outside the EU. The Japanese Agricultural Chemicals Regulation Act of 1948 includes provisions allowing priority registration for agricultural chemicals that are deemed "especially safer" than other products targeting similar pests under comparable conditions of use. Similarly, the Canadian Environmental Protection Act incorporates

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prioritisation mechanisms aimed at reducing or eliminating the use of more harmful chemicals. In the United States, the National Environmental Policy Act requires federal agencies to assess reasonable alternatives when evaluating actions with significant environmental effects, a requirement that can include consideration of alternative pest control methods in the context of pest management programmes.

Together, these examples indicate that the use of comparative reasoning, either explicitly or implicitly, is a common feature of regulatory frameworks addressing pest control and environmental protection.

Identification of comparators informing GDMI risk assessment

As discussed in the preceding sections, comparators serve two distinct but complementary purposes in risk assessment. First, as articulated in Directive 2001/18/EC, comparison with a relevant counterpart is used to identify differences between a GMO and its non-modified equivalent, in order to determine whether any observed differences could pose risks to human health or the environment. Second, comparators are used to contextualise identified risks or potentially harmful characteristics by situating them relative to other activities or control methods that pursue similar objectives.

Given that GDMIs currently under development and are relevant for the EU and its OMTs are largely intended either to eradicate agricultural insect pests or to control insect vectors of human diseases, it is feasible to identify a limited but relevant set of comparators for future assessments.

For GDMIs intended for population suppression, appropriate comparators include existing control methods used against the target organism within the EU and its OMTs. These include chemical control methods, such as insecticide applications, as well as biological control approaches, including the release of other insects. Physical and environmental control measures may also be relevant, such as the use of bed nets, acknowledging that these are often insecticide-treated, or habitat modification to remove breeding sites, which historically played a major role in the eradication of malaria from parts of southern Europe.

For GDMIs intended for population replacement, it may also be informative to compare the GDMI with alternative organisms or control approaches that would occupy a similar ecological niche if the target population were reduced or altered through other means. In both population suppression and replacement scenarios, comparisons should consider not only the nature and magnitude of potential adverse effects, but also the relative effectiveness of the different approaches in achieving their intended public health or agricultural objectives.

Importantly, the use of comparators in this context does not presuppose that GDMIs will replace existing control methods, nor that different methods are mutually exclusive. Rather, the purpose of comparative assessment is to place the proposed use of a GDMI within a broader risk context, allowing potential effects on human health and the environment to be understood relative to other pest management activities that are already accepted or under consideration.

4.3.1.8 SQ3.1.4 – For each identified GDMI, what activities, processes or methods used for a similar purpose might be used as comparators for risk assessment?

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For GDMIs relevant to the EU and its OMTs, comparators can be identified at different stages of the risk assessment process. These include comparators used for MC, to identify any novel traits relevant to harm, as well as comparators used during ERA and PMEM, where risk is contextualised relative to alternative control methods pursuing similar goals.

Aedes aegypti

Work on GDMI *Aedes aegypti* has primarily focused on population suppression strategies. For MC, the most relevant comparator is the wild-type *Aedes aegypti*, either originating from the laboratory strain used to generate the GDMI or from the receiving population. The objective of this comparison is to identify any differences between the GDMI and its comparator that could plausibly result in harm to human health or the environment. Comparisons should focus on characteristics identified during problem formulation as relevant to risk, rather than on all observed differences.

Previous problem formulation exercises for GDMI mosquitoes (including *Aedes aegypti*) have identified plausible PTHs linked to environmental protection goals, including biodiversity (through ecological interactions), water quality (owing to the aquatic larval life stage), and human health (primarily via disease transmission and blood-feeding behaviour) (Roberts et al., 2017; Teem et al., 2019; Devos et al., 2020; Connolly et al., 2021; Hayes and Hosack, 2025). Case-specific assessments would necessarily refine these pathways based on the characteristics of the specific GDMI under consideration.

For ERA, contextual comparators broaden to include alternative control methods used to achieve the same public health or vector control objectives. In the EU and its OMTs, control of *Aedes aegypti* typically relies on integrated approaches, including reduction of breeding sites, public awareness campaigns, and insecticide application. Insecticidal control encompasses larvicides, fogging, indoor residual spraying, and surface treatments. Biological control comparators may include the use of predators or pathogens (e.g., fish, copepods, *Bacillus thuringiensis israelensis* (Bti), *Toxorhynchites* spp.) and the use of *Wolbachia*-infected mosquitoes (OECD, 2018). Other genetic control approaches, such as classical SIT, non-driving non-driving GMIs for suppression, and *Wolbachia*-based IIT, are also relevant contextual comparators (Lee et al., 2009; Bargielowski et al., 2011; Buchori et al., 2022; Spinner et al., 2022).

For PMEM, the primary comparator remains the wild-type or established receiving population. Monitoring outcomes may also be contextualised by comparison with observed environmental effects of other control measures, including chemical insecticides, alternative non-driving GMIs or *Wolbachia*-based interventions.

Drosophila suzukii

Drosophila suzukii is an agricultural pest for which GDMI development has largely focused on population suppression. Although explicit, published problem formulation is less developed than for mosquito GDMIs, available analyses identify plausible harms relating to agricultural productivity, agroecosystem functioning, and biodiversity, including potential gene transfer to closely related *Drosophila* species (Romeis et al., 2020).

For MC, the appropriate comparator is the wild-type *Drosophila suzukii*, derived either from the laboratory strain used in GDMI development or from the local population in the area of intended release.

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For ERA, contextual comparators include existing pest management practices, notably chemical insecticides and physical control measures (e.g., netting or physical barriers over fruit). Outside the EU, baited traps are also commonly employed, particularly in protected cultivation systems (Garcia et al., 2022). Biological control approaches under investigation include larval parasitoids (Girod et al., 2018a,b; Wang et al., 2018), while genetic control strategies such as SIT, *Wolbachia*-based IIT, and other non-driving GMIs are also being explored (Cattel et al., 2018; Nikolouli et al., 2018; Homem et al., 2022; Regalado et al., 2023).

For PMEM, the comparator is the wild-type *Drosophila suzukii* population established in the receiving environment.

Plutella xylostella

Plutella xylostella is a major pest of cruciferous crops. For MC, wild-type *Plutella xylostella* populations provide the appropriate comparator to identify genetic or phenotypic changes potentially relevant to harm.

Explicit problem formulation for GDMI *Plutella xylostella* has not yet been published, but given its agricultural role, relevant protection goals are likely to align with those identified for *Drosophila suzukii*, notably biodiversity, ecosystem services, and agricultural productivity. Control currently relies heavily on chemical insecticides, although resistance has developed to multiple active substances, including *Bacillus thuringiensis* formulations. Biological control comparators include parasitoids and generalist predators, and classical biological control has been proposed (Sarfraz et al., 2005; Cock et al., 2021). Self-limiting GM *Plutella xylostella* intended for SIT-like applications have been field-tested, primarily for comparative studies of dispersal and fitness relative to wild-type populations (Shelton et al., 2020).

For ERA, appropriate contextual comparators therefore include chemical insecticides, microbial control agents, classical biological control, and non-driving GMIs where these are deployed. For PMEM, the comparator is the wild-type population present in the receiving environment.

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Table 30: Identification of potential comparators

GDMI species	Intended use	Molecular Characterisation (MC)	ERA – Water quality	ERA – Human health / disease vectoring	ERA – Biodiversity & ecological harms	ERA – Agricultural / agro-ecological harms	PMEM comparator
Aedes aegypti	Population suppression	Laboratory strain or wild-type population established in the receiving environment	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control	Not applicable	Wild-type or native *Aedes aegypti* population
Aedes aegypti	Population replacement	Laboratory strain or wild-type population established in the receiving environment	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control; other mosquito species	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control; other mosquito species	Wild-type or established *Aedes aegypti* population; chemical insecticides; alternative *Aedes aegypti* non-driving GMI or biological control; other mosquito species	Not applicable	Wild-type or native *Aedes aegypti* population
Drosophila suzukii	Population suppression	Laboratory strain or wild-type population already established in the receiving environment	Not applicable	Not applicable	Wild-type or established *Drosophila suzukii* populations; chemical insecticides	Wild-type or established *Drosophila suzukii* populations; chemical insecticides; alternative non-driving GMI or biological control*	Wild-type or established *Drosophila suzukii* population

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GDMI species	Intended use	Molecular Characterisation (MC)	ERA – Water quality	ERA – Human health / disease vectoring	ERA – Biodiversity & ecological harms	ERA – Agricultural / agro-ecological harms	PMEM comparator
Plutella xylostella	Population suppression	Laboratory strain or wild-type population established in the receiving environment	Not applicable	Not applicable	Wild-type or established *Plutella xylostella* populations; chemical insecticides; alternative non-driving GMI or biological control*	Wild-type or native *Plutella xylostella* populations; chemical insecticides; alternative non-driving GMI or biological control*	Wild-type or native *Plutella xylostella* population

* Biological control includes microbial agents (e.g., *Bacillus thuringiensis*), parasitoids, predators, and classical biological control approaches, where applicable.

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4.3.2 AQ3.2 – What are the strengths and weaknesses of the identified comparators under AQ1?

4.3.2.1 SQ3.2.1 – For each identified potential comparator or type of comparator, identify the strengths of using that comparator for risk assessment

For all three GDMIs that have been identified as potentially relevant for the EU and its OMTs, the comparators can be grouped into three categories:

- The laboratory strain from which the GDMI is derived (near-isogenic line)
- The wild type or natural population(s) of the insect species in the receiving environment where the GDMI will be released
- Alternative control methods

Laboratory strains

Wild-type or natural populations of the target insect species present in the receiving environment represent a complementary and, in many respects, more ecologically realistic comparator. Laboratory strains are known to differ genetically and phenotypically from wild populations, and such differences can be relevant for key traits such as survival, mating competitiveness, dispersal and interactions with other organisms (Hoffmann and Ross, 2018; Leftwich et al., 2021). These traits are directly relevant both to the effectiveness of biocontrol strategies and to potential environmental impacts.

Using receiving environment populations as comparators is therefore particularly valuable for ERA, especially for strategies involving population replacement, as proposed for some *Aedes aegypti* GDMIs. In these cases, the wild-type population represents the actual population that would be suppressed or replaced following release. This makes it an appropriate benchmark for identifying GDMI characteristics that might be undesirable from an environmental or public health perspective.

Strengths of this comparator include its relevance for assessing fitness-related traits associated with persistence, spread and invasiveness, as well as traits affecting the ability to manage or control the GDMI once released, such as susceptibility to insecticides or other control measures. Comparisons with local wild-type populations are also particularly informative when assessing vector competence or other characteristics relevant to disease transmission, allowing a direct comparison of the GDMI's performance relative to the population it is intended to replace or suppress.

Alternative control methods

Alternative control methods constitute a third category of comparators and include all approaches currently used or reasonably available to control the target insect species in the relevant geographic areas. These may include chemical insecticides, biological control agents, traps, habitat modification, physical barriers, or other integrated pest or vector management measures.

Although this category is broad and heterogeneous, its strength lies in its ability to place the risks associated with GDMI use into a real-world management context. Comparisons with alternative control methods allow potential harms associated with GDMIs to be evaluated relative to those arising from established practices that pursue the same control objectives

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(EFSA, 2020). This is particularly useful for contextualising risks rather than identifying novel hazards.

Alternative control methods can serve as informative comparators for a wide range of endpoints, including impacts on non-target organisms and food webs, effects on protected environmental characteristics such as water quality, and occupational or public health risks associated with the implementation of control programmes. They may also be relevant for broader evaluations that consider effectiveness, sustainability, and, where appropriate, cost–benefit considerations. In this way, alternative control methods provide a practical reference frame that can support balanced and proportionate risk characterisation of GDMIs.

4.3.2.2 SQ 3.2.2 – For each identified potential comparator or type of comparator, identify the weaknesses of using that comparator for risk assessment

Laboratory strains

While laboratory strains are well characterised and highly comparable to the GDMI at the genetic level, their relevance as comparators for assessing impacts associated with deliberate environmental release is limited. Insects reared under laboratory conditions are known to diverge rapidly from wild-type populations, sometimes within only a few generations, due to genetic drift, adaptation to artificial rearing conditions, and relaxed natural selection pressures.

As a result, laboratory strains are often poorly representative of the environmental performance of insects following release. This limits their usefulness for assessing interactions between the GDMI and environmental components, including non-target organisms, competitors, predators, parasites, or, where relevant, humans. Laboratory conditions also fail to replicate ecological complexity, making it difficult to extrapolate laboratory-based findings to real-world environments. Consequently, while laboratory strains are valuable for MC and early-tier comparison, they are a weak comparator for higher-tier ERA and PMEM questions that relate to environmental or ecological outcomes.

Wild type or natural populations in the receiving environment

Wild-type or natural populations constitute a realistic comparator for assessing interactions between a GDMI and the environment or human populations in the intended receiving area. However, several practical and methodological challenges limit their use in comparative risk assessment.

Direct use of field-collected insects in laboratory-based assays is often problematic. Field collections may result in damaged individuals, disrupted development, or altered behaviour, and typically lack comprehensive information on health status, age structure, mating history, and life-history traits. High variability within and among natural populations further complicates controlled comparison.

These challenges generally necessitate the establishment of laboratory colonies derived from wild-type or natural populations. However, the process of colony establishment itself introduces genetic and phenotypic changes, even over a relatively small number of generations. Founder effects, genetic bottlenecks, and early adaptation to laboratory conditions reduce the ecological realism of these populations and weaken their representativeness as comparators. As a result, comparisons with laboratory-adapted

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derivatives of wild populations inevitably involve a trade-off between experimental control and ecological relevance.

Natural populations are therefore more informative for assessing ecological and, where relevant, human health impacts at a conceptual or observational level. However, available field data will often need to be interpreted alongside laboratory and modelling outputs, particularly because field trial data for GDMIs may be limited or unavailable due to the self-propagating nature of engineered gene drive systems. This constrains the direct comparability between GDMI performance and field-derived observations from wild populations.

Alternative control methods

The principal weakness of alternative control methods as comparators lies in their diversity and heterogeneity. Chemical, biological, physical and environmental control measures differ substantially in mode of action, spatial and temporal scale, exposure pathways and types of associated hazards. As a result, direct, item-by-item comparison between a GDMI and a specific alternative control method is often not meaningful.

For example, comparing a population suppression GDMI *Aedes aegypti* directly with a chemical larvicide does not provide useful information unless the comparison is framed in terms of effects on defined protection goals, such as human health, non-target organisms, or environmental compartments like water bodies. Meaningful comparison therefore requires abstraction to shared endpoints, rather than direct comparison of the control methods themselves.

As with wild-type populations, much of the information available for alternative control methods is derived from field observations, whereas risk assessment information for GDMIs is likely to be based predominantly on laboratory studies and modelling, particularly prior to deliberate environmental release. This asymmetry in data type and availability introduces uncertainty when attempting to contextualise GDMI risks relative to existing practices.

Use of comparators across GDMI case examples relevant to the EU and its OMTs

Applying the above considerations to GDMIs identified as relevant to the EU and its OMTs illustrates how weaknesses in comparator choice manifest in practice. While the overall categories of comparators are similar across the three insect species considered, their usefulness varies depending on the nature of the harm caused by the target species.

For *Aedes aegypti*, which acts as a vector of human disease, comparisons with wild-type or local populations are particularly important for assessing traits related to vector competence and disease transmission. This is especially relevant for population replacement strategies, where the GDMI may persist in the environment for extended periods. However, acquiring robust, comparable data for such traits remains challenging due to limitations in both field sampling and experimental realism.

By contrast, *Drosophila suzukii* and *Plutella xylostella* do not pose direct human health risks through disease transmission but are significant agricultural pests. Comparisons for these species should therefore focus on traits related to crop damage, population dynamics and interactions within agroecosystems. While wild-type comparators remain conceptually appropriate, variability among agricultural settings and management practices limits the extent to which findings can be generalised.

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For all three species, comparisons with wild-type or local populations in relation to susceptibility to chemical or biological control methods are important to ensure that any released GDMI remains controllable should mitigation measures be required.

In all cases, differences in rearing history, genetic background and environmental exposure complicate comparisons between GDMI and wild-type comparators and must be carefully accounted for in risk assessment design and interpretation.

Table 31: Exemplifying the use of comparators for gene drive modified insect (GDMI) cases relevant to the European Union (EU) and its outermost territories (OMTs)

GDMI Case Example	Comparator	Purpose	Examples of endpoints of comparison	Notes
Aedes aegypti	Laboratory strain	Molecular characterisation (MC) to assess intended and unintended alterations	Phenotypic comparisons designed to identify both intended and unintended effects of the genetic modification	Most relevant for early-tier assessment and MC
	Wild-type or local population relevant to the release area	Phenotypic comparison for characteristics relevant to environment and human health	Vector competence; insecticide susceptibility; fitness-related characteristics (e.g., growth, fecundity, developmental timing); tolerance to abiotic environmental conditions; tolerance to biotic environmental conditions (e.g., predators, pathogens, competitors)	Especially relevant for GDMIs for population modification
	Other control systems or methods	Contextual comparison of potential adverse effects on the environment and human health	Adverse effects on abiotic environment (e.g., water quality, soil health, air quality); adverse effects on biotic environment; human health impacts (including workers engaged in control activities, public health outcomes)	Comparators should reflect control methods in use or reasonably expected to be used
Drosophila suzukii	Laboratory strain	MC to assess intended and unintended alterations	Phenotypic comparisons designed to identify both intended and unintended effects of the genetic modification	Primarily informative for identifying intended and unintended changes
	Wild-type or local population relevant to the release area	Phenotypic comparison for characteristics relevant to environment and human health	Ability to damage agricultural products; insecticide susceptibility; fitness related characteristics (e.g., growth, fecundity, developmental timing); tolerance to abiotic environmental conditions; tolerance to biotic environmental conditions	Focused on agricultural and agro-ecosystem impacts

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GDMI Case Example	Comparator	Purpose	Examples of endpoints of comparison	Notes
	Other control systems or methods	Contextual comparison of potential adverse effects to the environment and human health	Potential adverse effects on the abiotic environment (e.g., water quality, soil health, air quality); potential adverse effects on biotic environment; human health impacts (including workers engaged in agriculture and control activities)	Useful for contextualising GDMI impacts relative to existing pest management
Plutella xylostella	Laboratory strain	MC to assess intended and unintended alterations	Phenotypic comparisons designed to identify both intended and unintended effects of the genetic modification	Relevant for early-stage assessment
	Wild-type or local population relevant to the release area	Phenotypic comparison for characteristics relevant to environment and human health	Ability to attack and damage agricultural products; insecticide susceptibility; fitness-related characteristics (e.g., growth, fecundity, developmental timing); tolerance to abiotic environmental conditions; tolerance to biotic environmental conditions (including predators, pathogens and competitors)	Reflects key protection goals for agricultural pests
	Other control systems or methods	Contextual comparison of potential adverse effects on the environment and human health	Potential adverse effects on abiotic environment (e.g., water quality, soil health, air quality); potential adverse effects on biotic environment; human health impacts Workers engaged in agriculture and control activities	Supports contextual risk characterisation and comparison with established practices

4.3.3 AQ 3.3 – What comparators (single or multiple) are recommended for the risk assessment of GDMIs?

General recommendations

Comparators have long been used in risk assessment to address a variety of questions, and the risk assessment for GMOs has explicitly relied on comparative approaches (OECD, 1986;SCBD, 2000). Directive 2001/18/EC requires comparison of a GMO with the "non-modified organism from which it was derived and its use under corresponding situations". While this definition provides a clear baseline, it represents a relatively narrow interpretation of comparator use.

Risk assessment of GDMIs is expected to benefit from the application of a broader and more flexible range of comparators, as previously recognised in international and EU level guidance (EFSA, 2013, 2020). The utility of any specific comparator depends on the risk hypotheses being addressed, the biology of the organism, and the intended use and scale of deployment of the GDMI (Hayes et al., 2018)

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For the GDMIs most relevant to the EU and its OMTs, three insect species have been identified: one insect vector of human disease and two insect pests of agriculture. In all three cases, GDMIs are being developed either to suppress insect populations or to reduce the ability of insects to spread disease. The recommendations presented here are therefore tailored to these use cases. While similar comparator strategies may be applicable to other GDMI applications, comparator selection should always be considered on a case-by-case basis, taking into account species-specific biology and the purpose of GDMI introduction. These recommendations are not intended to preclude the use of additional comparators where these may provide further relevant context for assessing risk.

Comparators for molecular and phenotypic characterisation

Laboratory strain from which the GDMI was derived

Although no fully developed GDMI currently proposed has been identified as directly relevant to the EU and its OMTs, it is anticipated that any GDMI would be derived from a laboratory-reared insect population. In such cases, the classic comparative approach described in Directive 2001/18/EC can be applied, using the unmodified laboratory strain as the primary comparator.

This comparator is particularly appropriate for MC, allowing assessment of intended and unintended genetic changes associated with the genetic modification. It is also useful for phenotypic comparisons addressing characteristics identified during problem formulation as relevant to risk assessment, such as susceptibility to control measures (e.g., insecticides), life-history traits, reproductive characteristics, and measurable fitness-related attributes.

However, comparisons to laboratory strains alone are unlikely to be sufficient to inform all aspects of ERA, given the adaptation of laboratory strains to artificial rearing conditions and their limited ecological realism.

Wild-type or local population in the receiving environment

For all three GDMI case examples relevant to the EU and its OMTs, the target insect species are already established in certain regions, and the purpose of the deliberate environmental release of the GDMI would be to control or modify these existing populations. In such contexts, comparison of the GDMI with wild-type or local populations in the receiving environment can provide critical information for risk assessment.

Comparisons should focus on phenotypic characteristics that influence interactions with the environment, including susceptibility to existing control measures, survival under abiotic and biotic stressors, dispersal, and other fitness-related traits. While such comparisons are often informative for evaluating the effectiveness of the GDMI, they are equally valuable for identifying potential risks associated with persistence, spread or unintended ecological effects.

These comparisons are particularly important for GDMI intended for population replacement, which are expected to persist in the environment over extended timeframes. They also provide useful insight into potential consequences in scenarios where a population-suppression GDMI fails to achieve its intended outcome and persists in the environment.

Comparisons for ERA

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Other control systems or methods

Because all GDMIs identified as relevant to the EU and its OMTs target pest species, each is subject to existing or potential control programmes. For GDMIs intended to control insect populations, comparison with other control systems or methods provides essential context for characterising risk.

This category includes chemical, biological, physical and environmental control measures and is therefore inherently broad. Selection of relevant comparators should be made on a case-by-case basis, focusing on control methods that are currently used or reasonably expected to be used in the receiving environment.

Comparisons with alternative control methods are most informative when framed around shared protection goals and outcomes, rather than direct functional equivalence. These comparisons typically focus on environmental and human health impacts, including potential adverse effects on non-target organisms, impacts within and beyond the treated area, and human health risks for workers engaged in control programmes and for local populations.

In most cases, data from experience with alternative control methods will need to be compared with predictions of GDMI impacts derived from laboratory studies and modelling. This requires explicit consideration of uncertainties arising from differences in data type, scale and availability, particularly where field data for GDMI are limited or unavailable prior to deployment.

Comparisons for PMEM

Wild type or local population in the receiving environment

Although PMEM is not generally a comparative activity, population replacement applications such as those identified for *Aedes aegypti* may benefit from such comparisons. It is useful to compare the abundance and distribution of the GDMI to the population it is intended to replace in order to identify if there are changes and those changes impact an identified environmental protection goal.

4.4 ToR4 – Review factors affecting engineered gene drive dynamics

4.4.1 ASQ4.1 – Review of molecular, biological and ecological factors affecting the spread, persistence and invasiveness of engineered gene drives

This AQ addresses which molecular, biological and ecological factors influence the spread and persistence of engineered gene drives, as well as their potential invasiveness in target and non-target populations or species. To this end, a systematic literature search was conducted to identify relevant modelling and conceptual studies addressing these aspects.

Following removal of duplicates, 258 publications were initially identified through literature searches using predefined keywords. An initial screening step, focusing on publications aligned with the ToR and giving priority to modelling and conceptual approaches, reduced the dataset to 177 publications considered relevant for further analysis. These publications were then categorised according to their primary contribution. The vast majority of publications

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(>95%) were classified under "mapping existing models", while only a very small proportion (<1%) addressed "approaches to model validation".

Within these categories, publications were further characterised according to the biological dimensions addressed. Most publications focused primarily on genetic aspects of engineered gene drive systems (97%), reflecting the central role of molecular design, inheritance patterns and fitness effects in determining gene drive behaviour. A substantial subset of publications (32%) integrated both genetic and ecological components, for example by incorporating population structure, dispersal, mating systems or ecological interactions alongside genetic assumptions. As expected, none of the modelling studies focused exclusively on ecological processes, since ecological analyses inherently depend on explicit assumptions about the underlying genetic mechanisms and gene drive dynamics.

The full results of the literature screening and categorisation, including study characteristics and classifications, are provided in Annex 2.

4.4.2 AQ4.2 – Mapping existing models used to investigate engineered gene drive dynamics

This AQ aims to map and critically assess existing models used to investigate the dynamics of engineered gene drives, with particular attention to their strengths and limitations. Specifically, models are evaluated in terms of their underlying assumptions, computational approaches, parameterisation, representation of biological and ecological processes, and their capacity to predict ecological effects relevant to ERA and PMEM.

In this section, the use of mathematical models to predict gene drive persistence, efficacy, biosafety and potential environmental risks to humans and wider biodiversity is reviewed. In response to the specific SQs, the assessment distinguishes between models primarily developed to investigate genetic efficacy and persistence of gene drives, and those that address ecological factors influencing biosafety, here understood as potential impacts on non-target organisms, ecosystem processes and/or human health.

Although the task is framed as a "mapping of existing models", it is important to note that each publication identified through the literature search describes a specific modelling exercise, characterised by particular modelling approaches, gene drive systems and assumptions about genetic and ecological processes. In this sense, each publication represents a distinct model instance producing its own set of outcomes. However, rather than assessing each individual model in isolation, greater insight is gained by comparing models across the literature to identify shared structures, recurring assumptions, common methodological choices and key differences. These similarities and contrasts are more informative for understanding the overall modelling landscape and its relevance for regulatory risk assessment than a purely publication-by-publication appraisal.

The publications identified through the literature review protocol are listed in Annex 2, while general descriptive information on the modelling approaches employed is summarised in Table 32. The analysis presented below considers the body of literature as a whole, drawing on selected examples to illustrate major modelling approaches, their assumptions and limitations, and their applicability to ERA and/or PMEM of GDMIs.

Table 32: Overview of publications on modelling identified in the literature search*

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Category	Sub-category	Number of publications
Model study type	Genetic	172
	Ecological	32
	Validation	2
Gene drive call modelled	Low-threshold gene drives	81
	High-threshold gene drives	55
Model characteristics	Deterministic simulations	83
	Stochastic simulations	68
	Overlapping generations	30
	Non-overlapping generations	103

*Overview of modelling publications related to engineered gene drives identified through the literature search conducted according to the agreed protocol. Publications are categorised by primary study focus (genetic, ecological, validation), gene drive class modelled (low- or high-threshold), and general modelling characteristics (deterministic versus stochastic simulations, and overlapping versus non-overlapping generations). Categories are not mutually exclusive; individual studies may contribute to more than one category.

4.4.2.1 SQ4.2.1 – What are the expected limits of, and variability in, model performance?

To provide the necessary background for this SQ, this section first outlines how mathematical models are formulated and used, and describes the general expectations and inherent limitations associated with mathematical modelling. These broader considerations are then illustrated using examples drawn from modelling applications to engineered gene drives.

Mathematical models are simplifications of real biological systems that are designed to represent dynamic processes operating over time and/or space. In the context of engineered gene drives, models have been used to explore and predict a wide range of relevant processes, including gene expression dynamics (e.g., Farasat and Salis, 2016), inheritance patterns and genetic outcomes (e.g., Deredec et al., 2011), within-species ecological dynamics such as population suppression or modification (e.g., Alphey and Bonsall, 2014), and interactions between species, including potential indirect ecological effects (e.g., Vollans and Bonsall, 2021). Collectively, these models aim to improve understanding of how engineered gene drives may spread, persist or fail under different biological and environmental conditions.

Mathematical models may be formulated in continuous time, typically using systems of differential equations, or in discrete time, using recursion equations. For example, at a basic ecological level, a continuous time model describing the spread or growth of a single population Nmay Nmay be written as:

$$\frac{dN}{dt} = rN(t)(1)$$

where $\frac{dN}{dt}$ represents the rate of change in population size over time, and $r$is the intrinsic rate of population increase. When $r$is positive and density-independent, population growth is exponential. Alternatively, in discrete time, population growth can be described as:

$$N_{t+1} = \lambda N_t(\qquad (2)$$

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where population size at the next time step (N_{t+1}) depends on the current population size (N_t) and the finite rate of increase.(λ) Analogous formulations are widely used in gene drive models, where population size may be replaced or complemented by genotype or allele frequencies.

A key limitation of many mathematical models is that they are deterministic, meaning that they describe average system behaviour under fixed parameter values and typically do not account explicitly for random variation. Deterministic models often assume constant environmental conditions, homogeneous populations and time-invariant parameters, which can limit their ability to capture stochastic processes such as demographic fluctuations, random mating events, mutation, or environmental variability. To address these limitations, some gene drive models adopt stochastic formulations, allowing random effects to be explicitly incorporated, particularly when considering small population sizes, invasion thresholds or early establishment dynamics.

Further approaches to exploring model behaviour and performance include analytical techniques from dynamical systems theory (e.g., stability and bifurcation analyses; Strogatz, 2024), as well as computer-based simulation methods that permit more complex assumptions, non-linearities and higher dimensionality. These approaches can help identify parameter sensitivities, thresholds for spread or loss, and conditions under which qualitatively different outcomes may emerge.

A central consideration for GDMI risk assessment is how model outcomes are defined and measured. For gene drive modelling, it is essential that the chosen measures reflect the biological or ecological endpoints of interest and are compatible with the modelling framework. Outcomes may be expressed in terms of allele or genotype frequencies, absolute numbers of individuals, population densities (e.g., individuals per unit area), or other derived metrics relevant to efficacy, persistence or ecological impact. The choice of measurement units influences how processes are parameterised and interpreted, and therefore affects both model performance and comparability across studies.

Overall, the expected limits and variability in the performance of mathematical models used to assess engineered gene drives depend primarily on three interrelated choices: (1) the modelling framework adopted (e.g., recursion equations versus differential equations); (2) whether gene drive dynamics are represented deterministically or stochastically; and (3) how gene drive outcomes are defined, quantified and translated into model parameters and assumptions. These choices determine the scope, realism and predictive capacity of models, and thus shape their applicability and limitations for ERA and PMEM.

4.4.2.2 SQ4.2.2 – What do models predict in terms of frequency, persistence, spread and impacts on the target organism population?

Mathematical models: predictions of molecular and genetic efficacy

Mathematical models developed to assess the performance of engineered gene drives targeting pest populations originate largely from the extensive theoretical literature on selfish

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genetic elements (reviewed by Burt and Trivers, 2006). These models typically rely on discrete time population genetic recursion frameworks to predict changes in the frequency of genotypes or alleles over successive generations. In their simplest form, such models have been adapted from classical population genetics theory (e.g., Gillespie, 1998) to explore the invasion, spread and persistence of low-threshold engineered gene drives (e.g., Deredec et al., 2011).

Early modelling work has demonstrated that relatively simple genetic recursion models can capture key processes influencing gene drive frequency dynamics and allow identification of critical parameters shaping spread and persistence. In particular, modelling studies consistently highlight three main factors influencing the performance of low-threshold engineered gene drives: selection acting against the gene drive construct, fitness effects in heterozygotes, and the efficiency with which wild-type alleles or genotypes are converted into gene drive variants (Deredec et al., 2011; Alphey and Bonsall, 2014). Together, these parameters determine whether an gene drive increases in frequency, reaches fixation, persists at intermediate levels or is lost from the population.

The majority of modelling studies addressing gene drive spread and efficacy use deterministic genetic recursion models (often analogous to Equation 2), implemented computationally to explore a wide parameter space. While rooted in population genetics theory, many of these models prioritise numerical simulation over analytical derivation in order to generate broad predictive insights for gene drive design. Such studies have examined not only homing-based low-threshold gene drives, but also alternative designs, including underdominant systems, high-threshold gene drives and more complex architectures such as daisy chain gene drives.

Selfish genetic elements bias their own inheritance relative to Mendelian expectations, and from a purely genetic perspective this creates the potential for global spread, as observed for naturally occurring elements such as the P-element in *Drosophila melanogaster*. This property has stimulated interest in the development of alternative gene drive designs aimed at achieving spatial confinement and limiting unintended spread. Consequently, a substantial body of modelling work has focused on threshold-dependent systems, including underdominance and split gene drive approaches, which theoretically require introduction above a critical frequency in the target organism population before spreading.

Similarly to low-threshold gene drives, modelling of underdominant gene drive systems has concentrated on predicting invasion thresholds, persistence and spatial containment. Classical and contemporary studies have used discrete time population genetic models to investigate whether engineered underdominance could function as a feasible pest management strategy (e.g., Curtis, 1968; Davis et al., 2001; Magori and Gould, 2006). For example, Davis et al. (2001) developed a standard recursion model describing two engineered constructs that together produce underdominance. In this system, individuals carrying both constructs survive because each construct suppresses the lethal effect of the other, whereas individuals carrying only a single construct suffer lethality. Modelling results show that such designs generate underdominant dynamics characterised by an invasion threshold and, if that threshold is exceeded, local fixation. Importantly, these properties imply spatially restricted spread, as gene drive constructs introduced below the threshold are predicted to be lost.

This modelling framework has been further extended to toxin-antidote (or "killer-rescue") systems, which have attracted substantial attention over the past two decades. Magori and Gould (2006), building on the work of Davis et al. (2001), used deterministic simulation

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models of independently segregating lethal constructs that mutually suppress each other to estimate the minimum release size required for successful introgression. Their analyses demonstrate that increasing fitness costs associated with the constructs raises the release threshold, and that more complex designs involving multiple constructs further increase the number of released individuals required for establishment.

More recent modelling work has explored hybrid or modular gene drive architectures designed to combine local confinement with strong population level impacts. For instance, Dhole et al. (2020) proposed the tethered homing drive, which links a homing-based gene drive to a spatially restricted underdominant system. Using deterministic genetic recursion simulations, these authors show that tethered homing drives exhibit threshold-type dynamics, resulting in localised spread once the threshold is exceeded, while still being capable of imposing substantial fitness costs on the target organism population, potentially leading to population suppression.

Across these modelling studies, predictions concerning target organism populations typically focus on changes in allele or genotype frequencies, the likelihood of fixation or loss, the expected persistence of gene drive constructs over time and, where relevant, reductions in population size or viability resulting from fitness costs imposed by the drive. These predictions provide insight into the potential efficacy and durability of different gene drive designs, as well as their capacity for containment.

Advantages and limitations of models for predicting genetic efficacy

The dominance of deterministic, discrete time recursion models in gene drive modelling confers several advantages. These frameworks are computationally tractable and can be implemented using well-established linear algebraic methods. Complex inheritance patterns, mating outcomes and genotype frequencies can be efficiently represented using matrix formulations, allowing exploration of a wide range of gene drive designs and parameter combinations.

However, recursion-based models also have notable limitations. First, time is treated as discrete, often corresponding to non-overlapping generations, which restricts the ability to incorporate age structure, seasonal dynamics or more realistic population structures. Second, deterministic formulations do not capture stochastic variability in biological processes, such as random mating, demographic fluctuations or environmental variability. As a result, given a fixed set of initial conditions and parameters, model outcomes are always identical.

While deterministic models are valuable for identifying general patterns, thresholds and qualitative behaviour, the limited use of systematic sensitivity analyses in many studies constrains their applicability for ERA and PMEM. Without thorough exploration of parameter uncertainty, it is difficult to assess the robustness of predictions or to identify worst-case scenarios relevant for risk assessment.

Differential equation approaches offer alternative ways to model gene drive dynamics, particularly when continuous time, overlapping generations or spatial structure need to be represented using ordinary or partial differential equations. In principle, these models permit similar analytical treatments to recursion models and can better align with biologically realistic time scales. Nevertheless, they are less commonly used in the gene drive literature and are

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frequently explored only through numerical simulations. Their implementation can require sophisticated numerical methods, especially for spatially explicit models, which may limit accessibility and comparability across studies.

Importantly, many of the limitations associated with differential equation approaches parallel those of recursion models when used deterministically. In particular, the lack of stochasticity and limited application of formal validation or sensitivity analyses constrain their predictive reliability for ERA and PMEM purposes. Overall, while existing models provide valuable insights into expected frequency dynamics, persistence, spread and impacts on target organism populations, their predictions are contingent on simplifying assumptions and parameter choices that define the limits and variability of model performance.

4.4.2.3 SQ4.2.3 – What do models predict in terms of relevant release requirements and necessary field conditions to achieve target performance?

Mathematical models have been widely used to inform release requirements for insects (including non-driving GMIs) and to explore the field conditions necessary to achieve specific target performance objectives. In this context, model predictions are typically concerned with how release size, release frequency and spatial scale interact with gene drive properties and ecological conditions to determine whether a desired outcome, such as population suppression, modification or containment, can be achieved.

A critical prerequisite for deriving meaningful release requirements from mathematical models is a clear and explicit definition of target performance. Model formulations and predictions depend strongly on whether the intended goal is local eradication, long-term suppression, population modification or broader-scale elimination. For example, outcomes defined at a local scale differ fundamentally from those relevant to regional or global objectives, and model assumptions must be aligned accordingly. Even within local applications, target performance must be specified in quantitative terms, such as the degree of population reduction, duration of suppression or probability of elimination, in order for model outputs to be interpretable in a regulatory or management context.

Modelling studies addressing disease vector suppression illustrate how release requirements depend on the chosen performance endpoint. For instance, models developed for insect vectors of human disease (e.g., Alphey et al., 2011) indicate that, where the objective is to reduce disease transmission rather than to eradicate the vector species, complete local elimination of the target population may not be necessary. Instead, maintaining vector abundance below a critical threshold required to sustain transmission can be sufficient. Under such scenarios, release requirements are predicted to be lower than those needed for eradication, and the time frame and intensity of releases can be optimised accordingly.

Model predictions further indicate that the efficacy and speed of achieving target performance depend on interactions between release intensity, the ability of the gene drive to spread and persist, and ecological factors such as population size, dispersal and reproduction rates. Higher release numbers or repeated releases can accelerate suppression or replacement, but these effects are mediated by gene drive efficiency, fitness costs and mating dynamics. Consequently, models often explore combinations of release strategies and biological parameters to identify conditions under which target outcomes can be reliably achieved.

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Release requirements also vary substantially across different gene drive designs. Low-threshold engineered gene drives are generally predicted to require smaller release sizes to establish and spread in the target organism population (e.g., Sinkins and Gould, 2006). In contrast, high-threshold systems, such as underdominant gene drives, require the introduced constructs to exceed a critical frequency before spreading. Models consistently predict that these systems need larger and more spatially coordinated releases to surpass the threshold and achieve establishment (e.g., Leftwich et al., 2018).

In addition to release size and frequency, models highlight the importance of field conditions for achieving target performance. Population structure, migration, mating patterns and environmental heterogeneity can all alter predicted outcomes. For example, immigration of wild-type individuals into the release area may counteract suppression or prevent fixation, while strong spatial structure may enhance localisation for threshold-dependent gene drives. As a result, release requirements derived from idealised models are often sensitive to assumptions about field conditions, underscoring the need for scenario-specific parameterisation.

Overall, mathematical models predict that relevant release requirements and necessary field conditions are highly context dependent, shaped by clear definitions of target performance, the properties of the gene drive system employed and the ecological characteristics of the target population. While models provide valuable guidance for exploring feasible release strategies, their predictions must be interpreted within the limits imposed by underlying assumptions and uncertainties, particularly when extrapolated to real-world field conditions relevant for ERA and PMEM.

4.4.2.4 SQ4.2.4 – What do models predict in terms of expected population interactions with non-target organism populations?

Mathematical models: predictions of ecological outcomes

Understanding the broader ecological outcomes of GDMI releases is essential for the development of robust ERA and PMEM schemes. In this context, ecological outcomes refer primarily to potential effects of gene drive releases on non-target organisms, wider biodiversity and, where relevant, human health.

Engineered gene drive technologies under development are mainly targeted at insect agricultural pests or insect vectors of infectious diseases. For agricultural pest management, ecological outcomes are typically framed in terms of impacts on non-target species, community structure and ecosystem functioning. While similar considerations apply to gene drives targeting disease vectors, additional emphasis is placed on possible indirect ecological effects mediated through changes in disease transmission dynamics and human health outcomes. Despite these broader concerns, most modelling work to date has focused on gene drive efficacy and dynamics within target populations, with limited attention to downstream ecological interactions.

Consistent with this observation, only a relatively small proportion of studies identified by the literature search incorporated ecologically relevant components into their modelling frameworks. Approximately 18% of the reviewed publications considered ecological processes alongside genetic dynamics. These studies typically focused on how ecological feedbacks

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influence gene drive spread and persistence within target populations rather than explicitly modelling interactions with non-target organisms.

Several core ecological processes are nonetheless recognised as central to understanding how GDMI releases may indirectly affect non-target populations. These include interspecific competition, trophic interactions with predators and prey, local and regional population dynamics, and processes such as Allee effects that operate at low population densities. Modelling studies incorporating such processes suggest that ecological context can substantially shape gene drive outcomes and therefore influence potential exposures and interactions with non-target organisms.

As highlighted by Dhole et al. (2020), density dependence, spatial structure and population structure are critical ecological determinants of gene drive dynamics. Density dependence, typically expressed through intraspecific competition, reflects the reduction in per capita birth rates or increase in mortality as population density rises (Begon et al., 2006). The form and strength of density-dependent regulation can affect equilibrium population sizes (Bellows, 1981) and, consequently, the genetic load imposed by an engineered gene drive (Alphey and Bonsall, 2014; Dhole et al., 2020). Density dependence may also give rise to Allee effects, whereby population growth rates decline at low densities. Such effects can influence the likelihood of successful suppression, the spatial spread of gene drives and the probability of recolonisation by wild-type individuals following population reduction (Tanaka et al., 2017; Dhole et al., 2020).

Environmental and demographic variability further complicate ecological outcomes. Natural habitats are heterogeneous in both space and time, and this heterogeneity can alter gene drive dynamics. Recent modelling work indicates that increasing environmental variation can, under some circumstances, accelerate the spatial spread of gene drives (Champer et al., 2021; Kim et al., 2023). Using a stepping-stone framework, Kim et al. (2023) demonstrate that spatial variability can facilitate faster and broader spread. However, ecological interactions at local scales may also constrain or redirect spread, highlighting the context-dependent nature of these outcomes.

The interface between released GDMI individuals and surrounding wild-type populations is particularly important for understanding potential interactions with non-target organisms. Many gene drive models assume well-mixed populations, but spatially explicit interactions at release boundaries strongly influence outcomes relevant for ERA and PMEM. Extensive theoretical work on the spatial spread of selfish genetic elements predates current gene drive applications (e.g., Barton, 1979; Barton and Hewitt, 1985; Rouhani and Barton, 1987), and more recent publications have adapted these concepts to GDMI contexts (e.g., Tanaka et al., 2017;North et al., 2019; Girardin and Débarre, 2021).

Spatial models distinguish between different modes of gene drive spread. At the leading edge of a release area, where drive alleles are initially rare, models predict that a selective advantage is required to "pull" the front forward (Barton and Turelli, 2011). Alternatively, when population densities are low at the frontier, spread may be "pushed" by higher densities behind the leading edge (Tanaka et al., 2017). Which mechanism dominates depends on the interaction between genetic parameters and ecological dynamics, and these interactions can influence spatial containment and, indirectly, exposure pathways for non-target organisms.

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Population structure adds further complexity. Age- or stage-structured models show that density-dependent regulation acting at different life stages can alter population dynamics and gene drive performance (Alphey and Bonsall, 2014). In some scenarios, gene drive success depends on timing relative to density-dependent feedbacks, with poorly timed releases potentially leading to failure or even population increases. Such ecological feedbacks can also restrict spatial spread by imposing additional barriers that GDMI individuals must overcome (Girardin and Débarre, 2021). Mating system structure, such as polygynous or polyandrous mating, has likewise been shown to influence outcomes. For example, Wilkins et al. (2018) demonstrate that assuming random mating can lead to overly optimistic predictions of eradication when reproductive structure and Allee effects are present.

Despite these advances, it is notable that none of the modelling studies identified in the present literature review explicitly evaluated interspecific competition, trophic interactions, impacts on wider biodiversity or epidemiological outcomes linking GDMI releases to disease spread or reduction. This reflects a major limitation of current modelling approaches, which remain largely focused on within-species genetic efficacy rather than ecosystem level impacts. As a result, the application of mathematical models to fully support ERA and PMEM of gene drive systems is still underdeveloped, and further work is required to extend modelling frameworks to explicitly address interactions with non-target organism populations and broader ecological consequences across different gene drive constructs.

4.4.2.5 SQ4.2.5 – What do models predict in terms of resistance, reversal and recall?

A critical component of GDMI risk assessment concerns the potential emergence of resistance, as well as the feasibility of reversing or recalling an engineered gene drive once released. Mathematical modelling has played a central role in exploring these issues, as resistance evolution can substantially affect gene drive efficacy, persistence and spatial spread, with possible consequences for non-target interactions and long-term ecological outcomes.

The emergence of resistance alleles is a key determinant of gene drive performance. Using population genetic recursion models, Unckless et al. (2017) demonstrate that the dominant factor governing resistance evolution is the rate at which resistance alleles arise, for example, in CRISPR/CAS based homing gene drives, resistance alleles arise primarily through mutation or DNA repair processes such as NHEJ during gene drive activity. Their modelling indicates that parameters often considered critical for gene drive success, such as conversion efficiency, fitness costs of the drive construct and initial introduction frequency, have comparatively little influence on whether resistance evolves. Instead, even low rates of resistant allele formation can undermine long-term persistence if resistance alleles confer a selective advantage relative to the drive construct.

Mathematical models have also been used to explore strategies for reversing or mitigating GDMI releases. Edgington and Alphey (2019), using discrete time genetic recursion frameworks, examined reversal mechanisms for underdominant engineered gene drive systems. Their analysis shows that accumulation of mutations can compromise drive function and that loss-of-function mutations may ultimately lead to elimination of an underdominant gene drive. In addition to passive loss through mutation, models have evaluated active reversal strategies. One commonly explored approach is the release of wild-type individuals, which can dilute gene drive frequencies and, under certain conditions, reduce them below invasion thresholds. Modelling studies suggest that wild-type releases can be effective,

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although they may require large release numbers and could be economically or logistically prohibitive at scale (Alphey and Bonsall, 2014; Champer et al., 2016).

Alternative reversal approaches have also been examined. For underdominant systems, Edgington and Alphey (2019) show that releasing individuals carrying only the suppressor component of the original construct may, under particular parameter combinations, drive transgene frequencies below the threshold required for persistence. Once below this threshold, the remaining modified insects are predicted to be eliminated due to their associated fitness costs relative to wild-type individuals. These results illustrate that reversal strategies are highly design-specific and depend strongly on the genetic architecture and threshold properties of the original gene drive.

Beyond population genetic recursion models, differential equation frameworks have been developed to investigate resistance and reversal mechanisms at finer molecular scales. For example, Farasat and Salis (2016) model the biophysical dynamics of CRISPR/Cas9-based gene drives using systems of ordinary differential equations. These models describe how guide RNA sequences, DNA concentrations and Cas9 expression patterns influence binding and cleavage dynamics, which in turn affect gene conversion efficiency and the likelihood of resistance formation. Model predictions were generated using numerical integration and parameterised with experimental data, with model performance assessed using goodness-of-fit metrics based on relative error rather than formal likelihood-based approaches. While these models do not directly predict population level outcomes, they provide mechanistic insight into molecular processes driving resistance emergence.

Given regulatory concerns associated with uncontrolled or global spread of engineered gene drives, substantial modelling effort has focused on understanding localised persistence and spatial containment, which are closely linked to recall and reversibility. Reaction-diffusion models, formulated as systems of partial differential equations, have been used to describe both the genetic dynamics ("reaction") and spatial spread ("diffusion") of gene drive alleles. These models treat time and space as continuous and represent genetic change through frequency-based dynamics coupled to spatial dispersal processes. For low-threshold gene drives, Tanaka et al. (2017) demonstrate that selection against the gene drive must fall within a specific range to prevent indefinite spatial spread. Outside this range, gene drive alleles are predicted to propagate across space, limiting the feasibility of recall through local interventions.

Reaction-diffusion modelling has further revealed distinct modes of spatial spread, such as "pushed" versus "pulled" waves, which influence containment potential and resistance dynamics. In pushed waves, spread is maintained by high frequency regions behind the leading edge, suggesting that reducing gene drive densities locally may not be sufficient to halt expansion. These insights underscore that resistance, reversal and recall cannot be assessed independently of spatial ecology and dispersal processes.

Overall, mathematical models predict that resistance to engineered gene drives is likely to emerge unless resistant allele formation rates are extremely low, and that resistance evolution is often largely independent of release size or conversion efficiency. Reversal and recall are theoretically possible for certain gene drive designs, particularly threshold-dependent systems, but require carefully defined and sometimes resource-intensive intervention strategies. Spatially explicit models further indicate that containment, resistance evolution and reversibility are tightly coupled processes. While current modelling frameworks

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provide valuable conceptual guidance, their application to ERA and PMEM remains limited by simplifying assumptions and by the need for more integrated models linking molecular mechanisms, population dynamics and spatial processes.

4.4.3 AQ4.4 – Recommendations on the choice and further development of models to support ERA and PMEM of GDMIs

Models used to study engineered gene drive dynamics are typically developed in a bespoke manner, tailored to specific academic, technical or regulatory questions. Consequently, there is no single modelling framework capable of meeting all requirements for ERA or PMEM of GDMIs. Rather than relying on a universal model, modelling should be used as a flexible, complementary tool that augments qualitative and empirical risk assessment approaches.

The sections below provide recommendations on how existing modelling strategies can support ERA and PMEM, identify key gaps in current approaches, and outline propositions for advancing modelling frameworks to improve their regulatory utility.

4.4.3.1 SQ4.4.1 – How useful are current modelling strategies for ERAs of GDMIs?

The modelling strategies most commonly used to investigate engineered gene drive dynamics are deterministic, discrete time population genetic recursion models describing changes in gene or genotype frequencies across generations. These models provide tractable and transparent mathematical frameworks for evaluating gene drive efficacy under a wide range of assumptions related to gene drive design (e.g., low-threshold, underdominant or high-threshold systems), inheritance patterns, sex-specific effects and population level spread.

Such models are useful for ERA in early-tier assessments, where the objective is to understand fundamental properties of engineered gene drive systems, identify invasion thresholds, explore persistence and compare alternative gene drive architectures under standardised conditions. However, several key assumptions limit their direct applicability for full ERAs. First, the discretisation of time into generations restricts the representation of complex life histories, overlapping generations and continuous ecological feedbacks. Second, deterministic formulations do not account for variability in demographic, genetic or environmental parameters, limiting their ability to address uncertainty and rare but consequential outcomes. While these frameworks could, in principle, be extended to stochastic analogues, this has rarely been done in a way directly relevant to ERA.

Third, and most critically, these models have been developed primarily to assess outcomes for the target organism, rather than to evaluate potential impacts on non-target organisms, wider biodiversity or ecosystem functioning. As such, although they are valuable for characterising gene drive performance, they do not by themselves provide comprehensive frameworks for ERA.

Nonetheless, modelling approaches could play a valuable complementary role in ERA if explicitly extended to address risk hypotheses. For example, stochastic population genetic models incorporating ecological feedbacks could be developed to explore how gene drive spread and suppression of target populations might indirectly affect competitors, predators

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or ecosystem structure. Similarly, models developed to quantify genetic efficacy of gene drives targeting disease vectors could be extended to explore potential impacts on disease transmission dynamics and human health endpoints.

4.4.3.2 SQ4.4.2 – How useful are different modelling approaches for PMEM of GDMIs?

The majority of modelling studies identified in the literature search focus on predicting efficacy and spread prior to release and are not directly designed to support PMEM. PMEM requires predefined strategies for data collection, analysis and interpretation, with an emphasis on detecting deviations from expected outcomes rather than forecasting idealised scenarios.

Mathematical models can nonetheless play an important role in PMEM when embedded within appropriate statistical and inferential frameworks. Beyond standard regression-based approaches, models such as occupancy, detection and mark-release-recapture frameworks are already widely used to estimate population abundance and trends in ecological monitoring contexts. When coupled with genetic and ecological models of gene drive dynamics, these approaches could be used to evaluate observed changes in genotype frequencies, population size or spatial distribution following environmental releases.

In this context, models become tools for hypothesis testing and interpretation rather than prediction alone. Comparing observed PMEM data with model-based expectations can help to identify unanticipated spread, early signs of resistance, changes in persistence or deviations in ecological outcomes. In particular, spatially explicit models that integrate genetic dynamics with dispersal and landscape structure could be valuable in assessing ongoing efficacy, containment and spatial exposure relevant to PMEM.

4.4.3.3 SQ4.4.3 – What gaps exist in the use of modelling for ERA and PMEM of GDMIs?

A central gap identified in the literature is the absence of modelling approaches explicitly designed to support ERA and PMEM of GDMIs. Current models are largely focused on demonstrating gene drive efficacy and theoretical behaviour rather than addressing regulatory risk questions.

In particular, there is a lack of modelling frameworks that integrate genetics, ecology and uncertainty in a manner aligned with risk hypotheses. Existing models seldom incorporate stochasticity in a way that allows probabilistic assessment of adverse outcomes, nor do they routinely link predictions to empirical data through formal statistical validation.

To bring mathematical modelling credibly into ERA and PMEM, dedicated risk-focused tools are required. These should be developed to support the full lifecycle of gene drive technologies, from early-stage development and confined testing through to post-release monitoring. Such tools must be explicitly linked to clearly articulated assessment endpoints, protection goals and data collection strategies.

Overall, there is a need for modelling approaches that combine deterministic or stochastic genetic and ecological models with statistical, probabilistic and inferential methods. These tools should enable evaluation of outcomes such as persistence, resistance, unanticipated

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spread, impacts on non-target organisms, and potential consequences for wider biodiversity or human health.

4.4.3.4 SQ4.4.4 – Propositions and solutions to address modelling gaps and recommendations for ERA and PMEM of GDMIs

A key conclusion from the literature search is that no single modelling framework can serve the diverse needs of ERA and PMEM for GDMIs. Instead, independent, bespoke modelling tools should be developed to address specific categories of risk questions.

Several core principles should guide the development and selection of models for regulatory use. First, models intended to support ERA and PMEM should, wherever possible, incorporate stochastic formulations of genetic and/or ecological processes in order to quantify uncertainty and variability in outcomes. Second, model development should be closely aligned with data requirements, ensuring that parameters can be informed by laboratory, semi-field, field or monitoring data. Third, models should be embedded within appropriate statistical frameworks that allow formal evaluation of model fit, comparison of alternative hypotheses and iterative updating as new data become available.

In addition, modelling tools should allow robust sensitivity and uncertainty analyses to identify parameters and assumptions that most strongly influence predicted outcomes. This is essential for prioritising data collection, identifying worst-case scenarios and supporting transparent regulatory decision-making.

Conclusions

This literature search highlights that the current body of mathematical modelling for GDMIs has been developed primarily to explore the genetic efficacy, invasion dynamics and theoretical performance of engineered gene drive systems. The majority of studies focus on population genetic outcomes in target organisms, often under idealised and deterministic assumptions. While these models have been instrumental in advancing scientific understanding of gene drive behaviour and in informing technology design, their direct applicability to ERA and PMEM is limited.

In particular, there is a clear imbalance between modelling efforts aimed at demonstrating gene drive efficacy and those addressing ecological interactions, non-target organism effects, wider biodiversity impacts and human health considerations. Models that explicitly incorporate ecological complexity, stochasticity, spatial structure and uncertainty remain relatively rare, despite their relevance for regulatory risk assessment. As a result, current modelling approaches only partially address the types of risk hypotheses that underpin ERA and PMEM, such as unintended spread, persistence beyond intended spatial or temporal scales, resistance evolution, reversibility and indirect ecological effects.

The literature search further indicates that modelling has so far been used predominantly as a predictive and exploratory tool prior to environmental release, rather than as an integrative component of post-release monitoring and adaptive risk management. For PMEM in particular, there is considerable unrealised potential for models to support interpretation of monitoring data, hypothesis testing and early detection of deviations from expected outcomes. Realising this potential will require closer integration of modelling with designed data collection, statistical inference and iterative updating as empirical evidence accumulates.

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Overall, the findings support the conclusion that modelling should not be viewed as a single, definitive solution for ERA or PMEM of GDMIs, but rather as part of a broader, tiered and weight-of-evidence approach. To strengthen the role of modelling in regulatory contexts, future efforts should prioritise the development of bespoke, risk-focused modelling tools that explicitly address uncertainty, incorporate relevant ecological processes and are aligned with defined protection goals and assessment endpoints. Embedding such models within transparent statistical and probabilistic frameworks will be essential to ensure credibility, interpretability and regulatory usefulness.

In conclusion, while existing mathematical models provide a strong conceptual foundation and valuable insights into engineered gene drive dynamics, substantial further development is needed for them to effectively support ERA and PMEM of GDMIs. Advancing modelling in this direction will improve its capacity to complement empirical evidence, support informed decision-making and enhance the robustness of environmental risk governance for gene drive technologies.

4.5 ToR5 – Review monitoring approaches

4.5.1 AQ5.1 – What monitoring objectives are relevant for the PMEM of GDMIs?

Under EU legislation, applications for the placing on the market of GMOs, including GDMIs, are required to include a PMEM plan. This plan forms an integral part of the final authorisation and is intended to ensure continued oversight once a GMO is deliberately released into the environment. PMEM aims to identify changes in the environment and trends in specific indicators that could be caused by the exposure to an approved substance (such as a GMO) showing no cause for concern in the ERA (Anastassiadou et al., 2025).

In this context, PMEM serves as an early warning system to identify outcomes that deviate from those anticipated in the ERA, thereby enabling timely implementation or adjustment of risk mitigation and management measures from a pre-defined set of options. In case a risk has been realised, it may trigger the application of remedial measures that are already known to be effective, such as the use of insecticides or sterile insects (EFSA, 2020; Devos et al., 2022).

Annex VII of Directive 2001/18/EC (EC, 2001), describes two distinct but complementary objectives for monitoring of GMOs, case-specific monitoring (CSM) and general surveillance (GS).

The objective of CSM is to gather targeted, measurable data to verify the conclusion of the ERA and to assess the validity of predictions and assumptions underpinning the regulatory decision-making process. The overall conclusions of the ERA provide the basis for determining whether CSM is required and, if so, for defining its scope and design. CSM focuses on specific potential risks to human and animal health and the environment that have been identified in the ERA as requiring follow-up. In addition, CSM may be used to reduce the residual scientific uncertainties highlighted during the ERA, particularly those related to effects that may only manifest under large-scale or long-term environmental exposure. CSM can also be used to evaluate the effectiveness of risk mitigation measures implemented as part of the authorisation. In this way, CSM may complement the ERA by contributing to the further reduction and management of remaining scientific uncertainty after authorisation (Devos et al., 2022). As such, CSM is hypothesis-driven and should be designed to target the specific

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assessment endpoints and protection goals identified in the ERA conclusions as being at risk or characterised by critical uncertainty (EFSA, 2013).

The objective of GS is to address residual uncertainty related to potential adverse environmental effects that could not be anticipated during the ERA. GS is not limited to predefined hypotheses and is intended to capture unexpected changes that may occur following the introduction of a GMO. GS relies on existing monitoring/surveillance networks that operate at the EU, national and local levels to measure a range of natural resources and environmental characteristics related to protection goals, such as biodiversity, and water and air quality, independent of the factors influencing them (Anastassiadou et al., 2025). Importantly, not all observed deviations from intended or expected outcomes are necessarily adverse. Therefore, GS includes the challenge of determining whether an observed effect is harmful, whether it can be causally linked to the GMO, and whether regulatory action is required. When adverse effects are detected in a timely manner, appropriate corrective or risk mitigation measures can be implemented (Sanvido et al., 2005).

Given their distinct objectives, CSM and GS are designed to be complementary. Moreover, they are conceptually and operationally interlinked. In principle, GS may identify unanticipated adverse effects that appear to be associated with the GMO. Where such signals are detected, further investigation may be warranted to explore potential cause-effect relationships. In case the results of these investigations conclude the adverse effect is due to the particular GMO, the results may feed back into the ERA of the GMO and, where appropriate, lead to modifications of existing CSM measures or the introduction of new, targeted monitoring activities.

4.5.1.1 SQ5.1.1 – What are the different objectives for which insect monitoring is conducted?

The deliberate environmental release of GM insects, and particularly those carrying engineered gene drives, represents a novel technological development for which experience outside contained research settings remains very limited (EFSA, 2020). Therefore, there is currently no direct practical experience with PMEM of such organisms following environmental release. In the absence of an established PMEM track record for GDMIs, it is therefore informative to draw on experience gained from other insect control technologies that have been implemented in open environments and for which different forms of insect monitoring have long been conducted.

This section reviews monitoring objectives associated with a range of existing insect control strategies, including CBC, ABC, SIT, GMIs carrying a dominant (female) lethal gene [(fs)RIDL], IIT, and Wolbachia-mediated pathogen interference (PI) approaches (Flores and O'Neill, 2018; EFSA, 2020; Romeis et al., 2020; WHO, 2021). While these approaches differ substantially in their mechanisms of action, regulatory status and intended outcomes, they share a reliance on insect monitoring to support deployment, assess performance and identify potential adverse effects.

Across these technologies, insect monitoring has typically served several interrelated objectives. A primary objective has been to verify that the control strategy performs as intended under field conditions. This includes monitoring insect population densities, spatial distribution and temporal dynamics in order to assess establishment, persistence, dispersal and, where relevant, population suppression or replacement effects. For example, in SIT and

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(fs)RIDL programmes, monitoring is essential to confirm mating competitiveness, population suppression efficiency and the continued effectiveness of mass rearing and release strategies. In CBC and ABC, monitoring is used to document establishment and spread of biological control agents and to assess their contribution to pest regulation.

A second major objective of insect monitoring has been to support adaptive management of the control intervention. Monitoring data are used to inform operational decisions such as release rates, spatial targeting, timing of interventions and, where necessary, the implementation of corrective or remedial measures. In this context, monitoring functions as an early warning system, detecting deviations from expected outcomes that may require adjustments to management practices. This operational feedback role is particularly well developed in SIT, IIT and *Wolbachia*-based PI programmes, where continuous or periodic monitoring is integral to programme effectiveness.

A further objective has been the identification and assessment of unintended or adverse effects on non-target organisms, ecosystem functions or broader environmental attributes. In biological control, for example, post-release monitoring has been used to assess host specificity and potential non-target impacts, complementing pre-release risk assessment. Similarly, monitoring associated with *Wolbachia*-based approaches may include observations of ecological interactions, persistence beyond target areas, or unforeseen effects on local insect communities. This objective aligns closely with the concept of post-authorisation environmental oversight and is particularly relevant when uncertainties remain regarding long-term or large-scale ecological consequences.

In addition, insect monitoring has served to generate empirical data that feed back into risk assessment frameworks and improve scientific understanding of insect ecology and control technologies. Monitoring outcomes have been used to refine models of population dynamics, dispersal and resistance development, and to reassess assumptions made during prospective risk assessments. In this sense, monitoring contributes not only to risk management but also to the iterative improvement of regulatory science.

Given the novelty of engineered gene drives and the limited empirical evidence available from previous deliberate environmental releases, these established monitoring objectives provide valuable reference points for defining PMEM objectives for GDMIs. While GDMIs raise specific challenges related to persistence, spread and potential transboundary effects, the underlying purposes of insect monitoring—verification of intended effects, detection of unanticipated outcomes, support for adaptive management and reduction of residual uncertainty—remain highly relevant.

In addition to insights from existing insect control technologies, guidance on insect monitoring objectives has been developed by international organisations and regulatory authorities in different jurisdictions. Advice from bodies such as the World Health Organization, the North American Plant Protection Organization, the Secretariat of the Convention on Biological Diversity and the New Zealand Environmental Protection Authority provides further perspectives on how monitoring objectives can be articulated, prioritised and operationalised in regulatory contexts. Together, these sources offer a foundation for defining robust, proportionate and scientifically grounded monitoring objectives for the PMEM of GDMIs.

Experience with other insect control technologies

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Given the novelty of engineered gene drives and the absence of practical experience with PMEM of GDMIs, it is informative to examine monitoring practices associated with other insect control technologies that have been deployed in open environments. These approaches differ in mechanisms, regulatory treatment and intended outcomes, but all rely, to varying degrees, on insect monitoring to assess performance, guide management and identify potential unintended effects.

Classical Biological Control

CBC involves the intentional introduction of natural enemies, typically sourced from the geographical region of origin of an invasive arthropod pest, with the aim of establishing permanent populations that provide long-term control of the target pest and, in some cases, contribute to its local eradication. In several jurisdictions, post-release monitoring is a prerequisite for the release of exotic biological control agents (BCAs), for example under guidance from the North American Plant Protection Organization (NAPPO, 2015a,b), and is also recommended by other bodies such as EPPO (2014).

The main objectives of monitoring in CBC programmes are to assess the efficacy of the control intervention and to understand the ecological interactions that emerge following release. Monitoring typically focuses on the establishment and spread of the BCA, population dynamics over time, rates of attack on target organisms, interactions with non-target species, and broader effects on population and community-level processes or structures (Barratt et al., 2006; Romeis et al., 2020). These objectives align with both operational evaluation and environmental impact assessment.

In practice, however, most jurisdictions do not impose formal monitoring requirements once a BCA has been released. Post-release studies are often conducted by independent researchers rather than regulatory authorities or applicants, and the available evidence base remains limited, largely due to insufficient long-term funding. Consequently, adverse effects, if they occur, may go undetected or unreported (Barratt et al., 2006).

An illustrative example is the release of the parasitoid wasp *Microctonus aethiopoides* (Hymenoptera: Braconidae) in New Zealand for the control of the non-native weevil *Sitona obsoletus* (Coleoptera: Curculionidae). Post-release research demonstrated that the parasitoid had become established within native weevil communities in grassland ecosystems, but parasitism rates were generally low, typically below 10%. No conclusions were drawn regarding potential adverse effects on native weevil populations, and the regulatory approval did not prescribe any management action even if adverse effects were to be identified (Ferguson et al., 2024). This example highlights both the value and the limitations of post-release monitoring in CBC.

Augmentative Biological Control

ABC involves the regular release of mass-reared natural enemies to supplement existing populations and suppress pest outbreaks. ABC is typically applied in relatively contained environments, such as commercial glasshouses, where exposure beyond the target system is limited. ABC is regulated under some jurisdictions (Barratt et al., 2021).

Monitoring under ABC programmes is primarily operational in nature and aims to assess the efficacy of releases and inform short-term management decisions. Objectives typically include tracking pest and natural enemy populations and adjusting release strategies accordingly.

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Monitoring is not intended to detect environmental effects beyond the production system and does not address long-term or landscape-level impacts.

Sterile Insect Technique

SIT is a biological control method based on the mass, inundative release of sterile male insects that compete with wild males for mates, thereby reducing reproductive output and leading to population suppression. Repeated releases over successive breeding cycles can, in principle, result in local population elimination (Kapranas et al., 2022). SIT has been widely applied in Europe and elsewhere to control agricultural pests such as the Mediterranean fruit fly (*Ceratitis capitata*; Diptera: Tephritidae), olive fruit fly (*Bactrocera oleae*; Diptera: Tephritidae), codling moth (*Cydia pomonella*; Lepidoptera: Tortricidae), as well as disease vectors such as *Aedes albopictus* (Bourtzis and Vreysen, 2021).

In most jurisdictions, SIT insects are not considered novel organisms because they are derived from existing local populations. Consequently, they are generally not subject to ERA or PMEM requirements and are regulated primarily through phytosanitary frameworks where applicable (Enkerlin and Pereira, 2022). Nevertheless, insect monitoring is widely recognised as essential for SIT programmes, albeit for operational rather than regulatory purposes.

Monitoring objectives in SIT focus on assessing and managing releases, measuring population suppression, optimising release strategies and demonstrating programme effectiveness (Flores and O'Neill, 2018; Kapranas et al., 2022; Ogoyi et al., 2024). Environmental monitoring beyond the target population is rare and usually undertaken by third-party researchers rather than programme operators.

One example of such monitoring is a study conducted during the release of sterile tsetse flies (*Glossina palpalis gambiensis*; Diptera: Glossinidae) in Senegal, which evaluated potential environmental effects by monitoring selected fruit-feeding insect taxa as indicators of ecosystem health. The study found very limited impacts on insect densities or biodiversity, suggesting no detectable adverse environmental effects associated with the releases (Ciss et al., 2019).

(fs)RIDL GM insects

GM insects carrying dominant or female-specific lethal constructs ((fs)RIDL) are released in an inundative manner similar to SIT. Modified males mate with wild females, and inheritance of the lethal gene causes female offspring to die before reaching maturity, thereby reducing population size. From a population control perspective, this approach closely resembles SIT, although it relies on genetic mechanisms rather than irradiation-induced sterility (Flores and O'Neill, 2018; Ogoyi et al., 2024; Zhu et al., 2024).

Open releases with (fs)RIDL *Aedes aegypti* mosquitoes to suppress wild-type populations have taken place in Brazil, the Cayman Islands, Malaysia, and Panama, while activities have been planned in Florida (USA) and India (EFSA, 2020). From 2018 on, open releases with the second generation (fs)RIDL *Aedes aegypti* were started in Brazil, the US, Marshall Islands and activities were planned in Australia (Ogoyi et al., 2024; CSIRO, 2024). In Brazil, the regulatory authority (CTNBio) required post-release monitoring to determine the proportion of the mosquito population carrying the genetic construct, with monthly monitoring during the first year and annual monitoring thereafter. The primary objective was to assess the stability of the genetic marker over time (Zhu et al., 2024). Similarly, approvals by the US

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Environmental Protection Agency included monitoring requirements focused on the spread, persistence, stability and efficacy of the genetic construct (Ogoyi et al., 2024). In both cases, monitoring objectives were limited to construct performance and population suppression, rather than broader environmental effects.

Wolbachia-mediated Incompatible Insect Technique

In Wolbachia-mediated IIT, male mosquitoes infected with the endosymbiotic bacterium *Wolbachia pipientis* are released to induce cytoplasmic incompatibility. When infected males mate with uninfected females, embryos fail to develop, resulting in population suppression. IIT has been implemented in several countries, including China, Thailand, Singapore and the United States, primarily to control mosquitoes transmitting dengue fever.

Post-release monitoring under IIT has largely focused on assessing suppression efficacy by tracking mosquito population densities (Flores and O'Neill, 2018; Bansal et al., 2024; Lim et al., 2024a, 2024b). Only limited examples exist of monitoring beyond this objective. One study included periodic genome sequencing of both the bacterium and host mosquito, but the purpose was to assess genomic stability rather than to identify adverse environmental effects (Wimalasiri-Yapa et al., 2023).

Wolbachia-mediated Pathogen Interference

Wolbachia-mediated PI involves the release of both male and female *Wolbachia*-infected mosquitoes, leading to population modification rather than suppression. Infected females can reproduce successfully with both infected and uninfected males, allowing Wolbachia to spread through the population. *Wolbachia* infection reduces the capacity of mosquitoes to transmit pathogens such as dengue virus. Large-scale PI programmes have been implemented mainly in the Asia–Pacific region and Latin America (Mushtaq et al., 2024).

Post-release monitoring of *Wolbachia*-infected mosquito releases has been conducted, for example, in northern Queensland, Australia. Monitoring objectives focused on confirming the durability, spatial spread and long-term persistence of *Wolbachia* infection in local *Aedes aegypti* populations, thereby verifying the continued effectiveness of disease transmission reduction (Ryan et al., 2020).

Overall insights for PMEM

Overall, experience with other insect control technologies indicates that most regulatory jurisdictions require little or no formal PMEM once releases are authorised. Although monitoring is often recognised as important in principle, it is frequently limited or absent in practice, largely due to funding constraints and the absence of explicit regulatory obligations (Paterson et al., 2024; Abram et al., 2026). Where monitoring is conducted, objectives are predominantly operational and include assessing efficacy, population suppression, spread, persistence and genetic or microbial stability of the released organisms. Dedicated environmental monitoring, particularly aimed at detecting unintended or adverse ecological effects, has been relatively uncommon and is most evident in the context of classical biological control.

These observations provide important context for defining monitoring objectives for GDMIs, where higher levels of uncertainty and potential for long-term or wide-scale effects may justify a broader and more structured PMEM approach.

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4.5.1.2 Advice on PMEM outside the EU

To identify how monitoring objectives for insect releases are addressed beyond the EU, guidance from other jurisdictions and advisory organisations was examined. These included the World Health Organization (WHO), the North American Plant Protection Organization (NAPPO), the Secretariat of the Convention on Biological Diversity (CBD), and the New Zealand Environmental Protection Authority (NZ EPA).

WHO provides only very general guidance on post-implementation monitoring in its Guidance Framework for Testing Genetically Modified Mosquitoes. The framework does not define specific monitoring objectives, but emphasises procedural agreement, stating that "if post-implementation monitoring is required, parties should agree on what is to be monitored, to what extent, how, by whom, and how the data will be collected and analysed" (WHO, 2021). No further specification is given regarding environmental or ecological monitoring endpoints.

More substantive guidance on post-release monitoring objectives is provided by NAPPO and by the CBD Secretariat in the context of BCAs (NAPPO, 2015a,b; Sheppard et al., 2019). This guidance outlines several key objectives that a PMEM plan for BCAs should address:

- Confirmation of establishment of the BCA through documented observation and measurement of population density and spatial spread;
- Confirmation of the impact on the target species, based on observations of population density, distribution and trends over time;
- Validation of expectations identified in the risk assessment regarding impacts on target and non-target species, including host preference, development, and changes in growth, survival or reproduction;
- Survey of non-target species not identified in the risk assessment within the establishment area, with the aim of identifying unexpected impacts. This includes characterising interactions between the BCA and non-target species, as well as any induced changes at the community level, such as shifts in species composition or diversity;
- Assessment of BCA performance in relation to the intended outcomes of the release programme.

These objectives are relatively broad. To further explore potential monitoring endpoints, information requirements used in regulatory risk assessment frameworks were also examined. For example, applications to release BCAs in New Zealand are structured around a set of questions that implicitly define monitoring-relevant concerns (Vieglais and Harrison, 2004). These include:

- Surveys for displacement of native species within natural habitats;
- Surveys for significant deterioration of natural habitats;
- Surveys for significant adverse effects on human health or safety;
- Assessment of adverse effects on genetic diversity within the establishment area;
- Surveillance for novel diseases, parasites or pest associations arising following release.

Taken together, these two sets of objectives provide comprehensive coverage of post-release monitoring aims and align well with the overarching monitoring objectives identified under AQ5.1. Combined, they point to the following monitoring objectives:

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- Confirmation of establishment of the released organism;
- Confirmation of impact, including efficacy of the control intervention;
- Validation of risk assessment expectations regarding impacts;
- Detection of displacement of other species;
- Identification of significant deterioration of natural habitats;
- Detection of significant adverse effects on human health or safety;
- Assessment of adverse effects on genetic diversity within the receiving environment;
- Surveillance for novel diseases, parasites or pest associations.

Confirmation of establishment and confirmation of impact (e.g., efficacy) are common to PMEM for all insect release programmes and are routinely addressed where monitoring is conducted. In contrast, the remaining objectives—particularly those relating to environmental, ecological and genetic effects—are consistently addressed on a case-by-case basis. Their relevance depends on the characteristics of the organism, the intended use, and the receiving environment.

Importantly, the objectives associated with environmental monitoring of BCAs outside the EU closely align with the objectives of CSM under the EU PMEM framework. Beyond assessing efficacy (establishment and impact), CSM aims to validate the assumptions made during the ERA and to address critical uncertainties identified in the ERA. As such, CSM is inherently framed by the ERA and implemented on a case-by-case basis, in the same way as environmental monitoring objectives for BCAs outlined above.

It is therefore concluded that post-release monitoring of BCAs outside the EU, where environmental monitoring is conducted, corresponds conceptually to CSM within the EU regulatory framework. By contrast, the objective of GS as defined under EU legislation—namely the systematic detection of unanticipated adverse effects independent of predefined hypotheses—is not explicitly required or routinely implemented in PMEM frameworks outside the EU.

4.5.1.3 SQ5.1.2 – Considering the examples of GDMIs most relevant for the EU, what are the environmental protection goals for which PMEM approaches would be useful?

In 2022, Decision (EU) 2022/591 of the European Parliament and of the Council on a General Union Environment Action Programme to 2030 entered into force (EC, 2022). The Decision establishes six interlinked thematic priority objectives, of which one is of particular relevance for the PMEM of GDMIs, namely the objective of protecting, preserving and restoring biodiversity. This includes halting and reversing biodiversity loss, improving ecosystem structure and function, safeguarding ecosystem services, and improving the quality of environmental compartments such as air, water and soil.

Building on EU legislation and its implementation at national level, the EFSA GMO Panel identified two overarching environmental protection goals relevant for GMO risk assessment and monitoring (EFSA, 2013, 2010). These are biodiversity conservation, with a focus on species of conservation or cultural value, including red-listed species, and ecological functions, with a focus on the preservation and sustainable use of soil, water, production systems and plant health. These protection goals are consistent with the biodiversity-related objectives set out in the 2022 Environment Action Programme. The Panel also emphasised the need to define assessment endpoints that are representative of the environmental components and functions requiring protection under EU legislation.

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Directive 2001/18/EC establishes the overarching protection goal of safeguarding human and animal health and the environment. For the purposes of ERA, this protection goal is operationalised into specific "areas of risk" that must be addressed for all GMOs (EC, 2001). For GMIs, including GDMIs, these areas of risk include, among others, human and animal health, plant health, non-target organisms (including species of conservation concern and species delivering ecosystem services), and environmental microbiomes (e.g., soil and aquatic systems). These areas of risk, and the associated pathways to harm, are reflected in the problem formulation and summarised in Table 27 (ToR1).

The overall conclusions of the ERA determine whether CSM is required and, if so, define its objectives. CSM is focused on assessment endpoints and protection goals identified in the ERA conclusions as being at potential risk or characterised by critical uncertainty. In line with the problem formulation approach, CSM is targeted at key steps in identified pathways to harm and is therefore inherently hypothesis-driven. Consequently, CSM objectives for GDMIs are defined on a case-by-case basis and depend on the specific GDMI, its biology and ecology, its intended use, and the characteristics of the receiving environment(s) in which release is foreseen.

By contrast, GS aims to identify adverse effects on human health or the environment that were not anticipated in the ERA. GS is therefore not hypothesis-driven and is intended to be generic in nature, applying to all authorised GDMIs irrespective of their specific traits or intended uses. Based on ToR1, the GDMI species currently considered most relevant for potential deliberate environmental release within the EU and its OMTs are *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella*. These species include one human disease vector and two agricultural insect pests. All three are considered non-native to the EU and its OMTs, are invasive and are established in the EU and its OMTs. The receiving environments for these species differ markedly and are described in Tables 24-26 (ToR1), encompassing urban and domestic habitats with humid sub-tropical climates for *Aedes aegypti* and agricultural ecosystems with temperate climates for *Drosophila suzukii* and *Plutella xylostella*.

4.5.2 AQ5.2 – Considering the objectives identified in AQ5.1 and GDMIs identified under ToR1, what monitoring approaches would achieve these objectives?

As concluded in SQ5.1, PMEM of GDMIs in the EU comprises two distinct but complementary components: CSM and GS. These two components reflect different monitoring objectives and require different monitoring approaches for insect monitoring.

CSM is hypothesis-driven and aims to confirm that the assumptions, predictions and conclusions made in the ERA regarding the occurrence and magnitude of potential adverse effects of a GMO or its use are correct. GS, in contrast, aims to identify adverse effects on human health or the environment that were not anticipated in the ERA and is therefore non-hypothesis-driven and generic in nature.

Experience reviewed in SQ5.1.1 demonstrated that, across a wide range of insect control technologies (CBC, ABC, SIT, GMIs and *Wolbachia*-based approaches), insect monitoring has most commonly been conducted to assess efficacy of the control method, particularly establishment, spread and population suppression or modification. Environmental monitoring aimed at detecting adverse effects has been relatively rare and has mainly been associated with classical biological control. Where monitoring objectives beyond efficacy were defined,

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they focused on validating assumptions made during pre-release risk assessment and investigating potential impacts on non-target organisms or habitats.

In SQ5.1.1.2, guidance from non-EU organisations and jurisdictions was examined. This guidance consistently identified two main monitoring objectives: confirmation of efficacy and validation of risk assessment expectations of impacts. Examples of monitoring aspects proposed on a case-by-case basis included displacement of other species, deterioration of natural habitats, adverse effects on human health or safety, effects on genetic diversity, and the emergence of novel diseases, parasites or pest associations. These objectives align closely with the EU concept of CSM, which focuses on confirming ERA assumptions and addressing critical uncertainties. Notably, GS as defined in EU legislation is not explicitly included in monitoring recommendations outside the EU.

In SQ5.1.2, the GDMI species considered most relevant for potential release in the EU and its outermost regions were identified as *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella*. Based on their ecology, intended use and receiving environments, the environmental protection goals most relevant for PMEM were defined as human health for *Aedes aegypti*, and plant/crop health and agrobiodiversity for *Drosophila suzukii* and *Plutella xylostella*.

Monitoring approaches for CSM

CSM represents the primary monitoring approach to achieve the objectives identified in AQ5.1 related to confirmation and validation of ERA assumptions. Because CSM is framed by ERA conclusions and problem formulation, its design and implementation are necessarily case-by-case (Abram et al., 2026).

For *Aedes aegypti*, where human health is the key protection goal, CSM should focus on monitoring endpoints relevant to disease vector control and potential human health impacts. Monitoring approaches may include confirmation of GDMI establishment and persistence in the target area, assessment of population suppression or replacement efficacy, and verification that the intended health-related outcomes are achieved without giving rise to adverse effects linked to ERA assumptions. Given the close association of this species with humans and the limited exposure of other environmental components, CSM would primarily address human health-related pathways to harm identified in the ERA.

For *Drosophila suzukii* and *Plutella xylostella*, where protection goals relate to plant health and agrobiodiversity in agricultural ecosystems, CSM should focus on confirming the effectiveness of pest control and validating expectations regarding interactions with non-target organisms and agro-ecosystem functions. Monitoring approaches may include assessing establishment and spread of the GDMI, evaluating impacts on target pest populations, and verifying ERA assumptions regarding non-target arthropods, pollinators, natural enemies or other organisms providing ecosystem services. Where the ERA identifies critical uncertainties, such as potential indirect ecological effects, CSM may also address these through targeted measurements linked to specific pathways to harm.

Across all three GDMI species, CSM therefore represents the appropriate monitoring approach to achieve objectives related to efficacy, confirmation of ERA assumptions and reduction of remaining scientific uncertainty. This aligns with monitoring practices observed for other insect control technologies and with international guidance on post-release monitoring of BCAs.

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Monitoring approaches for general surveillance (GS)

GS represents the monitoring approach intended to achieve the objective of identifying adverse effects of a GDMI or its use that were not anticipated during the ERA. Unlike CSM, GS is not hypothesis-driven.

For the three GDMI species considered relevant for the EU, GS should therefore be designed to take into account all environmental protection goals applicable to their receiving environments. This includes human and animal health, the environment in a broad sense (including plant health), and the ecological functions supplied by urban and agricultural ecosystems. GS may rely on existing monitoring systems, observational networks or reporting mechanisms and aims to act as an early warning system for unexpected effects.

While GS has no direct equivalent in post-release monitoring frameworks outside the EU, its inclusion in PMEM reflects a precautionary approach to addressing residual and uncharacterised uncertainty associated with the environmental release of GMOs, including GDMIs.

Overall conclusion

In conclusion, the monitoring objectives identified in AQ5.1 are best achieved through a combination of CSM and GS. CSM is the appropriate monitoring approach to confirm assumptions made in the ERA for the specific GDMI species relevant to the EU, focusing on human health for *Aedes aegypti* and on plant/crop health and agrobiodiversity for *Drosophila suzukii* and *Plutella xylostella*. GS complements CSM by providing a generic mechanism to identify potential adverse effects not foreseen in the ERA, taking into account the full range of environmental protection goals relevant to the receiving environments of these GDMIs.

4.5.2.2 SQ5.2.1 What are the attributes and strengths and weaknesses of these approaches in achieving the monitoring objectives?

PMEM of GDMIs poses particular challenges, as the potential impacts of deliberate environmental releases may not be spatially or temporally confined. In addition, any unexpected changes in the behaviour, performance or stability of a transgenic construct may require timely management interventions. These challenges are especially pronounced for self-sustaining engineered gene drives, where both spatial and temporal scales of exposure can be substantially larger than for self-limiting approaches. As a consequence, PMEM for engineered gene drives needs to be dynamic, spatially explicit and capable of tracking spread, persistence and effects over extended periods and across areas that may extend beyond the initially intended release zone (EFSA, 2020; Abram et al., 2026).

CSM

CSM is framed by the conclusions of the ERA and focuses on critical steps in plausible PTHs, as identified through problem formulation (Table 27, ToR1). These critical steps determine the most relevant assessment endpoints and measurable indicators to be monitored. As a result, CSM is targeted, hypothesis-driven and tailored to the specific GDMI, its intended use and the receiving environment.

For GDMI *Aedes aegypti*, CSM would focus on assessment endpoints and indicators relevant to PTHs associated with human health. For GDMI *Drosophila suzukii* and *Plutella xylostella*,

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CSM would focus on PTHs related to plant and crop health and agrobiodiversity, including interactions with non-target organisms within agricultural ecosystems. Monitoring would be conducted in the receiving environments where releases occur and, in the case of self-sustaining gene drives, potentially beyond these areas, reflecting expected spatial and temporal dynamics.

A key strength of CSM is that it is fit-for-purpose. By focusing on specific assessment endpoints and indicators derived from ERA, the data collected are directly relevant to regulatory decision-making. This targeted nature makes CSM comparatively efficient and often more cost-effective than untargeted monitoring approaches. In addition, because CSM focuses on critical steps in PTHs, it has a strong potential to detect emerging adverse effects at an early stage. This allows CSM to function as an early warning system for specific, anticipated risks associated with a given GDMI. The flexibility of CSM also enables adaptation over time, for example through refinement of indicators or expansion of monitoring scope if new information becomes available, and supports the timely implementation of mitigation or remedial measures where necessary.

The main limitation of CSM lies in its inherent dependence on the ERA. Effects that fall outside the assumptions, scenarios or PTHs considered during risk assessment may not be detected through CSM, as they are not explicitly targeted. Thus, while CSM is strong in confirming and validating ERA-based assumptions, it is less suited to detecting completely unanticipated effects.

GS

GS is intended to identify adverse effects on human health, animal health or the environment, including plant health and ecological functions, that were not anticipated in the ERA. GS is therefore not hypothesis-driven and is not framed by pre-defined PTHs. For the three GDMI cases (insect species) considered relevant for the EU, GS would in principle require a monitoring system capable of detecting unexpected adverse changes across their receiving environments, and potentially beyond, without prior specification of assessment endpoints.

The conceptual strength of GS lies in its role as a potential "safety net", addressing residual uncertainty by providing a mechanism to detect unanticipated adverse effects. If a relevant effect or change were to be detected through GS, the underlying cause–effect relationship could, in theory, be investigated further through targeted studies, with outcomes feeding back into ERA and, if necessary, prompting adaptations to CSM or risk management measures.

In practice, however, GS has significant limitations when applied to GDMIs. Because GS is untargeted and not guided by ERA conclusions, it is inherently unfocused. Detecting meaningful adverse effects against a background of natural variability and environmental change is challenging, and attributing observed effects to a specific GDMI is even more difficult. The absence of clear hypotheses, assessment endpoints or indicators makes GS difficult to design, implement and interpret in a scientifically robust manner.

Moreover, practical guidance on how to establish a fit-for-purpose GS system capable of detecting adverse effects of GMOs that were not foreseen during ERA is limited (EFSA, 2020). Developing such systems would likely be resource-intensive, time-consuming and not cost-effective. Due to its untargeted nature, GS is also unlikely to function as an effective early warning system, as signals may be detected late, ambiguously or not at all.

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Overall assessment

In summary, CSM has clear strengths in achieving the monitoring objectives identified for GDMIs. Its targeted, hypothesis-driven and flexible nature allows efficient data collection, early detection of anticipated adverse effects, and effective support for adaptive risk management. Its main limitation is that it may not capture effects entirely outside the scope of the ERA.

GS, while conceptually attractive as a catch-all safety mechanism for unforeseen effects, suffers from major practical weaknesses. Its lack of focus, difficulties in attribution, absence of operational guidance and high resource requirements limits its effectiveness and feasibility. On this basis, GS is considered an unsuitable approach for monitoring potential adverse effects of GDMIs in practice, whereas CSM represents the most robust and proportionate monitoring approach within the EU PMEM framework.

The strengths and weaknesses of both approaches are summarised in Table 33.

Table 33: Strengths and weaknesses of case-specific monitoring (CSM) and general surveillance (GS) as monitoring approaches for gene drive modified insects (GDMIs) relevant for the European Union (EU) and its outermost regions (OMTs)

Case-specific monitoring (CSM)		**General surveillance (GS)**	
Strengths	**Weaknesses**	**Strengths**	**Weaknesses**
Hypothesis-driven and explicitly focused on potential risks and critical uncertainties identified in the environmental risk assessment (ERA) for GDMI *Aedes aegypti*, *Drosophila suzukii* and *Plutella xylostella*	Limited to risks and assumptions identified during ERA; unforeseen effects completely outside the ERA framework may not be detected	Focused on identifying adverse effects not foreseen during ERA; can theoretically function as a "safety net"	Not hypothesis-driven; absence of predefined assessment endpoints makes design and implementation difficult
Targeted to critical steps in pathways to harm, taking into account the specific GDMI construct, intended use, receiving environment(s), and relevant spatial and temporal scales	May require updating or extension if new pathways to harm (PTH) are identified post-authorisation	Generic and intended to detect adverse effects resulting from any GDMI release	Untargeted nature makes data interpretation difficult and causal linkage to a specific GDMI release highly uncertain
Fit-for-purpose data collection based on clearly defined assessment endpoints and measurable indicators derived from ERA	Depends on the quality and completeness of problem formulation and ERA	Potentially broad coverage of environmental compartments and protection goals	Data collection may be open-ended, unfocused and resource-intensive due to lack of specification

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Case-specific monitoring (CSM)		General surveillance (GS)	
Strengths	**Weaknesses**	**Strengths**	**Weaknesses**
Flexible and adaptable over time to reflect new information, refined hypotheses or changes in the release context	Requires continuous scientific oversight to ensure relevance	Can, in principle, trigger follow-up investigations if unexpected effects are detected	Limited flexibility, as GS is not tailored to specific GDMIs or release scenarios
Observed adverse effects can be directly linked to a specific GDMI due to the targeted design of monitoring	—	If a signal is detected, it can be further investigated through targeted studies such as CSM, with feedback into ERA	Difficult to attribute observed adverse effects to a GDMI rather than other environmental stressors
Can function as an effective early warning system, enabling timely implementation of mitigation or management measures	—	—	Poor suitability as an early warning system, as relevant effects may be detected late or ambiguously
Relatively cost-effective due to focused, hypothesis-based data collection	—	—	Generally not cost-effective due to unfocused and potentially extensive data collection requirements

4.5.3 AQ5.3 – What monitoring and assessment approaches are recommended for use in PMEM of GDMIs?

Scope and assumptions

This section outlines monitoring and assessment approaches recommended for PMEM of GDMIs for *Aedes aegypti*, *Drosophila suzukii*, and *Plutella xylostella*. As no specific GDMI products have yet been developed for these species, it is assumed for the purposes of this report that both engineered self-sustaining and self-limiting gene drive constructs may be developed (see ToR1). The most likely intended uses are population suppression (reduction of vector or pest populations) and, for *Aedes aegypti*, population modification (reducing disease transmission). The PMEM approaches described below therefore focus on these intended uses.

Overall approach

Building on AQ5.2, CSM is considered the most suitable PMEM approach for the relevant GDMIs, targeting the most critical steps in the plausible PTHs for each species as described in ToR1 (Table 27). Illustrative monitoring elements are provided per species. In addition to biosafety, PMEM should track GDMI efficacy, including persistence and spread of the GDMI (or its gene drive construct), genetic efficacy in the target population, and—for *Aedes*

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aegypti—epidemiological efficacy (impact on disease incidence), building further on existing surveillance systems (Connolly et al., 2024; Okumu et al., 2026). Monitoring for resistance development to the GDMI should be part of any PMEM.

GDMI development is expected to follow a phased testing pathway (NASEM, 2016; James et al., 2018), which incrementally increases human and environmental exposure while meeting predefined safety and efficacy criteria. By the PMEM implementation stage (phase 4), a full ERA will have been completed; studies will have been conducted under contained and (semi-) confined conditions; and targeted safety and efficacy monitoring will have occurred in field trials. Plausible PTHs addressed prior to open release include genetic and phenotypic stability and potential differences from the non-GM comparator (e.g., morphology, fitness traits such as development and reproduction, disease-related characteristics such as vector competence, potential allergenicity or toxicity, and resistance to commonly used insecticides). In later phases with open releases, stability, efficacy, and biosafety can be further assessed under realistic conditions (e.g., WHO, 2021; Connolly et al., 2024). Each phase is monitored, and results should iteratively shape PMEM in the post-implementation phase. PMEM should therefore (further) assess efficacy and biosafety (including human health), focusing on major risks (Ogoyi et al., 2024) and on critical PTH steps that could not be fully challenged earlier and/or for which uncertainties remain under realistic conditions and potentially enlarged spatial and temporal scales.

Pre-release modelling can extrapolate results from earlier phases to realistic field conditions and address the potentially wide spatial and long temporal scales of GDMI effects in the receiving environments prior to release. PMEM can then be used to confirm model predictions and support model validation and calibration for future regulatory purposes, creating a dynamic interplay between pre-release modelling and PMEM (Devos et al., 2022; Okumu et al., 2026).

PMEM is especially important for GDMIs because potential impacts may not be spatially or temporally constrained, and any changes to the transgenic construct may require rapid management intervention. This is particularly relevant for self-sustaining GDMIs, where spatial and temporal scales may extend beyond the initial release area (EFSA, 2020). Useful lessons can be drawn from control methods such as classical biological control and *Wolbachia* (for population modification), and from SIT/RIDL (for population suppression) (see SQ 5.1.1). CSM should be adaptive and targeted at the most important risks, functioning as an early warning system to enable timely intervention (EFSA, 2020).

What to monitor

Efficacy. Monitoring should verify that the GDMI behaves as intended by following spread and persistence of the GDMI (and/or the GD construct) in the environment, effects on the target population, and the emergence and dynamics of resistance to the gene drive. The primary focus should be on practical performance indicators that reflect the spread of the transgenes as well as the desired outcomes (Okumu et al., 2026). For population suppression, priorities include target species population size, presence and abundance of non-target vector species, prevalence of drive-resistant alleles, and persistence of intact drive alleles (Rašić et al., 2021). These apply to GDMI cases of *Aedes aegypti*, *Drosophila suzukii*, and *Plutella xylostella*. For *Aedes aegypti* population modification, efficacy monitoring should focus on persistence of intact drive alleles, prevalence of drive-resistant alleles, and continued effectiveness of the effector gene (Rašić et al., 2021). Existing surveillance systems provide a workable foundation

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for efficacy monitoring, for example high-throughput molecular assays for quantifying genetic allele frequencies and any emerging resistance to the gene drives could be integrated into existing workflows (Okumu et al., 2026)

Biosafety. PMEM should target the most important risks identified in the ERA for which critical uncertainty have been identified, focusing on critical PTH steps. For *Aedes aegypti*, emphasis includes PTHs with potential adverse effects on human health, for example due to loss of GDMI efficacy leading to more pathogenic target populations, or niche replacement by a more harmful vector (e.g., *Aedes albopictus*). Monitoring here can be integrated with, or is already covered by, existing surveillance programs (e.g., ECDC) for *Aedes aegypti* and *Aedes albopictus*.

For *Drosophila suzukii* and *Plutella xylostella*, plausible PTHs include adverse effects on non-target organisms via loss of food resources (e.g., predators or parasitoids) and niche replacement by competitor or invasive species more harmful to plant health (Table 27, Fig. 15, ToR1). A critical step is to identify non-target organisms in the receiving environments with strict dependency on *Drosophila suzukii* or *Plutella xylostella* as a food source. If such non-target organisms are known, monitoring should prioritise them. Where dependencies are uncertain, indicator or focal species representing the relevant ecosystem services can be prioritised and selected (e.g., EFSA, 2010; Puig-Girones and Real, 2022; Abram et al., 2026). Additional considerations for choosing and monitoring indicator/focal species are provided in SCBD (2025) and Abram et al. (2026).

Potential adverse effects due to niche replacement of *Drosophila suzukii* or *Plutella xylostella* will likely become evident through monitoring of plant health and damage at and around release sites. Larger-scale effects may also be detected through agricultural surveillance (e.g., integrated pest management, IPM) (see "How to monitor").

How to monitor

Surveillance programs for human disease vectors such as *Aedes aegypti* are already in place in the EU and its OMTs. The spread and persistence of agricultural pests such as *Drosophila suzukii* (Nair and Peterson, 2023; CABI, 2025) and *Plutella xylostella* (Richardson et al., 2020; Li et al., 2021) are also tracked. Methods used in these programs are directly relevant to GDMI monitoring of genetic efficacy and resistance development. Additional experience can be leveraged from releases targeting *Aedes aegypti* population suppression (SIT, RIDL) and population modification (*Wolbachia*). Potential increases in pathogenic target populations due to loss of GDMI efficacy can be monitored indirectly via trends in ika, dengue, and chikungunya incidence in release areas, using existing EU/OMT reporting systems (e.g., ECDC).

Monitoring potential impacts to non-target organisms due to food-resource loss in fruit/berry agroecosystems (*Drosophila suzukii*) and Brassicaceae systems (*Plutella xylostella*) depends on the selected indicators, their variability, specificity, sensitivity, and ability to signal adverse change (SCBD, 2025). Much of the required entomological monitoring can use established methods (WHO, 2021). The bycatch of entomological surveillance programmes or the use of new methodologies such as environmental DNA (eDNA) approaches may provide a promising and cost-effective complement to detect community changes and non-target organism responses (Connolly et al., 2024; Okumu et al., 2026). Methodology should specify sampling

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design, collection and analytical methods, and data-analysis plans in sufficient detail (SCBD, 2025).

For niche replacement leading to increased plant damage or plant health risks, data can come from dedicated monitoring at initial release sites and from other sources, including surveys, questionnaires, field observations, and ongoing/existing programs for IPM, biological control, pesticide resistance monitoring, and invasive pest surveillance (e.g., as coordinated by EU Member States with EFSA; see https://www.efsa.europa.eu/en/topics/pest-surveillance). Effective identification, detection, and traceability of GDMIs in the likely receiving environments must also be ensured (SCBD, 2025).

Where to monitor

PMEM should begin in representative areas where deliberate releases occur. Early monitoring can focus on or near initial release sites where GDMI concentrations are expected to be highest, then adapt spatially and temporally as informed by modelling and observed spread—especially for self-sustaining GDMIs (EFSA, 2020; WHO, 2021; Abram et al., 2026).

For *Aedes aegypti*, initial release and monitoring are expected in human habitats within humid subtropical areas. For *Drosophila suzukii*, releases will likely occur in major fruit and berry production regions (e.g., Spain, Poland, Greece, Italy). For *Plutella xylostella*, releases will likely occur in major Brassica-growing areas (e.g., Germany, Poland, Hungary). Depending on species biology and gene drive type (self-limiting vs self-sustaining), monitoring may need to extend to similar agroecosystems identified through pre-release modelling.

Dispersal characteristics will shape monitoring scales. *Aedes aegypti* typically disperses over short distances (~200 m), so GDMI spread may proceed gradually across contiguous human habitations; unintentional long-distance dispersal via transport (e.g., boats, aircraft) must be considered. *Drosophila suzukii* can actively disperse among fruit-producing regions and orchards, warranting monitoring in neighbouring production areas based on dispersal range, life cycle, and drive characteristics; passive dispersal via infested fruit may extend PMEM far beyond intended receiving environments. *Plutella xylostella* is highly migratory, with wind-borne adults travelling long distances to colonise Brassica crops across regions, countries, and continents; consequently, monitoring areas may need to be much more extensive. Here, pre-release modelling will be especially helpful to anticipate GDMI spread and prioritise surveillance.

Other relevant PMEM aspects are described in WHO (2021), SCBD (2025) and Abram et al. (2026).

Duration of monitoring

PMEM duration depends on the endpoints (e.g., spread, intended changes, resistance development), biosafety concerns (critical PTH steps), human health outcomes (disease incidence), gene drive type (self-limiting vs self-sustaining), and intended use (population suppression vs modification) (e.g., Rašić et al., 2021). Duration is also influenced by the frequency, number, and periodicity of observations required to reliably detect change (time to signal) and by the magnitude and type of change of interest (SCBD, 2025).

For GDMI *Aedes aegypti* (suppression and/or modification), monitoring of spread and persistence should continue as long as the GDMI (or its construct) is detectable. PMEM

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addressing human health risks—such as more pathogenic target populations due to loss of efficacy or increased disease incidence due to niche replacement by more harmful species—may need to continue longer, but can be integrated into existing surveillance programs (WHO, 2021), including those operated by ECDC.

For *Drosophila suzukii* and *Plutella xylostella* (assumed population suppression), monitoring of spread and persistence should likewise continue while the GDMI remains detectable. Monitoring of potential adverse effects on non-target organisms will be most informative when suppression is at or near its peak; it can be reduced in frequency or stopped if no significant effects are detected. Monitoring for increased plant damage due to niche replacement by more harmful species may need to continue over longer periods, but can be embedded in agricultural surveillance programs that track (invasive) plant pests.

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5 Conclusions

The concept of gene drives is not new. Nature provides many examples of selfish genetic elements that bias inheritance and promote their own transmission through populations. However, recent advances in molecular biology have renewed interest in engineering gene drives to address complex biological challenges. Proposed applications include controlling or eradicating invasive or problematic species, reducing populations of disease vectors, and spreading beneficial traits, such as disease resistance, through wild populations. These potential applications have stimulated significant interest among public and private researchers and prompted extensive discussions on how, when and why such technologies could be used. In parallel, risk assessment and regulatory authorities have undertaken a range of initiatives to evaluate whether existing regulatory frameworks are capable of addressing the unique characteristics of GDMIs and ensuring their safe and sustainable deployment.

This report attempts to address information gaps identified in EFSA 2020, and is based on a review of the literature aimed at identifying molecular mechanisms proposed for engineered gene drives and currently under investigation in insects, as well as assessing which GDMIs may realistically be deployed in the near future in the EU and its OMTs. The review identified a wide range of molecular mechanisms and techniques that remain at an early stage of laboratory research, including conceptual development and proof of concept studies in model organisms. Only a limited number of these approaches are being actively developed in insect species of relevance to the EU and its OMTs, and no GDMI has yet been released anywhere in the world.

Against this background, a review of existing EFSA guidance applicable to molecular characterization (MC) and ERA of GMAs indicates that current guidance provides an adequate basis for identifying and generating data relevant to ERA. Although some sections may require further interpretation or additional detail to address the specificities of individual GDMIs, the considerable diversity of molecular mechanisms and the uncertainty surrounding future GDMI applications relevant to the EU and its OMTs argue for maintaining a flexible and adaptable approach. Such flexibility is essential to accommodate the broad range of potential gene drive systems that may emerge. In this context, the development of dedicated guidance specifically for MC of GDMIs appears premature. Nevertheless, the analysis confirms that the existing guidance framework provides a robust foundation for future developments.

A review of insect species currently under consideration for GDMI development identified four species of relevance to the EU and its OMTs: *Aedes aegypti*, *Anopheles* spp. (including *Anopheles stephensi, Anopheles gambiae and Anopheles coluzzii*), *Drosophila suzukii* and *Plutella xylostella*. Importantly, the most advanced GDMI development efforts focus on *Anopheles* species for malaria control. However, given the availability of effective conventional malaria control measures in the EU and its OMTs, the release of such GDMIs in these regions is considered highly unlikely. Therefore, *Anopheles* species were excluded from further consideration. The remaining species include one vector of human disease (*Aedes aegypti*) and two agricultural pests (*Drosophila suzukii* and *Plutella xylostella*).

Problem formulation proved central for identifying plausible pathways to harm and defining relevant risk hypotheses for future GDMI ERA. The analysis demonstrated that ERA for GDMIs cannot rely on generic or uniform data requirements alone, given the diversity of possible

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molecular mechanisms, intended uses and deployment strategies. Instead, data requirements should be tailored to the biology and ecology of the target organism, the characteristics of the engineered gene drive system, the receiving environment, the intended management objective and the anticipated spatial and temporal scale of spread and persistence. This supports a targeted, risk hypothesis-driven and proportionate approach to assessment.

The work furthermore confirmed the importance of adopting a broad and context-dependent approach to comparators. Because all species considered are already subject to existing management or control programmes, the comparative approach in ERA should not be restricted to comparisons with non-modified insects of the same species. Comparisons with alternative control strategies may also be relevant and informative for contextualising environmental risks and benefits. Different comparators may therefore be appropriate for different assessment questions and stages of decision-making.

The review of modelling approaches highlighted that most existing studies focus primarily on the inheritance dynamics of engineered gene drives, whereas comparatively few incorporate ecological processes influencing spread, establishment, persistence and potential environmental consequences under realistic conditions. In addition, modelling addressing ecological endpoints beyond spread and persistence remains very limited. This identifies an important gap between current modelling capabilities and future regulatory needs for ERA and PMEM. Future modelling frameworks supporting regulatory assessment should therefore integrate genetic, ecological and environmental processes, adequately characterise uncertainty, and allow evaluation against empirical data. Stochastic and spatially explicit modelling approaches embedded within appropriate statistical frameworks are likely to be particularly important.

Finally, PMEM remains an essential component of any future GDMI environmental release. Although there is currently no direct operational experience with released GDMIs, valuable lessons can be drawn from analogous insect management approaches, including biological control, SIT programmes, releases of GMIs for sterile male strategies, and *Wolbachia*-based methods. These experiences highlight the importance of CSM designed to verify assumptions made during ERA, and address identified uncertainties. PMEM should therefore be considered an integral continuation of the ERA rather than a generic post-authorisation requirement.

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6 Recommendations

- Existing EFSA guidance documents provide a robust foundation for future risk assessments of GDMIs.
- The development of dedicated guidance for the MC of GDMIs is currently premature due to the diversity of molecular mechanisms under investigation and the uncertainty surrounding which approaches and applications may ultimately become relevant to/deployed in the EU and its OMTs.
- GDMIs relevant to the EU and its OMTs are most likely to target insect species that are either vector diseases affecting humans, animals or plants, or act directly as agricultural pests. The relevance of future GDMI applications for the EU and its OMTs should be evaluated not only on the basis of scientific feasibility, but also considering realistic deployment needs, existing management options and the wider regulatory context.
- ERA should maintain flexibility in the interpretation of guidance and in data requirements, allowing assessments to be adapted to the characteristics of the specific GDMI, the receiving environment and the intended use.
- ERA should adopt a targeted, risk hypothesis-driven approach grounded in robust problem formulation, including clearly defined pathways to harm and testable risk hypotheses.
- Data generation should be proportionate and focused on information relevant for decision-making, rather than following a generic or one-size-fits-all framework.
- ERA of GDMIs should adopt a broad approach to comparators, enabling the selection of comparators most appropriate for addressing specific risk hypotheses.
- Modelling frameworks supporting ERA and PMEM should extend beyond inheritance dynamics and incorporate ecological processes, environmental variability, spatial structure and uncertainty.
- Models used to predict the spread and persistence of GDMIs should be based on stochastic genetic and/or ecological frameworks that quantify uncertainty and are embedded within suitable statistical frameworks allowing comparison with empirical data.
- Distinct modelling approaches may be needed for different regulatory purposes, including pre-release ERA, monitoring design, post-release evaluation and interpretation of PMEM data.
- PMEM should be case-specific, hypothesis-driven and directly linked to uncertainties and assumptions identified during ERA.
- Monitoring strategies should build, where appropriate, on experience gained from existing insect management and monitoring programmes, while recognising the novel aspects associated with the spread and persistence of some GDMIs.
- The adequacy of the current guidance framework should be periodically revisited as scientific knowledge, technological maturity and potential applications of GDMIs continue to evolve.

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