1

2

COVER PAGE	
Metadata element	**Value**
Output category	Draft Opinion of the Scientific Committee/Scientific Panel
Date adopted/endorsed	29 January 2020
Complete author list (names or ORCID)	EFSA Panel on Genetically Modified Organisms (GMO), Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Ewen Mullins, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann, Fabio Veronesi, Michael Bonsall, John Mumford, Ernst A Wimmer, Yann Devos, Konstantinos Paraskevopoulos and Leslie George Firbank
Doi	xxxx/yyyy
Requestor	European Commission
EFSA article id	xxxx/yyyy
Question number	EFSA-Q-2018-00619
Correspondence	GMO@efsa.europa.eu
Short title (header)	Adequacy of existing EFSA guidelines for the risk assessment of gene drive modified insects
Panel members	Hanspeter Naegeli, Jean-Louis Bresson, Tamas Dalmay, Ian Crawford Dewhurst, Michelle M Epstein, Leslie George Firbank, Philippe Guerche, Jan Hejatko, Francisco Javier Moreno, Ewen Mullins, Fabien Nogué, Nils Rostoks, Jose Juan Sánchez Serrano, Giovanni Savoini, Eve Veromann and Fabio Veronesi
Copyright exclusion	/
Acknowledgments	EFSA wishes to acknowledge the contribution of the Gene Drive ERA Working Group experts (Michael Bonsall, Andrea Crisanti, John Mumford, Fabien Nogué and Ernst A Wimmer) to this opinion, as well as that of Ana Martin Camargo and Barbara Gallani. John Mumford resigned as ad hoc expert from the Gene Drive ERA Working Group on 4 February 2020
Minority opinion	/
Competing interests	/

Evaluation of existing EFSA guidelines for their adequacy for the molecular characterisation and environmental risk assessment of genetically modified insects with synthetically engineered gene drives

EFSA GMO Panel

Abstract

Recent advances in molecular and synthetic biology are enabling the engineering of gene drives that spread genes of interest through interbreeding populations at a frequency greater than the rate expected by simple Mendelian inheritance, even if they incur a fitness cost. At present, insects represent the most likely cases of gene drive modified organisms for deliberate release into the environment. The application of synthetically engineered gene drives is expected to complement and substantially expand the existing range of genetic methods for insect vector/pest control, especially for population replacement. While gene drive modified insects (GDMIs) have been tested experimentally in the laboratory, none has been assessed in small-scale confined field trials, or in open release trials yet. As a proactive measure and due to the potential for gene drives to spread through populations, persist in the environment, and potentially cause irreversible effects on organisms and ecosystems, the European Food Safety Authority (EFSA) has been requested by the European Commission to review whether its previously published guidelines for the risk assessment of genetically modified animals (EFSA, 2012 and 2013) are adequate for the molecular characterisation (MC) and environmental risk assessment (ERA) of gene drive modified disease-spreading mosquitoes and agricultural insect pests for deliberate release into the environment. The considerations/requirements given in the guidelines are broadly adequate for the GDMIs addressed in this GMO Panel Scientific Opinion, confirming that the ERA of GDMIs can build on the existing risk assessment frame for non-GDMIs. Given the non-food/feed uses of GDMIs and the self-replicating nature of gene drives, the guidelines would benefit from revisions particularly focussing on MC, the assessment of persistence and invasiveness, modelling and post-market environmental monitoring. Consistent with EFSA (2013), the ERA of GDMIs should begin with an explicit problem formulation that follows the case-by-case approach, and that is framed by relevant protection goals and experience gained with existing insect vector/pest control strategies. Enhanced dialogue between risk assessors, risk managers and stakeholders is advocated to define clear protection goals and decision-making criteria for the ERA of GDMIs.

Keywords

Deliberate release, harm, problem formulation, replacement drive, risk assessment, self-limiting drive, self-sustaining drive, suppression gene drive, threshold dependent drive, threshold independent drive

Summary

A summary will be prepared after the public consultation.

Table of Contents

Introduction

In the European Union (EU), including its special territories, the use of genetically modified organisms (GMOs) is subject to risk assessment and regulatory approval. In this process, the role of the European Food Safety Authority (EFSA) is to assess and provide scientific advice to risk managers on any plausible risk that the deployment of a GMO may pose to human and animal health, and the environment. The decision on the level of acceptable risk, given the potential for appropriate risk management, and thus whether the use of a GMO ought to be permitted, is taken by risk managers (the European Commission and EU Member States).

Potential future applications for the placement of GMOs on the market, including public use, in the EU may include the deliberate release of GMOs with synthetically engineered gene drives (referred to hereafter as gene drive modified organisms [GDMOs]) into the environment (referred to hereafter as deliberate release[1]). As a proactive measure, EFSA has been requested by the European Commission to assess, through a problem formulation exercise, whether: (1) the deliberate release of GDMOs could pose potential new hazards and risks to human/animal health and the environment, considering relevant comparators; (2) the scientific considerations/requirements given in its previously published guidelines for the risk assessment of genetically modified animals (GMAs) (EFSA, 2012, 2013) are adequate for the molecular characterisation (MC) and environmental risk assessment (ERA) of GDMOs; and (3) there is a need for updated guidance in relation to previous documents (EFSA, 2012, 2013; see also Section 1.1). This advice is expected to support the EU in its work under the Convention on Biological Diversity[2] and the Cartagena Protocol on Biosafety.[3] The Cartagena Protocol and its Nagoya–Kuala Lumpur Supplementary Protocol on Liability and Redress[4] aim to ensure safe handing, transport, and use of living modified organisms resulting from modern biotechnology that may have adverse effects on biodiversity, also taking into account risks to human health. These multinational agreements bear direct relevance for the governance of GDMOs (Marshall, 2010; Brown, 2017; James et al., 2018; Rabitz, 2019).

Any genetic element[5] that is inherited at a higher frequency than predicted by Mendelian laws of inheritance can be referred to as a gene drive. The idea of harnessing naturally occurring gene drives to address challenges related to disease vectors (e.g. mosquitoes, ticks), agricultural pests (e.g. pigweed, screwworm, desert locust), invasive species (e.g. mice, rats, other mammals, cane toads, some invasive plant species) and conservation is not new (e.g.

[1] Terminology as defined by the Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC

[2] The Convention on Biological Diversity is a multilateral treaty under the auspices of the United Nations Environment Program. Its major goals are the conservation of biodiversity, sustainable use of the components of biodiversity, and fair and equitable sharing of benefits arising from genetic resources stemming from biodiversity

[3] The Cartagena Protocol on Biosafety to the Convention on Biological Diversity was adopted on 29 January 2000, and entered into force on 11 September 2003. The Cartagena Protocol presently has 171 contracting parties, excluding large LMO exporters such as Argentina, Canada and the United States

[4] The Nagoya–Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety was adopted on 15 October 2010, and entered into force on 5 March 2018). The Supplementary Protocol presently has 43 contracting parties, chiefly from the European and African regions

[5] Also termed: Selfish genes, ultra-selfish genes, selfish DNA, self-promoting elements, parasitic DNA and genomic outlaws

Curtis, 1968; Esvelt et al., 2014; Ledford, 2015; Webber et al., 2015; Harvey-Samuel et al., 2017; Min et al., 2018; Scott et al., 2018; Rode et al., 2019; Serr et al., 2020). However, the classical genetic approaches attempted have until recently either not been sufficiently flexible to construct efficient gene drive systems, or difficult to engineer (Rasgon and Gould, 2005; Champer et al., 2016; NASEM, 2016; Burt and Crisanti, 2018; James et al., 2018; Min et al., 2018). Advances in molecular and synthetic biology, including the discovery of homing endonuclease genes (HEGs) and the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9) system[6], have delivered molecular and computational tools that enable the design and development of a wide range of synthetically engineered gene drive systems in diverse organisms (Burt, 2003, 2014; Champer et al., 2016; NASEM, 2016; Godfray et al., 2017). The CRISPR-Cas9 system enables the insertion, deletion, or replacement of specific genes in many species, but also provides a molecular tool to engineer novel HEGs. Preliminary evidence, from laboratory studies, indicates that CRISPR-Cas9-based gene drives could push genes of interest through nearly 100% of a given population of yeast, fruit flies and mosquitoes (NASEM, 2016). These developments suggest that a practical application of gene drive systems could be more readily achievable than previously believed in insects (Esvelt et al., 2014; Burt et al., 2018). Although no market registration application for the deliberate release of gene drive modified insects (GDMIs) has been submitted for regulatory approval yet, the technology could in principle be ready for use in mosquitoes in the near future (Scudellari, 2019). This GMO Panel Scientific Opinion therefore focuses on GDMIs, as they represent the most likely cases for deliberate release into the environment at present.

The nature of potential GDMI applications may be demonstrably different from other GMO applications, which are generally intended to be limited to specific uses in controlled environments (as is the case with genetically modified (GM) crops for agriculture or farm-raised GM fish), or limited in exposure over space and time (as is the case with the release of sterile GM insects [GMIs]). Gene drive applications require the spread of genes of interest for achieving intended outcomes (e.g. fixation or high frequency in the target population). Some gene drive systems may enable: (1) rapid and non-localised spread of genes of interest through interbreeding populations from low initial introductions, even if they incur a fitness cost on their

[6] CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) DNA sequences and associated Cas9 (CRISPR associated protein 9) constitute an adaptive immunity system in certain bacteria. Cas9 enzymes compose a family of RNA-guided DNA endonucleases that use the CRISPR sequences as a guide to recognise and cleave DNA from viruses. The Cas9 endonuclease, when associated with a single guide RNA (sgRNA), can be used as a genetic engineering tool to edit a specific locus in a given genome (Doudna and Charpentier, 2014; Sternberg and Doudna, 2015). CRISPR-Cas9 can be used to drive a genetic modification through a population at higher than normal rates of inheritance (Scudellari, 2019). Once a gene drive is engineered into the genome of an organism, the organism's offspring inherits one allele containing the gene drive element from the transgenic parent and one wild type allele from its other parent. During early development, the Cas9 endonuclease cuts at the corresponding wild type allele—its target prescribed by an independently expressed guide RNA (gRNA)—producing a double-strand break (Jinek et al., 2012). This break is then repaired either through homology-directed repair (HDR), producing a second copy of the gene drive construct, or through a non-homologous repair pathway (non-homologous end joining, NHEJ, or microhomology-mediated end joining, MMEJ), which typically introduces a mutation at the target site (Cong et al., 2013; Mali et al., 2013). The former repair mechanism leaves the offspring with two copies of the modification. Thus, CRISPR-based gene drive systems function by converting heterozygotes for the gene drive allele into homozygotes in the late germline or early embryo (Gantz and Bier, 2015; Scudellari, 2019). A CRISPR gene drive cassette comprises several elements: (1) a gene encoding a gRNA that can recognise a specific target DNA sequence; (2) a *Cas9* gene encoding a Cas9 endonuclease that can cut DNA at the site specified by the gRNA; (3) sequences at the extremities that are homologous to sequences flanking the target site, so that the gene drive cassette can copy itself at the cleavage site via HDR; and (4) optional cargo/payload genes conferring trait(s) of interest

host; (2) indefinite persistence of genes of interest in target populations or until those populations are locally eliminated; (3) change the genetic makeup of wild type populations (Burt, 2003; Marshall and Hay, 2012b; Alphey and Bonsall, 2014; NASEM, 2016; Simon et al., 2018; Noble et al., 2018). These features have raised questions about the desirability and ethics of synthetically engineered gene drive drives (Pugh, 2016; Thompson, 2018; Jones et al., 2019; Sandler, 2019) and prompted a consortium of non-governmental organisations to call for a moratorium on gene drive field tests, as they argue that the deployment of synthetically engineered gene drives may lead to undesired side effects and alter organisms and ecosystems in irreversible ways (Callaway, 2016; 2018; CSS–ENSSER–VDW, 2019). Others have called for a better understanding of the ecological and evolutionary impacts of such releases (e.g. Scott et al., 2002; Esvelt et al., 2014; Lindholm et al., 2016; NASEM, 2016; Courtier-Orgogozo et al., 2017; Esvelt and Gemmell, 2017; Giese et al., 2019; Snow, 2019), and the establishment of different forms of governance that include, among others, mechanisms that facilitate the effective engagement of all concerned parties/stakeholders (Oye et al., 2014; Caplan et al., 2015; NASEM, 2016; Adelman et al., 2017a,b; Emerson et al., 2017; Najjar et al., 2017; James et al., 2018; Barnhill-Dilling et al., 2019; Bartumeus et al., 2019; Brossard et al., 2019; Buchthal et al., 2019; George et al., 2019; Hartley et al., 2019; Kofler et al., 2019; Kuzma, 2019; Rabitz, 2019; Singh, 2019; Thizy et al., 2019; Serr et al., 2020). This has led to the establishment of several recommendations for the safe, responsible and sustainable deployment of the technology (e.g. WHO, 2014; NASEM, 2016; James et al., 2018). Since it is expected that gene drives may eventually spread across national borders, regional approaches that would facilitate multi-country/international regulatory oversight and governance have been suggested (Marshall, 2010; Brown, 2017; James et al., 2018; Rabitz, 2019).

1.1 Background and Terms of Reference as provided by the requestor

In accordance with Article 29(1) of Regulation (EC) No 178/2002, the European Commission has mandated EFSA to deliver "*an opinion on genetically modified organisms engineered with gene drives (gene drive modified organisms) and their implications for risk assessment methodologies*".[7]

In particular, "*through a problem formulation exercise providing the foundation for the environmental risk assessment*", EFSA is requested:

- "*To identify potential risks in terms of impact on human and animal health and the environment that gene drive modified organisms could pose. In this respect EFSA is also asked to identify potential novel hazards of gene drive modified organisms, considering relevant comparators, where appropriate*";
- "*To determine whether the existing guidelines for risk assessment are adequate and sufficient for gene drive modified organisms or whether there is a need for updated guidance*";
- "*To identify the specific areas where such updated guidance is needed*".

[7] registerofquestions.efsa.europa.eu/roqFrontend/ListOfQuestionsNoLogin (EFSA-Q-2018-00619)

Under this mandate, EFSA is not requested "*to develop guidelines for the risk assessment of gene drive modified organisms*".

EFSA is also requested "*to provide technical and scientific expertise on risk assessment of gene drive modified organisms to support the EU in the work under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety*".

1.2 Interpretation of the Terms of Reference

Following discussions with the European Commission (Directorate-General for Health and Food Safety), it was agreed to limit the scope of the mandate to insects, as they represent the most likely cases of GDMOs moving to practical application/for deliberate release into the environment. Although the use of synthetically engineered gene drive systems is considered in mammals (Leitschuh et al., 2018; Conklin, 2019; Godwin et al., 2019; Grunwald et al., 2019; Manser et al., 2019) and for agricultural weed management (Neve, 2018; Barrett et al., 2019), basic technical challenges need to be overcome before a gene drive will be possible in these taxa (NASEM, 2016; Godwin et al., 2019; Pixley et al., 2019; Scudellari, 2019).

In insects, the most likely gene drive cases for deliberate release into the environment application are expected to be those that are directed at human, livestock and wildlife disease vectors and agricultural and horticultural pests. The potential for gene drives to self-replicate opens new opportunities for area-wide insect management. Current area-wide control depends economically on concentration of areas where high benefits could be achieved relative to control effort, to justify the continuous costs (Brown et al., 2019). However, GDMIs could be used in areas with much lower pest concentrations and that are not easily managed, given their lower ongoing costs of implementation. Since disease vectors and agricultural pests can affect human or animal health by transmitting diseases, or are a threat to agricultural production and biodiversity, humans have aimed at controlling or eradicating them through a variety of methods including the use of biological or chemical insecticides, resistant crop varieties, biological control, and genetic control methods such as the sterile insect technique (SIT) or incompatible insect technique (IIT) (reviewed by Ritchie and Staunton, 2019; Romeis et al., 2020). Controlling disease transmission by mosquitoes is a long-standing public health goal, and the eradication of these human diseases would have tremendous economic and social benefits (Feachem et al., 2019; Masterson, 2019). However, current methods of vector control, including removal of standing water, use of insecticides delivered via bed-nets and indoor residual spraying, and the mass release of sterile males, have not been entirely effective in combatting the spread of mosquito-vectored diseases worldwide (Ritchie and Staunton, 2019). Consequently, novel vector control strategies, including genetic-based approaches that utilise GM mosquitoes with synthetically engineered gene drives, are under development/test for future deployment (Gantz et al., 2015; Windbichler et al., 2007, 2008, 2011; Hammond et al., 2016; Kyrou et al., 2018; Buchman et al., 2019). Likewise, increasing challenges associated with the invasion of non-native insect species, and increasing resistance to commonly used insecticides drive the development and deployment of novel insect control techniques, including genetic techniques (Alphey, 2014; Alphey and Bonsall, 2018). Consequently, this GMO Panel Scientific Opinion focuses on insect pest species, in particular disease vectors and agricultural pests. It does not address the use of synthetically engineered gene drives for biodiversity

conservation purposes or the enhancement of agricultural production systems, as no concrete applications are currently in the pipeline for such purposes (e.g. NASEM, 2006; Rode et al., 2019).

The scope of the mandate focuses on the MC and ERA of GDMIs for deliberate release into the environment; such releases are non-confined[8] and not intended for food/feed uses. Since synthetically engineered gene drives are intended to spread genes of interest through interbreeding wild type/target populations occurring in the environment, the deliberate release of GDMIs will be non-confined, and not covering GMIs for food/feed uses. Consequently, the mandate excludes confined and semi-confined GDMI releases and the deliberate release of GDMIs for food/feed uses (if any).

In summary, the scope of the mandate covers:

- The non-confined release of GDMIs into the environment for non-food/feed uses;
- The MC and ERA, including the problem formulation process and its function in ERA, of GDMIs for deliberate release into the environment;
- The use of synthetically engineered gene drives to control harmful insects such as disease-transmitting mosquitoes and agricultural pests.

EFSA is not mandated to provide advice on ethical and socio-economic aspects and possible benefits associated with gene drive technology. Some of these aspects are expected to be addressed by the European Group on Ethics, which has been requested by the European Commission to deliver an advice on GDMOs.[9]

2 Data and Methodologies

2.1 Data

In delivering its Scientific Opinion, the GMO Panel, along with its Gene Drive expert Working Group (together referred to hereafter as GMO Panel), took into account the considerations/requirements given in the GMO Panel Scientific Opinions that provide guidance for the risk assessment of GMAs, including GMIs (EFSA, 2012, 2013), Directive 2001/18/EC on the deliberate release into the environment of GMOs and the Commission Directive (EU) 2018/350 amending Directive 2001/18/EC, where appropriate, and relevant information reported in the scientific literature.

EFSA (2012, 2013) serve as the reference documents for the MC and ERA of GMAs, respectively. These guidelines assist applicants in the preparation and presentation of their registration applications by describing the elements and information requirements for a structured risk assessment of GMAs.

- EFSA (2012) covers the risk assessment of food/feed containing, consisting of, or produced from GMAs, as well as the health and welfare assessment of these animals,

[8] The terms 'confined', 'semi-confined' and 'non-confined' are defined in EFSA (2013)
[9] https://ec.europa.eu/info/sites/info/files/research_and_innovation/ege/letter_chair_of_the_ege_group.pdf

within the framework of Regulation (EC) No 1829/2003 on GM food/feed. EFSA (2012) focuses on husbandry animals, fish, crustaceans and molluscs, and does not consider insects and other arthropods. EFSA (2012) addresses the MC, which provides information on the structure and expression of the insert(s) and on the stability of the intended trait(s); the toxicological assessment, which addresses the possible impact of biologically relevant change(s) in the GMA and/or derived food/feed, the allergenicity assessment of the novel protein(s), as well as of the whole food derived from the GMA; and the nutritional assessment to evaluate whether food/feed derived from a GMA is as nutritious to humans and/or animals as food/feed derived from traditionally-bred animals. EFSA (2012) also addresses the scientific requirements for the assessment of health and welfare of GMAs bred for food/feed use. EFSA (2012) does not cover the ERA of GMAs, which is addressed in EFSA (2013);

- EFSA (2013) provides guidance for the ERA of living GMAs, namely fish, insects and mammals and birds, to be placed on the EU market in accordance with Regulation (EC) No 1829/2003 or Directive 2001/18/EC. EFSA (2013) provides guidance for assessing potential effects of GMAs on animal and human health and the environment and the rationales for data requirements for a comprehensive ERA. EFSA (2013) follows Annex II of Directive 2001/18/EC, considering specific areas of risk to be addressed by applicants and risk assessors during the ERA of GM fish, GMIs and GM mammals and birds. Each specific area of risk must be considered in a structured and systematic way following the six successive steps for ERA: (1) problem formulation including hazard and exposure identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) an overall risk evaluation. In addition, EFSA (2013) describes several generic cross-cutting considerations (e.g. choice of comparators, use of non-GM surrogates, experimental design and statistics, long-term effects, uncertainty analysis) that need to be accounted for throughout the whole ERA.

The GMO Panel notes that the development of EFSA (2012, 2013) called for a general approach, as the European Commission mandated EFSA to develop guidelines for the risk assessment of GMAs that would address both the food/feed safety assessment and ERA, including animal health and welfare aspects, and cover the ERA of broad range of taxa ranging from GM fish to insects, mammals and birds. Consequently, EFSA (2013) provides a non-exhaustive list of potential issues to consider, but without necessarily clarifying how these issues should be addressed concretely for the ERA of GMAs, including insects. Although GDMIs are mentioned in EFSA (2013), little emphasis is given to them.

2.2 Methodologies

2.2.1 Working group

EFSA established an *ad hoc* expert Working Group of the GMO Panel on the MC and ERA of GDMIs that met regularly to address the mandate of the European Commission.[10]

[10] http://www.efsa.europa.eu/sites/default/files/wgs/gmo/wg-gene-drive-era.pdf

2.2.2 Assessment

A section-by-section approach has been followed to examine whether the considerations/requirements given in EFSA (2012, 2013) are adequate for the MC and ERA of GDMIs, respectively. This evaluation is reported in Section 7 for each of the relevant headings and subheadings of EFSA (2012, 2013).

The adequacy evaluation of EFSA (2012, 2013) has been performed on the basis of relevant information reported in the scientific literature and practical developments of GDMIs (see Section 3.3).

In addition, the potential for novel hazards/risks associated with GDMIs for deliberate release into the environment was addressed, and specific areas potentially requiring updated/revised guidance were identified.

In contrast to the adequacy evaluation of EFSA (2012, 2013), the practical applicability of the considerations/requirements given in EFSA (2012, 2013) for a specific GDMI must be assessed on a case-by-case basis as part of the problem formulation process. Such an assessment has not been conducted in this GMO Panel Scientific Opinion, as the GMO Panel has not been mandated by the European Commission to assess a concrete GDMI for regulatory approval. Moreover, no GDMI application has been submitted for regulatory approval at present.

2.2.3 Consultations

Considering the current societal debate on the potential applications of gene drive, given the need for greater dialogue, and in line with its policy on openness and transparency, EFSA organised two consultations at different development stages of the GMO Panel Scientific Opinion to collect input from its stakeholders (including EU Member States) and other interested parties. One, in the shape of a stakeholder workshop, took place early in the development process and the other, in the shape of an online public consultation, was carried out at a later stage in the development of this GMO Panel Scientific Opinion.

2.2.3.1 Stakeholder workshop "Problem formulation for the environmental risk assessment of gene drive modified insects" (15 May 2019, Brussels)

Through an open workshop, EFSA aimed to engage with stakeholders to discuss potential environmental risks associated with the deliberate release into the environment of GDMIs. To focus the discussions, participants were invited to contribute to an example problem formulation to:

1. Identify relevant broad protection goals and make them operational for use in ERA;
2. Formally devise examples of plausible pathways to harm that describe how the deployment of GDMIs could be harmful;
3. Formulate example risk hypotheses about the likelihood and severity of such events;
4. Identify possible information that would be useful to test these risk hypotheses;
5. Identify how to acquire new data for hypothesis testing when existing information is deemed insufficient for regulatory decision-making.

The problem formulation exercise was run for two hypothetical case studies in two separate discussion groups:

1. Self-sustaining low threshold gene drives to control disease-spreading mosquitoes (*Aedes albopictus,* the Asian tiger mosquito);
2. Self-sustaining low threshold gene drives to control agricultural pests (*Drosophila suzukii,* the spotted-wing *Drosophila*).

The two case studies were selected representing species relevant for the EU.

1. *Aedes albopictus*, the Asian tiger mosquito, is an aggressive biting mosquito native to Asia that has colonised all continents, except Antarctica, during the last ~30-40 years. The species is of great public health concern as it can transmit several arboviruses, including dengue, chikungunya and Zika viruses (Lounibos, 2002). With climate change, the *Ae. albopictus* transmission potential is likely to increase substantially for most of Europe even in the short term (Ryan et al., 2019);
2. *Drosophila suzukii*, commonly known as the spotted-wing *Drosophila*, is a highly invasive pest that has recently and rapidly expanded out of its native range, in Southeast Asia, to Europe and both North and South America, where it causes significant economic damage to the fruit sector (Ørsted and Ørsted, 2019). Females lay eggs inside ripening soft-skinned fruits, and larvae feed inside the fruit, which becomes soft and rots (e.g. Schetelig et al., 2018; Romeis et al., 2020).

The outcomes of the two discussion groups were presented and further developed in a final plenary session, during which the conclusions of the workshop were drawn.

The goal of the workshop was not to produce a comprehensive and detailed ERA of the two GDMI case studies, but rather to familiarise the participants with the problem formulation process and its function in ERA, and to gather feedback on this approach.

Points raised by the workshop participants, on defining protection goals, formulating specific pathways to harm and on structuring risks, were considered by the GMO Panel during its deliberations, and are listed in Appendix A. Any points raised by workshop participants should not necessarily be interpreted as comprising substantiated hazards or risks associated with the two hypothetical GDMI case studies that are supported by evidence from the scientific literature.

The workshop materials supplied by EFSA and speakers (i.e. agenda and briefing notes for participants, list of participating stakeholders and presentations) are available on EFSA's website.[11]

2.2.3.2 Online public consultation

EFSA also consulted the public and its stakeholders via an online public consultation. Between 17 February and 17 April 2020, interested persons were invited to submit their comments on

[11] https://www.efsa.europa.eu/en/events/event/190515

the draft GMO Panel Scientific Opinion.[12] Following this consultation process, the document was revised by the GMO Panel.

The outcome of the online public consultation and associated workshop will be reported in a technical report for publication on EFSA's website, together with the final Scientific Opinion as adopted by the GMO Panel.

3 Explaining gene drives

A gene drive can be described as any system in which genes bias their own inheritance to gain a transmission advantage over the rest of the genome (e.g. Burt and Trivers, 2006; Schenkel and Leggewie, 2015; NASEM, 2016; ZKBS, 2016; AAS, 2017; EASAC, 2017; HCB, 2017; SAM, 2017; High-Level African Panel on Emerging Technologies, 2018; Leftwich et al., 2018; Royal Society, 2018; Ethics Council of the Max-Max-Planck-Gesellschaft, 2019; Hurst, 2019; North et al., 2019; Redford et al., 2019; Wedell et al., 2019). During sexual reproduction of diploid organisms, each of the two alleles of a gene present in each parent has a 50% chance of being inherited by offspring according to the Mendelian laws of inheritance. Gene drives increase this probability and are transmitted to subsequent generations at a frequency greater than the 50% expected by Mendelian inheritance. This super-Mendelian mode of transmission allows gene drive systems to rapidly spread in sexually reproducing populations, increasing their prevalence and that of any genetically linked cargo/payload genes[13], even if they incur a fitness cost on their host. This is because individuals with a gene drive element will produce more offspring carrying the gene drive allele than without it (Champer et al., 2016).

NASEM (2016) reported differences in the use of terminology and definitions, with terms often having overlapping definitions depending on the historical period and the scientific context in which they are used. Since gene drive research is evolving very quickly, it may potentially result in differences in definitions and terminology, and in the way each may conceptualise and interpret gene drive strategies among stakeholders (see Section 3.2). Although the nuances of different definitions, interpretations and classifications can be valuable, there may be a need to address the existing ambiguity to improve comparability. This will promote consistency, transparency and transferability. The development of a common set of definitions and terminology – a "standard lexicon" – if generally accepted, would help to frame gene drive-related discussions.

3.1 Mechanisms

Researchers have studied naturally occurring gene drive systems for more than a century (reviewed by Burt and Trivers, 2006). First reported in the 1920s, gene drives have been observed in a variety of organisms, and encompass a variety of different mechanisms: transposable elements[14] that insert copies of themselves at other places in the genome; homing

[12] Published at xxx
[13] Also termed: Effector genes
[14] Also termed: Jumping genes

endonuclease genes that copy themselves at targeted genomic sites; segregation distorters[15] that destroy competing chromosomes during meiosis; gametic killers that eliminate gametes not carrying the drive element; the Medea (maternal effect dominant embryonic arrest) system that confers maternal-effect lethality to all offspring that does not have a copy of the M (Medea) element; and *Wolbachia* endosymbionts that favour offspring of infected females (e.g. Beeman et al., 1992; Burt and Trivers, 2006; Sinkins and Gould, 2006; Champer et al., 2016; Hammond and Galizi, 2017; Ågren and Clark, 2018; Collins, 2018; Rüdelsheim and Smets, 2018; Cash et al., 2019a,b; Frieß et al., 2019). The study of natural gene drive systems over the last century has provided considerable theoretical and empirical insights into how gene drives work and how they spread (Courret et al., 2019; Dyer and Hall, 2019; Finnegan et al., 2019; Larner et al., 2019; Lea and Unckless, 2019; Price et al., 2019; Wedell et al., 2019). This can provide baseline information for the design of synthetically engineered gene drives, and in some cases, for the risk assessment of GDMIs.

Selfish genetic elements use three main mechanisms to achieve super-Mendelian inheritance: (1) over-replication; (2) interference; and (3) gonotaxis (Burt and Trivers, 2006).

1. Over-replicating selfish genetic elements (such as transposable elements and homing elements) increase their copy number in the genome by replicating more often than other genes in the genome. For example, homing endonucleases use over-replication by copying themselves (causing breakage and self-insertion) onto the homologous target sequence (a process termed homing), resulting in most or all offspring inheriting the gene drive allele. Many of the currently discussed and most advanced gene drive strategies are based on synthetically engineered HEGs (see Section 3.3);
2. Interfering genetic elements (such as meiotic gene drives and chromosomal translocations) disrupt the transmission of other gene variants through the distortion of meiosis or gamete development,[16] or interference with offspring survival. Pre-gametic gene drives distort transmission ratios during meiosis, so that gametes carrying the drive allele have a higher probability of being produced. Post-gametic gene drives accomplish segregation distortion via mechanisms that render gametes inviable after meiosis has taken place. Reducing the viability of gametes that inherit the wild type allele gives the wild type allele a fitness disadvantage compared to the gene drive allele. Besides gamete killers, there are also maternal effect killers such as Medea, where all offspring dies unless the selfish genetic element is inherited. Currently developed synthetically engineered gene drives based on interference include Medea, killer-rescue, or cleave and rescue systems (see Section 3.3);
3. Gonotaxis refers to selfish genetic elements that bias Mendelian segregation by moving away from dead-end polar bodies into the functional egg during oogenesis (e.g. some plant B-chromosomes or heterochromatic knobs of A-chromosomes). Since polar bodies do not become functional gametes, the selfish gene is transmitted to more than 50% of

[15] Also termed: Meiotic drive elements
[16] Also termed: Transmission distorters

the offspring. The process is not well understood molecularly and currently there are no synthetically engineered gene drives proposed based on gonotaxis.

3.2 Strategies

Scientists are working to harness gene drives, either by repurposing naturally occurring systems or by synthetically engineering (redesigning) them, so that they can be used to spread desired genetic elements through wild populations over many generations (Redford et al., 2019).

Gene drive strategies, including their design, can be differentiated based on the following dimensions: (1) the intended outcome; and (2) the ability of the gene drive to establish, spread and persist[17] in target populations (see Table 1).

3.2.1 The intended outcome

Depending on the intended outcome of the deliberate release of a GDMI, gene drives and their associated cargo/payload genes can be designed either to suppress target populations, or to replace them with a new desired genotype. This can be achieved either through the introduction of a new (engineered) genetic trait in a target population, or by the inactivation of an endogenous gene.

3.2.1.1 Population suppression[18]

Population suppression strategies aim to reduce a target population by imposing a substantial fitness cost via the inactivation of important genes involved in the survival (non-developing offspring) or reproduction of the target population (e.g. reducing fertility of offspring, bias of the sex ratio toward males), or through the introduction of a new gene or genes that reduce(s) lifespan or bias(es) sex ratios (Buchman et al., 2018b; James et al., 2018). Modified target insects are expected to decrease to low numbers over the period of a few generations as the overall target population is reduced. This may result in population decline or even collapse. Suppression drives are being developed for suppressing populations of human/animal disease vectors and agricultural pests. Strategies aiming for population suppression from a single release would require the modification to persist. Alternatively, strategies could use self-limiting gene drives (see Section 3.2.2.1), which could require repeated releases over time to maintain suppression.

3.2.1.2 Population replacement[19]

Population replacement strategies are used to replace a current genotype with one less able to transmit disease (disease refractory/impaired vector competence), or that is more resistant to pathogen infection (Franz et al., 2006; Mathur et al., 2010; Hedge and Hughes, 2017; Jupatanakul et al., 2017; Carballar-Lejarazú and James, 2017; Buchman et al., 2019, 2020; Pham et al., 2019). These strategies are based on the inactivation of a gene or genes involved in pathogen survival in the insect , or that are required for the target organism to transmit the pathogen (e.g. a tendency to feed on humans in the case of mosquitoes) (see Section 3.3 for

[17] Remain active in a population in the long-term
[18] Also termed: Population reduction
[19] Also termed: Population modification, population alteration, population transformation, or population conversion

examples). They can also involve the introduction of a new gene or genes, such as those that produce molecules that block pathogen development, or that kill the pathogen in the insect (Lejarazú and James, 2017; James et al., 2018; Buchman et al., 2019, 2020). To perform successfully, such introduced genes must be genetically linked to the gene drive. Strategies aiming for population replacement require the modification to persist (James et al., 2018).

3.2.2 The ability of the gene drive to establish, spread and persist in target populations

Gene drives differ in their intended ability to establish, spread and persist in target populations. Based on these characteristics, gene drives fall into different categories: (1) self-sustaining[20] vs. self-limiting[21] drives; and (2) low vs. high threshold drives.[22]

3.2.2.1 Self-sustaining vs. self-limiting gene drives

Self-sustaining gene drives are designed to cause desirable genes to increase in frequency in a target population and ideally become fixed in the population. These drives can sustain the high frequency of the desirable gene indefinitely in the target population unless actions are taken to reverse the impact and/or frequency of the drive through release of another transgenic strain.

Self-sustaining gene drives can be designed to be spatially unrestricted and move to any population that has gene flow with the population where the drive was released. Examples of spatially unrestricted gene drives include some homing endonuclease drives, especially CRISPR-Cas9, and Medea drives which are expected to have very low thresholds for release (Chen et al., 2007; Simoni et al., 2014; Gantz et al., 2015; Buchman et al., 2018b; Oberhofer et al., 2019).

Several genetic strategies have been proposed and designed to reduce the spread of gene drives over a limited period of time or within a limited area, possibly reducing their frequency in the target population over the course of several generations (Dhole et al., 2018; Marshall and Akbari, 2018). This would restrict gene drives spatially (Marshall and Hay, 2012a; Akbari et al., 2014; Buchman et al., 2018b), temporally (Gould et al., 2008), or both spatially and temporally (Esvelt and Gemmell, 2017; Burt and Deredec, 2018; Leftwich et al., 2018; Noble et al., 2019; Li et al., 2020a). Such self-limiting gene drives constitute a form of biological or molecular confinement that could supplement physical and ecological confinement (James et al., 2018).

Gene drives can be designed to only spread within a single population or geographic region. These are referred to as spatially restricted gene drives. Generally, spatially restricted gene drives are not expected to establish themselves at high frequency in neighbouring populations when migration rates are low (Dhole et al., 2019). Examples of spatially restricted gene drives are underdominance drives[23] and split drives[24], which are being developed to have high

[20] Also termed: Self-propagating drives
[21] Also termed: Self-exhausting drives
[22] Also termed: Threshold-independent drives and threshold-dependent drives, respectively
[23] Underdominance refers to a situation where heterozygotes are less fit than either of the two homozygotes and thus selected against within a population
[24] In split gene drives, the gene drive components (for example, Cas9, gRNA, and the donor template) are supplied separately to the organism

thresholds for establishment (Alphey, 2016; Davis et al., 2001; Edgington and Alphey, 2017, 2018; Li et al., 2020b).

Self-limiting gene drives can be designed to increase the frequency of desirable genes in a population for a limited number of generations, after which the frequency of these genes in the population decreases and they are then lost from the population.[25] The desirable genes could either be those that change harmful population characteristics or suppress population density. This type of gene drive is referred to as a temporally restricted drive. Examples (see Section 3.3 for more details) are daisy-chain drives (Noble et al., 2019) and split killer-rescue drives (Gould et al., 2008).

Other proposed approaches include intentional genetic modifications that aim to limit the temporal or spatial scale over which a gene drive is expected to remain functional (see Section 3.3).

3.2.2.2 Low vs. high threshold gene drives

Inherent in many gene drive systems is the requirement for individuals to be released above a certain threshold frequency before they will drive the genetic change through the population (Alphey, 2014; Leftwich et al., 2018; Backus and Delborne, 2019). This threshold refers to the proportion of GDMIs with respect to the total target population that will reliably initiate spread of the genetic modification. Below that threshold, the gene drive will die out (Warner et al., 2019).

Gene drives with a high threshold frequency only spread if the number of gene drive modified individuals reaches a high proportion in the target population, requiring a larger introduction (or proportion) of transgenic individuals to be successful. Examples of high threshold drives include: double-Medea systems (Akbari et al., 2013; Wimmer, 2013), split homing drives (López del Amo et al., 2019, 2020; Noble et al., 2019; Li et al., 2020a), or split killer-rescue drives (Webster et al., 2019). These types of drives enable local confinement and may be eliminated from a population through being diluted below the threshold frequency. Such threshold-dependent GDMIs are expected to be reversible (Warner et al., 2019).

In contrast, low threshold gene drives are able to spread from very low initial population frequencies, requiring only a small number of gene drive modified individuals to be released to spread, independent of whether the drive is based on over-replication by synthetic homing elements (Hammond et al., 2016; Kyrou et al. 2018), or by interference by killer-rescue elements (Oberhofer et al., 2019). These types of drives have a higher potential to spread into neighbouring populations and are typically considered invasive (Champer et al., 2016).

[25] Assuming no residual fitness benefit

583 **Table 1. Overview of gene drive strategies**

Intended outcomes	Ability of the gene drive to establish, spread and persist in target populations		
	Self-limiting drives		Self-sustaining drives
	Threshold dependent (high threshold)	Threshold independent (low threshold)	Threshold independent (low threshold)
Population suppression	Spatially restricted (e.g. sex-linked underdominance drives based on double Medea[26])	Temporally restricted (e.g. daisy-chain drives)	Spatially and temporally unrestricted, though may locally self-extinguish before the drive is able to spread to new target populations (e.g. homing endonuclease drives)
Population replacement	Spatially restricted (e.g. underdominance drives based on double Medea, double cleave and rescue drives, split homing endonuclease drives, split killer-rescue drives)	Temporally restricted (e.g. daisy-chain drives)	Spatially and temporally unrestricted (e.g. Medea drives, cleave and rescue drives, homing endonuclease drives, killer-rescue drives)

584

585 ## 3.3 Approaches for gene drive modified insects

586 Research on gene drive and its applications in insects are moving at a fast pace, though it is
587 generally accepted that it will take several years for technological developments to move to
588 practical applications for deliberate release into the environment. Drawing inspiration from
589 systems that exist naturally, a variety of synthetically engineered gene drives, which are
590 integrated into the host nuclear genome, have been developed in recent years (Sinkins and
591 Gould, 2006; Champer et al., 2016; NASEM, 2016; Hammond and Galizi, 2017; Macias et al.,
592 2017; Burt and Crisanti, 2018; Rüdelsheim and Smets, 2018; CSS–ENSSER–VDW, 2019; Frieß et
593 al., 2019). They encompass (see Table 1):

594 1. HEG-based gene drives, for either population suppression or replacement strategies;
595 2. Sex-linked meiotic interference gene drives (Y-linked X-shredder) for population
596 suppression strategies;
597 3. Medea (toxin-antidote) gene drives for population replacement strategies;
598 4. Underdominance gene drives for spatially restricted high threshold strategies;
599 5. Other self-limiting gene drives for spatially/temporally restricted strategies.

600 GDMI approaches and applications will likely continue to expand as gene editing tools become
601 more refined (NASEM, 2016; Holman, 2019). Consequently, the previously reported "prototype"

[26] Also termed: Medusa (Marshall and Hay, 2014)

gene drives may not necessarily be representative of the gene drive systems that are currently under development and expected to be more specific, stable and controllable systems.

At present, GDMIs with synthetically engineered gene drives are either in development or have been tested experimentally in the laboratory; however, none has been assessed in small-scale physically and/or ecologically confined field trials, or in open release trials (Rüdelsheim and Smets, 2018).[27]

3.3.1 Homing endonuclease gene-based gene drives

HEG-based gene drive systems can be used either to spread cargo/payload gene(s) in interbreeding populations, or disrupt a target gene by homing into it, which leads to recessive lethality or sterility. HEGs may also be designed to manipulate populations by targeting other desirable genes, such as genes to reduce lifespan, bias sex ratios, impede host seeking, block pathogen development, or to block the ability of the modified organism to act as a vector for pathogens (Champer et al., 2016).

Several proof-of-principle studies have demonstrated the feasibility of using synthetically engineered HEG-based gene drive systems under laboratory settings. Substantial research investments have been made in mosquitoes for malaria control (*Anopheles stephensi* and *Anopheles gambiae*). The most advanced gene drive systems for *Anopheles* vectors have been tested under laboratory settings, and prevent reproduction in *An. gambiae* [I-SceI: Windbichler et al. (2011); CRISPR-Cas9: Hammond et al. (2016) and Kyrou et al. (2018)]. A second HEG-based gene drive system in development prevents *Plasmodium falciparum* malaria infection in *An. stephensi* [CRISPR-Cas9: Gantz et al. (2015)]. Early evidence suggests that this gene drive system might also be effective in *Anopheles coluzzi* and *Anopheles arabiensis* (Feachem et al., 2019). Further modification and current ongoing research is required before these abovementioned gene drive modified mosquitoes can be tested under small-scale physically and/or ecologically confined field settings (NASEM, 2016; Scudellari, 2019). Such self-sustaining gene drives are expected to be highly invasive provided that the evolution of resistance alleles can be minimised (Hammond and Galizi, 2017; Unckless et al., 2017). HEG-based gene drive systems for *An. gambiae* and *An. stephensi* based on CRISPR-Cas9 might become available for roll-out by 2030 (Feachem et al., 2019), subject to resolution of regulatory, ethical and community issues.

Other research efforts have focused on developing synthetically engineered HEG-based gene drive systems in the model organism *Drosophila melanogaster* [I-SceI: Chan et al. (2011, 2013a); I-Onul: Chan et al. (2013b); CRISPR-Cas9: Gantz and Bier (2015); transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs): Simoni et al. (2014)].

3.3.2 Sex-linked meiotic interference gene drives (Y-linked X-shredder)

Meiotic interference gene drives bias the transmission of certain alleles during meiosis, resulting in increased frequencies of those alleles in the gametes, and hence in the offspring. Many types

[27] According to the WHO (2014) testing phases

of meiotic interference gene drive systems are found in nature, including sex-linked meiotic drive elements, which function through altering the sex ratio of offspring of affected individuals (Cha et al., 2006; Champer et al., 2016).

X-chromosome shredding gene drives located on the Y-chromosome (Y-linked X-shredders) have been proposed as tools to suppress insect populations by biasing the sex ratio of the wild population toward males, thus reducing its natural reproductive potential (e.g. Windbichler et al., 2007, 2008; Klein et al., 2012).

Steps have been taken towards engineering a Y-linked X-shredder in *An. gambiae*. A single-copy autosomal integration of the I-PpoI megaendonuclease on the Y-chromosome enabled to shred the paternal X-chromosome during meiosis, resulted in fertile males producing >95% male offspring (Bernardini et al., 2014; Galizi et al., 2014). This approach suppressed small caged populations of mosquitoes under a multiple-release strategy (Galizi et al., 2004). While the use of I-PpoI as an Y-linked X-shredder in *An. gambiae* holds much promise, it only functions in the few organisms that have an X-chromosome with repeated I-PpoI target sequences and thus may not be portable across species (Champer et al., 2016). In the same species, Galizi et al. (2016) developed a CRISPR-Cas9 sex-distortion system, using a CRISPR-Cas9 nuclease that targets an X-linked rDNA sequence that is different from the previously utilised I-PpoI target site and that is conserved among the *An. gambiae* complex, yet absent from more distantly related species. This CRISPR-Cas9 system achieved a male bias of between 86% and 95% (Galizi et al., 2016).

Synthetically engineering X-shredders based on CRISPR, the selection of gRNA targets, in the form of high-copy sequence repeats on the X-chromosome of a given species, is challenging, since such repeats are not accurately resolved in genome assemblies and cannot be assigned to chromosomes with confidence (Papathanos and Windbichler, 2018).

3.3.3 Medea (toxin-antidote) gene drives[28]

The Medea system confers maternal-effect lethality to all offspring that do not have a copy of the M (Medea) element. Although the molecular underpinnings of the natural Medea system remain unknown (Champer et al., 2016), multiple versions of the Medea inheritance pattern have been synthetically reverse engineered and shown to act as robust gene drives in *D. melanogaster* (Chen et al., 2007; Akbari et al., 2014) and *D. suzukii* (Buchman et al., 2018b). These synthetically engineered Medea systems in the *Drosophila* spp. utilise an RNA interference (RNAi)-based toxin-antidote combination. The Medea element have been synthetically engineered based upon a maternal oogenesis-expressed micro RNA (miRNA) toxin that silences a gene essential in embryo development. The developmental defect is rescued only in those embryos that inherit the Medea element and thus carry an early embryogenesis-expressed miRNA-insensitive version of the target gene. These two components are placed adjacent to each other in the genome and enable to rapidly drive a linked cargo/payload gene

[28] Killer-rescue gene drives use independent toxin and antitoxin genes to spread cargo/payload genes associated with the antitoxin (Gould et al., 2008)

through a population (Huang et al., 2009; Hay et al., 2010; Guevara-Souza and Vallejo, 2011; Ward et al., 2011).

As Medea uses elements that are specific to *Drosophila*, attempts to develop mosquitoes and other species with functional synthetically engineered Medea elements have, to date, been unsuccessful (Champer et al., 2016).

3.3.4 Underdominance gene drives[29]

Underdominance occurs when heterozygotes (or their offspring) have a lower fitness than parental homozygotes (Champer et al., 2016). Since underdominant systems require a high introduction threshold to spread through a population, they are likely to be spatially restricted, and they can be removed completely by the release of large numbers of wild type organisms (Champer et al., 2016). Underdominance can be achieved using: a toxin-antidote mechanism; reciprocal chromosomal translocations; and cytoplasmic incompatibility (CI) (Burt and Crisanti, 2018).

Strategies to engineer synthetic underdominant gene drives using combinations of toxins and antidotes have been proposed (Gould and Schliekelman, 2004) and implemented in *D. melanogaster*, both as a proof-of-principle system (Reeves et al., 2014), and as fully functional systems capable of invading wild populations (Akbari et al., 2013). Akbari et al. (2013) used two constructs, each consisting of a maternally expressed toxin (multimers of miRNAs that act to suppress the corresponding gene via a mechanism of RNAi) and a zygotically expressed antidote (resistant mRNAs). Another design in *D. melanogaster* introduced gene constructs on different chromosomes, one having RpL14.dsRNA targeting RNAi to a haplo-insufficient gene RpL14 and the other an RNAi insensitive RpL14 to rescue (Reeves et al., 2014). Both approaches were successfully tested under laboratory settings.

Recently, Buchman et al. (2018a) created a synthetically engineered reciprocal chromosome translocations gene drive in *D. melanogaster*, using homing endonuclease genes that carried a cargo/payload gene, and tested them under laboratory settings. The strains showed frequency-dependent spread in laboratory populations. The spread of such drives can be hindered by fitness costs and resistance due to naturally occurring genetic variation and associated (Buchman et al., 2018a).

3.3.5 Other self-limiting gene drives

The development of self-limiting gene drive systems (alone or in combination with other types of gene drives) with limited spatial and temporal spread are ongoing (e.g. Huang et al., 2007; Gokhale et al., 2014), but mostly at the theoretical level; some have been tested under laboratory settings.

CRISPR genome editing technology accelerated the development of self-limiting gene drive systems. Li et al. (2020a) have developed split HEG-based gene drives in *Ae. aegypti* that could

[29] Also known as heterozygote inferiority

enable local restriction of the drive. López del Amo et al. (2019, 2020) demonstrated the possible usefulness of a split/trans-complementing gene drive system in *D. melanogaster*.

To limit the temporal exposure of a population to the effect of a gene drive, a self-exhausting form of a HEG-based gene drive, called a "daisy-chain gene drive", has been designed and modelled, which will indirectly also lead to local restriction of the drive (Noble et al., 2019). In a daisy-chain gene drive, the CRISPR components are split up in a way that none of them can be effective on its own, and they are distributed throughout the genome. The components are functionally arranged in a linear daisy-chain and act similar to the booster stages of a rocket: components at the base promote the drive of the next component, which promotes the drive of the next higher component. Since the components cannot promote their own drive and probably carry some cargo/payload gene, they will be successively lost again. Therefore, after a certain amount of time, the gene drive will stop operating, and the drive components will be lost again from the population. The spread of the cargo/payload gene(s) will depend both on the release ratio and the number of links to the daisy chain.

Champer et al. (2019a) developed a new form of CRISPR-Cas9-based gene drive, the toxin-antidote recessive embryo (TARE) drive, which limits resistance by targeting a recessive lethal gene while providing a recoded sequence to rescue only drive-carrying individuals. Other designs for so-called killer-rescue (toxin-antidote-based) systems exist (Gould et al., 2008; Marshall, 2011; Marshall and Hay, 2011, 2012a, 2014; Marshall et al., 2011; Oberhofer et al.; 2019). The inverse Medea system relies on a toxin that takes effect in the zygote unless it receives a maternally delivered antidote (Marshall and Hay, 2011). The Merea system functions similarly to Medea, but the antidote to the maternal toxin is recessive (Marshall, 2011). The Semele system, conversely, uses a paternal semen-based toxin and a maternally delivered antidote (Marshall, 2011; Marshall et al., 2011). Marshall and Hay (2012a, 2014) have also proposed several additional variants utilising toxin and antidote combinations, including the Medusa system, which induces a population crash by using a pair of sex-linked toxins and antidotes (Marshall and Hay, 2014).

Oberhofer et al. (2019) have demonstrated a killer-rescue system (referred to as *CleaveR* [Cleave and Rescue (*ClvR*)] for population replacement in *D. melanogaster*. *ClvR* comprises two linked chromosomal components: one, germline-expressed Cas9 and gRNAs – the cleaver – cleaves and thereby disrupts endogenous copies of a gene whose product is essential, while the other, a recoded version of the essential gene resistant to cleavage and gene conversion with cleaved copies – the rescue – provides essential gene function. *ClvR* enhances its transmission, and that of linked genes, by creating conditions in which progeny lacking *ClvR* die because they have no functional copies of the essential gene (Oberhofer et al., 2019). Split killer-rescue systems are currently tested in *D. melanogaster* for locally restricted self-limiting gene drive strategies (Webster et al., 2019). It is expected that RNA-guided nucleases will further contribute to the development of each of these systems in diverse species (Champer et al., 2016). In addition, allelic drives could contribute to the development of new efficient synthetically engineered gene drive systems (Guichard et al., 2019).

3.4 State of the art

To summarise, gene drive research is currently focused on the following main areas:

1. Identifying, developing and testing desirable cargo/payload genes that may be spread by gene drive systems (e.g. Franz et al., 2006; Khoo et al., 2010; Criscione et al., 2016; Jupatanakul et al., 2017; Mathur et al., 2010; Buchman et al., 2019, 2020; Duvall et al., 2019);
2. Developing synthetically engineered gene drives and pairing them with desirable cargo/payload gene (e.g. Chen et al., 2007; Akbari et al., 2014; Simoni et al., 2014; Gantz et al., 2015; Hammond et al., 2016; Galizi et al., 2016; Buchman et al., 2018a,b; Kandul et al., 2019; Oberhofer et al., 2019);
3. Studying the nature of target site resistance to mitigate its eventual occurrence (e.g. Basu et al., 2015; Beaghton et al., 2017a,b, 2019; Champer et al., 2017, 2018, 2019b; Hammond et al., 2017; Marshall et al., 2017; Noble et al., 2017; Unckless et al., 2017; KaramiNejadRanjbar et al., 2018; Kyrou et al., 2018; Oberhofer et al., 2018; Bull et al., 2019; Champer et al., 2019; Guichard et al., 2019; Marshall et al., 2019);
4. Mitigating the spreading potential of gene drives (e.g. Gould et al., 2008; Altrock et al., 2010; Marshall, 2011; Marshall and Hay, 2011, 2012a, 2014; Marshall et al., 2011; Akbari et al., 2013, 2014; Champer et al., 2016, 2019a; Esvelt and Gemmell, 2017; Tanaka et al., 2017; Buchman et al., 2018a,b; Burt and Deredec, 2018; Dhole et al., 2018; Leftwich et al., 2018; Marshall and Akbari, 2018; López del Amo et al., 2019, 2020; Noble et al., 2019; Webster et al., 2019; Li et al., 2020a);
5. Mathematical modelling to determine ideal gene drive characteristics, predict their behaviour at population and landscape level, and understand their potential environmental impacts and associated uncertainties (e.g. Rasgon and Gould, 2005; Deredec et al., 2011; de Jong, 2017; Eckhoff et al., 2017; Godfray et al., 2017; Haller and Messer, 2017; Lambert et al., 2017; Noble et al., 2017; Dhole et al., 2018, 2019; Khamis et al., 2018; Noble et al., 2018; Beaghton et al., 2019; Courtier-Orgogozo et al., 2019; Edgington and Alphey, 2019; Nash et al., 2019; North et al., 2019; Sánchez et al., 2019);
6. Assessing the applicability of existing risk assessment frameworks and in which areas of such frameworks refinements may be needed for GDMOs (e.g. WHO, 2014; NASEM, 2016; Adelman et al., 2017a; HCB, 2017; Roberts et al., 2017; Krishnan and Gillum, 2017; Lunshof and Birnbaum, 2017; Benedict et al., 2018; Hayes et al., 2018; Meghani and Kuzma, 2018; Rüdelsheim and Smets, 2018; van der Vlugt et al., 2018; CSS–ENSSER–VDW, 2019; Kuzma, 2019; Teem et al., 2019; Warner et al., 2019; Mitchell and Bartsch, 2020);
7. Assessing the applicability of existing regulatory frameworks and in which areas of such frameworks refinements may be needed for GDMOs (Rabitz, 2019);
8. Developing pathways/recommendations to/for responsible and sustainable deployment of the technology (e.g. Oye et al., 2014; WHO, 2014; Akbari et al., 2015; NASEM, 2016; Adelman et al., 2017a,b; Emerson et al., 2017; Esvelt and Gemmell, 2017; Lunshof and Birnbaum, 2017; James et al., 2018; Thompson, 2018; Backus and Delborne, 2019; Bartumeus et al., 2019; Kuzma, 2019; Cisnetto and Barlow, 2020);

9. Developing guidance/best practices on societal/stakeholder engagement and communication (e.g. Bartumeus et al., 2019; Brossard et al., 2019; Buchthal et al., 2019; George et al., 2019; Hartley et al., 2019; Singh, 2019; Thizy et al., 2019; MacDonald et al., 2020; Serr et al., 2020);
10. Developing effective management and implementation of vector control programmes (e.g. Feachem et al., 2019).

4 Ecology and population dynamics

The potential environmental impact of a gene drive cannot be completely evaluated without a detailed understanding of the ecology impact of species carrying these modified traits (e.g. NASEM, 2016). The deliberate release of a GDMI will alter the ecology in the receiving environment and it is important that the key ecological considerations pertinent to the efficacy and safety of gene drives are properly evaluated. Key considerations need to focus on the principles of population dynamics, the effects of seasonality, dispersal, within-species and between-species competition, spatial heterogeneity and invasiveness. Advances in our conceptual approaches to understanding the novel evolutionary and ecological couplings and feedbacks that GMDI generate (NASEM, 2016) requires better focus on theory, mathematical modelling and empirical ecological studies.

4.1 Insect population dynamics

Insect population dynamics are based on principles of births, deaths and dispersal (e.g. Varley et al., 1973; Begon et al., 2005). These population-level processes affect factors that determine the steady state (limitation) and factors that influence the return to or departure from steady state (regulation). Equilibrium is the state achieved in a population when the births, deaths and dispersal all balance with the environment in which a species finds itself. Limitation comprises the abiotic and biotic processes that determine the level of the equilibrium and regulation is the population-level processes that return a population to an equilibrium.

For insect vector/pest control and gene drives, the abovementioned ecological concepts are critical. The efficacy and achieved outcomes of a gene drive to replace or reduce a vector or pest population is, to a major degree, dependent on the ecological as much as the genetic dynamics. Understanding the spatial and temporal spread of a gene drive requires understanding the factors affecting births, deaths and dispersal that influence the population-level equilibrium and process of limitation and regulation. Disrupting these ecological processes that maintain the vector/pest population and lead to the reduction, elimination or eradication is the goal on integrated pest management plans. Therefore, understanding how the ecological feedbacks affect population dynamics is critical to risk assessment. Of these, seasonality, dispersal and within and between species competition are key aspects in evaluating the risks posed by novel gene drive technologies for insect vector/pest control.

4.1.1 Seasonality dispersal, and intraspecific and interspecific competition

Seasonality is a critical ecological factor in the dynamics of many insects. For instance, for mosquitoes the necessary requirement for aquatic habitats for larval development and the

seasonal availability of water has important consequences for mosquito abundance and dynamics. Understanding seasonality and the survival capabilities of wild type and GM mosquitoes is an important population-level characteristic necessary to evaluate the potential success of, or risk associated with, the deliberate releases of gene drive modified mosquitoes. For example, understanding the variability in temporally seasonal condition is critical for the success and timing of releases of gene drive modified mosquitoes (Lambert et al., 2018). Further, knowing how mosquitoes survive dry or shorter wet periods (e.g. egg aestivation) and the consequences for potential control using gene drive constructs is poorly understood but is essential for the risk assessment of genetic-based controls that are expected to lead to long-term spatial and/or temporal spread.

Intraspecific competition is the ecological process that determines how individuals within a species compete for limited resources. In mosquitoes, this is predominantly for detritus-based food in the larval aquatic habitat. Unfortunately, precise details on the magnitude of this competition is often lacking. However, from different mathematical modelling approaches for different vector species, intraspecific competition is known to be a critical process in the success of any integrated vector management control programme (Rogers and Randolph, 1984; Yakob et al., 2008a,b; Alphey and Bonsall, 2014). The timing of the genetic control with respect to the intraspecific competition can influence the outcome. For example, SIT is a control intervention that acts early in the life cycle of an insect by disrupting egg production. Rogers and Randolph (1984) showed that this sort of control can even lead to enhanced vector/pest population sizes as the imposed (control-based) mortality alleviates the strength of intraspecific competition, allowing surviving individuals unrestricted access to resources and mating opportunities, which can lead to unwanted population level increases rather than decreases in abundance. Mitigation of these unwanted environmental risks of increased pest/vector abundance is only possible with appropriate ecological knowledge. For gene drive systems, a much more detailed understanding of the timing of ecological processes such as intraspecific competition with respect to the gene drive effects is required to avoid exacerbating pest/vector population sizes (Deredec et al. 2011; Alphey and Bonsall, 2014).

Interspecific competition is where two species which potentially share the same ecological niche compete for limiting resources. Behavioural interactions such as heterospecific (between species) matings (Paton and Bonsall, 2019) and/or resource competition (Juliano, 2009, 2010) can all be considered forms of interspecific competition that operates in mosquitoes. Again, it has been shown that the timing of these effects on vector control and coexistence patterns are essential to the success of genetic-based approaches for vector control to avoid exacerbating vector population sizes (Bonsall et al., 2010; Paton and Bonsall, 2019).

Understanding aspects of interspecific competition is important in niche replacement with GMIs (Bonsall et al., 2010). As control operates, it reduces population size in the target species population and this can lead to unexpected, novel interactions between closely related species. It is well-established in vector control epidemiology that reduction rather than elimination of vectors can be quite sufficient to break transmission cycles and lower disease burdens. Again, understanding these ecological factors is central to ERAs and allow ecological knowledge to inform on risk mitigation strategies.

4.1.2 Dispersal and spatial heterogeneity

The critical ecological process in the establishment, spread, efficacy and environmental risk of GDMIs is dispersal. Without a thorough and comprehensive understanding of dispersal, the outcomes of a spatially-spread gene drive modified insect through time cannot be understood. Dispersal is the ecological process of individuals moving between different habitats, but not necessarily returning to a natal patch (as opposed to migration which is movement back and forth between two different habitats).

Dispersal will affect the outcome of gene drives at different spatial scales. Within patches (which also need careful investigation), dispersal is critical to vector redistributions and dynamics. For instance, Manoranjan and van Driessche (1986) modelled the efficacy of vector control under a self-limiting SIT control. They concluded that the number of mosquitoes required to eliminate the population was dependent on: (1) mosquito demography of births, deaths and movement; (2) the dimensions of the spatial and, most critically (3) the initial spatial population distribution. More recently, Ferreria et al. (2008) showed that in spatially-heterogeneous environments vector elimination under self-limiting control is difficult to achieve and can dependent on the optimal timing of the genetic-based control (Yakob and Bonsall, 2008).

At broader spatial scales, dispersal heterogeneity in relation to key environmental features (such as breeding sites) affects heterogeneity in the environmental impact effects (e.g. vectorial capacity). At these spatial scales, difference in species-specific dispersal is critical to the efficacy of a gene drive technology. For example, *Aedes* are typically short-dispersing species (with a large proportion of species not necessarily moving large distances from the natal sites) (e.g. Harrington et al., 2005; Hemme et al., 2010). In contrast, *Anopheles* disperse much more widely (e.g. Taylor et al., 2001; Thomson et al., 1995; Dao et al., 2014; Huestis et al., 2019). For slowly dispersing species (like *Aedes*) local elimination and/or eradication may be achievable, but this may not be the case for fast dispersing species where repopulation of wild type vectors may be strong. Spatial control depends critically on the combination of the genetics of control and the ecological aspects of dispersal.

At landscape scales, non-random distribution of insects can limit the success of control programmes (Yakob et al., 2008a,b; North et al., 2013) as higher density patches may not receive the critical threshold of modified insects necessary for control to be successful (Barclay, 1992). Connectivity networks and landscape structures and the coverage proportion (propensity of released modified insects to inhabit patches occupied by wild type vectors) is crucial to vector outcomes. If patches are highly clustered, isolated patches or pockets of vector/pest insect persistence are likely to occur as they have reduced probability of colonisation and hence control (Yakob et al., 2008b).

4.1.3 Invasiveness

Invasiveness is the ecological concept that allows a species to spread from rare as the species has positive population growth (that the change in numbers from one time step to the next are greater than zero). This is critically determined by the genetics, demography and receiving environment. For some gene drives, the inherent expectation is that invasiveness is achieved

simply by genetic modification and release of a small number of GDMIs. However, anticipating the spatial establishment and spread of GDMIs (distinct features of invasiveness) also requires an ecological understanding. Spatial establishment requires that modified insects are able to reproduce and is associated with the demography of the GDMI compared to the wild type. In a naive environment, the spatial spread of a gene drive is, as noted above, associated with dispersal with the upper limit to spread determined by dispersal, demography (the intrinsic rate of population increase) and genetics (the drive rate of the genetic construct) (e.g. Shigesada and Kawasaki, 1997; Beaghton et al., 2016).

4.2 Heterogeneity of receiving environments

Depending on the degree of heterogeneity in the receiving environments and the strength of the gene drive, there may be barriers to full establishment of the intended trait(s) in the population. For example, isolated populations may not be exposed to a spreading gene drive in the wider population. This could affect the overall impact of the gene drive on the target organisms and could be a factor influencing the efficacy of the GDMI. Understanding the heterogeneity of receiving environments requires approaches that consider the ecological processes at broader regional and national scales (e.g. North et al., 2019).

5 Familiarity with/experience from existing insect vector/pest control strategies

Although, in many ways, the use of synthetically engineered gene drives for insect vector/pest control is novel, it does have similarities with some well-established insect vector/pest control strategies, including sterile insect releases and classical biological control programmes. It is appropriate to draw on the familiarity with/experience from existing insect vector/pest control strategies, seek precedence in the potential hazards, exposures and risks identified for more or less similar situations, and use this familiarity/experience to inform/frame the ERA of GDMIs (EFSA, 2013; Webber et al., 2015; Murray et al., 2016; Roberts et al., 2017; Hayes et al., 2018; James et al., 2018; Ritchie and Staunton, 2019; Romeis et al., 2020).

5.1 Genetic control strategies

5.1.1 Release of artificially reared radiation-sterilised males

SIT uses the mass release of artificially reared radiation sterilised male insects (sterilised using e.g. ionizing radiation) that prevents them to produce viable offspring when mating with wild type females. This strategy has enabled the suppression of populations of several insect pests of agricultural and veterinary importance (Benedict et al., 2010). Effectiveness of SIT is associated with the fitness of the sterilised males as related to their dispersal ability, longevity, and ability to compete with wild type males for mating wild type females (Romeis et al., 2020). Despite various open release trials, SIT has not been widely used against mosquitoes because of the difficulty of irradiating males without reducing their mating competitiveness and survival (Dame et al., 2009; Helinski et al., 2009; Lees et al., 2015).

In general, no formal ERA procedures are in place for SIT (HSCP, 2018; Romeis et al., 2020).

5.1.2 Release of artificially reared males with dominant/female specific lethality

At present, open release trials with GMIs mostly involved the release of male insects carrying either a dominant lethal (RIDL) or female-specific lethal (fsRIDL) transgene for the suppression of insect pest populations. Through the introduction of a repressible lethal genetic system, the RIDL technology results in non-viable offspring, thereby decreasing the reproductive potential of the wild type population (Phuc et al., 2007; Alphey et al., 2010; Benedict et al., 2010; Beech et al., 2012; Slade and Morrison, 2014). If sufficient numbers of wild type females mate with RIDL males over time, then the population collapses. This self-limiting technology has been tested since 2009 in open release trials with the RIDL GM mosquito *Ae. aegypti* (strain OX513A) to suppress wild type populations in Brazil, Cayman islands, Malaysia and Panama (Alphey and Beech, 2012; Harris et al., 2012; Lacroix et al., 2012; Neira et al., 2014; Carvalho et al., 2015; Gorman et al., 2015; GeneWatch-TWN-ACB, 2019; Williams et al., 2020), while activities have been planned in Florida (USA) and India (Slade and Morrison, 2014; Romeis et al., 2020).

The fsRIDL technology only leads to female-specific lethality, which enables additional mating cycles to reduce target populations. Since male offspring is not impacted by the transgene, fsRIDL males continue to emerge and pass on the self-limiting gene for a few subsequent generations. After releases cease, the self-limiting gene declines to extinction, decreasing each generation by half (Harvey-Samuel et al., 2015). Field cage studies were performed with GM mosquito *Ae. aegypti* (strain OX3604C) (Facchinelli et al., 2013). In 2018, open release trials with the fsRIDL GM mosquito *Ae. aegypti* (strain OX5034) were started in Brazil (Slade and Morrison, 2014; GeneWatch-TWN-ACB, 2019).

fsRIDL technology is under development/test to suppress wild type *Ae. aegypti*, *Ae. albopictus*, *Anopheles albimanus* and *An. stephensi*, (Fu et al., 2010; Wise de Valdez et al., 2011; Labbé et al., 2012; Slade and Morrison, 2014) and agricultural pests such as the diamondback moth (*Plutella xylostella*; strain OX4319L; Harvey-Samuel et al., 2015; Bolton et al., 2019), fall armyworm (*Spodotera frugiperda*; strain OX4319; Jin et al., 2013), pink bollworm (*Pectinophora gossypiella*; strains OX3402C, OX4135 and OX4319; Morrison et al., 2012; Jin et al., 2013), Mediterranean fruit fly (*Ceratitis capitata*; strain OX3864A; Leftwich et al., 2014; Asadi et al., 2019) and olive fly (*Bactrocera olea*; strain OX3097D; Ant et al., 2012; Turner et al., 2018). These strains also express the fluorescent protein marker, DsRed, to permit the effective monitoring of the presence of such strains in the field. Recently, a series of open release trials took place in Geneva (NY, USA) with adult male fsRIDL GM diamondback moths (strain OX4319L) and wild-type counterparts to test dispersal, persistence and field survival of the local diamondback moth population in a cabbage field (Shelton et al., 2020). Further open release trials are recommended to assess suppression efficacy. Previous glasshouse experiments demonstrated the effectiveness of this approach (Harvey-Samuel et al., 2015).

Although (fs)RIDL-based SIT approaches to suppress insect pest population do not require radiation sterilisation, they typically require inundative releases of large numbers of sterile individuals (Beech et al., 2009, 2012; Mumford, 2012; Reeves and Phillipson, 2017), which can be laborious and expensive, and impede scalability and large scale adoption (Buchman et al., 2019).

Like any other GMO, the deliberate release into the environment of GMIs is regulated in almost all jurisdictions under specific GMO legislation. Consequently, regulatory and ERA experience has been gained in jurisdictions where actual releases have taken place. In all cases, potential adverse effects on the environment, including effects on human and animal health, have been assessed as part of the ERA, which is conducted before GMIs can be deliberately released into the environment. Moreover, guidelines for the risk assessment of GMIs have been developed over the last few years (reviewed by HCB, 2017; Glandorf, 2017; Romeis et al., 2020).

5.2 Biological control strategies

5.2.1 Release of *Wolbachia*-infected individuals

Wolbachia are intracellular, maternally inherited endosymbionts that manipulate the reproduction of their host in various ways to favour their own maternal transmission (reviewed by Nikolouli et al., 2018). This can result in an increase of the frequency of infected females in the host population, either by inducing a female biased sex ratio in the offspring of infected females, or by reducing viable egg production in uninfected females. *Wolbachia* occur naturally in many insects, and have been introduced experimentally into others.

Wolbachia has been deployed to: (1) suppress vector/pest populations through the release of *Wolbachia*-infected males that are incompatible with the wild type (uninfected) females (Turelli and Hoffmann, 1991; Sinkins et al., 1995; O'Connor et al., 2012; Alphey et al., 2013; Mains et al., 2016; Zheng et al., 2019); and (2) alter/replace a population of the target species with *Wolbachia*-infected disease-refractory individuals (Hoffmann et al., 2011, 2014; Bourtzis et al., 2014; Shaw et al., 2016; Callaway, 2019; Servick, 2019). Particular *Wolbachia* strains have been reported to reduce the susceptibility of the individuals that they infect to pathogens such as dengue and chikungunya (e.g. *w*Mel and *w*MelPop strains transinfected *Ae. aegypti* and *Ae. albopictus* [Moreira et al., 2009; Blagrove et al., 2012]), reducing their ability to transmit disease (also known as pathogen interference (PI)). The mechanism of *Wolbachia*-induced pathogen-blocking is not well understood (Marshall et al., 2019). Yet, this feature, along with the gene drive-*like* inheritance pattern of *Wolbachia*, has been harnessed in replacement strategies to limit disease transmission by mosquito populations (Hoffmann et al., 2011, 2014, 2015; Walker et al., 2011; Schmidt et al., 2017; O'Neill, 2018; Nazni et al., 2019; O'Neill et al., 2019).

Both approaches rely on CI induced by *Wolbachia*. CI is commonly expressed as embryonic lethality in crosses between infected males with uninfected females (unidirectional CI), all other crosses being fertile. However, infected females can successfully mate with infected and uninfected males and thus have a reproductive advantage. Consequently, the *Wolbachia* infection will spread through the population (Alphey, 2014). In bi-directional CI, crosses between individuals infected with different (incompatible) *Wolbachia* strains are sterile. In this case, only matings between females and males carrying the same *Wolbachia* strain will result in offspring (Bourtzis et al., 2014).

Population suppression is based on *Wolbachia* infections that cause bidirectional CI or unidirectional CI if the target population is uninfected. In this strategy, infected males are

repeatedly introduced into a population and the introduced *Wolbachia* type does not establish within the targeted population. Due to the similarity with the classical SIT, the CI strategy is often referred to as IIT.

Population replacement is based on unidirectional CI. Females[30] are introduced that are infected with a *Wolbachia* type that shows a pattern of unidirectional CI with individuals in the targeted population. Above a critical threshold, the introduced infection can establish and spread. In this scenario, the *Wolbachia* infection, directly or indirectly, reduces pathogen transmission, and the outcome is a vector population less able to cause disease.

Artificially acquired strains of *Wolbachia* have been shown to be effective in suppressing populations of different species of mosquitoes, or replacing them with disease-refractory strains, when tested under small-scale physically and/or ecologically confined field settings, and/or in open release trials (e.g. De Barro et al., 2011; Hoffmann et al., 2011; Walker et al., 2011; O'Connor et al., 2012; Atyame et al., 2015; Mains et al., 2016; Schmidt et al., 2017; Waltz, 2017; Nazni et al., 2019; O'Neill et al., 2019; Zheng et al., 2019; Williams et al., 2020). Moreover, successful population suppression has been observed in physically confined laboratory experiments with *Wolbachia*-infected strains of the Mediterranean fruit fly *C. capitata* (Zabalou et al., 2004, 2013), and the transmission of the bacterial endosymbiont has been studied in ants (Pontieri et al., 2017).

The possibility of transferring *Wolbachia* mechanically into novel hosts (transinfection) to create associations not restricted by mating barriers has greatly increased the possibilities for application of this technology (Hughes and Rasgon, 2014).

While *Wolbachia*-based population suppression IIT strategies can be effective, they require inundative releases of large numbers of individuals (Armbruster, 2019; Buchman et al., 2019). Moreover, *Wolbachia* has been reported to enhance certain flavivirus infections (Dobson et al., 2014; Amuzu et al., 2018; King et al., 2018). This approach can also be undermined by the accidental release of females infected with the same *Wolbachia* strain as the released males. An advantage of IIT is that *Wolbachia*-based sterilisation has little or no effect on male mating competitiveness and survival (Chambers et al., 2011; Zhang et al., 2015; Atyame et al., 2016).

Developments are on-going to combine IIT and SIT, so that any residual females that are not separated from the released males are sterilised using low dose irradiation without affecting the male mating competitiveness or survival (Zheng et al., 2019).

Wolbachia has been proposed as a drive for synthetically engineered gene constructs, but thus far it has not proved amenable to transformation (Champer et al., 2016; Macias et al., 2017). However, the flexibility of RNA-guided endonucleases may change this, potentially enabling the development of improved strains of *Wolbachia* with enhanced disease-refractory properties and a reduced fitness impact on their host, allowing them to propagate more rapidly throughout an

[30] Sex sorting is less of an issue for population replacement strategies, so both infected females and males can be released. The released males are expected to contribute to the population replacement process, as their matings with non-infected wild type females prevent the latter from having offspring

insect population (Champer et al., 2016). *Wolbachia* should be seen as a natural gene drive that is cytoplasmically inherited, and thus would not fall within the GMI category.

Regulatory and ERA experience with the release of *Wolbachia*-infected insects has so far only been gained with mosquitoes (Romeis et al., 2020). Currently deployed mosquito suppression and replacement strategies based on the mass release of *Wolbachia*-transinfected individuals, which are not considered GMOs, have been subjected to an ERA that evaluates potential risks to human and animal health and the environment resulting from their deliberate release (e.g. Murphy et al., 2010; Murray et al., 2016; US EPA, 2017). This assessment falls under different regulatory frameworks depending on the jurisdiction where the releases take place. For instance, in the USA, *Wolbachia*-transinfected strains are regulated as biopesticides, whereas in Australia they are evaluated as veterinary chemical products, i.e., considering *Wolbachia* as a substance by the Pesticides and Veterinary Medicines Authority (De Barro et al., 2011). In the EU, *Wolbachia* could be regulated as a microbial agent under the appropriate biocide legislation.

5.2.2 Classical biological control

There is substantial experience with releasing organisms (and their genomes) into new environments. Releasing biological control agents (BCA) such as predators and parasitoids to control insect pests is an important pest management tool. There are two principle applications of BCA: (1) augmentative biological control; and (2) classical biological control (CBC) (Romeis et al., 2020).

In augmentative biological control, native or exotic species are mass-reared and repeatedly released in the field or the greenhouse; wider dispersal and establishment are not intended. The aim is a short-term or season-long suppression of the target pest. In the case of CBC, natural enemies of invasive arthropod pests are (typically) introduced from the area of origin of the pest. They are released with the aim to establish and provide long-term control of the target pest potentially even leading to the eradication of the exotic pest. Consequently, potential environmental effects caused by such releases are likely to be irreversible. However, since classical biocontrol is generally used against exotic pests, this irreversible effect of reducing the target organism is to revert to the ecosystem back to a state without the insect pest species. A major consideration in risk assessment and regulatory approval for classical biocontrol is the host specificity of any biocontrol agent to ensure that the CBC agent will not adversely affect any native host (Shaw et al., 2011; Marchante et al. 2017). Therefore, the application of CBC could thus serve as a model for ERA of GDMIs. These experiences provide a suitable basis to identify and assess many potential risks of GDMIs (Romeis et al., 2020).

Shaw et al. (2011) provide lessons on the application of EU and Member State plant health regulations and risk assessment procedures to license the field release of a CBC agent in the United Kingdom. Marchante et al. (2017) note that such releases are rare in Europe (there have been three approved intentional biocontrol releases) and they outline a series of Portuguese and European level applications, reviews and approvals before their introduction was allowed. An EPPO/COST-SMARTER (2015) report noted the lack of uniform guidance on how the regulations, developed for other purposes, should be applied for biocontrol releases. This report recommended that a distinction should be made between self-sustaining and self-limiting

biocontrol agents, and that benefits should be assessed alongside risks to establish net benefit or harm.

6 Potential new hazards/risks associated with gene drive modified disease-spreading mosquitoes and agricultural pests

Existing genetic methods for insect vector/pest control are self-limiting and mostly used to suppress target populations (Table 2). Synthetically engineered gene drives are expected to complement and substantially expand the range of existing genetic vector/pest control methods, especially for population replacement. Owing to their potential to self-replicate, synthetically engineered gene drives may enable: (1) rapid and non-localised spread of genes of interest through interbreeding populations from low initial introductions, even if they incur a fitness cost on their host; (2) indefinite persistence of genes of interest in a target population or until this population is locally eliminated; and (3) changing the genetic makeup of wild type populations. These features may introduce additional complexity to the risk assessment of some GDMIs compared with non-GDMIs.

However, similar forms of environmental harm are anticipated from the deliberate release into the environment of GDMIs that have been encountered before, whether from the use of non-GDMIs or other existing insect vector/pest control strategies. These include among others: the potential negative consequences of removing the target organism from the environment (e.g. Fang, 2010); and human health consequences if a disease is removed from the environment only to return after local immunity is reduced. These are important considerations as part of any control effort, but they should not be linked exclusively to any particular gene drive technology (NASEM, 2016; Roberts et al., 2017; James et al., 2018; Romeis et al., 2020).

No additional unintended effects due to the genetic transformation process are expected for GDMIs than for non-GMIs, as similar approaches (e.g. based on transposable elements or CRISPR-Cas9) are typically used for genetic transformation in insects (e.g. Alphey and Alphey, 2014; Macias et al., 2017; Anderson et al., 2019; Paulo et al., 2019; Sim et al., 2019; Zhao et al., 2019; Li et al., 2020b). Unintended effects could also occur through mutations on the gene drive sequence, the cargo/payload sequence, or some related or unrelated off-target sequences. Random off-target mutations are likely to disappear naturally in a gene drive if they do not confer any fitness advantage. Mutations biased to occur with greater frequency when the drive mechanism occurs, however, could be maintained in a population. For replacement drives, there may be such off-target effects, but the likelihood, viability and impact of any such mutations is not known. The rate of phenotype and genotype changes in GDMIs could be checked by whole genomic sequencing if reference genome data are available. NASEM (2016) indicated that the optimisation of gRNA design, endonuclease cutting efficiency, and homology-directed repair (HDR) vs. non-homologous end joining (NHEJ) activity may enable to achieve high specificity and thus reduce the potential for off-target effects (see also Thomas et al., 2019).

While the molecular complexity of some GDMIs may be higher than that of non-GDMIs, especially for multi-locus gene drive approaches, tools and approaches from computing and

1154 engineering such as mathematical modelling and computer-aided design are typically employed
1155 to inform and predict the outcomes of different engineering strategies. These tools and
1156 approaches might similarly aid and improve the MC and ERA of GDMIs.

1157 **Table 2. Overview of existing genetic and biological vector/pest control strategies**

Intended outcomes	Ability of the gene drive to establish, spread and persist in target populations		
	Self-limiting approaches		Self-sustaining approaches
	Threshold dependent (high threshold)	Threshold independent (low threshold)	Threshold independent (low threshold)
Population suppression	-Release of artificially reared radiation-sterilised males [SIT] -Release of *Wolbachia*-infected males that are incompatible with the wild type (uninfected) females [IIT] -Release of artificially reared male non-GDMIs with dominant/female specific lethality [(fs)RIDL] -Release of GDMIs with spatially restricted gene drives	-Release of GDMIs with temporally restricted gene drives	-Release of GDMIs with spatially and temporally unrestricted gene drives
Population replacement	-Release of GDMIs with spatially restricted gene drives	-Release of GDMIs with temporally restricted gene drives	-Release of *Wolbachia*-infected disease-refractory females that are compatible with the wild type (un- and infected) males [PI] -Release of GDMIs with spatially and temporally unrestricted gene drives

1158 Abbreviations: fsRIDL: release of male insects carrying a female-specific lethal transgene; GDMIs: gene
1159 drive modified insects; IIT: incompatible insect technique; PI: pathogen interference; RIDL: release of
1160 male insects carrying either a dominant lethal transgene; SIT: sterile insect technique

1161

7 Evaluation of EFSA (2012, 2013) for their adequacy for the molecular characterisation and environmental risk assessment of gene drive modified insects

The adequacy evaluation of the considerations/requirements given in EFSA (2012, 2013) for the MC and ERA of GDMIs, respectively, is reported below for each of the relevant headings and subheadings of EFSA (2012, 2013).

7.1 EFSA (2013)

7.1.1 Scope of EFSA (2013) [Section 1]

This adequacy evaluation of EFSA (2013) is limited to the use of synthetically engineered gene drives to control insect pest species such as disease-transmitting mosquitoes and agricultural pests. Such GDMIs are expected to be deliberately released into the environment, and thus are not confined or semi-confined animals as defined in Section 1 of EFSA (2013). Consequently, the scope of this adequacy evaluation focuses on non-confined GDMI releases and excludes food/feed uses of GDMIs.

7.1.2 Strategies for the environmental risk assessment of genetically modified animals [Section 2]

The strategies for the ERA of GMAs (covering the case-by-case approach, the step-by-step approach, the problem formulation approach, the comparative approach, and the consideration of intended and unintended effects, previous knowledge and experience, and familiarity) given in Section 2 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

The nine specific areas of risk identified for GMIs are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

7.1.2.1 Different steps of the environmental risk assessment [Section 2.1]

The stepwise approach for the ERA of GMIs given in Section 2.1 of EFSA (2013) is adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Step 1: Problem formulation including identification of hazard and exposure pathways [Section 2.1.1]

The problem formulation approach for the ERA of GMIs described in Section 2.1.1 of EFSA (2013) is broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, in light of the points raised by the participants of EFSA's workshop "Problem formulation for the environmental risk assessment of gene drive modified insects" (see Section 2.2.3.1 and Appendix A), the practical implementation of problem formulation requires further consideration for GDMIs that are addressed below.

Problem formulation is considered a key procedure to frame the ERA of potential GDMI applications on a case-by-case basis, and to ensure that existing knowledge is organised and

used efficiently. Therefore, a robust ERA must begin with an explicit problem formulation, as it helps to identify what should be assessed, why it should be assessed, and how it should be assessed.

Different gene drive strategies will pose different levels of risk. Consequently, the information required for the ERA of GDMIs will be case-specific, as it will vary depending on the biology and ecology of the insect species under consideration, the gene drive design and strategy, the introduced traits, the intended uses of the GDMI, the scale and frequency of the deliberate release, the receiving environments (covering both the receiving environments where the GDMIs will be released and where they will interact with the wild type/target populations), and the interactions amongst these variables. Problem formulation offers more flexibility to address the broad array of potential gene drive applications in a proportionate manner, than pre-set mandatory information/data requirements.

In evaluating efficacy and biosafety of gene drives, ecological attributes are expected to be more critical than might be the case under self-limiting RIDL, fsRIDL or SIT approaches. Consequently, in the problem formulation process, more weight needs to be given to ecological processes, such as trophic interactions, intraspecific competition, density dependence, niche replacement, assortative mating, etc., to frame the ERA of gene drive-based vector/pest control.

Transparency in how a problem formulation is conducted is important to all stakeholders. Thus, sufficient detail about the methods, data, assumptions and uncertainties must be reported to promote transparency, facilitate an appropriate assessment of the quality of the problem formulation, ensure relevance, and enable reproducibility.

Experience gained from jurisdictions and domains where pre-submission exchange between applicants and risk assessment bodies is a well-established process shows that such an exchange can be helpful to frame the problem formulation by clarifying policy goals (including protection goals), decision-making criteria and information requirements, advise on study designs and navigate the regulatory process.

Problem formulation involves among other steps: (1) identifying relevant broad protection goals and making them operational for use in ERA; (2) formally devising plausible pathways to harm that describe how the deliberate release of the GDMI could be harmful; (3) formulating risk hypotheses about the likelihood and severity of such events; (4) identifying the information that would be useful to test the risk hypotheses; and (5) developing a plan to acquire new data for hypothesis testing should tests with existing information be insufficient for decision-making (e.g. US EPA, 1998, Raybould, 2006, 2007, 2010; Gray, 2012; Tepfer et al., 2013; Wolt et al., 2010; Raybould and Macdonald, 2018; Devos et al., 2019a).

Identifying relevant broad protection goals and making them operational: Protection goals determine the nature of harm to be assessed from releases of GDMIs and any predicted or observed changes that result from a release should be assessed in relation to these goals. Consequently, a crucial step in problem formulation is to define what qualifies as harm under the relevant regulations. This requires the delineation of the environmental components

that are valued and must be protected (e.g. species, ecosystem services, habitats), where and over what time period, and the maximum tolerable impact. As such, protection goals establish the context for ERA by describing the components of ecosystems and the environment that should be protected and thus considered during ERA. These protection goals can vary among jurisdictions, but their overall aim is to reduce the harm to the environment, including biodiversity and ecosystems, caused by human activity.

Legislative frameworks generally define protection goals broadly. Consequently, refinement is required to make them operational for use in ERA – they need to be translated into specific, operational goals (also termed specific protection goals or assessment endpoints) (Nienstedt et al., 2012; Garcia-Alonso and Raybould, 2014; Devos et al., 2014, 2015, 2016, 2019b; Van den Brink et al., 2018). EFSA has recommended the use of an ecosystem services (ES) approach for setting operational protection goals for several regulated stressors connected to food/feed production, such as GMOs, plant protection products and feed additives (EFSA, 2010a,b, 2016; Nienstedt et al., 2012; Devos et al., 2015, 2019b; Maltby et al., 2017a, 2018). This framework has been shown to be potentially applicable to other stressors (Maltby et al., 2017b). EFSA's ES approach to defining operational protection goals follows three sequential steps: (1) identifying relevant ES potentially impacted by the use of regulated products; (2) identifying service-providing units – structural and functional components of biodiversity – that provide or support these ES; and (3) specifying the level of protection for these service-providing units. The level of protection is then defined by the ecological entity (e.g. a functional group) of the service-providing unit and its attributes, as well as the maximum magnitude and spatial and temporal scale of tolerable impacts (EFSA, 2016).

Instead of generating operational protection goals on a case-by-case basis, the US Environmental Protection Agency (US EPA) defined generic assessment endpoints that are valid for all regulated stressors, as this ensures consistency between regulated stressors when protecting the environment from harm (Suter, 2000; Suter et al., 2004). These generic assessment endpoints were subsequently expanded to encompass ES (Munns et al., 2009, 2015, 2017). The application of ES-based generic assessment endpoints in ERA can provide an improved means of communicating risks and informing management decisions because incremental changes in the endpoints directly or indirectly benefit humans (Selck et al., 2017).

As with any other ERA for a new technology, it will be important for risk managers to define clear protection goals and decision-making criteria (e.g. definition of protection goals and what constitutes harm, limits or thresholds of concern, trigger values for action or acceptability of risk, judging the sufficiency of scientific knowledge and the extent to which uncertainty should be reduced for decision-making) that are needed to guide the interpretation of scientific information (Devos et al., 2019a,c). In this respect, an important consideration is whether the proposed activity may lead to new harms, or only to different ways of causing harms that already result from current practice. Hence, reaching agreement on protection goals and decision-making criteria is a prerequisite for producing ERAs that address them. Collected data and their interpretation can then be directed towards evaluating the impact of any observed effect on what is desirable to protect. Consequently, enhanced dialogue between risk assessors

and risk managers is advocated to clarify how ERA can address protection goals and decision-making criteria.

In addition, active stakeholder engagement on problem formulation (including the setting of protection goals and assessment endpoints) can improve the value of ERA, as it may help to ensure that ERA are meaningful and informative to the environmental decisions that affect them (e.g. Nelson et al., 2009; NASEM, 2016; Kuzma, 2019; Burgess et al., 2019). In the context of the potential deployment of a gene drive as part of a malaria eradication strategy, researchers, donor organisations and stakeholders, ethicists, health professionals, government regulators in the fields of environment health and biosafety as well as government policymakers have embarked on a series of consultations, workshops and public engagements aimed at problem formulation for the use of gene drive modified mosquitoes to reduce malaria incidence (e.g. Roberts et al., 2017; James et al., 2018; Teem et al., 2019). These types of consultation provide a helpful format to identify relevant protection goals (Craig et al., 2017; Hokanson et al., 2018) and frame ERA (Murphy et al., 2010; Kolopack et al., 2015; Murray et al., 2016). If risk managers consider such an engagement useful to define protection goals, they may want to explore how it should be best designed, and whether it should be performed on single applications, groups of applications, or on the technology per se.

Since it is expected that gene drives may eventually spread across national borders, a point requiring further consideration is whether ERA should be framed by the protection goals established by the jurisdictions that would host the release, or go beyond this to capture the target release area and the potential for transboundary movements.

Devising plausible pathways to harm:

To further frame the ERA, plausible pathways to harm[31] are devised in the problem formulation process to describe how the deliberate release of a GDMI could lead to possible harm to operational protection goals. A pathway can be the function of a simple linear chain of events, or a complex one that is branched. An ERA may include many pathways, because the proposed activity could lead to different harms, or because a particular harm could arise in different ways, or both. Moreover, there may be multiple interconnected pathways to consider that may share some of the same steps.

Adequately identifying multiple, complex pathways to harm over long time period, a wide area, and/or a heterogenous environment is challenging. Different techniques may be used to postulate pathways to harm (e.g. Wolt et al., 2010; Gray, 2012; Roberts et al., 2017; Hayes et al., 2018; Teem et al., 2019). The nature and formality of this exercise is case-dependent and may reflect preferences and approaches of the responsible authority. In principle, only plausible and consequential ones should be carried forward into the analysis. It is thus recommended to: (1) determine the validity and (when possible) the plausibility of pathways to harm based on the available evidence published in the scientific literature; and (2) ensure that they are at least potentially consequential enough to merit further consideration. If the magnitude of a potentially realised harm would be negligible or well below the range of maximum tolerable

[31] A pathway to harm is a causal chain of events that need to occur for a harm to be realised

impacts, then it would not necessarily be worth investigating the pathway to harm further, even if the pathway was plausible.

If the plausibility of a pathway is uncertain, one can either expand the efforts to consider existing knowledge or gather additional information through experimentation for the most critical step(s) of the pathway, depending on the potential of a pathway to cause harm. Since problem formulation is iterative, this information could be used to revisit the level of certainty about the plausibility of the pathway. In all cases, a rationale justifying why specific pathways to harm are not considered sufficiently plausible and consequential should be reported transparently.

Several relevant pathways to harm associated with the deliberate release into the environment of gene drive modified mosquitoes for malaria control and gene drive modified *D. suzukii* carrying a suppression drive have been reported by Roberts et al. (2017) and Teem et al. (2019), and Romeis et al. (2020), respectively, and can be considered further when devising plausible pathways to harm.

Formulating risk hypotheses:

The steps in a pathway to harm enable the formulation of risk hypotheses that can then be tested to characterise risk. Thus, each step in the pathway leads to a risk hypothesis that harm will not arise (Figure 1). The precise form of risk hypotheses will depend on how harm is defined and how decisions on the acceptability of risk will be made.

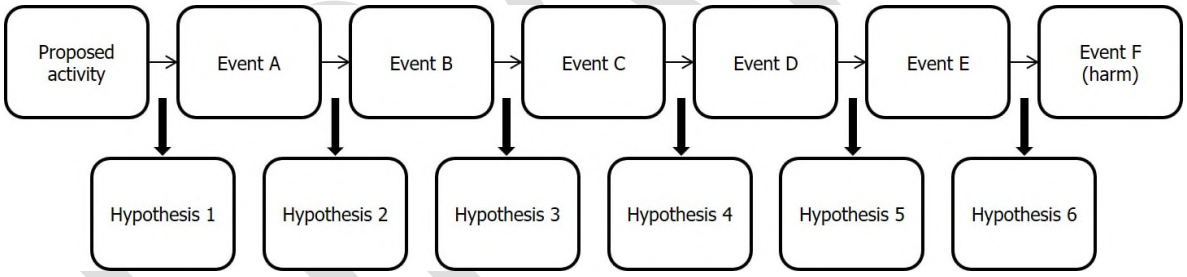

Figure 1: Pathway to harm and risk hypotheses (reprinted from Devos et al. (2019a))

Corroboration of risk hypotheses will build confidence that risk is appropriately assessed via the pathway in question, and corroboration following a rigorous test gives greater confidence than does a weak test. A careful first scrutiny of the pathway can usually help identify which of the step(s) may be the most decisive or easiest to test in attempting to disrupt the pathway with the highest degree of certainty. A particularly useful feature of this strategic analysis is that it decisively determines with sufficient confidence that a single (critical) step is highly unlikely, and so conclude that the likelihood that harm will result via the pathway is negligible and that no other step will require analysis.

In this process, it is important to link hazard to an exposure, and not to confuse hazard or exposure with risk.

Identifying relevant information to test risk hypotheses and developing a plan to acquire new data:

Risk hypotheses may be tested with existing information, which can come from many sources and does not necessarily require experimentation. Some risk hypotheses may be difficult to test in practice, or testing may not produce definitive conclusions regarding the likelihood of a particular step in a pathway. As part of the ERA, this uncertainty may be addressed through an iterative, stepwise/staged/tiered-based testing approach[32], by consideration of multiple lines of evidence (including modelling), and/or by new studies being undertaken (WHO, 2014; NASEM, 2016; Hayes et al., 2018; James et al., 2018; Romeis et al., 2020). If uncertainties remain and depending on the nature of the identified risk, risk mitigation options could be proposed for reducing the overall risk of a particular pathway to harm to a more acceptable level. There is also the possibility to design and implement a post-market environmental monitoring (PMEM) plan to detect or confirm the absence of adverse outcomes. In this respect, it is worth exploring how much weight can be put on PMEM as a complementary tool to ERA to manage uncertainties (see Section 7.1.5).

Step 2: Hazard characterisation [Section 2.1.2]

The considerations on the hazard characterisation for the ERA of GMIs given in Section 2.1.2 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Step 3: Exposure characterisation [Section 2.1.3]

The considerations on the exposure characterisation for the ERA of GMIs given in Section 2.1.3 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Step 4: Risk characterisation [Section 2.1.4]

The considerations on the risk characterisation for the ERA of GMIs given in Section 2.1.4 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Step 5: Risk management strategies [Section 2.1.5]

The considerations on the risk management strategies for the ERA of GMIs given in Section 2.1.5 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Since self-limiting gene drives constitute a form of biological or molecular confinement that could supplement physical and/or ecological confinement (see Section 3.2.2.1), these drives could represent a potential risk management strategy in contrast to self-sustaining gene drives that are designed to be spatially and/or temporally unrestricted.

[32] As a GDMI progresses through the phased testing and deliberate release pathway, the spatial and temporal scales of the concomitant risk assessment studies increase, and the suite of tools used to identify hazards and their potential associated adverse effects changes. Relevant data gathered under controlled, contained conditions provide confidence that the GDMI can safely progress to the next testing phase (NASEM, 2016; Hayes et al., 2018; James et al., 2018)

Step 6: Overall risk evaluation and conclusions [Section 2.1.6]
The considerations on the overall risk evaluation for the ERA of GMIs given in Section 2.1.6 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

7.1.2.2 Information to identify potential unintended effects [Section 2.2]

The considerations given in Section 2.2 of EFSA (2013) to identify potential unintended effects through the molecular, phenotypic and compositional characterisation of the GDMIs and comparisons of biotic and abiotic interactions are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

In line with EFSA (2013), the extent of the compositional and phenotypic analysis of GDMIs (i.e. the type and number of components and phenotypic parameters to consider), which are not intended for food/feed uses, is case-specific, and thus may vary with the nature of the animal and the genetic modification. In addition, the intended outcome of the deliberate GDMI release (population suppression vs. replacement) and level of environmental exposure should be considered as part of the problem formulation, and hence the need for compositional and/or phenotypic data for the ERA of GDMIs.

7.1.2.3 Structural overview of EFSA (2013) [Section 2.3]

The structural overview of EFSA (2013) given in Section 2.3 is adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

7.1.3 Cross-cutting considerations [Section 3]

7.1.3.1 Receiving environments [Section 3.1, including subheadings]

The considerations given in Section 3.1 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

EFSA (2013) is appropriate in highlighting the need for evaluating risks of GMIs across receiving environments and that these risks may differ in different environments. As noted in EFSA (2013), the receiving environment will vary in spatial scale, even when the deliberate release is not intended.

Characteristics of receiving environments highlighted in EFSA (2013) [in Section 3.1.2] are broadly adequate for GDMIs. However, given the expected extended spatial and temporal extent of gene drive systems, the scope of what is deemed an accessible ecosystem (i.e. the environment into which a GDMI is intended for release compared to where it might spread to) will require careful consideration as release and spread into novel accessible environments might be an anticipated outcome (with different risk evaluation and mitigation) following a deliberate release.

Selection of relevant sites for deliberate releases into the receiving environment requires much more scrutiny and assessment than is described in EFSA (2013). The expectation in EFSA (2013) is that applicants need to consider the full geographic range of a GMA which will depend on the context of the deliberate release. Yet, for GDMIs, this may be unfeasible. It will depend on the type of gene drive system, the selection of sites for deliberate release and the potential for range expansion. The emphasis on additional tools (such as mathematical modelling) to

evaluate the choice of receiving environments and inform ERA is briefly mentioned in EFSA (2013). However, with GDMI systems, these tools may play a much more prominent role. The need to develop proportionate ERAs for GDMIs in each receiving environment needs substantial rethinking beyond that covered in EFSA (2013), in order to provide operational ERAs on the application of gene drive technologies.

7.1.3.2 Experimental environment [Section 3.2]

The considerations given in Section 3.2 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

EFSA (2013) emphasises that an appropriate experimental environment for GMIs should focus on the appropriate spatial scale associated with the experimental units. This is broadly in line with that required for the deliberate release of a GDMI.

EFSA (2013) highlights that suitable confinement measures should be in place, but for unconstrained GDMIs the ultimate aim is for spatial and temporal spread. The use of small-scale physically and/or ecologically confined field trials compared to open release trials will thus involve different experimental environments and confinement measures (NASEM, 2016; Hayes et al., 2018; James et al., 2018). Confinement measures will likely vary as a GDMI progresses through phased testing and deliberate release pathways, and they may need to be relaxed to increase the scale and realism of the experimental environment, if a decision is made to proceed to the next phase of testing/implementation (Hayes et al., 2018).

EFSA (2013) highlights the need for evaluation of the potentially different receiving environments for GMAs intended for release into the environment. For GDMIs, ERAs across different environments, particularly for experiments/trials, should focus on the extent to which variation in ecological and environmental conditions might influence the environmental risks associated with the persistence and efficacy of the gene drive (e.g. persistence over inhospitable seasons).

7.1.3.3 Choice of comparators [Section 3.3, including subheading 3.3.2]

The considerations/requirements given in Section 3.3 of EFSA (2013) for the choice of comparators are adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, the concept of comparators could be further extended to include the range of gene drive applications in insects, and put more emphasis on the purpose of the risk assessment-related studies conducted. As a GDMI progresses through the phased testing and release pathway, the range of risk assessment studies and their purpose changes (Hayes et al., 2018). Consequently, there will often not be a single comparator for a given proposed deliberate release into the environment of a GDMI, but a range of comparators. Depending on the study purpose, appropriate comparators may include other insect vector/pest control systems such as biological pest management, use of pesticides and control of invasive aliens, and may not necessarily be limited to the non-GMI of the same species with a genetic background that is as close as possible to that of the GDMI. For the characterisation of a GDMI, the appropriate comparator would be the non-GMI from which the GDMI is derived. For the ERA of GDMIs, comparisons at both the organismal and (management) systems level may be relevant. The most appropriate comparisons will depend on the GDMI application and may consist of the

conventional counterpart as comparator (i.e. the non-GMI with a genetic background as close as possible and relevant to that of the GDMI) and comparison with alternative management scenarios (e.g. insecticides) of the non-GMIs. At the systems level, gene drive applications may need conventional comparator systems that also operate over large areas and long-time scales, such as area-wide control programs, extensive bed-net campaigns or large-scale environmental management programs such as land drainage.

As GDMI systems will operate at an ecosystem level the definition of comparator may need to be broadened from endpoints that solely measure genetic and phenotypic changes to those that can be indicative of potentially harmful ecosystem impacts.

Guidance on the selection of comparators should consider issues relevant to offspring of the GDMI, targeting species complexes with differential effects within the complex. Malaria vector populations consist of an extensive species complex and may derive from considerable distances (Huestis et al., 2019), making it difficult to select a static comparator population.

At the pre-release stage of laboratory populations of GDMIs (referred to hereafter as a colony) with breeding selection in the laboratory colony, comparators should take account whether the colony has reached a generation stable enough for comparisons with a wild type. This would require an introgression history and the background refreshing rate for the colony. Consideration should be given to the selection pressure on a colony based on the nature of the gene drive, so for example a male bias colony will have very high selection pressure from low proportion of females each generation. There may need to be a comparison between an early generation colony and later generations (to test potential effects early in release vs. later after release when many generations have passed). Any changes in trait expression over generations is likely to mainly affect interactions with target organisms that relate to efficacy, but indirectly may affect (non-)target organism interactions.

7.1.3.4 The use of non-genetically modified surrogates [Section 3.4]

The considerations given in Section 3.4 of EFSA (2013) are broadly adequate for GMA releases that are limited in space and time.

Gene drive systems can use non-GM control systems as comparators. For suppression gene drive systems other non-GM suppression systems can be considered, particularly those that are relatively species-specific and where their use occurs on a regular basis over time (such as SIT, bed-nets or selective breeding site removal). Use of irradiated sterile males may tell us something about the effect on reduction of the target organism in the receiving environment, without any (or at least not many) competing effects (due to their specificity). For replacement strategies, *Wolbachia*-based systems are probably the closest comparator, but they have some differences, such as the use of concurrent multiple strains, in some cases.

However, the selection of a comparable non-GM surrogate may be difficult, because of unknown fitness comparisons (e.g. irradiated sterile males will have high fitness cost, not the same as the GM equivalent), so may not tell us about the likely behaviour of the gene drive releases.

Moreover, for some gene drive strategies, the scale in space and time makes experimental study difficult, even with a non-GM surrogate. It depends on the nature of the harm of concern, so if concern is about spread in space and time, it may not be practical to carry out non-GM surrogate studies in the field.

7.1.3.5 Experimental design and statistics [Section 3.5, including subheadings]

The considerations given in Section 3.5 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

The aim of designing experiments is to ascertain the environmental harms associated with the release of GMAs. This needs: (1) clear risk-based hypotheses; (2) appropriate experimental design; and (3) appropriate statistical tools.

However, with GDMIs, the classic short-term ecological experiment to compare different treatment effects (through the use of linear statistical models such as analysis of variance) might not be appropriate. As outlined in EFSA (2013), comparative analyses are required to assess similarities and differences between GMAs and non-GMAs. However, the experimental design and analysis will depend on the risk hypothesis, whether the focus is on biosafety or efficacy, and what the expected differences should be between the GM target organism and the non-GM target organism.

The use of open release trials and experiments with GDMIs will differ from those in EFSA (2013). Measurement endpoints set around thresholds or limits of concern (following EFSA, 2010) should reflect plausible environmental harms from the release of GDMIs. Depending on the expected outcome of the release of a GDMI, limits of concern will differ if the goal is population suppression versus population replacement. Further, given the expected increase of spatial and temporal extent of these organisms, the use of small-scale physically and/or ecologically confined field trials may be less informative than post-market environmental monitoring (PMEM).

The use of multiplicative effect sizes (as outlined in EFSA (2013)) may be of limited use when the control of target organisms is the goal of a deliberate GDMI release. This needs more scrutiny. EFSA (2013) adequately considers a range of statistical principles such as the importance of phenotypic similarities and differences for comparative analyses, the importance of differences between laboratory, small-scale physically and/or ecologically confined field trials and open release trials. However, the limits of confined space and environmental responses might be context-dependent and highly non-linear for GDMIs. As such, the focus on ANOVA is probably an inappropriate statistical principle to base risk evaluation of GDMIs around and stratified sampling through time and across space, developing temporal and spatial approaches (e.g. Cressie and Wikle, 2011), would be better approaches to the statistical methodologies required to evaluate the environmental harms associated with GDMIs.

The requirements pertaining to statistical analysis (Section 3.5.3 in EFSA (2013)) are too prescriptive to be of benefit in assessing the environmental harms of GDMIs. Appropriate statistical analyses should be reflected through the specific choices of experimental designs and data collected.

7.1.3.6 Long-term effects [Section 3.6, including subheadings]

The considerations on potential long-term effects of GMAs given in Section 3.6 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, but could be made more specific on the information that is required to support risk assessment. In particular, EFSA (2013) does not provide sufficient consideration of multiple generations, and the gene drive potential to establish, spread and persist in target populations, which may be relevant for the deliberate release of GDMIs. This section of EFSA (2013) implies long-term effects arising from exposure to an increasing presence of GMAs and provides examples of delayed effects of invasive species, in which there is an increase in density over time. Further examples could be provided that are more relevant to population suppression strategies with GDMIs, in which populations would be expected to decline, causing exposure over time to diminish. Also, for a gene drive-based replacement strategy, the long-term effect would be due to the proportion of the population with gene expression rather than the density of the population (which would be expected to remain similar). For a replacement strategy, the density may increase if other control efforts aimed at suppression stop. Effects of interbreeding could occur quite quickly in gene drive systems that have a high potential to establish, spread and persist.

7.1.3.7 Further guidance on modelling [Section 3.7]

The considerations on mathematical modelling given in Section 3.7 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Mathematical modelling has an important role to play in each step of the phased testing and release pathway of GDMIs (James et al., 2018). Mathematical modelling can provide a valuable contribution to the weight of evidence (rather than final proof) of aspects associated with performance characteristics, environmental harm and effectiveness of risk mitigation measures. Mathematical modelling is likely to be more important with GDMIs than other GMIs due to the complexity of empirical studies. As there may be difficulties in validating model predictions, greater emphasis should be placed on the identification of key parameters. Moreover, the sensitivity of mathematical model predictions to the sensitivity of parameters is critical.

Appropriate and clear definition of model goals and assumptions (e.g. the limited ecology, temporal scales and spatial scales) for GDMIs go beyond those covered in EFSA (2013). Ecological outputs (e.g. changes in population numbers of an insect) may be less relevant than other metrics such as its vectorial and economic capacity.

It is expected that there will be a greater reliance of mathematical modelling to cope with increased spatial and temporal scales of GDMI releases. Case-specific monitoring will need more validity that in EFSA (2013) for the evaluation of model assumptions/predictions. Mathematical models should be given more value in designing appropriate release strategies, and ERA and PMEM schemes for the deliberate release of GDMIs.

The GMO Panel notes that EFSA has published guidance on good modelling practices (EFSA, 2014) that is relevant for the risk assessment of GDMI applications.

7.1.3.8 Uncertainty analysis [Section 3.8, including subheadings]

The considerations given in Section 3.8 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

7.1.3.9 Health and welfare aspects of genetically modified insects [Section 3.9, including subheading 3.9.3]

The considerations given in Section 3.9 of EFSA (2013) are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Since the European legislation related to health and welfare aspects of animals focuses on farmed animals and, only in exceptional cases, on wild animals, the GMO Panel considers that no additional welfare risk assessment is needed for the GDMIs considered in this GMO Panel Scientific Opinion.

7.1.4 Specific areas of risk for the environmental risk assessment of genetically modified insects [Section 4.2]

The scope of the adequacy assessment of EFSA (2013) is limited to the use of synthetically engineered gene drives to control harmful insect species such as disease-transmitting mosquitoes and agricultural pests, and excludes the use of such gene drives for biodiversity conservation purposes or the enhancement of production systems.

7.1.4.1 Persistence and invasiveness of genetically modified insects, including vertical gene flow [Section 4.2.1, including subheadings]

Several considerations/requirements on persistence and invasiveness, including vertical gene flow, given in Section 4.2.1 of EFSA (2013) are not adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

As indicated by the title, Section 4.2.1 of EFSA (2013) focusses on the overall fitness of the GMI and how the intended trait(s) contribute to it. However, Section 4.2.1 does not address key aspects of the mechanisms enabling gene spread, establishment and persistence by GDMIs. Due to the selfish nature of gene drives, cargo/payload genes linked to the gene drive will spread through a target population, even if they incur a fitness cost on their host. Suppression gene drives typically incur a fitness cost by mediating e.g. female lethality or sterility. Therefore, besides the fitness of the individuals bearing the cargo/payload genes, also the potential of the gene drive to spread, establish and persist in target populations must be carefully discussed, independently of the effect on its individual host.

A variety of phenomena affect the potential of a gene drive to spread, establish and persist, e.g. the gene drive design, target population structure, migration rates, density dependence, environment, costly resistance, local ecology, and even mating incompatibilities between some laboratory strains and wild type individuals (Noble et al., 2018). Consequently, different gene drives will have different potential to spread, establish and persist. For example, population suppression drives may locally self-extinguish before they are able to spread to further populations.

Since the gene drive can spread into a target population with the introduction of only a small quantity of additional genomic material from the released GDMIs, the analysis and evaluation should be conducted on a population level rather than on an organismal level. In this evaluation, it is important to make a distinction between the gene drive construct and the genetic background of the released and target insects, as they are inherited independently.

When deploying GDMIs, the spread, establishment and persistence of the genetic elements in target populations are intended, and can by themselves not be considered a harm. Should the spread go beyond the target population, one can speak of invasiveness. Therefore, the assessment needs to consider the selfish genetic elements, which are intended to persist in the target population and may have the potential to invade other populations or closely related species. Moreover, the invasiveness of the transformed populations should be considered. While Section 4.2.1 of EFSA (2013) covers the evaluation of released individuals, it does not cover the other two dimensions of potential GDMI applications: independent spread of the gene drive, and potentially changed characteristics of transformed populations.

The routes of exposure in Section 4.2.1 of EFSA (2013) are in principle described correctly but focus on the fitness of individuals carrying a transgene. However, with GDMIs, the transgene may confer a reduced fitness on their host, though the gene drive might still spread. Therefore, the necessary description of the exposure of wild populations is not addressed adequately.

Several strategies have been proposed for limiting the spatial and temporal spread of gene drives (see Section 3.3.5). Especially for local restriction, threshold-dependent gene drives have been described in the scientific literature. Threshold (in)dependency will have different impacts in terms of the persistence and invasiveness of the "factory genomes"[33] in the wild population. In this respect, it should be noted that also non-GMI comparators such as classic SIT approaches lead to the introduction of factory genomes into the wild population. High threshold gene drives, which are intended for spatially-restricted uses, will bring in a relatively large amount of factory genome into the wild population. In contrast, low threshold gene drives will bring very little factory genome into the population, though the gene drive (and its linked cargo/payload genes) might spread uncontrolled and widely. Ideally, the mass rearing process should be designed in such a way that it ensures consistency in the produced GDMIs. In addition, quality control should not be limited to the individual GDMIs for deliberate release, but also consider subsequent generations/offspring in the release area (e.g. through PMEM) to monitor, whether the intended spread of the gene drive element performs as modelled before.

Regarding the potential of GMIs to persist or invade EU receiving environments, EFSA (2013) focuses on the distribution, occurrence and fitness of the parental or wild type of the GMI species, and the establishment and spread of the GMI. However, in the case of GDMIs, establishment and spread are necessary for achieving intended outcomes (e.g. population replacement) and thus cannot be considered a harm.

Whether a GDMI will have an altered persistence and invasive potential depends on the nature of the intended traits of the cargo/payload genes, as well as the ability of the gene drive to

[33] The genetic background derived from the rearing colonies used for releases

spread the intended traits. In most scenarios, GDMIs are likely to be less persistent and invasive than the target populations, as the intended traits confer a fitness costs on their host. This is especially the case for population suppression strategies.

Regarding the potential of GMIs to hybridise with compatible relatives to produce viable and fertile offspring, it should be noted that cross-species fertilisation is rare in insects and hybrids are rarely fertile. Thus, only closely related species can have fertile offspring with often reduced fitness. While this aspect is not different between GMI and GDMIs, once such a hybridisation occurs the presence of a gene drive element might enhance the further transmission of the selfish genetic element, since endonuclease-mediated double strand breaks (DSBs) might increase its spread (Courtier-Orgogozo et al., 2019). Laboratory experiments could be informative to study whether the gene drive construct would drive in related species, or whether vector competence would be impacted.

Regarding the potential for increased fitness of a population carrying the GM trait, the effect of a gene drive element will depend on the GDMI application. While suppression drives are very unlikely to convey increased fitness of those organisms that carry it, replacement drives might potentially lead to increased fitness.

Regarding the habitat and/or geographic range, issues will be case-specific and thus dependent on the GDMI application. For replacement gene drives, wide spread is likely to be intended and thus form part of the rationale of release in self-sustaining drives. The risk to biodiversity may differ in areas where the species is invasive or where the gene drive affects species in their native range.

The exposure characterisation will depend very much on the GDMI application. Self-sustaining low threshold drives will require only a small number of gene drive modified individuals to be released to spread. However, such drives are designed to cause desirable genes to increase in frequency in a population and be spatially unrestricted. In contrast, the spread of the gene drive will remain limited with self-limiting high threshold drives despite the release of a high number of transgenic individuals.

EFSA (2013) does not explicitly consider gene drive threshold mechanisms. Consideration of mechanisms helps specify the evidence needed, as part of the case-by-case risk assessment. Low threshold GDMI scenarios are based on extensive spread and impact from a relatively low density and low cost initial release. In self-sustaining low threshold gene drives, if risk management was required it would need some external mitigation to prevent spread from a release area. Exposure would be reduced in cases of high-threshold or self-limiting gene drives, and the high-threshold mechanism would reduce the need for additional risk management measures.

7.1.4.2 Horizontal gene transfer [Section 4.2.2, including subheadings]

The considerations/requirements given in Section 4.2.2 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, but could be made more specific on the information that is required to support risk assessment, especially for GDMIs with site directed nuclease (SDN)-based gene drives (e.g. CRISPR-Cas9).

The considerations for the assessment of the probability and frequency of horizontal gene transfer (HGT) from insects to insects or from insects to microorganisms are based on the assumption that gene drive systems may increase the likelihood of rare HGT events becoming established in new host populations.

Concerning the release, stability and degradation routes of GMI DNA in the receiving environments, exposure to the GDMI should be assessed on a case-by-case basis. For example, GDMIs developed for population suppression are not supposed to persist in the environment, and thus exposure can be considered temporally restricted, compared to replacement gene drives that may persist in the environment.

For GDMIs with HEG-based gene drives, information on the molecular elements of the transgene (gene drive plus cargo/payload gene, if any) is considered important for assessing the potential for HGT. By definition, the gene drive itself can affect the mobility of the associated transgene, and may, in theory, increase the potential for HGT compared to a classical GMI. When the gene drive target sequence and flanking homologous sequences are present in a non-target organism, the potential for HGT could be increased at two ways. First, induction of a double-stranded DNA break in the homologous sequence of the non-target genome could increase the probability for integration of the gene drive construct in this locus (Yamamoto and Gerbi, 2018). Second, the pre-existence of this locus in the receiving non-target population may facilitate the establishment and persistence of the gene drive in the new host population.

If a hazard is identified, the exposure characterisation should consider characteristics of the recombinant DNA, the number of insertions or modifications, the levels and routes of exposure related to the hazard, and the scope of the gene drive strategy (e.g. population replacement vs. population suppression).

In addition to a possible positive selection conferred by the horizontally transferred recombinant DNA and as described above, it is important to consider that the applied gene drive strategy itself can increase the probability of occurrence of an HGT event by affecting the mobility of its associated cargo/payload genes.

7.1.4.3 Pathogens, infections and diseases [Section 4.2.3, including subheadings]

The considerations/requirements given in Section 4.2.3 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion but could be made more specific on the information that is required to support risk assessment. Moreover, they should take into account the longer potential exposure arising with GDMIs. EFSA (2013) focusses on short-term effects arising from rearing processes and genetic insertions, and the effects of these in the immediate generations after release.

Section 4.2.3 of EFSA (2013) is relevant for disease vectors.

It is unlikely, following gene drive modification that species would become susceptible to new pathogens or symbionts as host-pathogen interactions are so complex. The close superimposition of phylogenetic trees of host-pathogen and host-symbiotic species supports this conclusion and indicates that individual genetic modifications are unlikely to modify the complex

molecular interactions that depend on the genetics of distinct organisms subjected to different selection forces.

Since GDMIs may operate at large scale and over a long term, the problem formulation should consider whether all diseases that can be transmitted by a vector should be taken into account or only the ones circulating in the particular receiving environment and when species relationship justify this possibility.

Different selective pressure is likely to be placed on the pathogen and its vector insect with some GDMIs; the selective pressure will be particularly high in replacement strategies due to long-term exposure which may impact pathogen-insect interactions. Risks will thus differ between GDMIs and GMI (for which there is no replacement at present). Long-term exposure may lead to the pathogen overcoming the gene drive. This is considered a new dimension when compared with GMIs.

For disease vectors a comparator system for a replacement strategy could be a widespread vaccine campaign that reduces disease transmission.

7.1.4.4 Interactions of genetically modified insects with target organisms [Section 4.2.4, including subheadings]

The considerations/requirements given in Section 4.2.4 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, but could be made more specific on the information that is required to support risk assessment.

As part of the problem formulation, it is critical to specify intended uses and mechanisms for gene drives, as stated in EFSA (2013). Target organisms may include a species complex or a set of partially reproductively connected species. The extent of the set of target organisms should be defined by the applicant in relation to the intended effects of a GDMI.

Wild type populations are expected to be genetically diverse, and so interactions between transgene and genetic background may be complex and difficult to predict. With GDMIs intended to spread over wide areas, this diversity of interactions is likely to be greater than anticipated in EFSA (2013) and this should be addressed explicitly.

Gene drives are expected to undergo unintended evolutionary responses from target organisms (Bull, 2015; Marshall et al., 2019). The likelihood that resistance will evolve in the target species in response to the gene drive will vary between different types of gene drives. In most cases, low resistance is desirable, unless resistance is part of a scheme to confine the gene drive to a smaller geographical area (Champer et al., 2016). It is important that the potential for resistance evolution is addressed so that resistance can be managed. In the case of synthetically engineered gene drives, the two main avenues of resistance evolution are: (1) resistance to the gene drive that slows or prevents its ability to be preferentially inherited (Burt, 2003; Sinkins and Gould, 2006; Ward et al., 2011); and (2) resistance against the cargo/payload genes themselves (Beaghton et al., 2017; Bull et al., 2019).

1. Resistance evolving to the gene drive is not addressed in EFSA (2013). For HEG-based gene drives, the mechanism of resistance is determined in large part by DNA repair

pathways activated by the endonuclease (Basu et al., 2015; Champer et al., 2017, 2018; Hammond et al., 2017; Marshall et al., 2017; Noble et al., 2017; Unckless et al., 2017; KaramiNejadRanjbar et al., 2018; Kyrou et al., 2018; Oberhofer et al., 2018). Such gene drives inherently rely on HDR pathways. However, alternative repair pathways such as NHEJ typically introduce mutations at the target site (Cong et al., 2013; Mali et al., 2013). Because of the sequence specificity of the nucleases, such mutations generally result in resistance to future cutting by the gene drive. Thus, the allele converts from a wild type to resistant allele if it undergoes repair by a pathway other than HDR. In instances where the gene drive allele is associated with a fitness cost, resistant alleles are expected to be positively selected, and therefore quickly impede the spread of the HEG-based gene drive in a population. Moreover, gene drive-resistant alleles are expected to exist in wild populations simply due to standing genetic variation (Drury et al., 2017; Unckless et al., 2017);

2. Resistance evolving to the cargo/payload genes is not specific to GDMIs and would be similar to deliberate releases of self-limiting GMIs such as RIDL or fsRIDL. Mechanisms involved would typically consist of modifying, inactivating or losing the cargo/payload genes altogether. The gene drive would remain operational, but would then drive an inefficient genetic load into the target population (Barrett et al., 2019).

It is relevant for both mechanisms of resistance to be addressed, distinguishing between the gene drive and cargo/payload genes. This may require knowledge of mutation rate and rate of gene drive failure. Depending on the gene drive strategy, resistance evolution to the gene drive and associated cargo/payload genes can be delayed by using multiplexed gRNA that target different target DNAs as resistance would require mutations at several target sites (e.g. Oberhofer et al., 2018; Champer et al., 2019), targeting ultra-conserved target genes (e.g. Burt, 2003; Champer et al., 2019b), or stacking multiple cargo/payload (inhibitory) genes in the same host individual (e.g. Ganz et al., 2015).

For suppression releases of GDMIs:

(a) Measurement endpoints should address size, density, age structure and sex ratio of the target population, but also the penetrance of the gene drive construct, in addition to the EFSA (2013) paragraph on endpoints. The gene drive itself can be used as an identifier to ensure that the modified individuals can be distinguished from the wild type target organism;

(b) Resurgence of an intrinsically harmful target organism due to gene drive failure or resistance to either the gene drive or its cargo/payload genes (for example, through assortative mating) could cause harm. Consequently, a consideration for the risk assessment could include the risk that the population developing from the released GDMI at some point has different effects on the target population than intended, for example due to loss of efficacy. Gene drive once released does not have a sustained quality control function, unlike continually reared and released systems. The nature of the target organism affects the type of harm – public health, invasive species, or pest outbreak (though the latter may have only economic harm, outside the scope of an environment and health risk assessment). There may be larger space and longer time

issues for the measurement endpoint of efficacy of releases. Defining efficacy, and hence its failure, may be difficult over the variable spatial and temporal dimensions relevant to some types of gene drive;

(c) An extreme result of narrow diversity in the deliberate release step for GDMIs is that assortative mating may occur. Measurement endpoints should address changes in interactions between released GDMIs and wild type populations over time and space. A meaningful description of the genetic history of a release colony of gene drives may be needed, with the original source diversity and the ensuing selection pressures during colony maintenance and production stages (after 50, 100 generations, etc, as relevant). The gene drive construct can be checked over generations, but it may not be clear what other aspects of the population genetics in a contained colony population may be changing due to selection, and what effects may result. This may be different with selection pressures operating on some gene drive mechanisms, for example where continual wild type backcrosses are or are not needed.

For permanent replacement releases of GDMIs:

(a) EFSA (2013) is adequate in relation to target organism population parameters, fitness and behaviour that may result in adverse effects;
(b) Reduction in efficacy may lead to harm to human health when controlling target organisms that are disease vectors and should be addressed in relation to the purpose of the gene drive;
(c) EFSA (2013) is adequate in relation to changes in interactions with the target organisms arising from an altered genetic diversity of a reared GMI population that may result in adverse effects;
(d) Relevant quality measures need to be determined.

For the assessment of effects on the target organism population the comparator should be related to the nature of the effect on the population or system and to a time dimension. So, for example, a conventional control system such as insecticide treated bed-nets could be a suitable comparator for a vector suppression GDMI for a period over which bed-nets are used. A drug treatment programme could be a suitable comparator for a replacement GDMI system.

Guidance should cover the release and subsequent self-sustaining generations, over increasing spatial range – not just the release generation. It needs to consider longer time periods and uncontrolled self-replication in the wild.

For both suppression or replacement GDMIs, the initial release number is most relevant when it may affect meeting a threshold for establishment, or the initial rate of spread or penetrance. The use of mathematical models and modelling scenarios of spread may support release rate decisions and suggest strategies for PMEM.

The terms "suppression" and "replacement" used in EFSA (2013) do not adequately cover the range of mechanisms and types of gene drive applications.

The conclusion to Section 4.2.4 (EFSA, 2013) is adequate.

7.1.4.5 Interactions of genetically modified insects with non-target organisms [Section 4.2.5, including subheadings]

The considerations given/requirements given in Section 4.2.5 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, but could be made more specific on the information that is required to support risk assessment. This is because EFSA (2013) lists potential impacts, rather than focussing on potential quantifiable harm to protection goals. The challenge is to distinguish between ecological change and harm to protection goals, in order to avoid disproportionate open-ended data collection exercises which do not shed light on environmental risks. The choice of comparator is critical here; for example, it may be appropriate to compare environmental risks to those that are already arising from current management systems, including the use of pesticides.

For replacement releases, the effects of replacement will depend on the intended traits that are being introduced. These may be different from any seen in GMIs to date.

7.1.4.6 Environmental impacts of the specific techniques used for the management of genetically modified insects [Section 4.2.6, including subheadings]

The considerations given/requirements given in Section 4.2.6 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, but could be made more specific on the information that is required to support risk assessment.

EFSA (2013) underlines the importance of comparing the impacts of management techniques associated with the release of the GMI, which again raises the importance of the selection of appropriate comparators. EFSA (2013) notes that the management techniques include the process of developing the GMI populations (e.g. the production of wastes) as well as management once released (e.g. changes to insecticide use). The importance of scale of the release is noted (Step 3). EFSA (2013) notes the value of analogous situations from insect vector/pest control and mathematical models for providing data. Gene drive operates over larger space and longer time. Risk characterisation based on modelled scenarios would be particularly appropriate for GDMIs. .

7.1.4.7 Impacts of GM animals on human and animal health [Section 4.2.7, including subheadings]

The considerations/requirements given in Section 4.2.7 of EFSA (2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, except those pertaining to the food/feed safety assessment of GMIs. The latter are only adequate if they specifically address: the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities.

The deliberate release into the environment of GDMIs considered in this GMO Panel Scientific Opinion is not intended for food/feed uses. Since ingestion or intake of GDMIs or parts of them by humans or livestock would be accidental, exposure is expected to be extremely low. Based on current knowledge, the GMO Panel is of the opinion that variations in the level of compound(s) in GMOs are generally not large enough to impact the nutritional or safety characteristics of an ingredient even under low exposure conditions (EFSA, 2017).

Consequently, a compositional analysis is not considered necessary for the GDMIs considered in this GMO Panel Scientific Opinion.

However, there may be plausible pathways to harm for humans in particular cases, e.g. blood-feeding mosquitoes through biting. This is particularly true for GDMIs that express antiparasitic or antiviral agents in the salivary glands.

In the case of replacement, the extended temporal dimension of GDMIs should be considered.

7.1.5 Post-market environmental monitoring [Section 5]

Several considerations/requirements on PMEM given in Section 5 of EFSA (2013) are inadequate for the GDMIs considered in this GMO Panel Scientific Opinion.

In line with Directive 2001/18/EC, EFSA (2013) explains the formal requirements for PMEM, but provides little specific information to guide the PMEM of GDMIs. More direction is needed to ensure that PMEM is fit-for-purpose and provides evidence that can feed back into the ERAs of future deliberate releases. This is particularly important due to the nature of possible GDMI applications. Moreover, the stepwise/staged/tiered-based testing approach, even if complemented by mathematical modelling, will still leave some uncertainty before open field testing or field implementation of a GDMI. Decisions to proceed to open field testing or to implementation will need to consider the extent of such uncertainty and potential mitigation options. This will include consideration of the scale and effectiveness of post-release monitoring, and consequently, more focus on PMEM is likely to be needed for GDMIs.

In addition, spatial and temporal scales will be greater with gene drive applications than other GMI applications, and reversibility may be an issue, depending on the nature of the gene drive. The point about the large-scale and long-term use is particularly relevant to gene drive because temporal/spatial scales are increased. Consequently, gene drive will have an evolving post-release phase over space and time.

Guidance should be practical. In particular, appropriate tools are needed to easily distinguish between wild type, GDMIs and hybrids (especially several generations after the release, as well as between wild type native and immigrants in a given area).

7.1.5.1 Case-specific monitoring [Section 5.1]

EFSA (2013) explains the basis of case-specific monitoring (CSM) correctly. However, the clear description of CSM is even more important for GDMIs than for other GMIs, as the potential impacts of the releases may not be time-constrained and any changes to the gene drive construct may require rapid management intervention.

CSM is used to confirm that any assumptions regarding the occurrence and impact of potential adverse effects of the GMI or its use characterised in the ERA are correct (EFSA, 2013). This would apply to GDMIs as to other GMI applications.

Monitoring is more important with gene drive applications than other GMI applications as the tiered phases of testing may not be fully achievable before final release in some cases. Post-release monitoring is the basis of any further management actions. Mathematical modelling will

be important as a design tool for sampling protocols to define expectations of intended outcomes, deviations, and responses. There should be clear triggers for management responses, based on modelling, for particular monitoring results/events. There is also a need to monitor changes in the challenge presented by the target organisms over time and space – due to changing conditions of climate, land use, immunity, pathogen load, pesticide resistance prevalence, etc. For GDMIs (compared to other GMIs), there is a strong and compelling case for mathematical modelling approaches, scenarios and sensitivity analyses to evaluate such changes.

Monitoring strategies may need to be organised in broad zones based on target organism challenges by location or season. Managers will need clear rules for action, with appropriate triggers for those actions. The likely scale of management will determine the scale of monitoring, both in space and time. The heterogeneity of penetrance could greatly affect the spatial scale of monitoring. Over time, patterns of population dynamics may indicate critical or less critical timing of monitoring.

The transboundary issues of monitoring and response need to be addressed, planned and resourced (Rabitz, 2019).

CSM is the basis of assessment of the success of the releases, and for any further management actions. There need to be clear triggers for responses by managers, based on mathematical modelling, for particular monitoring results/events. It may need to be dynamic and spatially explicit, tracking the evolving post-release phase over space and time, including areas beyond the expected range of the release, and possibly across national boundaries. The dynamics of GDMIs take place in a dynamic context, with changes in (e.g.) climate, land use, immunity, pathogen load, pesticide resistance prevalence. Therefore, CSM must explain both the approach to data acquisition and data interpretation.

CSM is likely to be adaptive in nature, focussing resources in the light of data. Evidence should be provided of the capacity to undertake adaptive, targeted monitoring that leads to management interventions. The capacity to undertake such interventions should be demonstrated, especially as much of the current development work is being undertaken by academic consortia of limited lifetimes: it must be clear which organisation will be liable to implement management responses, which may be required urgently should the gene drive break down and other forms of management have been stepped back, risking a resurgence of harm to human health in the case of vector control.

7.1.5.2 General surveillance [Section 5.2]

General surveillance (GS) as outlined in Section 5.2 of EFSA (2013) is too generic to be well suited to capture the potential environmental impacts of the GDMIs considered in this GMO Panel Scientific Opinion.

In light of Directive 2001/18/EC on the deliberate release into the environment of GMOs and the Commission Directive (EU) 2018/350 amending Directive 2001/18/EC, EFSA (2013) identifies that GS is required, and that the ERA should list the GS tools to be applied, including monitoring networks, literature reviews and questionnaires. Inevitably such GS is not specifically

targeted at particular indicators relevant to either assumptions in the ERA or to some particular harm to the environment. EFSA (2013) highlights challenges to GS, including the difficulty of detecting change, determining harm and associating change with the GMO. GS depends on the resources available for surveys in the receiving environment. With gene drive systems, the spatial and temporal scale of potential adverse environmental effects are likely to be much greater for self-sustaining systems than for self-limiting ones, and this will exacerbate the practical efficiency of GS in the longer term and at greater distances from a release. The ERA should specifically seek to identify the objectives and the efficiency of GS in a particular case, which may mean limiting its applicability to localised monitoring for a limited period after release, rather than expecting open-ended GS.

7.2 EFSA (2012)

The GDMIs considered in this GMO Panel Scientific Opinion are not intended to be deliberately released into the environment for food/feed uses. Thus, the evaluation of EFSA (2012) for its adequacy for the MC of GDMIs is tailored towards ERA needs. Besides the MC-related considerations/requirements given in Sections 2.1.1 and 2.1.2 of EFSA (2012), those laid down in Section II of Annex III A of Directive 2001/18/EC have been considered.

7.2.1 Information relating to the recipient or (where appropriate) parental animals [Section 2.1.1]

The considerations/requirements given in Section 2.1.1 of EFSA (2012) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion, except those that are explicitly tailored to the food/feed safety assessment of GMAs. The latter are only adequate if they specifically address: the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities (see Section 7.1.2.2).

The considerations/requirements given in Section 2.1.1 of EFSA (2012) are intended to support the risk assessment of food/feed containing, consisting of, or produced from GMAs, and thus are not tailored to the ERA of GMAs. Therefore, specific areas of further consideration for the ERA of GDMIs include: the assessment of persistence and invasiveness, and the potential for resistance evolution to the gene drive.

To assess the persistence and invasiveness potential of a GDMI (see Section 7.1.4.1), a thorough description and understanding of the biology of the target insect species (e.g. potential for interbreeding with other species, polymorphism in the population, vector competence, etc.) is required. This is consistent with the requirements outlined in Directive 2001/18/EC (e.g. organisms with which transfer of genetic material is known to occur under natural conditions, pathological, ecological and physiological traits, nature of indigenous vectors, etc).

To assess the potential for resistance to the gene drive to evolve, the following aspects can be considered, depending on the gene drive system:

- Possible occurrence of parthenogenetic individuals in the recipient insect that would escape sexual reproduction and thus the action of some gene drive systems;
- Possible polyploidy in the population that would have consequences on the number of targeted genes in the target insect population;
- Existence of polymorphisms in terms of sequence for the target gene(s) in the target insect population, rate of the presence of these "gene drive resistant" insects (see Section 7.1.4.1);
- Possible biased repair of the SDN-mediated DSBs via NHEJ rather than homologous recombination (HR). Relevant data on the general mechanism of repair of DSBs (NHEJ vs. HR ratio) in the target insect population could be informative (specific repair of the target sequence is addressed in Section 7.2.2.2).

7.2.2 Molecular characterisation [Section 2.1.2]

The considerations/requirements given in Section 2.1.2 of EFSA (2012) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. The information on the genetic modification will enable the identification of the nucleic acid intended for transformation and related vector sequences potentially delivered to the recipient insect, and the characterisation of the DNA actually inserted in the GDMI including its expression and genetic stability. Although not mentioned in Section 2.1.2 of EFSA (2012), it is important that the nature and mechanism of the gene drive system are clearly described.

7.2.2.1 Information relating to the genetic modification [Section 2.1.2.1]

Description of the methods and vectors used for the genetic modification [Section 2.1.2.1.1]
The considerations/requirements given in Section 2.1.2.1.1 of EFSA (2012) and Section II B of Annex III A of Directive 2001/18/EC are adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Source and characterisation of nucleic acid intended to be inserted [Section 2.1.2.1.2]
The considerations/requirements given in Section 2.1.2.1.2 of EFSA (2012) and Section II C.1 of Annex III A of Directive 2001/18/EC are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, those that are explicitly tailored to the food/feed safety assessment of GMAs (i.e. information on the history of consumption of the gene product(s) arising from the regions intended for insertion, and data on the possible relationship of the gene products with known toxins, anti-nutrients, allergens and other compounds with potential adverse health effects) are only relevant in conjunction with the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities.

Specific areas of further consideration for the ERA of GDMIs to characterise the GDMI include:

- Information on the gene drive system and its design covering both the underlying mechanisms involved (e.g. CRISPR-Cas9) and their (multiple) components (e.g. Cas9 protein and sgRNA);
- The assessment of the stability and specificity of the expression of the gene drive system;

- Information on any cargo/payload gene(s) linked to the gene drive, and their function;
- Information on the molecular approaches used to detect and follow the intended and unintended spread, establishment and persistence of the gene drive in interbreeding populations.

7.2.2.2 Information relating to the genetically modified animal [Section 2.1.2.2]

General description of the trait(s) and characteristics introduced or modified [Section 2.1.2.2.1]
The considerations/requirements given in Section 2.1.2.2.1 of EFSA (2012) and Section II C.2 of Annex III A of Directive 2001/18/EC are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion.

Specific areas of further consideration for the ERA of GDMIs to characterise the introduced/modified traits/characteristics include:

- Information on the target sequence (including any available information on the polymorphism in the population targeted);
- Information on the nature of the target sequence (e.g. within a conserved domain of a particular protein);
- Information on the ratio of NHEJ versus HR repair resulting from the cleaving of the targeted sequence(s);
- The characterisation of the NHEJ repair step following the cleaving of the targeted sequence (e.g. whether the targeted gene remains functional);
- The pre-existence of resistance alleles to the cargo/payload genes in the target population;
- Information on the possible occurrence of resistance alleles to the gene drive itself;
- Information on the size of the homologous sequences used for homing;
- Information on single/multiple target sites (within the same gene or in multiple genes);
- Cleave 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 [Section 2.1.2.2.2]
The considerations/requirements given in Section 2.1.2.2.2 of EFSA (2012) and Section II C.2 of Annex III A of Directive 2001/18/EC are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, those pertaining to the food/feed safety assessment of GMAs are only relevant in conjunction with the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities.

The need for bioinformatic analyses of open reading frames (ORFs) present within the insert and spanning the junctions to investigate possible similarities with known toxins or allergens, in

order to inform the ERA of GDMIs, will depend on the intended outcome of the gene drive strategy used (see Section 7.1.4.5).

For SDN-based gene drives a possible cause for unintended sequence modifications in GDMIs is off-target activity of the gene drive (e.g. Sander and Joung, 2014; Taning et al., 2017). Any sequence changes in the genome of the target population induced by off-target activity of the gene drive would be less than those occurring with most mutagenesis techniques (e.g. irradiation used for the sterilisation of male mosquitoes). Furthermore, where such changes occur, they would be of the same nature as spontaneous mutations. Taking these characteristics into consideration and the fact that GDMIs considered in this GMO Panel Scientific Opinion are not intended for food/feed uses, the likelihood for off-target effects in the GDMI raising significant concerns for additional risks is likely to be low. Consequently, information supporting the assessment of possible off-targets in GDMIs (e.g. *in silico* approaches to predict off-targets) may be needed on a case-by-case basis only.

Information on the expression of the inserted/modified sequence [Section 2.1.2.2.3]
The considerations/requirements given in Section 2.1.2.2.3 of EFSA (2012) and Section II C.2 of Annex III A od Directive 2001/18/EC are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, those pertaining to the food/feed safety assessment of GMIs are only relevant in conjunction with the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities.

The use of information on the expression of the inserted/modified sequences to inform the ERA of GDMIs will depend on the intended outcome of the gene drive strategy used. Information on the expression of the inserted sequences can inform the ERA as regards the potential impact on other organisms (e.g. toxicity on non-target organisms), or on the level of nuisance caused by the modified insect (e.g. allergenicity due to mosquito bites) (Sections 7.1.4.4 and 7.1.4.5). Therefore, the level and site of expression of the gene drive system components (e.g. Cas9 and sgRNA(s)) and the cargo/payload genes linked to the gene drive (if any) can be informative. Information on the expression of the modified sequences (gene(s) situated in the vicinity of the gene drive cassette insertion locus or gene(s) targeted by the gene drive) can also inform the assessment of the potential impact on other organisms (e.g. non-target organisms). For gene drive systems that are designed to achieve the desired trait through multiple interactions (see section below) additional information might 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 [Section 2.1.2.2.4]
Several considerations/requirements given in Section 2.1.2.2.4 of EFSA (2012) and Section II C.2 of Annex III A of Directive 2001/18/EC are not adequate for the GDMIs considered in this GMO Panel Scientific Opinion. In particular, due to the super-Mendelian inheritance of gene drives and linked cargo/payload gene(s), the concepts of inheritance and genetic and phenotypic stability as outlined in Section 2.1.2.2.4 of EFSA (2012) need further consideration to address the broad array of possible GDMI applications and their intended

outcomes. For example, phenotypic stability of a suppression gene drive will be linked to reduced fitness (leading to mortality) of the individuals bearing the gene drive module, whereas for replacement drives the phenotypic stability will be linked to the trait(s) conferred by the cargo/payload gene(s). In addition, some gene drive systems can be designed to target multiple genes and the products of those genes themselves may interact to produce the desired trait. In some cases, genetic elements can be segregated out intentionally as part of the gene drive strategy (e.g. daisy-chain strategy). These features will complexify the definition of genetic and phenotypic stability as stated in EFSA (2012) and can also challenge the concept of "transformation event" as currently implemented for GMOs.

7.2.2.3 Conclusions of the molecular characterisation [Section 2.1.2.3]

The considerations/requirements given in Sections 2.1.1 and 2.1.2 of EFSA (2012) and laid down in Section II of Annex III A of Directive 2001/18/EC are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, those pertaining to the food/feed safety assessment of GMAs are only relevant in conjunction with the accidental ingestion or intake of GMAs or parts of them by humans or livestock, or exposure of persons to the GMA and derived material as part of their professional activities.

Specific MC-related areas of further consideration for the ERA of GDMIs include:

- The MC of the gene drive system, including the underlying mechanisms and their aim;
- Proof of the efficiency, stability and inheritance (as defined for GDMIs) of the gene drive system;
- An assessment of possible interactions between the multiple gene drive components, if the gene drive construct is composed of multiple elements that can segregate out intentionally as part of the gene drive strategy.

8 Conclusions

The GMO Panel considers it both timely and appropriate to evaluate its existing risk assessment guidelines for their adequacy for the MC and ERA of gene drive modified disease-spreading mosquitoes and agricultural insect pests for deliberate release into the environment.

It is timely because:

- The practical application of gene drive mechanisms in disease-spreading mosquitoes and agricultural pests is close to deliberate release into the environment, though not necessarily in the EU;
- International discussions on the risk assessment and regulatory oversight of GDMOs are on-going under the Convention on Biological Diversity and the Cartagena Protocol on Biosafety.

It is appropriate because:

- The current EFSA (2012, 2013) guidelines were generic across all GMAs, and guidance more focused to GDMIs can be more specific, making it more relevant and efficient for

risk assessors, risk managers and applicants to collect, assess and act on the required information/data in a timely and proportionate manner;

- The scientific understanding of gene drives has advanced greatly in recent years, and we are, therefore, more able to provide case-specific considerations relevant to the MC and ERA of GDMIs than in the past.

The conclusions below are organised according to the five main points of the mandate from the European Commission: (1) role of problem formulation; (2) potential for novel hazards/risks on human and animal health and the environment; (3) relevant comparators; (4) adequacy of existing EFSA guidelines for risk assessment; and (5) need for updated guidance in specific areas.

Although the scope of this GMO Panel Scientific Opinion focuses on the use of synthetically engineered gene drives to control harmful insects such as disease-transmitting mosquitoes and agricultural pests, some of its principles would be applicable to the potential use of synthetically engineered gene drives for biodiversity conservation or the enhancement of agricultural production systems.

8.1 Role of problem formulation for the environmental risk assessment of gene drive modified insects for deliberate release into the environment

- As with any technology, true understanding of the potential risks to human/animal health and the environment should be informed by a case-specific risk assessment that is framed by relevant protection goals, not only a generalised view of the technology. Evaluating harm will vary depending on the specifics of the gene drive design and strategy, the GDMI release, the receiving environments, and the spatial and/or temporal scale;
- Robust ERAs should begin with an explicit problem formulation where protection goals, plausible and relevant exposure scenarios and the potential adverse effects from those exposures are identified on a case-by-case basis. Risk can then be characterised by testing specific hypotheses about the probability that harm will occur and the severity of that harm if it occurs;
- Enhanced dialogue between risk assessors and risk managers along with stakeholder/societal engagement is required to define protection goals, decision-making criteria and the identification of pathways to harm for the ERA of GDMIs;
- The following aspects require specific consideration as part of the problem formulation process of GDMIs:
    - The description of the mechanisms and objectives for GDMI applications and the stability of the gene drive, as they are important components in assessing likely levels of exposure in space and time;
    - The specification of possible interactions between the multiple gene drive components, if the gene drive construct is composed of multiple elements that can segregate out intentionally as part of the gene drive strategy;

- Due weight should be given to ecological processes (such as trophic interactions, density dependence, competition, niche replacement, assortative mating, etc.) to frame the ERA of gene drive-based vector/pest control;
- There should be greater specification of the receiving environment, especially if there are likely to be dynamic management responses to population suppression and replacement that will have environmental impacts (e.g. reduction in pesticide applications);
- The distinction between "harm" and "efficacy" should be addressed in more detail, as well as the definition of target organism and populations, as they could apply to a wider species complex of populations that have varying degrees of reproductive isolation. As a result, intended effects would be different across the spectrum of such a complex;
- The deliberate release of any GDMI should be compared to a range of comparators (including alternative solutions) to allow harms to be appropriately quantified.

8.2 Potential novel hazards/risks associated with gene drive modified disease-spreading mosquitoes and agricultural pests

- Similar forms of environmental harm are anticipated from the deliberate release into the environment of GDMIs that have been encountered before, whether from the use of non-GDMIs or other existing insect vector/pest control strategies;
  - The most direct impact of GDMIs aimed at suppression will be the reduction of the target pest organism population, with an effect that is expected to be similar to the target population reduction effect of conventional insect vector/pest management;
  - GDMIs aimed at population replacement are not intended to have a direct impact on target population density;
  - The levels of environmental exposure are potentially high for self-sustaining gene drives for population replacement, because they are not constrained in time or in space. For self-sustaining gene drives for population suppression exposure is expected to diminish over time, but would increase over space.

8.3 Relevant comparators

- The concept of comparators could be further extended to include the range of gene drive applications in insects and put more emphasis on the purpose of the risk assessment-related studies conducted;
- Depending on the case, relevant comparators could be the unmodified target organism with similar or different genetic background as that of the GDMI and other insect vector/pest control systems (e.g. bed-nets, pesticide use, biological pest management, drug-interventions) to enable comparisons at both the organismal and (management) systems level.

8.4 Adequacy and sufficiency of existing guidelines for the molecular characterisation and environmental risk assessment of gene drive modified disease-spreading mosquitoes and agricultural pests

- The risk assessment approach for GDMIs can build on the existing comparative risk assessment paradigm for GMOs, which follows the case-by-case principle and an iterative, stepwise/staged/tiered-based testing approach, and which considers different lines of evidence in a weight of evidence approach;
- The considerations/requirements given in EFSA (2012, 2013) are broadly adequate for the GDMIs considered in this GMO Panel Scientific Opinion. However, the following aspects require further consideration in terms of the adequacy of the guidelines:
    - Part of the MC-related considerations/requirements given in EFSA (2012) is designed to support the risk assessment of food/feed containing, consisting of, or produced from GMAs, and thus not necessarily tailored to the ERA needs of GMIs, including gene drive modified ones, that are not intended for food/feed uses. Although those considerations/requirements are adequate their applicability/relevance should be assessed on a case-by-case as part of the problem formulation process, like any other adequate consideration/requirement;
    - The assessment of persistence and invasiveness focuses on the fitness of the individuals carrying a transgene and does not sufficiently address the inheritance of the selfish genetic element and its effect at the population level;
    - The stepwise/staged/tiered-based testing approach may leave some uncertainty before open field testing or field implementation of a GDMI, as it may be challenging to collect meaningful data from experimental systems that would be applicable to populations at the ecosystem scale where the gene drive construct is designed to function. This makes the use of mathematical modelling and the design and conduct of PMEM particularly important;
        - More extensive use of mathematical models may be needed to address the long temporal scale and wide spatial scale of many GDMI applications. ERAs will need to rely on modelled systems to describe expected outcomes;
        - Monitoring GDMIs will pose practical challenges and the design and interpretation of monitoring schemes will depend heavily on models of expected outcomes;
- Some aspects of EFSA (2012, 2013) do not adequately define the case-specific information that is required to support risk assessment. This can be addressed in the problem formulation process and through the use of examples.

8.5 Specific areas where updated guidance is needed

Specific areas where updated guidance is needed include:

- Since some of the MC-related considerations/requirements given in EFSA (2012) are not necessarily tailored to the ERA needs of GDMIs, additional ones may be required that account for the potential novel characteristics of particular cases;

- The concepts of inheritance, genetic and phenotypic stability, and persistence and invasiveness need further consideration due to the modified inheritance pattern of GDMIs;
- The greater use of mathematical modelling to address the long temporal scale and wide spatial scale of many GDMI applications requires guidance on model design, quality assurance, interpretation and validation;
- Further guidance will be required on the design, conduct and interpretation of CSM to ensure that the data add to our understanding of large scale and long term processes. Moreover, further consideration is needed for the design and implementation of GS to identify potential unanticipated adverse effects in a proportionate manner.

9 Documentation as provided to EFSA

- Request for an EFSA opinion on genetically modified organisms engineered with gene drives. June 2018. Submitted by the European Commission (Directorate-General for Health and Food Safety);
- Acknowledgement of receipt of the mandate. August 2018. Submitted by the European Food Safety Authority;
- Reception of the mandate. October 2018. Submitted by the European Food Safety Authority;
- Acknowledgement of receipt of EFSA's reception letter of the mandate. November 2018. Submitted by the European Commission (Directorate-General for Health and Food Safety).

10 References

AAS (Australian Academy of Sciences), 2017. Synthetic gene drives in Australia: implications of emerging technologies. Canberra. Available from: https://www.science.org.au/support/analysis/reports/synthetic-gene-drives-australia-implications-emerging-technologies

Adelman ZN, Pledger D and Myles KM, 2017a. Developing standard operating procedures for gene drive research in disease vector mosquitoes. Pathogens and Global Health, 111,436–447.

Adelman Z, Akbari O, Bauer J, Bier E, Bloss C, Carter SR, Callender C, Denis A C-S, Cowhey P, Dass B, Delborne J, Devereaux M, Ellsworth P, Friedman RM, Gantz V, Gibson C, Hay BA, Hoddle M, James AA, James S, Jorgenson L, Kalichman M, Marshall J, McGinnis W, Newman J, Pearson A, Quemada H, Rudenko L, Shelton A, Vinetz JM, Weisman J, Wong B and Wozniak C, 2017b. Rules of the road for insect gene drive research and testing. Nature Biotechnology, 35, 716–718.

Ågren JA and Clark AG, 2018. Selfish genetic elements. PLoS Genetics, 14, e1007700.

Akbari OS, Matzen KD, Marshall JM, Huang H, Ward CM and Hay BA, 2013. A synthetic gene drive system for local, reversible modification and suppression of insect populations. Current Biology, 23, 671–677.

Akbari OS, Chen CH, Marshall JM, Huang H, Antoshechkin I and Hay BA, 2014. Novel synthetic Medea selfish genetic elements drive population replacement in *Drosophila*; a theoretical exploration of Medea-dependent population suppression. ACS Synthetic Biology, 3, 915–928.

Akbari OS, Bellen HJ, Bier E, Bullock SL, Burt A, Church GM, Cook KR, Duchek P, Edwards OR, Esvelt KM, Gantz VM, Golic KG, Gratz SJ, Harrison MM, Hayes KR, James AA, Kaufman TC, Knoblich J, Malik HS, Matthews KA, O'Connor-Giles KM, Parks AL, Perrimon N. Port F, Russell S, Ueda R and Wildonger J, 2015. Safeguarding gene drive experiments in the laboratory. Science, 349, 927–929.

Alphey L, 2014. Genetic control of mosquitoes. Annual Review of Entomology, 59, 205–224.

Alphey L, 2016. Can CRISPR-Cas9 gene drives curb malaria? Nature Biotechnology, 34, 149–150.

Alphey L and Alphey N, 2014. Five things to know about genetically modified (GM) insects for vector control. PLoS Pathogens, 10, e1003909.

Alphey L and Beech C, 2012. Appropriate regulation of GM insects. PLoS Neglected Tropical Diseases, 6, e1496.

Alphey N and Bonsall MB, 2014. Interplay of population genetics and dynamics in the genetic control of mosquitoes. Journal of the Royal Society Interface, 11, 20131071.

Alphey N and Bonsall MB, 2018. Genetics-based methods for agricultural insect pest management. Agricultural and Forest Entomology, 20, 131–140.

Alphey L, Benedict M, Bellini R, Clark GG, Dame DA, Service MW and Dobson SL, 2010. Sterile-insect methods for control of mosquito-borne diseases: an analysis. Vector Borne Zoonotic Diseases, 10, 295–311.

Alphey L, McKemey A, Nimmo D, Neira Oviedo M, Lacroix R, Matzen K and Beech C, 2013. Genetic control of *Aedes* mosquitoes. Pathogens and Global Health, 107, 170-179.

Altrock PM, Traulsen A, Reeves RG and Reed FA, 2010. Using underdominance to bi-stably transform local populations. Journal of Theoretical Biology, 267, 62–75.

Amuzu HE, Tsyganov K, Koh C, Herbert RI, Powell DR and McGraw EA, 2018. *Wolbachia* enhances insect-specific flavivirus infection in *Aedes aegypti* mosquitoes. Ecology & Evolution, 8, 5441–5454.

Anderson ME, Mavica J, Shackleford L, Flis I, Fochler S, Basu S and Alphey L, 2019. CRISPR/Cas9 gene editing in the West Nile Virus vector, *Culex quinquefasciatus* Say. PLoS ONE, 14, e0224857.Ant T, Koukidou M, Rempoulakis P, Gong H, Economopoulos A, Vontas J and Alphey L, 2012. Control of the olive fruit fly using genetics-enhanced sterile insect technique. BMC Biology, 10, 51.

2362 Armbruster PA, 2019. Tiger mosquitoes tackled in a trial. Nature, 572, 39–40.

2363 Asadi R, Elaini R, Lacroix R, Ant T, Collado A, Finnegan L, Siciliano P, Mazih A and Koukidou M,
2364 2019. Preventative releases of self-limiting *Ceratitis capitate* provide pest suppression and
2365 protect fruit quality in outdoor netted cages. International Journal of Pest Management,
2366 doi:10.1080/09670874.2019.1601293

2367 Atyame CM, Cattel J, Lebon C, Flores O, Dehecq JS, Weill M, Gouagna LC and Tortosa P, 2015.
2368 *Wolbachia*-based population control strategy targeting *Culex quinquefasciatus* mosquitoes
2369 proves efficient under semi-field conditions. PLoS ONE, 10, e0119288.

2370 Atyame CM, Labbé P, Lebon C, Weill M, Moretti R, Marini F, Gouagna LC, Calvitti M and Tortosa
2371 P, 2016. Comparison of irradiation and *Wolbachia* based approaches for sterile-male strategies
2372 targeting *Aedes albopictus*. PLoS ONE, 11, e0146834.

2373 Backus GA and Delborne JA, 2019. Threshold-dependent gene drives in the wild: spread,
2374 controllability, and ecological uncertainty. BioScience, 69, 900–907.

2375 Barclay HJ, 1982. The sterile release method with unequal male competitive ability. Ecological
2376 Modelling, 15, 251–263.

2377 Barnhill-Dilling SK, Serr M, Blondel DV and Godwin J, 2019. Sustainability as a framework for
2378 considering gene drive mice for invasive rodent eradication. Sustainability, 11, 1334.

2379 Barrett LG, Legros M, Kumaran N, Glassop D, Raghu S and Gardiner DM, 2019 Gene drives in
2380 plants: opportunities and challenges for weed control and engineered resilience. Proceedings of
2381 the Royal Society B: Biological Sciences, 286, 20191515.

2382 Bartumeus F, Costa GB, Eritja R, Kelly AH, Finda M, Lezaun J, Okumu F, Megan Quinlan M,
2383 Thizy DC, Paré Toé L and Vaughan M, 2019. Sustainable innovation in vector control requires
2384 strong partnerships with communities. PLoS Neglected Tropical Diseases, 13, e0007204.

2385 Basu S, Aryan A, Overcash JM, Samuel GH, Anderson MAE, Dahlem TJ, Myles KM and Adelman
2386 ZN, 2015. Silencing of end-joining repair for efficient site-specific gene insertion after
2387 TALEN/CRISPR mutagenesis in *Aedes aegypti*. Proceedings of the National Academy of Sciences
2388 of the United States of America, 112, 4038–4043.

2389 Beaghton A, Beaghton PJ and Burt A, 2016. Gene drive through a landscape: reaction–diffusion
2390 models of population suppression and elimination by a sex ratio distorter. Theoretical Population
2391 Biology, 108, 51–69.

2392 Beaghton A, Beaghton PJ and Burt A, 2017a. Vector control with driving Y chromosomes:
2393 modelling the evolution of resistance. Malaria Journal, 16, 286.

2394 Beaghton A, Hammond A, Nolan T, Crisanti A, Godfray HCJ and Burt A, 2017b. Requirements
2395 for driving antipathogen effector genes into populations of disease vectors by homing. Genetics,
2396 205, 1587−1596.

Beaghton AK, Hammond A, Nolan T, Crisanti A and Burt A, 2019. Gene drive for population genetic control: non-functional resistance and parental effects. Proceedings of the Royal Society B: Biological Sciences, 286; 20191586.

Beech CJ, Vasan SS, Quinlan MM, Capurro ML, Alphey L, Bayard V, Bouaré M, McLeod MC, Kittayapong P, Lavery JV, Lim LH, Marrelli MT, Nagaraju J, Ombongi K, Othman RY, Pillai V, Ramsey J, Reuben R, Rose RI, Tyagi BK and Mumford J, 2009. Deployment of innovative genetic vector control strategies: progress on regulatory and biosafety aspects, capacity building and development of best-practice guidance. Asia-Pacific Journal of Molecular Biology and Biotechnology, 17, 75–85.

Beech CJ, Koukidou M, NI Morrison and Alphey L, 2012. Genetically modified insects: science, use, status and regulation. Collection of Biosafety Reviews, 6, 66–124.

Beeman RW, Friesen KS and Denell RE, 1992. Maternal-effect selfish gene in flour beetles. Science, 256, 89–92.

Begon M, Townsend CR and Harper JL, 2005. Ecology: from individuals to ecosystems. Wiley-Blackwell, Oxford.

Benedict M, Eckerstorfer M, Franz G, Gaugitsch H, Greiter A, Heissenberger A, Knols B, Kumschick S, Nentwig W and Rabitsch W, 2010. Defining environment risk assessment criteria for genetically modified insects to be placed on the EU market. EFSA Supporting Publication, 7, EN-71, 1–200.

Benedict MQ, Burt A, Capurro ML, De Barro P, Handler AM, Hayes KR, Marshall JM, Tabachnick WJ and Adelman ZN, 2018. Recommendations for laboratory containment and management of gene drive systems in arthropods. Vector-Borne Zoonotic Diseases, 18, 2–13.

Bernardini F, Galizi R, Menichelli M, Papathanos P-A, Dritsou V, Marois E, Crisanti A and Windbichler N, 2014. Site-specific genetic engineering of the *Anopheles gambiae* Y chromosome. Proceedings of the National Academy of Sciences, 111, 7600–7605.

Blagrove MSC, Arias-Goeta C, Failloux A-B and Sinkins SP, 2012. *Wolbachia* strain *w*Mel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. Proceedings of the National Academy of Sciences, 109, 255–260.

Bolton M, Collins HL, Chapman T, Morrison NI, Long SJ, Linn CE and Shelton AM, 2019. Response to a synthetic pheromone source by OX4319L, a self-limiting diamondback moth (Lepidoptera: Plutellidae) strain, and field dispersal characteristics of its progenitor strain. Journal of Economic Entomology, 112, 1546–1551.

Bonsall MB, Yakob L, Alphey N and Alphey L, 2010. Transgenic control of vectors: the effects of interspecific interactions. Israel Journal of Ecology and Evolution, 56, 353–370.

Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M, Moreira LA, Bossi HC, Moretti R, Baton LA, Hughes GL, Mavingui P, and Gilles JRL, 2014. Harnessing mosquito-*Wolbachia* symbiosis for vector and disease control. Acta Tropica, 132, S150–S163.

Brossard D, Belluck P, Gould F and Wirz CD, 2019. Promises and perils of gene drives: navigating the communication of complex, post-normal science. Proceedings of the National Academy of Sciences, 116, 7692–7697.

Brown Z, 2017. Economic, regulatory and international implications of gene drives in agriculture. Choices, 32, 1–8.

Brown ZS, Jones M and Mumford JD, 2019. Economic principles and concepts in area-wide genetic pest management. In: Onstad D and Crain P (eds). The Economics of Integrated Pest Management of Insects. CABI, Wallingford, UK. pp. 96–121.

Buchman AB, Ivy T, Marshall JM, Akbari O and Hay BA, 2018a. Engineered reciprocal chromosome translocations drive high threshold, reversible population replacement in *Drosophila*. ACS Synthetic Biology, 7, 1359–1370.

Buchman A, Marshall JM, Ostrovski D, Yang T and Akbari OS, 2018b. Synthetically engineered *Medea* gene drive system in the worldwide crop pest *Drosophila suzukii*. Proceedings of the National Academy of Sciences of the United States of America, 115, 4725–4730.

Buchman A, Gamez S, Li M, Antoshechkin I, Lic H-H, Wang H-W, Chen C-H, Klein MJ, Duchemin J-B, Paradkar PN and Akbari OS, 2019. Engineered resistance to Zika virus in transgenic *Aedes aegypti* expressing a polycistronic cluster of synthetic small RNAs. Proceedings of the National Academy of Sciences of the United States of America, 116, 3656–3661.

Buchman A, Gamez S, Li M, Antoshechkin I, Lee S-H, Wang S-W, Chen C-H, Klein MJ, Duchemin J-B, Crowe JE, Paradkar PN and Akbari O, 2020. Broad dengue neutralization in mosquitoes expressing an engineered antibody. PLoS Pathogens, 16, e1008103.

Buchthal J, Evans SW, Lunshof J, Telford III SR and Esvelt KM, 2019 Mice against ticks: an experimental community-guided effort to prevent tick-borne disease by altering the shared environment. Philosophical Transactions of the Royal Society B: Biological Sciences, 374, 20180105.

Bull JJ, 2015. Evolutionary decay and the prospects for long-term disease intervention using engineered insect vectors. Evolution, Medicine and Public Health, 2015, 152–166.

Bull JJ, Remien CH, Gomulkiewicz R and Krone SM, 2019. Spatial structure undermines parasite suppression by gene drive cargo. PeerJ, 7, e7921.

Burgess MM, Mumford JD and Lavery JV, 2018. Public engagement pathways for emerging GM insect technologies. BMC Proceedings, 12, 12.

Burt A, 2003. Site-specific selfish genes as tools for the control and genetic engineering of natural populations. Proceedings of the Royal Society B: Biological Sciences, 270, 921–928.

Burt A, 2014. Heritable strategies for controlling insect vectors of disease. Philosophical Transactions of the Royal Society B: Biological Sciences, 369, 20130432.

Burt A and Crisanti A, 2018. Gene drive: evolved and synthetic. ACS Chemical Biology, 13, 343–346.

Burt A and Deredec A, 2018. Self-limiting population genetic control with sex-linked genome editors. Proceedings of the Royal Society B: Biological Sciences, 285, 20180776.

Burt A and Trivers RL, 2006. Genes in Conflict. Belknap Press of Harvard University Press, Boston, MA.

Burt A, Coulibaly M, Crisanti A, Diabate A and Kayondo JK, 2018. Gene drive to reduce malaria transmission in sub-Saharan Africa. Journal of Responsible Innovation, 5, S66–S80.

Callaway E, 2016. 'Gene drive' moratorium shot down at UN biodiversity meeting. Nature Biotechnology, doi:10.1038/nature.2016.21216

Callaway E, 2018. Ban on 'gene drives' is back on the UN's agenda — worrying scientists. Nature, 563, 454–455.

Callaway E, 2019. Modified mosquitoes reduce cases of dengue fever. Nature, doi:10.1038/d41586-019-03660-8

Caplan AL, Parent B, Shen M and Plunkett C, 2015. No time to waste-the ethical challenges created by CRISPR. EMBO Reports, 16, 1421–1426.

Carballar-Lejarazú R and James AA, 2017. Population modification of Anopheline species to control malaria transmission. Pathogens and Global Health, 111, 424–435.

Carvalho DO, McKemey AR, Garziera L, Lacroix R, Donnelly CA, Alphey L, Malavasi A and Capurro ML, 2015. Suppression of a field population of *Aedes aegypti* in Brazil by sustained release of transgenic male mosquitoes. PLoS Neglected Tropical Diseases, 9, e0003864.

Cash SA, Lorenzen MD and Gould F, 2019a. The distribution and spread of naturally occurring *Medea* selfish genetic elements in the United States. Ecology and Evolution, 9, 14407–14416.

Cash SA, Robert MA, Lorenzen MD and Gould F, 2019b. The impact of local population genetic background on the spread of the selfish element *Medea-1* in red flour beetles. Ecology and Evolution, 00, 1–12. doi:10.1002/ece3.5946

Cha S-J, Mori A, Chadee DD and Severson DW, 2006. Cage trials using an endogenous meiotic drive gene in the mosquito *Aedes aegypti* to promote population replacement. American Journal of Tropical Medicine and Hygiene, 74, 62–68.

Chambers EW, Hapairai L, Peel BA, Bossin H and Dobson SL, 2011. Male mating competitiveness of a *Wolbachia*-introgressed *Aedes polynesiensis* strain under semi-field conditions. PLoS Neglected Tropical Diseases, 5, e1271.

Champer J, Buchman A and Akbari OS, 2016. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. Nature Reviews Genetics, 17, 146–159.

Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG and Messer PW, 2017. Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLOS Genetics, 13, e1006796.

Champer J, Liu J, Oh SY, Reeves R, Luthra A, Oakes N, Clark AG and Messer PW, 2018. Reducing resistance allele formation in CRISPR gene drive. Proceedings of the National Academy of Sciences of the United States of America, 115, 5522–5527.

Champer J, Lee YL, Yang E, Liu C, Clark AG and Messer PW, 2019a. A toxin-antidote CRISPR gene drive system for regional population modification. bioRxiv. doi:https://doi.org/10.1101/628354

Champer J, Yang E, Lee YL, Liu J, Clark AG and Messer PW, 2019b. Resistance is futile: a CRISPR homing gene drive targeting a haplolethal gene. bioRxiv. doi:https://doi.org/10.1101/651737

Champer SE, Oh SY, Liu C, Wen Z, Clark AG, Messer PW and Champer J, 2019. Computational and experimental performance of CRISPR homing gene drive strategies with multiplexed gRNAs. bioRxiv. doi:https://doi.org/10.1101/679902

Chan YS, Naujoks DA, Huen DS and Russell S, 2011. Insect population control by homing endonuclease-based gene drive: an evaluation in *Drosophila melanogaster*. Genetics, 188, 33–44.

Chan YS, Huen DS, Glauert R, Whiteway E and Russell S, 2013a. Optimising homing endonuclease gene drive performance in a semi-refractory species: the *Drosophila melanogaster* experience. PLoS ONE, 8, e54130.

Chan YS, Takeuchi R, Jarjour J, Huen DS, Stoddard BL and Russell S, 2013b. The design and in vivo evaluation of engineered I-OnuI-based enzymes for HEG gene drive. PLoS ONE, 8, e74254.

Chen CH, Huang H, Ward CM, Su JT, Schaeffer LV, Guo M and Hay BA, 2007. A synthetic maternal-effect elfish genetic element drives population replacement in *Drosophila*. Science, 316, 597–600.

Cisnetto V and Barlow J, 2020. The development of complex and controversial innovations. Genetically modified mosquitoes for malaria eradication. Research Policy, 49, 103917.

Collins JP, 2018. Gene drives in our future: challenges of and opportunities for using a self-sustaining technology in pest and vector management. BMC proceedings, 12, 9.

Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA and Zhang F, 2013. Multiplex genome engineering using CRISPR/Cas systems. Science, 339, 819–823.

Conklin BR, 2019. On the road to a gene drive in mammals. Nature, 66, 43–45.

Courtier-Orgogozo V, Morizot B and Boëte C, 2017. Agricultural pest control with CRISPR-based gene drive: time for public debate: should we use gene drive for pest control? EMBO Reports, 18, 878–880.

Courtier-Orgogozo V, Danchin A, Gouyon P-H and Boëte C, 2019. Evaluating the probability of CRISPR-based gene drive contaminating another species. bioRxiv. doi:https://doi.org/10.1101/776609

2543 Courret C, Chang C-H, Wei KH-C, Montchamp-Moreau C and Larracuente AM, 2019. Meiotic
2544 drive mechanisms: lessons from *Drosophila*. Proceedings of the Royal Society: Biological
2545 Sciences, 286, 20191430.

2546 Craig W, Ndolo DO and Tepfer M, 2017. A Strategy for integrating science into regulatory
2547 decision-making for GMOs. In: Adenle AA, Morris EJ and Murphy DJ (eds.) Genetically Modified
2548 Organisms in Developing Countries: Risk Analysis and Governance. 1st Edition. Cambridge
2549 University Press, Cambridge, UK. pp. 26–38.

2550 Cressie N and Wikle CK, 2011. Statistics for spatiotemporal data. Wiley.

2551 Criscione F, Qi Y and Tu Z, 2016. GUY1 confers complete female lethality and is a strong
2552 candidate for a male-determining factor in *Anopheles stephensi*. eLife, 5, e19281.

2553 CSS–ENSSER–VDW (Critical Scientists Switzerland – European Network of Scientists for Social
2554 and Environmental Responsibility – Vereinigung Deutscher Wissenschaftler), 2019. GENE
2555 DRIVES. A report on their science, applications, social aspects, ethics and regulations. Available
2556 from: https://genedrives.ch/wp-content/uploads/2019/10/Gene-Drives-Book-WEB.pdf

2557 Dao A, Yaro AS, Diallo M, Timbine S, Huestis DL, Kassogue Y, Traore AI, Sanogo ZL, Samake D
2558 and Lehmann T, 2014. Signatures of aestivation and migration in Sahelian malaria mosquito
2559 populations. Nature, 516, 387–390.

2560 Dame DA, Curtis CF, Benedict MQ, Robinson AS and Knols BG, 2009. Historical applications of
2561 induced sterilisation in field populations of mosquitoes. Malaria Journal, 8, S2.

2562 Davis S, Bax N and Grewe P, 2001. Engineered underdominance allows efficient and economical
2563 introgression of traits into pest populations. Journal of Theoretical Biology, 212, 83–98.

2564 De Barro PJ, Murphy B, Jansen CC and Murray J, 2011. The proposed release of the yellow
2565 fever mosquito, *Aedes aegypti* containing a naturally occurring strain of *Wolbachia pipientis*, a
2566 question of regulatory responsibility. Journal of Consumer Protection and Food Safety, 6, S33-
2567 S40.

2568 de Jong TJ, 2017. Gene drives do not always increase in frequency: from genetic models to risk
2569 assessment. Journal of Consumer Protection and Food Safety, 12, 299–307.

2570 Deredec A, Godfray HCJ and Burt A, 2011. Requirements for effective malaria control with
2571 homing endonuclease genes. Proceedings of the National Academies of Science USA, 108,
2572 E874−E880.

2573 Devos Y, Sanvido O, Tait J and Raybould A, 2014. Towards a more open debate about values in
2574 decision-making on agricultural biotechnology. Transgenic Research, 23, 933–943.

2575 Devos Y, Romeis J, Luttik R, Maggiore A, Perry JN, Schoonjans R, Streissl F, Tarazona JV and
2576 Brock TCM, 2015. Optimising environmental risk assessments – Accounting for biodiversity and
2577 ecosystem services helps to translate broad policy protection goals into specific operational
2578 ones for environmental risk assessments. EMBO Reports, 16, 1060–1063.

Devos Y, Gaugitsch H, Gray AJ, Maltby L, Martin J, Pettis JS, Romeis J, Rortais A, Schoonjans R, Smith J, Streissl F and Suter GW II, 2016. Advancing environmental risk assessment of regulated stressors under EFSA's remit. EFSA Journal, 14, s0508, 1–14.

Devos Y, Craig W, Devlin RH, Ippolito A, Leggatt RA, Romeis J, Shaw R, Svendsen C and Topping CJ, 2019a. Using problem formulation for fit-for-purpose pre-market environmental risk assessments of regulated stressors. EFSA Journal, 17, e170708, 1–31.

Devos Y, Munns WR Jr, Forbes VE, Maltby L, Stenseke M, Brussaard L, Streissl F and Hardy A, 2019b. Applying ecosystem services for pre-market environmental risk assessments of regulated stressors. EFSA Journal, 17, e170705, 1–24.

Devos Y, Elliott KC, Macdonald P, McComas K, Parrino L, Vrbos D, Robinson T, Spiegelhalter D and Gallani B, 2019c. Conducting fit-for-purpose food safety risk assessments. EFSA Journal, 17, e170707, 1–16.

Dhole S, Vella MR, Lloyd AL and Gould F, 2018. Invasion and migration of spatially self-limiting gene drives: a comparative analysis. Evolutionary Applications, 11, 794–808.

Dhole S, Lloyd AL and Gould F, 2019. Tethered homing gene drives: a new design for spatially restricted population replacement and suppression. Evolutionary Applications, 12, 1688–1702.

Dodson BL, Hughes GL, Paul O, Matacchiero AC, Kramer LD and Rasgon JL, 2014. *Wolbachia* enhances West Nile virus (WNV) infection in the mosquito *Culex tarsalis*. PLoS Neglected Tropical Diseases, 8, e2965.

Doudna JA and Charpentier E, 2014. The new frontier of genome engineering with CRISPR-Cas9. Science, 346, 1258096.

Drury DW, Dapper AL, Siniard DJ, Zentner GE and Wade MJ, 2017. CRISPR/Cas9 gene drives in genetically variable and nonrandomly mating wild populations. Science Advances, 3, e1601910.

Duvall LB, Ramos-Espiritu L, Barsoum KE, Glickman JF and Vosshall LB, 2019. Small-molecule agonists of *Ae. Aegypti* neuropeptide Y receptor block mosquito biting. Cell, 176, 687–701.

Dyer K and Hall D, 2019. Fitness consequences of a non-recombining sex-ratio drive chromosome can explain its prevalence in the wild. Proceedings of the Royal Society: Biological Sciences,286, 20192529.

EASAC (the European Academies' Science Advisory Council), 2017. Genome editing: scientific opportunities, public interests and policy options in the European Union. EASAC policy report, 31 March 2017. Available from: https://easac.eu/publications/details/genome-editing-scientific-opportunities-public-interests-and-policy-options-in-the-eu/

Eckhoff PA, Wengera EA, Godfray HCJ and Burt A, 2017. Impact of mosquito gene drive on malaria elimination in a computational model with explicit spatial and temporal dynamics. Proceedings of the National Academies of Science USA, 114, E255–E264.

Edgington MP and Alphey LS, 2017. Conditions for success of engineered underdominance gene drive systems. Journal of Theoretical Biology, 430, 128–140.

Edgington MP and Alphey LS, 2018. Population dynamics of engineered underdominance and killer-rescue gene drives in the control of disease vectors. PLoS Computational Biology, 14, e1006059.

Edgington MP and Alphey LS, 2019. Modeling the mutation and reversal of engineered underdominance gene drives. Journal of Theoretical Biology, 479, 14–21.

EFSA (European Food Safety Authority), 2010a. Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal, 8, 1–55.

EFSA (European Food Safety Authority), 2010b. Report on the PPR stakeholder workshop Protection goals for environmental risk assessment of pesticide: What and where to protect? EFSA Journal, 8, 1–46.

EFSA (European Food Safety Authority), 2012. Scientific Opinion on the Guidance on the risk assessment of food and feed from genetically modified animals and animal health and welfare aspects. EFSA Journal, 10, 1–43.

EFSA (European Food Safety Authority), 2013. Guidance on the environmental risk assessment of genetically modified animals. EFSA Journal, 11, 1–190.

EFSA (European Food Safety Authority), 2014. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. EFSA Journal, 12, 3589. doi:10.2903/j.efsa.2014.3589

EFSA (European Food Safety Authority), 2016. Guidance to develop specific protection goals options for environmental risk assessment at EFSA, in relation to biodiversity and ecosystem services. EFSA Journal, 14, 4499. doi:10.2903/j.efsa.2016.4499

EFSA (European Food Safety Authority), 2017 Scientific Opinion on guidance for the risk assessment of the presence at low level of genetically modified plant material in imported food and feed under Regulation (EC) No 1829/2003. EFSA Journal, 15, 5048 [19 pp.] doi:10.2903/j.efsa.2017.5048

Emerson C, James S, Littler K and Randazzo F, 2017. Principles for gene drive research. Science, 358, 1135–1136.

EPPO/COST-SMARTER (European and Mediterranean Plant Protection Organization), 2015. Workshop on the evaluation and regulation of the use of biological control agents in the EPPO Region. Available from https://www.eppo.int/MEETINGS/2015_meetings/wk_biocontrol

Esvelt KM and Gemmell NJ, 2017. Conservation demands safe gene drive. PLoS Biology, 15, e2003850.

Esvelt KM, Smidler AL, Catteruccia F and Church GM, 2014. Concerning RNA-guided gene drives for the alteration of wild populations. eLife, 3, e03401.

Ethics Council of the Max-Max-Planck-Gesellschaft, 2019. Discussion paper focusing on the scientific relevance of genome editing and on the ethical, legal and societal issues potentially involved. Available from: https://www.mpg.de/13811476/DP-Genome-Editing-EN-Web.pdf

Facchinelli L, Valerio L, Ramsey JM, Gould F, Walsh RK, Bond G, Robert MA, Lloyed AL, James AA, Alphey L and Scott TW, 2013. Field cage studies and progressive evaluation of genetically-engineered mosquitoes. PLoS Neglected Tropical Diseases, 7, e2001.

Fang J, 2010. A world without mosquitoes. Nature, 466, 432–434.

Feachem RGA, Chen I, Akbari O, Bertozzi-Villa A, Bhatt S, Binka F, Boni MJ, Buckee C, Dieleman J, Dondorp A, Eapen A, Feachem NS, Filler S, Gething P, Gosling R, Haakenstad A, Harvard K, Hatefi A, Jamison D, Jones KE, Karema C, Kamwi RN, Lal A, Larson E, Lees M, Lobo NF, Micah AE, Moonen B, Newby G, Ning X, Pate M, Quiñones M, Roh M, Rolfe B, Shanks D, Singh B, Staley K, Tulloch J, Wegbreit J, Woo HJ and Mpanju-Shumbusho W, 2019. Malaria eradication within a generation: ambitious, achievable, and necessary. The Lancet, 394, 1056–1112.

Ferreira CP, Yang NM and Esteva L, 2008. Assessing the suitability of sterile insect technique applied to *Aedes aegypti*. Journal of Biological Systems, 16, 565–577.

Finnegan SR, White NJ, Koh D, Camus MF, Fowler K and Pomiankowski A, 2019. Meiotic drive reduces egg-to-adult viability in stalk-eyed flies. Proceedings of the Royal Society: Biological Sciences, 286, 20191414.

Franz AWE, Sanchez-Vargas I, Adelman ZN, Blair CD, Beaty BJ, James AA and Olson KE, 2006. Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. Proceedings of the National Academy of Sciences of the United States of America, 103, 4198–4203.

Frieβ JL, von Gleich A and Giese B, 2019. Gene drives as a new quality in GMO releases – a comparative technology characterization. PeerJ, 7, e6793.

Fu G, Lees RS, Nimmo D, Aw D, Jin L, Gray P, Berendonk TU, White-Cooper H, Scaife S, Kim Phuc H, Marinotti O, Jasinskiene N, James AA and Alphey L, 2010. Female-specific flightless phenotype for mosquito control. Proceedings of the National Academy of Sciences of the United States of America, 107, 4550–4554.

Galizi R, Doyle LA, Menichelli M, Bernardini F, Deredec A, Burt A, Stoddard BL, Windbichler N and Crisanti A, 2014. A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nature Communications, 5, 1–8.

Galizi R, Hammond A, Kyrou K, Taxiarchi C, Bernardini F, O'Loughlin SM, Papathanos PA, Nolan T, Windbichler N and Crisanti A, 2016. A CRISPR-Cas9 sex-ratio distortion system for genetic control. Scientific reports, 6, 31139.

Gantz VM and Bier E, 2015. The mutagenic chain reaction: a method for converting heterozygous to homozygous mutations. Science, 348, 442–444.

Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E and James AA, 2015. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proceedings of the National Academy of Sciences of the United States of America, 112, E6736–E6743.

Garcia-Alonso M and Raybould A, 2014. Protection goals in environmental risk assessment: A practical approach. Transgenic Research, 23, 945–956.

GeneWatch-TWN-ACB (GeneWatch UK, Third World Network, African Centre for Biodiversity), 2019. Oxitec's failed GM mosquito releases worldwide: forewarnings for Africa and the Target Malaria project. Available from http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Oxitec_failed_GM_mosquito_releases_worldwide_Forewarnings_for_Africa_and_the_Target_Malaria_project.pdf

George DR, Kuiken T and Delborne JA, 2019. Articulating free, prior and informed consent (FPIC) for engineered gene drives. Proceedings of the Royal Society: Biological Sciences, 286, 20191484.

Giese B, Frieß JL, Barton NH, Messer PW, Débarre F, Schetelig MF, Windbichler N, Meimberg H and Boëte C, 2019. Gene drives: dynamics and regulatory matters—a report from the workshop "Evaluation of Spatial and Temporal Control of Gene Drives," April 4–5, 2019, Vienna. BioEssays, 1900151.

Glandorf DCM, 2017. Technical evaluation of a potential release of OX513A *Aedes aegypti* mosquitoes on the island of Saba. RIVM Letter report 2017-0087. Available from: https://www.rivm.nl/bibliotheek/rapporten/2017-0087.pdf

Godfray HCJ, North A and Burt A, 2017. How driving endonuclease genes can be used to combat pests and disease vectors. BMC Biology, 15, 81.

Godwin J, Serr M, Barnhill-Dilling SK, Blondel DV, Brown PR, Campbell K, Delborne J, Lloyd AL, Oh KP, Prowse TAA, Saah R and Thomas P, 2019. Rodent gene drives for conservation: opportunities and data needs. Proceedings of the Royal Society: Biological Sciences, 286, 20191606.

Gokhale CS, Reeves RG and Reed FA, 2014. Dynamics of a combined *Medea*-underdominant population transformation system. BMC Evolutionary Biology, 14, 98.

Gorman K, Young J, Pineda L, Márquez R, Sosa N, Bernal D, Torres R, Soto Y, Lacroix R, Naish N, Kaiser P, Tepedino K, Philips G, Kosmanna C and Cáceres L, 2015. Short-term suppression of *Aedes aegypti* using genetic control does not facilitate *Aedes albopictus*. Pest Management Science, 72, 618–628.

Gould F and Schliekelman P, 2004. Population genetics of autocidal control and strain replacement. Annual Review of Entomology, 49, 193–217.

Gould F, Huang Y, Legros M and Lloyd AL, 2008. A killer-rescue system for self-limiting gene drive of anti-pathogen constructs. Proceedings of the Royal Society: Biological Sciences, 275, 2823–2829.

Gray AJ, 2012. Problem formulation in environmental risk assessment for genetically modified crops: A practitioner's approach. Collection of Biosafety Reviews, 6, 10–65.

Grunwald HA, Gantz VM, Poplawski G, Xu XRS, Bier E and Cooper KL, 2019. Super-Mendelian inheritance mediated by CRISPR–Cas9 in the female mouse germline. Nature, 566, 105–109.

Guevara-Souza M and Vallejo EE, 2011. Computer simulation on disease vector population replacement driven by the maternal effect dominant embryonic arrest. Advances in Experimental Medicine and Biology, 696, 335–343.

Guichard A, Haque T, Bobik M, Xu X-RS, Klanseck C, Kushwah RBS, Berni M, Kaduskar B, Gantz VM and Bier E, 2019. Efficient allelic-drive in *Drosophila*. Nature Communications, 10, 1640.

Haller BC and Messer PW, 2017. SLiM 2: flexible, interactive forward genetic simulations. Molecular Biology and Evolution, 34, 230–240.

Hammond AM and Galizi R, 2017. Gene drives to fight malaria: current state and future directions. Pathogens and Global Health, 111, 412–423.

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S, Burt A, Windbichler N, Crisanti A and Nolan T, 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotechnology, 34, 78–83.

Hammond AM, Kyrou K, Bruttini M, North A, Galizi R, Karlsson X, Kranjc N, Carpi FM, D'Aurizio R, Crisanti A and Nolan T, 2017. The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito. PLoS Genetics, 13, e1007039.

Harris AF, McKemey AR, Nimmo D, Curtis Z, Black I, Morgan SA, Oviedo MN, Lacroix R, Naish N, Morrison NI et al., 2012. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. Nature Biotechnology, 30, 828–830.

Harrington LC, Scott TW, Lerdthusnee K, Coleman RC, Costero A, Clark GG, Jones JJ, Kitthawee S, Kittayapong P, Sithiprasasna R and Edman JD, 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. The American Journal of Tropical Medicine and Hygiene, 72, 209–220.

Hartley S, Thizy D, Ledingham K, Coulibaly M, Diabaté M, Dicko B, Diop S, Kayondo J, Namukwaya A, Nourou B and Paré Toé L, 2019. Knowledge engagement in gene drive research for malaria control. PLoS Neglected Tropical Diseases, 13, e0007233.

Harvey-Samuel T, Morrison NI, Walker AS, Marubbi T, Yao J, Collins HL, Gorman K, Davies TGE, Alphey N, Warner S, Shelton AM and Alphey L, 2015. Pest control and resistance management through release of insects carrying a male-selecting transgene. BMC Biology, 13, 49.

Harvey-Samuel T, Ant T and Alphey L, 2017. Towards the genetic control of invasive species. Biological Invasions, 19, 1683–1703.

Hay BA, Chen C-H, Ward CM, Huang H, Su JT and Guo M, 2010. Engineering the genomes of wild insect populations: challenges, and opportunities provided by synthetic Medea selfish genetic elements. Journal of Insect Physiology, 56, 1402–1413.

Hayes KR, Hosack GR, Dana GV, Foster SD, Ford JH, Thresher R, Ickowicz A, Peel D, Tizard M, De Barro P, Strive T and Dambacher JM, 2018. Identifying and detecting potentially adverse ecological outcomes associated with the release of gene-drive modified organisms. Journal of Responsible Innovation, 5, S139–S158.

HCB (Haut Conseil des Biotechnologies), 2017. Scientific Opinion in response to the referral of 12 October 2015 concerning use of genetically modified mosquitoes for vector control. Available from: http://www.hautconseildesbiotechnologies.fr/sites/www.hautconseildesbiotechnologies.fr/files/file_fields/2018/04/10/hcbscopinionmosquitoes170607entranslation180228.pdf

Hegde S and Hughes GL, 2017. Population modification of *Anopheles* mosquitoes for malaria control: pathways to implementation. Pathogens and Global Health, 111, 401–402.

Helinski ME, Parker AG and Knols BG, 2009. Radiation biology of mosquitoes. Malaria Journal, 8, S6.

Hemme RR, Thomas CL, Chadee DD and Severson DW, 2010. Influence of urban landscapes on population dynamics in a short-distance migrant mosquito: evidence for the *Aedes aegypti*. PLoS Neglected Tropical Diseases, 4, e634.

High-Level African Panel on Emerging Technologies, 2018. Gene drives for malaria control and elimination in Africa. Available from: https://www.nepad.org/publication/gene-drives-malaria-control-and-elimination-africa

Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y, Cook H, Axford J, Callahan AG, Kenny N, Omodei C, McGraw EA, Ryan PA, Ritchie SA, Turelli M and O'Neill SL, 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature, 476, 454–457.

Hoffmann AA, Iturbe-Ormaetxe I, Callahan AG, Phillips B, Billington K, Axford JK, Montgomery B, Turley AP and O'Neill SL, 2014. Stability of the *w*Mel *Wolbachia* infection following invasion into *Aedes aegypti* populations. PLoS Neglected Tropical Diseases, 8, e3115.

Hoffmann AA, Ross PA and Rašić G, 2015. *Wolbachia* strains for disease control: ecological and evolutionary considerations. Evolutionary Applications, 8, 751–768.

Hokanson KE, Ellstrand N and Raybould A, 2018. The integration of science and policy in regulatory decision-making: observations on scientific expert panels deliberating GM crops in centers of diversity. Frontiers in Plant Science, 9, 1157.

Holman L, 2019. Evolutionary simulations of *Z*-linked suppression gene drives. Proceedings of the Royal Society B: Biological Sciences, 286, 20191070.

HSCP (Haut Conseil de la Santé Publique), 2018. Avis relatif à l'élaboration de recommandations pour autoriser le lâcher de moustiques stériles à des fins de lutte anti-vectorielle. Haut Conseil de la Santé Publique, France, 28 juin 2018, 35pp. Available from: https://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=687

Huang Y, Magori K, Lloyd AL and Gould F, 2007. Introducing transgenes into insect populations using combined gene-drive strategies: modeling and analysis. Insect Biochemistry and Molecular Biology, 37, 1054–1063.

Huang Y, Lloyd AL, Legros M and Gould F, 2009. Gene-drive in age-structured insect populations. Evolutionay Applications, 2, 143–159.

Huestis DL, Dao A, Diallo M, Sanogo ZL, Samake D, Yaro AS, Ousman Y, Linton Y-M, Krishna A, Very L, Krajacich BJ, Faiman R, Florio J, Chapman JW, Reynolds DR, Weetman D, Mitchell R, Donnelly MJ, Talamas E, Chamorro L, Strobach E and Lehmann T, 2019. Windborne long-distance migration of malaria mosquitoes in the Sahel. Nature, 574, 404–408.

Hughes GL and Rasgon JL, 2014. Transinfection: a method to investigate *Wolbachia*-host interactions and control arthropod-borne disease. Insect Molecular Biology, 23, 141–151.

Hurst LD, 2019. A Century of bias in genetics and evolution. Heredity, 123, 33–43.

James S, Collins FH, Welkhoff PA, Emerson C, Godfray HCJ, Gottlieb M, Greenwood B, Lindsay SW, Mbogo CM, Okumu FO, Quemada H, Savadogo M, Singh JA, Tountas KH and Touré YT, 2018. Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in Sub-Saharan Africa: recommendations of a scientific working group. The American Journal of Tropical Medicine and Hygiene, 98, 1–49.

Jin L, Walker AS, Fu G, Harvey-Samuel T, Dafa'alla T, Miles A, Marubbi T, Granville D, Humphrey-Jones N, O'Connell S, Morrison NI and Alphey L, 2013. Engineered female-specific lethality for control of pest Lepidoptera. ACS Synthetic Biology, 2, 160–166.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA and Charpentier E, 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 337, 816–821.

Jones MS, Delborne JA, Elsensohn J, Mitchell PD and Brown ZS, 2019. Does the U.S. public support using gene drives in agriculture? And what do they want to know? Science Advances, 5, eaau8462.

Juliano SA, 2009. Species interactions among larval mosquitoes: context dependence across habitat gradients. Annual Review of Entomology, 54, 37–56.

Juliano SA, 2010. Coexistence, exclusion, or neutrality? A meta-analysis of competition between *Aedes albopictus* and resident mosquitoes. Israel Journal of Ecology and Evolution, 56, 325–351.

Jupatanakul N, Sim S, Angleró-Rodríguez YI, Souza-Neto J, Das S, Poti KE, Rossi SL, Bergren N, Vasilakis N and Dimopoulos G, 2017. Engineered *Aedes aegypti* JAK/STAT pathway-mediated immunity to dengue virus. PLoS Neglected Tropical Diseases, 11, e0005187.

Kandul NP, Liu J, Buchman A, Gantz VM, Bier E and Akbari OS, 2019. Assessment of a split homing based gene drive for efficient knockout of multiple genes. G3: GENES, GENOMES, GENETICS. doi:10.1534/g3.119.400985

KaramiNejadRanjbar M, Eckermann KN, Ahmed HMM, Sánchez CHM, Dippel S, Marshall JM and Wimmer EA, 2018. Consequences of resistance evolution in a Cas9-based sex conversion-suppression gene drive for insect pest management. Proceedings of the National Academy of Sciences of the United States of America, 115, 6189–6194.

Khamis D, El Mouden C, Kura K and Bonsall, MB, 2018. Ecological effects on underdominance threshold drives for vector control. Journal of Theoretical Biology, 456, 1–15.

Khoo CCH, Piper J, Sanchez-Vargas I, Olson KE and Franz AWE, 2010. The RNA interference pathway affects midgut infection- and escape barriers for *Sindbis* virus in *Aedes aegypti*. BMC Microbiology, 10, 130.

King JG, Souto-Maior C, Sartori LM, Maciel-de-Freitas R and Gomes MGM, 2018. Variation in *Wolbachia* effects on *Aedes mosquitoes* as a determinant of invasiveness and vectorial capacity. Nature Communications, 9,1483.

Klein TA, Windbichler N, Deredec A, Burt A and Benedict MQ, 2012. Infertility resulting from transgenic I-PpoI male *Anopheles gambiae* in large cage trials. Pathogens and Global Health, 106, 20–31.

Kofler N, 2019. Gene drives: yelling match drowns out marginalized voices. Nature, 565, 25.

Kolopack PA, Parsons JA and Lavery JV, 2015. What makes community engagement effective?: lessons from the eliminate dengue program in Queensland Australia. PLoS Neglected Tropical Diseases, 9, e0003713.

Krishnan P and Gillum D, 2017. Gene drive 101: a basic guidance resource for biosafety professionals. Applied Biosafety, 22, 181–184.

Kuzma J, 2019. Procedurally robust risk assessment framework for novel genetically engineered organisms and gene drives. Regulation & Governance. doi:10.1111/rego.12245

Kyrou K, Hammond AM, Galizi R, Kranjc N, Burt A, Beaghton AK, Nolan T and Crisanti A, 2018. A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged *Anopheles gambiae* mosquitoes. Nature Biotechnology, 36, 1062–1066.

Labbé GMC, Scaife S, Morgan SA, Curtis ZH and Alphey L, 2012. Female-specific flightless (fsRIDL) phenotype for control of *Aedes albopictus*. PLoS Neglected Tropical Diseases, 6, e1724.

Lacroix R, McKemey AR, Raduan N, Kwee Wee L, Hong Ming W, Guat Ney T, et al., 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS ONE, 7, e42771.

Lambert B, North A, Burt A and Godfray HCJ, 2018. The use of driving endonuclease genes to suppress mosquito vectors of malaria in temporally variable environments. Malaria Journal, 17, 154.

Larner W, Price T, Holman L and Wedell N, 2019. An X-linked meiotic drive allele has strong, recessive fitness costs in female *Drosophila pseudoobscura*. Proceedings of the Royal Society: Biological Sciences, 286, 20192038.

Lea JK, and Unckless RL, 2019. An assessment of the immune costs associated with meiotic drive elements in *Drosophila*. Proceedings of the Royal Society: Biological Sciences, 286, 20191534.

Ledford H, 2015. CRISPR, the disruptor. Nature, 522, 20–24.

Lees RS, Gilles JR, Hendrichs J, Vreysen MJ and Bourtzis K, 2015. Back to the future: the sterile insect technique against mosquito disease vectors. Current Opinion Insect Science, 10, 156–162.

Leftwich PT, Koukidou M, Rempoulakis P, Gong H, Zacharopoulou A, Fu G, Chapman T, Economopoulos A, Vontas J and Alphey L, 2014. Genetic elimination of field-cage populations of Mediterranean fruit flies. Proceedings of the Royal Society B: Biological Sciences, 281, 20141372.

Leftwich PT, Edgington MP, Harvey-Samuel T, Carabajal Paladino LZ, Norman VC and Alphey L, 2018. Recent advances in threshold-dependent gene drives for mosquitoes. Biochemical Society Transactions, 46, 1203–1212.

Leitschuh CM, Kanavy D, Backus GA, Valdez RX, Serr M, Pitts EA, Threadgill D and Godwin J, 2018. Developing gene drive technologies to eradicate invasive rodents from islands. Journal of Responsible Innovation, 5, S121–S138.

Li M, Yang T, Kandul NP, Bui M, Gamez S, Raban R, Bennett J, Sánchez HM, Lanzaro GC, Schmidt H, Lee Y, Marshall JM and Akbari OS, 2020a. Development of a confinable gene drive system in the human disease vector, *Aedes aegypti.* eLIFE, 9, e51701.

Li M, Li T, Liu N, Raban RR, Wang X and Akbari OS, 2020b. Methods for the generation of heritable germline mutations in the disease vector *Culex quinquefasciatus* using clustered regularly interspaced short palindrome repeats-associated protein 9. Insect Molecular Biology. doi:10.1111/imb.12626

Lindholm AK, Dyer KA, Firman RC, Fishman L, Forstmeier W, Holman L, Johannesson H, Knief U, Kokko H, Larracuente AM, Manser A, Montchamp-Moreau C, Petrosyan VG, Pomiankowski A, Presgraves DC, Safronova LD, Sutter A, Unckless RL, Verspoor RL, Wedell N, Wilkinson GS and Price TAR, 2016. The ecology and evolutionary dynamics of meiotic drive. Trends in Ecology & Evolution, 31, 315–326.

López Del Amo V, Bishop AL, Sánchez HM, Bennett JB, Feng X, Marshall JM, Bier E and Gantz VM, 2019. Split-gene drive system provides flexible application for safe laboratory investigation and potential field deployment. bioRxiv. doi:https://doi.org/10.1101/684597

López Del Amo V, Bishop AL, Sánchez HMC, Bennett JB, Feng X, Marshall JM, Bier E and Gantz VM, 2020. A transcomplementing gene drive provides a flexible platform for laboratory investigation and potential field deployment. Nature Communications, 11, 352.

Lounibos LP, 2002. Invasions by insect vectors of human disease. Annual Review of Entomology, 47, 233–266.

Lunshof JE and Birnbaum A, 2017. Adaptive risk management of gene drive experiments: biosafety, biosecurity, and ethics. Applied Biosafety, 22, 97–103.

MacDonald EA, Balanovic J, Edwards ED, Abrahamse W, Frame B, Greenaway A, Kannemeyer R, Kirk N, Medvecky F, Milfont TL, Russell JC and Tompkins DM, 2020. Public opinion towards gene drive as a pest control approach for biodiversity conservation and the association of underlying worldviews. Environmental Communication, doi:10.1080/17524032.2019.1702568

Macias VM, Ohm JR and Rasgon JL, 2017. Gene drive for mosquito control: where did it come from and where are we headed? International Journal of Environmental Research and Public Health, 14, 1006.

Mains JW, Brelsfoard CL, Rose RI and Dobson SL, 2016. Female adult *Aedes albopictus* *s*uppression by *Wolbachia*-infected male mosquitoes. Scientific Reports, 6, 33846.

Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE and Church GM, 2013. RNA-guided human genome engineering via Cas9. Science, 339, 823–826.

Maltby LL, Duke C and van Wensem J, 2017a. Ecosystem services, environmental stressors and decision making: How far have we got? Integrated Environmental Assessment and Management, 13, 38–40.

Maltby L, Jackson M, Whale G, Brown AR, Hamer M, Solga A, Kabouw P, Woods R and Marshall S, 2017b. Is an ecosystem services-based approach developed for setting specific protection goals for plant protection products applicable to other chemicals? Science of the Total Environment, 580, 1222–1236.

Maltby L, Van den Brink PJ, Faber JH and Marshall S, 2018. Advantages and challenges associated with implementing an ecosystem services approach to ecological risk assessment for chemicals. Science of the Total Environment, 621, 1342–1351.

Manoranjan VS and van den Driessche P, 1986. On a diffusion model for sterile insect release. Mathematical Biosciences, 79, 199–208.

Manser A, Cornell SJ, Sutter A, Blondel DV, Serr M, Godwin J and Price TAR, 2019. Controlling invasive rodents via synthetic gene drive and the role of polyandry. Proceedings of the Royal Society B: Biological Sciences, 286, 20190852.

Marchante H, Lopez-Nunez FA, Freitas H, Hoffmann JH, Impson F and Marchante E, 2017. First report of the establishment of the biocontrol agent *Trichilogaster acaciaelongifoliae* for control of invasive *Acacia longifolia* in Portugal. EPPO Bulletin, 47, 274–278.

Marshall JM, 2010. The Cartagena Protocol and genetically modified mosquitoes. Nature Biotechnology, 28, 896–897.

Marshall JM, 2011. The toxin and antidote puzzle: new ways to control insect pest populations through manipulating inheritance. Bioengineered Bugs, 2, 235–240.

Marshall JM and Akbari OS, 2018. Can CRISPR-based gene drive be confined in the wild? A question for molecular and population biology. ACS Chemical Biology, 13, 424–430.

Marshall JM and Hay BA, 2011. Inverse Medea as a novel gene drive system for local population which replacement: a theoretical analysis. Journal of Heredity, 102, 336–341.

Marshall JM and Hay BA, 2012a. General principles of single-construct chromosomal gene drive. Evolution, 66, 2150–2166.

Marshall JM and Hay BA, 2012b. Confinement of gene drive systems to local populations: a comparative analysis. Journal of Theoretical Biology, 294, 153–171.

Marshall JM and Hay BA, 2014. Medusa: a novel gene drive system for confined suppression of insect populations. PLoS ONE, 9, e102694.

Marshall JM, Pittman GW, Buchman AB and Hay BA, 2011. Semele: a killer-male, rescue-female system for suppression and replacement of insect disease vector populations. Genetics, 187, 535–551.

Marshall JM, Buchman A, Sánchez HM and Akbari OS, 2017. Overcoming evolved resistance to population-suppressing homing-based gene drives. Scientific Reports, 7, 3776.

Marshall JM, Raban RR, Kandul NP, Edula JR, León TM and Akbari OS, 2019. Winning the tug-of-war between effector gene design and pathogen evolution in vector population replacement strategies. Frontiers in Genetics, 10, 1072.

Masterson K, 2019. Mosquitoes, war and power. Murderous trail of the mosquito. Nature, 572, 310–311.

Mathur G, Sanchez-Vargas I, Alvarez D, Olson KE, Marinotti O and James AA, 2010. Transgene-mediated suppression of dengue viruses in the salivary glands of the yellow fever mosquito, *Aedes aegypti*. Insect Molecular Biology, 19, 753–763.

Meghani Z and Kuzma J, 2018. Regulating animals with gene drive systems: lessons from the regulatory assessment of a genetically engineered mosquito. Journal of Responsible Innovation, 5, S203–S222.

Min J, Smidler AL, Najjar D and Esvelt KM, 2018. Harnessing gene drive. Journal of Responsible Innovation, 5, S40–S65.

Mitchell H and Bartsch D, 2020. Regulation of GM organisms for invasive species control. Frontiers in Bioengineering and Biotechnology, 7, 454.

2977 Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin
2978 S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den Hurk AF, Ryan PA and
2979 O'Neill SL, 2009. A *Wolbachia* Symbiont in *Aedes aegypti* Limits Infection with Dengue,
2980 Chikungunya, and *Plasmodium*. Cell, 139, 1268–1278.

2981 Morrison NI, Simmons GS, Fu G, O'Connell S, Walker AS, Dafa'alla T, Walters M, Claus J, Tang
2982 G, Jin L, Marubbi T, Epton MJ, Harris CL, Staten RT, Miller E, Miller TA and Alphey L, 2012.
2983 Engineered repressible lethality for controlling the pink bollworm, a lepidopteran pest of cotton.
2984 PLoS ONE, 7, e50922.

2985 Mumford JD, 2012. Science, regulation, and precedent for genetically modified insects. PLoS
2986 Neglected Tropical Diseases, 6, e1504.

2987 Munns WR Jr, Helm RC, Adams WJ, Clements WH, Cramer MA, Curry M, DiPinto LM, Johns DM,
2988 Seiler R, Williams LL and Young D, 2009. Translating ecological risk to ecosystem service loss.
2989 Integrated Environmental Assessment and Management, 5, 500–514.

2990 Munns WR Jr, Rea AW, Suter, GW II, Martin L, Blake-Hedges L, Crk T, Davis C, Ferreira G,
2991 Jordan S, Mahoney M and Barron M, 2015. Ecosystem services as assessment endpoints for
2992 ecological risk assessment. Integrated Environmental Assessment and Management, 12, 522–
2993 528.

2994 Munns WR Jr, Poulsen V, Gala WR, Marshall SJ, Rea AW, Sorensen MT and von Stackelberg K,
2995 2017. Ecosystem services in risk assessment and management. Integrated Environmental
2996 Assessment and Management, 13, 62–73.

2997 Murphy B, Jansen C, Murray J and De Barro P, 2010. Risk Analysis on the Australian release of
2998 *Aedes aegypti* (L.) (Diptera: Culicidae) containing *Wolbachia*. CSIRO Report. Available from:
2999 http://www.eliminatedengue.org/LinkClick.aspx?fileticket=nMtZNaIayzw%3dandtabid=3911

3000 Murray JV, Jansen CC and De Barro P, 2016. Risk associated with the release of *Wolbachia*-
3001 infected *Aedes aegypti* mosquitoes into the environment in an effort to control dengue.
3002 Frontiers in Public Health, 4, 43.

3003 Najjar DA, Normandin AM, Strait EA and Esvelt KM, 2017. Driving towards ecotechnologies.
3004 Pathogens and Global Health, 111, 448–458.

3005 NASEM (National Academies of Sciences Engineering and Medicine), 2016. Gene drives on the
3006 horizon: Advancing science, navigating uncertainty, and aligning research with public values.
3007 Washington (DC): The National Academies Press. https://doi.org/10.17226/23405.

3008 Nash A, Urdaneta GM, Beaghton AK, Hoermann A, Papathanos PA, Christophides GK and
3009 Windbichler N, 2019. Integral gene drives for population replacement. Biology Open, 8,
3010 bio037762.

3011 Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, Kamarul GMR, Arif
3012 MAK, Thohir H, NurSyamimi H, ZatilAqmar MZ, NurRuqqayah M, NorSyazwani A, Faiz A, Irfan F-
3013 RMN, Rubaaini S, Nuradila N, Nizam NMN, Irwan SM, Endersby-Harshman NM, White VL, Ant
3014 TH, Herd CS, Hasnor AH, AbuBakar R, Hapsah DM, Khadijah K, Kamilan D, Lee SC, Paid YM,

Fadzilah K, Topek O, Gill BS, Lee HL and Sinkins SP, 2019. Establishment of *Wolbachia* strain *w*AlbB in Malaysian populations of *Aedes aegypti* for dengue control. Current Biology, 29, 4241–4248.

Neira M, Lacroix R, Cáceres L, Kaiser PE, Young J, Pineda L, Black I, Sosa N, Nimmo D, Alphey L and McKemey A, 2014. Estimation of *Aedes aegypti* (Diptera: Culicidae) population size and adult male survival in an urban area in Panama. Memórias do Instituto Oswaldo Cruz, 109, 879–886.

Nelson KC, Andow DA and Banker MJ, 2009. Problem formulation and option assessment (PFOA) linking governance and environmental risk Assessment for technologies: a methodology for problem analysis of nanotechnologies and genetically engineered organisms. Journal of Law, Medicine and Ethics, 37, 732–748.

Neve P, 2018. Gene drive systems: do they have a place in agricultural weed management? Pest Management Science, 74, 2671–2679.

Nienstedt KM, Brock TCM, van Wensum J, Montforts M, Hart A, Aagaard A, Alix A, Boesten J, Bopp SK, Brown C, Capri E, Forbes V, Köpp H, Liess M, Luttik R, Maltby L, Sousa JP, Streissl F and Hardy AR, 2012. Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Science of the Total Environment, 415, 31–38.

Nikolouli K, Colinet H, Renault D, Enriquez T, Mouton L, Gibert P, Sassu F, Cáceres C, Staufer C, Pereira R and Bourtzis K, 2018. Sterile insect technique and *Wolbachia* symbiosis as potential tools for the control of the invasive species *Drosophila suzukii*. Journal of Pest Science, 91, 489–503.

Noble C, Olejarz J, Esvelt KM, Church GM and Nowak MA, 2017. Evolutionary dynamics of CRISPR gene drives. Science Advances, 3, 3–10.

Noble C, Adlam B, Church GM, Esvelt KM and Nowak MA, 2018. Current CRISPR gene drive systems are likely to be highly invasive in wild populations. eLife, 7, e33423.

Noble C, Min J, Olejarz J, Buchthal J, Chavez A, Smidler AL, DeBenedictis EA, Church GM, Nowak MA and Esvelt KM, 2019. Daisy-chain gene drives for the alteration of local populations. Proceedings of the National Academy of Sciences of the United States of America, 116, 8275–8282.

North A, Burt A and Godfray HCJ, 2013. Modelling the spatial spread of a homing endonuclease gene in a mosquito population. Journal of Applied Ecology, 56, 1216–1225.

North AR, Burt A and Godfray HCJ, 2019. Modelling the potential of genetic control of malaria mosquitoes at national scale. BMC Biology, 17, 26.

O'Connor L, Plichart C, Sang AC, Brelsfoard CL, Bossin HC and Dobson SL, 2012. Open release of male mosquitoes infected with a *wolbachia* biopesticide: field performance and infection containment. PLoS Neglected Tropical Diseases, 6, e1797.

O'Neill SL, 2018. The Use of *Wolbachia* by the World Mosquito Program to Interrupt Transmission of *Aedes aegypti* Transmitted Viruses. In: Hilgenfeld R and Vasudevan SG (eds.). Dengue and Zika: Control and Antiviral Treatment. Advances in Experimental Medicine and Biology, 1062. Springer Nature, Singapore, pp. 355-360.

O'Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, Dong Y, Kenny N, Paton CJ, Ritchie SA, Brown-Kenyon J, Stanford D, Wittmeier N, Jewell NP, Tanamas SK, Anders KL and Simmons CP, 2019. Scaled deployment of *Wolbachia* to protect the community from dengue and other *Aedes* transmitted arboviruses. Open Research, 2, 36.

Oberhofer G, Ivy T and Hay BA, 2018. Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs. Proceedings of the National Academy of Sciences, 115, E9343–E9352.

Oberhofer G, Ivy T and Hay BA, 2019. Cleave and Rescue, a novel selfish genetic element and general strategy for gene drive. Proceedings of the National Academy of Sciences, 116, 6250–6259.

Ørsted IV and Ørsted M, 2019. Species distribution models of the Spotted Wing Drosophila (*Drosophila suzukii*, Diptera: Drosophilidae) in its native and invasive range reveal an ecological niche shift. Journal of Applied Ecology, 56, 423–435.

Oye KA, Esvelt K, Appleton E, Catteruccia F, Church G, Kuiken T, Lightfood SB-Y, McNamara J, Smidler A and Collins JP, 2014. Regulating gene drives. Science, 345, 626–628.

Papathanos PA and Windbichler N, 2018. Redkmer: an assembly-free pipeline for the identification of abundant and specific X-Chromosome target sequences for X-shredding by CRISPR endonucleases. CRISPR Journal, 1, 88–98

Paton RS and Bonsall MB, 2019. The ecological and epidemiological consequences of reproductive interference between the vectors *Aedes aegypti* and *Aedes albopictus*. Journal of the Royal Society Interface, 16, 20190270.

Paulo DF, Williamson ME, Arp AP, Li F, Sagel A, Skoda SR, Sanchez-Gallego J, Vasquez M, Quintero G, Pérez de León AA, Belikoff EJ, Azeredo-Espin AML, McMillan WO, Concha C and Scott MJ, 2019. G3: Genes, Genomes, Genetics, 9, 3045–3055.

Pham TB, Phong CH, Bennett JB, Hwang K, Jasinskiene N, Parker K, Stillinger D, Marshall JM, Carballar-Lejarazú R and James AA, 2019. Experimental population modification of the malaria vector mosquito, *Anopheles stephensi*. PLoS Genetics, 15, e1008440.

Phuc HK, Andreasen MH, Burton RS, Vass C, Epton MJ, Pape G, Fu G, Condon KC, Scaife S, Donnelly CA, Coleman PG, White-Cooper H and Alphey L, 2007. Late-acting dominant lethal genetic systems and mosquito control. BMC Biology 5, 11.

Pixley KV, Falck-Zepeda JB, Giller KE, Glenna LL, Gould F, Mallory-Smith CA, Stelly DM and Stewart CN JR. 2019. Genome editing, gene drives, and synthetic biology: will they contribute to disease-resistant crops, and who will benefit? Annual Review of Phytopathology, 57, 165–188.

Pontieri L, Schmidt AM, Singh R, Pedersen JS and Linksvayer TA, 2017. Artificial selection on ant female caste ratio uncovers a link between female-biased sex ratios and infection by *Wolbachia* endosymbionts. Journal of Evolutionary Biology, 30, 225–234.

Price T, Verspoor R and Wedell N, 2019. Ancient gene drives: an evolutionary paradox. Proceedings of the Royal Society: Biological Sciences, 286, 20192267.

Pugh J, 2016. Driven to extinction? The ethics of eradicating mosquitoes with gene-drive technologies. Journal of Medical Ethics London, 42, 578.

Rabitz F, 2019. Gene drives and the international biodiversity regime. RECIEL, 00, 1–10.

Rasgon JL and Gould F, 2005. Transposable element insertion location bias and the dynamics of gene drive in mosquito populations. Insect Molecular Biology, 14, 493–500.

Raybould A, 2006. Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environmental Biosafety Research, 5, 119–125.

Raybould A, 2007. Ecological versus ecotoxicological methods for assessing the environmental risks of transgenic crops. Plant Science, 173, 589–602.

Raybould A, 2010. The bucket and the searchlight: Formulating and testing risk hypotheses about the weediness and invasiveness potential of transgenic crops. Environmental Biosafety Research, 9, 123–133.

Raybould A and Macdonald P, 2018. Policy-led comparative environmental risk assessment of genetically modified crops: Testing for increased risk rather than profiling phenotypes leads to predictable and transparent decision-making. Frontiers in Bioengineering and Biotechnology, 6, 43.

Redford KH, Brooks TM, Macfarlane NBW and Adams JS (eds.), 2019. Genetic frontiers for conservation: an assessment of synthetic biology and biodiversity conservation. Technical assessment. Gland, Switzerland: IUCN. xiv + 166pp.

Reeves RG, Bryk J, Altrock PM, Denton JA and Reed FA, 2014. First Steps towards Underdominant Genetic Transformation of Insect Populations. PLoS ONE, 9, e97557.

Reeves R and Phillipson M, 2017. Mass releases of genetically modified insects in area-wide pest control programs and their impact on organic farmers. Sustainability, 9, 59.

Ritchie SA and Staunton KM, 2019. Reflections from an old Queenslander: can rear and release strategies be the next great era of vector control? Proceedings of the Royal Society B: Biological Sciences, 286, 20190973.

Roberts A, Paes de Andrade P, Okumu F, Quemada H, Savadogo M, Amir Singh J and James S, 2017. Results from the workshop "Problem Formulation for the Use of Gene Drive in Mosquitoes". The American Journal of Tropical Medicine and Hygiene, 96, 530–533.

Rode NO, Estoup A, Bourguet D, Courtier-Orgogozo V and Débarre F, 2019. Population management using gene drive: molecular design, models of spread dynamics and assessment of ecological risks. Conservation Genetics, 20, 671–690.

Rogers DJ and Randolph SE, 1984. From a case study to a theoretical basis for tsetse control. International Journal of Tropical Insect Science, 5, 419–423.

Romeis J, Collatz J, Glandorf DCM and Bonsall MB, 2020. The value of existing frameworks for the environmental risk assessment of agricultural pest control using gene drives. Environmental Science & Policy

Royal Society, 2018. Gene drive research: why it matters? Available from: https://royalsociety.org/-/media/policy/Publications/2018/08-11-18-gene-drive-statement.pdf

Rüdelsheim PLJ and Smets G, 2018. Experience with gene drive systems that may inform an environmental risk assessment. COGEM Report CGM 2018-03. Available from: https://www.cogem.net/index.cfm/en

Ryan SJ, Carlson CJ, Mordecai EA and Johnson LR, 2019. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. PLoS Neglected Tropical Diseases, 13, e0007213.

SAM (Scientific Advice Mechanism), 2017. New techniques in agricultural biotechnology. Directorate-General for Research and Innovation. Available from: https://ec.europa.eu/research/sam/pdf/topics/explanatory_note_new_techniques_agricultural_biotechnology.pdf#view=fit&pagemode=none

Sánchez CHM, Wu SL, Bennett JB and Marshall JM, 2019. MGDrivE: a modular simulation framework for the spread of gene drives through spatially-explicit mosquito populations. Methods in Ecology and Evolution, 00, 1–11. doi:10.1111/2041-210X.13318

Sander JD and Joung JK, 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotechnology, 32, 347–355.

Sandler R, 2019. The ethics of genetic engineering and gene drives in conservation. Conservation Biology, 0, 1–8. doi:10.1111/cobi.13407

Sanvido O, Romeis J, Gathmann A, Gielkens M, Raybould A and Bigler F, 2012. Evaluating environmental risks of genetically modified crops – Ecological harm criteria for regulatory decision-making. Environmental Science & Policy, 15, 82–91.

Schenkel W and Leggewie GJ, 2015. New techniques in molecular biology challenge the assessment of modified organisms. Journal für Verbraucherschutz und Lebensmittelsicherheit, 10, 263–268.

Schetelig MF, Lee K-Z, Otto S, Talmann L, Stökl J, Degenkolb T, Vilcinskas A and Halitschke R, 2018. Environmentally sustainable pest control options for *Drosophila suzukii*. Journal of Applied Entomology, 142, 3–17.

Schmidt TL, Barton NH, Rašić G, Turley AP, Montgomery BL, Iturbe-Ormaetxe I, Cook PE, Ryan PA, Ritchie SA, Hoffmann AA, O'Neill SL and Turelli M, 2017. Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. PLoS Biology, 15, e2001894.

Scott MJ, Gould F, Lorenzen M, Grubbs N, Edwards O and O'Brochta D, 2018. Agricultural production: assessment of the potential use of Cas9-mediated gene drive systems for agricultural pest control. Journal of Responsible Innovation, 5, S98–S120.

Scott TW, Takken W, Knols BGJ and Boëte C, 2002. The ecology of genetically modified mosquitoes. Science, 298, 117–119.

Scudellari M, 2019. Self-destructing mosquitoes and sterilized rodents: the promise of gene drives. Nature, 571, 160–162.

Selck H, Adamsen PB, Backhaus T, Banta GT, Bruce P, Burton GA, Butts MB, Bøgh E, Clague JJ, Dinh KV, Doorn N, Gunnarsson J, Hauggaard-Nielsen H, Hazlerigg C, Hunka A, Jensen J, Lin Y, Loureiro S, Miraglia S, Munns WR Jr, Nadim F, Palmqvist A, Rämö RA, Seaby LP, Syberg K, Tangaa SR, Thit A, Windfeld R, Zalewski M and Chapman PM, 2017. Assessing and managing multiple risks in a changing world – the Roskilde Recommendations. Environmental Toxicology and Chemistry, 36, 7–16.

Serr ME, Valdez RX, Barnhill-Dilling KS, Godwin J, Kuiken T and Booker M, 2020. Scenario analysis on the use of rodenticides and sex-biasing gene drives for the removal of invasive house mice on islands. Biological Invasions, 00, 1–14. doi:10.1007/s10530-019-02192-6

Servick K, 2019. Mosquitoes armed with bacteria beat back dengue virus. Science, doi:10.1126/science.aba3223

Shaw RH, Tanner R, Djeddour D and Cortat G, 2011. Classical biological control of *Fallopia japonica* in the United Kingdom – lessons for Europe. Weed Research, 51, 552–558.

Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, Sawadogo SP, Dabire RK, Diabate A and Catteruccia F, 2016. *Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development. Nature Communications, 7, 11772.

Shelton AM, Long SJ, Walker AS, Bolton M, Collins HL, Revuelta L, Johnson LM and Morrison NI, 2020. First field release of a genetically engineered, self-limiting agricultural pest insect: evaluating its potential for future crop protection. Frontiers in Bioengineering and Biotechnology. doi:10.3389/fbioe.2019.00482

Shigesada N and Kawasaki K, 1997. Biological invasions: theory and practice. Oxford University Press, Oxford.

Sim SB, Kauwe AN, Ruano REY, Rendon P and Geib SM, 2019. The ABCs of CRISPR in Tephritidae: developing methods for inducing heritable mutations in the genera *Anastrepha*, *Bactrocera* and *Ceratitis*. Insect Molecular Biology, 28, 277–289.

Simmons GS, McKemey AR, Morrison NI, O'Connell S, Tabashnik BE, Claus J, Fu G, Tang G, Sledge M, Walker AS, Phillips CE, Miller ED, Rose RI, Staten RT, Donnelly CA and Alphey L, 2011. Field performance of a genetically engineered strain of pink bollworm. PLoS ONE, 6, e24110.

Simon S, Otto M and Engelhard M, 2018. Synthetic gene drive: between continuity and novelty. EMBO Reports, 19, e45760.

Simoni A, Siniscalchi C, Chan YS, Huen DS, Russell S, Windbichler N and Crisanti A, 2014. Development of synthetic selfish elements based on modular nucleases in *Drosophila melanogaster*. Nucleic Acids Research, 42, 7461–7472.

Sinkins SP, Braig HR and O'Neill SL, 1995. *Wolbachia pipientis*: bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus*. Experimental Parasitology, 81, 284–291.

Sinkins SP and Gould F, 2006. Gene drive systems for insect disease vectors. Nature Reviews Genetics, 7, 427–435.

Singh JA, 2019. Informed consent and community engagement in open field research: lessons for gene drive science. BMC Medical Ethics, 20, 54.

Slade G and Morrison N, 2014. Developing GM insects for sustainable pest control in agriculture and human health. BMC Proceedings, 8, O43.

Snow AA, 2019. Genetically engineering wild mice to combat Lyme disease: an ecological perspective. BioScience, biz080.

Sternberg SH and Doudna JA, 2015. Expanding the biologist's toolkit with CRISPS-Cas9. Molecular Cell, 58, 568–574.

Suter GW, 2000. Generic assessment endpoints are needed for ecological risk assessment. Risk Analysis, 20, 173–178.

Suter GW, Rodier DJ, Schwenk S, Troyer ME, Tyler PL, Urban DJ, Wellman MC and Wharton S, 2004. The US Environmental Protection Agency's generic ecological assessment endpoints. Human and Ecological Risk Assessment: An International Journal, 10, 967–981.

Tanaka H, Stone HA and Nelson DR, 2017. Spatial gene drives and pushed genetic waves. Proceedings of the National Academy of Sciences of the United States of America, 114, 8452–8457.

Taning CNT, Van Eynde B, Yu N, Ma S and Smagghe G, 2017. CRISPR/Cas9 in insects: applications, best practices and biosafety concerns. Journal of Insect Physiology, 98, 245–257.

Taylor C, Toure YT, Carnahan J, Norris DE, Dolo G, Traoré SF, Edillo FE and Lanzaro GC, 2001. Gene flow among populations of the malaria vector, *Anopheles gambiae*, in Mali, West Africa. Genetics, 157, 743–750.

Teem JL, Ambali A, Glover B, Ouedraogo J, Makinde D and Roberts A, 2019. Problem formulation for gene drive mosquitoes designed to reduce malaria transmission in Africa: results from four regional consultations 2016–2018. Malaria Journal, 18, 347.

Tepfer M, Racovita M and Craig W, 2013. Putting problem formulation at the forefront of GMO risk analysis. GM Crops and Food: Biotechnology in Agriculture and the Food Chain, 4, 1–6.

Thizy D, Emerson C, Gibbs J, Hartley S, Kapiriri L, Lavery J, Lunshof J, Ramsey J, Shapiro J, Singh JA, Pare Toe L, Coche I and Robinson B, 2019. Guidance on stakeholder engagement practices to inform the development of areawide vector control methods. PLoS Neglected Tropical Diseases, 13, e0007286.

Thomas M, Burgio G, Adams DJ and Iyer V, 2019. Collateral damage and CRISPR genome editing. PLoS Genetics, 15, e1007994.

Thompson PB, 2018. The roles of ethics in gene drive research and governance. Journal of Responsible Innovation, 5, S159–S179.

Thomson MC, Connor SJ, Quinones ML, Jawara M, Todd J and Greenwood BM, 1995. Movement of *Anopheles gambiae* s.l. malaria vectors between villages in The Gambia. Medical and Veterinary Entomology, 9, 413–419.

Turelli M and Hoffmann AA, 1991. Rapid spread of an inherited incompatibility factor in California *Drosophila*. Nature, 353, 440–442.

Turner G, Beech C and Roda L, 2018. Means and ends of effective global risk assessments for genetic pest management. BMC Proceedings, 12, 13.

Unckless RL, Clark AG and Messer PW, 2017. Evolution of resistance against CRISPR/Cas9 gene drive. Genetics, 205, 827–841.

US EPA (US Environmental Protection Agency), 1998. Guidelines for ecological risk assessment. Washington (DC), USA: USEPA Risk Assessment Forum. EPA/630/R-95/002F. Available from: http://rais.ornl.gov/documents/ECOTXTBX.PDF

US EPA (US Environmental Protection Agency), 2017. Final Registration Decision of the New Active Ingredient *Wolbachia pipientis* ZAP (*w*Pip) strain in *Aedes albopictus*. PC Code: 069035. Available from: https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0205-0034

Van den Brink PJ, Boxall AB, Maltby L, Brooks BW, Rudd MA, Backhaus T, Spurgeon D, Verougstraete V, Ajao C, Ankley GT, Apitz SE, Arnold K, Brodin T, Cañedo-Argüelles M, Chapman J, Corrales J, Coutellec M, Fernandes TF, Fick J, Ford AT, Giménez Papiol G, Groh KJ, Hutchinson TH, Kruger H, Kukkonen JV, Loutseti S, Marshall S, Muir D, Ortiz-Santaliestra ME, Paul KB, Rico A, Rodea-Palomares I, Römbke J, Rydberg T, Segner H, Smit M, van Gestel CA, Vighi M, Werner I, Zimmer EI and van Wensem J, 2018. Toward sustainable environmental quality: priority research questions for Europe. Environmental Toxicology and Chemistry, 37, 2281–2295.

van der Vlugt CJB, Brown DD, Lehmann K, Leunda A and Willemarck N, 2018. A framework for the risk assessment and management of gene drive technology in contained use. Applied Biosafety, 23, 25–31.

Varley GC, Gradwell GR and Hassell MP, 1973. Insect population ecology. An analytical approach. Blackwell Science, Oxford.

Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P, Lloyd AL, Ritchie SA, O'Neill SL and Hoffmann AA, 2011. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature, 476, 450–453.

Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McMeniman CJ, Leong YS, Dong Y, Axford J, Kriesner P, Lloyd AL, Ritchie SA, O'Neill SL and Hoffmann AA, 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature, 476, 450–453.

Waltz E, 2017. US government approves 'killer' mosquitoes to fight disease. Nature, doi:10.1038/nature.2017.22959

Ward CM, Su JT, Huang Y, Lloyd AL, Gould F and Hay BA, 2011. Medea selfish genetic elements as tools for altering traits of wild populations: a theoretical analysis. Evolution, 65, 1149–1162.

Warner CM, Lance RF, Crocker FH, Perkins EJ, Rycroft TE and Pokrzywinski KL, 2019. Synthetic biology: research needs for assessing environmental impacts. ERDC/EL TR-19-10 August 2019. Available from: https://apps.dtic.mil/dtic/tr/fulltext/u2/1078268.pdf

Webber BL, Raghu S and Edwards OR, 2015. Opinion: is CRISPR-based gene drive a biocontrol silver bullet or global conservation threat? Proceedings of the National Academy of Sciences of the United States of America, 112, 10565–10567.

Webster SH, Vella MR and Scott MJ, 2019. Development and testing of a novel Killer-Rescue self-limiting gene drive system in *Drosophila melanogaster*. bioRxiv. doi:https://doi.org/10.1101/680629

Wedell N, Price TAR and Lindholm AK, 2019. Gene drive: progress and prospects. Proceedings of the Royal Society B: Biological Sciences, 286, 20192709.

WHO (World Health Organization), 2014. Guidance framework for testing of genetically modified mosquitoes. Available from: https://www.who.int/tdr/publications/year/2014/guide-fmrk-gm-mosquit/en/

Williams AE, Franz AWE, Reid WR and Olson KE, 2020. Antiviral effectors and gene drive strategies for mosquito population suppression or replacement to mitigate arbovirus transmission by *Aedes aegypti*. Insects, 11, 52.

Wimmer EA, 2013. Insect biotechnology: controllable replacement of disease vectors. Current Biology, 23, R453–R456.

Windbichler N, Papathanos PA, Catteruccia F, Ranson H, Burt A and Crisanti A, 2007. Homing endonuclease mediated gene targeting in *Anopheles gambiae* cells and embryos. Nucleic Acids Research, 35, 5922–5933.

Windbichler N, Papathanos PA and Crisanti A, 2008. Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. PLoS Genetics, 4, e1000291.

Windbichler N, Menichelli M, Papathanos PA, Thyme SB, Li H, Ulge UY, Hovde BT, Baker D, Monnat RJ, Burt A and Crisanti A, 2011. A synthetic homing endonuclease-based gene drive system in the human malaria mosquito. Nature, 473, 212–215.

Wise de Valdez MR, Nimmo D, Betz J, Gong H-F, James AA, Alphey L and Black IV WC, 2011. Genetic elimination of dengue vector mosquitoes. Proceedings of the National Academy of Sciences of the United States of America, 108, 4772–4775.

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M and Wu F, 2010. Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Research, 19, 425–436.

Yakob L and Bonsall MB, 2009. Importance of space and competition in optimizing genetic control strategies. Journal of Economic Entomology, 102, 50–57.

Yakob L, Alphey L and Bonsall MB, 2008a. *Aedes aegypti* control: the concomitant role of competition, space and transgenic technologies. Journal of Applied Ecology, 45, 1258–1265.

Yakob L, Kiss IZ and Bonsall MB, 2008b. A network approach to modeling population aggregation and genetic control of pest insects. Theoretical Population Biology, 74, 324–331.

Yamamoto Y and Gerbi SA, 2018. Making ends meet: targeted integration of DNA fragments by genome editing. Chromosoma, 127, 405–420.

Zabalou S, Riegler M, Theodorakopoulou M, Stauffer C, Savakis C and Bourtzis K, 2004. *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. Proceedings of the National Academy of Sciences, 101, 15048-15045.

Zabalou S, Apostolaki A, Livadaras I, Franz G, Robinson AS, Savakis C and Bourtzis K, 2013. Incompatible insect technique: incompatible males from a *Ceratitis capitata* genetic sexing strain. Entomologia Experimentalis et Applicata, 132, 232–240.

Zhang D, Zheng X, Xi Z, Bourtzis K and Gilles JR, 2015. Combining the sterile insect technique with the incompatible insect technique: I impact of *Wolbachia* infection on the fitness of triple- and double-infected strains of *Aedes albopictus*. PLoS ONE, 10, e0121126.

Zhao S, Xing Z, Liu Z, Liu Y, Liu X, Chen Z, Li J and Yan R, 2019. Efficient somatic and germline genome engineering of *Bactrocera dorsalis* by the CRISPR/Cas9 system. Pest Management Science, 75, 1921–1932.

Zheng X, Zhang D, Li Y, Yang C, Wu Y, Liang X, Liang Y, Pan X, Hu L, Sun Q, Wang X, Wei Y, Zhu J, Qian W, Yan Z, Parker AG, Gilles JRL, Bourtzis K, Bouyer J, Tang M, Zheng B, Yu J, Liu J,

Zhuang J, Hu Z, Zhang M, Gong JT, Hong XY, Zhang Z, Lin L, Liu Q, Hu Z, Wu Z, Baton LA, Hoffmann AA and Xi Z, 2019. Incompatible and sterile insect techniques combined eliminate mosquitoes. Nature, 572, 56–61.

ZKBS (Zentrale Kommission für die Biologische Sicherheit), 2016. Position statement of the ZKBS on the classification of genetic engineering operations for the production and use of higher organisms using recombinant gene drive systems. Available from: http://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/02_Allgemeine_Stellungnahmen_englisch/general_subjects/Gene_drive_systems_2016.html;jsessionid=0A3A482247799626AF97D0420DE919D9.2_cid322?nn=8569924#download=1

11 Abbreviations

BCA: Biological control agent

Cas9: CRISPR associated protein 9

CBC: Classical biological control

CI: Cytoplasmic incompatibility

ClvR: Cleave and rescue

CRISPR: Clustered regularly interspaced short palindromic repeats

CSM: Case-specific monitoring

DSB: Double strand break

dsRNA: double stranded RNA

ES: Ecosystem service

EFSA: European Food Safety Authority

ERA: Environmental risk assessment

EU: European Union

fsRIDL: Female-specific lethal RIDL

GDMI: Gene drive modified insect

GDMO: Gene drive modified organsism

GM: Genetically modified

GMA: Genetically modified animal

GMI: Genetically modified insect

GMO: Genetically modified organism

GS: General surveillance

gRNA: Guide RNA

HDR: Homology-directed repair

HEG: Homing endonuclease gene

HGT: Horizontal gene transfer

HR: Homologous recombination

IIT: Incompatible insect technique

M element: Medea element

MC: Molecular characterisation

mRNA: Messenger RNA

miRNA: Micro-RNA

MMEJ: Microhomology-mediated end joining

NHEJ: Non-homologous end joining

ORF: Open reading frame

PI: Pathogen interference

PMEM: Post-market environmental monitoring

RIDL: Release of insects carrying either a dominant lethal

RNAi: RNA interference

SDN: Site directed nuclease

sgRNA: Single guide RNA

SIT: Sterile insect technique

TALEN: Transcription activator-like effector nuclease

TARE: Toxin-antidote recessive embryo

ZFN: Zinc finger nuclease

12 Appendices

Appendix A – Main participant comments raised at EFSA's stakeholder workshop "Problem formulation for the environmental risk assessment of gene drive modified insects" (Brussels; 15 May 2019)

1 Gene drive strategies

a) Criteria to categorise gene drives were addressed, as gene drives are not all the same and encompass different molecular mechanisms. Some participants suggested consideration of the following dimensions to categorise gene drives [The GMO Panel considered this point in Section 3.2]:
    - Spread characteristics (temporally or spatially restricted vs. unrestricted gene drives);
    - Impact (population replacement vs. suppression);
    - Threshold dependency or not;
b) A participant indicated that gene drives can change from a category to another as they spread within a target population. Reference was made to a hypothetical example of a replacement gene drive that would change the host finding behaviour of the target insect. Theoretically, this could result in individuals feeding on another plant species, leading to population decline and thus suppression [The GMO Panel took note of this point];
c) There was discussion on whether the use of heritable microorganisms such as *Wolbachia* endosymbionts should be considered a synthetically engineered gene drive, as neither the host organism nor *Wolbachia* are genetically modified. It was noted that *Wolbachia* has a gene drive-like inheritance pattern that has been harnessed in replacement strategies to limit disease transmission in some mosquito populations [The GMO Panel considered this point in Section 5.2.1].

2 Potential novel hazards/risks

a) Some participants indicated that the deliberate release into the environment of gene drive modified insects (GDMIs) would pose novel hazards/risks (in terms of their spatial and temporal scale, persistence, potential for self-replication, uncontrolled spread) with little or no opportunity for recall. They argued that applications for GDMIs are demonstrably different from other applications with genetically modified organisms (GMOs), as they deal with very heterogeneous and diverse natural systems and non-managed species, instead of controlled environments (such as agroecosystems). They also mentioned that gene drives may eventually spread over entire continents and establish across national borders, raising issues of transboundary movements and international governance [The GMO Panel considered these points in Sections 1 and 6];
b) Some other participants considered that GDMIs would not pose new harms compared with genetically modified insects (GMIs), but that such harms might be more likely due to their repeated cycles of reproduction, or might lead to more severe environmental effects [The GMO Panel considered this point in Sections 6 and 8];

c) Several participants did not consider concerns pertaining to the suppression of insect pest populations as novel; they argued that such an effect is not unique to gene drive technology. Humans have aimed at controlling or eradicating insect pests through a variety of methods for many years. Consequently, environmental impacts of GDMIs should be evaluated against those of alternative actions (i.e. sterile insect releases, classical biological control programmes), including no action. This experience is considered useful to inform the ERA of GDMIs and put risks in a broader perspective. In their view, the use of synthetically engineered gene drives should be seen as complementing the range of genetic methods of insect pest control [The GMO Panel considered this point in Section 6].

3 Risk assessment paradigm

a) Participants had opposing views on whether the existing framework for the risk assessment of GMOs would be sufficiently robust to assess the potential adverse effects associated with the deliberate release into the environment of GDMIs [The GMO Panel considered these points in Section 8]:
    - Some participants considered that the deliberate release into the environment of GDMIs will challenge the current environmental risk assessment (ERA) paradigm, as it will be difficult or impossible to predict their ecological impact, control any unintended effects, or to manage risks, especially with regard to potential long-term adverse effects. Moreover, they argued that the classical methods used in risk assessment such as the comparative and stepwise testing approach target crop plants and animals that typically do not spread on their own in the environment. With synthetically engineered gene drives, the intention is for them to spread into interbreeding populations in the environment. Consequently, the current ERA paradigm may be not generally appropriate for testing GDMIs. In addition, they felt that judging the sufficiency of scientific knowledge and the extent to which uncertainty should be reduced for decision-making would be impossible for gene drive applications;
    - Some other participants considered that the current ERA frame, pending revisions, should remain appropriate. They noted that the tiered-based testing, stepwise and weight of evidence approaches, and appropriately designed modelling and post-market environmental monitoring (PMEM) would provide the necessary safeguards to manage potential risks and uncertainty linked to the deliberate release into the environment of GDMIs.

4 Familiarity with/experience from existing insect vector/pest control strategies

a) Similarities between the use of synthetically engineered gene drives for insect vector/pest control and some well-established insect vector/pest control strategies (e.g. biological or chemical insecticides, resistant crop varieties, biological control, and genetic control methods such as the sterile insect technique (SIT) or incompatible insect technique (IIT)) were addressed. It was noted that substantial regulatory and ERA experience has been gained, which could be used to identify information/data

requirements for the ERA of GDMIs [The GMO Panel considered this point in Sections 1.1 and 5];

b) Some other participants did not consider existing vector/pest control methods such as *Wolbachia* and SIT suitable comparative systems to predict potential long-term effects associated with the deliberate release of GDMIs [The GMO Panel considered this point in Section 7.1.3.3].

5 Problem formulation

a) The usefulness of problem formulation as an approach to frame the ERA of GDMIs was addressed. Overall, most participants were in agreement that the problem formulation process is fit-for-purpose for GDMIs, but it was acknowledged that practical challenges may be encountered. Moreover, some participants indicated that it is complicated to apply problem formulation to a technology in a generic way; instead, it may be easier to apply problem formulation to concrete/specific cases [The GMO Panel considered this point in Section 7.1.2.1];

b) Participants raised the following points on the identification of relevant broad protection goals and how to make them operational [The GMO Panel considered these points in Section 7.1.2.1]:
   - Policy goals are defined broadly. Consequently, there is a need to translate policy goals into operational goals for use in ERA. Operational protection goals can be case-dependent. For example, the level of tolerable harm may differ depending on the pest status of the modified species (e.g. whether it is known to be invasive/harmful or protected in a specific jurisdiction);
   - The setting of protection goals involves normative considerations (e.g. about the tolerable level of harm). Given that risk assessors cannot define protection goals alone, an improved dialogue between risk managers and risk assessors, and stakeholder engagement for the definition of operational protection goals were advocated;
   - Since the overarching goal of ERAs conducted for regulated stressors (such as pesticides, GMOs, invasive species and biocides) is to protect the same environment, some participants considered that protection goals should be similar for all regulated stressors;
   - A list of protection goals, covering among others human and animal health, biodiversity, ecosystems, water quality, genomic purity, were briefly presented for the case studies used during the workshop. Some of these protection goals are not explicitly addressed by EU legislation (i.e. genomic purity of wild type/target organisms);

c) Participants raised the following points on the elaboration of pathways to harm [The GMO Panel considered these points in Section 7.1.2.1]:
   - Various pathways could lead to a range of harms (e.g. removal of target population, loss of efficacy due to resistance evolution), and they can vary depending on the gene drive characteristics;
   - It was noted that pathways to harm can be complex, as there may be more than one pathway to consider, while multiple pathways may share some of the same steps;

- Gene drive efficacy affects pathways to harm, so it was generally considered as a first step in any pathway to harm – Speed and success of suppression are inversely related to likelihood of harm;
- Some participants considered that pathways to harm would not differ between genetically modified (GM) mosquitoes and gene drive modified ones. They were of the opinion that the likelihood of already existing hazards would be increased, but no novel harms or new pathways would necessarily be associated with GDMIs. In contrast, some other participants argued that the intended persistence of self-sustaining gene drives will make the construct persist over the generations, which changes pathways to harm owing to increased exposure and the potential for evolutionary responses;

d) Participants raised the following points on the formulation of risk hypotheses about the likelihood and severity of possible harmful events [The GMO Panel considered these points in Section 7.1.2.1]:
   - Some participants questioned whether rare or unlikely events can be appropriately considered in the problem formulation process. Such events may potentially have substantial environmental consequences, especially in the case of self-sustaining and low threshold gene drives;
   - Some other participants noted that rare or unlikely events would not necessarily translate into harm; only those that may be harmful should be considered further in ERA. They therefore emphasised the need to link hazard to an exposure, and not to confuse hazard or exposure with risk;

e) Participants raised the following points on the identification of possible information that would be useful to test these risk hypotheses [The GMO Panel considered these points in Section 7.1.2.1]:
   - Should all possible pathways to harm be considered for testing, irrespective of their plausibility, or only the plausible ones? According to some participants the testing of all possible pathways to harm is the only way forward to avoid overlooking unintended effects and unknowns. Others considered that problem formulation is sufficiently robust to capture uncertainties by identifying issues that require further data for risk assessment purposes. Consequently, in their view, only plausible pathways should be taken into account, as it is unfeasible to test them all. They suggested to prioritise pathways based on their level of validity and plausibility, and transparently report the rationale justifying why specific pathways are not considered plausible (e.g. based on evidence from the scientific literature);
   - The comparative nature of risk assessments of GMOs was challenged by some participants, as they argued that absolute harms/risks should be quantified when conducting ERAs, instead of relative ones;
   - It was briefly discussed whether the risk assessment should consider if a proposed activity may lead to new harms/risks, or only to different ways of causing harm that already result from current practice, as this helps to put potential impacts in the context of those caused by existing practices.

6 Potential harms

a) Several harms, covering among others the loss of gene drive efficacy due to resistance evolution, dispersal of GDMIs beyond the target release area, loss of biodiversity due to

hybridisation, disruption of the food web due to the removal of the target organism, loss of immunity, altered immune response following mosquito biting, were briefly presented for the two case studies used during the workshop and further discussed. Some participants indicated that:

- It was questioned whether CRISPR-Cas9-based gene drives would fully replace or suppress wild populations due to the potential for resistance to the gene drive to evolve. Resistance evolution should be carefully considered in ERA. Modelling predictions and laboratory experiments suggest resistance to evolve to CRISPR/Cas9-based gene drives, which could slow or prevent the gene drive's ability to be preferentially inherited [The GMO Panel considered this point in Section 7.1.4.4];
- There are no clear indications that all gene drives would spread in a similar and uncontrolled manner after their release. Self-sustaining gene drives are expected to be highly invasive provided that the evolution of resistance alleles can be minimised [The GMO Panel considered this point in Section 3.3.2];
- Intermediate effects might take place if the goal of the gene drive is not achieved rapidly [The GMO Panel considered this point in Section 7.1.2.1];
- A better understanding of the ecological and evolutionary impacts of GDMIs for deliberate release into the environment is required due to the extended spatial scale and time scale at which gene drives may operate. This may allow for evolutionary processes to take place, a greater range of ecological interactions to occur and a higher potential of transboundary movement [The GMO Panel considered this point in Section 4];
- Uncertainty may be higher for population replacement strategies than for population suppression strategies, as they require the modification to persist in the environment. However, for both strategies it is expected that the GDMIs will interact with wild type populations that have heterogeneous genetic backgrounds [The GMO Panel considered this point in Section 3.2.1];
- In situations where there is both insufficient sterility and subsequent control b continuing SIT releases, the persistence and invasiveness of the factory genome in the wild type population may impact native/wild type genetic diversity. However, this would not be exclusive to GDMIs, as it could also happen with non-GMI comparators such as classic SIT approaches [The GMO Panel considered this point in Section 7.1.4.1];
- Potential interactions between different GDMIs intended to be deliberately released simultaneously into the environment should be considered, in order to address possible combinatorial effects [The GMO Panel took note of this point].

7 Comparators

a) The selection and suitability of comparators were discussed [The GMO Panel considered some of the below points in Section 7.1.3.3]. Some participants raised the following points:
    - For malaria-transmitting mosquitoes, comparators should be the unmodified mosquitoes in the presence of commonly used control measures (such as insecticides) – No comparison should be made in the absence of existing control measures;
    - Alternative control methods should be considered (i.e. organic farming);

- In some cases, no other control measures may be available (e.g. for *Drosophila suzukii* no native biological control agents have been found in Europe and insecticides may not always provide effective control);

b) Removing an invasive species from a receiving environment using gene drives would not necessarily lead to the situation that existed before, given that other measures that have been taken (netting, insecticides) and which can impact biodiversity could be kept in place even after the invasive species has been removed [The GMO Panel took note of this point].

8 Receiving environments

a) There are generic factors to consider when addressing the receiving environments [The GMO Panel took note of the below points]:
   - Genotype × environment interactions: Some participants questioned whether knowledge of organisms in a given receiving environment can be extrapolated to another receiving environment;
   - Possible interactions of the gene drive with other vector/pest control methods that might become more relevant in the context of climate change.

9 Risk management

During the workshop some participants raised the following risk management-related points [The GMO Panel took note of the below points, but did not consider them further, as they are not in the Panel's remit]:

a) Only self-limiting gene drives (which are restricted either spatially, temporally, or both) and reversal gene drives should be proposed for deployment. However, it was noted that reversal gene drives, which are designed to mitigate potential unintended consequences of another drive, may induce further changes that may undo a phenotypic alteration caused by the initial drive, so they may not restore the original modification to the wild type or redress fully ecological effects from the original drive;
b) The most plausible approach to the deliberate release into the environment of gene drive modified organisms is on islands due to the lower genetic drift, which would result in lower sequence variability of the targeted gene drive;
c) There is deep concern that gene drive technology would be used as a biological weapon for military purposes;
d) Both risks and benefits should be considered by risk managers. This requires the risk assessment to be completed with a benefit assessment;
e) For homing endonuclease gene (HEG)-based gene drives some participants indicated that the inserted sequence would be the only traceable element for traceability purposes when the drive moves through the target population;
f) Possible delays encountered in the regulatory process should be avoided, as by the time one gets clearance to deliberately release a GDMI into the environment, the receiving environment considered during the ERA may have changed. For example, an invasive species might have been outcompeted or got established. Some other participants indicated that this should not be a concern, as applicants are typically asked to keep their ERA up to date;

g) Dialogue with risk managers from the very early stages of gene drive development would be useful to explore if potential adverse effects associated with their use (in comparison with existing insect pest control strategies) are acceptable or not;
h) The business model to deliberately release into the environment of gene drive systems for commercial purposes would be driven by the potential for resistance to evolve and thus allow applicants to market gene drives every few years. Other participants sensed the business model would be more similar to that of vaccines, given their potential to protect whole nations, but that the approach followed would be case-specific;
i) The amount and nature of risk assessment information/data required for systems designed to suppress pest populations with insecticides, crop resistance, mechanical or habitat modification are not the same as for GDMIs, though such control systems may have similar long-term population suppression effects on target organisms, achieved through different mechanisms;
j) The precautionary principle does not provide sufficiently definite guidance on how to balance potential risks of GDMIs for deliberate release into the environment with the protection of the environment. Some participants considered that the deployment of gene drive strategies in insects can be compatible with the precautionary principle, as it states that "*where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation*". However, since GDMIs designed for self-sustaining vector/pest control can have effects that may be unlimited in space and time, without an obvious way of containing or reversing environmental impacts, some other participants argued that the application of the precautionary principle would preclude the deliberate release of GDMIs;
k) Self-sustaining gene drives may eventually spread over entire continents and establish across national borders, raising issues of transboundary movements and international governance to address under the Convention on Biological Diversity and its Cartagena Protocol on Biosafety.

10Post-market environmental monitoring

a) For HEG-based gene drives some participants indicated that the inserted sequence would be the only traceable element for monitoring purposes when the drive moves through the target population. Some other participants indicated that molecular markers could be used such as a fluorescent marker [The GMO Panel took note of this point];
b) Some participants considered that it is necessary to establish baselines in the context of monitoring, as this will enable us to check whether an ecosystem has shifted or not [This point is addressed in EFSA (2013)].