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Study on Risk Assessment Application of annex I of decision CP 9/13 to living modified organisms containing engineered gene drives

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On behalf of the Secretariat of the Convention on Biological Diversity

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Disclaimer

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Executive summary

Gene drives allow for a trait to be distributed across generations deviating from the laws of Mendelian inheritance. Active in sexually-reproducing species, they are powerful tools to "drive" a gene, *i.e.* increase its frequency, independent of external selection pressure. They have been proposed as offering solutions for challenges in public health, agriculture, conservation and others. They have inspired researchers to use gene drives to combat diseases transmitted by insects such as malaria, dengue and Zika. For decennia attempts have been made to use or modify naturally occurring gene drive mechanisms. In recent years advances in genetics have allowed for modifying natural gene drive systems and the development of synthetic gene drive systems (or engineered gene drives). With these new developments, in particular those based on genetic modification, concerns on safety and potential negative impacts on the environment and biodiversity have been raised.

This study is intended to inform the application of Annex I of decision CP-9/13 to LMOs containing engineered gene drives for the process for recommending and prioritizing specific issues of risk assessment for consideration under the Cartagena Protocol on Biosafety mentioned on paragraph 6 of that decision. It is intended to be presented to the open-ended online forum and to serve as one of the inputs to the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management.

Information was collected from scientific publications, stakeholder meetings and communications, interviews with authorities and risk assessors, available risk assessment processes and finally from the on-line discussions on the first draft of this report. The report attempts to reflect the broad range of considerations on the (potential) applications of gene drives and related risk assessment.

To date, research on LMOs with engineered gene drives has remained limited to laboratory, contained environments and population modelling. However, some applications are close to being released in trials.

The diversity of gene drive systems as well as the possible applications necessitate a case-by-case approach. However, a number of considerations on risk assessment were identified in relation to engineered gene drive systems and their most advanced applications:

- **Relating to applications of genetically engineered gene drives**

  Unlike LMOs that have been selected, tested and presented as finished and well-characterized products; engineered gene drives imply the release of a tool that will continue to trigger further genetic modification of individuals in the target species.

  The most advanced applications of engineered gene drives target non-domesticated or wild species, for which the choice of comparators may be challenging. At the same time, the most advanced engineered gene drive applications target releases in non-managed or uncontrolled environments, for which there is less experience in conducting risk assessments.

The predicted ease of spread of engineered gene drive organisms, in particular for low threshold systems[1], combined with the possibility that an introduction is irreversible calls for an extremely thorough evaluation under careful confinement before deciding on release into a hospitable environment. This may require in particular cases rethinking the limitations of the development phases in a stepwise approach.

- **Relating to effects on the gene drive-bearing organism**

Off-target effects within the recipient organism is a concern for engineered gene drive systems that are based on gene editing techniques and RNAi methods. Gene driver-cargo systems[2] risk to unlink the payload gene, *e.g.* that targets a pathogen, and the elements of the drive system. Finally, the vector organism may show a modified competency for transmission of other pathogens.

- **Relating to biodiversity**

Regarding the potential effects on the environment a distinction should be made between population suppression and population replacement drives. Suppression gene drives may result in the removal of a local population of the target organism or the extinction of that species. The extent of the effects on the ecosystem depends on whether the target organism is a “keystone” species in the environment, and/or whether there are ecological equivalents present.

Since gene drives are based on mating potential, the potential for exchange with related species is very species specific. Unintentional transfer of an engineered gene drive into a beneficial, threatened, endangered, neutral, or valued species could lead to its extinction.

Extinction, or reduction of abundance, of the gene drive-carrying species can have consequences for *e.g.* predators, competitors, prey, due to its ecological role, such as resource, consumer, competitor, or disease vector.

- **Relating to resistance development**

The presence or development of resistance against an engineered gene drive system, will reduce its efficiency in the host population, but will also limit the potential impact. Resistance may also be used deliberately as part of a scheme aiming to confine the engineered gene drive to a limited geographical area or a certain time period.

- **Relating to effects beyond the target area**

The spread of an engineered gene drive outside the intended geographical area could potentially have environmental impacts well beyond the site of its introduction. Especially low threshold drives may have widespread consequences across national borders. Concerns arise from the accidental release of just a few organisms containing engineered gene drive systems. Technical solutions are being explored aiming to confine engineered gene drives.

[1] A low threshold gene drive needs only a small amount of gene drive-bearing organisms to replace or suppress a population, whereas high threshold gene drives need a large amount relative to the target population.

[2] A gene drive system that contains, next to the genetic elements that comprise the driving mechanism, also one or more genes (cargo or payload genes) that will induce the intended effect on the target population.

- **Relating to perspectives of indigenous peoples and local communities**

  In particular when the broad spread of an LMO with a genetically engineered drive is likely, it will be challenging for instance, to obtain the free, prior and informed consent of IPLCs, as covered in decision 14/19 on Synthetic Biology. Several recent papers have provided examples and proposed tools to define acceptance.

Annex I of decision CP-9/13 provides criteria for which a structured analysis is required. As basis for this structured analysis, the obtained information was reviewed in the light of the specified criteria for the case of engineered gene drives. The following summarizes the findings per criterion:

- **Issue identified by Parties as priority**

  Given the type of application, many developing countries and countries with economies in transition are confronted with or anticipate to be soon confronted with possible applications of engineered gene drive systems, if not directly than possibly by via transboundary movement. Contributors from countries with less experience in LMO risk assessment indicated to be uncertain on how to evaluate certain aspects (*e.g.* gene flow), and although they are relevant for all LMOs, they are considered essential for engineered gene drives.

- **Issue within the scope and objective of the Cartagena Protocol**

  LMOs with engineered gene drives result from modern biotechnology, as defined in the Cartagena Protocol. All interviewees acknowledged that LMOs with engineered gene drives may have adverse effects on the conservation and sustainable use of biological diversity and that a thorough risk assessment should precede any decision on intended introduction in the environment.

- **Issue poses challenges to existing risk assessment frameworks**

  The interviewees differentiated between challenges to the risk assessment methodology and challenges relating to obtaining information required to inform the risk assessment.

  Most interviewees anticipated that it will be possible to use existing risk assessment methodology for evaluating LMOs with engineered gene drives, which is largely based on problem formulation methodology and provides a structured and systematic approach for addressing risk assessment. At the same time, different proposals have been made to further improve the methodology for conditions of high uncertainty.

  Although no actual releases have been performed, there is globally a lot of preparatory activity involving international expert meetings, problem formulation workshops and development of guidance Examples cited in this report are listed at the end of this summary. At the same time, most of the considerations that were raised in literature and identified during this study, are not specific for LMOs with an engineered gene drive. Nevertheless, the fact that some engineered gene drive systems are seen to have the power to result in an irreversible impact on a species at global level, requires international understanding on common protection goals. Some interviewees highlighted the lack of clarity on the level of acceptable risks; *e.g.* the discussion on outcrossing of LMOs has not clarified what level of outcrossing would be acceptable in this context.

While the risk assessment methodology may still be applicable, different aspects are essentially distinguishing engineered gene drive-bearing organisms from LMOs assessed so far and these differences may require new approaches to provide information for the risk assessment. These technical and methodological challenges will likely render the risk assessment for engineered gene drive applications more detailed and more complex, also requiring public consultation.

The following possible consequences of these differences have been indicated by interviewees and need to be considered on a case-by-case basis:

- some of the assessment principles such as the comparative approach may not be fit for purpose anymore;
- a "stepwise" approach may not be applicable since the smallest scale introduction (*e.g.* field trial) of an LMO with a low threshold gene drive might result in spread and a permanent impact. This would limit the ability to do field tests, which are however required to obtain information for the risk assessment in order to decide on subsequent introductions;
- using robust models to predict long-term and ecosystem effects are required to support risk assessment;
- concepts like the "receiving environment" must be revisited in function of the release of wild species as opposed to domesticated species, such as crop plants or livestock that are to a significant degree controlled by man. Information on the ecological context of wild populations is required to feed the risk assessment. Only patchy knowledge is available, not in line with the complexity of the potential broad temporal and geographical scope. This context is also needed to have reliable predictive modelling.

- **Issue for which the challenges are clearly described**

Although several considerations have been identified, the only way to clearly describe specific issues is in relation to specific cases. Generic discussions are confounded by extrapolation of specific cases. Most of the considerations are relevant for the host organism, the introduced trait or the receiving environment.

- **Specific issues for engineered gene drives**

All interviewees agreed that LMOs with engineered gene drives have the potential to cause serious and/or irreversible adverse effects on biodiversity. Some considered that such an impact could only be envisaged in very specific, worst-case scenarios. Others expressed concern that the available information is insufficient to judge on what would lead to a worst-case scenario.

Once released, there is a potential to disseminate across borders. Again, this is not a characteristic *per se* of gene drives, rather of the host organism. However, as engineered gene drive applications today mostly target non-domesticated species, there will be less (or no) possibility for preventing crossing national borders. Whereas the introduction of a domesticated species is largely driven by market realities and controlled by humans, the distribution of non-domesticated species follows ecological habitats.

Finally, a stock-taking exercise was undertaken to determine if resources on similar issues have been developed by national, regional and international bodies. Resources identified during this study are listed and briefly described how they may provide a background for risk

assessments for releases into the environment of LMOs with engineered gene drives. Some of these examples are: work by the Australian Academy of Science, EFSA, High Council for Biotechnology in France, NASEM, workshops convened by the FNIH, workshops in Africa organized by the New Partnership for Africa's Development.

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List of abbreviations

ACB	African Centre for Biosafety
CBD	Convention on Biological Diversity
COGEM	the Netherlands Commission on Genetic Modification
COP-MOP	Conference of the Parties serving as the meeting of the Parties
CRISPR/Cas	Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR associated protein
DNA	Deoxyribonucleic acid
EFSA	European Food Safety Authority, EU
ERA	Environmental risk assessment
FNIH	Foundation for the National Institutes of Health, USA
GMO	Genetically modified organism
HCB	High Council for Biotechnology, France
HDR	Homology-directed repair
HEG	Homing endonuclease gene
IIT	Incompatible insect technique
IPLCs	Indigenous peoples and local communities
LMO	Living modified organism
MEDEA	Maternal-effect dominant embryonic arrest
NASEM	National Academies of Sciences Engineering and Medicine, USA
NGO	Non-governmental organization
OGTR	Office of the Gene Technology Regulator, Australia
PRRAF	Procedurally Robust Risk Assessment Framework
R&D	Research and Development
RNA	Ribonucleic acid
SIT	Sterile insect technique
TALEN	Transcription activator-like effector nuclease
TWN	Third World Network
UN	United Nations
UNEP	United Nations Environment Programme
ZFN	Zinc finger nuclease
ZKBS	Zentrale Kommission für die Biologische Sicherheit, Germany

1 Introduction

Annex III of the Cartagena Protocol provides an outline for a risk assessment methodology for activities with living modified organisms (LMOs). Furthermore, the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management with input from the Online Forum Risk Assessment and Risk Management, produced the voluntary "Guidance on Risk Assessment of Living Modified Organisms" (UNEP/CBD/BS/COP-MOP/8/8/Add.1). At the same time, the Conference of the Parties serving as the meeting of the Parties (COP/MOP) to the Cartagena Protocol on Biosafety, through decision CP-VIII/12 adopted at its eighth meeting in 2016, invited Parties to submit to the Executive Secretary:

a) information on their needs and priorities for further guidance on specific topics of risk assessment of living modified organisms,
b) proposals on criteria, including the technical justification, that may facilitate the selection of topics for the development of further guidance,
c) views on perceived gaps in existing guidance materials.

The ninth meeting of the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety decided on a process for recommending and prioritizing specific issues of risk assessment for consideration. The process should include a structured analysis of a set of criteria defined in Annex I of decision CP-9/13 as well as a stock-taking exercise of resources on similar issues. The COP-MOP also decided to consider at its tenth meeting, whether additional guidance materials on risk assessment are needed for living modified organisms containing engineered gene drives, and requested the Executive Secretary to commission a study informing the application of annex I to living modified organisms containing engineered gene drives, to facilitate the process referred to in paragraph 6 of the decision, and present it to the open-ended online forum and Ad Hoc Technical Expert Group on Risk Assessment and Risk Management.

This study is intended to inform this evaluation process, focussing on to the criteria defined in Annex I of decision CP-9/13 as well as the stock-taking exercise applied to LMOs containing engineered gene drives. It is intended as input for the discussions of the open-ended online forum and for the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management.

The authors present this report as a reflection of the broad range of communications on the (potential) application of engineered gene drives and related risk assessment, realizing that a selection of the available information was required for the report to be concise and comprehensive. The selection was guided by inclusivity aiming to provide a view on a broad range of considerations, rather than presenting detailed opinions.

2 Methodology

The detailed workplan of the project contained different components as summarized in Figure 1.

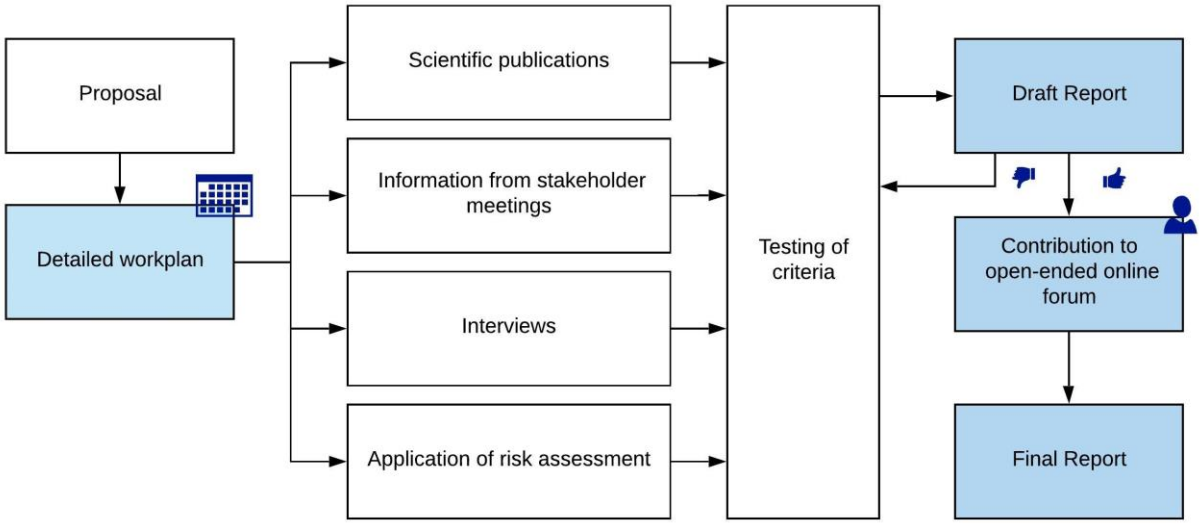

Figure 1 Schematic representation of the different elements of the study and the deliverables (blue boxes)

2.1 Comprehensive, systematic study of scientific publications

A systematic literature review was undertaken compiling scientific information related to engineered gene drives. The literature review built upon research done by Perseus in the context of another study on gene drive systems (Rüdelsheim and Smets, 2018). A pre-specified protocol, including research question, search strategy, inclusion/exclusion criteria for the articles and methods for the analysis, was developed before the beginning of the study. The setup of the review is described in Annex 1 and was done according to the typical consecutive steps of a systematic review.

2.2 Information from stakeholder meetings

2.2.1 CBD Fora

Different activities under the Cartagena Protocol provide valuable input on considerations expressed by a diversity of stakeholders. In particular we mention:

- Submissions made following the decision on risk assessment from COP-MOP 8 (decision CP-VIII/12 from 2016)

  Submissions made by Austria, Belarus, Bolivia, Bulgaria, Finland, France, Republic of Moldova, Republic of South Africa mention engineered gene drives, mostly in connection with synthetic biology.

- Related discussions in an online forum.

Table 1 lists the number of interventions that refer to engineered gene drives in the specific online forums.

Table 1. CBD online fora that were reviewed in this study

Topic of the online forum	# Replies	
	Total	Related to gene drives
Experiences on risk assessment of living modified organisms	26	1
Information and views on existing guidance materials on risk assessment	28	0
Perceived gaps in existing guidance materials on risk assessment, and proposals to address any gaps identified	56	17

- Submissions made following the COP-MOP 9 decision on risk assessment relevant to the work to be undertaken in the 2019-2020 inter-sessional period.

  Paragraph 10 in decision CP 9/13, COP-MOP invited Parties, other Governments, indigenous peoples and local communities, and relevant organizations to submit to the Executive Secretary information relevant to the work of the online forum and the Ad Hoc Technical Expert Group. Submissions were invited on information related to:

  - Experience in undertaking risk assessment of living modified organisms containing engineered gene drives and living modified fish (detailing how and for which cases); or else, lack of experience in doing so;
  - Challenges experienced or foreseen in undertaking risk assessment of living modified organisms containing engineered gene drives and living modified fish;
  - Specific needs (if any) to properly undertake risk assessment of living modified organisms containing engineered gene drives.

  Submissions were made by the African Centre for Biosafety (ACB), Australia, Austria, Bangladesh, Bolivarian Republic of Venezuela, Brazil, Bulgaria, Côte d'Ivoire, Czech Republic, Environmental Health Safety Consultancy Ltd. (Kenya), Ethiopia, European Union, Finland, France, Germany, Global Industry Coalition, Islamic Republic of Iran, Japan, Malaysia, Mexico, Netherlands, Nigeria, Republic of South Africa, Spain, Sweden, Testbiotech, Third World Network (TWN), United States of America and Zimbabwe

All submissions and interventions were analysed and summarized as part of this study.

2.2.2 Consultative meetings, workshops and symposia

Consultative meetings, workshops and symposia that addressed the environmental risk assessment of engineered gene drive technology were identified as an additional source of information. In as much as information (presentations, proceedings) was publicly available, it was accessed. Such information was handled with care as it may reflect the position of a particular speaker and/or organizer and is not peer reviewed. Nevertheless, it was considered as supportive material to help identify different considerations relevant for the purpose of the study. Table 2 lists the meetings, workshops and symposia that were reviewed in preparing this study.

Table 2. Consultative meetings, workshops and symposia that were reviewed in this study

Meeting (Main organizer(s))	Location / Dates
Gene Drives to Control Insect-Borne Human Disease and Agricultural Pests: A Workshop to Examine Regulatory and Policy Issues (J. Craig Venter Institute, UC San Diego)	San Diego, CA, USA 20 21 January 2016
Roadmap to Gene Drives: A Deliberative Workshop to Develop Frameworks for Research and Governance (Genetic Engineering and Society (GES) Center, North Carolina State University)	Raleigh, NC, USA 24 – 26 February 2016
Problem Formulation for the Use of Gene Drive Technology in Mosquitoes (Foundation for the National Institutes of Health)	Reston, VA, United States 25 – 27 May 2016
West Africa Consultative Meeting on Gene Drive Technology (New Partnership for Africa's Development (NEPAD) Agency).	Accra, Ghana 16 – 19 October 2016
Gene Drive: Regulatory, Legal and Ethical Issues (Centre for Science & Policy / Centre for the Study of existential risk – University of Cambridge)	18 October 2016

Challenges for the Regulation of Gene Drive Technology (Lorentz Center)	Oort, The Netherlands 20 - 24 March 2017
East Africa Consultative Meeting on Gene Drive Technology (New Partnership for Africa's Development (NEPAD) Agency).	Nairobi, Kenya 20 – 22 June 2017
Southern Africa Consultative Meeting on Gene Drive Technology (New Partnership for Africa's Development (NEPAD) Agency).	Gaborone, Botswana 26 – 28 June 2017
Central Africa Consultative Meeting on Gene Drive Technology (New Partnership for Africa's Development (NEPAD) Agency).	Libreville, Gabon 19 - 22 February 2018
Kick-off meeting for launching the Association for Responsible Research and Innovation in Genome Editing (ARRIGE)	Paris, France 22-23 March 2018
Exploring Stakeholder Perspectives on the Development of a Gene Drive Mouse for Biodiversity Protection on Islands (Genetic Engineering and Society (GES) Center, North Carolina State University, Keystone Policy Center, Consortium for science, Policy & Outcomes (Arizona State University)	Raleigh, NC, USA 7 – 8 March 2019
The science and ethics of gene drive technology (Scientific Foresight Unit (STOA) of the European Parliament	Brussels, Belgium 19 March 2019
15th ISBR Symposium of the International Society for Biosafety Research (ISBR)	Tarragona, Spain 1 – 4 April 2019
Workshop Evaluation of Spatial and Temporal Control of Gene Drives (Institute of Safety/Security and Risk Sciences (ISR))	4 – 5 April, 2019 Vienna
Gene Drive-Mosquito Monitoring and Surveillance: A Scenario-Oriented Workshop (Foundation for the National Institutes of Health)	Washington, D.C., USA April 24 - 26, 2019
Workshop on the problem formulation for the environmental risk assessment of gene drive modified insects (European Food safety Authority (EFSA))	Brussels, Belgium 15 May 2019
Gene Drives Symposium - Interdisciplinary symposium with a focus on scientific, ethical, socio-economic and regulatory aspects (Critical Scientists Switzerland (CSS); European Network of Scientists for Social and Environmental Responsibility e.V. (ENSSER), Federation of German Scientists)	Bern, Switzerland 24 May 2019
Gene Drive Modelling Conference (International Life Sciences Institute (ILSI) Research Foundation)	Washington, D.C., USA June 11 - 12, 2019

2.2.3 Other stakeholders

A search was made for publications and statements by a selection of stakeholder organizations that had already communicated on this subject. These standpoints and views were expected to broaden the collection of views on the subject.

Table 3 summarizes the consulted networks.

Table 3 Views from other stakeholders

Organization	Type of publication
African Centre for Biodiversity[3]	Briefing papers, press releases, video
ETC Group[4]	Briefing paper, news item
European Network of Scientists for Social and Environmental Responsibility (ENSSER)[5]	Report
Food First Information and Action Network (FIAN) International[6]	Report
Friends of the Earth International[7]	Blog
Testbiotech[8]	News item
Third World Network[9]	Briefing paper
Royal Society Te Apārangi Gene Editing Panel[10]	Technical paper

[3] https://www.acbio.org.za/en/publications?search_api_fulltext=gene+drive&sort_by=search_api_relevance
[4] http://www.etcgroup.org/content/reckless-driving-gene-drives-and-end-nature
[5] http://www.db.zs-intern.de/uploads/1558973988-Gene%20Drives%20Report.pdf
[6] http://bch.cbd.int/database/attachment/?id=18977
[7] https://foe.org/permanently-changing-species-go-wrong/
[8] https://www.testbiotech.org/en/limits-to-biotech/gene-drive/basic_paper
[9] https://biosafety-info.net/wp-content/uploads/2019/11/Biosafety-briefing_From-lab-to-wild.pdf
[10] https://royalsociety.org.nz/assets/Uploads/Gene-editing-in-pest-control-technical-paper.pdf

2.3 Interviews

Interviews with biosafety competent national authorities and stakeholders were a key component of the study, as they were expected to allow collecting specific views and challenges on risk assessment. Given the scope of the study, it was decided that the emphasis of the interviews could be placed on competent national authorities and risk assessors. The considerations of other stakeholders (scientists, developers, NGOs) were expected to be captured via the survey of literature and the stakeholder meetings as described above.

To ensure a broad coverage, a double approach was followed: selection of a core group of interviewees and an open call for contribution.

1) Preparation

A basic set of questions was established as well as an information sheet that introduced the purpose and methodology of the study, as well as the importance of providing input. This was finalized upon review by CBD staff.

Furthermore, an introductory letter from the Executive Secretary of the CBD was provided. A similar letter was distributed on August 29 as an open call for interest to the competent national authorities of the Cartagena Protocol. National focal points were copied on the letter as well.

2) Selection initial targeted group & reaction to the open call

The detailed workplan included a proposal of potential interviewees aiming at involving interviewees from a broad range of experience with LMO risk assessment, potential application of engineered gene drives or experience with similar systems. This list was further expanded based on feedback from the CBD staff to ensure a broad geographical scope and diverse expertise on the matter (*i.e* risk assessment). In total 30 contacts were identified.

All identified contacts from the initial target group were contacted by e-mail and invited to either contribute via an interview or via a written reply. A reminder was sent in case of no reaction. In some cases, the contacted person either referred to other individuals or preferred to submit a written input. In other cases, interviews were scheduled by phone or by Skype.

In total 18 indications of interest were received for the open call, although not all of them eventually contributed. Every respondent was invited to provide either an interview or a written submission.

3) Interviews and written input

Interviews were conducted along a predefined interview outline, thus allowing the interviewee to be optimally prepared. Interviews lasted on average 45 minutes. Following each interview a summary report was provided to the interviewee for verification of correctness. These summary reports only served to prepare this report and were not shared with any other party.

Combined with the information provided as written contributions, all data were further processed anonymously. In total, 8 interviews and 12 written contributions were recorded (See Annex 2)

2.4 Review of the application of existing risk assessment processes.

The identification of exiting risk assessments relating to environmental releases of LMOs with engineered gene drives was based on 3 approaches:

- Verification of databases in particular the Biosafety Clearing-House sections on “Country’s Decisions and other Communications” and “Risk Assessments”;
- Indications found in literature of R&D trials and largescale releases;
- Inquiry during interviews with selected stakeholders.

In contrast to several mentions of gene drives in news items, we have not found any confirmation of an environmental release of LMOs with engineered gene drives. On the contrary there have been

releases with non-engineered gene drives and preparatory trials with LMOs (predominantly arthropods) in which gene drives may be deployed.

3 Background

Gene drives are genetic mechanisms that allow for a trait to be propagated throughout a population beyond Mendelian inheritance. Active in sexually-reproducing species, they are powerful tools to "drive" a gene, *i.e.* increase its frequency, independent of external selection pressure. They have been proposed as offering solutions for many challenges in public health, agriculture, conservation and others. They have inspired researchers to use gene drives to combat diseases transmitted by insects such as malaria, dengue and Zika.

For decades, attempts have been made to use or modify naturally occurring gene drive mechanisms. Given that this study is limited to LMO containing engineered gene drives, the use of naturally occurring gene drive systems (*e.g.* the *Wolbachia* system) will not be covered in this section. In recent years advances in genetics have allowed for co-opting natural gene drive systems and the development of synthetic gene drive systems. Especially, since the discovery of the gene editing capabilities of the CRISPR/Cas9 system and the ability to use it as a gene drive system, interest for gene drives has increased as the technology significantly enhances the abilities to engineer gene drives. CRISPR/Cas-based drives turn out to be very efficient, at least in the laboratory. At the same time concerns were raised regarding the safety of their use.

Gene drives may be used in two ways:

- Suppression drive:
  Used to suppress populations of human and animal disease vectors, to control agricultural invertebrate pests such as fruit flies, moth pests, thrips and mites, and to eliminate invasive species.
- Replacement drive (also termed modification drive):
  Used to provide an extra trait to the target population, *e.g.* to block pathogen development, or to enforce populations (also called rescue drive), *e.g.* in endangered species or crop and livestock breeding.

The environmental impact of an organism carrying a gene drive system is determined by the type of gene drive system on the one hand and the effector, the trait or payload gene(s) that it carries, on the other hand. In some cases, these two factors are combined: if the effect of a CRISPR/Cas-based gene drive is induced by the location where the insertion has occurred, the gene drive directly induces the effector. However, in other cases, the CRISPR/Cas-based gene drive will be linked with a payload gene(s) that induces the effect.

Gene drives come with a fitness cost for the hosting organism. The type of gene drive and the accompanying fitness cost will determine how large the invading population needs to be relative to the target population (low level vs. high level threshold).

Until now, research on engineered gene drives was limited to laboratory experiments and modelling.

3.1 Types of engineered gene drives

Gene drives generally move in one direction, *i.e.* driving into a population until fixation (unidirectional). Or they can be bidirectional, *i.e.* moving away from an unstable equilibrium in either direction, all wild-type or all gene drive hosting).

An example of a bidirectional gene drive is the underdominance system, in which the heterozygote individuals are less fit than the wild-type or homozygote engineered individuals. They are high threshold-dependent gene drives that act locally and can be theoretically removed through dilution of the population with wild-type individuals.

Unidirectional systems are meiotic drives where the transmission of certain alleles is biased during meiosis, leading to increased frequencies of those alleles in the gametes, and therefore in the

offspring. The maternal-effect dominant embryonic arrest (MEDEA) systems disturb the normal inheritance pattern during embryo development. Homing endonuclease genes (HEGs) and especially CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR associated protein) systems interrupt specific gene sequences (target sequence), combined with a rapid spread in the population. They are low threshold gene drives, meaning that only a low amount of drive-bearing individuals are needed to replace or suppress a population.

More information on the types of gene drives is presented in Annex 3.

3.1.1 Comparison of different engineered gene drive systems

To perform a risk assessment the characteristics of the specific gene drive system and its application need to be considered on a case-by-case basis. Gene drives can be categorized in relation to their application for either suppression use and/or replacement use (Table 4). In this table only ideal-case scenarios are compared to emphasise intrinsic differences of the various types of drives. However, characteristics are variable and depend on a range of factors (*e.g.* ecology of the target species, population distribution, movement patterns, fitness costs, payload characteristics, …). Consequently, the same drive system can be used for different uses.

Table 4 Summary of characteristics of several engineered gene drive systems (adapted from Champer et al., 2016)

	Underdominance	Meiotic drive	MEDEA	Homing-based drive (including CRISPR/Cas)
Type	Replacement -	- Suppression	Replacement -	Replacement Suppression
Rate of spread	Slow	Moderate	Moderate	Fast
Locally confined?	Yes	No	No, if low fitness cost[1]	No
Resistance allele generation rate	Moderate	Low	Low	High
Reversible?*	Yes	Yes	Yes	Yes
Removable with wild type?**	Yes	No[2]	No, if low fitness cost[1]	No[2]

[1] High fitness costs may make these systems locally confined and removable with the release of large numbers of wild-type organisms.

[2] Suppression types that proceed to fixation and eliminate a population will remove the gene drive system, allowing replacement with wild-type organisms.

* Reversibility is the ability to replace an existing gene drive system with another gene drive

** Removability is the ability to completely remove a gene drive system from a population via the release of wild-type organisms.

3.2 Status of application of LMO with engineered gene drives

The WHO presented guidelines on phased testing of GM mosquitoes and these are also relevant for gene drives in insects in general (WHO, 2014).

1 Table 5

Table 5 Consecutive phases in a step-wise approach (based on WHO, 2014) and tentative indication of status of different applications to date.lists the different phases in a step-wise approach. Based on the literature survey, we have tentatively indicated at which stage the most advanced applications of engineered gene drives are.

Table 5 Consecutive phases in a step-wise approach (based on WHO, 2014) and tentative indication of status of different applications to date.

Phase	Description	Status of application of engineered gene drives
0	Preparation for Research	Rats and rabbits
1	Laboratory-Based Research	Fruit fly (*Drosophila* sp.) Mosquitoes (*Aedes* sp., *Anopheles* sp.) House mouse (*Mus musculus*)
2	Field-Based Research	-
3	Staged Environmental Release	-
4	Post-Release Surveillance	-

Possibilities for exploiting engineered gene drives in agricultural weeds, fall armyworm, ticks, diamondback moth, soybean looper and invasive bivalves have been proposed, yet we have not found reports on concrete experiments and they are therefore not included in the status table.

Each of the phases allows for knowledge to be gained that will either force to return to a previous phase (feedback loops) or allow to advance to the next phase. Safeguards are in particular of concern for engineered gene drive systems that are not spatially and/or temporally self-limiting.

This step-wise approach was also recommended in the NASEM report as the pathway for research on a gene-drive modified organisms (NASEM, 2016). Hayes and colleagues further elaborated on the approach (Hayes et al., 2018).

A first step in Phase 0 may be to inventory all available biological, genetic and ecological information to evaluate knowledge gaps on certain species and to evaluate whether they would be suitable for a gene drive approach (Moro et al., 2018). Next, a set of standards or acceptability criteria for the gene drive performance should be defined (Hayes et al., 2018). Also, a prospective technology characterization may be a useful tool in Phase 0 for an estimation of the risk potential associated with the different gene drive options (Frieβ et al., 2019). The suggested approach is based on the technological power (drive) and range (exposure) of the technology, the intensity of intervention (size and number of releases), the reliability of the technology (probability of failure) and the corrigibility or limitation of damage in case of failure (*e.g.* overwriting drive).

In Phase 1 laboratory experiments will actually develop all components and “manufacture” the gene drive organism. In lab cage experiments the effectivity, fitness and behaviour can be studied. Structured hazard analysis tools at this stage can be used to prepare the field stages or conclude to abandon the particular approach. However, field research is needed to validate lab-based findings and improve models (NASEM, 2016).

Before entering the field, semi-field cages may be used to mimic the environmental conditions, while still in containment. If this results in a positive outcome, small localized field trials may be conducted (biologically or geographically contained) and may eventually lead to staged environmental releases. In all open releases, a stringent monitoring effort would be required.

Throughout the different phases, information from other risk assessments can help to inform the process. Organisms with non-engineered gene drives may not be subject to a formal risk assessment and regulatory oversight. Nevertheless, there is one example that might inform the risk assessment of engineered gene drives. The *Wolbachia*-system, although not a gene drive in the strict sense, does behave like one. *Wolbachia* are intracellular bacterial endosymbionts of many arthropods that are maternally inherited. The *Wolbachia* genome normally does not integrate into the host’s chromosomes as in engineered gene drives, but infected females can mate with either infected or uninfected males and produce almost 100% infected viable progeny. *Wolbachia*-infected males do not produce viable offspring when mating with uninfected females. Therefore, *Wolbachia*-infected females have a reproductive advantage relative to uninfected females allowing infection to spread rapidly through host populations to a high frequency in spite of fitness costs. *Wolbachia* may be used to combat virus diseases transmitted by arthropods (*e.g.* dengue, Chikungunya, Japanese encephalitis virus, West

Nile virus and Zika virus) by increasing the arthropods' resistance to viruses. Before *Wolbachia*-infected *Aedes aegypti* were released in Australia to fight dengue, a risk assessment was conducted. This was the first time risks were formally assessed for organisms bearing a gene drive system. The Risk Analysis Framework developed by the Australian Office of the Gene Technology Regulator (OGTR) was followed (Murphy et al., 2010). Although this gene drive system is not an LMO, the methodology was found suitable for an organism with a novel trait. Because of the novelty of this specific gene drive, the lack of data on potential hazards and likelihoods was captured by expert opinions.

Also prior to releases of *Wolbachia*-infected *Aedes aegypti* in Vietnam a similar risk assessment was performed (Hoc et al., 2011). The overall risk of the release of *Aedes aegypti* containing *Wolbachia* resulting in more harm than that currently caused by naturally occurring *Aedes aegypti* over a 30 year timeframe was estimated to be negligible.

Recently, Zheng *et al.* (2019) communicated that a combination of the radiation-based sterile insect technique with the incompatible insect technique based on *Wolbachia* enabled near elimination of field populations of *Aedes albopictus* from two experimental field sites, islands in a river in Guangzhou, China. While there may not be a formal risk assessment since no genetic engineering was involved, the experience of such trials seems relevant for LMOs with engineered gene drives.

The final step can be post-release surveillance once a gene drive bearing organism is introduced in the environment. An example of a successful introduction and accompanying monitoring programme is reported by O'Neill and colleagues on the introduction of the non-LMO *Wolbachia*-infected *Aedes aegypti* in Australia (O'Neill et al., 2019).

4 Considerations for risk assessment

The number of communications on potential applications of engineered gene drives, and in particular those introduced via genetic engineering, is rapidly increasing. This report therefore aims to reflect the state of the art and identify considerations relevant for the risk assessment on the basis of a broad input. Where relevant it builds on our preceding study (Rüdelsheim and Smets, 2018). These findings were reviewed and adapted based on the updated literature information, inputs from stakeholders and consultation of a wider range of sources as indicated in the methodology. This section provides a summary and a more detailed review is presented in Annex 4.

4.1 Applications

4.1.1 Broad diversity

Gene drives can be assembled for a variety of applications:

- human disease (tackling vector organisms),
- conservation (eradication of invasive species) and
- agriculture (disease/pest reduction, weed control).

The engineered gene drive mechanism in itself may have no influence on the performance and/or the characteristics of the host organism. This will depend on the payload gene that is combined with the gene drive. In other cases, the engineered gene drive combines the drive function with the introduction of the intended effect trait. In many of the interviews it was stressed that this can only be addressed at this state of knowledge by maintaining the case-by-case approach.

The broad range of organisms in which engineered gene drives may be deployed, as well as the need to differentiate between suppression and replacement gene drive applications, further justify a case-by-case approach. It must therefore be stressed that considerations indicated in this section may be relevant for certain engineered gene drives in certain organisms and are not applicable in a generic way for all gene drive applications.

4.1.2 Introducing a modification tool rather than a finished product

Simon et al (2018) point out that as the modification introduces a gene drive system, the entire CRISPR/Cas toolbox is inherited. Although the gene drives are constructed in the laboratory, the drive is designed to genetically modify organisms in the wild, over and over again.

Whereas so far LMOs have been selected during R&D, tested and presented as finished and well characterized products; engineered gene drives imply the release of a tool that will continue to trigger further genetic modification of individuals in the target species once released. Non-intended effects, such as resistance or off-target effects, are difficult to predict and characterize before the release into the environment, particularly in genetically diverse wild populations.

4.1.3 Targeting non-domesticated species

Most of the experience with risk assessment of LMOs has been gained with environmental releases of modified domesticated -or at minimum cultivated- plants. In addition, a limited number of animal species, also cultured, like pigs and fish have been subject of environmental risk assessments. This allowed risk assessors to refer to experience with the introduction of non-engineered organisms and thus rely on the comparative approach as the foundation for the risk assessment.

In contrast, gene drives target non-domesticated or wild species. It has been pointed out that the choice of comparators may be challenging. Experience with introduction of wild populations may be limited. Also relevant data on population dynamics as well as ecosystem functions and services may not be available. On the other hand, the risk assessment of insects with engineered gene drive

systems can build on existing knowledge and experience with vector control programs using insects that do not contain gene drives (*e.g.* sterile insect technique (SIT); incompatible insect technique (IIT)).

4.1.4 Targeting non-managed environments

Whereas domesticated species are introduced in human-managed environments, the most advanced engineered gene drive applications target releases in non-managed (also perceived as uncontrolled) environments, for which there is less experience in conducting risk assessments.

4.1.5 Managing a stepwise approach

In line with the precautionary approach, scientific uncertainty must be reduced in order to advance through R&D. In the stepwise pathway to deployment, experience gained and data established during preceding steps is used as the basis for the risk assessment of the next, less limited step. As pointed out by James and Tountas (2018) *"it should be emphasized that continued research is the only way to decrease the uncertainties that underlie the perception of risk".*

Nevertheless, the predicted ease of spread of engineered gene drive organisms, in particular for low threshold systems, combined with the perception that an introduction is irreversible calls for an extremely thorough evaluation under careful confinement before a decision on release into a hospitable environment. To confine potential releases, geographically isolated location and self-limiting constructs that constitute a form of biological or molecular confinement may be used.

4.2 Effect on the gene drive-bearing organism

4.2.1 Off-target modifications

Off-target effects within the recipient organism is a concern for gene drive systems that are based on a gene editing technique and RNAi methods. Off-target cutting by a HEG gene drive may lead to the loss or modification of native traits with potentially effects on the survival, behaviour and breeding success of the organism.

4.2.2 Stability of the gene drive system

Gene driver-cargo systems risk to unlink the payload gene, *e.g.* that targets a pathogen, and the elements of the drive system (Alphey, 2014). The driver may then continue to spread without having the desired effect on the population.

4.2.3 Modified susceptibility

Another concern, theoretically, may be the ability of the vector organism to have modified competency for pathogen transmission (Benedict et al., 2008; David et al., 2013). The vector organism may in theory become a more susceptible host to another existing or new virus that harms human health (NASEM, 2016).

4.3 Considerations for biodiversity

Regarding the potential effects on the environment, a distinction should be made between population suppression and population replacement drives. They may have the same ultimate goal, *e.g.* eradication of an insect-borne pathogen, but they have different implications for potential environmental interactions. In a population replacement drive the GM trait is intended to persist in the environment.

The extent of the effects on the ecosystem depends on whether the target organism is a "keystone" species in the environment, and/or whether there are ecological equivalents present.

A gene drive may be used to eradicate an invasive species. Although the intent may be the re-establishment of the original species diversity, the elimination of an invasive species may not restore the original ecosystem.

4.3.1 Target organism

Suppression gene drives may result in the extinction of (a local population of) the target or host organism. Although the target species may be locally affected (even eradicated), this must be seen in the light of other control techniques, but may be expanded and more effective with powerful gene drives.

4.3.2 Non-target organisms

Potential non-target effects are often raised as concern. However, since gene drives are based on mating potential, the potential for exchange with related species is very species specific (Alphey, 2014).

Effects on non-targets organisms may act directly, *e.g.* due to hybridization between related species, or act indirectly *e.g.* due to trophic relationships. In contrast to a genetically modified domesticated species, for engineered gene drive organisms, inheritance and spread of the transgene is a required prerequisite for their functionality. Unintentional transfer of a gene drive into a beneficial, threatened, endangered, neutral, or valued species could lead to its extinction.

Another aspect is the reduction of the target organism that may increase the population of other species (niche replacement). Technologies for population replacement instead of population suppression are therefore likely to induce less ecological harm.

4.3.3 Other trophic levels

Extinction (or reduction of abundance) of the gene drive-carrying species can have consequences for *e.g.* predators, competitors, prey, due to its ecological role, such as resource, consumer, competitor, or disease vector. These links create dynamic feedbacks that affect the relative abundances of different species. However, indirect effects on food webs or ecological functions are not specific for gene drives. They are equally important in classical biocontrol strategies and these may provide information for risk assessment.

4.3.4 Alternative protection mechanisms and herd immunity

In an area with high malaria incidence, people acquire immunity after several attacks of malaria. A gene drive suppressing the insect vector could result in the loss of the acquired immunity when they stop contracting malaria. Whenever the insect vector recovers, this can lead to a higher disease prevalence since the susceptible population has increased.

4.4 Resistance development

4.4.1 Resistance to the engineered gene drive system

The presence or development of resistance against a gene drive system will reduce its efficiency in the host population, but will also limit the potential impact. It is therefore relevant for the risk assessment to acknowledge that gene drive systems may be particularly susceptible to resistance development.

HEGs in general are prone to the development of resistance alleles that are insensitive to conversion by the drive system (Hammond et al., 2017).

Natural sequence polymorphisms in the population and *de novo* mutation of wild-type alleles could also prevent HEGs to drive (Unckless et al., 2017).

Furthermore, any HEG that reduces the fitness of its host will face the potential evolution of resistance (de Jong, 2017; Godfray et al., 2017; Unckless et al., 2017). Thus, even though a gene drive may initially spread to high frequency in the population, its ultimate fate will depend on whether resistant alleles have emerged during this process. To prevent the spread of resistant alleles, it will be necessary to target genomic sites that cannot tolerate changes, *e.g.* active sites of proteins, conserved regions in genes (Deredec et al., 2011; Godfray et al., 2017).

Another path to gene drive resistance would be that the gene drive containing organism develops a method of specifically inhibiting the drive endonuclease (Bull, 2015; Esvelt et al., 2014).

Resistance may be part of a scheme to confine the engineered gene drive to a smaller geographical area or a certain time period (a number of generations), when short-term population transformations are the objective (Champer et al., 2016; Esvelt et al., 2014; Unckless et al., 2017).

Also, with other drive mechanisms, such as MEDEA, resistance is observed (Akbari et al., 2014; Buchman et al., 2018).

Another factor is behavioural resistance. The target population structure in space and time, its mating system and density-dependence, age and social structure may hinder the gene drive to invade (Rode et al., 2019)

4.4.2 Resistance to an effector

Resistance development to an effector is a common phenomenon that can occur in each species, *e.g.* a pest developing resistance against a management strategy. Resistance can occur by selection of resistance of the target pathogen to the effector gene, or the selection of increased virulence of the target pathogen (Alphey, 2014; Franz et al., 2009; James, 2005). It is undesired and, under certain legislative frameworks, resistance development is also considered as an environmental concern.

4.5 Effects beyond the target

The spread of an engineered gene drive outside the intended geographical area could potentially change the environmental landscape well beyond the site of its introduction. A drive to eradicate an invasive species may be accidently introduced in the species' original environment, *i.e.* where it is endemic, where it has a function in the local ecosystem or is important as a human food source. Gene flow to other populations of the same species depends on the mode of dispersal between populations: human assisted, as a result of a disruptive event (*e.g.* fire, hurricane), normal movement of organisms, or as a result of habitat unsuitability (*e.g.* crowding, no nesting sites) (NASEM, 2016).

4.5.1 Dispersal

Especially low threshold drives may have widespread consequences across national borders. Concerns arise from the release, deliberate or accidental, of just a few organisms containing gene drive systems. Also, dispersal may create political tensions with bordering countries that may not have approved the technology (Macias et al., 2017).

Using high threshold drives help confine the spread of a gene drive to a local breeding population. Underdominance systems display high migration thresholds, next to a high release requirement.

Technical solutions are explored to confine engineered gene drives, such as a 'daisy chain' CRISPR/Cas gene drive, a split drive with a molecularly unlinked endonuclease, or a daisy quorum drive (Li et al., 2019; Min et al., 2017; Noble et al., 2019). This would limit the capacity of the gene drive to spread. It would be a way to temporarily and locally replace a population.

A CRISPR-based gene drive could also be used to block the spread of other gene drives by recoding sequences targeted by the unwanted drive ('immunising' drive) (Esvelt et al., 2014).

4.6 Perspectives of indigenous peoples and local communities

The rights of indigenous peoples and local communities (IPLCs) are embedded in different UN treaties. Decision 14/19 welcomes the outcome of the Ad Hoc Technical Expert Group on Synthetic Biology that "given the current uncertainties regarding engineered gene drives, the free, prior and informed consent of IPLCs might be warranted when considering the possible release of organisms containing engineered gene drives that may impact their traditional knowledge, innovation, practices, livelihood and use of land and water."

Within the context of this study, attention was paid to identify specific aspects that may require a different approach. Respondents highlighted the diversity of IPLCs, resulting in divergent views. This is typically addressed via public consultations and countries with IPLCs already include these in their decision-making process. In order to obtain insights on the fundamental elements, consultation needs to be accompanied by information.

4.6.1 Value of biodiversity

Introducing a "foreign" gene in a species or directly influencing the population dynamics of a species may be seen as anthropocentric intervention on life. The release of organisms targeting unmanaged environments may trigger concerns of disrupting a pristine balance. On the other hand, using a gene drive to control an invasive species which in itself has a negative impact on the native biodiversity might be welcomed.

For localized gene drive applications, it would be possible to involve the local IPLCs. On the other hand, there is no clear mechanism for transboundary considerations for applications of engineered gene drives that can span several countries.

4.6.2 Right for self-determination

Different papers refer to the intrinsic right for self-determination of peoples and the possible infringement when introducing LMOs with engineered gene drives (*e.g.* Meghani, 2019). Several recent papers have provided examples and proposed tools to define acceptance (*e.g.* Kolopack and Lavery, 2017, Singh, 2019; Godwin et al. 2019; Buchthal et al. 2019; Farooque et al. 2019, Hudson et al. 2019).

Again, in case of an application that would not remain localized, it will be more difficult to scope the consultation of possible stakeholders and IPLCs across borders.

For the large-scale deployment of the earlier mentioned *Wolbachia* in *Aedes aegypti* to eliminate dengue in Australia a public acceptance model was developed that formed the basis for obtaining community support for the research activities. (O'Neill et al., 2019). It consisted of four key components:

- Raising awareness by providing information on the programme),
- Quantitative surveys that measured community awareness and acceptance,
- An issues management system that allowed community members to easily contact the program with questions or concerns,
- A community reference group that consisted of respected community members.

5 Informing the application of Annex I of decision CP-9/13

5.1 Information summarized on the Annex I criteria

Annex I of decision CP-9/13 lists criteria that are part of a structured analysis that forms the basis of the process for recommending specific issues of risk assessment for consideration by the Conference of the Parties serving as the meeting of the Parties to the Cartagena Protocol on Biosafety. The annex also indicates that a stock-taking exercise should be done to determine if resources on similar issues have been developed and if so, whether these resources could be revised or adapted to the objective of the Protocol, as appropriate. The purpose of this study is to inform the application of this annex and we will review the information of previous sections in the light of the specified criteria.

5.1.1 Priorities identified by Parties

(a) *They are identified by Parties as priorities, taking into account the challenges to risk assessment, particularly for developing country Parties and countries with economies in transition;*

Based on the information collected in different CBD processes (see section 2.2.1), several Parties, including developing country Parties and countries with economies in transition, have indicated LMOs with engineered gene drives as a priority topic.

From the interviews, it can be concluded that:

- All interviewees pointed out specific features of gene drives that present challenges to the current risk assessment paradigms (see 5.1.3);
- Given the type of application, many developing countries and countries with economies in transition are confronted with or anticipate to be soon confronted with possible applications of gene drive systems, if not directly than possibly by via transboundary movement;
- Interviewees from countries with less experience with risk assessments, some being developing country Parties and countries with economies in transition, expressed uncertainty on how to evaluate certain aspects (*e.g.* gene flow), and although they are relevant for all LMOs, they are considered essential for engineered gene drives.
- Interviewees from countries with a long experience (over 10 years) in conducting LMO risk assessments, stressed that -while the same risk assessment approach can be used- gene drives with low thresholds will require a comprehensive risk assessment much earlier in the staged introduction.

5.1.2 Scope and objective of the Cartagena Protocol

(b) *They fall within the scope and objective of the Cartagena Protocol;*

Article 1 of the Cartagena Protocol describes the objective as contributing to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.

Article 4 of the Cartagena Protocol describes the scope as applying to the transboundary movement, transit, handling and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.

LMOs with engineered gene drives result from modern biotechnology, as defined in the Cartagena Protocol. All interviewees acknowledged that LMOs with engineered gene drives may have adverse effects on the conservation and sustainable use of biological diversity and that a thorough risk assessment should precede any intended introduction in the environment.

5.1.3 Challenges to existing risk assessment frameworks

(c) They pose challenges to existing risk assessment frameworks, guidance and methodologies, for example, if the issue at hand has been assessed with existing risk assessment frameworks but poses specific technical or methodological challenges that require further attention;

The interviewees differentiated between challenges to the risk assessment methodology and challenges relating to obtaining information required to inform the risk assessment.

Most interviewees anticipated that it will be possible to use existing risk assessment methodology for evaluating LMOs with engineered gene drives. The risk assessment methodology is largely based on problem formulation methodology, providing a structured and systematic approach for addressing risk assessment, subsequent risk management and provides a tool for risk communication. NASEM (2016) calls for the use of probabilistic (ecological) risk assessment approaches for organisms with a gene drive, as it identifies and quantifies the ecological and human health risks. Further refinements continue to be proposed, *e.g.* recently Kuzma (2019) published the Procedurally Robust Risk Assessment Framework (PRRAF) with a set of principles and criteria for assessing and enhancing risk assessment protocols under conditions of high uncertainty.

All interviewees pointed out that no case of an actual release of an LMO with engineered gene drives has been assessed, leaving the definition of issues speculative. Nevertheless, it is relevant to note the multitude of preparatory efforts to tailor the risk assessment questions. This is marked by international expert meetings, problem formulation workshops and development of guidance as highlighted in other sections of this report.

Most of the considerations that were raised in literature and identified during this study, are not specific for LMOs with an engineered gene drive. *E.g.* outcrossing and dispersal of an organism/trait is already an element of the risk assessment to date. However, this has usually been considered as of limited likelihood, whereas gene drives are designed to force the inheritance patterns. In case of population suppression this might have a direct impact on entire populations and consequently on biodiversity and ecosystem services (depending on the species).

The fact that some engineered gene drive systems are seen to have the power to result in an irreversible impact on a species at global level, requires international understanding on common protection goals. Some highlight the lack of clarity on the level of acceptable risks. *E.g.* the discussion on outcrossing of LMOs has not clarified what level of outcrossing would be acceptable in this context.

While the debate on protection goals is not new, and in fact broader than only for gene drives, the introduction of gene drives makes it more urgent to address these points. Devos et al. (2019) stress that regulators and governments have to clearly identify their environmental policy goals (protection priorities) as a prerequisite for the environmental risk assessment (ERA) to address them (problem formulation).

Various features, in effect, distinguish organisms with engineered gene drives from LMOs assessed so far:

- the modified inheritance pattern,
- targeting non-managed environments,
- targeting non-domesticated species, and
- threat of an irreversible impact at a scale exceeding the intended release.

While the risk assessment methodology may still be applicable, these differences may require new approaches to inform the risk assessment, which is a technical and methodological challenge.

As an example it was discussed that whereas so far plants have been modified to protect them against a pest, engineered gene drives allow modifying the pest species. This may have an effect on "wild" populations and since neither the temporal nor geographical scale can be controlled, it may even have an effect on an entire species, ecosystems and, generally, biodiversity.

It can therefore be expected that a risk assessment for engineered gene drive applications will be more detailed and more complex, and this would in any case also require a public consultation.

Depending on the case, specific questions may be required and the data requirements may need to be adapted to reflect the type of organism, gene drive system, payload gene, environment of the release and the type of application. While much of the focus of discussion appears to be on highly invasive (*i.e.* low threshold) gene drives, generalizations regarding those types of drives may not be applicable to other types.

Furthermore, depending on the case, more information may be required on specific aspects. *E.g.* a self-propagating gene drive application may require more stringent management considerations than the proposed self-limiting applications.

Most contributors stressed that the risk assessment methodology for LMOs with an engineered gene drive will be the same as for other LMOs. In many cases this methodology is embedded in a national legal framework, with reference to international guiding principles such as Annex III of the Cartagena Protocol.

While the methodology may be the same, the questions will need be tailored to what is distinctive. The following points have been indicated by interviewees and need to be considered on a case-by-case basis:

- some of the assessment principles such as the comparative approach may not be fit for purpose in this context;
- the "stepwise" approach may not be applicable since the smallest introduction (*e.g.* field trial) of an LMO with a low threshold gene drive might result in spread and a permanent impact. This would limit the ability to do field tests, which are however required to obtain information for the risk assessment in order to approve subsequent introductions;
- using robust models to predict long-term and ecosystem effects are required to support risk assessment;
- concepts like the "receiving environment" must be revisited in function of the release of wild species as opposed to domesticated species, such as crop plants or livestock that are to a significant degree controlled by man. Information on the ecological context of wild populations is required to feed the risk assessment. Only patchy knowledge is available, not in line with the complexity of the potential broad temporal and geographical scope. This context is also needed to have reliable predictive modelling.

5.1.4 Clearly described issues

(d) The challenges in addressing the specific issue are clearly described;

Although above several considerations that are likely to be relevant for organisms containing engineered gene drives have been identified in general, it must be reminded that the only way to clearly describe specific issues is in relation to specific cases. Generic discussions are confounded by extrapolation of specific cases. Most -if not all- of the considerations that were identified are not related to engineered gene drives *per se*. Rather they were relevant for the host organism, the introduced trait or the receiving environment.

5.1.5 Specific issues for engineered gene drives

(e) The specific issues concerning living modified organisms that:

i. Have the potential to cause adverse effects on biodiversity, in particular those that are serious or irreversible, taking into account the urgent need to protect specific aspects of biodiversity, such as an endemic/rare species or a unique habitat or ecosystem, taking into account risks to human health and the value of biological diversity to indigenous peoples and local communities;

ii. May be introduced into the environment either deliberately or accidentally;

iii. Have the potential to disseminate across national borders;

iv. Are already, or are likely to be, commercialized or in use somewhere in the world;

All interviewees agreed that LMOs with engineered gene drives have the potential to cause serious and/or irreversible adverse effects on biodiversity. Still some pointed out that such an impact can only be envisaged in very specific, worst-case scenarios, whereas others expressed concern that the available information is insufficient to judge on what would lead to a worst-case scenario.

The special interest of IPLCs has been briefly discussed before. However, more information may be needed to better understand the potential implications of the release of organisms containing engineered gene drives for IPLCs. In particular when the broad spread of an LMO with a genetically engineered drive is likely, it will be challenging for instance, to obtain the free, prior and informed consent of IPLCs. It is also recognized that different gene drive applications may have different potential impacts, and therefore, information on each potential application will be key for any consultation process with IPLCs.

Different authorities have already indicated the need for containment measures for preventing unintended releases of LMOs with an engineered gene drive. On the other hand, applications in vector control or control of invasive species will require deliberate introduction in the environment.

Once released, there is a potential to disseminate across borders. Again, this is not a characteristic *per se* of gene drives, rather of the host organism. However, as gene drive applications today mostly target non-domesticated species, they will not be bound by national borders or territorial agreements. Whereas the introduction of a domesticated species is largely driven by market realities and controlled by humans, the distribution of non-domesticated species follows ecological habitats. The potential for transboundary movement of gene drives in *e.g.* arthropods which are the most advanced applications, makes international discussion essential and the need for joint decision-making, operationalising prior informed consent for all potentially affected countries was stressed by some.

Although no releases of LMOs with engineered gene drives have been performed, preparatory steps have been taken and largescale deployment might be envisaged.

5.2 Information on stock-taking exercise related to existing guidance

Annex I of decision CP-9/13 furthermore refers to a stock-taking exercise to determine if resources on similar issues have been developed by national, regional and international bodies and, if so, whether such resources may be revised or adapted to the objective of the Cartagena Protocol, as appropriate.

Several interviewees referred to statements that have been made by official bodies, *e.g.* in Australia (OGTR, 2019), Germany (ZKBS, 2016), The Netherlands (Westra et al., 2016) and Japan (Tanaka et al., 2019). Nevertheless, these statements relate to conducting research with engineered gene drives in containment, emphasizing the need to avoid release in the environment, and therefore were deemed less relevant for this study.

During this study the following resources were identified by interviewees as providing indications for risk assessments for releases into the environment of LMOs with engineered gene drives:

- EFSA GMO Panel (2013)

  This document provides guidance for the environmental risk assessment (ERA) of living GM animals, namely fish, insects, mammals and birds, to be placed on the EU market. It describes the six sequential ERA steps (1) problem formulation including hazard and exposure identification; (2) hazard characterization; (3) exposure characterization; (4) risk characterization; (5) risk management strategies; and (6) an overall risk evaluation. It includes indications on assessing gene drive systems.

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

  In decision CP-VIII/12, the COP-MOP took note of the voluntary "Guidance on Risk Assessment of Living Modified Organisms". In the section of the Guidance on specific types of LMOs and traits, the risk assessment of living modified mosquitoes species that act as vectors of human and animal diseases is presented. Considerations on engineered gene drives are included.

- NASEM (2016)

  This consensus study report outlines the state of knowledge relative to the science, ethics, public engagement, and risk assessment as they pertain to research directions of gene drive systems and governance of the research process. It aims to offer principles for responsible practices of gene drive research and related applications for use by investigators, their institutions, the research funders, and regulators.

- Australian Academy of Science (2017) "Discussion paper – Synthetic gene drives in Australia: Implications of emerging technologies"

  The paper discusses environmental hazards, social and economic issues (including trade implications) and how the technology can be managed within Australia's governance arrangements. It highlights the potential benefits and hazards of possible applications, emphasizing the need to eventually consider these within a risk assessment framework.

- High Council for Biotechnology (HCB), France (2017) Scientific Opinion of the High Council for Biotechnology concerning use of genetically modified mosquitoes for vector control in response to the referral of 12 October 2015 (Ref. HCB-2017.06.07).

  Report (in French) by a working group of experts in response to a request for clarifications on the use of genetically modified mosquitoes, including the use of engineered gene drives. Activities ranging from controlled laboratory conditions to deliberate release into the environment are considered.

- Roberts et al. (2017)

  This report of a workshop convened by the FNIH has been indicated as illustrating a problem formulation approach to identify plausible risks using case studies in malaria vector control in sub-Saharan Africa.

- James et al. (2018)

  Guidance on best practices for development of gene drive LMO mosquitoes, including some considerations for risk assessment.

- Rüdelsheim and Smets (2018)

  Commissioned by the Netherlands COGEM, this report summarized experience with gene drive systems, naturally occurring as well as introduced via genetic engineering, in order to better understand the potential consequences for human health and the environment of gene drive use.

- Teem et al. (2019)

  Report on 4 workshops in Africa, to introduce problem formulation as a tool to the stakeholder community, and to serve as a starting point for conducting systematic environmental risk assessments in the future, identifying protection goals related to gene drive mosquitoes that are particular to African stakeholders.

Furthermore, 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”. The results of the dedicated working group are expected in 2020. Also other countries have indicated that further guidelines are being developed.

Several articles describing modelling of gene drives have been published, such as the following:

- Bull J. J., Remien C. H., Gomulkiewicz R., Krone S.M. (2019). Spatial structure undermines parasite suppression by gene drive cargo. PeerJ 7:e7921. https://doi.org/10.7717/peerj.7921.
- Bull J. J., Remien C. H., Krone S.M. (2019). Gene-drive-mediated extinction is thwarted by population structure and evolution of sib mating. Evolution, Medicine, and Public Health 1, 66–81. Doi:10.1093/emph/eoz014.
- de Jong T.J. (2017) Gene drives do not always increase in frequency: from genetic models to risk assessment. Journal of Consumer Protection and Food Safety 12, 299-307.
- Marshall, J. M. and Akbari O. S. (2018). Can CRISPR-Based Gene Drive Be Confined in the Wild? A Question for Molecular and Population Biology. ACS Chem. Biol. 2018, 13, 424-430. Available at: https://pubs.acs.org/doi/abs/10.1021/acschembio.7b00923.
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Annex 1 Steps in the procedure of the systematic review

The literature search has been performed according to the methodology of a systematic review and is described by the following steps.

1) Formulation of the study question

The review question for the study was defined as:

> *"Do LMOs containing engineered gene drives present risk assessment considerations that may justify development of further guidance?"*

Or alternatively:

> *"Is the current GMO risk assessment methodology suitable to assess applications of gene drives?"*

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

In order to be able to formulate search strings, the following key words were chosen:

> *LMO, GMO, gene drive, risk assessment, ecological risk, guidance, problem formulation, field, CRISPR-Cas9, synthetic, biosafety, biodiversity, underdominance, MEDEA, vector control, meiotic drive, reciprocal chromosome translocation, sex-linked translocation, Mendelian inheritance, biased inheritance, segregation ratio, segregation distortion, sex ratio, sex ratio distorter, population suppression, population replacement, homing endonuclease, engineered, pest control, self-limiting gene drive, safeguarding, RNA-guide, environmental impact assessments*

These were then combined in typical search strings, as shown by the following example:

> *("gene drive*" OR CRISPR-Cas* OR synthetic OR underdominance OR MEDEA OR "meiotic drive" OR "chromosome translocation" OR "sex-linked translocation" OR "Mendelian inheritance" OR "biased inheritance" OR "segregation ratio" OR "segregation distortion" OR sex*ratio OR "population suppression" OR "population replacement" OR "homing endonuclease" OR RNA-guid*) AND (engineer* OR LMO OR GMO OR "genetic* modif*) AND ("risk assessment* OR guidance OR "ecolog* risk" OR "problem formulation" OR biosafe* OR safety OR biodiversity)*

3) Pilot testing

In a first Pilot testing using the search string above for the time period 2018-2019, many publications were retrieved in Web of Science™ core collection (6,881 records) and Scopus® (140 records). Most of these were not related to genetically engineered gene drives.

Replacing the first Boolean operator OR by AND reduced the number of papers for the period 2018-2019: 52 records in the Web of Science™ core collection and 7 records in Scopus®, resulting in a total of 56 records after removal of duplicates.

The final search string that was used, was simplified:

> ***("gene drive*" AND (engineer* OR LMO OR GMO OR "genetic* modif*) AND ("risk assessment* OR guidance OR "ecolog* risk" OR "problem formulation" OR biosafe* OR safety OR biodiversity))***

4) Eligibility/inclusion and Exclusion criteria:

In order to identify relevant publications, a set of selection criteria has been set to narrow down the number of retrieved publications. Both inclusion and exclusion criteria were predefined before any search was started. These were:

Eligibility/inclusion criteria:

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

Exclusion criteria:

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

If mixed topics were addressed, the inclusion criteria superseded the exclusion criteria. *E.g.* a report on population modelling in relation to risk assessment would be selected included.

5) Initial data collection

Two electronic bibliographic multi-disciplinary databases were chosen to search for relevant publications: Web of Science™ core collection[11], and Scopus®[12].

- Web of Science™ core collection consists of six online databases indexing scholarly books, peer reviewed journals, original research articles, reviews, editorials, chronologies, abstracts, as well as other items. Disciplines included in this index are agriculture, biological sciences, engineering, medical and life sciences, physical and chemical sciences, and many others. The database contains 1.4 billion cited references going back to 1900.
- Scopus® by Elsevier is an abstract and citation database of peer-reviewed literature, including scientific journals, books and conference proceedings, covering research topics across all scientific and technical disciplines, ranging from medicine and social sciences to arts and humanities. Scopus® is updated daily and includes over 71 million records and over 1.4 billion cited references after 1970.

The search was performed on September 19, 2019

An additional search string was used with the aim to find information of potential recent field experience (field trials or releases):

("Gene drive" AND field AND (risk OR hazard))

The searches were performed by one of the project team members and subsequently checked by a second team member.

6) Initial selection based on title and abstract

The title, abstract and keywords of these 91 publications were checked for relevance using predefined exclusion/inclusion criteria. Using these criteria the collection of publications was further narrowed down to 18 publications. For the selected ones a full copy was retrieved.

An additional publication published after our search was included as well.

[11] https://clarivate.com/products/web-of-science/databases/, accessed on June 25, 2019

[12] https://www.scopus.com, accessed on June 25, 2019

7) Detailed selection based on full content

All selected papers could be retrieved.

Two persons continued to examine the retrieved publications on the full content. The references of the included studies were manually screened to search for further papers. No language or publication restrictions were applied, and studies were not selected based on quality.

8) Detailed data extraction

The key findings of the selected, full text papers were summarized.

Annex 2 List of persons interviewed and/or providing written input

Name	Function	Country
Mr. Peter Thygesen	Principal Regulatory Scientist, Evaluation Branch, Office of the Gene Technology Regulator	Australia
Mr. Helmut. Gaugitsch	Head of Unit, Landuse & Biosafety, Environment Agency Austria	Austria[13]
Mrs. Marion Dolezel	Landuse & Biosafety, Environment Agency Austria	Austria[13]
Mrs. Galina Mozgova,	Head of the National Coordination Biosafety Centre, Biosafety expert, Institute of Genetics and Cytology, National Academy of Sciences of Belarus	Belarus
Mr. Felicien Amakape	Ministère du Cadre de Vie et du Developpement Durable	Benin
Mr. Jim Louter	Biotechnology Section ; Emerging Priorities Division Environment and Climate Change Canada / Government of Canada	Canada
Mrs. Sylvie Braibant	Servicio Nacional De Salud Animal – SENASA National Animal Health Service	Costa Rica
Mrs. Catherine Golstein	Senior scientific and European affairs officer at HCB (Haut Conseil des biotechnologies / High Council for Biotechnology)	France
Mr. Armin Baike	Bavarian Health and Food Safety Authority (LGL)	Germany
Mr. Daniel Lewis	Chief Agricultural Officer, Ministry of Agriculture & Lands National Biosafety Focal Point for Grenada	Grenada
Mrs. Carolin Alduvin	Comité Nacional de Biotecnología y Bioseguridad Agrícola (CNBBA)	Honduras
Mr. Behzad Ghareyazie	President, Biosafety Society of Iran / Cartagena Protocol on Biosafety National Focal Point	Iran
Ms. Keiko Okamoto	Wildlife Division, Nature Conservation Bureau Ministry of the Environment,	Japan
Mr. Josphat N. Muchiri	Chief Biosafety Officer, Biosafety Risk Evaluation Department, National Biosafety Authority	Kenya
Mrs. Bagayoko Mama Diarra	Chef /Section Biodiversité /Biosécurité Point Focal Biosafety Clearing House (BCH) Suppléante au Point Focal Protocole de Cartagena AEDD	Mali
Mrs. Kine Rautio Øverland	Senior Adviser Norwegian Environment Agency, Department for water resources and knowledge management, Section for invasive species and international trade	Norway
Mrs. Angela Lozan	Biodiversity Office, manager ENPI, Ministry of Agriculture, Regional Development and Environment	Republic of Moldova
Ms. Ntakadzeni Tshidada	Cartagena Protocol Primary NFP Department of Environmental Affairs;	Republic of South Africa
Mrs. D.C.M. Glandorf	Senior risk assessor and policy advisor; Dept. of Gene Technology and Biological Safety/GMO Office; National Institute of Public Health and the Environment (RIVM)	The Netherlands
Ms. Hazar Belli Abdelkefi	Cartagena Protocol Primary NFP, Ministry of local affairs and environment,	Tunisia
Mr. Jonathan Mufandaedza	Chief Executive Officer & Registrar, NEPAD SANBio Steering Chair, CBD CPB-NFP, BCH-NFP, National Biotechnology Authority	Zimbabwe

[13] Both representatives of Austria were interviewed at the same time

Annex 3 Types of gene drives

Since the thirties of last century natural gene drives have been studied. Gene drives are present in nature in a variety of organisms and no single molecular mechanism underlies all gene drives.

Applications of gene drives are reported since the early 1970s. Several types of gene drives have been assembled using genetic modification techniques. The most important ones are shortly described below.

1. Underdominance systems

Underdominance systems are an example of this bidirectional type. Underdominance is described as negative heterosis, where heterozygotes are less fit than homozygotes. When introduced in a population of wild types by regular releases, the population will evolve either into all wild-types or all gene drive-bearing individuals. They are high threshold-dependent gene drives that act locally and can be removed through dilution of the population with wild-type individuals (Altrock et al., 2010). Several situations have been modelled using different values for fitness cost and migration from neighbouring populations (Buchman et al., 2018a). High threshold gene drives are likely to be most effective in replacing populations in isolated conditions. manufacture

Examples of engineered underdominance systems are created in *Drosophila melanogaster* using a toxin–antitoxin mechanism (Akbari et al., 2013; Reeves et al., 2014).

2. Meiotic Drive

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

Engineered meiotic drive-based systems have been based on endonucleases that target and cut several locations on the X chromosome during spermatogenesis (X-shredder) (Galizi et al., 2014; Galizi et al., 2016).

3. Maternal-effect dominant embryonic arrest

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

The first engineered MEDEA gene drive system was based on an RNA interference (RNAi)-based toxin–antidote combination (Chen et al., 2007). It was later applied in *Drosophila suzukii*, a serious agricultural pest, especially for soft-skinned fruits (Buchman et al., 2018b).

4. Homing endonuclease genes

Burt first proposed to use homing endonuclease genes (HEGs) as gene drives (Burt, 2003). These endonucleases are able to selectively disrupt specific gene sequences (target sequence), combined with a rapid spread in the population. Homing can be defined as:

> *The process by which an endonuclease cleaves a specific DNA target sequence and copies itself, or ‘homes’, into this target sequence. Homing utilizes the cell’s homology-directed repair (HDR) machinery, which relies on sequences that flank the endonuclease and that are homologous to either side of the target sequence. The ultimate result of ‘homing’ is to generate an exact copy of the endonuclease in the target sequence (Champer et al., 2016).*

Double stranded DNA breaks activate highly conserved cellular repair mechanisms. The DNA ends can be reattached together primarily by either non-homologous end joining or by homology-directed repair. If the chromosome homologous to the one that is cut contains the HEG it can be included as a template to fill the gap. The latter is accomplished because the endonuclease gene has sequences on either side that are homologous to the target sequence. The phenomenon transforms a heterozygote organism into a homozygote for the gene drive (Figure 2Figure 2 The spread of homing endonuclease gene drives (adapted from Esvelt et al., 2014)). If this successfully happens anywhere in the lineage of the cells that will form the germline, the frequency of the changed allele in the progeny will be higher than expected according to Mendelian rules. Non-homologous end joining may induce resistance to the HEG, as the repair mechanism often joins incorrectly thereby changing the HEG target site. Transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) are candidate endonucleases. However, the target site must be relatively unique in the organism's genome as otherwise severe fitness costs will results from DNA cuts throughout the genome. Finding a meganuclease (restriction enzymes with large recognition sequences) that matches these requirements proved difficult as is reengineering the recognition sequence of previously discovered ones.

A special endonuclease is the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/ CRISPR associated protein). The advantage of CRISPR-Cas over other known nucleases is that it uses an easily re-engineerable guide RNA to find its target. Unlike meganucleases, ZFNs, or TALENs, not the sequence of the endonuclease gene, but the guide RNA needs to be adapted to the desired target site. The basic design of a CRISPR/Cas gene drive consists of the CRISPR endonuclease gene, one or more guide RNA sequences and depending on the application a payload gene. The system is often introduced on a plasmid with on either side of the drive cassette sequences homologous to the target site in order to induce homing.

Esvelt and colleagues first suggested the use of CRISPR/Cas9 as a gene drive mechanism (Esvelt et al., 2014). The proof came in the next year for yeast (DiCarlo et al., 2015) and fruit fly (Gantz and Bier, 2015). In addition, CRISPR gene drives have been introduced in *Anopheles gambiae* (Hammond et al., 2016), *Anopheles stephensi* (Gantz et al., 2015) and *Candida albicans* (Shapiro et al., 2018). Homing rates in these studies are often very high (greater than 95%) and maintained over several generations (4 for *Anopheles stephensi* (Gantz et al., 2015) and 5 for *Anopheles gambiae* (Hammond et al., 2016)).

The first proposed HEGs have limited applicability as a gene drive because of their specificity, but they possess high rates of drive and can be exploited for both population suppression and replacement. The CRISPR/Cas system is highly efficient with a low release threshold, and easy to engineer.

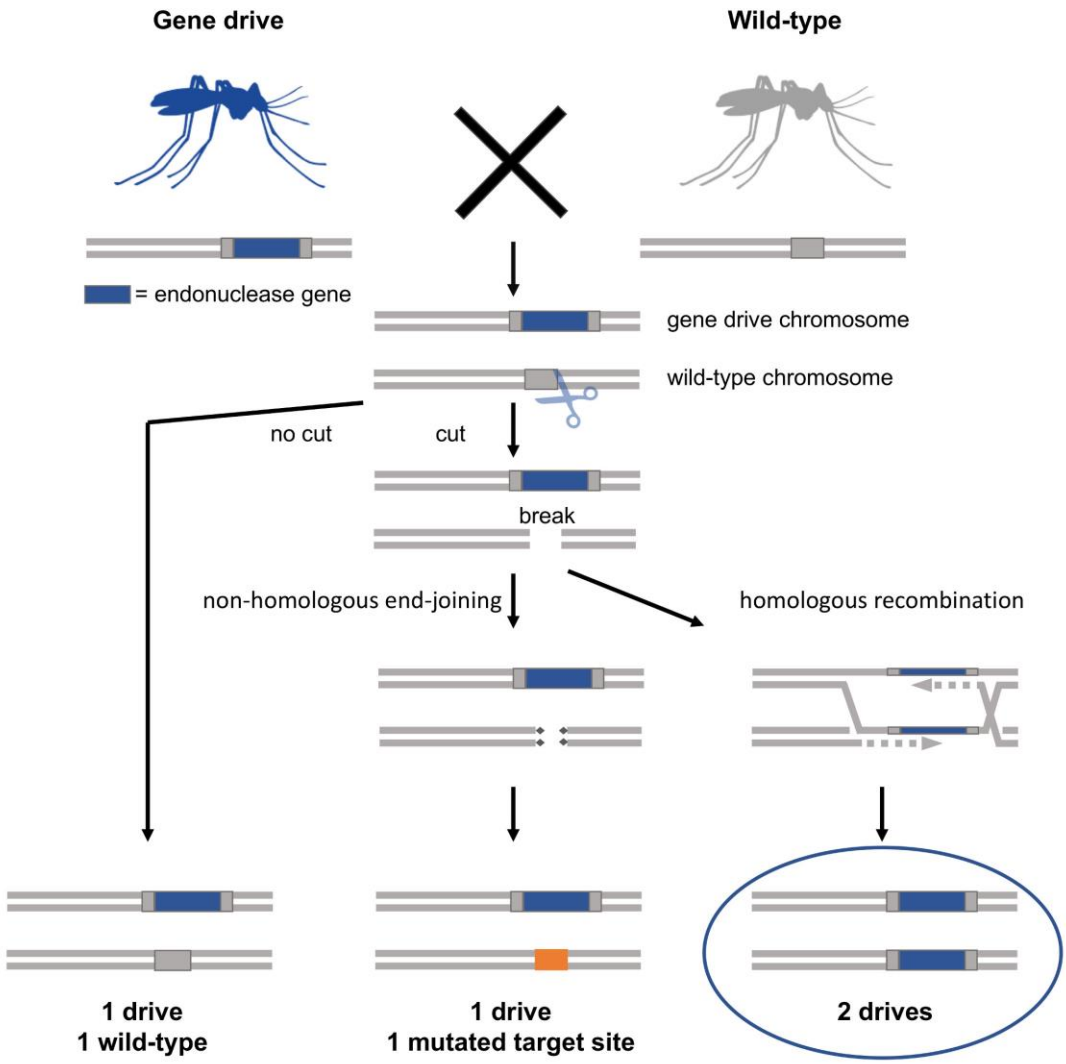

Figure 2 The spread of homing endonuclease gene drives (adapted from Esvelt et al., 2014)

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Annex 4 Elements for the risk assessment

This Annex provides more information to the summary presented in section 4 of the main text.

1. Applications

1.1 Broad diversity

In general, publications on gene drive applications focus on the efficacy of the system and influencing factors, and cover ecological effects only to a lesser extent. Irrespective, the selected papers deal with a variety of gene drive applications:

- human disease (tackling vector organisms),
- conservation (eradication of invasive species), and
- agriculture (disease/pest reduction, weed control).

It is not clear how one should categorize gene drives *per se*. In principle they represent a mechanism to bias inheritance independent of an external selective pressure. The mechanism in itself may have no influence on the performance and/or the characteristics of the host organism. This will on the other hand depend on the payload gene that is combined with the gene drive. In other cases, the gene drive combines the drive function with the introduction of the intended effect trait. In many of the interviews it was stressed that this can only be addressed at this state of knowledge by maintaining the case-by-case approach.

The broad range of organisms in which gene drives may be deployed, as well as the need to differentiate between suppression and replacement gene drive application, further justify a case-by-case approach. **It must therefore be stressed that considerations indicated in this section may be relevant for certain gene drives in certain organisms and are not applicable in a generic way for all gene drive applications**.

1.2 Introducing a modification tool rather than a finished product

Simon et al (2018) point out that as the modification introduces a gene drive system, the entire CRISPR/Cas toolbox is inherited. Although the gene drives are constructed in the laboratory, the drive is designed to genetically modify organisms in the wild, over and over again.

Whereas so far LMOs have been selected during R&D, tested and presented as finished and well characterized products; engineered gene drives imply the release of a tool that will continue to trigger further genetic modification of individuals in the target species once released. Non-intended effects, such as resistance or off-target effects, are difficult to predict and characterize before the release into the environment, particularly in genetically diverse wild populations.

1.3 Targeting non-domesticated species

Domesticated species do not seem to be the primary field of application of engineered gene drives. In domesticated species, man selects specific traits of the desired genotype via breeding and therefore somehow acts as the “gene driver”. Furthermore, cultivated populations are regularly replaced by man, which allows choice and adaptation to further improvements.

On the contrary, for non-domesticated species, engineered gene drives offer the possibility to reach into an entire population without having to replace each individual. Once released (taking into account the drive specific threshold), the trait will spread into the population.

Most of the experience with risk assessment of LMOs has been gained with environmental releases of modified domesticated -or at minimum cultivated- plants. In addition, a limited number of animal species, also cultured, like pigs and fish have been subject of environmental risk assessments. This

allowed risk assessors to refer to experience with the introduction of non-engineered organisms and thus rely on the comparative approach as the foundation for the risk assessment.

It has been pointed out that the choice of comparators may be challenging. Experience with introduction of wild populations may be limited. Also relevant data on population dynamics as well as ecosystem functions and services may not be available. On the other hand, the risk assessment of insects with engineered gene drive systems can build on existing knowledge and experience with vector control programs using insects that do not contain gene drives (*e.g.* sterile insect technique (SIT); incompatible insect technique (IIT)).

1.4 Targeting non-managed environments

Whereas domesticated species are introduced in managed environments and by nature of the species are mostly limited to man-managed environments, the most advanced engineered gene drive applications target releases not limited to specific environments. In fact, the gene drive would allow reaching into non-managed (also perceived as uncontrolled) environments, for which there is less experience in conducting risk assessments. While some risk assessors indicated that the possible impact on non – or less managed environments is part of risk assessments performed so far, these are however in most cases deemed of minor importance as domesticated species may not have an important role outside managed agricultural settings. For most of the initial gene drive applications this would be inherently different. Nevertheless, this would also be a valid consideration for other applications in a non-domesticated species and therefore is not specific for gene drives.

Some applications have been named “global drives”, indicating that they continue spreading until they affects a species globally. In case of a suppression drive this could theoretically lead to the eradication of a species. Local drives are likely to only work for a limited time, geography or number of generations.

1.5 Managing a stepwise approach

In line with the precautionary approach, scientific uncertainty must be reduced in order to advance through R&D. In the stepwise pathway to deployment, experience gained and data established during preceding steps is used as the basis for the risk assessment of the next, less limited step. As pointed out by James and Tountas (2018) *“it should be emphasized that continued research is the only way to decrease the uncertainties that underlie the perception of risk”.*

Nevertheless, the predicted ease of spread of engineered gene drive organisms, in particular for low threshold systems, combined with the perception that an introduction is irreversible calls for extremely thorough evaluation under careful confinement before release into an open environment.

Conducting trials in a geographically isolated location (*e.g.* the *Wolbachia* releases described by Zheng *et al.* (2019), can provide for an intermediate step. Still, a risk assessment for such release will have to take into account possible dispersal beyond the release site.

Some authors suggest to include a self-limiting step in the development pathway (James et al., 2018). Self-limiting constructs constitute a form of biological or molecular confinement, which would supplement physical and ecological confinement. Testing of a self-limiting intermediate could be a safer option before moving to the field with the “real” gene drive.

2. Effect on the gene drive-bearing organism

2.1 Off-target modifications

Off-target effects within the recipient organism is a concern for gene drive systems that are based on a gene editing technique and RNAi methods. Off-target cutting by a HEG gene drive may lead to the loss or modification of native traits with potentially effects on the survival, behaviour and breeding success of the organism. This concern is linked to possible unspecific recognition of target sites in the

genome and is not specific for gene drives. Off-target effects are often mentioned, but no data are available on their frequency.

Rather than assessing the possible impact of off-target effects of homing endonucleases, methods are pursued to minimize them including optimization of guide RNA design and of endonuclease cutting efficiency (Champer et al., 2016; Esvelt et al., 2014; Macias et al., 2017; NASEM, 2016). The former makes use of predictive software to identify other sequences that guide RNA may target (Bae et al., 2014; Tsai et al., 2015; Xie et al., 2014). The latter makes use of mutant versions of the endonucleases to address the efficacy and specificity (Davis et al., 2015; Slaymaker et al., 2016). A prerequisite is the availability of genome data sets from wild-caught mosquitoes or other target organisms (Macias et al., 2017). The Ag1000G international collaboration aims to provide a high-resolution view of genetic variation in natural populations of *Anopheles gambiae*[14]. Once a homing gene drive is introduced, off-target effects may be measured in several ways (Koo et al., 2015). The potential impact of off-target mutations is bigger in replacement gene drives compared to suppression gene drives, since gene drives aimed at eradicating a population will also eliminate unintended mutations.

2.2 Stability of the engineered gene drive system

Gene driver-cargo systems risk to unlink the payload gene, *e.g.* that targets a pathogen, and the elements of the drive system (Alphey, 2014). The driver may then continue to spread without having the desired effect on the population.

Modelling may help in determining the likelihood of adverse effects occurring including the probable spread of the transgene, mutation rates, and the effects on the phenotypic profile of the local insect population (Benedict et al., 2008). However, models are as precise as the designer is able to mimic natural situations. Nevertheless, models may be interesting in visualising the effect of changing parameters.

A model was constructed were male mosquitoes are introduced in a population that have a meiotic drive gene located on the Y-chromosome and a drive-insensitive response allele coupled to an antipathogen factor on the X-chromosome (Huang et al., 2007). When the cost for the drive gene and drive-insensitive response allele are weak the latter will go to fixation according to the model. Soon after the frequency of the drive gene in the population will diminish due to its fitness disadvantages over the non-drive gene. With high fitness cost the drive gene will quickly disappear after reaching its maximum. In the long run the population experiences an oscillation in frequencies of drive gene and a drive-insensitive response allele with the antipathogen gene. Still according to this model, in case more alleles exist for the response gene, the antipathogen factor will either disappear or find an equilibrium or exhibit stable periodic oscillations, depending on the sensitiveness of the other response alleles. Another complicating factor is the potential existence of a modifier gene that diminishes the response of the X-linked response allele to the drive gene. Again, depending on the fitness cost there will be a weak or strong selection for the modifier gene and the antipathogen gene will go extinct after a transient increase in frequency, or simply does not spread.

A recombination that might uncouple the drive-insensitive response allele and the antipathogen gene will reduce the effect of this strategy for disease control.

2.3 Modified susceptibility

Another concern, theoretically, may be the ability of the vector organism to have modified competency for pathogen transmission (Benedict et al., 2008; David et al., 2013). A mosquito that is modified so

[14] https://www.malariagen.net/projects/ag1000g

that it could not host the pathogenic virus (population replacement), may in theory become a more susceptible host to another existing or new virus that harms human health (NASEM, 2016).

3. Considations for biodiversity

Regarding the potential effects on the environment a distinction should be made between population suppression and population replacement drives. They may have the same ultimate goal, *e.g.* eradication of an insect-borne pathogen, but they have different implications for potential environmental interactions. In contrast to earlier GM applications (*e.g.* SIT), in a population replacement drive the GM trait is intended to persist in the environment.

The extent of the effects on the ecosystem depends on whether the target organism is a "keystone" species in the environment, or whether there are ecological equivalents present. Moreover, pathogen-host systems and predator-prey systems are co-evolving systems, *e.g.* removing a noxious weed may endanger pollinators depending on the plant considered to be a weed. Moreover, proteins introduced into organisms (including gene-drive components or markers) should be tested for toxicity to other species such as predators (Roberts et al., 2017).

A gene drive may be used to eradicate an invasive species. Although the intent may be the re-establishment of the original species diversity, the elimination of an invasive species may not restore the original ecosystem. Indeed, damage by the invasive species may have gone too far, inducing irreversible changes.

3.1 Target organism

Here "target organism" is used as a synonym for the gene drive host organism (as opposed to *e.g.* the pathogen that is targeted to be eliminated). Suppression gene drives may result in the extinction of (a local population of) the target organism. Although the target species may be locally affected (even eradicated), this must be seen in the light of other control techniques, but may be expanded and more effective with powerful gene drives.

3.2 Non-target organisms

Potential non-target effects are often raised as concern. Although knowledge is gradually increasing (*e.g.* Collins et al., 2019), there is hardly (field) information available. Also, since gene drives are based on mating potential, the potential for exchange with related species is very species specific (Alphey, 2014).

Effects on non-targets organisms may act directly, *e.g.* due to hybridization between related species, or act indirectly *e.g.* due to trophic relationships. While the transfer of a transgene to wild relatives maybe considered an undesirable effect in case of a genetically modified domesticated species, the likelihood is in most cases low and the impact limited. However, for gene drive organisms, inheritance and spread of the transgene is a required prerequisite (Simon et al., 2018). Unintentional transfer of an engineered gene drive into a beneficial, threatened, endangered, neutral, or valued species could lead to its extinction. *E.g.* a gene drive intended for eliminating/controlling a noxious weed could be transferred to a related food crop via vertical gene flow (*e.g*. pigweed in USA vs crop amaranth in Central and South America). The gene drive would not have an effect on the sexual compatibility of the species, which would still allow for understanding the limits of the genetic exchange.

Another aspect is the reduction of the target organism that may increase the population of other species (niche replacement). Elimination of a pest species may clear the way for another pest to fill in the niche. Removing one vector could allow another potentially harmful species to take its place. Again, these effects are not different from effects resulting from techniques that do not make use of gene drives, but are aiming as well at the eradication of a species. Technologies for population replacement instead of population suppression are therefore likely to induce less ecological harm. As the species is still present, no empty niche is created. Vertical gene transfer is still possible but is not likely to cause species elimination, since the gene drive is not lethal. However, in self-limiting strategies the gene

drive is expected to disappear over time, in this way also reducing the potential for *e.g.* vertical gene transfer.

Nonetheless, data are not available to check these considerations.

3.3 Other trophic levels

Extinction (or reduction of abundance) of the gene drive-carrying species can have consequences for *e.g.* predators, competitors, prey, due to its ecological role, such as resource, consumer, competitor, or disease vector. These links create dynamic feedbacks that affect the relative abundances of different species. On the other hand, an even transiently increased population size may potentially have long-lasting ecological consequences (Rode et al., 2019). However, indirect effects on food webs or ecological functions are not specific for gene drives. They are equally important in classical biocontrol strategies and these may provide information for risk assessment.

3.4 Alternative protection mechanisms and herd immunity

If the gene drive is only partially successful in suppressing *e.g.* an insect vector, the result could be loss of immunity, *i.e.* individuals within the population may become more susceptible to the disease as the vector recovers from the initial suppression (David et al., 2013; James, 2005). In an area with high malaria incidence people acquire immunity after several attacks of malaria. These people remain infectious, but may lose their acquired immunity when they stop contracting malaria.

Although a replacement drive may successfully eradicate a certain pathogen, it leaves the vector in place. If a resistant pathogen emerges it could spread back rapidly. Especially, if the temporary absence of that pathogen resulted in less strict use of other control measures, *i.e.* pesticides and bed nets. Population suppression of the vector may be safer in this regard.

4. Resistance development

4.1 Resistance to the engineered gene drive system

The presence or development of resistance against a gene drive system, will reduce its efficiency in the host population, but will also limit the potential impact. It is therefore relevant for the risk assessment to acknowledge that gene drive systems may be particularly susceptible to resistance development.

HEGs in general are prone to the development of resistance alleles that are insensitive to conversion by the drive system (Hammond et al., 2017). Most often this is caused by non-homologous end joining, resulting in disruption of the target site of the endonucleases (see Annex 3 Figure 2.). In *Anopheles gambiae* resistance to the homing endonuclease I-PpoI was observed in the low amount of female survivors due to misrepair and copy number variation of the ribosomal gene cluster (Galizi et al., 2014).

Already in the first publication on CRISPR/Cas9 gene drive resistance was mentioned (Gantz and Bier, 2015). A single-nucleotide change at the guide RNA cut site and an in-frame insertion-deletion (indel) most likely resulted from non-homologous end joining repair. These mutations appeared in the first generation after crossing. Resistance alleles also appeared in experiments with *Anopheles stephensi* (Gantz et al., 2015), *Anopheles gambiae* (Hammond et al., 2016) *and Drosophila melanogaster* (Champer et al., 2017; Champer et al., 2018).

Modelling several CRISPR/Cas9-based strategies to eliminate exotic mice from islands show that multiplex guide RNAs are needed to overcome resistance development due to non-homologous end joining to be successful (Marshall et al., 2017; Prowse et al., 2017). However, multiplexing guide RNAs in gene drives has only been experimentally studied with 2 guide RNAs (Champer et al., 2018). The drive conversion efficiency increased, but to a lower degree than theoretically expected. Possible causes may be the saturation of the Cas9 enzyme, the distance between the target sites and the simultaneous cutting of the 2 sites. The first experiments with mice, however, show that the non-

homologous end joining repair pathway already in the development phase may hinder the construction of a gene drive-bearing mouse (Grunwald et al., 2019).

Natural sequence polymorphisms in the population and *de novo* mutation of wild-type alleles could also prevent cutting (Unckless et al., 2017). Drury and co-authors modelled the effect of existing polymorphism in *Tribolium castaneum* (Drury et al., 2017). Even a non-cutting polymorphism at a low frequency can severely limit the spread of a very deleterious gene drive, such as one causing infertility. For a drive with low fitness cost it will take longer, but eventually the drive is predicted to disappear from the population.

Furthermore, any HEG that reduces the fitness of its host will face the potential evolution of resistance (de Jong, 2017; Godfray et al., 2017; Unckless et al., 2017). When resistant alleles are still functional, they will replace the costly drive allele. Thus, even though a gene drive may initially spread to high frequency in the population, its ultimate fate will depend on whether resistant alleles have emerged during this process. To prevent the spread of resistant alleles, it will be necessary to target genomic sites that cannot tolerate changes, *e.g.* active sites of proteins, conserved regions in genes (Deredec et al., 2011; Godfray et al., 2017).

Another path to gene drive resistance would be that the gene drive containing organism develops a method of specifically inhibiting the drive endonuclease (Bull, 2015; Esvelt et al., 2014). However, Esvelt et al. hypothesize that inhibitors of Cas9 are less likely to arise given the historical absence of RNA-guided nucleases from eukaryotes (Esvelt et al., 2014). Other mechanisms may be at play, *e.g.* overexpression of an RNA that competes with the guide RNA. Also, the driver construct itself may mutate preventing it from driving (Unckless et al., 2017). The zinc-finger nuclease and TALEN-based gene drives in *Drosophila* underwent recombination between repetitive sequences (Simoni et al., 2014). As a result only 75% and 40% of each respective drive was sufficiently intact after one copying event to catalyse a second round of copying. Because RNA-guided gene drives will not include such highly repetitive elements, they are likely to be more stable (Esvelt et al., 2014).

Resistance may be part of a scheme to confine the gene drive to a smaller geographical area or a certain time period (a number of generations), when short-term population transformations are aimed at (Champer et al., 2016; Esvelt et al., 2014; Unckless et al., 2017).

Also, with other drive mechanisms resistance is observed. Examples include:

- In an experiment with a synthetically engineered MEDEA gene drive system based on RNAi in *Drosophila suzukii* mutations were found in the miRNA target sites of a small number of wild-type offspring (Buchman et al., 2018). It was postulated that the efficiency of the miRNAs is influenced by naturally occurring genetic variation.
- In the synthetic MEDEA construct described in Annex 3 point 0, the toxin encoding miRNAs, or the promoter driving their expression, can mutate to inactivity, resulting in a non-functional drive (Akbari et al., 2014).

Another factor is behavioural resistance. The target population structure in space and time, its mating system and density-dependence, age and social structure may hinder the engineered gene drive to invade (Rode et al., 2019).

4.2 Resistance to an effector

Resistance development to an effector is a common phenomenon that can occur in each species, *e.g.* a pest developing resistance against a management strategy. Resistance can occur by selection of resistance of the target pathogen to the effector gene, or the selection of increased virulence of the target pathogen (Alphey, 2014; Franz et al., 2009; James, 2005). It is undesired and, under certain legislative frameworks, resistance development is also considered as an environmental concern. It was therefore included in this study.

5. Effects beyond the target

The spread of an engineered gene drive outside the intended geographical area could potentially change the environmental landscape well beyond the site of its introduction. A drive to eradicate an invasive species may be accidently introduced in its original environment, *i.e.* where it is endemic, where it has a function in the local ecosystem or is important as a human food source (*e.g.* eradication of exotic species from an island). Gene flow to other populations of the same species depends on the mode of dispersal between populations: human assisted, as a result of a disruptive event (*e.g.* fire, hurricane), normal movement of organisms, or as a result of habitat unsuitability (crowding, no nesting sites, …) (NASEM, 2016).

5.1 Dispersal

North and colleagues modelled the spatial spread of a HEG in *Anopheles gambiae* depending on the landscape characteristics (North et al., 2013). Landscapes were generated that differed in their densities of mosquito feeding and breeding sites. Where these mosquito resources are sparsely distributed (disconnected population structure), the HEG can drive the local population to extinction. But, wild-type mosquitoes can recolonize afterwards. Denser resources may lead to either extinction or population suppression depending on the HEG load. Seasonal variation, active or passive dispersal are not included and would make the model even more complex.

Especially low threshold drives may have widespread consequences across national borders. Concerns arise from the accidental release of just a few organisms containing gene drive systems. Also, dispersal may create political tensions with bordering countries that may not have approved the technology (Macias et al., 2017).

Using high threshold drives help confine the spread of a gene drive to a local breeding population (Australian Academy of Science, 2017). Marshall and Hay studied the possibility of replacement drives becoming established at their release site without spreading into neighbouring populations using a simple model where a drive is introduced in a population with exchanges with a neighbouring population (Marshall and Hay, 2012). Several gene drive types were examined. The invasive MEDEA gene drive could not be confined to an isolated population unless it is associated with a very large fitness cost. The same is true for the highly invasive HEGs. Transposable elements, when capable of spreading, show the same picture. Migration thresholds (below which no spread into the neighbouring population occurs) for these systems are unrealistically low. Underdominance systems display higher migration thresholds, next to a high release requirement.

A ‘daisy chain’ CRISPR/Cas gene drive where each genetic element drives the next is a gene drive that would stop after a few generations (Noble et al., 2019). This would limit the capacity of the engineered gene drive to spread. It would be a way to temporarily and locally replace a population.

Modelling may help to understand population dynamic effects (*e.g.* seasonal fluctuations, density dependency) that can directly influence control strategies (Alphey, 2014). Comparing one-locus underdominance, two-locus underdominance, and daisy-chain drive, modelling reveals that the daisy-chain drive is the least capable of remaining localized due to the low threshold frequency (Dhole et al., 2018). In contrast, using a split drive with a molecularly unlinked endonuclease, could allow to spatially and temporally confine the drive (Li et al., 2019).

An RNA-guided gene drive could also be used to block the spread of other gene drives by recoding sequences targeted by the unwanted drive (‘immunising’ drive) (Esvelt et al., 2014). A proof-of-principle was delivered for a Cas9-ablated chain termination system that functions as a brake (Wu et al., 2016). The cas9 sequence itself contains the target site for the guide RNA that becomes inserted in the gene and as a consequence expression of the endonuclease is lost. Any population containing cas9 may be stopped from driving.

A daisy quorum drive that combines daisy chain drive characteristics with underdominance could theoretically be used to limit the drive locally and allow for reversal by introducing wild-type individuals (Min et al., 2017).

Vella et al. modelled the effect of some proposed countermeasures for CRISPR/Cas drives (Vella et al., 2017). Replacement drives with synthetic resistance alleles and reversal drives are not guaranteed to eliminate a homing drive from a population due to the existence, in general, of a stable polymorphic equilibrium where both systems co-exist. An immunising reversal drive that targets both the homing drive and wild-type alleles has the best chance to remove the drive. However, cas9 gene and guide RNAs will remain in the population.

A strategy to confine a gene drive to suppress an invasive species might be to first introduce a standard drive to insert a unique sequence followed by a suppression drive that is targeted to that unique sequence (Esvelt et al., 2014). Alternatively, the invasive population may be made sensitive to pesticide using a gene drive. The application of the pesticide would then only affect the invasive species. Prowse et al. modelled four realistic CRISPR/Cas9 gene-drive strategies to eradicate exotic vertebrates (Prowse et al., 2017). They used heterozygotic XX sterility, heterozygotic XX sex reversal, homozygotic embryonic non-viability and homozygotic XX sterility in combination with multiplexed guide RNAs modelled in mice. Simulations reveal that only the latter two approaches lead to eradication in 4 to 5 years provided that 3 or more guide RNAs are used.

6.0 References

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